PRODUCING RENEWABLE CHEMICALS FROM FISHERY WASTE: CHITIN, CHITOSAN AND THEIR MONOMERS

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by

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ABSTRACT

Chitin, chitosan, and their monomers were investigated as renewable feedstocks to produce useful chemicals. These biopolymers are readily available, cheap, non-toxic, and environmentally benign. They are produced, degraded and reabsorbed in nature. They could in part replace depleting resources, such as fossil fuels, in useful chemicals production. To this end, microwave heating, catalysts and additives have been investigated. Factorial designs (FDs) were used as an effective approach to optimise the reaction conditions. Reaction products obtained were extracted using ethyl acetate and their amounts were determined using GC-MS. Although these biopolymers are difficult to solubilise, useful products were obtained in water under the reaction conditions described herein. Levulinic acid (LA) and 5-hydroxymethylfurfural (5-HMF) were produced from chitosan using $SnCl_4 \cdot 5H_2O$ which can be handled with ease and is a relatively inexpensive reagent. The medium for this reaction (water) is non-toxic, non-flammable, and readily available at low cost. The chitosan reaction mixture was heated to 200 °C under microwave irradiation for 30 min in a closed-vessel. Two different reaction concentrations were tested; the "concentrated reaction" yielded 23.9 wt% LA, and the "diluted reaction" yielded 10.0 wt% 5-HMF. Each reaction has a high selectivity for the desired product; chitin was also able to produce LA under the concentrated reaction conditions (12.7 wt%). This proof of concept study showed that amino-saccharides can be used as feedstocks for levulinic acid and 5-hydroxymethylfurfural typically obtained from cellulosic biomass. It was discovered that a nitrogen containing compound, 3-acetamido-5-acetylfuran (3A5AF), could also be produced from chitin and chitosan in small

amounts. However, by using *N*-acetylglucosamine (NAG) instead of chitin, a higher % yield of 3A5AF could be obtained, possibly due to limitations in the depolymerisation process of chitin. The transformation of NAG was dependent on the supplier of this amino-sugar and it was discovered that B- and Cl-containing impurities in one source were likely aiding the conversion process. Therefore, NAG was reacted in the presence of two additives: NaCl and $B(OH)_3$. After solvent-screening, the reaction was optimised in dimethylacetamide (DMA). The reaction mixture was heated using microwave irradiation to 220 °C for 15 min. 3A5AF production was quantified using GC-MS. The % yield of 3A5AF was determined to be ca 58. This study showed that this compound could potentially be produced cheaply and is a potential renewable feedstock for other N-containing molecules e.g. proximicins A, B and C (antibiotic and antitumor drugs). As an alternative to this method, a procedure was developed in ionic liquids (ILs) to produce 3A5AF. 3A5AF could be obtained in up to 60% yield by heating NAG in the presence of NaCl and $B(OH)_3$. In summary, the research described in this thesis demonstrates the potential of fishery waste to produce valuable chemicals (LA, 5-HMF, and 3A5AF). Mechanisms of product formation are also proposed and discussed.

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DEDICATION

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This thesis is a gift to the soul of my lovely mother (Yusra).

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List of Symbols and Abbreviations

α: alpha

Ac: acetyl

atm: atmosphere

A: pre-exponential factor in Arrhenius equation

β: beta

ca.: about (Latin circa)

^{13}C NMR: carbon nuclear magnetic resonance

°C: degree Celsius

Da: dalton

DD: degree of deacetylation

DoE: design of experiment

d_f: degree of freedom

δ: chemical shift in parts per million downfield from a standard

δ: delta

E_a: activation energy

etc.: and so forth (Latin et cetera)

EI: electron ionization

EMT: electron multiplier tube

equiv: equivalent

e.g.: for example (Latin exempli gratia)

FDs: factorial designs

γ: gamma

GC-MS: Gas Chromatography-Mass Spectrometry

GFC: gel filtration chromatography

GPC: gel-permeation chromatography

GHz: gigahertz (10^9 Hz)

GHG: greenhouse gases

h: hour

^{1}H NMR: proton nuclear magnetic resonance

IR: infrared spectroscopy

IL: ionic liquid

i.e.: that is (Latin id est)

J: coupling constant (NMR)

kDa: kilodalton

k: rate constant

LC-MS: Liquid Chromatography-Mass Spectrometry

LCFS: (California's) low carbon fuel standard

ME: margin of error

MS: mass spectrometry

m/z: mass to charge ratio

MALDI: matrix-assisted laser desorption ionization

m: median

μL: micro liter (10^{-6} L) min: minute

mL: milliliter (10^{-3} L)

MW: microwave

mol%: mole percent of a substance

NCW: near critical water

NMR: nuclear magnetic resonance

OAc: acetate

OFAT: one factor at a time

P_c: critical pressure

pK_a: negative logarithm of acid dissociation (ionization) constant

pH: negative logarithm of hydrogen ion concentration

PSE: pseudo standard error

R: gas constant

R_f: retention or retardation time

s: pseudo standard deviation of absolute values

s: second

scCO_2: supercritical fluid carbon dioxide

scH_2O: supercritical fluid water

T_c: critical temperature

t_R: retention time

T: temperature

vs: versus

VOCs: volatile organic compounds

v/v: volume per volume (volume of solute/volume of solution)

w/v: weight per volume (weight of solute/volume of solution)

wt%: weight per weight percentage (see section 1-16-1)

w/w: weight per weight (weight of solute/weight of solution)

% yield: percent yield (see section 1-16-2)

Chapter One

Introduction and Overview

Green chemistry is a key research area in global efforts toward achieving a clean environment and sustainable development. Green chemistry is made possible by designing chemical products and processes in which the use and generation of waste and hazardous substances are reduced or eliminated. To achieve this goal, chemists try to apply the twelve principles of green chemistry. Paul Anastas and John Warner introduced these principles, which state that (i) waste prevention is better than clean up, (ii) maximize atom economy (Equation 1-1) in a way that lets reactants be highly incorporated into the final product, (iii) perform less hazardous chemical synthesis, (iv) design safer chemicals, (v) use safer solvents and minimize auxiliaries, (vi) design for energy efficiency, (vii) use renewable feedstocks, (viii) reduce derivatives, (ix) use catalysts, (x) design for degradation, (xi) use real-time analysis for pollution prevention and (xii) perform inherently safer chemistry for accident prevention.

$$\text{Atom economy} = \frac{\text{Molecular weight of desired product(s)}}{\Sigma\,\text{Molecular weight of all reactants}} \times 100\% \qquad \textbf{Equation 1-1}$$

Green chemistry may be applied in many areas including: small scale research laboratories, large scale industrial processes, education (e.g., teaching laboratories) and public awareness.[1] In the early stage of any process or methodology, raw material are the first thing to be considered in the manufacture of a product. In this thesis, the tested raw materials are renewable feedstocks, including chitin, chitosan and their constituent sugars.

1-1 Renewable Feedstocks

A feedstock, also known as a raw material, is required in the manufacture of all products. There are two types of feedstocks: depleting and renewable. Depleting resources, such as fossil fuels including petroleum, natural gas and coal, need millions of years to be produced again. The rate of consumption of this type of feedstock is higher than its formation in nature. In short, the sun will continue to shine whilst petroleum runs out. Therefore, solar power is a source of renewable feedstocks. A renewable feedstock is a material that can be regenerated in nature easily and fast.[1] Recently, significant attention has been paid to renewable resources especially in the energy field and in the production of chemicals. Two driving forces are responsible for the inherent attraction of using renewable resources. The first one is the economic factor, which is related directly to the increasing price of petroleum resources. The second is the environmental impact of using depleting resources.[2] The burning of fossil fuels produces significant emissions of greenhouse gases (GHG) such as CO_2 and CH_4, which contribute to climate change.[3] Based on this fact, one of the options of the regulated parties to meet California's low carbon fuel standard (LCFS) and reduce GHG emissions is to encourage refiners to mix gasoline and diesel with ethanol.[3]

Examples of renewable feedstocks are plants including trees and animals, such as crabs. While cellulose is a biopolymer obtained from plants, chitin is a biopolymer found in crustacean shells; both can be used to produce useful products.[4] These polysaccharides are created, biodegraded and reabsorbed in nature, readily available, environmentally

benign and a source of renewable feedstocks. The solubility of chitin and chitosan in various solvents is an important factor in utilizing them to produce useful products.

1-2 Chitin and Its Isolation

1-2-1 Chitin Background

In 1811, Braconnot isolated chitin for the first time from different kinds of mushrooms. He named his impure product "fungine". He proposed that fungine was a new substance and it was different from the woody materials of plants. In 1823, the name chitin was given by Odier who isolated this biopolymer from the elytrum (a hard cover that is protecting the fragile wings) of the Cockchafer beetle. The word chitin comes from the Greek word meaning tunic.[5] Chitin is an amino polysaccharide made up of *N*-acetylglucosamine (NAG) (Figure 1-1). The IUPAC name for NAG is 2-acetamido-2-deoxy-β-D-glucose. In chitin, NAG monomers are connected through β (C1-C4) linkages. Chitin is the third most abundant biopolymer after cellulose and hemicellulose on earth and the most abundant biopolymer in the marine environment.[6] The primary difference in the structures of chitin and cellulose is the functional group on C2, which are acetamido and hydroxyl groups, respectively.

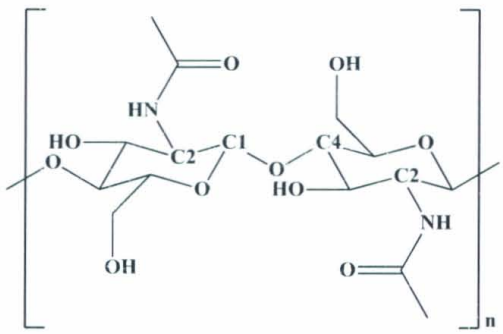


Figure 1-1. The molecular formula of chitin showing β-linkages and N-acetamido functionality.

Chitin is water insoluble material that can bind strongly with proteins and minerals such as calcium carbonate. This combination gives the rigid structure of the composite material, which forms crustaceans' shells. Chitin is available in three polymorphs. All of these polymorphs are obtained in nature in different packing configurations. The first one is antiparallel packing that is called α-chitin. The second one is called β-chitin in which chains of the biopolymer are packed in parallel. A mixture of α- and β-chitins is called γ-chitin, which is the last polymorph. The major one is α-chitin and the minor is γ-chitin.[7] Marine algae produce β-chitin. β-chitin is also the major polymorph in squid pens.[8] β-chitin is relatively rare and can be found in anhydrous and hydrate forms.[9] α-Chitin is the main component of crustaceans' shells such as crab, lobster and shrimp.[8] Chitin is also available in the exoskeletons of insects and molluscs and in the cell wall of fungi and algae.[9] In some insect species, chitin can reach 40% of the dry mass of cuticles. Chitin is biosynthesized, degraded and reabsorbed naturally. Many enzymes are responsible for the biosynthesis of chitin in insects.

The synthesis of chitin begins with trehalose, a major hemolymph sugar found in most insects (Figure 1-2). Trehalose changes into many different phosphate sugars until it forms the sugar, uridine diphosphate–NAG (UDP-NAG). Chitin synthase then converts UDP-NAG into the chitin biopolymer. Chitin degradation in insects is catalyzed by the presence of chitinases and chitinolytic enzymes such as poly [1,4-(*N*-acetyl-β-D-glucosaminide)] glycanohydrolase and β-*N*-acetylglucosaminidases. These types of enzymes hydrolyze chitin into oligomers.[10]

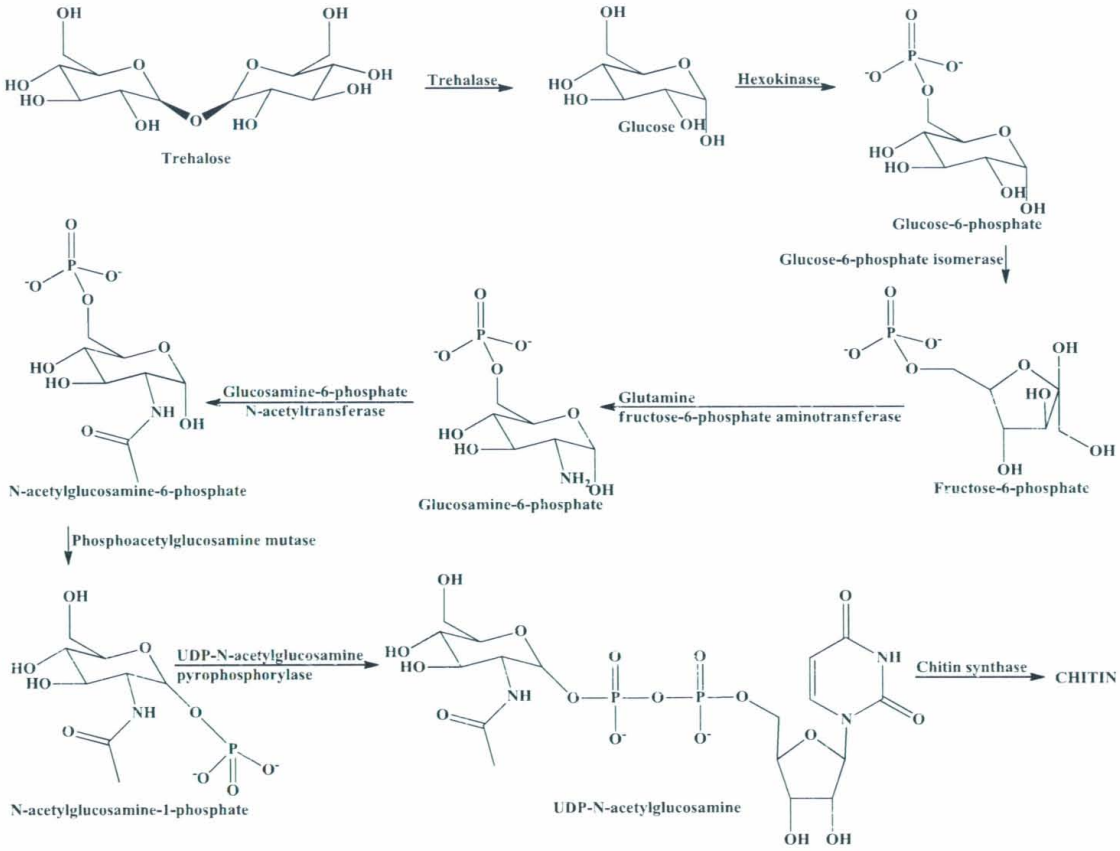


Figure 1-2. Chitin synthesis in insects starting from trehalose.

Chitin degradation in the oceans is an important pathway for cycling of nutrients. This process involves ocean-dwelling bacteria via a two-component regulatory system in which chitin is detected and then a gene that is responsible for the breakdown of chitin into its oligomers is activated.[11] Therefore, chitin is a biopolymer that is produced in nature, degraded and reabsorbed in a natural biocycle. The molecular weight of natural chitin is ca. 1-2 $\times$ 10^3 kDa.[12] The annual estimated amount of biosynthesized chitin (crustaceans, molluscs, insects and fungi) is 10^{10} to 10^{12} tons.[13,14] It is estimated that 14-35% (dry weight) of shellfish processing waste is chitin.[13] For example, shrimp are popular seafood that produce 40-50% wt% waste during their processing to be made ready for human consumption,[15] and 30-40% of this waste is chitin.

1-2-2 Chitin Isolation

Chitin from marine sources needs to be purified due to its association with proteins (15-40%, on a dry weight basis), minerals: $CaCO_3$ and $Ca_3(PO_4)_2$ (35-55%, on a dry weight basis), lipids (0-10%, on a dry weight basis), and pigments.[13] The production of chitin, Figure 1-3, involves three main steps after grinding, which are demineralization, deproteination and decolouration. In the first step, an acid such as hydrochloric acid, sulfuric acid, nitric acid or acetic acid can be used to solubilize the minerals, which are mainly calcium carbonate.[15,16] This step can be performed at room temperature in 1 to 3 h. Chitin hydrolysis also takes place in this acid demineralization step. However, ethylenediaminetetraacetic acid (EDTA) can be used instead of acids in the demineralization step to overcome the hydrolysis of chitin.[16] The second process is protein removal, which can be achieved using a basic medium such as sodium or

potassium hydroxide or carbonate, sodium sulfide, calcium hydrogen sulfite or sodium phosphate. This step is conducted between 65-100 °C for 30 min to 6 h.[16] In the shells of decapod crustaceans, a carotenoid pigment is present and is known as astaxanthin, which is the principal pigment in crustacean shells. This pigment is available in combination with a macromolecular protein complex called crustacyanin, which is responsible for giving the typical orange-red colour to these animals.[17] A bleaching agent such as hydrogen peroxide or potassium permanganate is used to oxidize the pigments to produce colorless chitin in the last step of the production process. Strong acids and bases damage the environment, are hazardous chemicals by themselves and, to a certain extent, can depolymerize chitin. An alternative process for chitin production is the use of a protease enzyme to remove protein.[18] Bacteria can be used to remove minerals, which produce lactic acid during fermentation.[15]

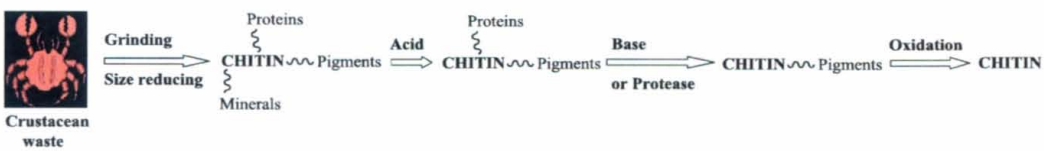


Figure 1-3. A schematic diagram of the chitin purification process.

Recently, it has been shown that chitinous biomass could be purified using ionic liquids (ILs), where 10 g of 1-ethyl-3-methyl-imidazolium acetate [EMIm]OAc (an IL), Figure 1-4, can dissolve 0.46 g of chitinous biomass heated in an oil bath at 100 ºC over 19 h.[19] Chitin was recovered from the IL by adding water to the mixture, as water is able to dissolve the IL, but not the chitin. The resulting chitin, in powder form, was washed several times using water and then dried in an oven at 80 °C. In a related procedure, using a microwave heating method, 0.3 g of chitinous biomass can be dissolved in 10 g of the

same IL after a 2 min exposure to microwave irradiation, greatly reducing the time in comparison to conventional heating.

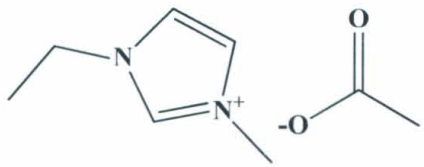


Figure 1-4. The ionic liquid 1-ethyl-3-methyl-imidazoium acetate, which can dissolve crustaceans' shells.

1-3 Chitosan and Its Preparation

Chitosan, Figure 1-5, is the deacetylated form of chitin (i.e., after the removal of the acetyl group (CH_3CO) from the nitrogen in chitin). In 1859, Rouget discovered chitosan by refluxing chitin with concentrated potassium hydroxide. At that time he named chitosan "modified chitin". The name chitosan was given in 1894 by Hoppe-Seyler.[5] Chitosan can also be called poly(glucosamine) or poly(2-amino-2-deoxy-β-D-glucose).

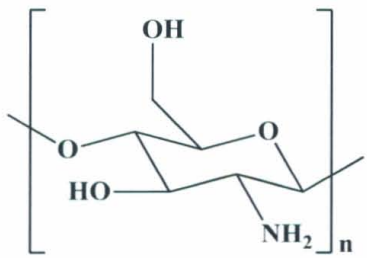


Figure 1-5. Molecular formula showing the deacetylated form of chitin (assuming DD is 100%), chitosan.

However, this is somewhat inaccurate as it is close to impossible to deacetylate chitin fully. To distinguish between chitin and chitosan, solubility is used, since chitosan is

soluble in dilute acid and forms viscous solutions, but chitin is insoluble. Chitosan is a moderately basic polysaccharide (pK_a = 6.3). The pK_a of its monomer glucosamine is 7.5.[12] The lower pK_a of chitosan is likely due to the hydrogen-bonding network within the polymer. This means that the lone pairs of the nitrogen atoms within the polymer are acting as hydrogen-bond acceptors to protons within a neighbouring polymer chain. Therefore, the $-NH_2$ group in chitosan is not freely available to act as a base and this leads to a lower pK_a and increased acidity. Chitosan is non-toxic, biodegradable, environmentally benign and readily available. The deacetylation process of chitin is mainly conducted using alkaline treatment.[20] For example, 50-55% (w/v) sodium hydroxide solution can be used at 95-110 °C for the deacetylation process followed by neutralization, filtration and washing steps.[21] Interestingly, the fungus *Mucor rouxii* is the first species from which the chitin deacetylase enzyme has been obtained. Therefore, use of chitin deacetylase could be an alternative method for deacetylating chitin to give chitosan.[22] This enzyme is a metalloenzyme that belongs to the carbohydrate esterase group, and can be extracted from fungi and some species of insects.[23] In short, chitosan is a natural polysaccharide that is available in some species and synthesized chitosan can be found in a wide range of molecular weights, 50-2000 kDa.[24]

1-4 Degree of Deacetylation (DD)

The process of removing the acetyl group from chitin leaving an amino group (NH_2) in place of the N-acetylamido group (NHAc) does not proceed 100% to completion. Therefore, chitosan, the deacetylated product, typically has acetyl groups at some sites within the polymer. The DD present within a sample of chitosan is the ratio of

glucosamine to NAG units × 100 and is given as a percentage. The DD value is an important factor when describing chitosan in terms of physical, chemical and biological properties.[14] Biodegradability is also affected by the DD.[25] Different methods have been used to determine the DD (%) including infrared (IR), nuclear magnetic resonance (NMR), mass spectrometry (MS), ultraviolet spectrometry, and gel-permeation chromatography (GPC).[20] The method used to determine this value should always be mentioned with the %DD number. The simplest technique to determine DD% is IR spectroscopy because it needs minimal sample preparation and is a quick analytical method. This method is based on recording the absorbance (A) at two frequencies (1655 and 3450 cm^{-1}), which represent the N-acetyl group (amide-I band) and the hydroxyl band, respectively.[26] An accurate measurement can be achieved by plotting a calibration curve of the ratio of A at 1655 cm^{-1} (A_{1655}) to A at 3450 cm^{-1} (A_{3450}) versus known DD (%) values of chitosan standards. The unknown DD% sample can be determined based on its ratio of A_{1655}/A_{3450}.[27] Equation 1-2, introduced by Roberts et al., can be used to calculate the DD value where 0.87 is the A_{1655}/A_{3450} ratio for chitin (fully N-acetylated groups).[28]

$$\%DD=100-[(A_{1655}/A_{3450})\times 100/0.87] \qquad \textbf{Equation 1-2}$$

When the DD of a biopolymer is greater than 75%, it is called chitosan.[20] Up to 98% DD chitosan can be obtained through deacetylation of chitin.

1-5 Applications of Chitin and Chitosan

Although chitin and chitosan have limitations in their reactivity and solubility, they possess several desirable properties such as biocompatibility, biodegradability and non-toxicity. Chitosan has been used in drug delivery applications for treating diseases associated with the colon[29] and for lowering cholesterol.[29,30] Chitosan has also been shown to improve drug absorption in nasal treatments. The DD and molecular weight of chitosan both play a role in absorption enhancement. For example, 95-99% DD chitosan of various molecular weights (low and high) gives the best absorption in nasal epithelia, but 65% DD requires the chitosan to be of high molecular weight (170 kDa) to act effectively. In transdermal drugs, cross-linked chitosan membranes can be used to deliver a drug into the skin. The pores in the membrane facilitate drug release into the skin and help control the drug delivery over a certain period of time. Because chitin and chitosan are non-toxic and non-carcinogenic, they have other medical applications such as in human implantable devices.[29] For example, implantable devices composed of chitosan and hyaluronic acid sodium salt can be used to release insulin into the human body.[31] Some of the properties of chitin and chitosan have led to their use in wound dressing materials, such as anti-inflammatory action, biodegradability, biocompatibility, antimicrobial activity, and retention of growth factors, release of glucosamine and NAG monomers and oligomers, and stimulation of cellular activities. Therefore, they are very useful biopolymers for wound healing and tissue engineering.[29]

Nowadays, many people prefer to consume food that contains no synthetic chemical preservatives. Chitin and chitosan are natural antimicrobial biopolymers, which work

against microorganisms. Because chitosan is more soluble, it has better antimicrobial activity than chitin. One of proposed mechanisms by which chitosan prevents toxin formation and microbial growth is its ability to chelate metals. Chitosan at pH < 6.0 forms a positively charged group on C2 that can also interact with the negatively charged microbial cell membrane. This interaction causes proteinaceous and other intracellular constituents to leak out and thus destroying the microbial cells.[29] There are many food applications of chitosan. For example, chitosan addition to meat products inhibits spoilage bacteria growth.[32] Treating industrial wastewater by removing metals improves the quality of water and reduces the impact of industry on health and the environment.[33] The NH_2 group on C2 can form coordinate covalent bonds with metals to produce complexes. Therefore, chitosan has the ability to act as a chelating agent. Chitosan has also been used to remove pesticides and polychlorinated biphenyls (PCBs) from contaminated water.[33]

Chitin and chitosan can also be used in other applications including agricultural materials (e.g., plant seed coating and fertilizer, feed additives, textiles and woven fabrics, papers, films and sponge materials). Chitosan has also found additional applications such as in food processing (e.g., sugar refining) materials, paints, dyeing and weaving.[34] Chitin, chitosan and their oligomers are also renewable feedstocks that can be used to produce useful value-added chemicals (more explanation is provided in sections 1-8 and 1-14).

1-6 Chitin and Chitosan Solubility

1-6-1 Solubility of Chitin

Chitin is insoluble in most solvents[35], Table 1-1, such as dilute acid solutions (e.g., HCl and acetic acid) and organic solvents (e.g., acetone and acetonitrile).[36] In α-chitin, the carbohydrate rings (hydrophobic faces) in the structure are arranged over each other; this is the cause of its low solubility.[9] The sheets of chitin interact with each other via non-covalent linkages such as hydrogen bonding between C=O amide···HN.[37] However, chitin is soluble under harsh and non-environmentally friendly conditions such as in hexafluoroisopropanol, and hexafluoroacetone.[38] Chloroalcohols, including 2-chloroethanol, 1-chloro-2-propanol, and 3-chloro-1,2-propanediol, can be used in combination with either mineral acids such as aqueous HCl or organic acids such as acetic acid to dissolve chitin. β-Chitin is the only polymorph that is soluble in anhydrous formic acid. However, chitin precipitates when water is added to dilute the formic acid solution.[38] Chitin is also soluble in hot concentrated solutions of some salts including CaI_2, $CaBr_2$ and $CaCl_2$.[5] Recent results have shown that suitably designed ionic liquids can be used to dissolve chitin.[42] For a discussion of chitosan solubility, see section 1-6-2.

