Synthesis of Azetidinium Salts and their Applications on Nano-cellulose

Master's thesis in Materials Chemistry

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Gothenburg, Sweden 2023
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Master's Thesis 2023

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Abstract

Cellulose is a biopolymer with many fantastic applications, ranging from its use as building material to its refined products making up paper and packaging or being used in medical applications. To further utilize cellulose and its composites, new methods are needed to modify its surface and chemistry. In this project, Azetidinium salts (Az-salts) are used to modify sulphate half-ester groups on the surface of cellulose nanocrystals (CNC). Az-salts are a form of cyclic amines and are especially reactive towards certain nucleophiles, which is utilized to make them conjugate to the sulfonated CNC. 17 different salts were investigated, aiming to deepen the understanding of these Az-salts. Of them, 10 were successfully synthesized using a standing procedure. Further, the surfactant Sodium Dodecyl Sulphate (SDS) was successfully used for the synthesis of long alkyl chains containing salts. The synthesis of four of the 17 salts was investigated using Fourier Transform Infrared Spectroscopy (FTIR) to gain intelligence of the reaction and its behaviour. It was concluded that all tested reactions need to run for longer than 5 hours at room temperature for full conversion. One salt was monitored using Nuclear Magnetic Resonance spectroscopy (NMR). The reaction was successfully monitored. Lastly, three salts were conjugated to sulphonated CNC.

Keywords: Azetidinium salt, nano crystalline cellulose, surface modification, synthesis.

List of abbreviations

Az-salt	Azetidinium salt
$CDCl_3$	Deuterated chloroform
CNC	Nanocrystalline Cellulose
DAA	Dialkyl amine
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
ECH	Epichlorohydrin
EtOAc	Ethyl acetate
EtOH	Ethanol
FTIR	Fourier Transform Infrared Spectroscopy
*i*PrOH	Isopropanol, 2-Propanol
MAA	Monoalkyl amine
MeCN	Acetonitrile
MeOD	Deuterated methanol
MeOH	Methanol
MWCO	Molecular weight cutoff
NMR	Nuclear Magnetic Resonance Spectroscopy

Acknowledgement

First and foremost, I would like to thank my supervisor Gunnar Westman for all the encouragement and support throughout this past year. It has been exciting to work with you and I am very happy that we will continue to work together throughout my PhD.

To my happy Free-Shavoca-Doo (you know who you are), thank you for all the coffee and lunch chats, random adventures, and times in the lab!

To Amit, thanks for all the help with the cellulose, lunches, and good jokes! I wish you good luck in Finland!

To the rest of the office-gang, thanks for the good spirit, happy dances, and music brakes (especially Agnes)! I hope I wasn't too loud and distracting, and August; I'm sorry.

And of course to my family and friends, thanks for being there when it wasn't all smooth sailing.

Jelka Feldhusen, Gothenburg, March 2023

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1. Introduction

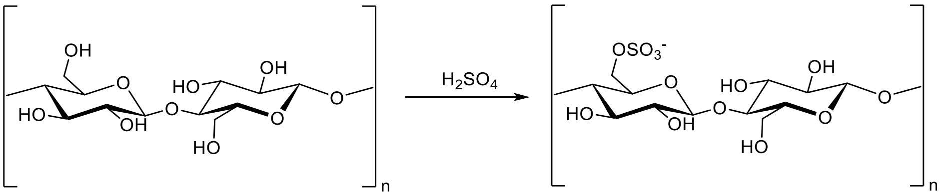
Cellulose is often stated as being the most abundant biopolymer on earth and has received much attention over the years. [1] Cellulose is found in many different places on earth, coming from obvious sources such as wood, plant residues, and algae, but also from animals and bacteria. [2] Not only do we use cellulose as a largely unrefined material such as wood for building, but the derivative forms are also used for paper, packaging, insolation, and medical applications. Several methods have been developed to acquire derivatives of cellulose to tune its properties and gain desired application possibilities. [1]–[7]

When cellulose is processed into paper, pulp fibres are extracted by separating it from lignin and other components. These extracted pulp fibres may be treated further, either mechanically or chemically, to produce finer particles like cellulose nanofibrils (CNFs) and cellulose nanocrystals (CNCs). The main difference between them is their structure, CNFs are more amorphous whereas CNCs are highly crystalline with a size of less than 500 nm. [4], [6] These CNCs are rod-shaped particles and are believed to have a hexagonal cross-section. When sulphuric acid is used to manufacture CNCs, sulphate groups are situated on the primary hydroxyl groups on the surface of the crystallites, Figure 1.These sulphate groups give the crystallites a very high colloidal stability, resulting in them being stable in water, creating suspensions of CNC [8].

Figure 1. Overview of the structures of CNCs. From left to right: Illustration of crystal with sulphur half-esters on the surface. Illustration of CNC crystal with yellow dots representing the sulphate groups. Hexagonal cross-section of the CNC crystals.

Nanocellulose and CNCs inherit the excellent properties found in cellulose, that is, being light weight, having high tensile strength, and having high specific surface area. From these nanoparticles, cellulose nano papers (CNPs) can be created, which are thin films with great physical-chemical properties like tuneable optical transmittance, high thermal stability, and good mechanical properties. [3] Even though these properties are desirable, and films made from CNCs are promising, they still have their challenges, for example being sensitive to moisture because of their hydrophilic nature. [9], [10]. One way to combat this could be to make them more hydrophobic which may be achieved by altering the surfaces of the CNCs. A method to do so is to convert the sulphate half-ester groups is by conjugation of Az-salts containing long alkyl chains.

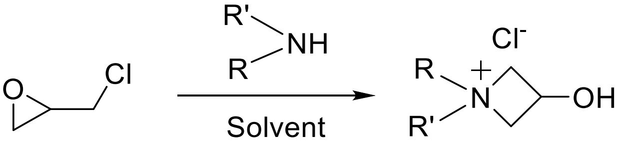
In this thesis, cellulose microfibrils are hydrolysed with sulphuric acid to produce sulphonated CNC. In addition to hydrolysing the amorphous part of the cellulose, an exchange of some surface hydroxyl groups found on the glucose units of cellulose also occurs. These are replaced by sulphuric half-ester groups, $-OSO_3^-$ and thereby forming charged nanocrystals, see Scheme 1. The number of sulphate-substituted hydroxyl groups can be varied, altering their solubility and their possibility to be functionalised. [11] The sulphate group on the cellulose can act as a handle, enabling further rection on the CNC altering its properties.



Scheme 1. Modification of CNC with sulfuric acid, replacing some -OH groups with -OSO_3^-. [11]

Organic chemistry often goes hand in hand with materials chemistry as one needs ways to produce materials and chemically alter them to achieve the desired characteristics. As one can imagine, there are a nearly infinite number of ways in which materials can be made and modified to fit certain applications. However, one must simultaneously consider that although possible, certain ways may not be the most feasible, mostly because aspects such as scalability, reaction efficiency, costs and waste management may make an excellent material unfeasible for practical purposes. Other limitations may lie in that the reactant(s) may be toxic or hazardous in some way. Hence, it has lately become a great focus in organic chemistry to simplify reactions and find greener and safer alternatives.

Azetidines, 4-membered cyclic amines, have been shown to have great applications in the pharmaceutical field and polymer chemistry. [12] Within these azetidines, azetidinium salts (Az-salts) have sparked interest as they may be just as versatile as, for example, ionic liquids. Az-salts are rather easily produced by reacting bulk chemicals epichlorohydrin (ECH) with a dialkyl amine (DAA) containing the desired alkyl chains, Scheme 2. They may also be made by reacting a monoalkyl amine (MAA) with ECH to form an azetidine ring, which can be quaternized with an alkyl halide [13], [14]. Because of this, Az-salts can also be chiral, making them of interest in the fields of pharmaceutical chemistry [15].



Scheme 2. Formation of Az-salt from epichlorohydrin and dialkyl amine.

Nonetheless, ECH is toxic, cancerogenic, flammable and corrosive, and must be used with care. Research has been conducted to produce epichlorohydrin from glycerol [16], [17], as the yearly demand for ECH is estimated to be around 2.77 million metric tons worldwide in the year 2029 [18]. Today, ECH is mostly produced by a multi-step synthesis of propylene and chlorine, producing 1,3-dichlorohydrin and 2,3-dichlorohydrin of a ratio of 3:1. The product mixture is lastly treated with an alkali to gain the desired ECH. Although widely used, the yield in terms of chlorine usage is low and because of the bad atom economy of the reaction, alternative synthesis pathways are explored, such as the production of ECH from glycerol. [17] As a result, different new methods are explored such as the reaction of glycerol with HCl and NaOH [17], or the use of a heterogeneous hydrotalcite-derived mixed oxide of Al and Mg [16], both showing promising results [16], [17]. Despite the production difficulties, ECH is still used as one of the primary reagents in this thesis as it has many advantages compared to its drawbacks.

A summary of the Az-salts explored within this study is illustrated in Figure 2. The salts are organized by functional groups and chain length. The salts were selected based on diversity, aiming to investigate Az-salts with different properties and solubilities to further investigate the standing synthesis procedure.

To find a universal method and monitor the reaction, *in situ* Fourier Transform Infrared Spectroscopy (FTIR) was used. This is based on inserting a probe into the flask in which the reaction occurs, tracking changes detected in the infrared region, thus measuring vibrations of bonds which may be formed or broken. The salts investigated further as "model compounds" for this are marked with "FTIR" under their structure. Furthermore, some of these Az-salts were studied as a possible reactant for the modification of cellulose and are denoted as "CNC".

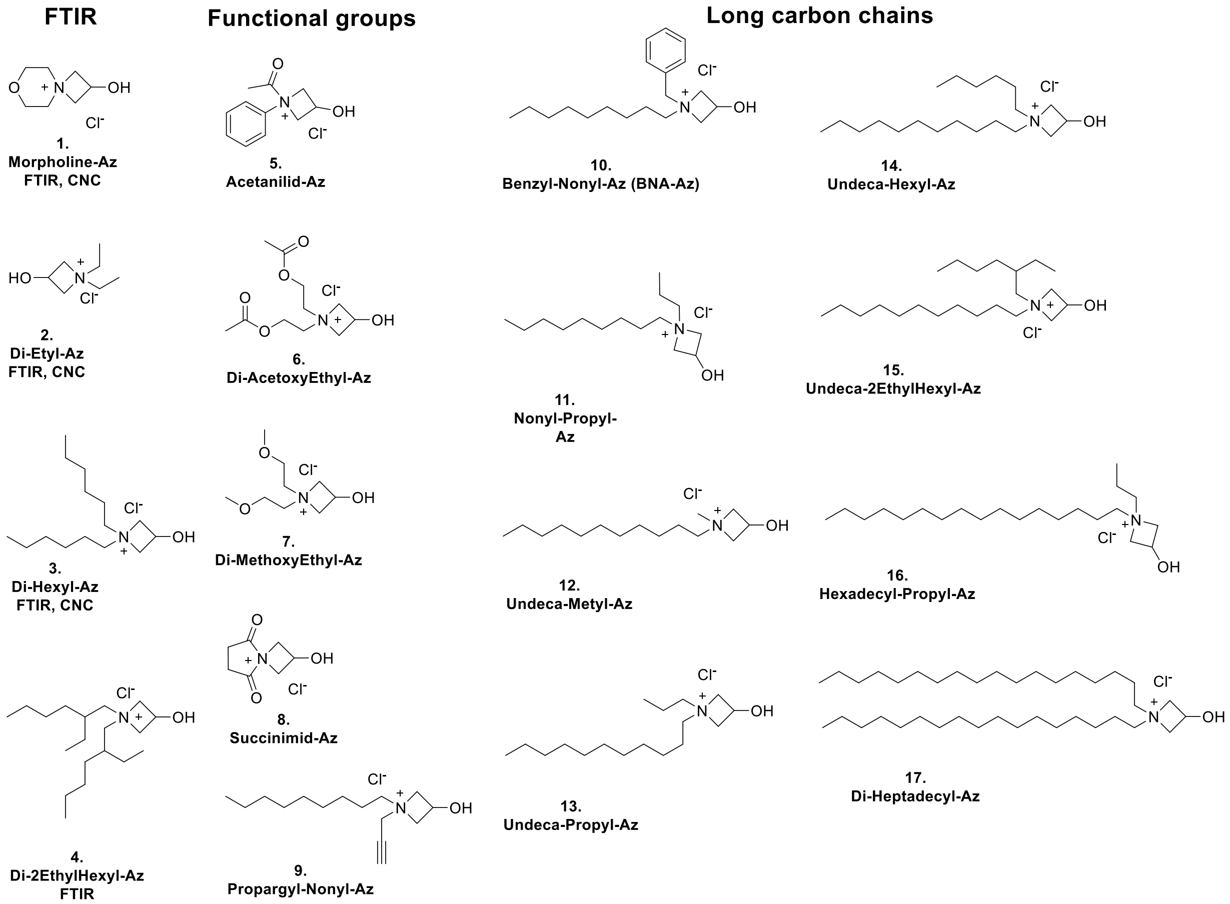
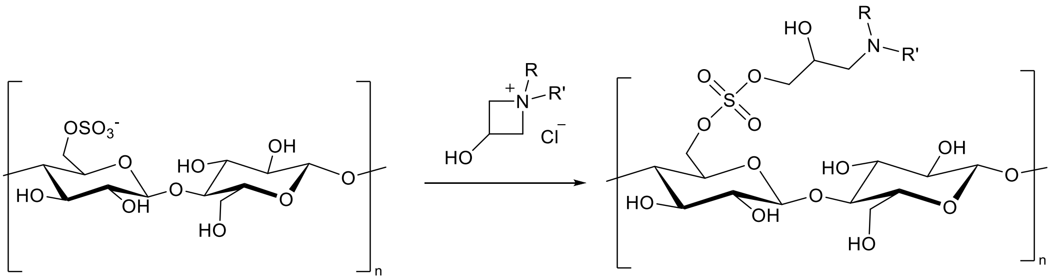


Figure 2. Structure and names of synthesized salts. "FTIR" after the name denotes that the reaction was followed in situ using FTIR. "CNC" denotes that the salt has been conjugated to CNC.

The conjugation of the Az-salts occurs at the carbonyl/sulphate groups on the CNC. This is as the carboxylates and sulphate esters behave like nucleophiles, enabling the opening of the Az-salts, Scheme 3. [8], [11] The conjugation results in a modification of the CNC and has been shown to affect the flexibility and transparency of films formed from CNC derivatives. Additionally, by conjugating salts, another linker may be introduced which may selectively react further. The conjugation is performed directly in the CNC-water suspension.



Scheme 3. Conjugation of Az-salt to sulphonated CNC. [10], [14]

1.1 Objective and Aim

This thesis aims to build upon already existing knowledge about the synthesis of Az-salts and their conjugation to CNC. The objective for this project thus becomes to deepen the understanding of the fabrication of different Azetidinium salts, and their reproducibility and to understand the challenges that arise with each salt in question. The new developments in this thesis are to explore the reaction rate spectroscopically, by IR and NMR. Further, this thesis aims to deepen the understanding of the reaction with dialkylamines with alkyl chains longer than 12 carbon, molecules that likely form micelles in water and low-carbon alcohols. Furthermore, the conjugation of different Az-salts to CNC is investigated, exploring more of the interaction between CNC and salt, thereby creating novel properties. The overall focus of this thesis is to use sustainable chemicals and as simple procedures as possible to minimize the negative environmental impacts.

1.2 Specific questions of interest

This project aims to understand more about the synthesis of a variety of different Az-salts. The specific questions to answer during this thesis were the following:

i. Is it possible to synthesize the azetidinium salts of interest in a scalable way, i.e. on a gram-scale?
ii. Is it possible to monitor reactions using FTIR and NMR, and develop a general protocol for the synthesis of azetidinium salts, independently of side chains in the DAA?
iii. Can these Az-salts be used to modify biopolymers?

1.3 Limitations

The scope of this thesis is to extend a current project in the Westman group. As an infinite number of salts may be investigated, the number was limited to an array of 17 salts containing linear and branched carbon chains of different lengths, and a variety of functional groups, as described previously in the introduction. The synthesis of four salts was selected to be investigated with *in situ* FTIR, chosen on a variety in chain length of the alkyl chains and functionality, as well as being bases on previous knowledge about the reaction. One reaction was investigated using NMR. Furthermore, only one concentration of sulphonated CNC was used for conjugation of Az-salt.

2. Theory

In this part, the theoretical background necessary for this thesis is described, starting with the definition and explanation of cellulose, to how Az-salts are synthesized and how important techniques function.

2.1 Cellulose and CNC

Cellulose is a complex biopolymer with many different hierarchical levels and an infinite number of applications. As stated, cellulose is present everywhere in society ranging from being an invaluable building material to being used in papers and pharmaceuticals, amongst many others. One comprehensive illustration of cellulose, by Chen C. and Hu L., [6], is provided in Figure 3. The illustration describes the many different levels where the complexity truly is visible.