Table 1-1. Solubility of chitin and chitosan

	Soluble in	Insoluble in
Chitin	• ILs including [EMIm]OAc • Hexafluoroisopropanol and hexafluoroacetone • Chloroalcohols including 2-chloroethanol, 1-chloro-2-propanol, and 3-chloro-1,2-propanediol, with either mineral acids such as aqueous HCl or organic acids such as acetic acid • Hot concentrated solutions of some salts including CaI_2, $CaBr_2$ and $CaCl_2$	• Water • Basic media • Dilute acid solutions including HCl and CH_3COOH • Organic solvents including acetone and acetonitrile
Chitosan	• ILs including [EMIm]OAc • Acidic solutions of pH less than 6.0 • DMF-N_2O_4	• Water • Basic media

1-6-2 Solubility of Chitosan

Although chitin and chitosan are insoluble in water, in general chitosan is more soluble than chitin under many conditions, Table 1-1. Chitosan is soluble in acidic solutions of pH less than 6.0[39] because of the protonation of NH_2 groups in the C2 position.[36] When the pH is increased up to ca. 6.0, chitosan will start to precipitate. In general, chitosan is insoluble in neutral or basic media. Its solubility depends on DD. The solubility of chitosan in water increases with a decrease in its molecular weight.[39] A mixture of dimethylformamide (DMF) and dinitrogen tetraoxide (DMF-N_2O_4) is able to dissolve chitosan in a ratio of 3:1 N_2O_4:chitosan.[5]

Use of safer solvents and auxiliaries is one of the principles of green chemistry. In some cases, low toxicity and recyclable solvents can be used as useful alternatives for nocuous solvents. For example, volatile organic compounds (VOCs) are toxic and flammable. VOCs can also contribute to ozone depletion and smog formation process. Some

reactions can be done without solvent (neat).[40] However, ionic liquids (ILs) have many useful properties that make them a solvent of choice (alternative solvent) for biomass transformations and green chemistry in general such as low vapor pressure, tunable solubility and thermal stability. 1-Butyl-3-methyl-imidazolium chloride ([BMIm]Cl) is an IL which can dissolve chitin and chitosan biopolymers.[41] These polysaccharides can also be dissolved in a mixture of ILs which includes 1-butyl-3-methylimidazolium acetate, 1,3-dibutylimidazolium acetate, and 1,3-dimethylimidazolium acetate.[42] ILs are able to dissolve polysaccharides by disrupting their inter- and intra-molecular hydrogen bonding between chains.[41] Although solubility is a problem in processing polysaccharides, chitin and chitosan can potentially be used to produce useful chemicals.

1-7 Saccharides

Biopolymers consist of three major classes: saccharides, proteins, and nucleic acids.[76] Saccharides, also known as carbohydrates, may be classified into three groups: monosaccharides, oligosaccharides, and polysaccharides.

1-7-1 Monosaccharides

Among the saccharide classes, monosaccharides are the simplest and the lowest in molecular weight. Monosaccharides are named based on the number of carbon atoms in the monosaccharide, the name ending with the suffix "ose". For example, the simplest monosaccharide consists of 3 carbon atoms and is called a triose. Similarly, monosaccharides composed of 4, 5, 6, and 7 carbon atoms are termed tetraose, pentose, hexose, and heptanose, respectively.[77] The monosaccharide structure contains many hydroxyl groups and/or a carbonyl group. Monosaccharides bearing an aldehyde or ketone functionality are known as aldoses and ketoses, respectively. Figure 1-6 shows the simplest aldose and ketose structures, namely glyceraldehyde and dihydroxyacetone, respectively.

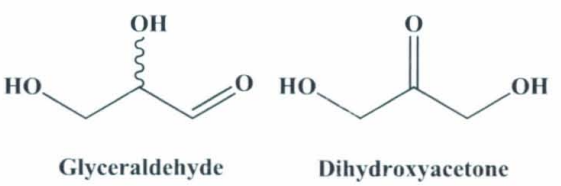
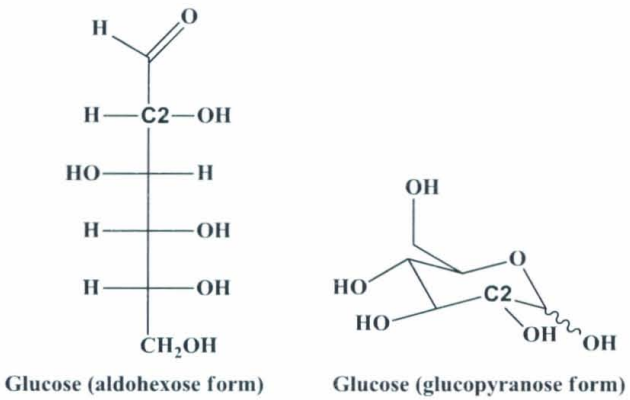


Figure 1-6. Structures of glyceraldehyde and dihydroxyacetone.

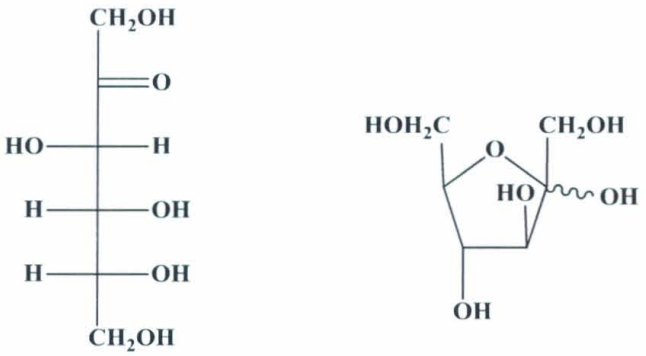
Glucose is a hexose type sugar. It can be formed as an aldohexose (acyclic) or pyranose (glucopyranose, cyclic). As depicted in Figure 1-7. if the hydroxyl group at the C2

position is replaced with an amine or acetamide group, it will be glucosamine or NAG.[77] Fructose is another example of a monosaccharide (Figure 1-11). It is available in many forms including ketose and furanose.



Glucose (aldohexose form) Glucose (glucopyranose form)

Figure 1-7. Glucose in aldohexose and glucopyranose forms.



Fructose (ketose form) Fructose (furanose form)

Figure 1-8. Structures of the two forms of fructose monosaccharide (Ketose and fructofuranose).

1-7-2 Oligosaccharides

An oligosaccharide is a short saccharide polymer consisting of 2 to 10 monosaccharides. Disaccharides contain two of either the same or different monosaccharide units. For

example, cellobiose is a dimer of two glucose molecules, and sucrose (table sugar) is a dimer of glucose and fructose (Figure 1-9). By replacing the OH group on the C2 position in cellobiose with an amine or acetamide group, this disaccharide will be chitobiose or *N,N'*-diacetylchitobiose, respectively. A huge number of disaccharides, 80 different sugars, can be formed from glucose and mannose.[76]

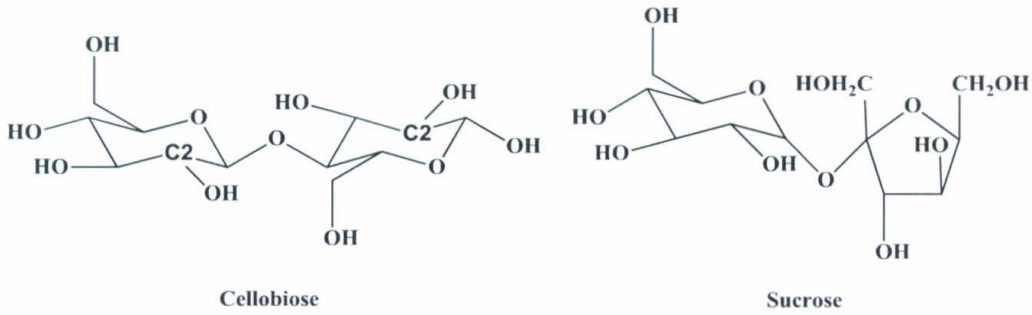


Figure 1-9. Structures of disaccharides (cellobiose and sucrose).

1-7-3 Polysaccharides

A polymer consisting of a large number of monosaccharides is a polysaccharide. There are several polysaccharides found in nature including cellulose, starch, chitin, and chitosan. [78] Polysaccharides are renewable biopolymers that are formed, degraded and reabsorbed in nature.

1-8 Processing of Chitin and Chitosan to Produce Chemicals

In comparison with cellulose and related sugars, investigations using chitin, chitosan and oligomers to produce biofuels and other useful products have been performed to a lesser extent. Greater efforts should be directed toward finding economical uses for these

biopolymers, as this would be better than dumping waste from the seafood industry directly into the sea or landfills. There are barriers to using chitin, chitosan and their oligomers, such as the cost of these resources (i.e., they are more expensive than cellulose). However, for regions where crustacean shells are readily available, the cost can be reasonable. Chitin, chitosan and their monomers can potentially be hydrolyzed and processed in many ways to produce chemicals for many different uses and processes involving these starting materials are growing in the scientific literature.

1-8-1 Enzymes

1-8-1-1 Chitinases

Organisms such as bacteria, fungi, insects and plants are a good source of chitinases. Chitinases from *Vibrio* and *Streptmyces* are very stable. They are able to hydrolyze chitin into different oligomers (e.g., 2 g chitin produced 20-25 mg chito-oligosaccharide).[43] Different size ranges of oligomers have been obtained by changing the temperature of the incubation process. The main product from such processes is the disaccharide, which is di-*N*-acetylchitobiose $(NAG)_2$. In this study, chito-oligomers were identified using TLC and purified by column chromatography. Colloidal chitin (treated with DMSO (10% LiCl (w/w)) has also been used to produce $(NAG)_2$. Colloidal chitin (20 g) undergoes hydrolysis using *Vibrio furnissii* chitinase (chi E, 89 kDa) in an ammonium bicarbonate solution incubated at 37 °C for 24 h producing 210 mg $(NAG)_2$.[44] It is worth noting that in these examples, the yields of products are relatively low (~1 wt%).

1-8-1-2 Hemicellulase, Lysozyme and Cellulase

Chitosan has been hydrolyzed using hemicellulase. The hydrolyzed products were then treated using acetic anhydride to add an acetyl group at each NH_2 group to produce $(NAG)_{1\text{-}7}$. 200 mg chitin produced 13 mg NAG, 28 mg $(NAG)_2$, 42 mg $(NAG)_3$, 48 mg $(NAG)_4$, 67.6 mg $(NAG)_5$, 81.1 mg $(NAG)_6$ and 19.7 mg $(NAG)_7$ when it was treated using hemicellulase.[43] In lysozyme, chitosan was acetylated and then treated with the enzyme. After reacetylating the products, the % yield of the chito-oligomers produced were higher compared to chitin treatment using lysozyme. These products were purified

using gel filtration chromatography (GFC). 100 mg of acetylated chitosan produced 16.3 mg $(NAG)_2$, 10.3 mg $(NAG)_3$, 18.0 mg $(NAG)_4$ and 1.7 mg $(NAG)_5$.[43] Cellulase is also an enzyme option that can be used to hydrolyze biopolymers. It can be used to hydrolyze chitin into NAG and good yields were obtained (40 wt%).[45]

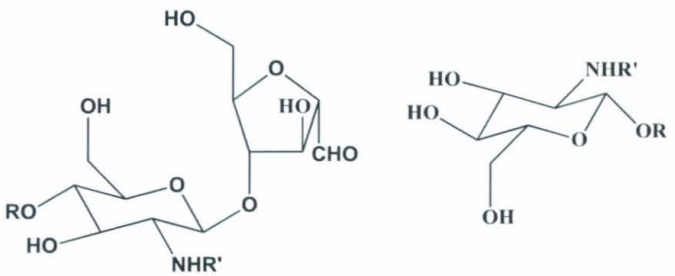
1-8-2 Acids

1-8-2-1 Hydrochloric Acid (HCl)

HCl has been used to produce chito-oligomers from chitin. $(NAG)_{2-7}$ have been reported as products from hydrolysis of chitin using HCl. 200 g chitin produced 12.1 wt% $(NAG)_2$, 11.8 wt% $(NAG)_3$, 11.6 wt% $(NAG)_4$, 10.8 wt% $(NAG)_5$, 4.8 wt% $(NAG)_6$ and 1.3 wt% $(NAG)_7$.[43] In another procedure, 300 g chitin was mixed with concentrated HCl and stirred at 45 °C for 3 h. After diluting the mixture with water followed by neutralization using NaOH, filtration, decolouration, salt removal and concentration, a mixture of NAG and chito-oligosaccharides was obtained. To separate NAG, the mixture was dissolved in water at 55 °C and cooled to 0 °C. 40.5 g NAG was obtained.[46]

1-8-2-2 Nitrous Acid (HNO_2)

Using nitrous acid, different kinds of products are obtained compared to the enzymatic and HCl methods previously mentioned. In chitosan, the NH_2 group reacts with HNO_2 but not the N-acetyl group. One mole of the NH_2 group reacts with one mole of HNO_2. After cleaving the biopolymer, a 2,5-anhydro-D-mannofuranose unit is formed at the reducing end of the cleavage.[43] After treating chitosan with concentrated HNO_2, twelve oligomers were formed (Figure 1-10). The trimer $(NAG)_2$-2,5-anhydro-D-mannofuranose ($(NAG)_2$–M) is an oligomer example.[47]



For each compound, **R(R')** are H(H), H(CH_3CO), NAG(H),NAG(CH_3CO), glucosamine(H) and glucosamine(CH_3CO).

Figure 1-10. The twelve oligomers produced after treating chitosan with HNO_2. Six of them with a 2,5-anhydro-D-mannofuranose unit at the end of the chemical structures.

1-9 Applications of Chito-oligomers $(NAG)_X$

Chitin and chitosan oligomers have antitumorigenic properties. For example, $(NAG)_{4-7}$ have been shown to have a significant effect on peritoneal exudate cells in mice and inhibit the growth of tumors. $(NAG)_6$ and $(glucosamine)_6$ are tumor growth inhibitors in the allogeneic (unlike genetically) and syngeneic (genetically identical) mice system. Chitin and chitosan oligomers have been shown to have a protective effect against

pathogenic infections in mice. These oligomers and their derivatives have also been shown to inhibit fungal and bacterial growth.[33]

1-10 Microwave Chemistry

In conventional heating methods, e.g., Bunsen burner, heating mantle, oven, oil and sand baths, the reaction mixture is heated gradually (slowly) from the outside inwards. The portion of the mixture that is in direct contact with the reaction vessel is exposed to high temperatures, which could lead to material decomposition. In microwave heating, however, the reaction mixture is not heated through contact with the surface of the reaction vessel but is heated from the inside out, Figure 1-11.[48] Microwave irradiation first penetrates the reaction vessel that is microwave-transparent (e.g., borosilicate glass, quartz, and Teflon). It then interacts with the mixture and heats it uniformly over a short period of time (seconds) via a number of mechanisms (see section 1-10-2).

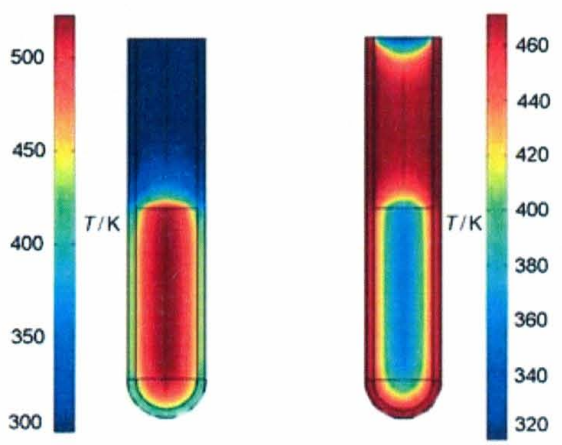


Figure 1-11. Microwave heating (left) shows more uniform heating of sample. [Reprinted with permission from *Angew. Chem., Int. Ed.,* 2004, **43**, 6250-6284. Copyright 2004 John Wiley & Sons, Inc.].

In Figure 1-7, conventional heating (right) shows that the temperature at the walls of the vessel is higher than the sample inside. Therefore, heating is not uniform (i.e., the heating is from the outside to the inside).[48]

The benefits of microwave over conventional heating, in many cases, are the shorter reaction times, higher product yields, and fewer side products (i.e., a product of higher purity can be obtained). The first two papers describing microwave heating were published in 1986 by Gedye et al.[49] and Giguere et al.[50] Nowadays, microwave-assisted chemistry has become a popular area of research and is an important technique in both academia and industry.

1-10-1 Background on Microwave-Assisted Chemistry

Within the electromagnetic spectrum, microwaves are located between infrared and radio frequencies between 0.3 to 300 GHz. The domestic (kitchen) and reactor (laboratory) microwaves operate at the same frequency (2.45 GHz). This frequency has been selected to avoid interference with other applications such as radar transmissions and telecommunications. Normal kitchen microwaves cannot be used to conduct chemical experiments for many reasons. Domestic microwave ovens can become corroded in the presence of acids because they are not designed for this type of work, and they are not designed with chemical safety considerations in mind. For example, if the pressure is very high inside a closed reaction vessel, the domestic microwave oven is unable to stop the microwave irradiation and cool the reaction quickly to avoid an explosion.[51] In many commercially available microwave reactors, sealed reaction vessels are used, which makes it possible to heat the reaction mixture to a temperature above a solvent's boiling point. This type of super heating is not readily possible using conventional heating methods and apparatus.[51]

1-10-2 Microwave Heating Mechanisms

Electromagnetic waves consist of electrical and magnetic field components. The component responsible for generating heat in microwave ovens or reactors is the electric field. There are two main types of mechanisms which describe the interaction of the electrical component with the reaction mixture (including solvents and reagents): dipolar polarization and ionic conduction.[52]

1-10-2-1 Dipolar Polarization Mechanism

Any substance that has a dipole moment is heated by the dipolar polarization mechanism in a microwave.[51] When such a substance is exposed to microwaves, it will rotate to align with the applied electric field, which is alternating. Therefore, the substance is always changing its alignment. During this process, the substance experiences molecular friction and collision that produces heat. The amount of heat produced is directly proportion to the ability of a molecule to align itself with the oscillating electrical component of the microwave irradiation. It should be noted that microwave irradiation cannot heat gases because the rotating molecules of a gas are too widely separated. Therefore, they are unable to experience molecular friction and collision.

1-10-2-2 Ionic conduction Mechanism

This heating mechanism can occur if the reaction mixture contains charged particles (ions).[51] The ions will move under the effect of the applied electric field of the microwaves. This movement causes the ions to collide with the surrounding molecules and atoms causing agitation or motion, which will produce heat. For example, ionic liquids (ILs) can be heated easily under microwave irradiation.[53] The ion conduction mechanism is generally more powerful than the dipolar polarization mechanism in terms of heat-generating capability because the ions in a solution could be freer to move in solution compared with molecules.

1-10-3 Enhancing Productivity Using Microwave Heating

There is no single explanation for the increased productivity that is obtained when reactions are performed using microwave heating. When microwave and conventional heating methods are compared at the same reaction temperature, the % yield of a product will usually be higher for the microwave method. One explanation is that, in microwave heating, the high reaction temperature can easily be reached (rapidly, seconds) compared with conventional heating and this means there is less chance for side-reactions to occur.[51] In most cases, accelerated rates are explained by the Arrhenius equation, Equation 1-3, where k is the rate constant of a chemical reaction, R is the gas constant and T is the temperature in Kelvin.

$k=Ae^{-Ea/RT}$ **Equation 1-3**

Most evidence supports thermal explanations for enhanced productivity in microwave-assisted chemistry, which means, as samples can often be heated to higher temperatures than in conventional methods, activation energies (E_a) are more easily overcome and higher rates can be achieved. For example, methanol can be heated in a microwave up to 165 °C resulting in a higher reaction rate compared with running the same reaction under reflux using conventional heating methods at 65 °C.

1-10-4 Conversion from Conventional to Microwave Heating

The two most important parameters to be considered in microwave heating and conventional heating methods are the temperature and the reaction time. In Equation 1-3, a rule of thumb states that the rate of a reaction is doubled with each 10 °C

increment in temperature. For example, to convert from a conventional reaction at 100 °C for 56 min into a microwave heated reaction at 110 °C or 130 °C, the time would be reduced to 28 min or 14 min, respectively.[51] A conversion table can be found in the literature or online.[51,54] In running a new reaction using microwave irradiation, it is advisable to begin with a temperature of 30-40 °C above the boiling point of the solvent employed, and a reaction time of 5-10 min. However, the best results will only be obtained through optimization of both parameters. Decomposition temperature of substances must be taken into consideration when performing microwave-assisted reactions.[51]

1-11 Solvents

Solvents are usually employed in their liquid form. There are many reasons for using solvents in chemical reactions such as for mass and heat transfer. They can also participate in the reaction mechanism, e.g., as intermediate stabilizers to increase the reaction rate and afford higher yields. Also, some solvents have acidic or basic properties. Although VOCs are widely used as solvents, it is prudent to find alternatives to replace them. VOCs can be toxic, flammable, cause ozone depletion, may be carcinogenic and can form low-level ozone and smog. The best solvents are those that have little or no impact on the environment and can be recycled.

1-11-1 Solvent-Free

The greenest reaction conditions in terms of solvent use are "neat" (or solvent-free). This means no solvent is added to the reaction mixture. Solid-state synthesis is an example of a

solvent-free reaction. In some reactions, solutions of reagents are used, which means that some of the reagent (or product) acts as the solubilizing agent. However, these reactions are still coined "solvent-free", and are greener than those where a solvent, e.g., CH_2Cl_2, is added to the reaction mixture because they produce less waste.[40] Mechanochemistry is another solvent-free reaction type in which reactants are mixed together in high-speed ball mills (HSBM).[55] In some cases, solvent-free reactions can be conducted under microwave irradiation.[56,57] From an industrial perspective, the quality, quantity and cost of a product are highly important factors in a production process. In terms of solvents, the cost of a solvent (including its price, cost of disposal, and its recyclability) are crucial in solvent selection. Therefore, any alternative solvent used should also be cost effective.

1-11-2 Water

Because most reactions are not possible in the absence of solvent, water is possibly the greenest choice of solvent. Water is non-flammable, non-toxic, abundant, renewable and available at a low cost. Furthermore, contamination in water can often be easily recognized (e.g., by colour and odour). The density of water is 1 g/mL and therefore, most organic substances can form biphasic systems with water, which aid in the extraction and separation of products. In a microwave, water can be heated above its normal boiling point (100 °C) in a sealed vessel. In the phase diagram of water (Figure 1-12), supercritical water (scH_2O) exists in the region beyond the critical point: critical temperature, T_c= 374 °C and critical pressure, P_c = 218 atm. A temperature between 100 °C and 374 °C is called high temperature, superheated or near-critical water (NCW). The properties of water above its boiling point differ from those of water at room temperature;

in the former case, it has a lower polarity, density, viscosity, and surface tension, all of which are similar to common organic solvents. For example, the density and polarity of water at 250 °C is similar to acetonitrile at room temperature. At higher temperatures, water is miscible with toluene. NCW has the ability to dissolve many organic compounds and possesses both acidic and basic properties. Water (at all temperatures) has disadvantages as a solvent. For example, some compounds are poorly soluble in water and deactivation of water-sensitive catalysts and reagents can occur. Following a reaction, contaminated water can be difficult to purify and care must be taken to prevent its release into the environment.[40]

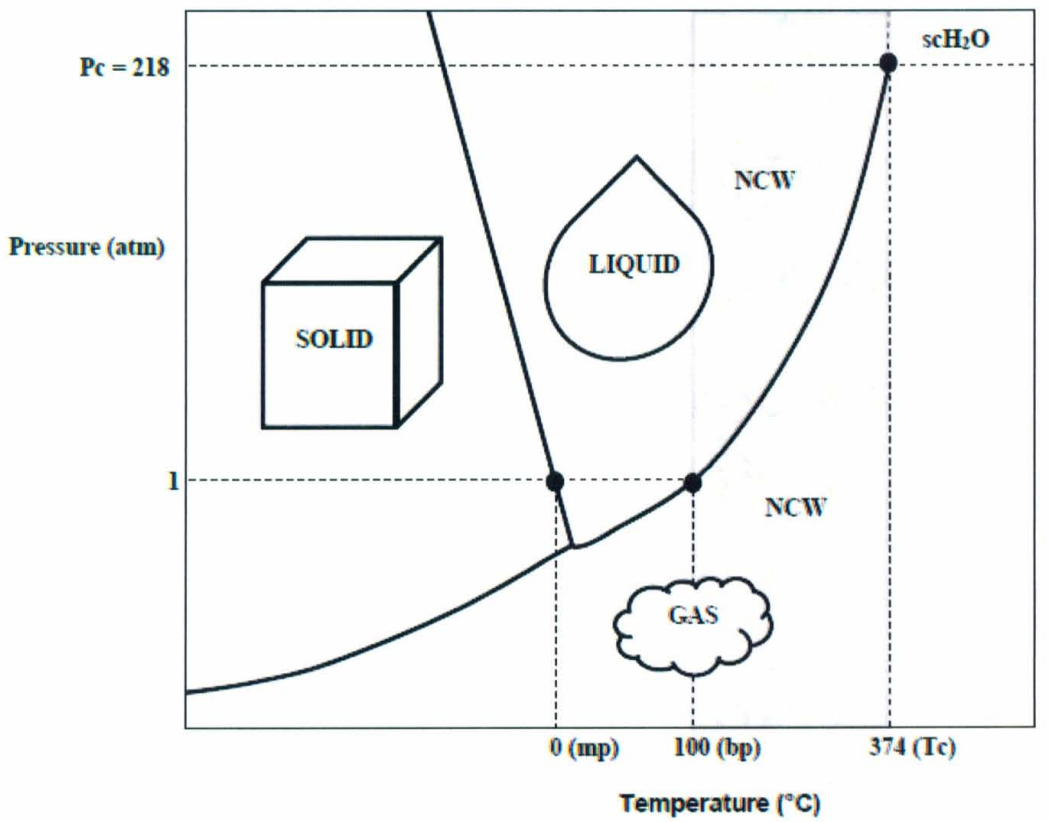


Figure 1-12. Phase diagram of water showing NCW, scH_2O, P_c and T_c.