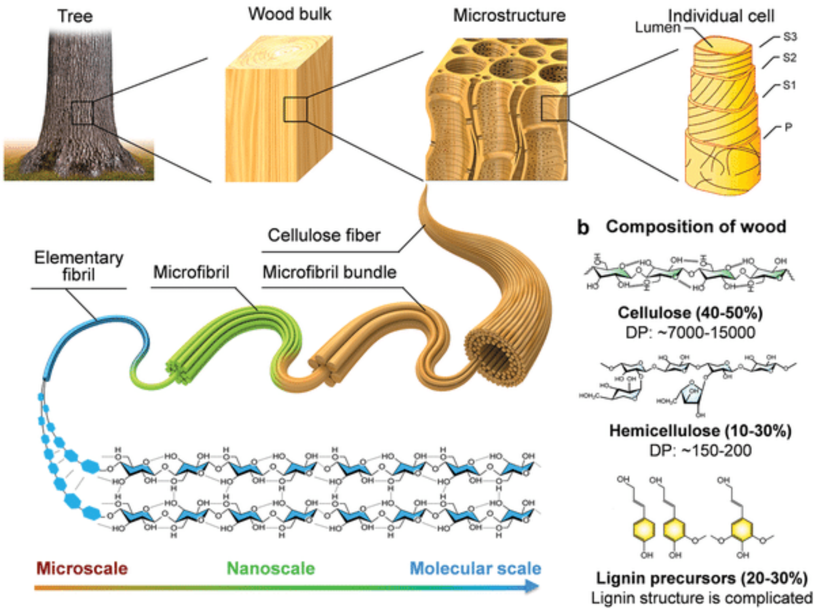


Figure 3. Illustrations of all levels in cellulose by Chen C. and Hu L. [6]

In this thesis, cellulose on the nanoscale is of interest, more specifically, nanocrystals of it. Wood cellulose is made up of a mixture of hemicellulose, lignin, and cellulose. The different components are utilized differently depending on the purpose but also require different methods of extraction and modification. As nano cellulose is far down in the hierarchy, many pre-treatments need to be made to extract it from the raw material, performed either chemically or mechanically.

As Figure 3 visualises, cellulose is a polymer consisting of β-D-glucopyranoses, sometimes referred to as anhydrous glucose units, which are linked by β-1,4-glycosidic bonds. Each unit contains hydroxyl groups on the C2, C3 and C6 units, enabling the formation of strong hydrogen bonds between and within the polymer chains, gaining a strong network containing crystalline and amorphous regions. [1]

To produce CNC, the amorphous parts in the cellulose must be removed, often achieved by chemical hydrolysis of these amorphous parts. [19] Depending on cellulose and the ratio between the amorphous and crystalline parts, different sizes of the crystals are obtained. Likewise, the hydrolysing method may additionally alter the surface of the crystals, for example, hydrolysis of microcellulose fibres with sulphuric acid results in charged sulphonated CNC crystals. The formation of sulphonated half-esters gains colloidal stability to the particles above their pKa. Further, sulphonated CNC can undergo self-catalysed desulphation, this may however me minimized by storing the CNC suspension at low temperatures. [8] When treating microfibrils of cellulose with sulphuric acid, factors such as reaction time, temperature and concentration of the acid are important. They all control the size and shape of the CNC, with higher concentration, longer reaction time and higher temperature resulting in finer and more crystalline CNCs. [20] The size and shape of the CNCs have a great impact on their rheological behaviours, especially at which point the solution becomes concentrated enough to produce a continuous network (the percolation threshold) and when the gelation point is reached. [8]

Classical ways for modification of CNC rely on the use of free hydroxyl groups for esterification, etherification, oxidation and silylation, using reagents prone to react mainly with alcohols and are performed in various organic solvents. Sulphate half-ester were long seen as unimportant but have since been proven to be exceptionally useful. As stated previously, the conjugation of Az-salts to sulphonated CNC was of interest in this thesis. The reaction occurs directly in the water suspension containing the CNC which is an advantage if the reaction is to be scalable. Additionally, as Az-salts are charged, these too are to a certain degree soluble in water, making the reaction feasible for large scale productions. Compared to other reactions, the use of Az-salts for the modification of the sulphonated CNC is thereby superior to other reactions. [8]

2.2 Synthesis of Az-salts

Azetidinium salts (Az-salts), are a type of cyclic amine with great versatility. [21] They are part of the Azetidine group, 4-membered heterocycles, which has grown to be invaluable for many pharmaceutical applications. Azetidines are generally produced by cyclization and cycloadditions and are generally difficult to work with due to their inherent ring strain and reactivity. Additionally, the ring possesses unique reactivity as its chemical properties partly resemble other cyclic compounds, such as aziridine (3-membered ring) and pyrrolidine (5-membered ring), which depend on reaction conditions and the electronic and steric environment of the molecule. [22] By the addition of two alkyl chains to the nitrogen in the azetidine ring, the corresponding azetidinium ion if formed, also known as an Az-salt with an associated counter ion. [23]

Az-salts started to become of interest in the 1960s which since then has grown. [14] Further research investigating their versatility has been performed, venturing into many different fields of applications such as the pharmaceutical field or polymer branching. Because of their ring strain, they may be used for alkylation by ring opening mechanisms towards C, N, S and O nucleophiles, [24] opening through an S_N2 mechanism at the 2’ and 4’ position in the ring. The selected Az-salts produced and utilized in this project have two side chains, R-groups, at the 1,1’-nitrogen position and a hydroxyl group at the 3’- position in the ring, Figure 4.

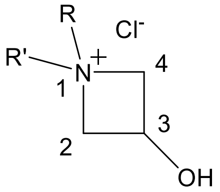
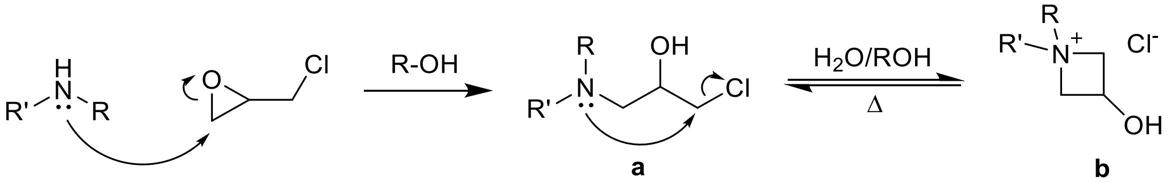


Figure 4. Illustration of Az-salt with two side chains at the 1,1'-nitrogen position and a hydroxyl group at the 3'-position in the ring.

There are of course many different types of Az-salts to be investigated, which all may be synthesized in different ways. Depending on the amine, different salt may be obtained with different applications.

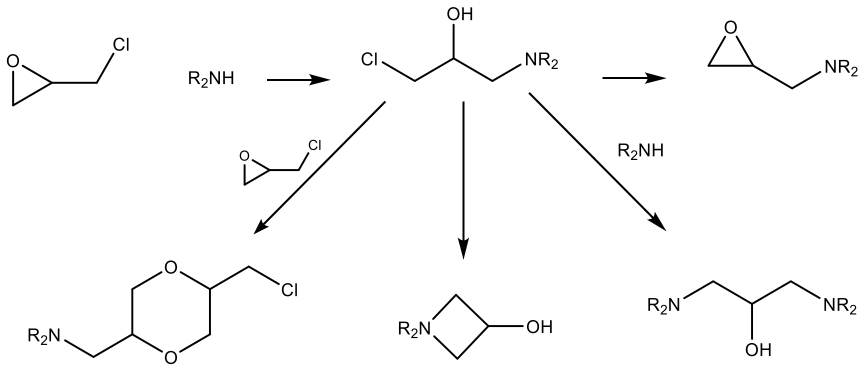
2.3 Synthesis of Az-salts

This thesis uses a method for the synthesis of Az-salts which is already established and published. As stated previously, the reaction is based on an ECH reacting with a DAA. Depending on which amine is used, different salts are obtained with varying properties which may further be utilized when being conjugated to the CNC. The reaction is illustrated in Scheme 4. Two products can be produced namely the ring-open form (a) and ring-closed form (b). In this reaction it is often possible to isolate the ring-open structure before closing it, however, some amines generate open and closed forms simultaneously.



Scheme 4. The mechanism for reaction of epichlorohydrin with a dialkylated amine. Formation of ring-open structure (a) and closed structure of Az-salt (b).

However, the reaction may not be as simple as illustrated as several papers have disclosed the occurrence of side reactions, yielding different products and in some cases polymerization, Scheme 5. [8], [25]



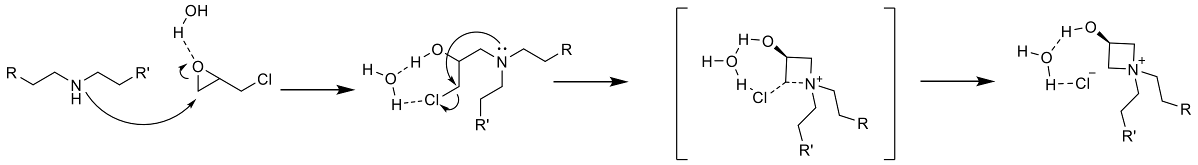
Scheme 5. Possible pathways for side reactions based on findings from Laguerre et.al and Börjesson. [8], [25]

The amines used in this thesis were often commercially available, often being symmetric amines meaning that the two R groups were identical.

2.3.1 Choice of solvents

Many reactions require solvents for them to work. The solvent chosen will often influence the speed of reaction, product quality and overall yield. As chemical solvents embody around 80% of chemicals used today, the search for greener solvents to replace conventional ones is important. [26] For the reaction of ECH and DAA, isopropanol (*i*PrOH), water and methanol (MeOH) were used as predominant solvents as they have been shown to provide adequate yields of the ring-open form structure and ring closure. The open form can be synthesized at room temperature, as the ring closes at elevated temperatures between 70 – 80 °C. The re-opening of the ring may occur at higher temperatures. The formation of the desired product may, in addition to the side chains, also be dependent on the choice of solvent. Solvent effects have previously been observed both specifically for the formation of Az-salts and for general S_N2-reactions [24], [27]. It has been shown that for the synthesis of Az-salts, polar protic solvents are the most suitable, possibly because of their ability to stabilize intermediate forms on the salt. [24]

Scheme 6, inspired by Sivo et al, shows the interactions of polar solvents with the intermediate open ring structure [24]. A hydroxy group may interact through hydrogen bonding with the oxygen in epichlorohydrin, enabling the amine to react faster. The ring-open form is also stabilized through a type of hydrogen bonding and interacts with the chloride atom, stabilizing the system further.



Scheme 6. Interaction of polar solvent during Az-salt synthesis, based on work by Sivo et al. [19]

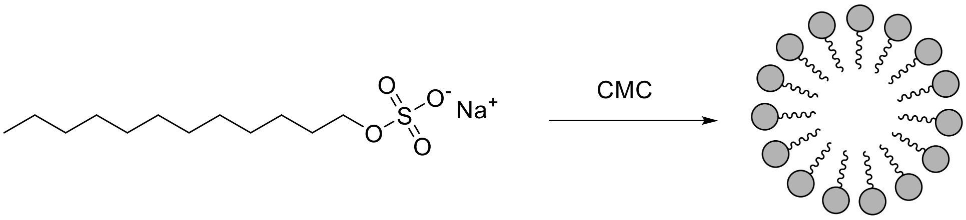
As one of the goals of this project is to investigate the scalability, the choice of solvent may be of even more importance regarding the environmental and toxicological effects. [28]

In this project, *i*PrOH is the primary solvent chosen, with *i*PrOH:water mixture (1:9) for the ring-closing reaction. MeOH was sometimes used as a substitute for water for easier isolation of the Az-salt, as the removal of water is energy and time-consuming. Further, solvent-residues in the product are generally disregarded as the later step, the conjugation to CNC, is performed in a water suspension. Solvents other than *i*PrOH, MeOH, and water may be used if needed, e.g. dissolving DAAs containing hydrophobic side chains for the formation of the ring-open product. This is especially important if the DAA is added directly to the reaction without being suspended in a solvent before addition. Without prior suspension, the DAA can then be seen as a micro-volume with high concentration and in large excess compared to the ECH, promoting deamination of the ECH, and hindering reaction. If *i*PrOH, MeOH or water did not work, dichloromethane (DCM), acetonitrile (MeCN), or ethyl acetate (EtOAc) were used instead.

Furthermore, for longer alkyl chains, for and above 16 carbons, Sodium Dodecyl Sulphate (SDS) at concentration forming micelles were used to promote the cyclization of salts on ring-open form intermediates.

2.3.1.1 *Sodium Dodecyl Sulphate, SDS*

Sodium Dodecyl Sulphate, SDS, is a common surfactant used within different fields of industry and academia. SDS is an amphiphilic molecule with a long hydrophobic carbon chain and a charged hydrophilic head group. This nature makes the SDS arrange itself into so-called micelle structure above a certain concentration, Scheme 7, in aqueous solvents. This is called the critical micelle concentration, CMC, and is generally 8 mM. for SDS in water [29]



Scheme 7. Illustration of SDS monomer and aggregation into micelles above CMC.

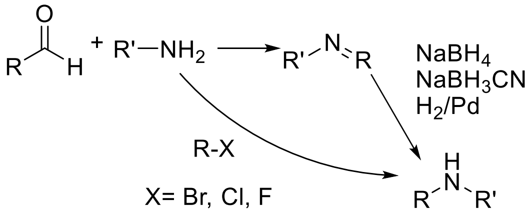
Surfactants in general have been used as catalysts for a long time, ranging from aiding oxidation and reduction reactions to hydrolysis and C-C coupling reactions. [29] In this thesis, SDS will be investigated in the ring-closing reaction of long-chained ring open structures, such as salt **16** and **17** from Figure 2.

2.4 Synthesis of starting materials

Different starting materials were required for the synthesis of Az-salts. Many were commercially available, however, some were synthesized by reductive amination or more complicated pathways, as described in the following sections.

2.4.1 Reductive amination

The formation of secondary amines is usually performed by reacting an aldehyde (with one desired R group) with an amine containing the other R-group. The aldehydes used were commercially available. The aldehyde proceeds to react with a primary amine producing an imine which is reduced to an amine. Scheme 8 describes the reactions.

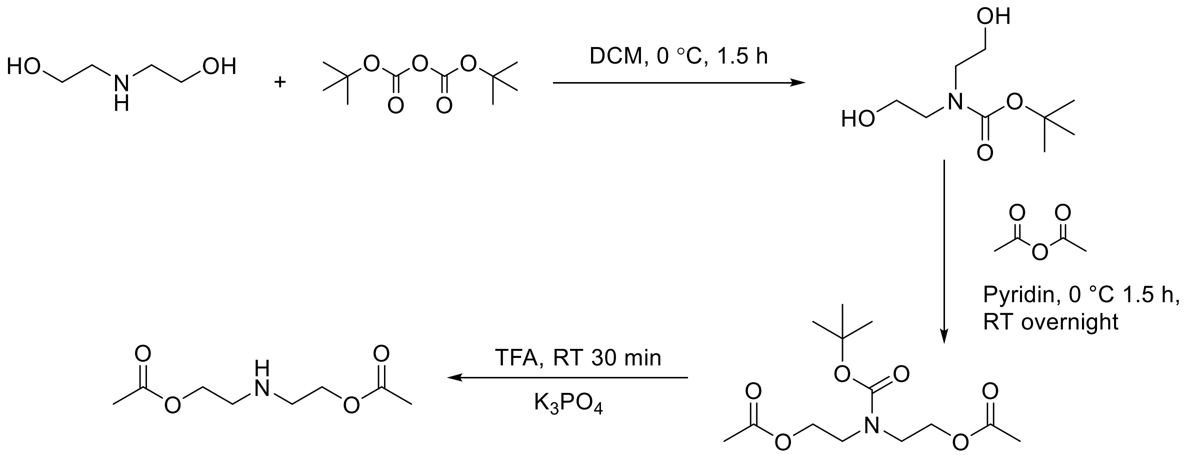


Scheme 8. Synthesis of secondary amines. X is a halide such as Br or Cl.

The reducing agent chosen for these processes was sodium borohydride, $NaBH_4$. Other reducing agents may be used, such as sodium cyanoborohydride, and $NaBH_3CN$, when functional groups such as ketones and aldehydes are present in the R-groups of the dialkylamine. The electron-withdrawing cyano- (CN) group makes it less reactive than $NaBH_4$ and reduces only the imine. One may also consider other reducing agents such as the use of platinum and hydrogen. [30] With that being said, as one aim of this thesis is to find more environmentally friendly and scalable reactions, the cost must be taken into consideration. As an example, the chemical company Alfa Aesar retails 100 g of $NaBH_4$ (98%) for approximately \$80 [31], compared to almost \$471 for 100 g of $NaBH_3CN$ (95%) [32]. Even if $NaBH_4$ may reduce other species than imines, the cost of purification may still be lower than the cost of the use of $NaBH_3CN$. However, this is strongly dependent on the chemistry performed. In this thesis, the use of $NaBH_4$ was seen as sufficient.

2.4.2 Esterification from alcohol

One of the DAAs was not possible to produce by reductive amination. For the synthesis of the diacetoxyethyl amine, a slightly longer synthetic pathway was required. The method used is based on H. Ocbiai et al [33] and Ruzié et al [34]. The reaction utilizes a Boc protective group to prevent the nitrogen from reacting with the acetic anhydride used in the second step to convert the alcohol to an ester. The pathway for this reaction is illustrated in Scheme 9 with reaction conditions.