1-11-3 Ionic Liquids (ILs)

Ionic liquids (ILs) are defined as salts in a liquid state below 100 °C. The first IL to be discovered was ethylammonium nitrate by Paul Walden in 1914.[59] Figure 1-13 shows several of the structural motifs for ILs. In general, a range of cations can be employed including phosphonium[60,61], pyridinium and piperidinium.[62] Anions include hexafluorophosphate, triflate,[62] and bis(trifluoromethylsulfonyl)imide (TFSI).[61]

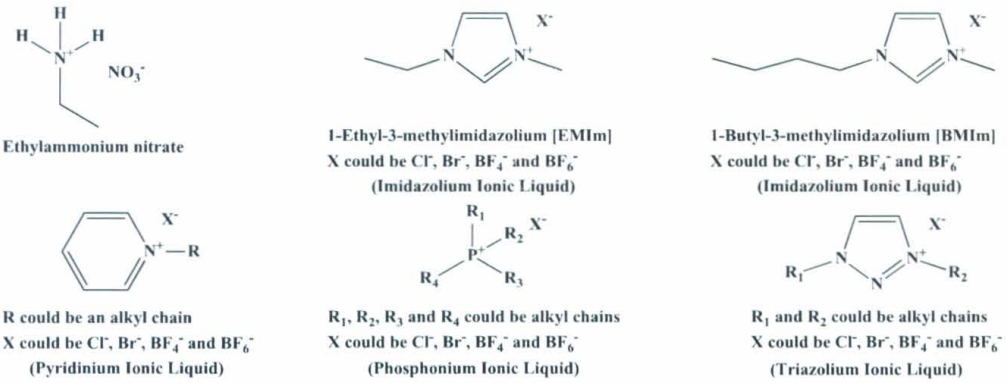


Figure 1-13. A wide range of structures showing ammonium, imidazolium, pyridinium, phosphonium, and triazolium ILs.

ILs are considered good alternative solvents due to a number of desirable properties;[63] e.g, they have low vapor pressures (i.e., they are non-volatile), which is ideal for volatile product isolation using vacuum or distillation. Also, many ILs are non-flammable and thermally stable; therefore, reactions can be performed over a wider temperature range compared with conventional solvents such as volatile organic compounds (VOCs). ILs are available in hydrophobic and hydrophilic forms and can be switched from one form to another by simply changing the anion or cation counterpart. Many catalysts and transition metal complexes are soluble in ILs. Interestingly, ILs can act as catalysts in some

reactions.[40] They have been used for many applications including extraction, electrochemistry, synthesis[40], as stationary phases in chromatographic separations[64,65] and as additives to the mobile phase in liquid chromatography.[66] There are also a range of other applications in analytical chemistry.[67] In terms of toxicity and biodegradation, the most commonly used ILs, such as 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF_4]), and BMIm hexafluorophosphate ([BMIm][PF_6]), are not biodegradable. However, they can be designed to be biodegradable by changing the anion group to octyl sulfate, allowing 25% biodegradability. Saccharin- and acesulfame- based ILs are also highly biodegradable.[40,68] For IL to be considered "readily biodegradable", it should release 60% of its theoretically calculated CO_2 within 28 days of incubation in activated sludge medium inside a closed vessel under a headspace of air.[69] For example, 1-methyl-3-(pentoxycarbonylmethyl)imidazolium saccharinate is readily biodegradable. On the other hand, the toxicity of ILs is a topic of controversy. Many of the toxicity studies involve organisms in aquatic media. These studies have shown that a longer alkyl chain on an imidazolium cation allows an IL to function as an antimicrobial agent (increasing toxicity).[70] A toxic IL that can be fully biodegraded into CO_2 and other benign chemicals is perhaps more desirable than a non-biodegradable solvent.[71] Interestingly, studies also report that increasing the alkyl chain length caused an enhancement in biodegradability. As a result, increasing the alkyl chain length is productive in terms of biodegradability, but is disadvantageous by increasing the toxicity of the IL.

Deep eutectic solvents such as mixtures of choline chloride ($HOCH_2CH_2N(CH_3)_3Cl$) with metal chlorides, carboxylic acids or urea are related to ILs. The urea-choline chloride

material is non-toxic, biodegradable, and can be prepared from renewable feedstocks. In general, many ILs are commercially available and can easily be prepared in the laboratory.[40] Other green alternatives include supercritical fluids and switchable solvents.[40] There are cases where alternative solvents do not perform better than conventional solvents and in such cases, one should attempt to use the least hazardous VOC available.

1-12 Extraction Protocol

Extraction is an important step following reaction completion, in which a product(s) is separated from the reaction mixture. The desired product is extracted using a suitable solvent to separate it from unreacted materials and other side products. VOCs such as carbon tetrachloride, benzene, hexanes, chloroform, and dichloromethane are widely used as they are able to extract a wide range of compounds. Another good feature is their volatility,[40] which allows them to be easily removed by evaporation from the desired product. Despite their advantages, however, their use imparts major environmental and health concerns. For example, pentane, and diethyl ether have low flash points, which are -49 and -40 °C, respectively. A flash point is the temperature at which a solvent produces enough vapour to start burning. Dichloromethane and other chlorinated solvents are carcinogenic. Polar aprotic solvents, such as DMF and *N*-methylpyrolidine-2-one (NMP), are toxic but are difficult to replace because of a lack of suitable alternatives. Undesirable solvents should be replaced with greener solvents whenever possible (Table 1-2).[40] For example, heptane is less toxic than hexane and pentane.

Table 1-2. Undesirable solvents and their suggested alternatives.[40]

Undesirable solvent	Alternative solvent
Pentane or hexane	Heptane
Ethers	2-methyl tetrahydrofuran (2-MeTHF) or methyl t-butyl ether (MTBE)
Dichloromethane	Ethyl acetate (EtOAc), MTBE, toluene or 2-MeTHF

Supercritical carbon dioxide ($scCO_2$; Tc = 31.0 °C and Pc = 72.8 atm) can also be a good replacement solvent for non-polar VOCs. After extraction, CO_2 can easily be released by reducing the pressure and leaves no residue behind. The polarity of $scCO_2$ can also be increased by adding a modifier such as methanol. This option is the best among the alternatives, which can replace solvents that have major environmental issues. The problem of using $scCO_2$ either in chromatographic or extraction techniques[40] is the high cost of equipment and operation.

1-13 Catalysts

A catalyst is a chemical substance that decreases the activation energy of a reaction (speeds up the process), while remaining unconsumed at the end of the reaction.[72] Catalysts are widely used in a range of industries including polymer, pharmaceutical, agrochemical, and petrochemical fields. Use of catalysts is emphasized as one of the twelve principles of Green Chemistry. Catalysis has led to many economic and environmental benefits.[72,73]

1-13-1 Homogeneous and Heterogeneous Catalysis

In homogeneous catalysis, the catalyst is present in a single phase along with the reactants, solvent, and products. When the catalyst is in a different phase, the catalysis is heterogeneous, where, in most cases, the catalyst is a solid. Heterogeneous catalysis is widely used in the petrochemical and bulk-chemical industries. It is the preferable scenario because the catalyst can be easily separated from a mixture (recyclable) by filtration, and this leads to easier and cheaper purification processes.[72] Consequently, the catalyst can be reused and so the operating costs will be reduced. In homogeneous catalysis, it is not easy to separate the catalyst for reuse, but it has other advantages over heterogeneous catalysis including higher selectivity and activity and minimizes mass transfer effects. Table 1-3 shows a comparison between homogeneous and heterogeneous catalysis.[73] In most cases, a homogeneous catalyst can influence a reaction to produce a particular compound. Therefore, it is more selective than a heterogeneous catalyst. A heterogeneous catalyst is generally a solid and all catalytically active atoms or groups are at the surface. This situation causes fewer active sites to be available for reaction compared with a homogeneous analog and this can affect the % yield of a product. This means that mass transfer between the reaction mixture and a catalyst is lower for heterogeneous types.

Table 1-3. Homogeneous and heterogeneous catalysis comparison.[73]

	Homogeneous catalysis	**Heterogeneous catalysis**
Active centers	All atoms	Atoms on surface
Selectivity	High	Low
Mass transfer	High	Low
High temperature stability	More	Less
Recyclability	Difficult	Easy

In some cases, the combination of using NCW as a solvent for a reaction in the presence of an organic catalyst can be valuable. In such cases, the organic catalyst behaves as a homogeneous catalyst in NCW because it can dissolve organic compounds (see section 1-11-2). After the reaction, the organic catalyst can easily be separated by cooling the reaction, since water at room temperature cannot dissolve organic compounds. In this way, the catalyst can be isolated and reused.[73]

1-13-2 Biocatalysis

Biocatalysts are natural catalysts. They are mainly exploited as isolated enzymes, but whole organisms can also be used. It should be noted that in comparison with homogeneous and heterogeneous catalysts, which achieve 10^2-10^4 catalytic cycles/h, biocatalysts are highly active and can reach 3.6×10^6 cycles/h. Another advantage of using biocatalysts is their specificity (i.e., they produce a specific product from a specific reactant, and their reactions are often enantioselective). This is very important for

pharmaceutical processes because the activity of a medicine depends on a specific enantiomer. Protein engineering methods can modify enzymes' properties to facilitate targeted objectives. Using enzymes as biocatalysts is also desirable in terms of sustainable green chemistry. Most enzymes are productive in water and can also tolerate organic solvents. For example, lipases are highly stable in organic solvents and have been used industrially in the synthesis of acrylates, which are used to produce plastics and adhesives.[74] Lipases have also been used with ILs.[75]

1-14 Converting Renewable Feedstocks to Useful Chemicals

Thinking green is a good tool in the production of new and useful products. In recent years, chemists have focused significant attention on using feedstocks such as cellulose, glucose and fructose to produce renewable chemicals 5-hydroxymethylfurfural (5-HMF) and levulinic acid (LA). The chemistry of chitin, chitosan, and their monomers (i.e., NAG and glucosamine) should be investigated as well because they are underexploited and less widely studied compared to cellulose and glucose. Moreover, converting these substances into useful products is a more useful strategy than dumping them into the sea. One can envisage that the sea food industries, in particular waste associate with crustacean waste, could be a future valuable source of chemicals for other industries as outlined in Figure 1-14.

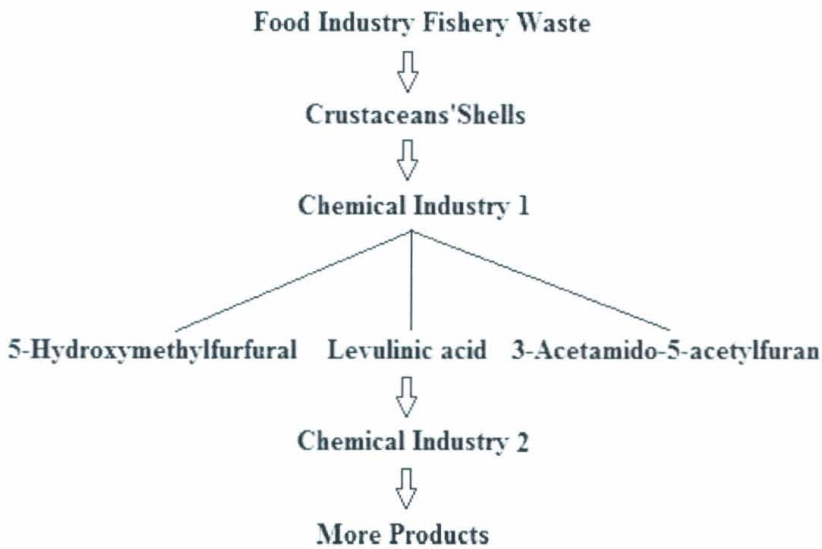


Figure 1-14. A potential chain of industries may develop to convert fishery waste into useful products. Products produced from "Chemical Industry 1" may lead to further industries. Levulinic acid was selected and shown for clarity.

1-14-1 5-Hydroxymethylfurfural (5-HMF)

1-14-1-1 Production

5-HMF can be obtained as a product of the Maillard reaction in which reducing hexoses react in the presence of amino acids or proteins during the thermal processing of food.[79] It is found in dried fruits, coffee, caramel, and honey. In terms of its toxicity, a daily dose (80-100 mg/kg) in animal experiments showed no ill effects. In addition, there is limited evidence of its carcinogenic potential.[80] In 1895, the first two reports of producing 5-HMF were published. Dull synthesized 5-HMF from inulin and Kiermayer used sugar cane in a similar procedure (i.e., heating one of these starting materials with oxalic acid under pressure).[81] Since then, many procedures have been developed to produce

reasonable amounts of 5-HMF using different types of solvents including water, dimethylsulfoxide (DMSO), DMF, dimethyl acetamide (DMA) and ILs,[82] and a wide range of catalysts including mineral acids, and acidic ion-exchange resins.[83] 73% yield of 5-HMF has been obtained from fructose using Amberlyst-15™ as a solid acid catalyst in DMF at 100 °C for 3 h.[84] In a microbatch reactor, a 92% yield of 5-HMF was produced by dehydration of fructose at 90 °C for 45 min in 1-H-3-methylimadizolium chloride, which acted as the solvent and an acid catalyst.[85,86] Under the same conditions, a 100% yield of 5-HMF was obtained from sucrose after 30 min.[86] A combination of boric acid ($B(OH)_3$) and NaCl has been used to dehydrate fructose into 5-HMF (60% yield) in water medium at 150 °C in 90 min. Using the same method, glucose produced only 1% yield 5-HMF.[87]

Glucose can be obtained from starch, cellulose, sucrose, and lactose. Industrially, it is produced from starch via a hydrolysis process using enzymes.[88,89,90] Corn is the main crop used to produce glucose, and glucose generally costs less than fructose. Therefore, financially it makes sense to produce 5-HMF from glucose rather than fructose.[89] In [EMIm]Cl with $B(OH)_3$, glucose produced 42% yield 5-HMF at 120 °C over 3 h. If sucrose was used, 66% yield 5-HMF was obtained after 10 h reaction time.[91] A 70% yield of 5-HMF has been obtained from glucose using $CrCl_2$ in 1-ethyl-3-methylimadazolium chloride ([EMIm]Cl) at 100 °C in 3 h.[92] This reaction provided higher yields than those using other catalysts such as H_2SO_4, Lewis acids, or other metal halides (< 10% yield 5-HMF). However, from a green perspective, the use of Cr-based catalysts is not desirable because of the potential formation of toxic hexavalent Cr species if released into the

environment. Glucose in 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm]BF_4) using $SnCl_4 \cdot 5H_2O$ as catalyst produced 62.3% yield at 100 °C for 3 h.[93] 91.4% yield of 5-HMF has been produced from fructose in a biphasic reaction system of ethyl acetate and choline chloride/citric acid, which ran for 1 h at 80 °C.[94] More than 90% yield was obtained from fructose in DMSO in presence of lanthanide ions.[85] In an attempt to decrease the amount of DMSO used, the DMSO was mixed with acetone (3:7 v/v). This reaction was performed using a strong cationic acid exchange resin (DOWEX 50WX8). The mixed solution was heated under microwave irradiation up to 150 °C for 20 min to produce 90% yield 5-HMF.[85] 60% yield of 5-HMF has been produced directly from cellulose in [EMIm]Cl in the presence of $CrCl_2$ and $RuCl_3$ as a catalyst mixture at 120 °C in 2 h. Under the same conditions, a 41% yield of 5-HMF was produced from a crude lignocellulosic raw material.[95] For a brief comparison of the 5-HMF productions identified above, see Table 1-4.

Table 1-4. A summary of 5-HMF production methods described in section 1-14-1-1.

Biomass	Conditions	5-HMF (%Yield)
Fructose	Amberlyst-15™ and DMF at 100 °C for 3 h.[84]	73
	Water at 150 °C for 90 min.[87]	60
	1-H-3-methylimadizolium chloride at 90 °C for 45 min.[85,86]	92
	Ethyl acetate and choline chloride/citric acid at 80 °C for 1 h.[94]	91
	1-H-3-methylimadizolium chloride at 90 °C for 30 min.[86]	100
Sucrose	[EMIm]Cl with $B(OH)_3$ at 120 °C for 10 h.[91]	66
	Water at 150 °C for 90 min.[87]	1
Glucose	$CrCl_2$ and 1-ethyl-3-methylimadazolium chloride ([EMIm]Cl) at 100 °C for 3 h.[92]	70
	$SnCl_4 \cdot 5H_2O$ and 1-ethyl-3-methylimidazolium tetrafluoroborate ([EMIm]BF_4) at 100 °C for 3 h.[93]	61
Cellulose	$CrCl_2$, $RuCl_3$ and [EMIm]Cl at 120 °C for 2 h.[95]	60
Crude lignocellulosic raw material	$CrCl_2$, $RuCl_3$ and [EMIm]Cl at 120 °C for 2 h.[95]	41

5-Chloromethylfurfural CMF (70-90%) can be produced from glucose, sucrose, cellulose, and corn in a biphasic reactor using HCl/$ClCH_2CH_2Cl$ at 80-100 °C for 3h. CMF can be converted into 5-HMF. 86.2 % of 5-HMF can be produced from CMF by boiling in water with fast stirring in 30 s followed by rapid cooling to room temperature.[96]

1-14-1-2 Mechanisms

Dehydration of hexoses occurs through the loss of three water molecules to produce 5-HMF. Amarasekara et al. proposed a mechanism for 5-HMF production from D-fructose using DMSO at 150 °C, Figure 1-15.[97]

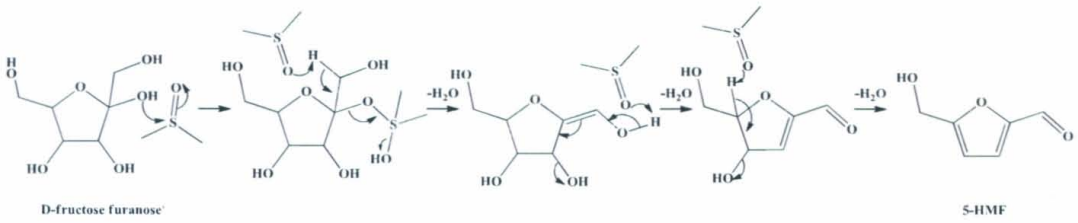


Figure 1-15. Proposed pathway: conversion of fructose (furanose form) into 5-HMF.

Pyrolysis of sucrose has produced 5-HMF. Figure 1-16 shows the proposed mechanism where the sucrose is cleaved into a fructofuranosyl cation at 350 °C. In this mechanism, 5-HMF is formed through loss of a hydrogen ion and dehydration processes. Fructose can also form a fructofuranosyl cation in the presence of H^+ and then produce 5-HMF. Also, the glucose formed undegoes dehydration to form 5-HMF through 3-deoxy-glucosone at 65 °C.[98]

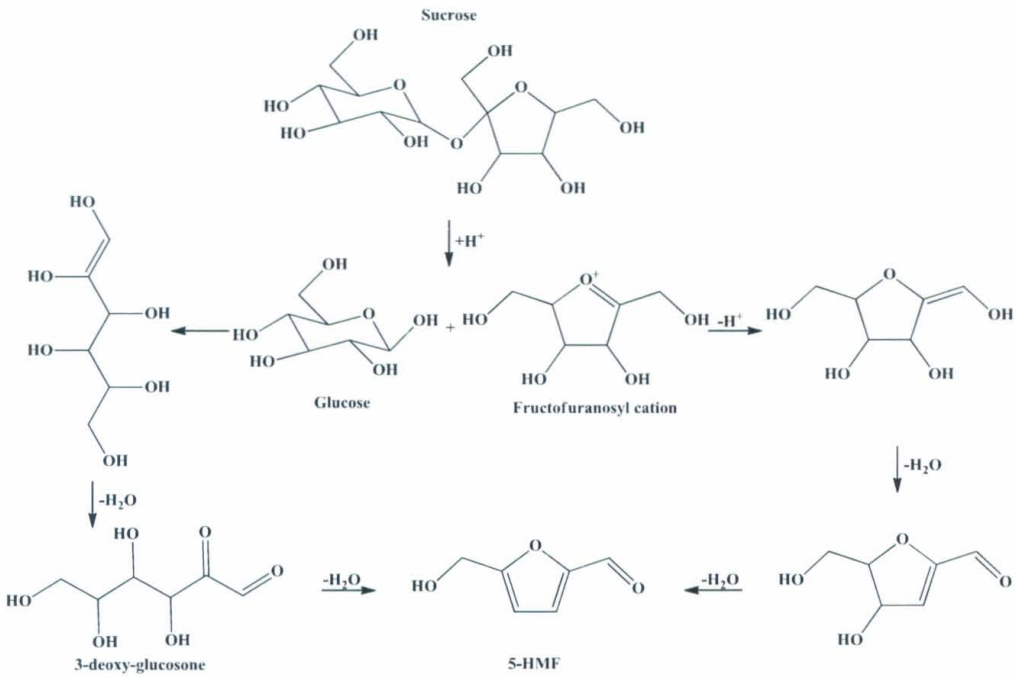


Figure 1-16. Proposed pathway: production of 5-HMF from sucrose.

In 2009, Han et al., proposed the mechanism of 5-HMF formation from glucose in $[EMIm]BF_4$ using $SnCl_4 \cdot 5H_2O$ as a catalyst, Figure 1-17. In $[EMIm]BF_4$, α-glucose exists in equilibrium with β-glucose. In this reaction, several interactions between glucose and the tin atom are proposed before 5-HMF is formed.[93] 61% yield of 5-HMF can be generated from glucose under the following reaction conditions: 100 mg glucose, 25 mol% $SnCl_4 \cdot 5H_2O$, 1 g $[EMIm]BF_4$, 100 °C over 3 h.

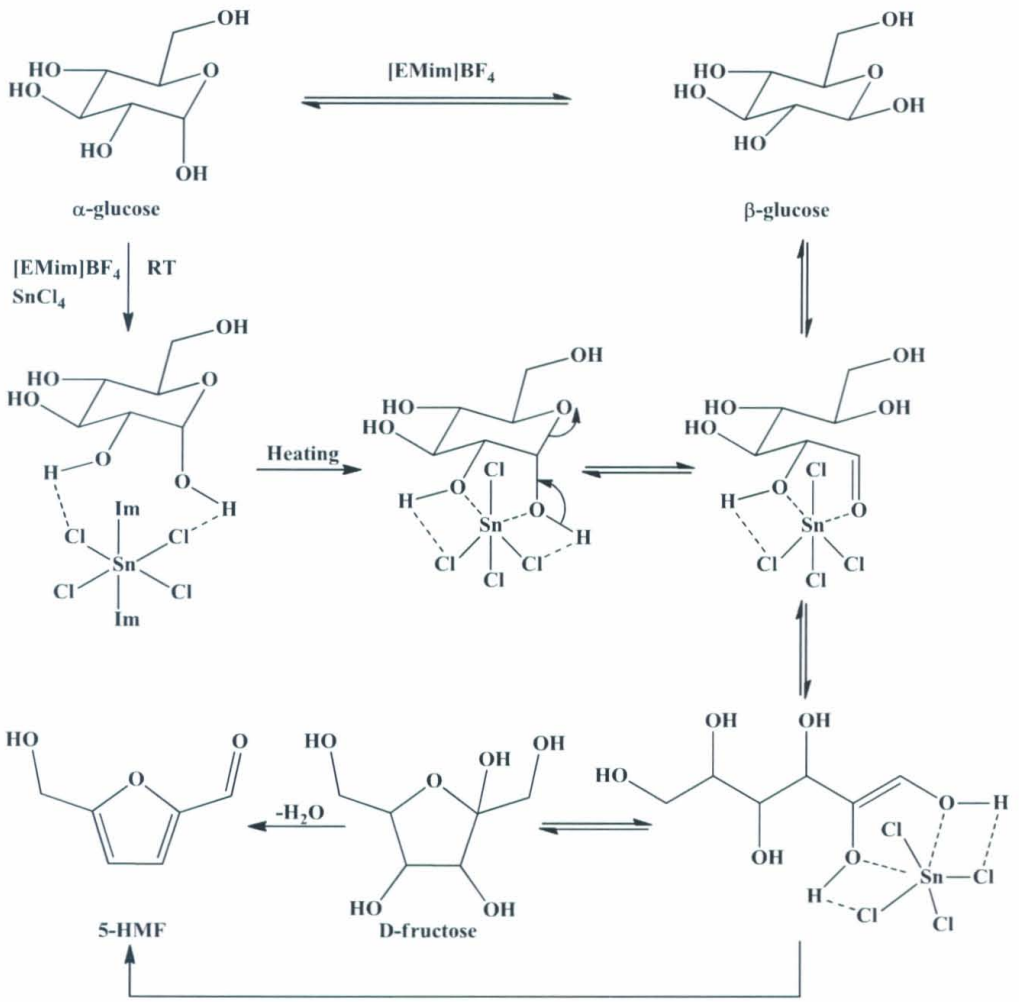


Figure 1-17. Proposed pathway: using glucose to produce 5-HMF.

In 2011, Riisager et al. showed that 5-HMF can be produced, from glucose, using $B(OH)_3$ and [EMIm]Cl in the absence of a metal catalyst. In this reaction pathway (Figure 1-18), glucose and $B(OH)_3$ formed a glucose-borate complex. This complex was then converted into fructose through several reaction steps. The chloride ion was shown to play an important role in this mechanism. It helped convert fructose into its enol form after losing

a water molecule. By losing two additional water molecules, 5-HMF was formed.[91] 42% and 66% yields of 5-HMF were produced from glucose and sucrose, respectively under the following reaction conditions: 1.0 g [EMIm]Cl, 100 mg glucose, Ca. 27.5 mg boric acid (for glucose) and 0.5 equiv of boric acid (for sucrose), 120 °C over 3 h (for glucose) and 10 h (for sucrose).

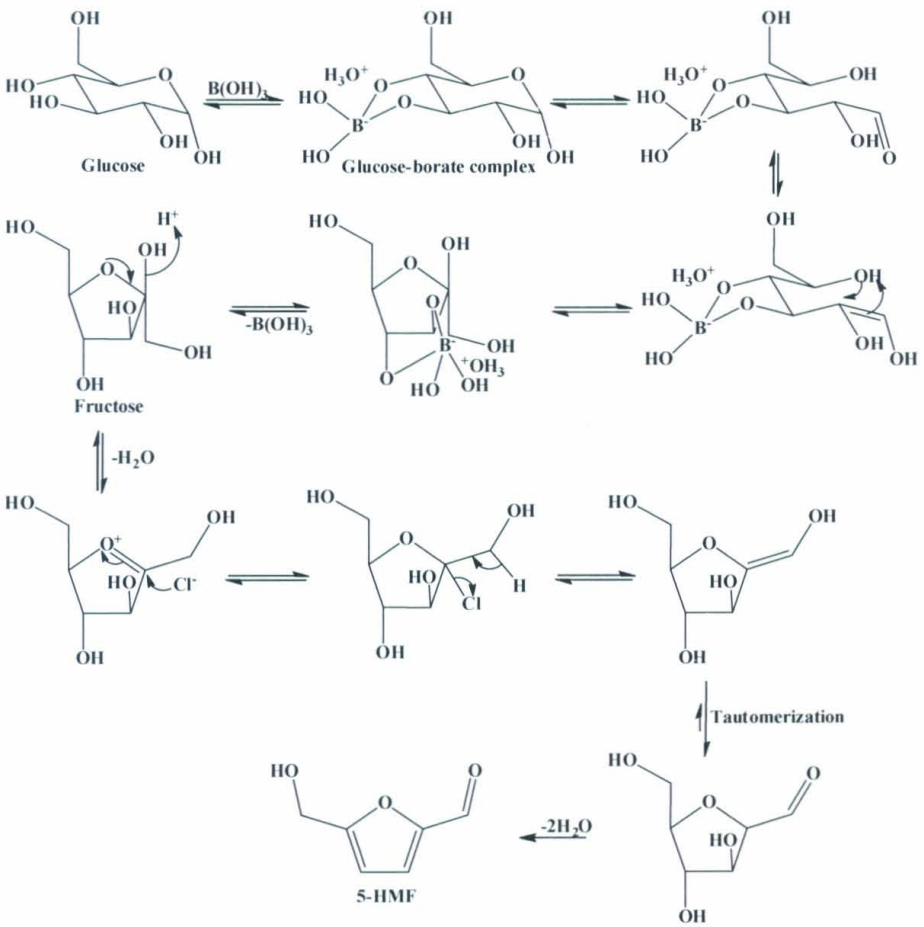


Figure 1-18. Proposed pathway: converting glucose into 5-HMF in an IL and $B(OH)_3$.

5-HMF can be prepared in DMF using a combination of acid and base. Ebitani et al. used sucrose, cellobiose, glucose, and fructose as starting materials in this process. For sucrose and cellobiose, the acid (Amberlyst-15™) was responsible for both the hydrolysis steps and also played a role in dehydration (i.e., it let fructose lose three water molecules to produce 5-HMF). The base (Mg–Al hydrotalcite) was responsible for catalyzing the isomerization between glucose and fructose, when glucose was used as the starting material. Figure 1-19 shows the proposed mechanism.[84] In this reaction 76, 58, 93, and 67% yields of 5-HMF were obtained from fructose, glucose, sucrose, and cellobiose, respectively. The reaction conditions were: 0.1 g substrate, 0.1 g Mg–Al hydrotalcite (0.2 g for glucose), 0.1 g Amberlyst-15™ and 3 mL DMF. The temperatures and run times were 100 °C over 3 h for fructose, 80 °C over 9 h for glucose and 120 °C over 9 h for sucrose and cellobiose over 3 h.

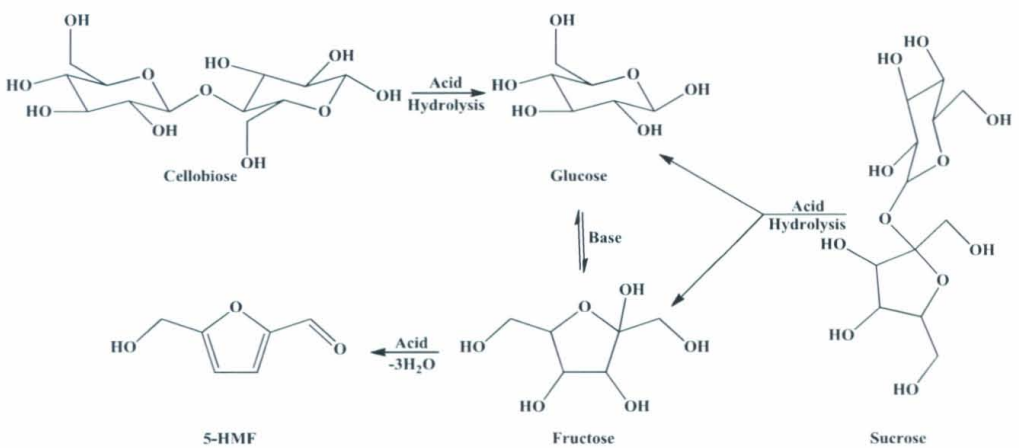


Figure 1-19. Proposed pathway: hydrolysis of sucrose and cellobiose into 5-HMF.

The mechanism of converting cellulose into 5-HMF in Figure 1-20 is similar to that shown in Figure 1-19 for the reaction of cellobiose. It starts with a hydrolysis step to

produce glucose (saccharification). The second step is isomerization of glucose into fructose. The dehydration processes form the final product (5-HMF).[95] Cho et al. produced 60% yield 5-HMF under the following reaction conditions: 10 mol% to cellulose of 4:1 ($CrCl_2$:$RuCl_3$), 50 mg cellulose, 500 mg [EMIm]Cl, 120 °C over 2 h.

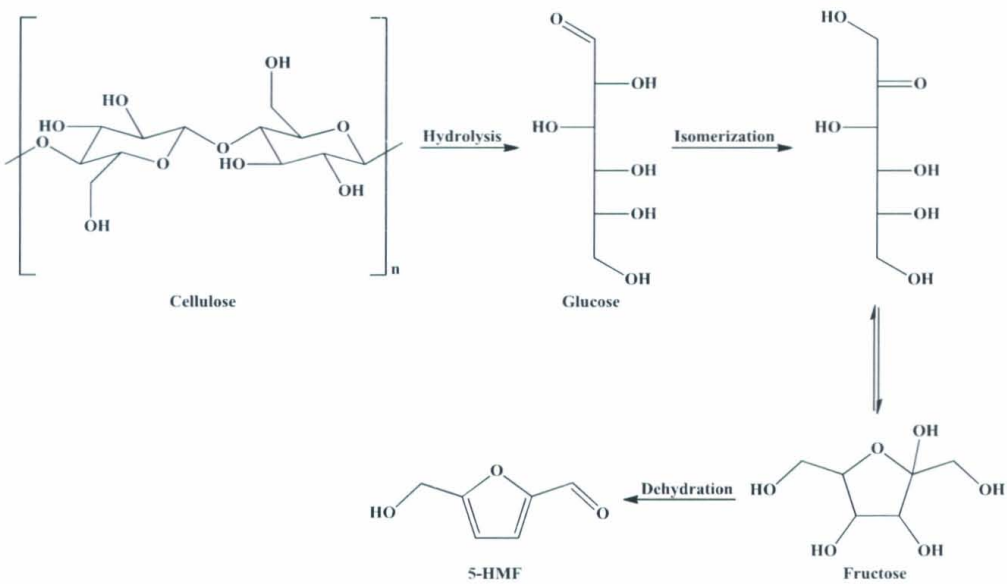


Figure 1-20. Proposed pathway: Cellulose hydrolysis into 5-HMF.

Polysaccharides such as starch and inulin behave similarly to cellulose. They undergo hydrolysis in the presence of acid as the first step in 5-HMF production. In Figure 1-21, inulin forms fructose, which then forms 5-HMF through dehydration processes.[99]

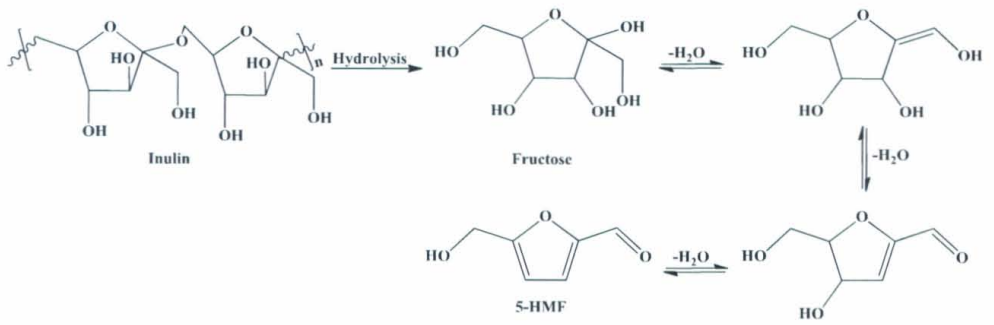


Figure 1-21. Proposed pathway: hydrolysis of inulin into 5-HMF.

1-14-1-3 Applications

It is well-known that the prices of fossil fuels worldwide are increasing with time, especially for oil. These sources are depleting and are from a practical sense non renewable. Therefore, there is a massive demand for replacement of these sources with renewable feedstocks. As a consequence, 5-HMF is a promising compound that can be produced from biomass materials. 5-HMF is an important precursor for the production of a range of biofuels and other useful products, which can be used in a wide range of applications (Figure 1-22).[85] One of these compounds is 2,5-furandicarboxylic acid (FDCA), which has been identified by the US Department of Energy as a biorefinery platform chemical (i.e., it can be used to produce a set of high-value bio-based chemicals).[93] FDCA can be used instead of terephthalic acid to produce polyesters.[99] FDCA can be prepared from 5-HMF in aqueous NaOH in the presence of supported Au/TiO_2 catalyst under a pressure of 690 kPa O_2.[100] 2,5-dimethylfuran (2,5-DMF) is another chemical that can be produced from 5-HMF. 2,5-DMF is a promising liquid transportation fuel[93] and can also be used as a fuel additive.[96] 5-HMF can also be

converted into 2,5-dihydroxymethylfuran and 2,5-bis(hydroxymethyl)tetrahydrofuran (HM-THF). These transformations of 5-HMF proceed through a variety of process oxidation, hydrogenation, hydrogenolysis or aldol condensation processes.[101,102] 5-HMF can be converted into long chain alkanes through multistep procedures including reactions with acetone, hydrogenation, and dehydration.[102]

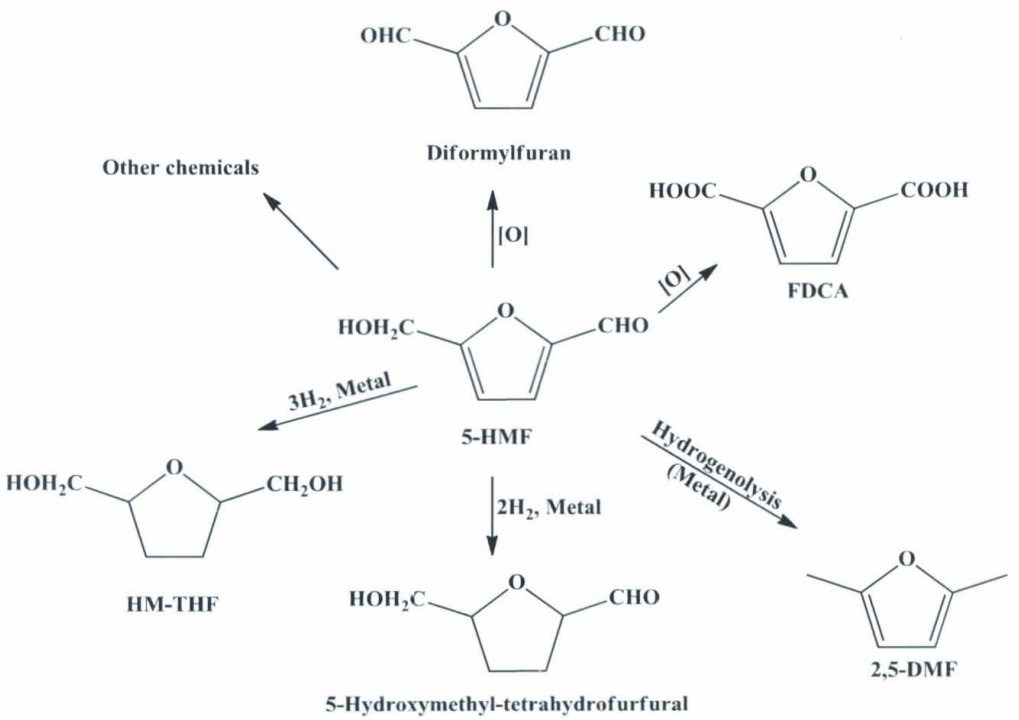


Figure 1-22. 5-HMF as a starting material in the production of a range of useful chemicals.

1-14-2 Levulinic Acid (LA)

1-14-2-1 Production

Levulinic acid (LA) belongs to the gamma-keto acid family.[103] It is also known as 4-oxopentanoic acid. It has also been identified by the US Department of Energy as a biorefinery platform chemical. LA can be obtained by treating hexoses with high concentrations of acid. During the reaction, 5-HMF is produced as an intermediate, which is converted into LA via a rehydration process.[85] Before biomass starting materials garnered popularity, one of the best methods to produce highly pure LA was using the petrochemical feedstock maleic anhydride.[103] It can also be formed via furfuryl alcohol hydrolysis. Production of LA can be achieved using different solvents and catalysts.[103] For example, Biofine Corporation has developed a hydrolysis process to manufacture LA industrially. Hexose sugars are treated in a reactor using 1-5% mineral acid at 210-230 °C for 13-25 s. In this step, 5-HMF is produced and transferred immediately and continuously into a second reactor. In this reactor, a hydrolysis process is conducted at 195-215 °C for 15-30 min to produce LA (minimum 60 %) from 5-HMF. Other starting materials can be used instead of hexoses such as paper mill sludge, urban waste paper, agricultural residues and cellulose fines from papermaking. The cost of LA based on this method is $ 0.04-0.10 USD per pound.[104] CMF can be hydrolyzed into LA (91.2% yield). In this process, an oil bath was used to heat up a mixture of CMF and water to 190 °C for 20 min.[96] Sodium chloride has a positive effect on the conversion of cellulose into LA via glucose as an intermediate. In this way, a 72% yield of LA could be produced from

cellulose in aqueous NaCl using Nafion as an acid catalyst. The reaction was run at 190-200 °C for 5 days.[105]

1-14-2-2 Mechanism of LA Formation

LA is produced from 5-HMF under certain conditions. The mechanism of this conversion passes through fructofuranosyl intermediates, Figure 1-23, and includes a number of dehydration and rehydration steps. The dehydration steps prefer high temperature conditions (endothermic reactions), whereas the rehydration steps to form LA are exothermic. Formic acid is a byproduct in this mechanism[103] and humic acids can be formed as side products.[89]

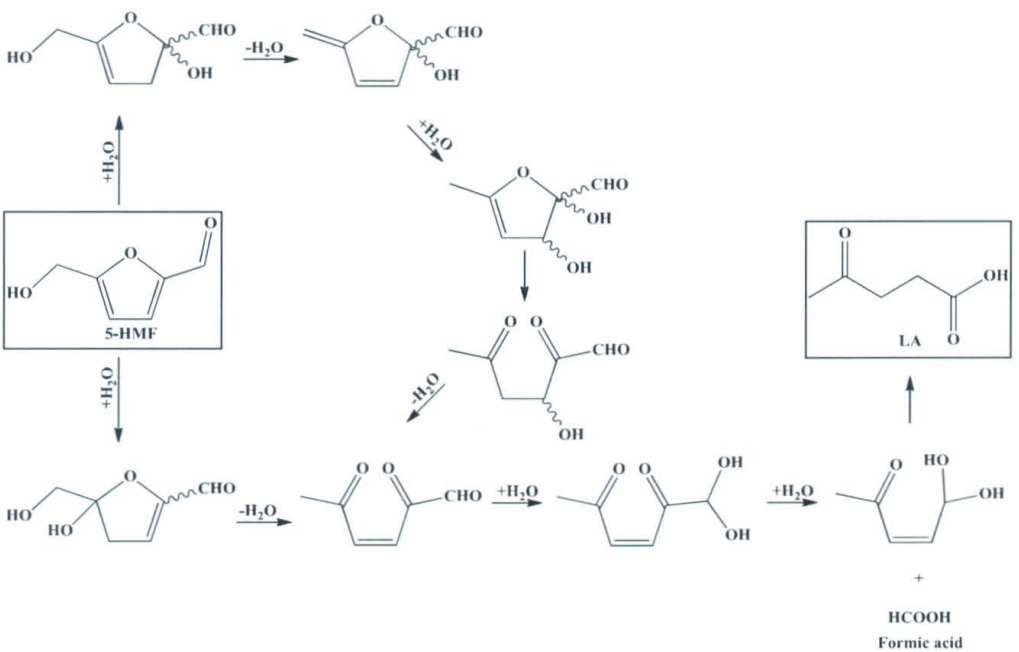


Figure 1-23. Proposed pathway: 5-HMF into LA.

Pentoses, e.g., xylose, can also produce LA. Dehydration, rehydration, and reduction (hydrogenation) processes occur during LA formation. Furfural is an intermediate in this pathway that is reduced to give furfuryl alcohol and then rehydrated to form LA. Figure 1-24 shows this production pathway, which has been proposed for when the reaction is performed in aliphatic ketone solvents.[103]

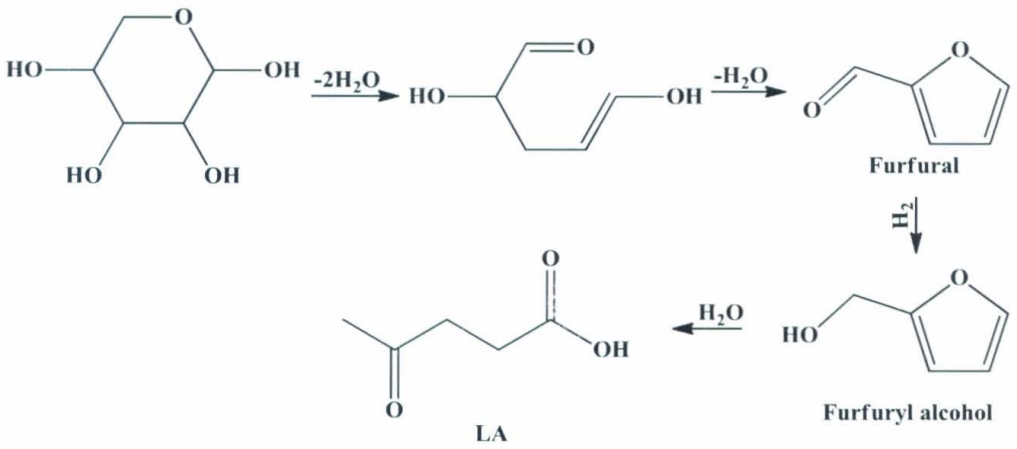


Figure 1-24. Proposed pathway: Converting pentose into LA.

1-14-2-3 Applications

A number of applications have been developed for LA derivatives including chiral reagents, biologically active materials, polymer chemistry, personal care products, lubricants, adsorbents, printing inks, coatings, electronics, photography, batteries, drug delivery and corrosion inhibitors.[104] In pharmaceutical industries, the synthesis of chiral building block molecules is an extremely important issue for production of specific enantiomers. For example, 3-hydroxyvalerate (3HV) is a chiral compound that can be used to produce poly(3-hydroxybutyrate-co-3-hydroxyvalerate),[106] which is applied in

skin regeneration practices.[107] 3HV can be biologically produced using *Pseudomonas putida* KT2440 and LA as the substrate.[106] LA can be converted into many useful chemicals, Figure 1-25.[103,104] Sodium levulinate can be used as an inhibitor of spoilage bacterial growth in fresh sausage, *Listeria monocytogenes* in turkey roll, and bologna (processed meat).[108] It can also be used as an antifreeze agent. Ethyl levulinate (EL) is an ester that can be used in the food flavoring and fragrance industries.[103] γ-Valerolactone (GVL), ethyl levulinate (EL), and 2-methyltetrahydrofuran (2-MeTHF) can be used as fuel additives. GVL can also be used to produce liquid high molecular weight hydrocarbon fuels that can be useful or added to gasoline, diesel, and jet fuels. 5-nonanone (dibutyl ketone (DBK)) is another diesel fuel component. DBK can be produced by converting GVL into valeric acid (pentanoic acid) and then onto DBK.[109] GVL has many more applications including its use as a food additive and a solvent. A catalytic hydrogenation of LA using diethyl ether (solvent) was applied to produce GVL at room temperature.[110] Preparation of GVL from LA can be achieved by hydrogenation of aqueous LA in the presence of a ruthenium supported catalyst and Amberlyst-15™ as a co-catalyst at low hydrogen pressure.[111] δ-aminolevulinic acid (DALA) is another LA derived chemical. It is a biodegradable herbicide[96] and insecticide.[112] DALA can also be used as a component in photodynamic therapy for cancer treatment.[104] β-acetylacrylic acid, diphenolic acid (DPA), and 1,4-pentanediol can be obtained from LA. These chemicals can be used as polymer building blocks.[96] For example, DPA, which is produced by reacting LA with phenol using heteropoly acids, can be used to produce polycarbonates and epoxy resins.[113] DPA can be used as a 'green' replacement for bisphenol A in polycarbonate production.[85,113]

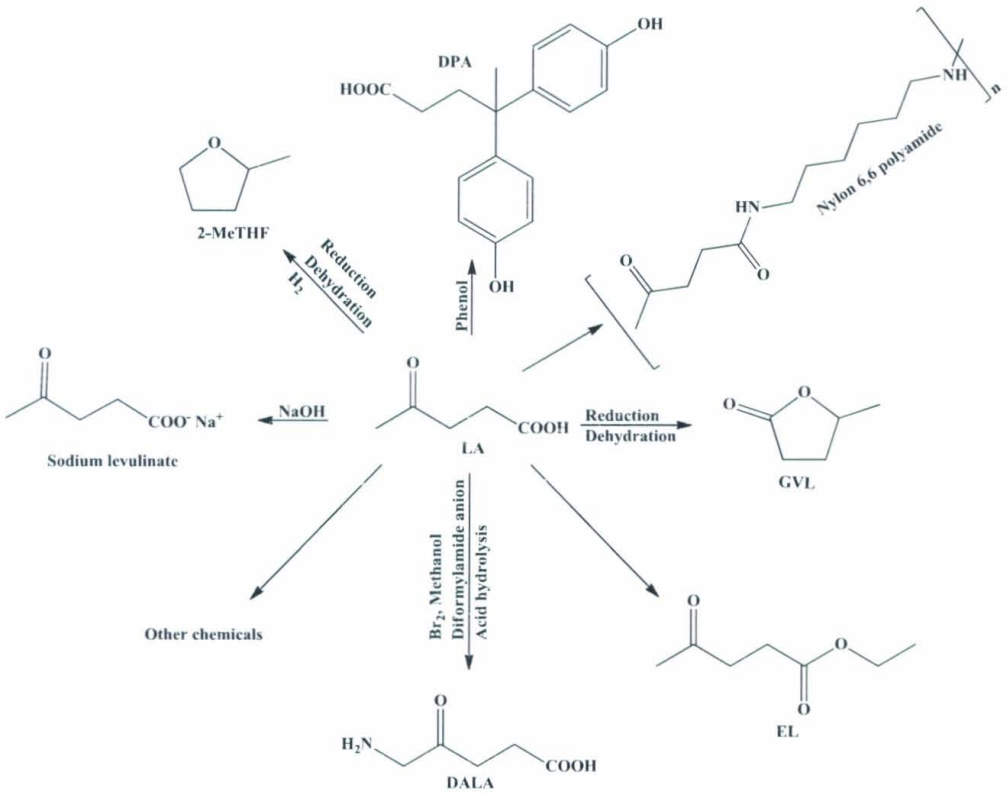


Figure 1-25. Conversion of levulinic acid into useful chemicals.

1-14-3 3-Acetamido-5-acetylfuran (3A5AF)

1-14-3-1 Production of 3A5AF

3-acetamido-5-acetylfuran (3A5AF) is an expensive compound to obtain commercially: 50 mg costing ca. $ 700 CAD. (Pricing from Atomax Chemicals Co., Ltd. 2010). This is likely due to an ineffective synthetic method. Limited exploitation and research involving 3A5AF has occurred to date because of its current price stifling research using it. The maximum amount of 3A5AF produced prior to research described in this thesis was only 2% molar yield from NAG.[114] In 1984, Franich et al. produced 3A5AF via a pyrolysis method. 3A5AF was prepared from a NAG solution (in water: methanol, 4:1 v/v) in a glass-tube apparatus, which was placed in the center of a platinum coil pyrolyser at 400 °C. For quantification purposes, the oven was interfaced with a gas chromatography mass spectrometry (GC-MS) instrument. Preparative pyrolysis was conducted using 2 g NAG in a glass tube apparatus inside a preheated oven at 400 °C. A condensed tar was formed. The tar components were eluted on a silica gel column using mixtures of chloroform and methanol. Fractions were collected using an absorbance detector at 280 nm for identification purposes. 2% molar yield 3A5AF was obtained via this preparative pyrolysis method.[114] In 1998, Ho et al. studied another pyrolysis method involving NAG under solvent-free conditions using an oil bath for heating:[115] In this way, ca. 4.4 g NAG were mixed with anhydrous disodium hydrogen phosphate and 50 g of quartz sand. This mixture was heated at 200 °C for 30 min in a sealed stainless steel vessel in an oil bath. After extraction using dichloromethane, 3A5AF was quantified using GC-MS. Only 0.04% yield of 3A5AF was produced.

1-14-3-2 Mechanism

The pyrolysis of NAG (as described above) is a thermal method by which 3A5AF may be prepared. In the production pathway (Figure 1-27), thermal rearrangement and hydration-dehydration steps were proposed to be the main and crucial steps in 3A5AF production. During the process, NAG is proposed to lose a water molecule to form 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose (anhydro sugar).[114]

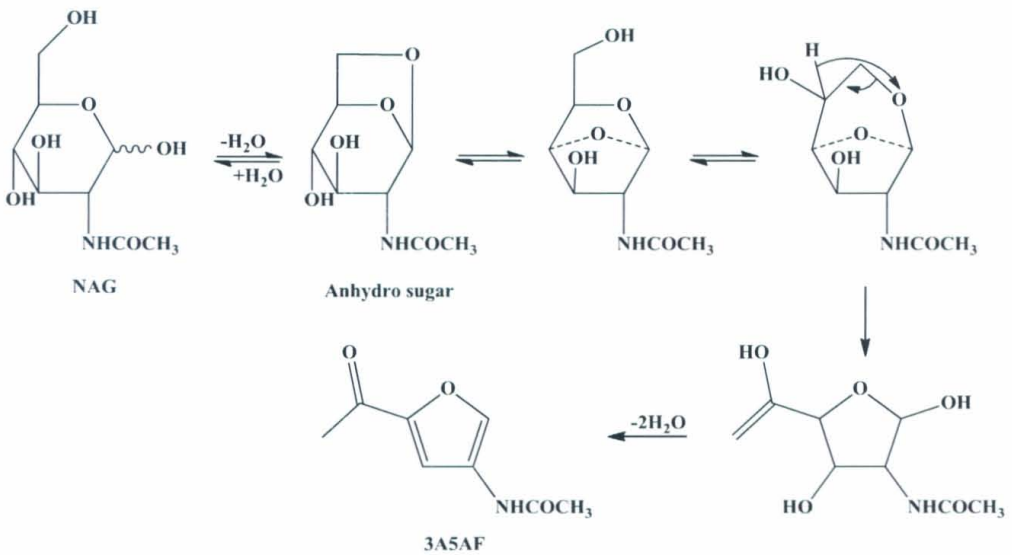


Figure 1-26. Proposed pathway: Converting NAG into 3A5AF.