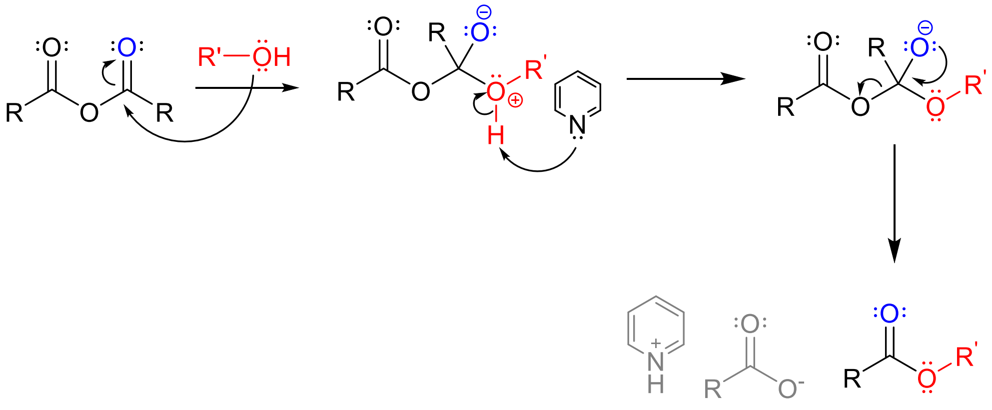


Scheme 9. Reaction scheme for the synthesis of di-acetoxyethyl amine. [33]

After the addition of the Boc protective group, acetic anhydride reacts with the hydroxyl groups on the di-ethanol amine, resulting in esterification. Pyridin is used as solvent acting as a base, first at low temperature for 1.5 h and at room temperature (RT) overnight. This reaction occurs in both alcohol groups. The Boc protective group is lastly removed using trifluoroacetic acid (TFA) and extracted with dipotassium hydrogen phosphate, K_3PO_4 and EtOAc.

2.4.2.1 The mechanism for esterification

As pyridine acts as a base, the reaction is said to be base-catalysed. Pyridine abstracts the hydrogen from the alcohol groups, making the oxygen reactive towards the carbonyl in the acetic anhydride. A general description of the mechanism is illustrated in Scheme 10.



Scheme 10. The mechanism for esterification of alcohol with pyridine acting as a base.

In the mechanism, the alcohol-group attacks one of the carbonyl carbons in acetic anhydride. This generates an intermediate from which pyridine can abstract a proton. The reaction continues with the rearrangement of electrons from the oxygen, resulting in the formation of an ester and a carboxylate anion.

2.5 Techniques

In this part, the most vital techniques used during this project are presented. FTIR has been one central method as it has been used in several forms, *in situ* for monitoring reactions and characterization of CNC films. Likewise, NMR has been used for monitoring one reaction and as the major characterization technique for all synthesized substrates.

2.5.1 Fourier Transform Infrared spectroscopy, FTIR

Fourier Transform Infrared spectroscopy, FTIR, is a method using radiation in the infrared region to study matter and materials. More specifically, the interaction of infrared radiation with electrical dipoles of chemical bonds. The frequencies of a bond can theoretically be calculated by using simple models describing the harmonic oscillations in a bond between two masses, also known as Hook's law. Equation 1 describes the proportional relationship between frequency and mass, where the frequency is given as ν [s^{-1}] and k is the force constant [kgs^{-2}]. [35]

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \tag{1}$$

The relationship of the masses m_1 and m_2 to the frequencies lies in the reduced mass μ [kg], which is given by the two atoms, equation 2.

$$\mu = \frac{m_1 m_2}{m_1 + m_2} \tag{2}$$

An illustration of two bodies of different masses connected by a spring is presented in Figure 5.

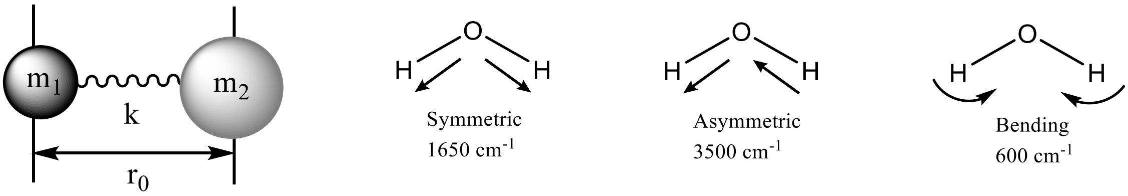


Figure 5. Left: Illustration of movement between two bodies, presented as a spring with a sprig constant k and distance r_0. Right: Examples of bond vibrations with corresponding frequencies.

A more accurate calculation of the frequencies may be obtained by utilizing software using quantum mechanics calculations instead. However, although this may be a more precise approximation, in practice molecules never show distinct frequencies. It is more common for them to present as spectral bands, or peaks of different widths, up to tens of reciprocal centimetres. This is especially true for molecules in solution and solids as widening usually is attributed to solvent/molecule and dipole-dipole interactions. As FTIR is used *in situ* to monitor the reaction, that is in solution, this becomes a slight difficulty. To be able to monitor a reaction process by FTIR the signals from formed or cleaved bond signals must be well separated from other signals, a challenge for this reaction.

There are many different types of FTIR used, depending on the specific application. Attenuated total reflectance, ATR-FTIR, was used for the measurement of samples such as cellulose films. This method provides the reflection of the different frequencies, 400 cm^{-1} to 4000 cm^{-1}, of a sample, providing information on chemical bonds present in the sample. [36] Further, ATR is ideal for the analysis of thicker samples which often produce strong signals. It measures the changes in internal reflections of the IR beam within the sample, reflecting it to a crystal which generates a spectrum.

2.5.2 Nuclear magnetic resonance spectroscopy, NMR

Nuclear magnetic resonance spectroscopy, NMR, is a method of analysis that provides information about the magnetic energy states of every unique atom in a molecule. This results in a type of fingerprint from which molecular structure and composition may be determined. NMR can be done in many ways, the most common ones being ^{1}H-NMR and ^{13}C-NMR. In addition, 2D NMR techniques like COSY, NOESY, HSQC and HMBC analysis are performed to provide further information on the coupling of the atoms in the molecule. The solvents used for analyses were both Chloroform-d ($CDCl_3$) and Dimethyl Sulfoxide (DMSO). Characteristic peaks for the closed structure are found around 6.4 ppm for the OH group of the ring closed structure and between 3.0-4.9 ppm for the hydrogens on the carbons in the ring.

2.5.3 Thin layer chromatography, TLC

Thin layer chromatography, TLC, was additionally used for monitoring reactions. TLC works by loading a sample onto a stationary phase, often silica, and eluting it in a mobile phase thereby separating different components in the sample based on for example polarity. Depending on the substance, different mobile and stationary phases may be chosen to accommodate the substance. Different stains were used for the visualization of compounds. $KMnO_4$ was used to visualize and distinguish different amines as tertiary amines are oxidized faster than secondary ones, enabling the detection of the product. In addition to $KMnO_4$, vanillin and phosphomolybdic acid were also used to differentiate compounds from each other from the developed colour after staining. The choice of eluent was MeOH in DCM or EtOAc in pentane.

2.5.4 Titration

Conductometric titration was used to determine the number of sulphate groups on the CNC surface. This works by measuring the pH and conductivity of the CNC solution as a function of time and concentration of NaOH added. The protons on the sulphate groups remaining from the synthesis will consume hydroxyl ions until the suspension is neutralised. After this, the pH in the solution will rise. Likewise, the conductivity of the suspension provides information on the sulphate content as a minimum will be reached when the least number of ions are present in the solution. After this point, the addition of NaOH will increase the conductivity. From these two points, the sulphate concentration can be determined.

3. Materials and Method

All reagents and solvents were purchased from Sigma-Aldrich and Alfa Aesar and used without any further purification unless specified.

3.1 Azetidinium salts

Az-salts were synthesized using different procedures. This section aims to describe the reactions and their setups.

3.1.1 Ring-open form – General procedure, not FTIR tracked

11 mmol (1.02 g, 0.86 mL) ECH was slowly added to 10 mL *i*PrOH. To this, 10 mmol DAA was slowly added. Depending on the amine used, a change in colour or slight precipitation could be observed. The solution was stirred at room temperature for 4-12 hours.

For amines with longer side chains, a total of 15 or more carbons, or aromatics DCM, EtOAc, or MeCN was used as the solvent. No purification was found to be necessary.

3.1.2 Ring-closed form – General procedure, not FTIR tracked

5 mL of *i*PrOH:water, ratio 1:9, were added into a round bottom flask, in which 5 mmol of ring-open form azetidinium were dissolved. The reaction was heated to 70-80 °C for 2 hours. The solvent was evaporated, and the product was analysed with NMR. Ring-closing reactions were additionally performed in *i*PrOH:MeOH, ratio 1:9.

3.1.3 NMR analysis of synthesized ring-open and ring-closed structures

^{1}H (400 MHz) and ^{13}C (101 MHz) NMR spectra were acquired on an Agilent NMR instrument at 25 °C. Other samples were recorded on the 600 MHz or 700 MHz at the NMR centre in Gothenburg. Chemical shifts were recorded as parts per million, relative to solvent peaks used as internal standards. For $CDCl_3$, ^{1}H NMR at δ 7.26 ppm and ^{13}C NMR at δ 77.16. For DMSO ^{1}H NMR at δ 2.50 ppm and ^{13}C NMR at δ 39.52. All coupling constants are reported in Hertz (Hz) and multiplicities are reported as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), ddd (doublet of doublets of doublets), triplet (t), dt (doublet of triplet), and m (multiplet). NMR data collected for the different compounds is presented in Appendix A. The monitoring of reaction using NMR was performed on a 500 MHz NMR with MeOD-d_4 as solvent.

3.1.4 FTIR monitoring

For tracking the reactions, the Mettler Toledo ReactIR 15 DiComp probe was used. The probe has an optical measuring range between 650 and 3000 cm^{-1}, with a blind spot between 1950–2250 cm^{-1}.

To a 250 mL two-necked round bottom flask equipped with a magnet, 10 mL of isopropanol was added. The FTIR probe was inserted through one of the necks and adjusted to not touch the flask or the magnet but submerged in the solvent. The recording of the reaction was started, recording one reading every minute. 11 mmol (1.02 g, 0.86 mL) ECH in 2 mL isopropanol was slowly added to the flask. Secondly, 10 mmol of dialkyl amine dissolved in 3 mL of isopropanol was added. The reaction was left to stir at room temperature for 4-6 hours. 2 mL of the reaction mixture was placed in a vial, TLC was performed. The solvent was evaporated, and the residue was collected for NMR analysis.

To the remaining reaction mixture with the FTIR probe still monitoring, 135 mL of distilled water was added so that the ratio of isopropanol to water became 1:9. The reaction was heated to 60-85 °C for 2 hours. The reaction was then taken off the heating plate. TLC was performed and the remaining solvent was evaporated, after which NMR was performed.

3.1.5 ^{1}H NMR monitoring – open ring structure

Two separate solutions were prepared before being added together and tracked in the NMR. To the first NMR tube, 0.1 mL morpholine (1.2 mmol) was added to 0.2 mL deuterated MeOH-d_4. A proton NMR spectrum was collected. In the second NMR tube, 0.1 mL epichlorohydrin (1.26 mmol) was added to 0.2 mL deuterated MeOH, and a proton NMR spectrum was collected. The morpholine-MeOH solution was thereafter added to the NMR tube containing epichlorohydrin-MeOH and resubmitted to the NMR. Spectra were collected every 30 minutes.

3.1.6 SDS mediated reactions

An 8 mM SDS solution was prepared by adding 0.587 g SDS to 250 mL of distilled water. This solution was used for all SDS-mediated reactions. To a vial, the desired mass of ring-open form salt was added. The Equivalent moles SDS were calculated, and the appropriate volume was added. A clear change could be observed as the solution became largely transparent. The solution was let to stir at 80 °C for 4 hours after which it was allowed to cool. To this, 1 g of resin (Amberlite IRA-400 (OH), Supelco Made) was added and left to stir over night at room temperature. The solution was filtered and concentrated in vacuo. NMR was performed on the final product.

3.2 Starting materials

In the following section the synthesis of the starting material is presented.

3.2.1 Reductive amination – synthesis of DAA

10 mmol of aldehyde was added to 15 mL of solvent, depending on the DAA synthesised. DCM, EtOAc, MeOH and EtOH were used for the different batches of synthesis for benzyl-nonyl amine and propargyl-nonyl amine. EtOH and MeOH were used for hexadecyl-propyl amine. To the solvent 10 mmol MAA were added. The reaction was left to stir at room temperature for 45 minutes to 2 hours. After TLC analysis, 3 mmol $NaBH_4$ was added, and the reaction was left to stir over night. The reaction mixture was mixed with 10 mL Na_2CO_3 (10 wt%) and extracted three times with DCM. Brine was added upon insufficient separation. The organic phase was concentrated by evaporation and NMR was performed on the obtained product. The solvents used depended on the synthesis of the amine. Different solvents were investigated for the synthesis of amines. DCM, MeCN and MeOH were used.

3.2.1.1 Purification of amines

Flash chromatography

For some amines, flash chromatography on silica gel was performed to remove impurities. Suitable solvent systems were found by TLC, among them ethyl acetate in pentane. Chromatography was performed for Propargyl-Nonyl amine, ranging from 1% - 10% Ethyl acetate in pentane. Each fraction was detected using TLC and concentrated in vacuo. NMR was performed.

Buffer extraction

Buffer extractions were performed for the synthesised hexadecyl-propyl amine, based on the separation of the primary and secondary mine. The NaH_2PO_3 buffer solution was based on a method by Ma and Jha [37], different amines were separated using buffer solutions of different pH. The buffer was prepared by mixing 25 mL of H_3PO_4 (2 M) and 25 mL NaOH (2 M) in a flask. The pH was adjusted to pH=8.

0.2 g amine-product was dissolved in 20 mL pentane and added to a separatory funnel. 10 mL NaH_2PO_3 buffer solution was added, shaken, and left to separate. The aqueous phase was removed, and the organic phase was washed additionally with 3x10 mL buffer solution. TLC was performed on both phases to confirm the separation of primary and secondary amine. The organic phase was concentrated in vacuo and NMR was performed.

3.3 Synthesis of CNC

CNC was synthesized by hydrolysis of microcrystalline cellulose, MCC by Avicel, using 64 wt% sulphuric acid. The 64 wt% sulphuric acid was prepared 2 days prior to the hydrolysis. For this, 750 mL 99 wt% concentrated sulphuric acid was added dropwise to 720 mL distilled water over the course of 15 hours. The solution was left to sit for an additional 36 hours before being used for hydrolysis. 1450 mL 64 wt% sulfuric acid was heated to 45 °C and 80 g MCC was slowly added to avoid aggregation. The mixture was left to stir at 45 °C for 2 hours before being quenched with 7500 mL of distilled water. A slight colour change was visible, from yellow to blueish. This suspension was then centrifuged for 15 minutes at 4300 rmp to separate the CNC from the supernatant. The supernatant was discarded, and more CNC suspension was added to the vessel. When all suspension had been centrifuged the remaining CNC pellets were washed thrice with distilled water, discarding the supernatant after each wash and mixing CNC with water in between. After this, all CNC was collected from the vessels and re-suspended in 2000 mL of distilled water before being transferred to dialysis tubes (12 – 14 kg/mol) and put into distilled water. The water was changed until the conductivity reached <5 µS. The solution was centrifuged again at 4300 rpm for 15 minutes, and the supernatant was removed. Samples for dry weight calculations and titration were removed. The remaining CNC slurry was weighed. By titration, the sulphate content was determined (mmol/g) and the equivalent moles of sodium hydroxide were added. The solution was diluted to a total of 1000 mL and stored at 4 °C until further use.

3.3.1 Titration

For the measurement, a 20 mL suspension with 0.5 wt% CNC was prepared. To this, 40 µL 0.5 M NaCl was added to make the sample conductive. Three samples were prepared and titrated for the determination of sulphate groups. The samples were titrated with 0.1 M NaOH and stirred during measurement at room temperature.

3.4 Conjugation of Azetidinium salts to CNC

To 20 mL of 2 wt% CNC (sulphate content of 300 µmol/g), the desired Az-salt was added. The amount of salt required was calculated to be 1:1 between salt and sulphate groups. A colour change may be observed dependent on the salt added. The mixture was left to stir for 2 hours at 80 °C before being allowed to cool to room temperature. The mixture was transferred to a dialysis tube, (12-14 kD MWCO) and put into distilled water. Conductivity was measured. The water was changed until the conductivity was below 5 µS, after which the CNC-Az was transferred into a vial. 0.5 mL of this solution was added to a petri dish and left to dry to generate a transparent but slightly milky film. ATR-FTIR was performed on the obtained film.

4. Results and Discussion

The results of the synthesis will be divided into several different parts, as the salts were synthesized in different ways, either batch-wise and monitored with FTIR, or stepwise. Additionally, different reaction conditions will be presented, together with the reaction outcome. All NMRs for the corresponding compounds are presented in the supplementary information.

4.1 Synthesis results

The results of the synthesis are separated into several parts as different methods were used for the synthesis of the different salts. The full assignment of each product can be found in Appendix A.

4.1.1 Synthesis of ring-open structures

The results of the synthesis for the open ring structure are presented in Table 1. The different salts were prepared using the same protocol, described in Materials and Methods. Depending on the salts, different solvents were used as solubility was proven to be one challenge in the synthesis. Some long-chained amines did not fully dissolve in *i*PrOH, leading to poor reaction outcomes. To investigate this further, reactions were additionally performed in DCM, EtOAc and MeCN. The reaction outcomes were evaluated based on the quality of the acquired NMR spectra as *i*PrOH and water were present in many samples. As these solvents however are used in following reaction steps and conjugation to CNC, the presence of solvent was disregarded. Further, the full removal of water and *i*PrOH was shown to be time and energy-consuming, and unnecessary if not important for the next steps in the reaction. Extractions of the salts were attempted for some salts, however, as one aim was to produce these salts in an environmentally friendly way, the use of organic solvent capable to extract these polar charged structures from water is unfeasible. Additionally, many polar solvents are soluble in *iPrOH* and water, making their use redundant.