In Figure 1-27, other pathways that have been proposed for the formation of 3A5AF from NAG are presented.[115]

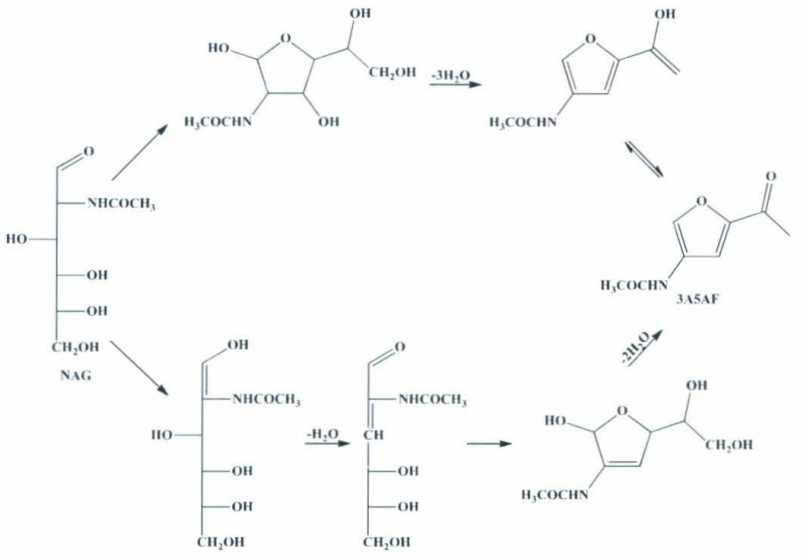


Figure 1-27. Proposed pathway: converting NAG into 3A5AF.

1-14-3-3 Applications

Due to the high price of 3A5AF, researchers have not studied the chemistry of 3A5AF extensively. One goal of the research presented herein was to develop a cost-effective method to produce 3A5AF. In terms of application, 3A5AF could be useful in proximicin A, B, and C syntheses (Figure 1-28). NAG might even be a biological precursor to proximicins, which are found in nature. Proximicins are new netropsin equivalents and have been isolated from marine actinomycete strains of the genus *Verrucosispora*. They are antibiotics and have antitumor activity.[116] Since proximicins are very important compounds, many researchers are interested in synthesising them. 3-Furfural aldehyde has been used as a major starting material in the total synthesis of proximicins published to date.[117]

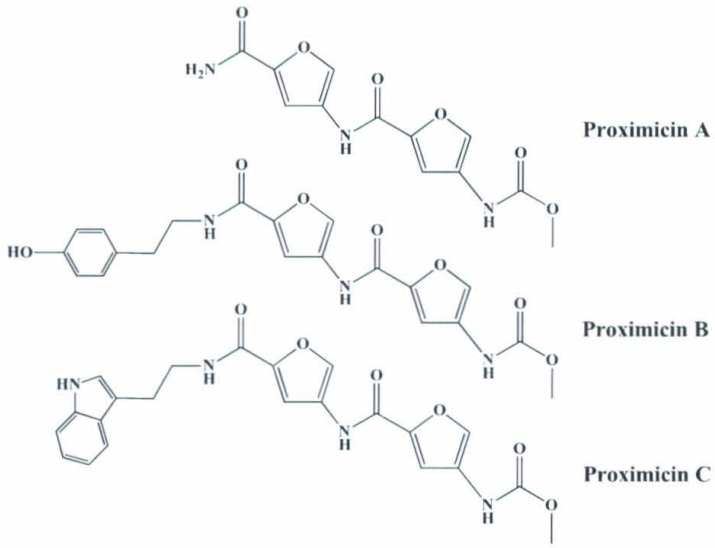


Figure 1-28. Proximicin A, B, and C structures.

1-15 Factorial Design

During the course of this PhD research, factorial design has been used to optimize the reactions conditions and achieve the desired goals. For any project or study, the person in charge is always planning, doing, studying, and acting in a cycle known as the "model for improvement'' (Figure 1-29). From this strategy, continuous improvement is achieved.[118]

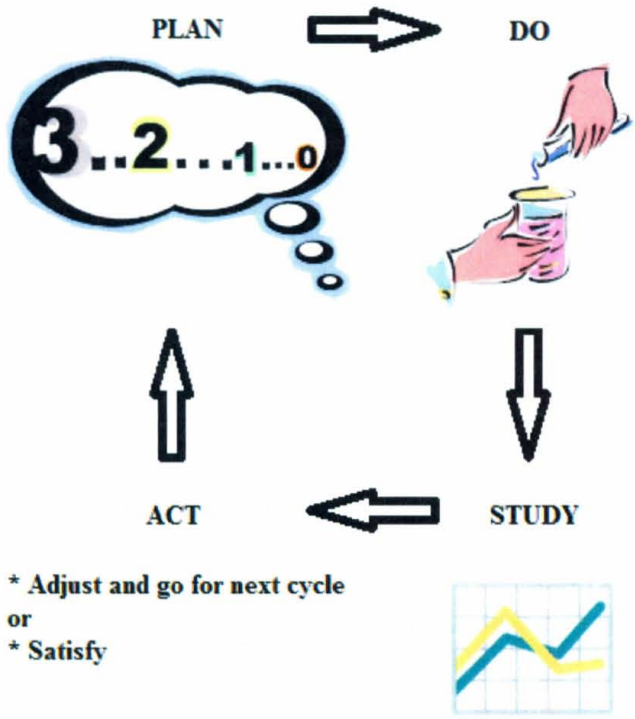


Figure 1-29. Plan, do, study, and act cycle strategy.

When a researcher begins to investigate a reaction process to produce a certain product(s), the researcher must discover the optimum conditions to achieve the highest yield in the shortest time with the least cost. Naturally, each process has several factors that can be investigated. An important question is "how can a strategy be developed to optimize the system"? The first approach is called one factor (or variable) at a time (OFAT). This method depends on changing one factor (A) while keeping others constant. After changing A, another cycle of reactions is performed and a different factor (B) is changed while other factors including A remain constant. This approach is the most commonly used one, but it has drawbacks. In particular, it neglects the interaction amongst factors.

In other words, if you increase a factor and decrease/increase another one, this may have an effect on the results too (i.e., synergistic or anti-synergistic effects).[118,119] Another disadvantage is that you end up with separate sets of data. Each set represents and gives interpretations for one factor only.[118] Another approach is called factorial designs (FDs), a type of design of experiments (DoE). FDs are alternative methods used to optimize and overcome many of the deficiencies of the "OFAT" approach outlined above. Using FDs, one factor at a time can be studied and factors can also be changed simultaneously (i.e., two, three…all factors can be varied). In this approach, each factor can be studied at two (or more) levels. If k is the number of factors and these are studied at 2 levels, 2^k is the number of experiments needed for FDs. For example, FDs for 3 factors at 2 levels (2^3 FDs) for each factor needs 8 experiments in total.[118,119] It should also be noted that the number of effects is equal to 2^k-1. For example, four factors at two levels offer 15 effects to study. Back to factors and levels, suppose that you have factors (A, B, C, etc.). Each factor has two levels; a low level (-) and a high level (+). There are three ways to present FDs. These forms are design matrix, tabular, and geometric displays as shown in Figure 1-30, Figure 1-31, and Figure 1-32, respectively.[118]

	Factors			
Run #	A	B	C	D
1	-	-	-	-
2	+	-	-	-
3	-	+	-	-
4	+	+	-	-
5	-	-	+	-
6	+	-	+	-
7	-	+	+	-
8	+	+	+	-
9	-	-	-	+
10	+	-	-	+
11	-	+	-	+
12	+	+	-	+
13	-	-	+	+
14	+	-	+	+
15	-	+	+	+
16	+	+	+	+

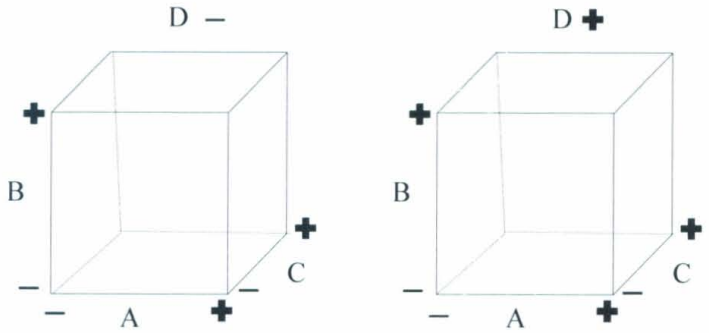
— 2^2, — 2^3 and the whole matrix display is for 2^4 FDs

Figure 1-30. Matrix display of FDs.

		-	C		+
D	B	- A	+ A	- A	+ A
-	-				
	+				
+	-				
	+				

— 2^2, — 2^3 and the whole tabular display is for 2^4 FDs

Figure 1-31. Tabular display for FDs.



The front facial square of any cube is 2^2, each cube without D is 2^3 and the whole geometric display (i.e., both cubes) is 2^4 FDs

Figure 1-32. Geometric display for FDs.

1-15-1 The Simplest Factorial Design (2^2 FD)

To show the difference between OFAT and FD, 2^2 factorial designs will be discussed as a simple example. For a chemical reaction that yields a product "X", two factors that could be studied are temperature (T) and time (t). Each factor would be investigated at two levels "Low (-) and high (+)". The matrix display of this example is shown in Table 1-5.

Table 1-5. 2^2 FD matrix display showing the % yield of product "X".

	T (-)	**T (+)**
t (-)	47% yield of product "X"	42% yield of product "X"
t (+)	25% yield of product "X"	44% yield of product "X"

The number of effects in this example is three. They are the individual effects (T and t) and the interaction effect (Tt). Each effect has a response that is the difference between the average of the yields of an effect at +1 and -1 (or the high and low levels i.e. the high and low temperatures), Table 1-5.

Table 1-6. The difference between the averages of %yields for each effect.

Effect	Average of %yields (+1)	Average of %yields (-1)	The difference
T	T(+): (42+44)/2=43	T(-): (47+25)/2=36	43-35=7
t	t(+): (25+44)/2=34.5	t(-): (47+42)/2=44.5	34.5-43.5=-10
Tt	T(-), t(-) and T(+), t(+): (47+44)/2=45.5	T(-), t(+) and T(+), t(-): (25+42)/2=33.5	44.5-43=12

The following points can be noted by interpreting Table 1-6:

- Increasing the T from a low to high level led to an increase in the yield of 7%.
- Increasing the t from a low to high level led to a decrease in the yield of 10%.
- The largest effect is for the interaction of T and t i.e. when both T and t are increased or decreased simultaneously, the %yield of product "X" increases.
- Increasing the T and t at the same time to even higher levels (greater than the high levels used in this example) may produce %yield greater than 47. This would be a starting point for future experiments with the aim of obtaining a greater yield of X.

This example 2^2 FD demonstrates that changing two factors simultaneously can have a greater effect than changing one at a time. If a researcher were looking at the same reaction using a OFAT approach, a maximum yield of 44% would likely be obtained. This is because the OFAT method neglects the synergistic effect between factors. To analyze complex FDs, software programs are available for quick, accurate analysis and interpretation of experimental data.

1-15-2 FDs Statistical Analysis

FDs can be interpreted using Minitab software.[120] In this program Lenth's analysis is used for interpretation. The most important effects in a study are termed to be significant. To determine these effects, a median of the absolute value of the ranges of effects (m) should be calculated. Then, the pseudo standard deviation of absolute values (s) equals $1.5 \times m$. The median of effects that are less than $2.5 \times s$ is m_o. Now, the pseudo standard error (PSE) equals $1.5 \times m_o$. A non-significant effect is an effect that lies within a 95% confidence interval. This interval is determined by calculating a margin of error (ME). To calculate ME, the t-distribution should be determined at α (1-95/100) and the degrees of freedom (d_f = number of effects/3). ME is a combination of many terms. However, ME equals to $t_{(1-\alpha/2),\ df} \times PSE$. A normal plot of the standardized effects using Minitab gives the significant and non-significant effects. To get better results, the significant effects must be tested.[121] For example, Chapter 2 (chitosan hydrolysis project) deals with a sample situation wherein 2^4 FD was applied and the significant effects were studied in more detail.

To show the statistical analysis manually for a 2^4 FD, results for the production of 5-hydroxymethylfurfural (5-HMF) from chitosan presented in Table 2-1 later in this thesis are used as sample data. The first step is to calculate the difference between the averages of %yields (the responses of effects) as in section 1-15-1, Table 1-7.

Table 1-7. The difference between the averages of %yields of 5-HMF production for each effect.

Effect[a]	Average of %yields (+1)	Average of %yields (-1)	The difference	Absolute value of the difference
A	3.22	1.58	1.63	1.63
B	1.06	3.74	-2.67	2.67
C	4.80	0.00	4.80	4.80
D	2.39	2.41	-0.02	0.02
AB	2.26	2.54	-0.28	0.28
AC	3.22	1.58	1.63	1.63
AD	2.60	2.20	0.40	0.40
BC	1.06	3.74	-2.67	2.67
BD	2.40	2.40	0.01	0.01
CD	2.39	2.41	-0.02	0.02
ABC	2.26	2.54	-0.28	0.28
ABD	2.29	2.52	-0.23	0.23
ACD	2.60	2.20	0.40	0.40
BCD	2.45	2.40	0.05	0.05
ABCD	2.29	2.52	-0.23	0.23

[a] A = Water volume, B = Amount of $SnCl_4.5H_2O$, C = Temperature, and D = Time

The median (m) of the absolute differences of the effects is 0.28. Let s equal 1.5 × m, which is 0.41. By excluding the effects that are greater than 2.5 × s (2.5 × 0.41 = 1.03), the new median (m_o) of the rest of effects will be 0.23. Then let PSE equal 1.5 × m_o, which is 0.35. After calculating the PSE of the results, ME can be determined by multiplying PSE with $t_{((1-\alpha/2),\ df)}$. $t_{((1-\alpha/2),\ df)}$ can be obtained from Student's t distribution table, which is in this case equal to 2.57. Therefore, ME equals 0.90. Any results that lay

within the ME are considered non significant because they are close to no response. In FDs, the significant effects are those that lay outside of the ME because they are the extremes that have the potential to produce the maximum %yield of 5-HMF. To wrap up a study of this kind, the significant effects determined through the FD approach should be studied further before drawing any firm conclusions. To show the distribution of the effects around the ME, the normal plot of the effects lets the significant effects be seen and assigned more easily. The normal plot of the effects is created based on the percentile (percent) of each effect against its difference between the averages of %yields. The percentile is a statistic term that means a certain percentage of observations are falling below an observation. To calculate the percentile, the differences in Table 1-7 should be sorted in descending order. The largest difference will have number 15 and the smallest is 1.The percentile of each difference is calculated as (the order of an effect – α) / total number of effects. For example, the percentile of the largest difference is for effect "C" equal $(15 - 0.5)/15 \times 100$ that is 97%. This means 97% of the observations fall below the effect "C".

Table 1-8. The percentiles of effects.

Effect	Order Number	The difference	Percentile
C	15	4.8	97
AC	14	1.63	90
A	13	1.63	83
ACD	12	0.4	77
AD	11	0.4	70
BCD	10	0.05	63
BD	9	0.01	57
CD	8	-0.02	50
D	7	-0.02	43
ABCD	6	-0.23	37
ABD	5	-0.23	30
ABC	4	-0.28	23
AB	3	-0.28	17
BCD	2	-2.67	10
B	1	-2.67	3

The normal plot of distribution for this data is presented in Figure 2-7 as a graph of the percentiles vs the difference of effects. Using Figure 2-7, it is easy to figure out the effects which significantly affect the %yield of 5-HMF production.

1-16 Data Analysis

A GC chromatogram shows individual peaks for each of the separated components, by their retention time (t_R). Each compound has a unique t_R. This t_R is usually highly reproducible, if the sample is re-injected using the same instrument parameters. Therefore, a compound is recognized based on its t_R. The challenge is that the analyst

cannot qualitatively identify the structure of a compound using the chromatogram (e.g., t_R) alone. By examining a mass spectrum, an analyst can study the fragmentation pattern of a given compound by its *m/z*. To quantify a compound, the analyst needs to form a calibration curve to correlate a peak area (from the chromatogram) and compound concentration. Thus, the concentration of an unknown compound can be inferred based on peak area.[122] GC-MS methods were employed for both qualitative and quantitative purposes in this thesis (Chapters 2, 3 and 4).

1-16-1 Wt% Expression

For the purpose of this work, wt% was defined as the number of mg a method can produce of a desired product from 100 mg raw material. For example, if a certain process gave 50 wt% of a specific product, this indicates that 100 mg of the raw material used produced 50 mg of the product. In Chapter 2, the produced amounts of LA and 5-HMF were determined using the wt% expression. Because chitosan cannot be produced in a fully deacetylated form (i.e., there is no 100% DD chitosan), it is better to use the wt% expression.

1-16-2 % Yield Expression

The % yield expression means: the actual yield of a desired product divided by the theoretical yield × 100%. The actual yield of the product is its quantity that is obtained experimentally. The theoretical yield is the maximum quantity of the product that can be produced based on the given quantities of reactants and the reaction stoichiometry.

In Table 2-3, 13 wt% of LA was produced from chitin using an optimized method (described in Chapter 2). The following is a sample calculation to show that 13 wt% is equivalent to 23% yield of LA. 13 wt% means 100 mg chitin produced 13 mg LA. Chitin formed from NAG monomers, where NAG loses one molecule of H_2O to form chitin. Therefore, the molecular weight of chitin can be approximated to be 221.2 g mol^{-1} (NAG, molecular weight) -18.0 g mol^{-1} (H_2O, molecular weight) = 203.2 g mol^{-1}. To calculate the theoretical mass of LA obtained from 100 mg chitin, complete conversion will be considered. 100 mg chitin = 100 mg/203.2 mg $mmol^{-1}$ = 0.492 mmol. Supposing that the reaction is 1:1 chitin:LA mol ratio. The theoretical mass of produced LA will be 0.492 mmol × 116.1 mg $mmol^{-1}$ = 57.1 mg. The % yield is calculated using Equation 1-4.

$$\% \text{ Yield} = \frac{\text{Actual mass of a desired product}}{\text{Theoritical mass of the desired product}} \times 100 \qquad \textbf{Equation 1-4}$$

As a result, the % yield of LA = 13.0 mg/57.1 mg × 100 = 22.8.

1-17 Summary

Chitin is a polysaccharide that is formed, degraded and absorbed naturally. Chitin, chitosan, and their monomers (glucosamine and NAG) are renewable feedstocks. Because of their availability and non-toxicity, increasing attention has been placed on them in both medical and industrial-based applications. On the other hand, the production of useful chemicals using these renewable feedstocks has only been discussed to a limited extent compared with fructose, glucose and cellulose. Most of the useful products from chitin and chitosan are oligomers that have associated medical applications. LA and 5-HMF have been produced from different sources including cellulose, starch inulin, glucose, fructose, and sucrose. Despite this, there are few studies related to the production of LA and 5-HMF from chitin and chitosan. These chemicals were presented and discussed from three angles: production methods, mechanistic features and potential applications. 3A5AF has been previously produced in two pyrolysis studies from NAG in low yields. The known chemistry of 3A5AF was discussed using the same three perspectives as for LA and 5-HMF. It is clear that there is significant scope to study the chemistry of 3A5AF further in the future when its current chemistry is compared with the related molecule 5-HMF. Factorial designs (FDs) are outlined in this introductory chapter and have been used to study and optimize most reactions presented in this thesis. GC-MS is briefly described above and was the major qualitative and quantitative analytical tool used to collect data presented in this thesis.

1-18 Objectives of Thesis

The essential objective of this thesis was to develop new methods to generate useful chemicals from biological sources, which are crustacean shells (chitin and its deacetylated form, chitosan). This represents a superior alternative to dumping chitin into the sea. There is a large amount of fishery waste (crustacean shells) produced from food processing industries, not only in the Atlantic region, but also worldwide. In Chapter 2, a green method for processing chitosan (in water) is presented. This method was based on microwave irradiation, in the presence of catalysts, resulting in the production of the useful platform chemicals LA and 5-HMF described above. In Chapters 3 and 4, two methods in different solvents were studied to produce 3A5AF from NAG. The yield reported herein is over 30 times more than that previously described in the literature. Statistical data were obtained using Minitab software.

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1-20 Co-Authorship Statement

This PhD thesis includes results of joint research that have been published in or submitted to peer reviewed journals in the form of two full papers and one communication, as follows:

Chapter Two: Hydrolysis of Chitosan to Yield Levulinic Acid and 5-Hydroxymethylfurfural in Water Under Microwave Irradiation

Authors: Khaled W. Omari, Jessica E. Besaw and Francesca M. Kerton

Journal: *Green Chem.*, 2012, **14**, 1870-1877

The principal author (Khaled W. Omari) contributed to all aspects of the project as the main researcher including: literature review, performing 90% of the experiments, collecting and analyzing most of the data, designing some new experiments, presenting and discussing the data with the corresponding author, mentoring Jessica E. Besaw as an undergraduate summer student, writing the first draft of the manuscript and preparing answers to the questions and comments of the peer reviewers.

The co-author (Jessica E. Besaw) ran around 10% of the experiments and data analysis, primarily those concerned with the reactions of glucosamine hydrochloride.

The corresponding author (Dr. Francesca M. Kerton) proposed the initial experiments and contributed to various aspects of the project including data analysis, design of new experiments, revision of the draft manuscript and submission to the journal, supervision both of the principal author (K. W. O.) and co-author (J. E. B.).

Chapter Three: A Simple One-Pot Dehydration Process to Convert *N*-acetyl-D-glucosamine into a Nitrogen-Containing Compound, 3-acetamido-5-acetylfuran

Authors: Khaled W. Omari, Linda Dodot and Francesca M. Kerton

Journal: *ChemSusChem,* 2012, **5**, 1767-1772.

The principal author (Khaled W. Omari) contributed to all aspects of the project as the main researcher including: literature review, performing 90% of the experiments, collecting and analyzing the data, designing new experiments, presenting and discussing the data with the corresponding author, mentoring Linda Dodot as an undergraduate summer student, writing the first draft of the manuscript and preparing answers to the questions and comments of the peer reviewers.

The co-author (Linda Dodot) ran around 10% of the experiments and data analysis, primarily running some duplicate reactions for assessing reproducibility of reaction conditions.

The corresponding author (Dr. Francesca M. Kerton) proposed the initial experiments and contributed to several aspects of the project including data analysis, design of new experiments, revision of the draft manuscript and submission to the journal, supervision both of the principal author (K. W. O.) and co-author (L. D.).

Chapter Four: Formation of a Renewable Amide, 3-acetamido-5-acetylfuran, via Direct Conversion of *N*-acetyl-D-glucosamine,

Authors: Marcus W. Drover, Khaled W. Omari, Jennifer N. Murphy and Francesca M. Kerton

Journal: *RSC Adv.*, 2012, **2**, 4642-4644

The principal author (Khaled W. Omari) contributed to all aspects of the project as the co-first author alongside Marcus Drover including: literature review, training, mentoring and advising Marcus Drover and Jennifer Murphy as undergraduate summer students, co-writing the first draft of the manuscript with Marcus Drover and preparing answers to the questions and comments of the peer reviewers. Day-to-day supervision and guidance of the two undergraduate students was provided by K. W. O. and F. M. K. assisted through biweekly meetings with the 3-member research team.

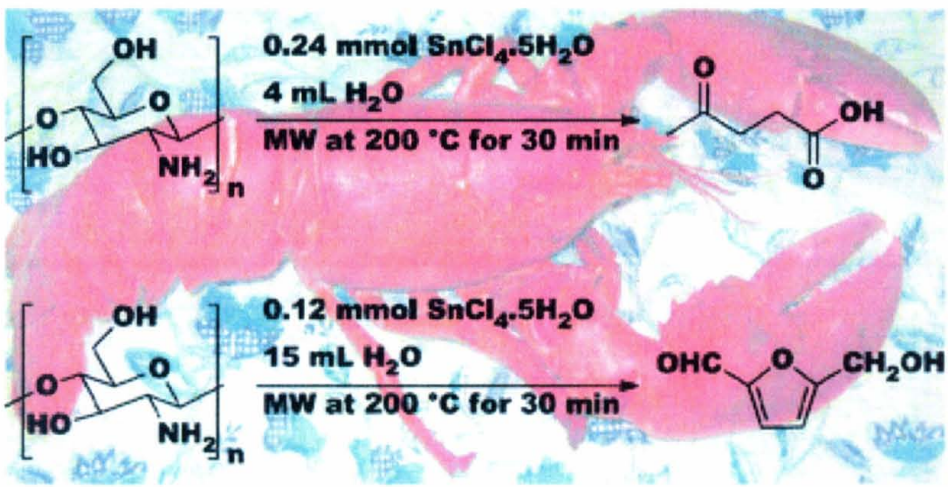
This project was initiated after the initial results presented in Chapter 3 were obtained. Therefore, quantification and characterization methods for 3-acetamido-5-acetylfuran used in this study were already established by K. W. O., as the key starting point in this project.

The co-first-author Marcus Drover ran around 65% of the experiments and worked on data analysis with K. W. O. and developed the NMR method used for kinetic studies. *Jennifer Murphy* ran about 10% of the experiments primarily running some duplicate reactions for assessing reproducibility of reaction conditions and preparing samples for the kinetic studies. M. W. D. and J. N. M. prepared some of the ionic liquids (ILs) used in this study but the majority of ILs were purchased.

The corresponding author (Dr. Francesca M. Kerton) proposed the initial experiments and contributed to several aspects of the project including data analysis, design of new experiments, revision of the draft manuscript and submission to the journal, supervision both of the co-principal authors (K. W. O. and M. W. D.) and co-author (J. N. M.).

Chapter Two

Hydrolysis of Chitosan to Yield Levulinic Acid and 5-Hydroxymethylfurfural In Water Under Microwave Irradiation



A version of this chapter has been published.

Khaled W. Omari, Jessica E. Besaw and Francesca M. Kerton*, Hydrolysis of Chitosan to Yield Levulinic Acid and 5-Hydroxymethylfurfural in Water Under Microwave Irradiation, *Green Chem.*, 2012, **14**, 1870-1877.

Some modifications were made to the original paper for inclusion as a chapter in this thesis. For example, the supporting information was incorporated in this chapter and some figures have been added.

2-1 Introduction

Chitin is an important biopolymer that can be sourced from the ocean and is the most abundant biopolymer on Earth after cellulose and hemicellulose, Figure 2-1.[1,2] The estimated annual production of chitin worldwide is about 1.5×10^5 tons.[2] It is mainly available from crustaceans' shells such as crab, lobster and shrimp, and as such it is an industrial waste material of fisheries and a renewable feedstock with much potential (see section 1-2-1). Three steps are used to purify chitin from crustacean's waste. These are (i) deproteinization using a strong base such as NaOH, (ii) demineralization using an acid such as HCl, and (iii) decolouration using a bleaching agent such as H_2O_2 (see section 1-2-2).[2] New methods are being developed that use green chemistry techniques in this field, for example, the use of ionic liquids.[3]

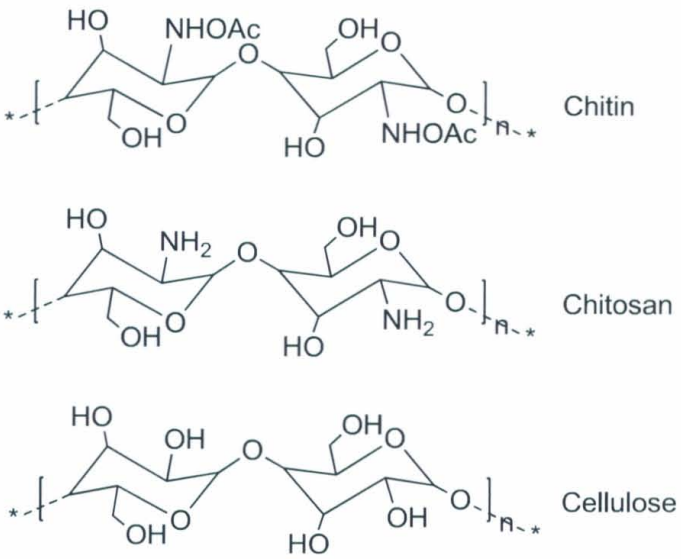


Figure 2-1. Comparison of Chitin, Chitosan, and Cellulose formulae.

Chitin is deacetylated under alkaline conditions to yield chitosan. 100% deacetylation cannot be achieved and therefore, chitosan is a copolymer of glucosamine and NAG.[4] It is readily available in a range of molecular weights and degrees of deacetylation (DD), 50 to 2000 kDa and 40 to 98%, respectively (for more details, see section 1-4).

In recent years, many useful chemicals have been produced from renewable feedstocks.[5,6] For example, catalytic conversions of cellulose,[7-10] fructose,[11-15] and glucose[16-21] into 5-HMF and LA have been reported (see section 1-14-1 and 1-14-2) but amino-sugars and carbohydrates have been overlooked. The chemistry of chitin and chitosan should be investigated in concert with current studies on cellulose to achieve maximum benefits from the most abundant bio-feedstocks available. The processing and usage of these N-containing polysaccharides has been somewhat restricted over the years because they contain many hydroxyl groups that are able to form strong intra- and inter-molecular

hydrogen bonds. Chitosan is insoluble in basic media and water.[22,23] Likewise, chitin is insoluble in water, most of organic solvents, dilute acidic, or basic solutions.[3]

Despite the currently limited industrial applications of these biopolymers, their hydrolysis has been quite widely studied. They can be hydrolyzed using enzymes such as cellulase, hemicellulase, lysozyme, papain, pectinases, and lipases to produce glucosamine, NAG, and oligomers.[24,25] Chitinases depolymerize chitin to produce chito-oligosaccharides consisting of one to six NAG sub-units.[25] High yields of diacetylchitobiose $(NAG)_2$ can be obtained through hydrolysis of colloidal chitin in dimethylsulfoxide and lithium chloride using *Vibrio furnissii chitinase*.[26] Chito-oligosaccharides can also be produced by chitin and chitosan cleavage using mineral acids. Depolymerization of chitosan in nitrous acid produces chito-oligosaccharides and 2,5-anhydro-D-mannofuranose (M).[25] Cleavage of chitin in sulfuric acid in the presence of acetic anhydride produces *N*-acetylchito-oligosaccharide peracetates.[25,27]

However, aside from the production of monosaccharides, disaccharides, and oligosaccharides, there have been few reports on the production of chemicals from chitosan or chitin. One published example is the conversion of chitin to 5-(chloromethyl)furfural and LA using aqueous HCl and large amounts of 1,2-dichloroethylene.[1] The trimer $(NAG)_2$-M has been prepared by treating chitosan (fraction of N-acetylated units = 0.59) in HNO_2.[28] This trimer was then reported to produce 1% 5-HMF upon further exposure to concentrated HNO_2. Conversion of gluocosamine to 5-HMF has been briefly reported by other researchers using organic acids in the presence of DMSO.[29] The current main use of glucosamine is as a dietary supplement and therefore

some studies concerning its stability have been performed. Pyrolysis of glucosamine at 200 °C in the solid-state yields a mixture of furans, pyridines, pyrroles, and pyrazines. The most abundant products, 2-acetylfuran and 2-(2-furyl)-6-methylpyrazine, were present at levels of only 0.063 mg per g of glucosmine pyrolyzed.[30] In aqueous solution, when glucosamine was heated to 100 °C, a similar mixture of products formed and around 20 mg of furan products were produced from 1 g of the sugar.[31] These results show that glucosamine has potential as a renewable feedstock for furans and N-containing heterocycles and that clearly, there is a need to further investigate the conversion of chitin and chitosan into useful renewable chemical building blocks.

Herein, attempts to hydrolyze chitosan in the absence of concentrated acids by generating superheated water under microwave conditions are described. The hydrolysis process was enhanced by use of a Lewis acid. Water is a clean, non-corrosive, non-flammable, renewable, readily available, cheap, and environmentally friendly solvent.[32,33] Some of the advantages of using microwave heating are that it reduces reaction times and can increase product yields compared with conventional heating methods.[34-36] Microwave heating is particularly efficient for chemical transformations in water as it can be superheated in sealed vessels.[35] In this study, different parameters were varied such as temperature, concentration, the Lewis acid used and reaction time. Two compounds were identified as the primary products from the hydrolysis of chitosan described herein: Levulinic acid (LA) and 5-hydroxymethylfurfural (5-HMF). In addition, chitin under similar conditions produced LA. LA can be used to produce many compounds such as ethyl levulinate and 2-methyltetrahydrofuran that can be used as miscible diesel biofuel

additives, δ-aminolevulinic acid, a herbicide, and β-acetylacrylic acid, diphenolic acid, and 1,4-pentanediol, which are polymer building blocks.[37] 5-HMF can yield other renewable building blocks such as 2,5-furandicarboxylic acid (FDCA), 2,5 dihydroxymethylfuran, 2,5-bis(hydroxymethyl)tetrahydrofuran, and 2,5-dimethylfuran. The latter is a promising liquid transportation fuel.[17,38] FDCA can be used in polyester production in the place of terephthalic acid.[39]

2-2 Results and Discussion

2-2-1 Catalysts Screening

Two series of reactions were performed initially to identify whether a catalyst was required to hydrolyze chitosan in superheated water under microwave conditions and whether an acidic or basic catalyst would give superior results. For each set of reactions, a control reaction (no catalyst) was performed and 21 catalysts were screened and the amounts of LA and 5-HMF produced from medium molecular weight chitosan were determined. The amount of chitosan processed under the two conditions was fixed at 100 mg. The first condition was 0.24 mmol catalyst and 4 mL deionized water. The second was 0.12 mmol catalyst and 20 mL deionized water. The potential catalysts that were assessed were anhydrous lanthanum trifluoromethanesulfonate $La(CF_3SO_3)_3$ (**1**), gadolinium trifluoromethanesulfonate hydrate $Gd(CF_3SO_3)_3 \cdot xH_2O$ (**2**), ytterbium trifluoromethanesulfonate hydrate $Yb(CF_3SO_3)_3 \cdot xH_2O$ (**3**), zinc perchlorate hexahydrate $Zn(ClO_4)_2 \cdot 6H_2O$ (**4**), Amberlyst-15™ hydrogen form (**5**) which is an acidic resin, anhydrous indium(III) chloride $InCl_3$ (**6**), hydrochloric acid HCl (**7**), iron perchlorate

hydrate $Fe(ClO_4)_3 \cdot xH_2O$ (**8**), nickel perchlorate hexahydrate $Ni(ClO_4)_2 \cdot 6H_2O$ (**9**), zirconyl chloride octahydrate $ZrOCl_2 \cdot 8H_2O$ (**10**), copper perchlorate hexahydrate $Cu(ClO_4)_3 \cdot 6H_2O$ (**11**), bismuth chloride $BiCl_3$ (**12**), chromium perchlorate hexahydrate $Cr(ClO_4)_3 \cdot 6H_2O$ (**13**), zirconium tetrachloride $ZrCl_4$ (**14**), tin chloride pentahydrate $SnCl_4 \cdot 5H_2O$ (**15**), manganese perchlorate hydrate $Mn(ClO_4)_2 \cdot xH_2O$ (**16**), yttrium trifluoromethanesulfonate $Y(CF_3SO_3)_3$ (**17**), acetic acid CH_3COOH (**18**), ammonia NH_3 (**19**), sodium hydroxide NaOH (**20**), and basic alumina Al_2O_3 (**21**). These were chosen because the metal complexes are generally stable in water[40] and some of the other species, e.g., Amberlyst-15™, have given good results for glucose/cellulose transformations.[18] HCl was studied because it is known to yield LA from chitin.[1] $SnCl_4 \cdot 5H_2O$ has been reported to yield 5-HMF from glucose in an ionic liquid.[17] Both the concentrated and dilute reaction mixtures were heated under microwave irradiation at 200 °C for 30 min. In the control reactions and in the presence of **16** to **21** neither LA nor 5-HMF was produced. For the concentrated reaction mixtures (0.24 mmol catalyst, 4 mL water), Figure 2-2 shows the weight percentages of LA and 5-HMF produced using catalysts **1** to **15**.

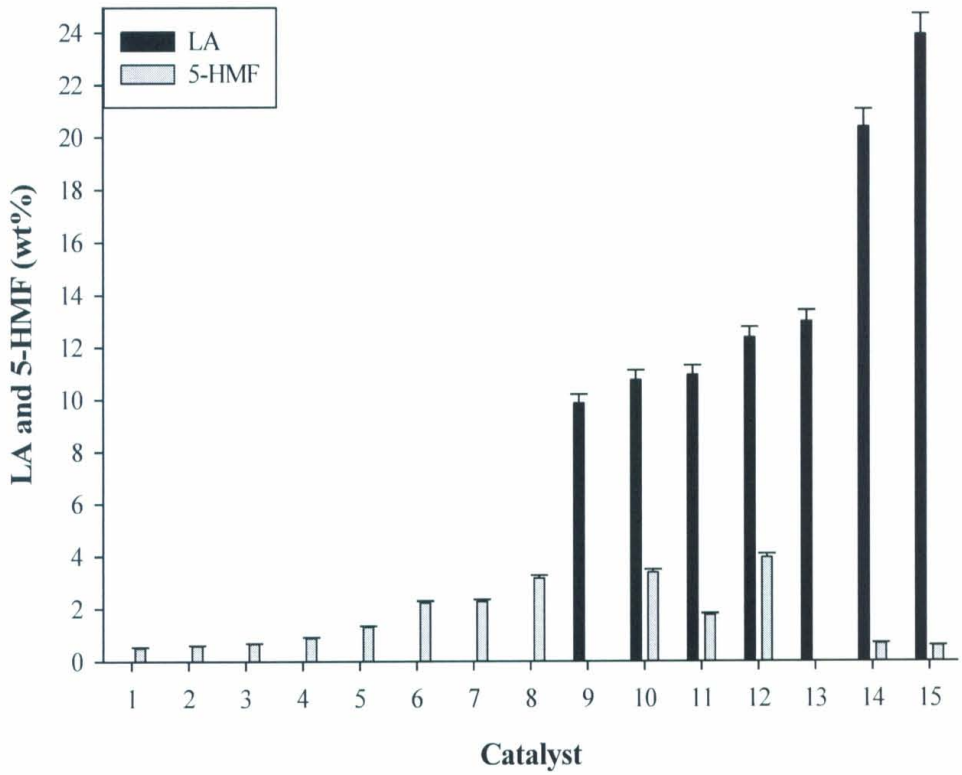
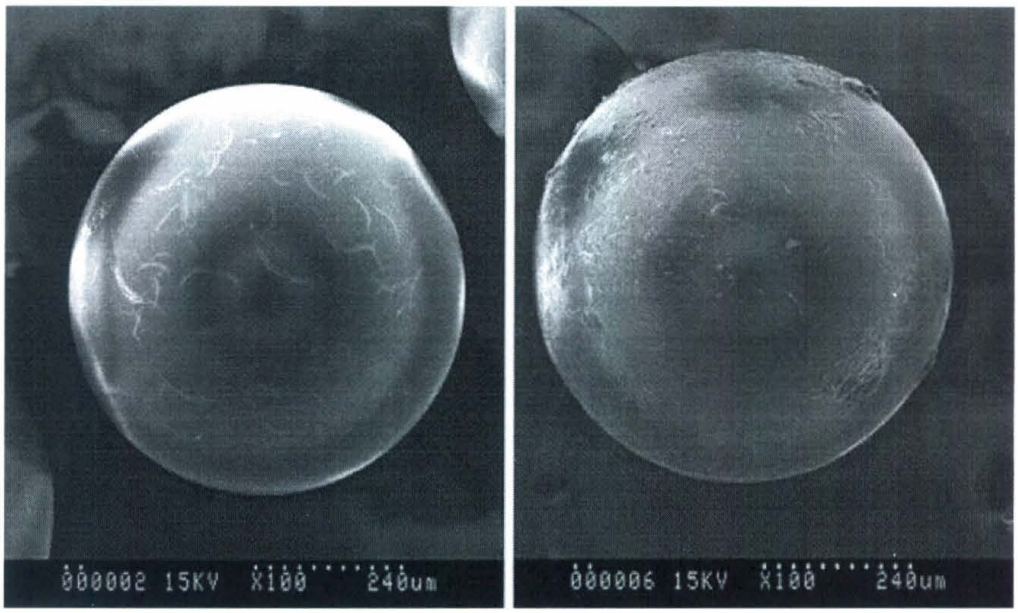


Figure 2-2. Comparison of weight percentages LA and 5-HMF produced under concentrated reaction conditions for a range of catalysts.

Generally, the metal-containing catalysts gave superior conversions when compared to the simple acids and bases. Acetic acid and the bases studied, **18-21**, produced no products, and HCl and Amberlyst-15™ produced only a small amount of 5-HMF (< 2.3%) and no LA. The reason for the poor performance of Amberlyst-15™ may be insufficient swelling of the resin in aqueous solvents and also deposition of biopolymer on the surface of the beads, which was evident through SEM studies (Figure 2-3).



(a) **(b)**

Figure 2-3. SEM analysis of Amberlyst-15™ (a) before microwave reaction, there are cracks on the surface of the beads (b) after microwave reaction, biopolymer deposited on the surface of the beads and filled the cracks.

Of the metal salts, the triflate catalysts were also ineffective for this process, **1** to **3** produced small amounts of 5-HMF (<0.7%) and **17** was completely inactive. This may be due to the non-coordinating nature of these anions compared with chloride. The metal perchlorate Lewis acid catalysts were generally a little more effective and in most cases produced LA and/or 5-HMF, however, the Mn(II) salt (**16**) produced neither LA nor 5-HMF. The Fe(III) salt (**8**) produced only 5-HMF (3.16 wt%) whereas the Ni(II) salt (**9**) and the Cr(III) salt (**13**) produced only LA (9.9 wt% and 13.0 wt%, respectively). The Cu(II) salt (**11**) was the only metal perchlorate that produced LA and 5-HMF simultaneously (10.9 wt% and 1.8 wt%, respectively). These results indicate that the

choice of metal ion is important in determining the selectivity of the hydrolysis reaction. However, in general, the overall yields of LA were strongly dependent on the anion and follow the reactivity trend: $CF_3SO_3^- < ClO_4^- < Cl^-$.

Under concentrated conditions, the metal chloride Lewis acid catalysts generally produced the largest amounts of LA and 5-HMF. $InCl_3$ (**6**) produced a small amount of 5-HMF (2.2 wt%) but no LA was produced. $BiCl_3$ (**12**) produced LA (12.4 wt%) and the largest amount of 5-HMF (4.0 wt%) under these conditions amongst the catalysts screened. $ZrCl_4$ (**14**) and $SnCl_4 \cdot 5H_2O$ (**15**) produced the largest amounts of LA (20.4 wt% and 23.9 wt%, respectively). $ZrCl_4$ is known to react with water to produce $ZrOCl_2 \cdot 8H_2O$ (**10**) and HCl (**7**).[41] As a result, catalyst **10** was examined and a moderate amount of LA and 5-HMF were produced (10.8 wt% and 3.4 wt%, respectively). However, neither $ZrOCl_2 \cdot 8H_2O$ nor HCl gave the largest amounts of LA and 5-HMF under the conditions studied. Thus indicating that this process is catalyzed more effectively by a suitable Lewis acid rather than a Brønsted acid. The largest amounts of LA (23.9 wt%) and 5-HMF (3.95 wt%) produced in this set of reactions came from those using $SnCl_4 \cdot 5H_2O$ (**15**) and $BiCl_3$ (**12**), respectively.

For the more dilute reaction condition screened (0.12 mmol catalyst, 20 mL water), Figure 2-4 summarizes the weight percentages of LA and 5-HMF produced using the catalysts screened. Under these conditions, a smaller number of catalysts were effective, namely, **3**, **6** to **8**, and **10** to **15**.

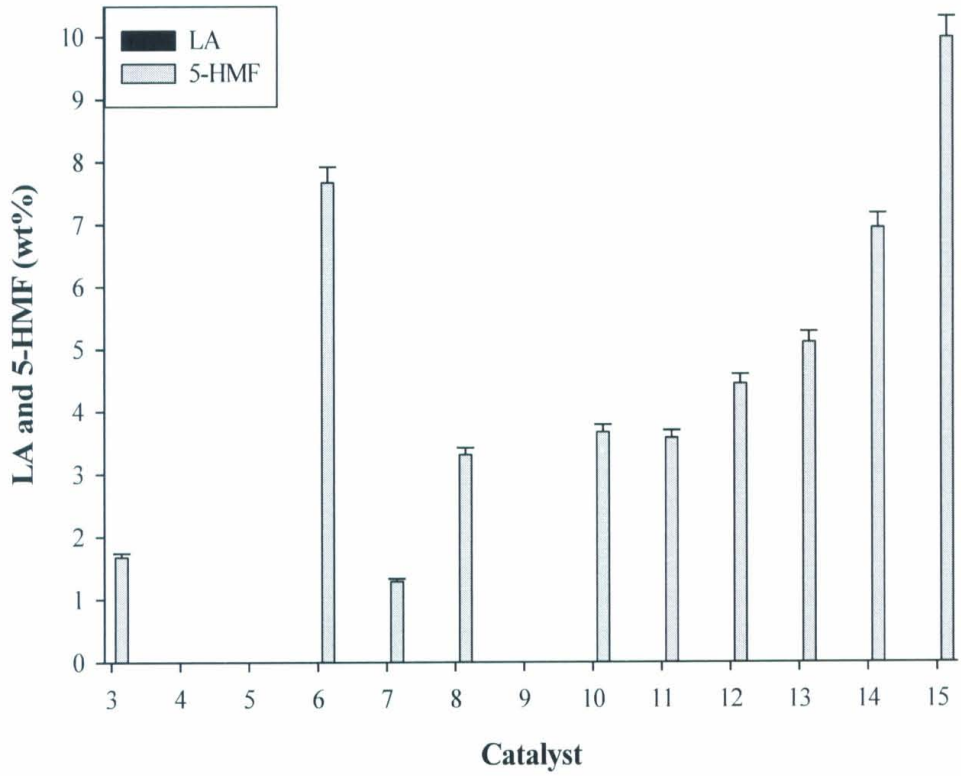


Figure 2-4. Comparison of weight percentages LA and 5-HMF produced under dilute reaction conditions for a range of catalysts.

Interestingly, under these dilute conditions, no catalyst was ever able to produce LA indicating that LA production is strongly dependent on reaction concentration. Catalysts **1**, **2**, **4**, **5,** and **9** although able to hydrolyze chitosan to 5-HMF or LA (in the case of **9**) under concentrated conditions, did not yield any 5-HMF under these more dilute conditions. HCl (**7**) was the only conventional (Brønsted) acid or base that produced 5-HMF (1.3 wt%) but this was a significantly smaller amount than that generated by some

of the Lewis acids studied. Also, $Yb(CF_3SO_3)_3 \cdot xH_2O$ (**3**) was the only metal triflate that produced 5-HMF (1.7 wt%) whereas under more concentrated conditions $La(CF_3SO_3)_3$ (**1**) and $Gd(CF_3SO_3)_3$ (**2**) were also active. Fe(III) (**8**), Cu(II) (**11**), and Cr(III) (**13**) perchlorates produced 5-HMF (3.3 wt%, 3.6 wt%, and 5.1 wt%, respectively) in similar quantities to those afforded under concentrated conditions. However, no LA was produced. Metal chloride Lewis acid catalysts yielded larger amounts of 5-HMF under dilute conditions compared with the more concentrated reaction mixtures. $InCl_3$ (**6**) in particular was a much more effective catalyst under dilute reaction conditions yielding 7.7 wt% 5-HMF compared with 2.2 wt% under concentrated conditions. Under both concentrated and diluted reaction conditions, $SnCl_4 \cdot 5H_2O$ (**15**) afforded the highest conversions to 23.9 wt% LA (concentrated conditions) and 10.0 wt% 5-HMF (diluted conditions). At this point it is worth noting that $SnCl_4 \cdot 5H_2O$ is a relatively cheap and easily handled reagent compared to some of the other species studied.

2-2-2 2^4 Full Factorial Designs

In general, several factors are varied to optimize experimental conditions. Chemists can use factorial design (FD) to understand the interactions between factors and thereby obtain a full picture of information and optimize a chemical reaction. If each factor is studied at two different levels (high and low), FD is expressed as 2^k, where k is the number of factors.[42] FD has been practiced in many catalytic research applications.[43-46]

To optimize the reaction conditions using $SnCl_4 \cdot 5H_2O$ (**15**), 2^4 FD was applied by varying the following four factors: volume of water, catalyst loading, temperature, and reaction time to study all effects and their interplay at once.

Table 2-1 shows weight percentages of LA and 5-HMF produced at different levels for each variable. The low and high levels of each factor were determined from preliminary experiments. 2^4 Factorial designs were studied using Minitab software. Lenth's analysis was employed to analyze this FD experiments (an alternative procedure to a normal plot). The number of studied effects in this chapter is 15. To determine the most significant effects of the 15 effects, pseudo standard error (PSE) should be calculated to determine the margin of error (ME). After calculating the median (m) of the absolute values of the effects, let $s = 1.5 \times m$. The median (m_o) of absolute values that are less than $2.5 \times s$ were calculated. PSE is then equal to $1.5 \times m_o$. ME is when an effect lies within 95% confidence interval. Therefore, ME is given by $t_{(1-\alpha/2),\, df} \times PSE$. Where t is t-distribution, $\alpha = 1-95/100$ and df is the degree of freedom that is equal to number of effects/3.[47]

Table 2-1. Weight percentage of LA and 5-HMF produced from 100 mg chitosan in the presence of $SnCl_4 \cdot 5H_2O$ (15) using 2^4 factorial designs.

Water Volume (mL)	$SnCl_4 \cdot 5H_2O$ (mmol)	Temperature (150 °C)		Temperature (200 °C)	
		Time (15 min)	Time (30 min)	Time (15 min)	Time (30 min)
		Yield LA (wt%)[a]			
4	0.12	ND	ND	10.2 (6.2)	10.9 (4.9)
	0.24	ND	ND	18.0 (1.0)	23.9 (0.6)
20	0.12	ND	ND	0 (8.8)	0 (10.0)
	0.24	ND	ND	16.8 (3.3)	21.6 (3.6)

[a] ND = not detected. Value in parentheses is the wt% 5-HMF produced concomitantly

2-2-2-1 Effects on LA Production

From Figure 2-5, the significant effects on LA production, when $\alpha = 0.05$, are catalyst amount (B) and temperature (C). It might seem, by inspecting Table 2-1, that water volume is a significant effect but statistical analysis at the $\alpha = 0.05$ level indicates that it is a non significant effect. Data for variation in A (water volume effect) leads to a point within the ME for this process (i.e. close to the blue line in Figure 2-5).

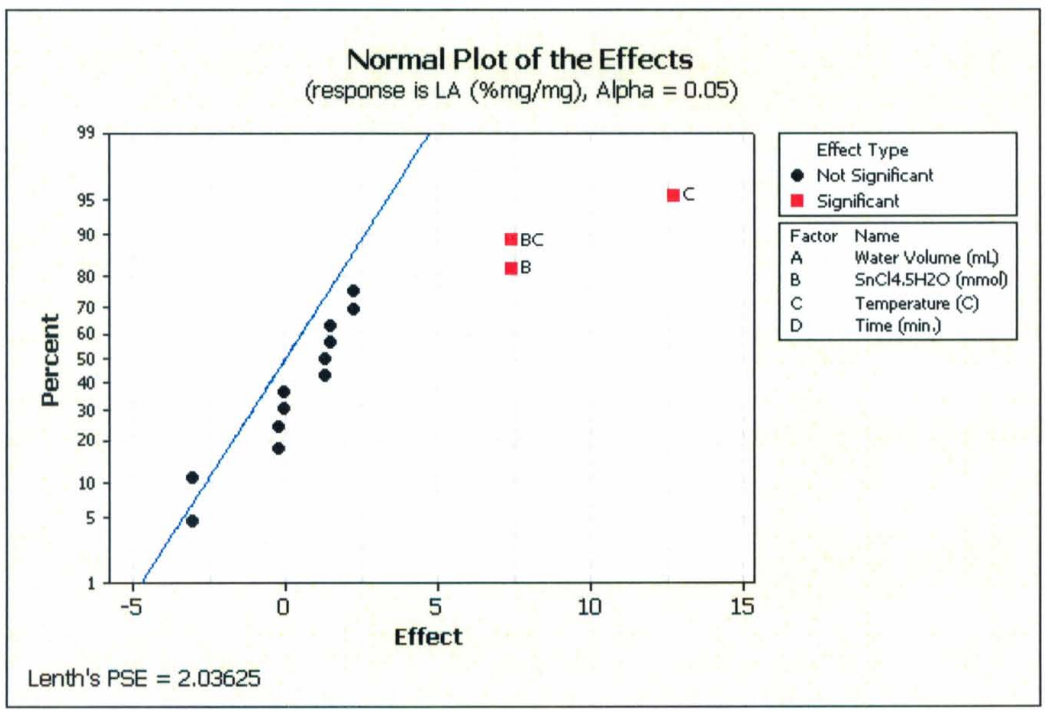


Figure 2-5. Normal plot of the effects on the weight percentage of LA.

Based on the data generated by the 2^4 FD experiments, the significant factors were changed while the water volume kept constant at 4 mL and the time constant at 30 min. Temperature was varied between 150 and 230 °C while the catalyst loading was kept constant at 0.24 mmol. Figure 2-6 shows that no LA was produced at lower temperatures

(150-170 °C). However, the amount produced increased almost linearly from 170 °C to 210 °C and then leveled out.