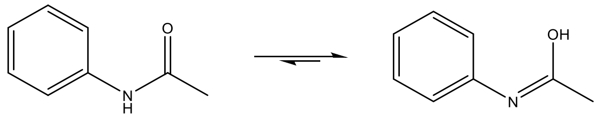
Table 1. Summary of reaction condition and outcome for ring-open structures.

Az-Salt: Ring-open structures	**Solvent**	**Open structure**
1. Morpholine	*i*PrOH	Quant. by NMR
2. Di-Ethyl	*i*PrOH	Quant. by NMR
3. Di-Hexyl	*i*PrOH	Complex mixture
4. Di-2EthylHexyl	*i*PrOH	Quant. by NMR
5. Acetanilid	*i*PrOH	No reaction
	DCM	Quant. by NMR
	EtOAc	No reaction
	MeCN	Quant. by NMR
6. Di-AcetoxyEthyl	*i*PrOH	Complex mixture
7. Di-MethoxyEthyl	*i*PrOH	Quant. by NMR
8. Succinimid	*i*PrOH	No product
	DCM	No product
9. Propargyl-Nonyl	*i*PrOH	Complex mixture
10. Benzyl-Nonyl	*i*PrOH	Quant. by NMR
11. Propyl-Nonyl	*i*PrOH	Quant. by NMR
12. Undeca -Methyl	*i*PrOH	Quant. by NMR
13. Undeca -Propyl	*i*PrOH	Quant. by NMR

14. Undeca -Hexyl	*i*PrOH	Quant. by NMR
15. Undeca -2EthylHexyl	*i*PrOH	Quant. by NMR
16. Hexadecyl-Propyl	*i*PrOH	No product
	DCM	Quant. by NMR
17. Di-Heptadecyl	*i*PrOH	No product
	DCM	Quant. by NMR

Two main types of salts where synthesized, ones containing functional groups and ones with carbon chains. The synthesis of salts with sidechains exclusively being carbon chains, that is salts **2-4** and **11-17**, works in *i*PrOH if the number of carbons does not exceed **15** in one or both of the side chains. The reaction of salt **14** yields ring-open product in *i*PrOH, however salt 16 and **17** do not. These reactions were repeated in DCM where the long-chained amines were more soluble, gaining product. Surprisingly, the synthesis of open form for salt **3**, Di-Hexyl, gained both open and closed form structures following the protocol.

The salt including different functional groups required more investigations regarding solvent systems. For acetanilide, salt **5**, the open form was successfully produced in DCM and MeCN, but not in *i*PrOH and EtOAc. The reason for this is believed to be that acetanilid reacts with the solvent, resulting in a type of keto-enol structure, Scheme 11, shifting more into the imidic acid form thereby making it unable to react further with ECH.



Scheme 11. Isomerism of acetanilid.

The synthesis of ring-open succinimide, salt **8**, was unsuccessful in both *i*PrOH and DCM, most likely due to the strong electron-withdrawing groups attached to the nitrogen, which limits the nitrogen to attack epichlorohydrin.

4.1.2 Synthesis of ring-closed structures

The reaction outcomes of the ring-closed structures are presented in Table 2. All reactions, except for the ring-closing reaction of salt **17** were performed in *i*PrOH:water (1:9) mixture at an elevated temperature. In some cases, water was substituted with MeOH for easier purification of the resulting salts. In most cases, the exchange of water for MeOH did not affect the reaction outcome.

Table 2. Summary of reaction for ring-closed structure.

Az-Salt: Ring-closed structures	**Solvent**	**Closed structure**
1. Morpholine	*i*PrOH:water (1:9)	Quant. by NMR
	MeOH:water (1:9)	Quant. by NMR
2. Di-Ethyl	*i*PrOH:water (1:9)	Quant. by NMR
3. Di-Hexyl	*i*PrOH:water (1:9)	Quant. by NMR
	MeOH:water (1:9)	Quant. by NMR
4. Di-2EthylHexyl	*i*PrOH:water (1:9)	Quant. by NMR
5. Acetanilid	*i*PrOH:water (1:9)	No reaction
	MeOH (100%)	Polymerisation
6. Di-AcetoxyEthyl	-	-

7. Di-MethoxyEthyl	*i*PrOH:water (1:9)	Quant. by NMR
8. Succinimid	-	-
9. Propargyl-Nonyl	*i*PrOH:water (1:9)	Complex mixture
10. Benzyl-Nonyl	*i*PrOH:water (1:9)	Complex mixture
11. Propyl-Nonyl	*i*PrOH:water (1:9)	Quant. by NMR
12. Undeca-Methyl	*i*PrOH:water (1:9)	Quant. by NMR
13. Undeca -Propyl	*i*PrOH:water (1:9)	Quant. by NMR
14. Undeca -Hexyl	*i*PrOH:water (1:9)	Quant. by NMR
15. Undeca -2EthylHexyl	*i*PrOH:water (1:9)	Quant. by NMR
16. Hexadecyl-Propyl	*i*PrOH:water (1:9)	No product
	SDS at CMC	Trace product
17. Di-Heptadecyl	MeOH:DMSO (20:1)	No product
	SDS at CMC	Trace product

The ring-closing reaction seemed not to work for acetanilid in *i*PrOH:water (1:9) and seemed to gain a polymer structure in pure MeOH, as described in the theory. As the synthesis for open form of succinimide was unsuccessful, no ring-closing reaction was performed. Likewise, the obtained open ring structure for propargyl-nonyl amine and di-acetoxyethyl resulted in complex reaction mixtures which were not able to be separated. The attempt for cyclization of ring-open structure **5**, acetanilide, gained polymerization, likely to gain product as presented in Scheme 5.

For sidechains only consisting of carbons, salts **2-4** and **11-15**, the ring-closing reaction was successful in *i*PrOH:water (1:9).

4.1.2.1 SDS-mediated ring-closing reaction

For salts **16** and **17**, the ring-closing reaction was challenging as a polar solvent is required for ring formation. As these salts have long hydrophobic chains, their solubility is limited. To mitigate this, the use of SDS was investigated to create micelles in which the ring-open form structures can diffuse into and close, Figure 6. The obtained product after ion-exchange of SDS to hydroxyl for the removal of SDS, showed promising results as small peaks in the characteristic region between 3 ppm and 6 ppm were visible. However, further studies must be done to verify the formation of the product as the signals from the long alkyl chains of the salt hide the characteristic peaks because of their size difference. Furthermore, no SDS could be detected in the NMR, indicating that the ion-exchange part of the reaction was successful.

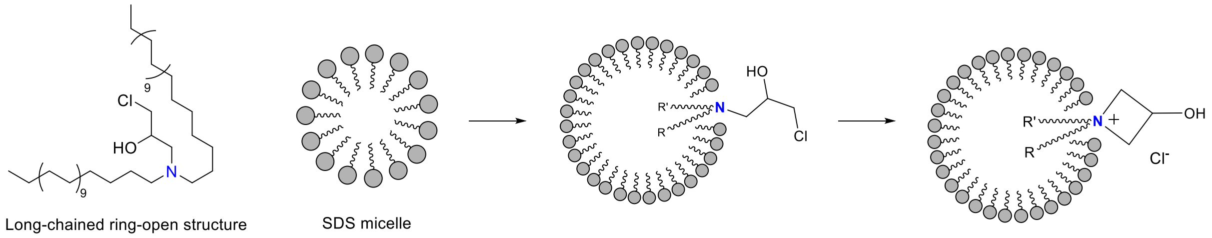


Figure 6. Suggested pathways for SDS-assisted ring-closing reaction.

4.1.3 Synthesis of DAA

Three amines were synthesized in this thesis to aid as starting materials for further reaction. The reaction of benzyl-nonyl amine was found to be the most successful in DCM, compared to EtOAc, *i*PrOH and MeOH. However, the reducing agent also reduced some of the remaining aldehyde resulting in nonanol. The synthesis of propargyl-nonyl amine was most successful in EtOAc, but also contained some impurities such as nonanol and remains of aldehyde. This amine was purified using flash chromatography as described in the method section. Lastly, hexadecyl-propyl-amine was synthesized in MeOH and ethanol (EtOH). The reaction gained purest product in MeOH, however, purification by buffer extraction was still necessary to gain the desired compound.

4.1.4 Characterizations of salts

Each salt was characterized using NMR. All spectra are presented in the supplementary information. As one example, the full characterisation of the morpholine-Az is described in this section. Figure 7 shows the ^{1}H NMR spectrum of open form for salt **1**, morpholine-Az. In the spectra, the peaks are marked with the corresponding proton in the spectrum. The peak at 1.2 ppm and 4.05 correspond to *i*PrOH solvent peaks. The characteristic peaks for the ring-open structure are visible, presenting as a pentet at 4 ppm, being a result of the four hydrogens on positions 3 and 5. Peak 1 lies next to the oxygen in the morpholine ring, resulting in a higher shift of the peak, in comparison to the protons in position 2. Likewise, the chemical shift for peak 5 is higher than for peak 3 because of its location next to the chlorine. The proton on the hydroxyl group is not detected as they may have a fast exchange with water and/or alcohol residues from the reaction. A full assignment of peaks including splitting and integration is provided in the supplementary information.

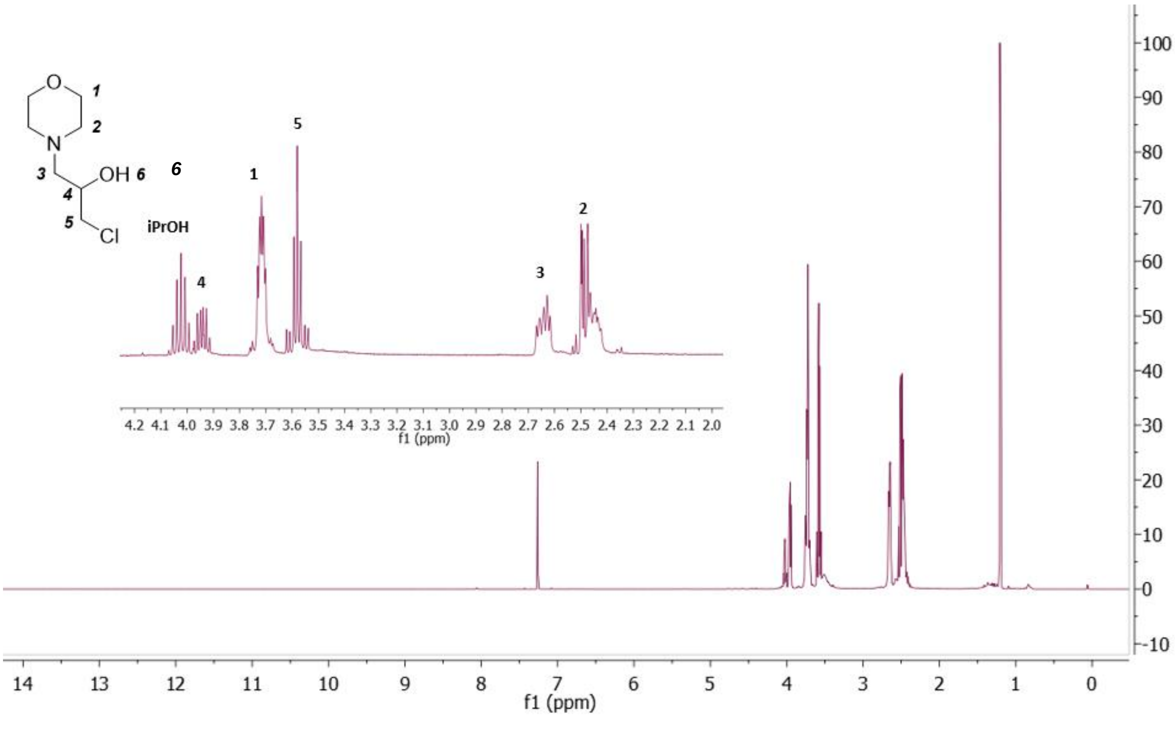


Figure 7. Morpholine-Az open structure ^{1}H NMR spectrum. Zoom to characteristic peaks between 2 and 4 ppm.

Figure 8 shows the ^{1}H NMR spectrum of the ring-closed structure. The peak at 3.4 ppm is water, remaining from the reaction and present in DMSO. In the spectrum, the hydroxyl can be detected at 6.6 ppm. The characteristic pentet, produced form the hydrogen in between the four hydrogens at positions 3 and 5 is detectable at approximately 5.65 ppm. The hydrogens in positions 3 and 5 are present at 4.6 and 4.2 ppm. Because of the symmetry of the molecule, the distinction between them is difficult. The characteristic peaks for morpholine are visible at 3.4-3.6 and 3.8 ppm.

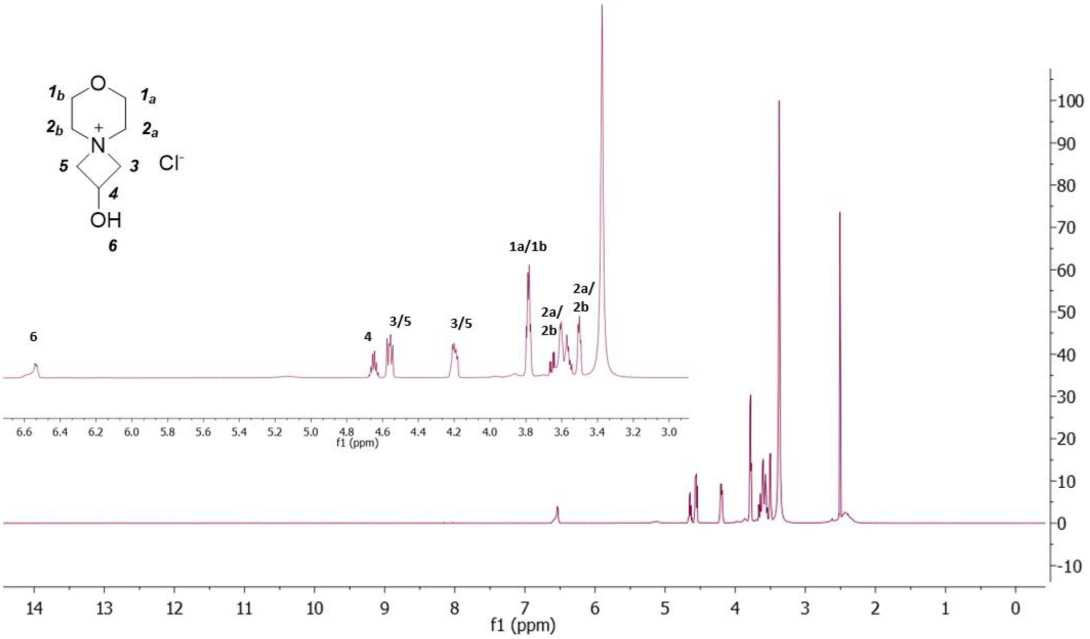


Figure 8. ¹H NMR spectrum for salt 1, Morpholine-Az closed structure. The peak at 2.5 ppm corresponds to DMSO, 3.4 ppm is a water peak.

The full assignment for salt **1**, morpholine-Az, of all carbons and hydrogens is provided in Table 3, alongside Figure 9, an illustration of the interaction from COSY (H-H interactions) and HMBC (H and next lying C or reversed). In some cases, the symmetry of the molecule leads to longer coupling ranges, meaning that hydrogens may interact with carbons being more than 2 bonds away.

Table 3. Full assignment of carbons and hydrogen in Morpholine-Az.

No.	**δ_H (Multiplicity, J Hz)**	**δ_C, Type**
1a	3.79 (q, *J* = 4.9 Hz)	61,40 CH_2 61.80 CH_2
1b	3.79 (q, *J* = 4.9 Hz)	61,40 CH_2 61.80 CH_2
2a - N	3.61 (q, *J* = 5.4, 4.8 Hz)	60.80 CN
2b - N	3.50 (t, *J* = 4.8 Hz)	59.00 CN
3	4.59 – 4.53 (m) 4.20 (dd, *J* = 11.7, 5.5 Hz)	71.30 CH_2
4	4.65 (dp, *J* = 12.2, 5.7 Hz)	58.39 CH
5	4.59 – 4.53 (m) 4.20 (dd, *J* = 11.7, 5.5 Hz)	71.30 CH_2
6 - OH	δ 6.55 (qd, *J* = 10.7, 7.4, 4.9 Hz)	

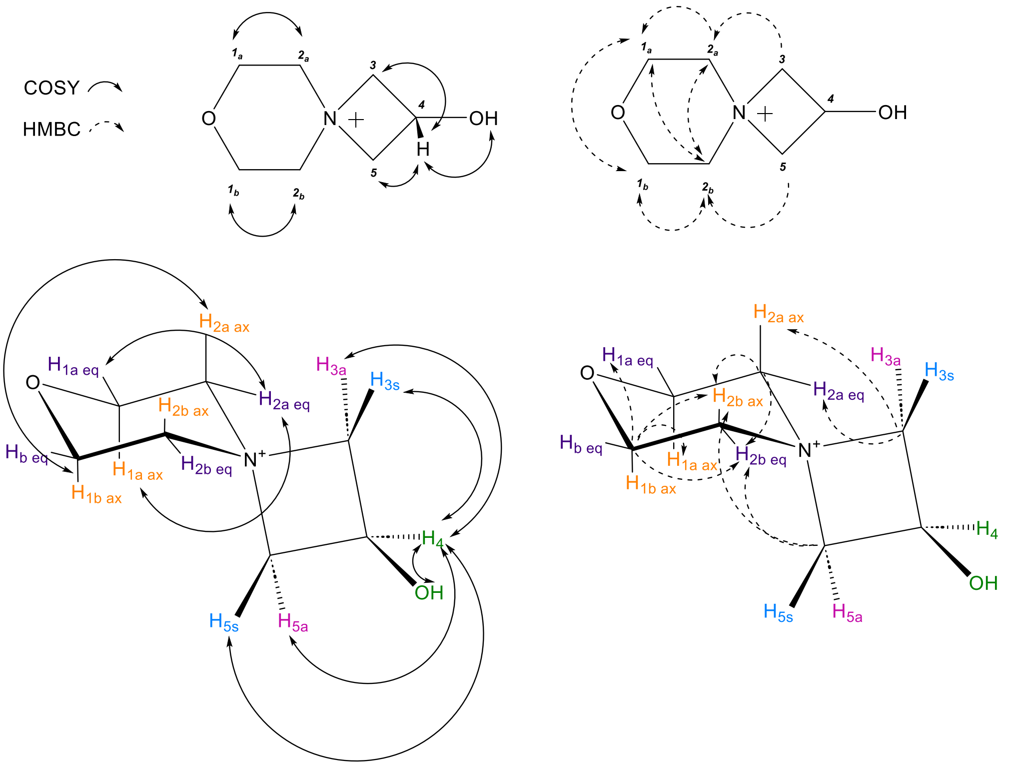


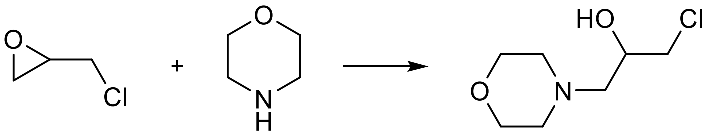
Figure 9. Illustration of COSY and HMBC couplings in salt 1, morpholine-Az. Equatorial hydrogens are assigned in purple, axial in orange, anti in pink, syn in blue, OH group and characteristic hydrogen in green.

4.2 Monitoring of the reactions

Reactions were monitored using *in situ* FTIR and NMR. For NMR, the reaction with morpholine was chosen as a model compound. For the monitoring of the reaction with FTIR, salts **1-4** were investigated and morpholine was again chosen as the model compound for the explanation of the results.

4.2.1 NMR

As to further investigate the reaction between epichlorohydrin and dialkyl-amines, one reaction was studied *in situ* using NMR. The reaction with morpholine was studied as the model compound as it also previously was investigated using FTIR. Figure 10 presents the NMR spectra collected from the starting materials, epichlorohydrin in deuterated MeOD-d_4, morpholine in deuterated MeOD-d_4 and reaction outcome after 30 minutes. Additionally, the spectra for the synthesis of the ring-open structure are presented after 60 minutes and 12 hours. The peaks in the spectra change with increasing time. As the concentrations of the reagent known for the reaction start and no other reagent or solvent is added, the intensity of the peaks may indicate the progress of the reaction. If the peak decreases it indicated that this peak is consumed, if an increase is visible, a different compound is produced. A visible change is presented around 3.0 ppm where the peak after 30 minutes is strong, however, has disappeared completely after 12 hours. This peak most likely corresponds to the peak of morpholine amine shifting in the open ring structure.



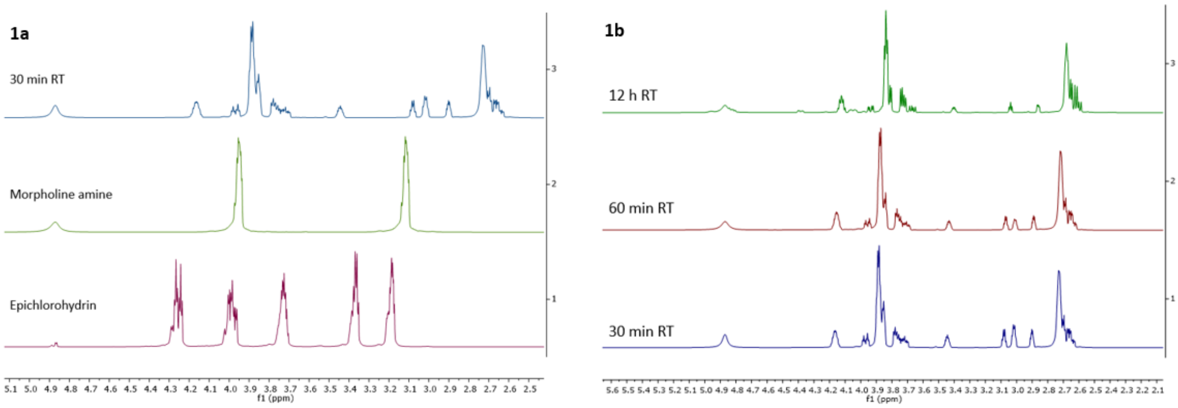
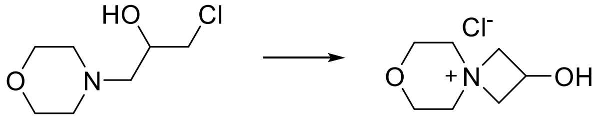


Figure 10. ¹H NMR spectra of 1a. 1 - Morpholine, 2- Epichlorohydrin, 3 - Reaction of them after 30 minutes. 1b. Reaction in room temperature after certain increments of time, 1- 30 min, 2 – 60 min, 3 – 12 hours.

After the synthesis of the open ring structure, the same NMR tube was heated to 50 °C to mediate ring closure. As the reaction occurred, one spectrum was collected every 15 minutes for two hours, starting with a zero-sample taken immediately as 50 °C was reached, Figure 11. From the spectra, an increase in the peak at 5.22 ppm is visible indicative of a proton corresponding to that growing signal strength. The location and splitting of peaks point towards the proton on the CH-OH carbon in the ring. Integrating it, using MeOD-d_4 as an internal standard, the integral of those peaks increases with time, further indicating that this peak is formed in the reaction.



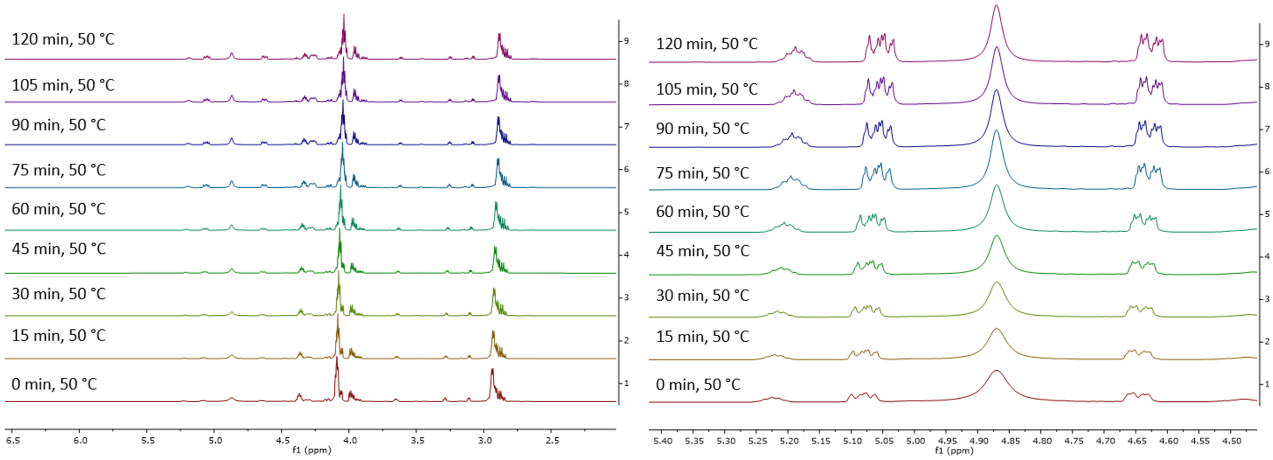


Figure 11 . ¹H NMR spectra were collected for ring-closing reaction, one spectrum was collected every 15 minutes for 2 hours.

To verify the continuous formation of the product, the peak at 5.22 ppm was integrated for each spectrum recorded, taking MeOD-d$_4$ as a reference peak, equal to one, Table 4.Table 4. Time stamps and relative integral, compared to the peak of MeOD-d$_4$ at 4.87 ppm.

Time after start [min]	Relative value of intergral
0	0.08
15	0.10
30	0.12
45	0.14
60	0.15
75	0.20
90	0.21
105	0.22
120	0.23

4.2.2 FTIR

Figure 12 illustrates a spectrum gained from the reaction between morpholine and epichlorohydrin in isopropanol. A clear change at around 800 cm^{-1} is observed as well as around 1000 cm^{-1}. After the formation of the open form, water is added for the closing of the ring, corresponding to the appearance of a broad water peak in the spectra at 1650 cm^{-1}. Additional changes may be observed throughout the spectra, however the characteristic peaks for *i*PrOH and water dominate. Previous studies have used a FTIR probe to follow the esterification of cellulose using allyl glycidyl ether, showing trends for the formation of esters on the cellulose. Additionally, it was found that the oxirane ring presented three distinct vibration regions, 1253 cm^{-1}, 810–950 cm^{-1} and 750–840 cm^{-1}. Although not identical with epichlorohydrin, these regions are still interesting for this thesis as the oxirane ring is present in both. [38]

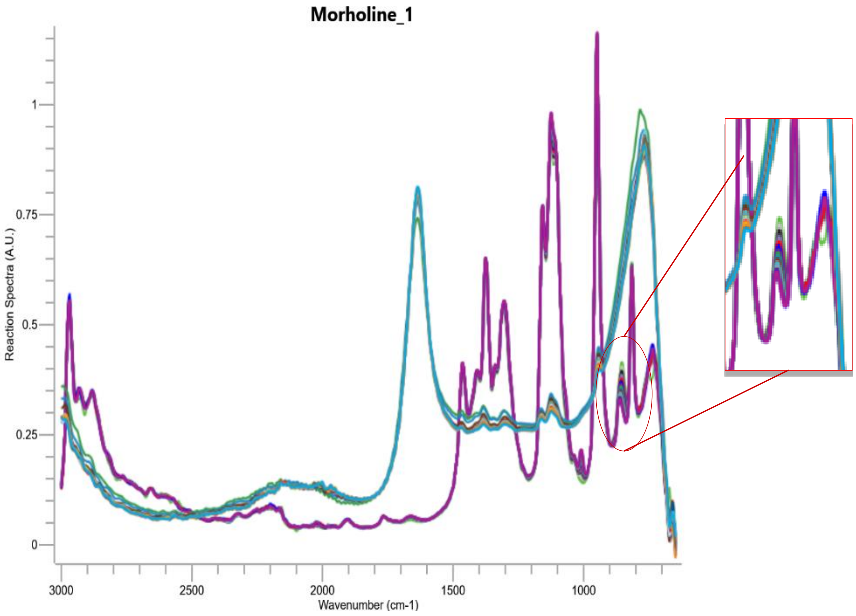


Figure 12. Resulting FTIR spectra for the formation of Morpholine salt, Az-salt 1. Zoom illustrates the region around 800 cm^{-1}. Each colour corresponds to one spectrum collected at a certain time stamp.

As mentioned before, significant signals can be identified from the spectra and their trends analysed using the provided software for the FTIR probe. From these trends, the formation and breaking of bonds may be recognized and the progress of the reaction predicted. The trends for the spectra in Figure 12 visualize different trends that may be observed, Figure 13.

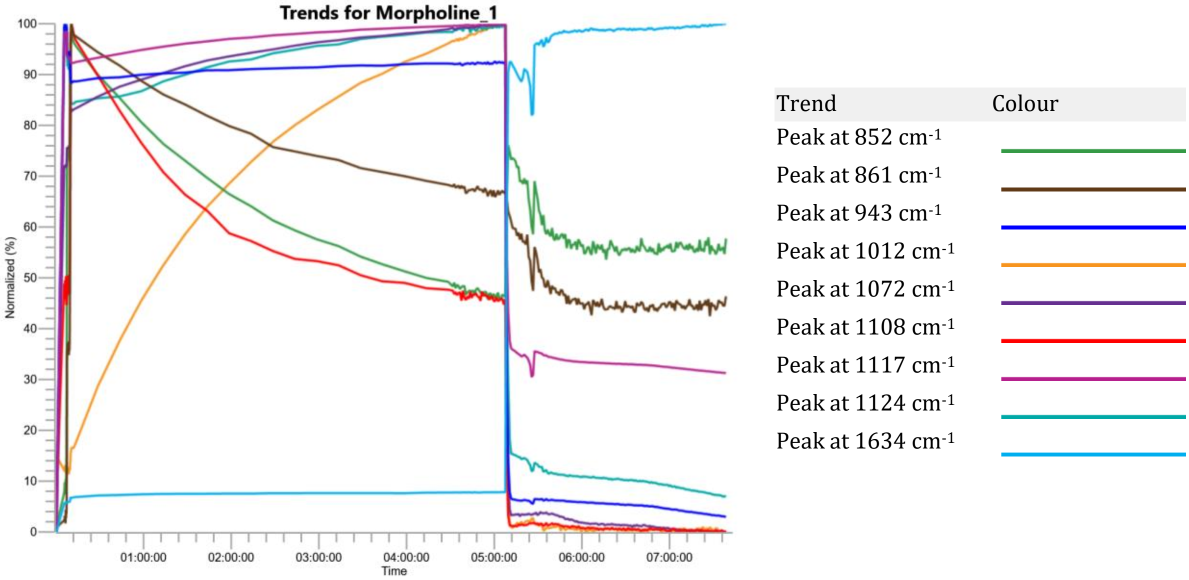


Figure 13. Resulting trends from spectra for Morpholine-Az when tracked with FTIR.

The trends drastically decrease and then stabilize at around 5 hours. This is due to the addition of water and heating to induce the ring-closing reaction. As the amount of water added is large in relation to the rest of the reaction mixture, many signals are drastically reduced and currently not possible to be further analysed. The detected signals and their corresponding frequencies were verified using ORCA, a programme able to calculate vibrational spectra for molecules based on their structure and symmetry. Simulations were executed for each species in the reaction mixture, that is dialkylamine, epichlorohydrin and the ring-open and ring-closed structures. Additionally, the effect of solvent may be added to the calculations, as frequencies have been shown to have a slight shift in the presence of different solvents or their absence. Hence, the ORCA should in this case be taken as a guide.

Combining the information provided by ORCA with the FTIR probe measurements, the formation and breaking of bonds can be determined and an estimate of the reaction progress obtained. For this example-reaction, their observed significant signals are listed in

Table 5, with corresponding theoretical vibrations calculated by ORCA. Note that only the significant vibrations are presented as the vibrations in the chains of the amine are consistent in the ring-open and ring-closed structure.

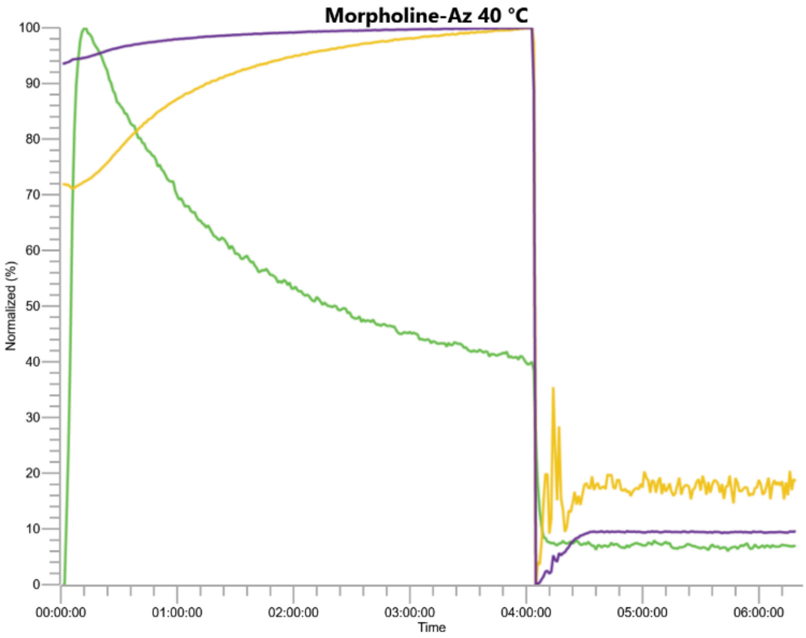
Table 5. Trends with corresponding chemical bonds for Morpholine Az-salt. Frequencies are compared between spectra, trends and ORCA simulations to assign the formation and breaking of bonds.