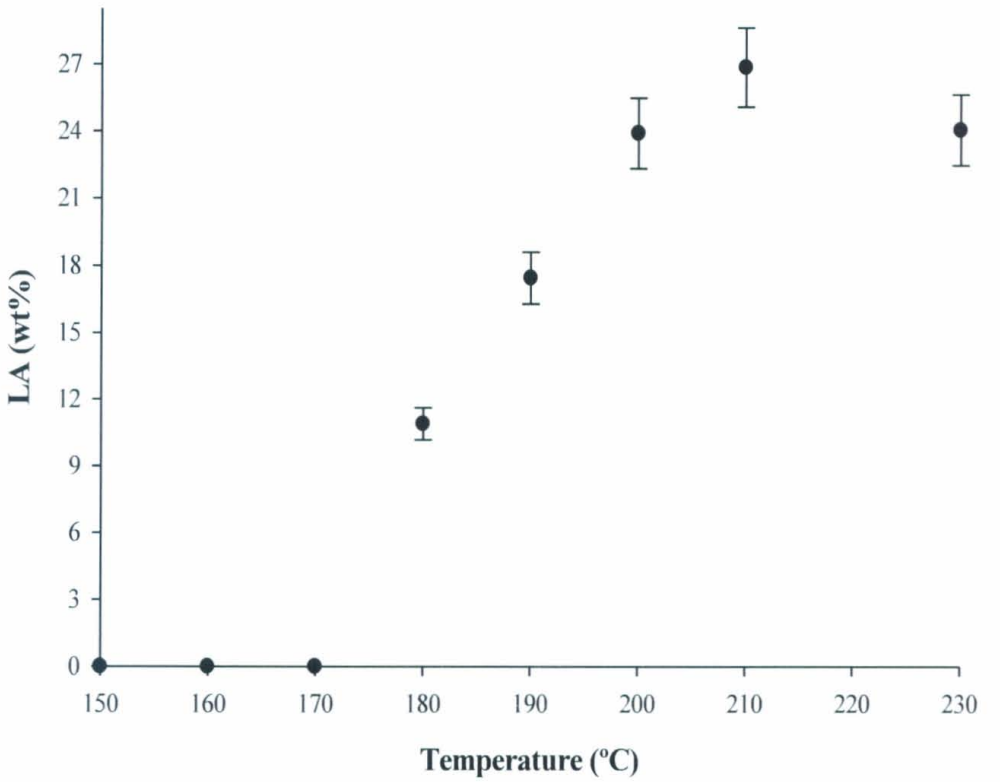


Figure 2-6. Effect of temperature on the weight percent LA produced from 100 mg chitosan in 4 mL water with 0.24 mmol of $SnCl_4 \cdot 5H_2O$.

Additional experiments were then performed to determine if any of the other variables would have a significant effect on the amount of LA obtained. Doubling the amount of catalyst to 0.48 mmol and performing the reaction at 200 °C yielded 26.6 wt% LA compared with 23.9 wt% at tin levels of 0.24 mmol. Performing the hydrolysis reaction at

210 °C at high tin levels (0.48 mmol), yielded 28.6 wt% LA. Actually, increasing the time did not have a positive effect on the wt% of LA. For example, the optimum condition at 30 min reaction time produced 23.9 wt% LA, which increased to reach 26.0 wt% at 60 min. Therefore, although close to 30 wt% LA could be obtained by increasing reaction time and the amount of tin used, the effects of these changes are not significant when compared with the effect of temperature as shown in Figure 2-6.

2-2-2-2 Effects on 5-HMF Production

A similar factorial design experiment was performed to optimize the reactions under dilute conditions and the production of 5-HMF from chitosan. The significant effects, when $\alpha = 0.05$, are volume of water (A), catalyst amount (B) and temperature (C) factors as shown in Figure 2-7.

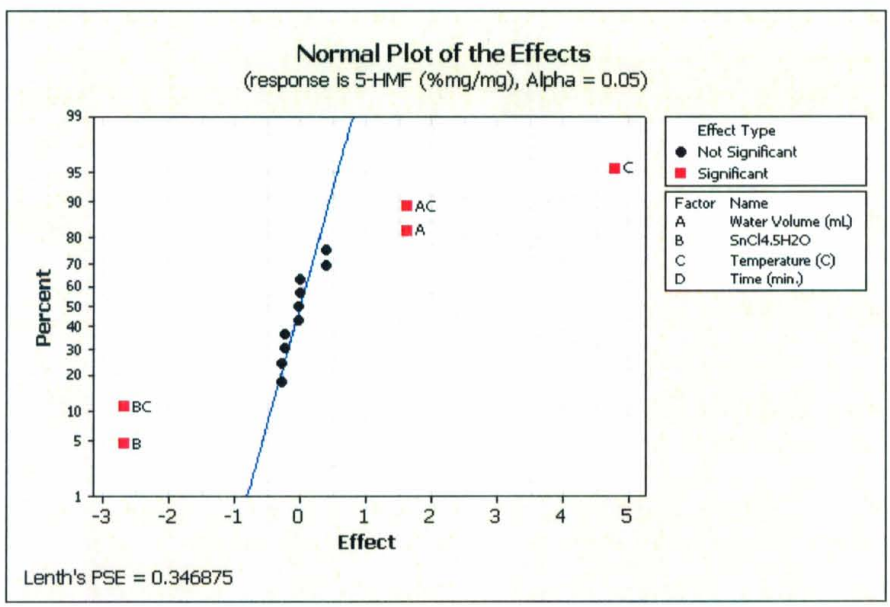


Figure 2-7. Normal plot of the effects of factors on the weight percentage of 5-HMF produced during microwave-assisted tin-catalyzed hydrolysis of chitosan.

Based on the data from the FD experiment, the significant factors were changed keeping the time constant at 30 min. The effect of temperature on the production of 5-HMF was investigated between 150 and 210 °C while the volume of water was kept constant at 20 mL and the catalyst amount maintained at 0.12 mmol. Figure 2-8 shows that no 5-HMF was detected for reactions conducted at 170 °C or lower. Between 170 and 200 °C, 5-HMF production increased linearly but decreased above this temperature. This decrease is likely due to more rehydration reactions occurring at higher temperatures to yield LA from the 5-HMF initially generated.

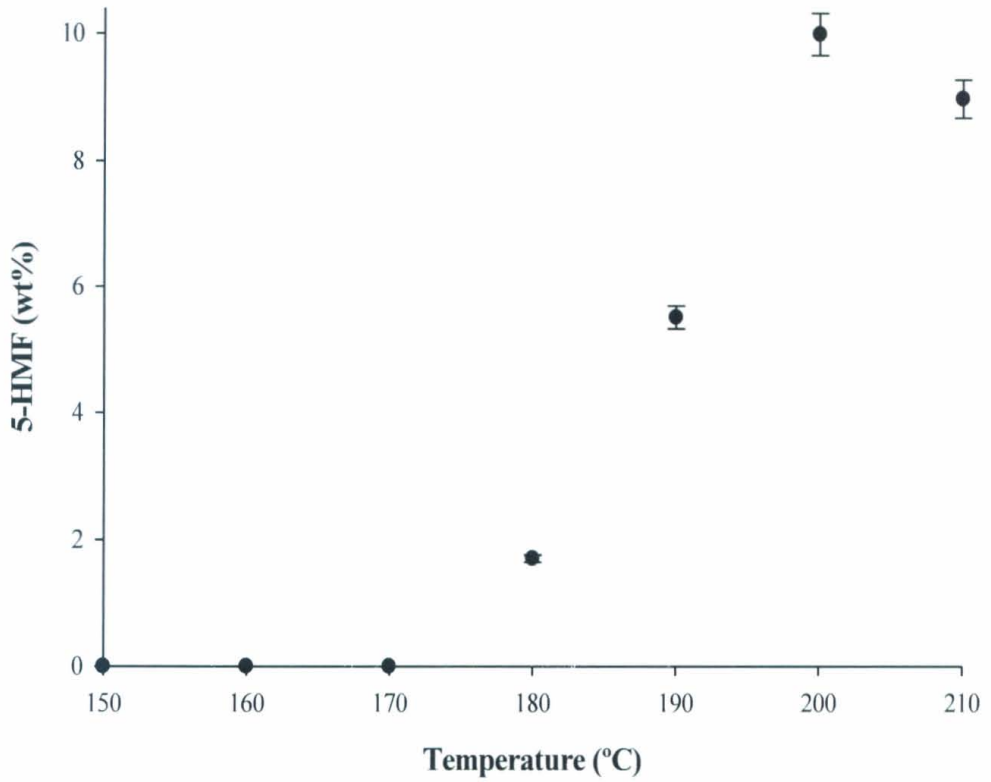


Figure 2-8. Effect of temperature on weight percent 5-HMF produced under dilute reaction conditions (100 mg chitosan, 20 mL water, and 0.12 mmol $SnCl_4 \cdot 5H_2O$).

Catalyst loading was studied at 0.06 and 0.24 mmol of Sn in 20 mL water at 200 °C and the percentages of 5-HMF were 6.3 and 3.6, respectively. This shows that the highest 5-HMF production was at 0.12 mmol, which yielded 10 wt% 5-HMF.

The effect of dilution on production of 5-HMF was studied by performing reactions in 4.0 to 30.0 mL water at a constant temperature of 200 °C and a catalyst loading of 0.12 mmol $SnCl_4 \cdot 5H_2O$. Figure 2-9 shows that the weight percent of 5-HMF produced increases

from 4 mL until 15.0 mL as conditions become more dilute and then levels out. When the volume of water used was 15.0 mL or 20.0 mL, the amount of 5-HMF produced was similar. Further dilution of reactions mixtures, e.g., 30 mL water, was detrimental in terms of wt% 5-HMF obtained.

To examine the significant effects proposed from FD analysis for 5-HMF production (i.e., AC, volume and temperature, and BC, [Sn] and temperature, Figure 2-7), the wt% of 5-HMF produced from simultaneously varying two factors were compared. Increasing the volume of water and temperature, whilst keeping [Sn] constant at 0.12 mmol, produced moderately less 5-HMF: 15 mL water at 200 °C yielded 10.0 wt% 5-HMF, Figure 2-9 and 20 mL water at 210 °C yielded 9.0 wt% 5-HMF, Figure 2-8. In contrast, simultaneously increasing the temperature from 200 to 210 °C and decreasing the catalyst loading from 0.12 to 0.06 mmol $SnCl_4 \cdot 5H_2O$ (in a constant volume of 15 mL H_2O) caused a significant decrease in the amount of 5-HMF observed (4.6 wt%) in line with changes predicted by FD.

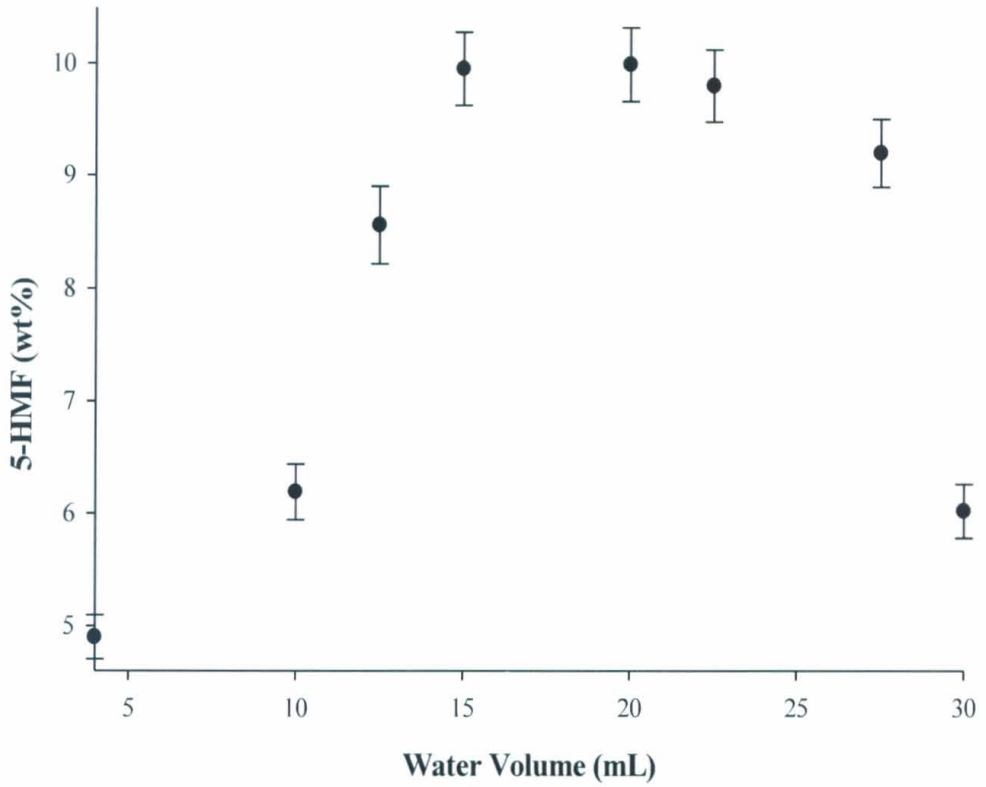


Figure 2-9. Effect of dilution on weight percent 5-HMF produced (100 mg chitosan, 0.12 mmol $SnCl_4 \cdot 5H_2O$).

In summary, the optimum conditions for 5-HMF production (9.9 wt%) were microwave irradiation of 100 mg chitosan in 15 mL water with 0.12 mmol $SnCl_4 \cdot 5H_2O$ at 200 °C for 30 min.

2-2-3 Conventional Heating Compared with Microwave Irradiation

As microwave heating, at present, is less widely used than conventional heating methods, efforts were made to study the reactions using conventional heating (stirrer-hotplate) with the same optimised catalyst loadings and volumes of water as described above. The reaction times and temperatures studied were chosen using the Biotage Prediction Chart and Time Converter in reverse,[48] and as such similar yields of products would be predicted. The two mixtures (concentrated and diluted) were heated at 100 °C in a round-bottomed flask equipped with a reflux condenser. This conventional heating process produced 12.1 wt% LA after 9 days from 100 mg chitosan, 4 mL water, 0.24 mmol $SnCl_4 \cdot 5H_2O$. This amount is almost half of that obtained via microwave irradiation. In attempts to yield 5-HMF using the optimum conditions from the microwave experiments, no product was obtained after 9 days of conventional heating. Reactions of chitosan at atmospheric pressure using microwave heating under both concentrated and diluted conditions were performed (100 °C, 2 h, CEM Discover SP system). Unfortunately, LA and 5-HMF were not produced. These results suggest a critical role for pressure in the reactions and they might be occuring under near-critical water conditions.

2-2-4 Studies Using Chitin as the Feedstock

In this study, chitin was tested and it was able to produce LA. Different sources of chitosan and chitin were tested under the optimum conditions of LA production that were determined using medium molecular weight chitosan. Table 2-2 summarizes these results. Chitin produced an average of 12.3 wt% LA and no differences were seen between chitin from crab or from shrimp. Similarly, the amount of LA produced from different sources

and molecular weight of chitosan resemble each other (average 24.2 wt%). This was somewhat surprising given the tough appearance of high molecular weight chitosan. Generally, it can be seen that chitosan produces two times more LA than chitin.

As shown in Table 2-2, 5-HMF was produced in similar amounts from chitosan of different sources and molecular weights (average 9.8 wt%). In contrast, chitin failed to produce 5-HMF, or in fact LA or any small EtOAc soluble organic compounds under the conditions studied (Table 2-2). In the absence of the catalyst, under the optimized conditions, chitosan produced neither LA nor 5-HMF, Table 2-2. Also, reduced catalyst loadings failed to yield the desired products under the conditions studied.

Table 2-2. Summary of results for different types of chitin and chitosan treated using the optimized procedures for generation of LA and 5-HMF.[a]

Biopolymer	LA (wt%)	5-HMF (wt%)
Low molecular weight Chitosan	24.7	10.3
Medium molecular weight Chitosan	23.9	10.0
High molecular weight Chitosan	25.1	10.3
Chitosan, without catalyst	ND	ND
S-189	24.1	9.3
S-190	23.0	8.8
Chitin	11.5	ND
T-187	12.7	ND
T-188	12.6	ND

[a] ND = not detected. Chitosan and chitin purchased from Aldrich and used as received. S-189 = shrimp chitosan, S-190 = crab chitosan, T-187 = crab chitin and T-188 = shrimp chitin from Newfoundland' fishery waste provided by ChitinWorks America LLC.

2-2-5 Proposed Mechanism

Our proposed mechanism for this process is shown in Figure 2-10. In the first step, chitosan is hydrolysed to yield glucosamine via cleavage of the glycosidic bond (C1→C4 linkage in the polymer) in the presence of $SnCl_4 \cdot 5H_2O$ and microwave irradiation. Microwave-assisted acid-catalysed biopolymer hydrolysis has previously been performed on starch to yield glucose.[49] This process occurs in a stepwise fashion and oligomers are formed initially. Evidence for the formation of chito-oligosaccharides has been obtained through mass spectrometric studies. No product is formed in the absence of catalysts. Therefore, coordination of the amine functional group to either a proton or a metal centre

facilitates this bond-breaking process by weakening the proximal C1-O bond and making it more amenable to hydrolysis. Furthermore by performing the reactions in water, both hydronium and hydroxide ions are readily available to react at this site. However, it is well known that an aqueous solution of $SnCl_4$ is mostly hydrolyzed and exists in equilibrium with colloidal tin(IV) oxide and HCl(aq).[50] Therefore, reaction mixtures prepared for this process are acidic. If colloidal tin oxide (Alfa Aesar) and HCl(aq) were used together rather than $SnCl_4 \cdot 5H_2O$, 100 mg of chitosan yielded 27.4 wt% LA under the optimized conditions described above. This is slightly greater than the yield under the same conditions using $SnCl_4 \cdot 5H_2O$ and significantly greater than yields obtained using either HCl(aq) or tin oxide alone.

For comparison and to provide some evidence for the last steps in the proposed mechanism, the reaction of glucosamine was studied under similar conditions. 32.0 wt% LA was produced from 100 mg glucosamine hydrochloride using 0.26 mmol $SnCl_4 \cdot 5H_2O$ and 20 mL water heated to 200 °C for 30 min. This corresponds to a yield of 59.4%. Unfortunately, attempts to date to detect the ammonia or any reducing sugars in the aqueous phase after extraction of the products from the reaction mixtures have been unsuccessful. Therefore, it is not feasible to provide unequivocal evidence for all steps in the proposed mechanism.

In recent literature, glucose is known to yield 5-HMF[17-21] and the subsequent conversion of 5-HMF to give LA is also known.[18,21] In such processes, glucose dehydrates to yield 5-HMF through loss of three water molecules - this process is also facilitated by the presence of a Lewis acid. Furthermore, in the presence of a high concentration of the

Lewis acidic catalyst, as in the optimum conditions for generation of LA from chitosan, 5-HMF is rehydrated into LA and formic acid through addition of two water molecules. To test the last step in the mechanism, namely rehydration of 5-HMF into LA, 5-HMF (26.0 mg) was processed under the optimum conditions for LA production from chitosan (i.e 4 mL water, 0.24 mmol $SnCl_4 \cdot 5H_2O$, T = 200 °C and t = 30 min). The weight percent yield of LA was 85.4 (or 92.7 mol%) thus providing evidence for this last step. In the absence of the Lewis acid, no LA was detected thus indicating the essential role of a metal centre in this last step.

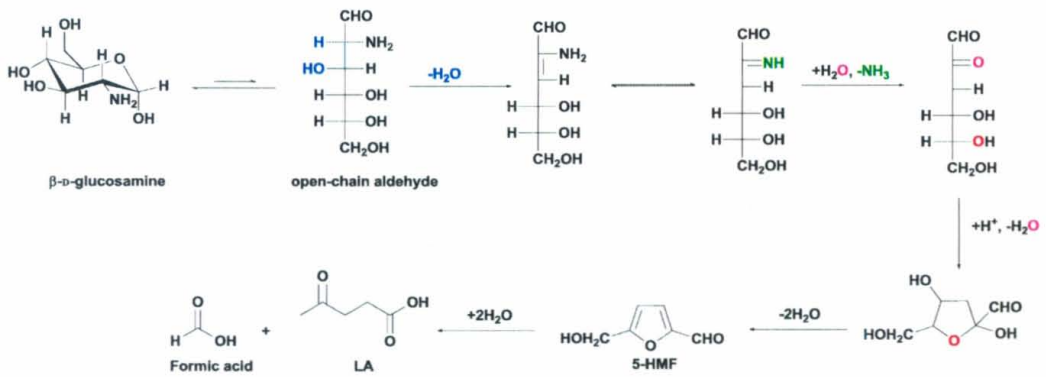


Figure 2-10. Proposed mechanism for 5-HMF and LA production from glucosamine. The glucosamine forms in situ via acid-catalysed hydrolysis of the biopolymer.

It should be noted that two volatile compounds (furan and 2-methylfuran) were also identified via head-space analysis of the gas phase inside the sealed reaction vessel. These have previously been observed when sugars are roasted.[51] These compounds have a lower degree of solubility in aqueous solution than LA and 5-HMF and therefore, they were not

found upon extraction of organics from the aqueous reaction mixture. Other hydrophilic or otherwise insoluble compounds would also be overlooked using the GC-MS method employed. MALDI-TOF mass spectra were obtained from aliquots of reaction mixtures in an attempt to observe intermediate depolymerization products and any compounds remaining in the aqueous phase. 2,5-dihydroxybenzoic acid (DHB) was used as the matrix, as it had been used previously in the study of chito-oligosaccharides.[52] For aliquots taken 15 min into the reaction, for both diluted and concentrated reaction mixtures, some interesting peaks were observed in the mass spectra. For concentrated reaction samples (4 mL water), peaks were observed at *m/z* 1188 and 543. These could be assigned to the oligosaccharides $(glucosamine)_6$-(NAG) and $(glucosamine)_2$-NAG, respectively. These peaks were not present in spectra from aliquots taken after 30 min reaction time. This suggests that the oligosaccharides are true intermediates, as they are formed at an early stage in the reaction and then consumed. For dilute reaction samples (15 mL water), a peak was observed at *m/z* 501. This could be assigned to the trisaccharide, $(glucosamine)_3$. This peak was also present in spectra of aliquots analyzed after 30 min reaction time. This indicates that incomplete degradation/hydrolysis of the biopolymer occurs under the dilute conditions studied.

2-2-6 Literature Comparison

This section compares this study with others in the literature. The criteria of the comparison are based on the % yield of LA and 5-HMF produced from chitin, chitosan, and their oligomers. Table 2-3 summarizes these results. In 2001, Tømmeraas et al. produced 1% yield of 5-HMF from $(NAG)_2$-M in the presence of concentrated HNO_2 but no LA was reported.[28] To this date, no literature studies have been found reporting a % yield of LA or 5-HMF using chitosan or glucosamine as feedstocks. In 2009, Mascal et al. used chitin to produce 29% yield of LA but no 5-HMF was observed.[1] This method used a lot of organic solvent (420 mL of 1,2-dichloroethane). 1,2-Dichloroethane is a toxic solvent and as such has a significant envionmental impact.[53] However, in this study, the medium used was water that is an environmentally benign solvent. Compared with the literature, this study (Chapter 2) reported the highest % yields of 5-HMF and LA from an aminocarbohydrate to date, which are ca 13% and 59% from chitosan and glucosamine, respectively. For chitin, Mascal et al. produced the largest amount of LA (29% yield) from this feedstock to date using a corrosive acid (HCl) and a large volume of 1,2-dichloroethane (a toxic solvent). In this study (Chapter 2), chitin produced ca 23% yield of LA (which corresponds to a yield of 13 wt%) in a much greener method than that of Mascal et al.. However, it should be noted that these yields are significantly less than some of those produced using cellulose as a feedstock described in Chapter 1.

Table 2-3. Comparison between this study and the literature for LA and 5-HMF productions from chitin, chitosan, glucosamine, and $(NAG)_2$-M.

Carbohydrate	Reference	LA (mol% yield)	5-HMF (mol% yield)
$(NAG)_2$-M	28	No data reported	1
Chitosan	No data available in the literature		
Chitosan	Chapter 2	35	13
Glucosamine	No data available in the literature		
Glucosamine	Chapter 2	59	None detected
Chitin	1	29	No data reported
Chitin	Chapter 2	23	None detected

2-3 Conclusions

This study demonstrates that chitosan can be used to produce LA and 5-HMF in water under microwave conditions. This work is a proof-of-principle that N-containing biopolymers can be degraded using green chemistry principles to give useful chemical building blocks in a similar way to ongoing research in the area of cellulosic feedstocks. The volume of water used and the loading of $SnCl_4 \cdot 5H_2O$ can be varied to produce either LA or 5-HMF with good selectivity. Factorial design was successfully employed to optimize the reaction conditions for this process. Microwave irradiation proved to be a more effective heating method for the generation of these small molecules compared with conventional heating, as 5-HMF could not be generated effectively under the conditions

studied using conventional heating. A mechanism for this process has been proposed based on the known chemistry of cellulose and glucose, and some studies using glucosamine. Furthermore, evidence for oligosaccharide intermediates has been obtained using MALDI-TOF mass spectrometry. The last step in the mechanism under microwave irradiation was performed to convert 5-HMF to LA in good yields in water. The method developed for chitosan could be extended to the more robust parent carbohydrate chitin from which LA can also be obtained albeit in smaller quantities. The results of this study will open the possibility of chemical and thermochemical transformations of the non-toxic and cheap biopolymers, chitosan and chitin, to yield useful sustainable chemicals with possible industrial applications. Studies in GCCG are ongoing using chitosan, chitin, and amino-sugars as feedstocks in different solvents (e.g., ionic liquids (ILs) and employing a wide range of catalysts).

2-4 Experimental

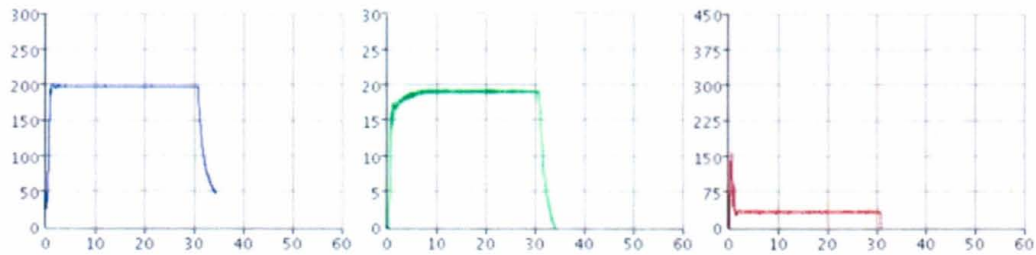
2-4-1 Materials

Chitosan of low, medium, and high molecular weight (75-85% deacetylated), chitin (from crab shell), tin (IV) chloride pentahydrate 98% and 2,5-dihydroxybenzoic acid (DHB) 98% were purchased from Aldrich. Further samples of chitin and chitosan from crab and shrimp were provided gratis by ChitinWorks America LLC. All other catalysts tested were purchased in 98% purity or greater from Fisher Scientific, Alfa Aesar, Aldrich or Strem Chemicals. (+/-)-3-hydroxy-gamma-butyrolactone 96%, 5-hydroxymethylfurfural 98% and levulinic acid (98%) were purchased from Alfa Aesar. Ethyl acetate (HPLC

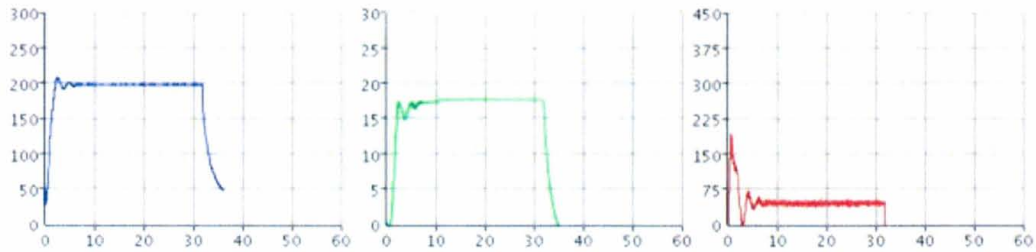
grade) 99.8% was purchased from EMD Chemicals Inc. Deionized water was obtained via a Nanopure II system (manufactured by Barnstead/Thermolyne, USA) using distilled water as a source for the inlet feed.