Trend four corresponding frequency [cm^{-1}]	**Frequencies matched in ORCA and corresponding bond**
852	Significant in the epoxy ring of epichlorohydrin
861	Significant in the epoxy ring of epichlorohydrin
934	Significant for Morpholine amine
1012	Significant for all Morpholine-species, C-OH stretch in the ring-open structure

1072	Significant for epichlorohydrin, C-C stretch and ring-open chain
1108	Significant in the epoxy ring of epichlorohydrin
1117	Significant in the epoxy ring of epichlorohydrin
1124	Significant in the epoxy ring as of epichlorohydrin
1634	Not relevant according to ORCA

From the information provided by ORCA, and the trends in Figure 13. Resulting trends from spectra for Morpholine-Az when tracked with FTIR.Figure 13, the increase of the yellow trend at 1012 cm^{-1} strongly indicates the formation of other morpholine species. The decrease for trends 852, 861 and 1108 cm^{-1} all indicate the consumption of epichlorohydrin. The trends for 1124, 1072, and 943 cm^{-1} all increase slightly indicating the formation of the ring-open structure. As no trends reach a plateau, it is concluded that the reaction had not gone to completion after 5 hours at room temperature.

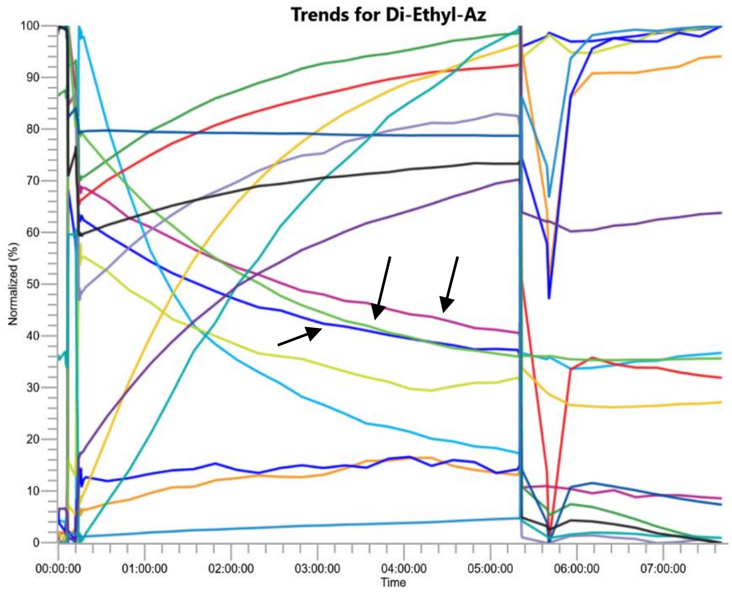
To further evaluate this, the reaction was repeated at 40 °C to investigate if the trends would plateau, in that case indicating that the reaction was done. For this, the peaks at 864, 1008 and 1068 cm^{-1} were analysed. In Figure 14, the trends for the reactions are presented. No plateau is reached, however, the trend for peak 864 cm^{-1} reaches a lower intensity, indicating that the reaction has proceeded further after 4 hours at 40 °C than after 5 hours at room temperature.



Trend	Colour
Peak at 864 cm^{-1}	🟩
Peak at 1008 cm^{-1}	🟨
Peak at 1068 cm^{-1}	🟪

Figure 14. Trends for reaction of morpholine at elevated temperatures to 40 °C.

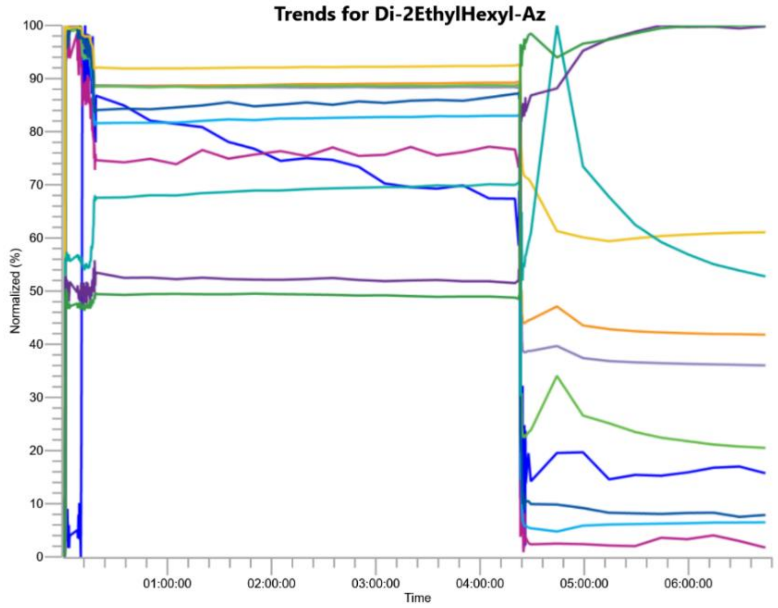
Salts **2**, **3** and **4** were also investigated *in situ* using FTIR. Like the morpholine-Az example, their trends were analysed, and it was concluded that no plateau was reached, meaning that the other reactions also likely take more than 5 hours at room temperature or 4 hours at 40 °C to conclude. As samples were taken from the rection vessel for further identification, the overall yield for the reactions is unreliable. Additionally, the samples collected continued to react in the vial before analysis was possible. Therefore, the results from the NMR are used as a qualitative determination of if the reaction was executed whilst tracing. As mentioned, salt **2**, Di-Ethyl-Az was also monitored using in situ FTIR. The trends are shown in Figure 15. The characteristic changes for the epoxy-ring in ECH at 1253 cm^{-1}, between 810–950 cm^{-1} and 750–840 cm^{-1}, are also visible in Figure 15, indicated with black arrows. The remaining trends correspond to vibrations for the ring-open structure and starting material. The drastic change after 5.5 hours corresponds to the addition of water.



Trend	Color
Peak at 848 cm^{-1}	🟪
Peak at 932 cm^{-1}	🟦
Peak at 942 cm^{-1}	⬛
Peak at 1052 cm^{-1}	🟦
Peak at 1101 cm^{-1}	🟦
Peak at 1126 cm^{-1}	🟦
Peak at 1127 cm^{-1}	🟪
Peak at 1204 cm^{-1}	🟨
Peak at 1270 cm^{-1}	🟩
Peak at 1304 cm^{-1}	🟩
Peak at 1341 cm^{-1}	🟪
Peak at 1410 cm^{-1}	🟥
Peak at 1465 cm^{-1}	🟨
Peak at 1635 cm^{-1}	🟦
Peak at 1768 cm^{-1}	🟧
Peak at 1903 cm^{-1}	🟦

Figure 15. Trends for the reaction of ECH with di-ethylamine at room temperature for 5 hours. Addition of water and heating at 5.5 hours. Characteristic trends for the opening of the epoxy-ring in ECH are marked with black arrows.

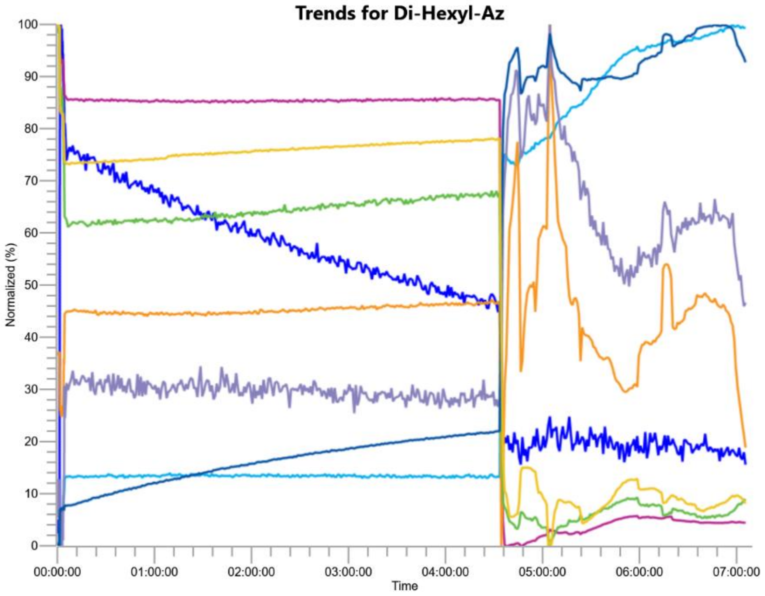
The trends for the reaction of salt **4**, Di-2EthylHexyl are presented in Figure 16. The trends for this reaction do not express any significant changes, except for the trend at 846 cm^{-1}, likely to correspond to the epoxy-ring disappearing of ECH. The shift in trends after 4.5 hours occurs as water is added, drowning the signals. The trends indicate that the reaction has not gone to completion after 4.5 hours at room temperature.



Trend	Color
Peak at 819 cm^{-1}	🟪
Peak at 846 cm^{-1}	🟦
Peak at 945 cm^{-1}	🟦
Peak at 1104 cm^{-1}	🟧
Peak at 1124 cm^{-1}	🟪
Peak at 1378 cm^{-1}	🟩
Peak at 1337 cm^{-1}	🟦
Peak at 1407 cm^{-1}	🟨
Peak at 1459 cm^{-1}	🟦
Peak at 1908 cm^{-1}	🟪
Peak at 1765 cm^{-1}	🟩

Figure 16. Trends for the reaction of ECH with di-2ethlylhexylamine at RT. Heating and addition of water after 4.5 hours

Similarly, the trends gained for the formation of Di-Hexyl-Az, salt **3**, visualize rather flat trends in Figure 17. These trends could be a result of the reaction not having fully converted to gain product, or due to the formation of both the ring-open and ring-closed structure as seen when following the standard protocol.



Trend	Color
Peak at 847 cm^{-1}	▬
Peak at 949 cm^{-1}	▬
Peak at 2856 cm^{-1}	▬
Peak at 1460 cm^{-1}	▬
Peak at 1408 cm^{-1}	▬
Peak at 1343 cm^{-1}	▬
Peak at 1098 cm^{-1}	▬
Peak at 1068 cm^{-1}	▬

Figure 17. Trends for the reaction of ECH with di-hexylamine at RT. Heating and addition of water after 4.5 hours.

4.3 Conjugation of Az-salts to CNC

The conjugation of Az-salt **1**, **2** and **3** was performed on 2 wt% CNC solution. The resulting films were analysed using FTIR, presented in Figure 18. A small change is visible in the regions around 820 cm^{-1}, characteristic of a change in the sulphate groups on the CNC. Further, the increase of intensity at peaks around 3300 cm^{-1} for increasing chain length indicates successful conjugation of Az-salt to CNC.

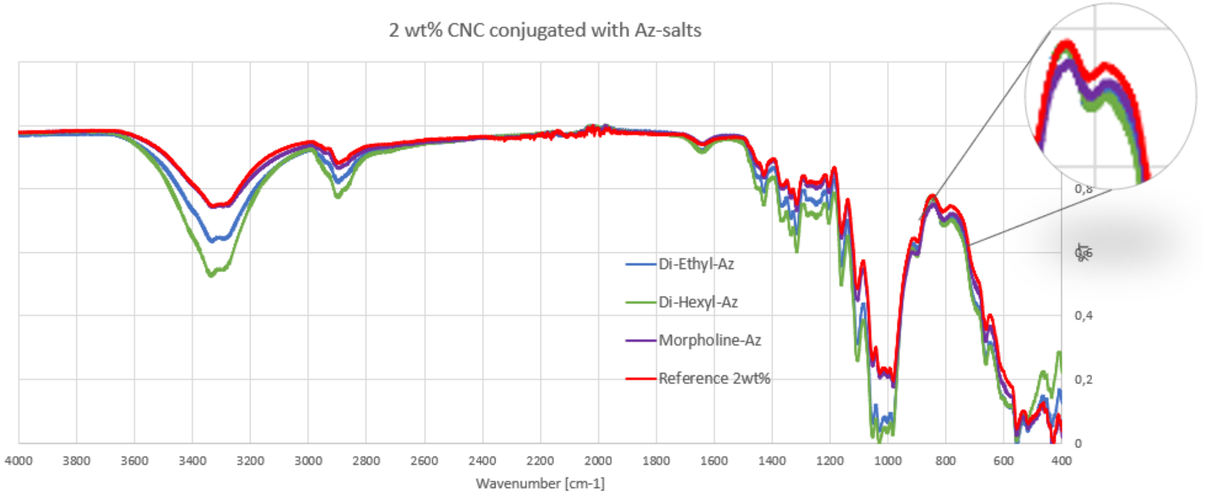


Figure 18. ATR-FTIR spectra for Az-salts conjugated to 2 wt% CNC

5. Conclusion

Several salts were successfully synthesized during this project. Of the **17** salts investigated, salts **1-4**, **7**, and **11-15** were synthesized using the standard procedure without modification. For salts **5**, **9**, and **10**, the product was a complex mixture, which was not separated. For salt **5**, the ring-open structure was successfully synthesized, using alternative solvents such as DCM, EtOAc, and MeCN. No ring-open structure was successfully synthesized for salt **8** because of their strong electron-withdrawing groups, hindering it to react with ECH. Salt **6** was not synthesized as the ring-open spectra showed a complex product mixture, unable to identify the product. Long-chained salts, numbers **16** and **17**, were successfully synthesized using SDS as a ring-closing agent. The SDS is thought to form micelle structures with the ring-open structures incorporated, making the salts soluble in an aqueous solution, and promoting the ring-closing reaction. Generally, the standing protocol for the synthesis of Az-salts is regarded to function well for salts containing alkyl chains shorter than 15 carbons on one or both sides. The reaction has been shown to be easy, robust, and rather safe in comparison to other pathways. The choice of solvent is critical for the reaction to occur and gain adequate yields. Green solvents such as *i*PrOH were shown to gain ring-open structures. Water was used for the cyclization reaction. Several salts such as salt 1 and 2, were synthesized in gram-scale and were determined pure without significant side product forming.

Salt **1** was monitored using ^{1}H NMR, in deuterated MeOD-d_4. For the ring-open structure, three spectra were collected. The first after 30 minutes, the second after 60 minutes, and the last after 12 hours. In the spectra, the appearance of several peaks could be followed as other peaks disappeared as the reactants formed the ring-open structure. The ring-closing reaction was monitored for 2 hours at 50 °C in MeOD-d_4, collecting one spectrum every 15 minutes, starting from zero. In the spectra, the appearance of several characteristic peaks was visible, indicating that the reaction is possible to monitor using NMR.

Four salts, salts **1-4**, were monitored using in situ FTIR. Theoretical frequencies of all bonds in all structures, DAA, ECH, ring-open and ring-closed structures were calculated using ORCA. Salt **1**, morpholine, was monitored at room temperature and 40 °C. The resulting FTIR showed that the reaction had not gone to completion after 5 hours at room temperature, or 4 hours at 40 °C as no plateau for the trends was reached. Similarly, no plateau was reached in any other reaction, indicating that all reactions take longer than 4 hours at room temperature to convert fully to the ring-open structure.

The conjugation of Az-salts to 2 wt% sulphonated CNC was successful for salt **1**, **2**, and **4** as the significant peak for the sulphur half-ester was shifted, verifying that the modification of biopolymers with Az-salts is possible.

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Supporting information

Appendix A

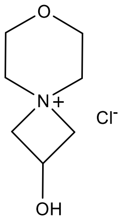
In this appendix, all NMR spectra for the synthesized Az-salts are presented. Ring open and closed structures are determined, and if applicable, the peaks for the synthesized amine are included. Lewis structures of the ring-closed structures are shown.

1. Morpholine-Az

Ring-open structure:

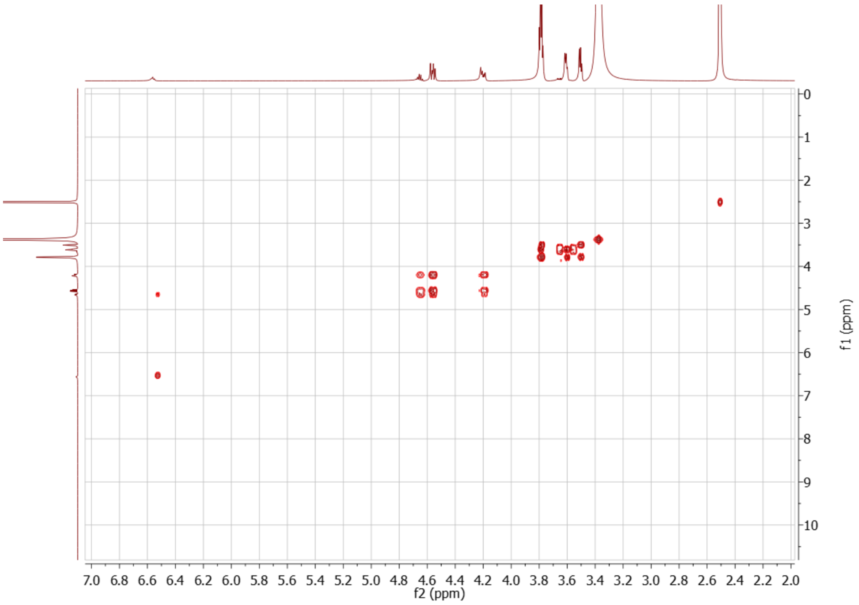
1-Chloro-3-morpholinopropan-2-ol. Following standard procedure, using 33 mmol (3.05 g, 2.59 mL) ECH and 30 mmol (2.61 g, 2.62 mL) morpholine. ^{1}H NMR (600 MHz, Chloroform-d) δ 2.57 – 2.40 (m, 4H), 2.70 – 2.60 (m, 2H), 3.63 – 3.54 (m, 2H), 3.77 – 3.69 (m, 4H), 3.96 (dq, *J* = 9.5, 4.8 Hz, 1H), 1.2 and 4.0 IPA peak

Ring-closed structure:



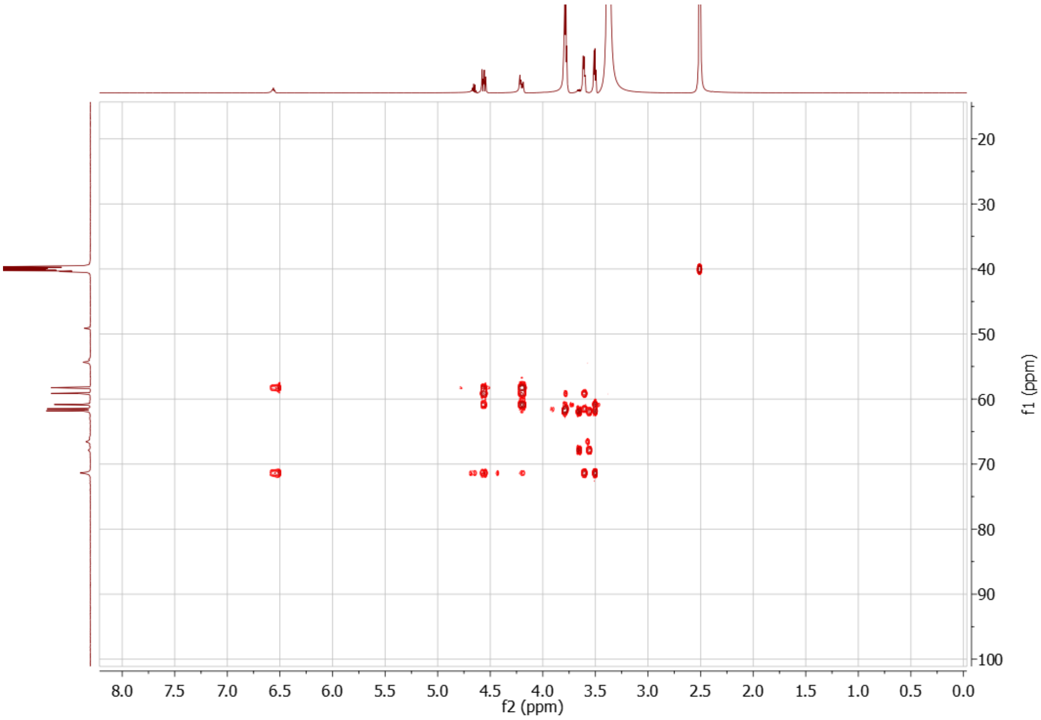
2-Hydroxy-7-oxa-4-azaspiro[3.5]nonan-4-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.50 (t, *J* = 4.8 Hz, 2H), 3.61 (q, *J* = 5.4, 4.8 Hz, 2H), 3.79 (q, *J* = 4.9 Hz, 4H), 4.59 – 4.53 (m, 2H), 4.65 (dp, *J* = 12.2, 5.7 Hz, 1H), 6.55 (qd, *J* = 10.7, 7.4, 4.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 58.25, 59.13, 60.85, 61.80, 71.37.

COSY:



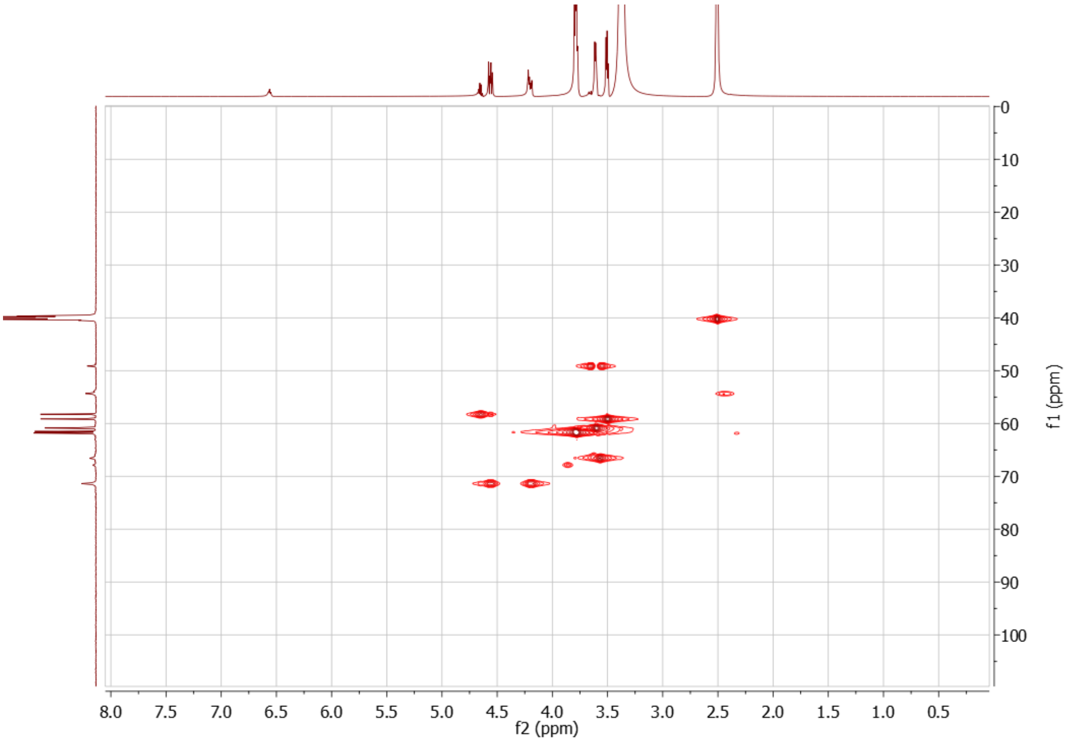
S.Figure 1. COSY spectra for salt 1, morpholine-Az

HMBC:



S.Figure 2. HMBC for salt 1, morpholine-Az.

HSQC



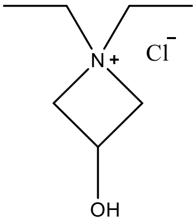
S.Figure 3. HSQC for salt 1, morpholine-Az.

2. Di-Ethyl-Az

Ring-open structure:

1-Chloro-3-(diethylamino)propan-2-ol. Following standard procedure, using 33 mmol (3.05 g, 2.59 mL) ECH and 30 mmol (2.20 g, 2.58 mL) di-ethylamine. ^{1}H NMR (400 MHz, Chloroform-d) δ 1.26, dt, J = 11.0, 7.2 Hz (6H), 2.60, ddd, J = 27.3, 13.4, 6.5 Hz (1H), 3.50, q, J = 7.2 Hz (2H), 3.78, q, J = 7.2 Hz (2H), 4.68 – 4.43, m (4H), 7.00, s (1H).

Ring-closed structure:



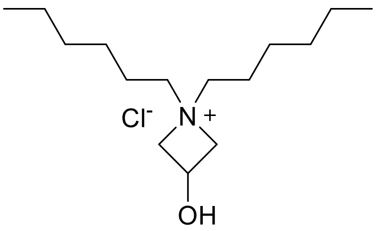
1-Chloro-3-(diethylamino)propan-2-ol. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 70 °C. ^{1}H NMR (400 MHz, DMSO) δ 1.11, t, J = 7.2 Hz (6H), 3.34 – 3.26, m (2H), 3.52 – 3.37 m (2H), 4.04, dd, J = 9.9, 4.7 Hz (2H), 4.45 – 4.34 m (2H), 4.60, q, J = 6.3 Hz (1H), 6.41, d, J = 30.1 Hz (1H) ^{13}C NMR (101 MHz, DMSO-d_6) δ 69.80 (d, J = 8.8 Hz), 57.46 (d, J = 9.0 Hz), 55.40, 53.39, 7.77 (d, J = 9.0 Hz), 7.38 (d, J = 9.3 Hz).

3. Di-Hexyl-Az

Ring-open structure:

1-Chloro-3-(dihexylamino)propan-2-ol. Following standard procedure, using 3.3 mmol (0.305 g, 0.259 mL) ECH and 3 mmol (0.56 g, 0.70 mL) dihexylamine. ^{1}H NMR (600 MHz, Chloroform-d) δ 3.83 (dq, J = 9.6, 4.9 Hz, 1H), 3.69 (dd, J = 9.2, 5.7 Hz, 1H), 3.60 – 3.50 (m, 2H), 3.34 – 3.19 (m, 1H), 2.92 – 2.33 (m, 6H), 1.80 (p, J = 7.8 Hz, 1H), 1.57 – 1.38 (m, 4H), 1.28 (tt, J = 9.5, 5.0 Hz, 12H), 0.88 (t, *J* = 6.9 Hz, 6H).

Ring-closed structure:



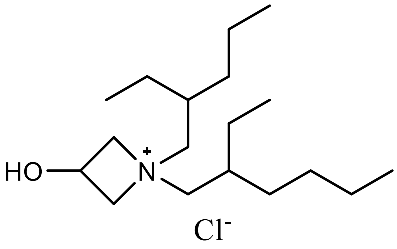
1,1-Dihexyl-3-hydroxyazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. 1H NMR (600 MHz, DMSO-d6) δ 0.86 (qd, *J* = 6.8, 2.8 Hz, 6H), 1.28 (d, *J* = 3.9 Hz, 12H), 1.70 – 1.38 (m, 4H), 3.29 – 3.19 (m, 2H), 3.50 – 3.27 (m, 2H), 4.11 (dd, *J* = 11.8, 5.6 Hz, 2H), 4.49 – 4.37 (m, 2H), 4.67 – 4.57 (m, 1H), 6.48 (dt, *J* = 6.5, 2.0 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d6) δ 13.88, 21.58, 22.23, 25.32, 26.20, 30.70, 31.07, 57.70, 58.70, 60.66, 70.93.

4. Di-Ethyl-Hexyl-Az

Ring open structure:

1-(Bis(2-ethylhexyl)amino)-3-chloropropan-2-ol. Following standard procedure, using 5.5 mmol (0.51 g, 0.43 mL) ECH and 5 mmol (1.21 g, 1.50 mL) bis-2ethylhexylamine. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 0.97 – 0.74 (m, 12H), 1.21 – 1.16 (m, 1H), 1.36 – 1.21 (m, 12H), 1.41 (tq, *J* = 10.7, 4.4, 3.4 Hz, 3H), 1.52 – 1.44 (m, 1H), 2.22 – 2.14 (m, 2H), 2.32 – 2.21 (m, 2H), 2.51 – 2.36 (m, 3H), 3.56 (dt, *J* = 5.0, 2.4 Hz, 2H), 3.82 (dq, *J* = 9.6, 4.9 Hz, 1H).

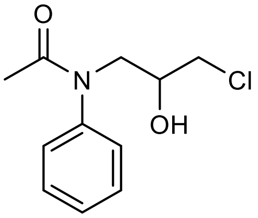
Ring-closed structure:



1-(2-Ethylhexyl)-1-(2-ethylpentyl)-3-hydroxyazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 70 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 – 0.64 (m, 12H), 1.27 – 1.05 (m, 12H), 1.43 – 1.29 (m, 4H), 2.21 – 2.02 (m, 5H), 2.44 – 2.27 (m, 2H), 3.58 – 3.44 (m, 1H), 3.75 – 3.61 (m, 2H), 4.92 (q, *J* = 4.8 Hz, 1H).

5. Acetanilid.

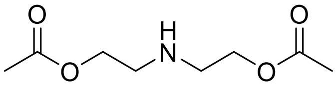
Ring-open structure:



N-(3-Chloro-2-hydroxypropyl)-N-phenylacetamide. Following standard procedure, using DCM as solvent. 11 mmol (1.01 g, 0.86 mL) ECH and 10 mmol (1.35 g) acetanilid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.17 (d, *J* = 2.2 Hz, 3H), 2.70 (dd, *J* = 4.8, 2.5 Hz, 1H), 2.90 (q, *J* = 3.2, 1.8 Hz, 1H), 3.36 – 3.13 (m, 1H), 3.57 (dd, *J* = 5.4, 1.9 Hz, 2H), 7.10 (t, *J* = 7.4 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H)

6. Di-Acetoxy-Ethyl-Az

Amine:



Azanediylbis(ethane-2,1-diyl) diacetate. Using the described procedure, starting with 10 mmol (1.0514 g, 0.956 mL) diethanolamine. The remaining reactants were scaled to fit. ^{1}H NMR (700 MHz, Chloroform-*d*) δ 1.62 (s, 6H), 2.07 (d, *J* = 10.6 Hz, 2H), 2.16 (d, *J* = 12.5 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 4H).

Ring-open structure: Mixed product

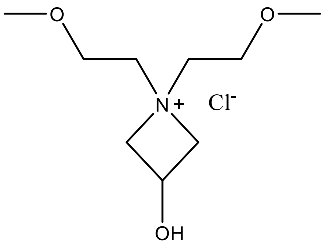
Ring-closed structure: Mixed product

7. Di-MethoxyEthyl-Az

Ring-open structure:

1-(Bis(2-methoxyethyl)amino)-3-chloropropan-2-ol. Following standard procedure, using 3.3 mmol (0.305 g, 0.259 mL) ECH and 3 mmol (0.40 g, 0.44 mL) dimethoxy amine. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.67 (tt, *J* = 13.4, 5.4 Hz, 1H) 2.83 (pd, *J* = 8.9, 6.0 Hz, 3H), 3.12 (t, *J* = 5.1 Hz, 1H), 3.34 (d, *J* = 2.4 Hz, 6H), 3.40 (s, 1H), 3.52 – 3.42 (m, 2H), 3.61 – 3.52 (m, 3H), 3.74 – 3.69 (m, 1H), 3.87 – 3.79 (m, 1H).

Ring-closed structure:



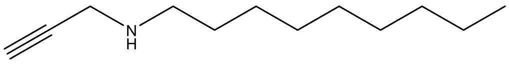
3-Hydroxy-1,1-bis(2-methoxyethyl)azetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 70 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.30 – 3.19 (broad, 12H), 3.64 – 3.58 (m, 1H), 3.70 – 3.65 (m, 3H), 3.73 (q, *J* = 3.7, 3.1 Hz, 1H), 4.26 (ddd, *J* = 11.4, 4.4, 1.9 Hz, 1H), 4.72 – 4.45 (m, 1H), 6.40 (t, *J* = 5.9 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 54.09, 58.55, 59.20, 61.20, 66.35, 66.52, 68.91, 73.39.

8. Suninimide-Az

No product from reaction

9. Propargyl-Nonyl-Az

Amine:



N-(Prop-2-yn-1-yl)nonan-1-amine. Following the procedure from the Materials and Method part. Nonanol is present in the reaction product. ^{1}H NMR (600 MHz, Chloroform-d) δ 0.92 – 0.83 (m, 3H), 1.37 – 1.18 (m, 12H), 1.53 – 1.43 (m, 2H), 2.20 (t, *J* = 2.4 Hz, 1H), 2.74 – 2.61 (m, 2H), 3.43 (d, *J* = 2.5 Hz, 2H). ^{13}C NMR (151 MHz, Chloroform-d) δ 14.26, 22.82, 27.46, 29.43, 29.61, 32.03, 38.32, 48.88, 71.28, 82.49.

Ring-open structure: Mixed product

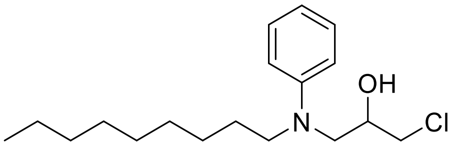
Ring-closed structure: Mixed product

10. Benzy-Nonyl-Az

Amine:

N-Benzylnonan-1-amine. Following the procedure from the Materials and Method part. DCM was used as the solvent, 5 mmol (0.54 g, 0.55 mL) benzylamine was used with 5.5 mmol (0.78 g, 0.95 mL). 1.5 mmol (0.057 g) $NaBH_4$ were used in the reducing step. Nonanol is present in the reaction product. ^{1}H NMR (600 MHz, Chloroform-d) δ 0.89 – 0.77 (m, 3H), 1.31 – 1.08 (m, 12H), 1.45 (p, *J* = 7.2 Hz, 2H), 2.57 (t, *J* = 7.3 Hz, 2H), 3.73 (s, 2H), 7.21 – 7.17 (m, 2H), 7.23 (d, *J* = 4.7 Hz, 1H), 7.30 – 7.26 (m, 2H).

Ring-open structure:



1-(Benzyl(nonyl)amino)-3-chloropropan-2-ol. Following standard procedure, using 5.5 mmol (0.51 g, 0.43 mL) ECH and 5 mmol (1.67 g) amine. ^{1}H NMR (600 MHz, Chloroform-d) δ 0.88 – 0.79 (m, 3H), 1.32 – 1.11 (m, 14H), 2.38 (ddd, *J* = 12.9, 8.8, 5.4 Hz, 1H), 2.52 – 2.45 (m, 2H), 2.55 (dd, *J* = 12.8, 4.4 Hz, 1H), 3.46 (d, *J* = 5.1 Hz, 2H), 3.72 (s, 1H), 3.70 (s, 1H), 3.79 (dq, *J* = 9.6, 5.0 Hz, 1H), 7.24 – 7.19 (m, 3H), 7.27 (dd, *J* = 8.2, 6.8 Hz, 2H).

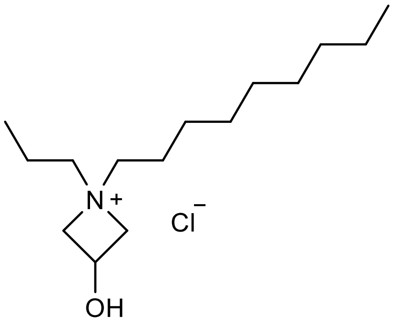
Ring-open structure: Mixed product

11. Nonyl-Propyl-Az

Ring-open structure:

1-Chloro-3-(nonyl(propyl)amino) propan-2-ol. Following standard procedure, using 1.1 mmol (0.10 g, 0.086 mL) ECH and 1 mmol (0.192 g) nonylpropyl amine. ^{1}H NMR (400 MHz, Chloroform-d) δ 0.88 (t, *J*=8Hz, 3H) 0.94, (t, *J*=8Hz, 3H) 1.20-1.36, (broad singlet, 12H), 1.37-1.57 (m, 4H), 2.35-2.55 (broad, 4H), 2.55-2.65 (dt, *J*=8Hz, 2H), 3.55 (d, *J*=8Hz, 2H), 3.83 (m, 1H). ^{13}C NMR (101 MHz, CDC3) δ 11.91, 14.27, 20.44, 22.82, 27.26, 27.52, 29.43, 29.73, 32.02, 47.29, 51.94, 54.50, 56.39, 57.80, 67.11.

Ring-closed structure:



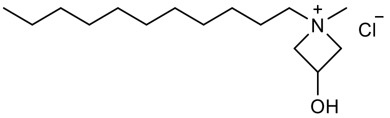
1-Hydroxy-1-nonyl-1-propylazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. ^{1}H NMR (400 MHz, Chloroform-d) δ 0.85 (dt, *J* = 7.2, 3.7 Hz, 6H), 1.16 – 1.54 (m, 16H), 2.23 – 2.80 (m, 6H), 4.20 – 4.30 (m, 1H), 4.46 (td, *J* = 8.2, 2.9 Hz, 1H), 4.63 – 4.74 (m, 1H).

12. Undeca-Methyl-Az

Ring-open structure:

1-Chloro-3-(methyl(undecyl)amino)propan-2-ol. Following standard procedure, using 0.55 mmol (0.051 g, 0.043 mL) ECH and 0.5 mmol (0.093 g) undecamethylamine. ^{1}H NMR (400 MHz, Chloroform-d) δ 0.88 (t, *J*=8Hz, 3H) 1.20-1.32 (broad singlet, 16H), 1.42-1.48 (broad, 2H), 2.26 (s, 3H), 2.32-2.40 (broad, 2H), 2.43-2.58 (broad, 2H), 3.55 (d, *J*=8Hz, 2H), 3.85 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-d) δ 14.28, 22.83, 27.40, 27.43, 29.48, 29.70, 29.77, 32.06, 42.29, 47.28, 58.24, 60.71, 67.06.

Ring-closed structure:



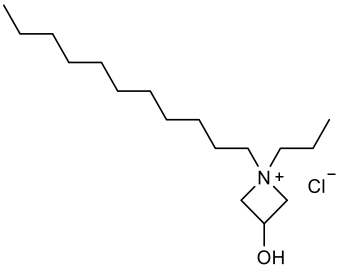
3-Hydroxy-1-methyl-1-undecylazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. ^{1}H NMR (400 MHz, DMSO) δ 0.81 (t, *J*=8Hz, 3H), 1.21-1.41 (m, 14H), 2.22-2.42 (m, 2H), 3.48-3.57 (m, 2H), 3.60-3.73 (m, 6H), 4.98 (d, *J*=9 Hz, 1H).

13. Undecyl-Propyl-Az

Ring-open structure:

1-Chloro-3-(propyl(undecyl)amino)propan-2-ol. Following standard procedure, using 1.1 mmol (0.11 g, 0.086 mL) ECH and 1 mmol (0.2 g) undecapropylamine. ^{1}H NMR (400 MHz, Chloroform-d) δ 0.88 (t, *J*=8Hz, 6H), 1.26 (broad singlet, 16H), 1.36-1.52 (broad, 4H), 2.32-2.60 (broad, 6H), 3.55 (d, *J*=8Hz, 2H), 3.82 (m, 1H).

Ring-closed structure:



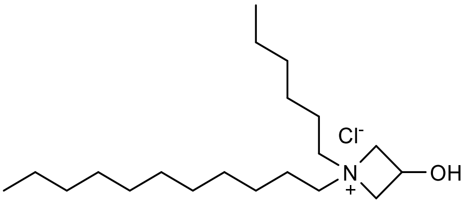
1-Hexyl-3-hydroxy-1-undecylazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. ^{1}H NMR (400 MHz, DMSO) δ 0.85 (t, *J*=8Hz, 3H), 0.89 (t, *J*=8Hz, 3H), 1.21 (m, 16H), 1.51 (broad, 4H), 3.18-3.37 8m, 4H), 4.02 (d, *J*=8Hz, 2H), 4.43 (dd, *J*=2, 8 Hz, 2H), 4.61 (d, *J*=4Hz, 1H).

14. Undeca-Hexyl

Ring-open structure:

1-Chloro-3-(pentyl(undecyl)amino) propan-2-ol. Following standard procedure, using 2.75 mmol (0.25 g, 0.22 mL) ECH and 2.5 mmol (0.2 g) undecapropylamine. ^{1}H NMR (400 MHz, Chloroform-d) δ 0.88 (t, *J*=8Hz, 6H), 1.25 (broad singlet, 22H), 1.35-1.55 (broad, 4H), 2.36-2.65, 2.61 (broad, 6H), 3.55 (d, *J*=8Hz, 2H), 3.81(m, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 14.21, 14.28, 22.79, 22.84, 27.19, 27.23, 27.26, 27.52, 29.49, 29.71, 29.77, 31.91, 32.06, 47.29, 54.48, 57.79, 67.10.

Ring-closed structure:



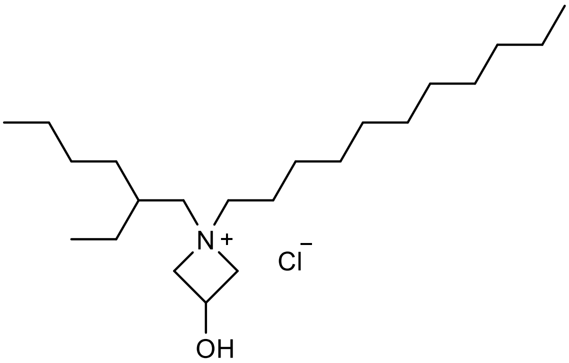
1-Hexyl-3-hydroxy-1-undecylazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. ^{1}H NMR (400 MHz, DMSO) δ 0.86 (t, *J*=8Hz, 6H), 1.21-1.26 (m, 22H), 1.51 (broad, 4H), 3.20-3.37 (m, 4H), 4.06 (d, *J*=8Hz, 2H), 4.42 (dd, *J*=2, 8 Hz, 2H), 4.61 (d, *J*=4Hz, 1H)

15. Undeca-2EthylHexyl-Az

Ring-open structure:

1-Chloro-3-(pentyl(undecyl)amino)propan-2. Following standard procedure, using 0.88 mmol (0.082 g, 0.69 mL) ECH and 0.8 mmol (0.23 g) undeca-2-ethyl-hexylamine. ^{1}H NMR (400 MHz, Chloroform-d) δ 0.87 (broad multiplet, 9H), 1.25 (broad singlet, 24H), 1.32-1.47, (broad, 3H), 2.20-2.73 (m, 6H) 3.55 (t, 8=Hz, 2H), 3.81 (m, 1H).

Ring-closed structure:



1-(2-Ethylhexyl)-3-hydroxy-1-undecylazetidin-1-ium chloride. Using the standard procedure, ring-closed with *i*PrOH:water (1:9), 80 °C. ^{1}H NMR (400 MHz, DMSO) δ 0.86 (broad, 9H), 1.21-1.26 (m, 24H), 1.55 (broad, 3H), 3.20-3.37 (m, 4H), 4.06 (d, *J*=8Hz, 1H), 4.15 (d, *J*=8Hz, 1H), 4.37 (d, *J*=8Hz, 1H), 4.52 (d, *J*=8 Hz, 1H), 4.67 (d, *J*=4Hz, 1H).

16. Hexadecyl-Propyl-Az

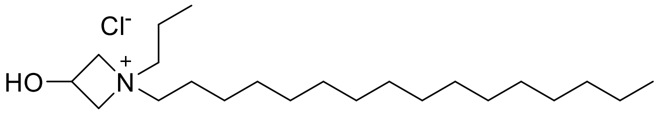
Amine:

N-Propylhexadecan-1-amine. Following the procedure from the Materials and Method part. MeOH was used as the solvent, and 10 mmol (2.41 g) hexadecyl amine was used with 10 mmol (0.58 g, 0.72 mL). 3.0 mmol (0.12 g) $NaBH_4$ were used in the reducing step. Further purified with buffer extraction. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 – 0.83 (m, 6H) 1.25 (s, 26H) 1.49 (dt, *J* = 14.7, 7.4 Hz, 2H) 1.57 (s, 18H) 2.57 (q, *J* = 7.7 Hz, 2H)

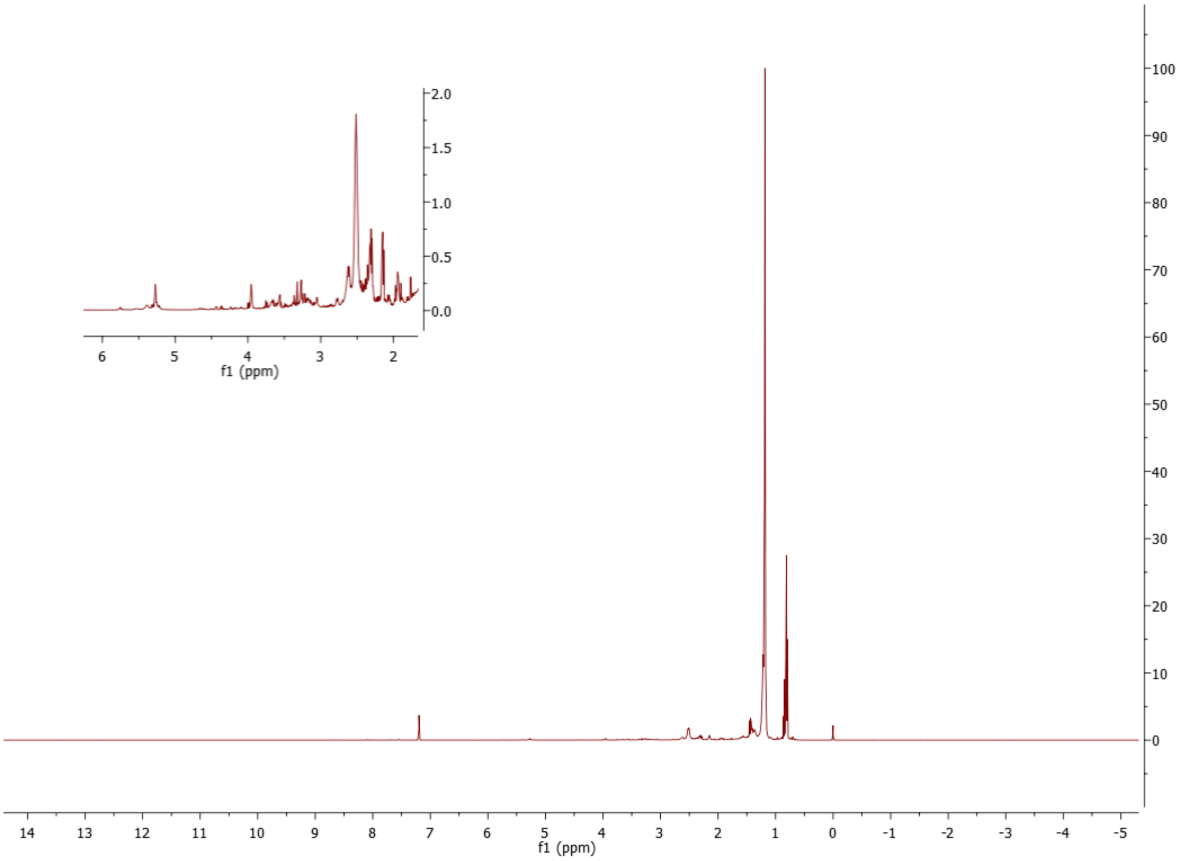
Ring-open structure:

1-Chloro-3-(hexadecyl(propyl)amino)propan-2-ol. Following standard procedure, using 0.55 mmol (0.051 g, 0.043 mL) ECH and 0.5 mmol (0.147 g) hexadecylproylamine in DCM. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (dt, *J* = 12.7, 7.2 Hz, 6H) 1.23 (s, 25H) 1.64 – 1.33 (m, 4H) 2.55 (dt, *J* = 8.5, 7.2 Hz, 2H) 2.66 (dd, *J* = 4.8, 2.4 Hz, 2H) 2.91 – 2.84 (m, 2H) 3.22 (dddd, *J* = 6.1, 4.9, 3.9, 2.5 Hz, 1H, OH) 3.56 (qd, *J* = 11.7, 5.4 Hz, 5H).

Ring-closed structure:



1-Hexadecyl-3-hydroxy-1-propylazetidin-1-ium chloride. Ring-closed using SDS reaction. Characteristic peaks between 2.0 – 6.0 ppm were detected, but small compared to the alkyl chain.



S.Figure 4. ^{1}H NMR spectra for Hexadecyl-Propyl-Az

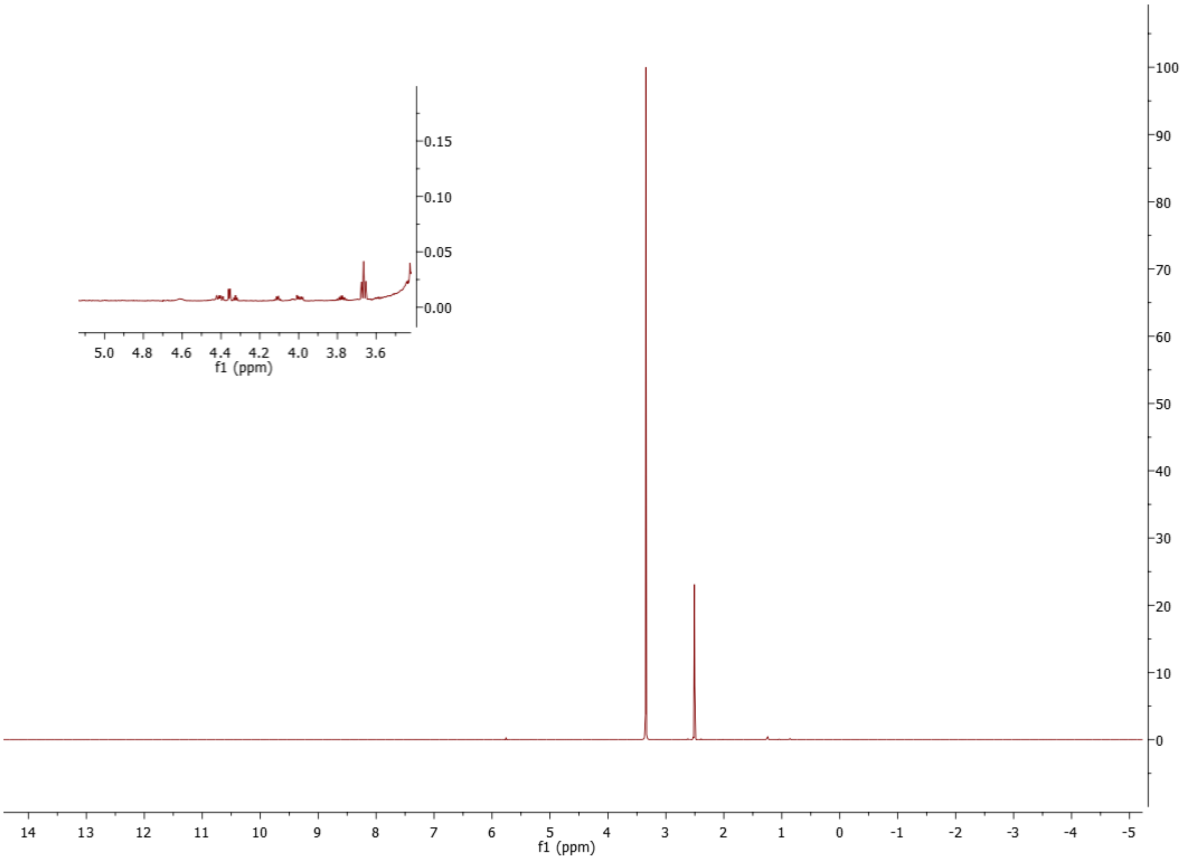
17. Di-Heptadecyl-Az

Ring open structure:

1-Chloro-3-(diheptadecylamino)propan-2-ol. Following standard procedure, using DCM as solvent. 11 mmol (1.01 g, 0.86 mL) ECH and 10 mmol (3.98 g) diheptadecylamine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 6H) 1.25 (s, 58H) 1.47 (q, *J* = 7.2 Hz, 4H) 2.58 (t, *J* = 7.3 Hz, 4H) 2.69 (dd, *J* = 4.9, 2.5 Hz, 1H) 2.90 (t, *J* = 4.4 Hz, 1H) 3.25 (dp, *J* = 5.5, 2.8 Hz, 1H) 3.58 (d, *J* = 5.4 Hz, 2H).

Ring-closed structure:

1,1-Diheptadecyl-3-hydroxyazetidin-1-ium chloride. Ring-closed using SDS reaction. Characteristic peaks between 3.6 – 4.6 ppm were detected, but small compared to the alkyl chain.



S.Figure 5. S. ^{1}H NMR spectra for Di-Heptaecyl-Az.

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