2-4-2 General Procedure for the Hydrolysis of Chitosan and Product Extraction

Chitosan (100 mg) was mixed with a specific volume of aqueous solution (or suspension in the case of Amberlyst-15™) of known concentration. The mixture was heated to the desired temperature under microwave irradiation using a Biotage initiator 2.5 instrument for a specific period of time. Figure 2-11 shows power, temperature, and time graphs of the concentrated and diluted optimum reactions conditions.



(a)



(b)

Figure 2-11. Microwave reactions graphs (a) LA and (b) 5-HMF optimum conditions. Each set consists of three graphs temperature (°C), pressure (bar), and power (W), respectively against time (min) on x-axes.

After this time, an aliquot of the reaction mixture (Concentrated reactions for LA analysis, V = 250 µL; Dilute reactions for 5-HMF analysis, V = 500 µL) was mixed with 3-gamma-hydroxy-butyrolactone (internal standard) and extracted with 3 × 2 mL ethyl acetate. After each addition of ethyl acetate, the mixture was vortex-mixed at high speed for 30 seconds and then centrifuged at 1500 rpm for 2 min. The combined ethyl acetate layers were transferred to a quartz tube and evaporated to dryness at 45 °C using a Radleys Greenhouse Blowdown Evaporator under a stream of nitrogen. The contents of the dried tube were reconstituted in 1 mL ethyl acetate for GC-MS analysis.

2-4-3 Identification of LA and 5-HMF

After hydrolysis of chitosan, extraction with ethyl acetate and evaporation of the solvent, the dried residue was dissolved in $CDCl_3$. ^{1}H-NMR spectra obtained using a Bruker AVANCE 500MHz spectrometer showed the presence of LA (Figure 2-12) and 5-HMF (Figure 2-13). GC-MS was also used for characterization and quantification of the products. Samples were compared with authenticated samples and with the NIST database. Figure 2-14 shows the spectra of LA, internal standard and 5-HMF.

LA: ^{1}H-NMR δ_H (298 K, 500MHz; $CDCl_3$; Me_4Si) 2.20 (s, 3H), 2.63 (t, 2H, *J* 6.4 Hz) and 2.75 (t, 2H, *J* 6.4 Hz).

MS *m/z* 116 (10%), 101 (13), 99 (9), 73 (28), 57 (7), 56 (100), 55 (37).

5-HMF: 1H-NMR δ_H (298 K, 500 MHz; $CDCl_3$; Me_4Si) 4.73 (s, 2H), 6.53 (d, 1H, *J* 3.5 Hz), 7.23 (d, 1H, *J* 3.5 Hz) and 9.61 (s, 1H).

MS *m/z* 126 (61%), 109 (9), 97 (100), 81 (6), 69 (31), 53 (16).

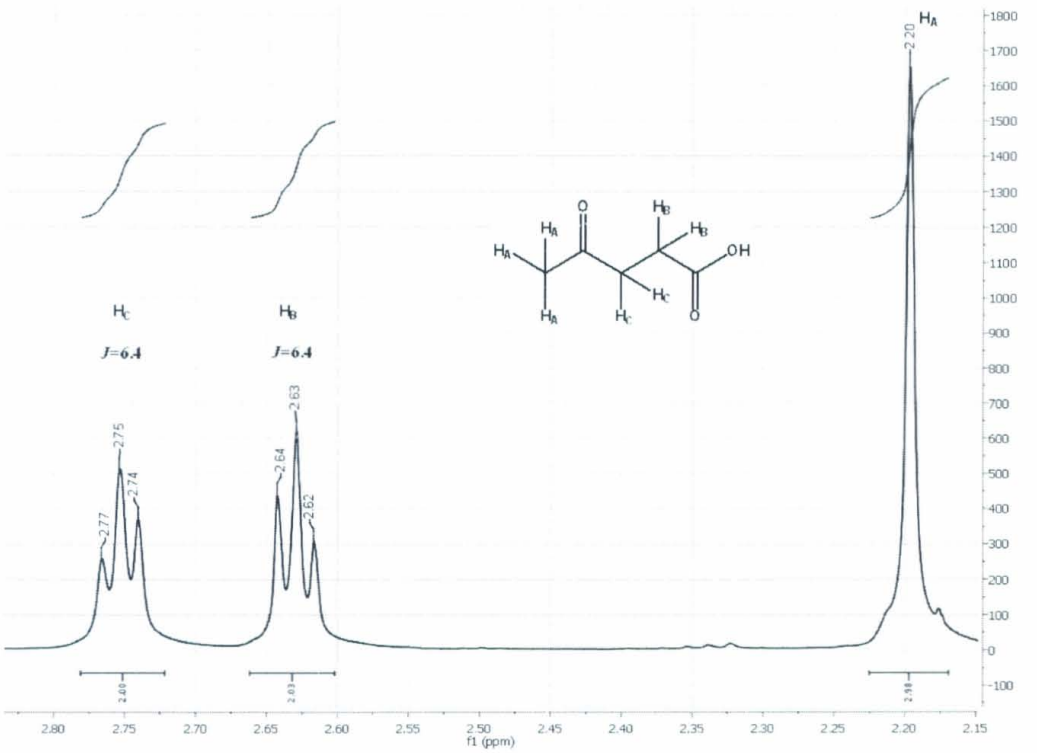


Figure 2-12. ^{1}H NMR spectrum of LA in $CDCl_3$.

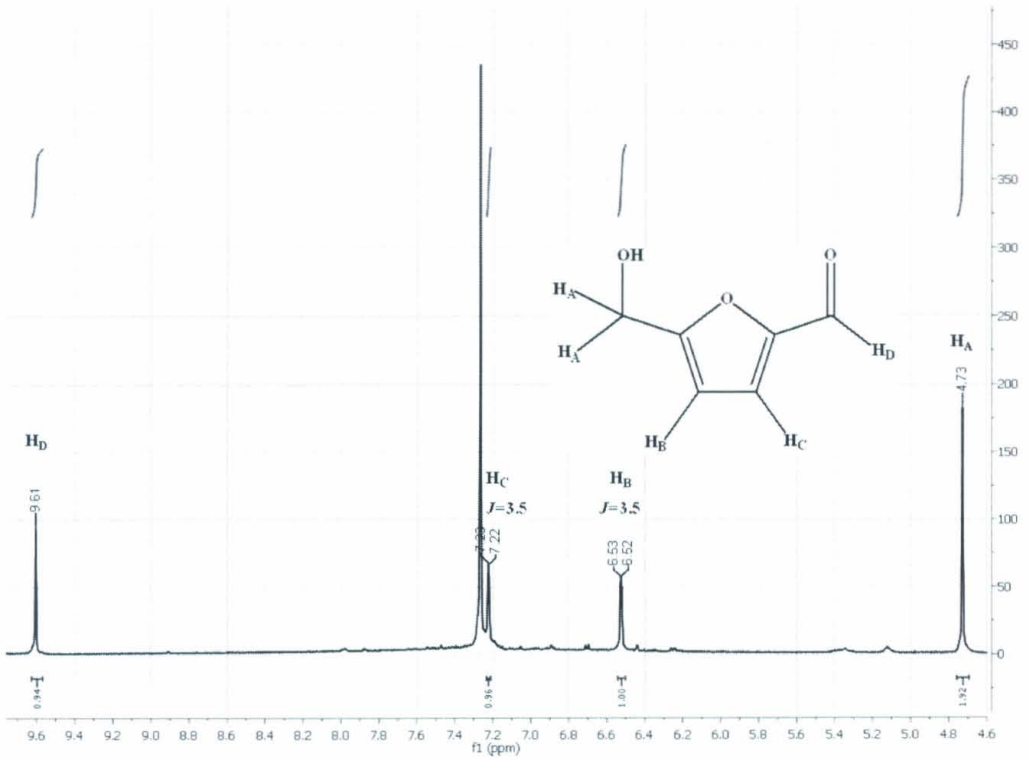


Figure 2-13. ^{1}H NMR spectrum of 5-HMF in $CDCl_3$.

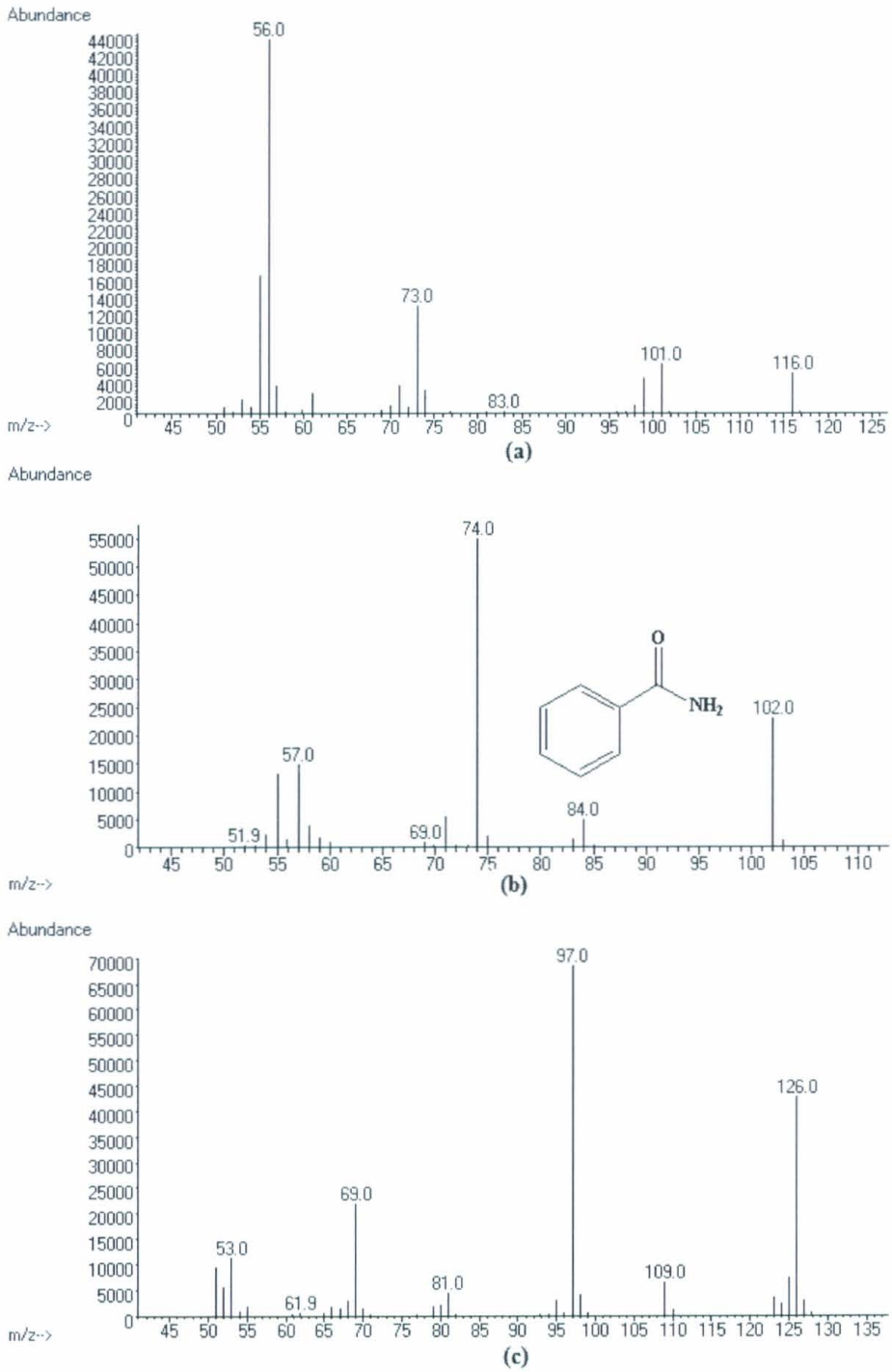


Figure 2-14. Mass spectra (a) LA, (b) Internal standard and (c) 5-HMF.

2-4-4 Gas Chromatography-Mass Spectrometry (GC-MS)

Determination of LA and 5-HMF

5-HMF and LA were analyzed using an Agilent Technologies 7890 GC with 5975 MSD. 1 μL of reconstituted sample was injected through a 7683B Series Injector using a split mode of 50%. The GC separation was done using a DB5 column at a flow rate of 1 mL/min He 99.999%. The oven temperature was programmed as follows: 50 °C (hold 1 min), 25 °C/min to 150 °C, 20 °C/min to 170 °C, and 80 °C/min to 250 °C for 3 min. (The total run time was 10 min). Products were detected at *m/z* 50-250 scan range. Under these conditions, the retention times of LA, 3-hydroxy-gamma-butyrolactone, and 5-HMF were 4.63, 5.27, and 5.71 min, respectively. Figure 2-15 shows the chromatograms of LA and 5-HMF under the optimum conditions. R^2 of LA and 5-HMF calibration curves were 0.9978 and 0.9990, respectively. Figure 2-16 shows the calibration curves of LA and 5-HMF. Accuracy was greater than 91% and RSD was less than 3.3% for both products. The limit of detection (LOD) and limit of quantitation (LOQ) of LA were 8 and 26 ppm, respectively. The LOD and LOQ of 5-HMF were 2 and 8 ppm, respectively.

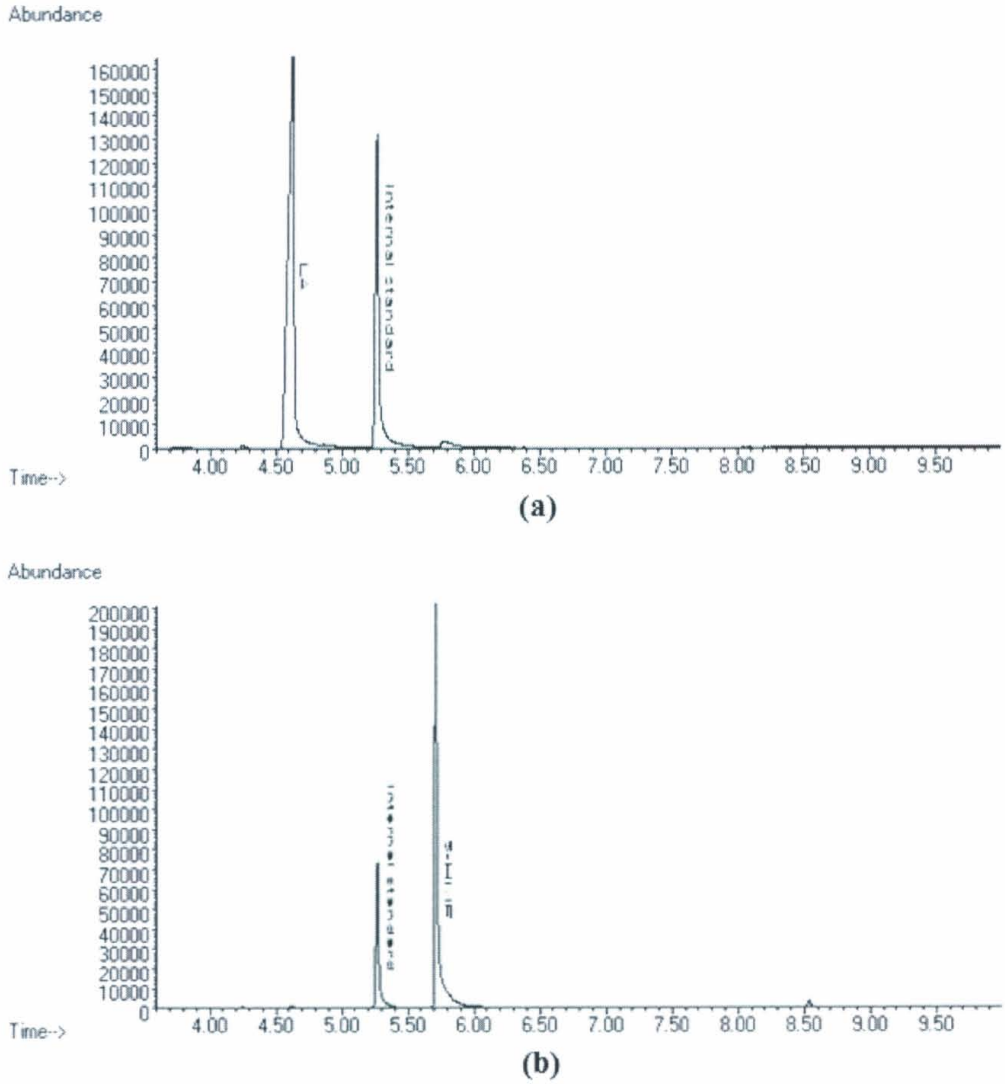


Figure 2-15. GC chromatograms (a) LA, t_R = 4.63 min and (b) 5-HMF, t_R = 5.71 min.

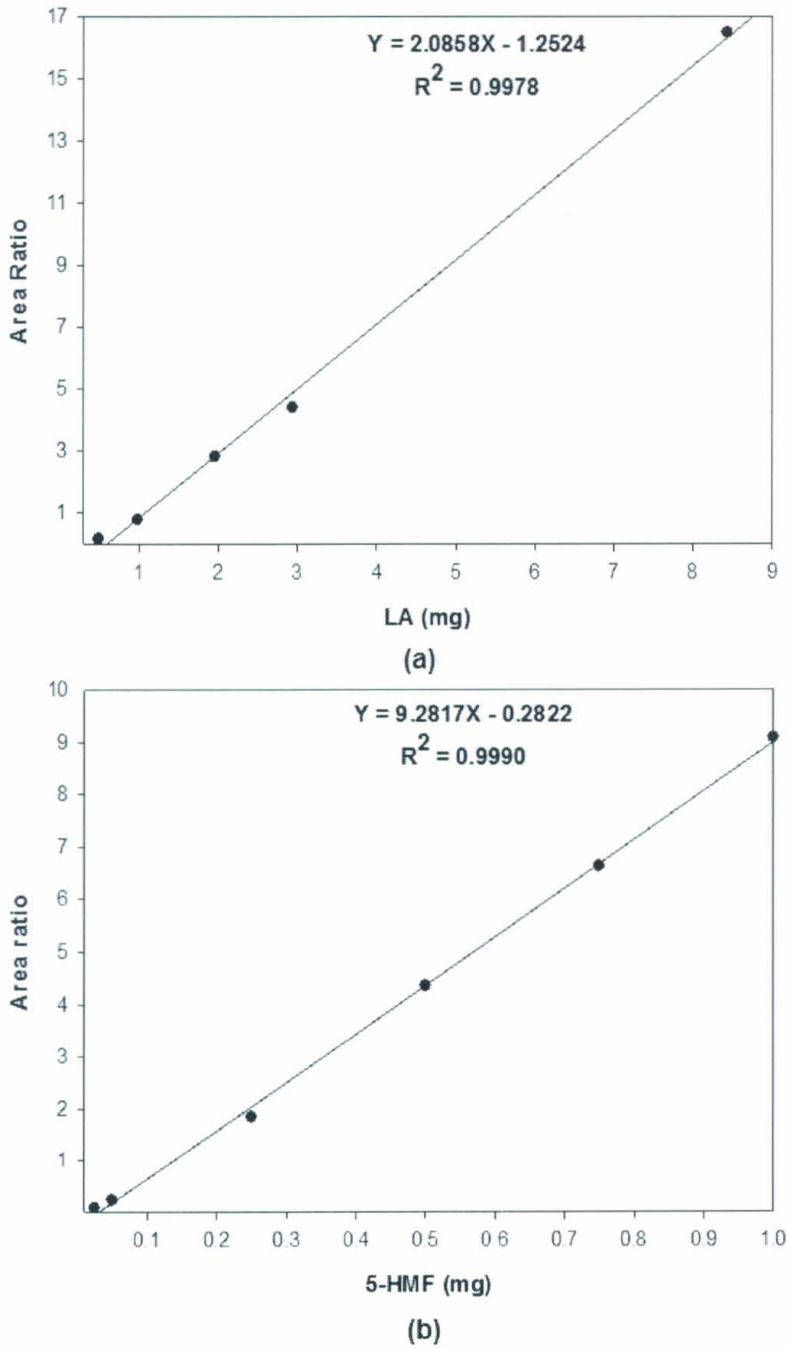


Figure 2-16. Calibration curves (a) LA (b) 5-HMF.

2-4-5 Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry

MALDI analysis was conducted using AB SCIEX 4800 MALDI TOF/TOF analyzer. 2,5-dihydroxybenzoic acid (DHB) was used as a matrix. DHB solution was prepared in water (10 mg/mL). DHB solution was mixed with a reaction mixture in 1:1 volume ratio. Then, 1 μL of the mixed solutions was spotted on the MALDI plate. The spot was dried at room temperature. Then, the plate was loaded into the instrument for analysis.

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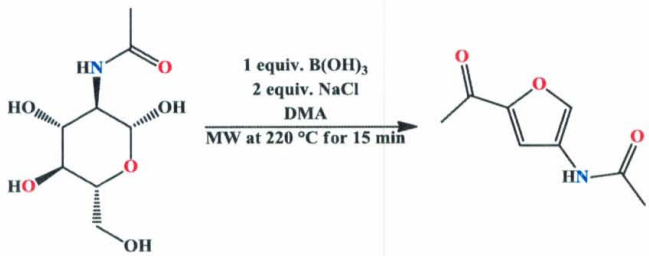
Chapter Three

A Simple One-Pot Dehydration Process to Convert *N*-acetyl-D-glucosamine into a Nitrogen-Containing Compound, 3-acetamido-5-acetylfuran



A version of this chapter has been accepted for publication.

Khaled W. Omari, Linda Dodot and Francesca M. Kerton*, A Simple One-Pot Dehydration Process to Convert *N*-acetyl-D-glucosamine into a Nitrogen-Containing Compound, 3-acetamido-5-acetylfuran, *ChemSusChem,* 2012, **5**, 1767-1772.

Some modifications were made to the original paper for inclusion as a chapter in this thesis. For example, the supporting information was incorporated in this chapter and some figures have been added.

3-1 Introduction

Renewable, bio-sourced feedstocks are now being widely studied for the production of both fuels and chemical precursors (see sections 1-8 and 1-14).[1-3] These processes typically afford chemicals containing only C, H, and O. There is a growing interest in the production of renewable chemicals that contain other heteroatoms. For example, recently, caprolactam has been prepared in a four-step process from 5-hydroxymethylfurfural (5-HMF) and ammonia.[4] Inspired by such research, the following question arose: could amino-sugars be used as precursors to new N-containing platform chemicals? When water was used as a medium (as in Chapter 2), the nitrogen atom was not present in the final products (LA and 5-HMF). To see if the nitrogen atom could be retained in the final product, other solvents were studied. The initial studies in this area are presented herein.

NAG, Figure 3-1a, is an amino sugar and also known as *N*-acetyl-2-amino-2-deoxy-D-glucose. Chitin is a polysaccharide made up of NAG monomers. It is available from crustaceans' shells such as crab, lobster, and shrimp (fisheries' waste). It is also found in the cell walls of fungi and insects.[5] Chitin is produced on a large scale annually from a range of sources (ca. 100 billion tons),[6] and is the third most abundant biopolymer after cellulose and hemicellulose. It is readily available, non-toxic, and environmentally benign. Also, it may be available as a feedstock in regions of the world that do not have easy access to waste cellulosic biomass feed. Partial hydrolysis of chitin in HCl followed by neutralization, filtration, decolourization, and salt removal affords 13.5 wt% NAG.[7] Chitin hydrolysis using enzymes is another approach to produce NAG, e.g., cellulase has been used to produce 40 wt% NAG from chitin.[8] Chitosan is the partially deacetylated

form of chitin, which is also non-toxic and readily available. Hydrolysis of chitosan (22% deacetylated) using hemicellulase can produce 6.5 wt% of NAG.[5] Two studies have been reported where NAG has been used as the starting material to yield low molecular weights organic products through decomposition pathways. Franich et al. produced 3-acetamido-5-acetylfuran (3A5AF), Figure 3-1b, from NAG.

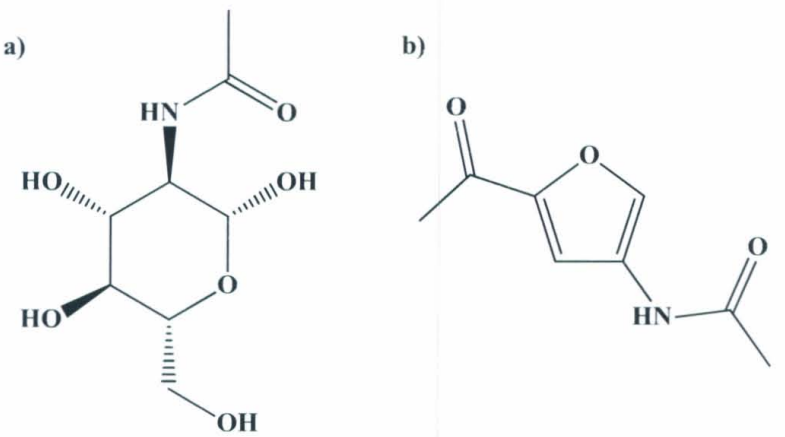


Figure 3-1. a) *N*-acetyl-D-glucosamine (NAG) and b) 3-acetamido-5-acetylfuran (3A5AF).

The pyrolysis of NAG was performed in a glass tube apparatus, which was heated in an oven at 400 °C. The yield of 3A5AF obtained through this process was 2%,[9] which is currently the highest reported in the literature. In another study, NAG was mixed with anhydrous disodium hydrogen phosphate and quartz sand in a stainless steel vessel. The pyrolysis of this mixture was performed in an oil bath at 200 °C for 30 min. After work-up, the major product was 3A5AF (0.04% yield).[10] In this study, a novel method was developed to produce 3A5AF from NAG using microwave irradiation to heat up the reaction mixture. It was also found that there is no need to adjust the pH of the mixture

prior to extracting the 3A5AF. The extraction procedure was performed using ethyl acetate, which is more environmentally friendly than chloroform, methanol, and dichloromethane that were used in previous studies.[11]

The method reported herein produces ca. 30 times the yield compared with the Franich et al. procedure. This novel process will hopefully allow 3A5AF to become available as a starting material for more complex chemical products. For example, polyamides proximicins A, B, and C, Figure 3-2, are naturally occurring compounds which have been isolated from marine actinomycete of the genus *Verrucosispora*.[12] Recently, proximicins A-C were studied as antitumor and antibiotic drugs.[13] Due to the similarity in structure between this furan product and sub-units in proximicins, it is though that NAG or other amino-sugars might be the biosynthetic precursors to such complex natural products.

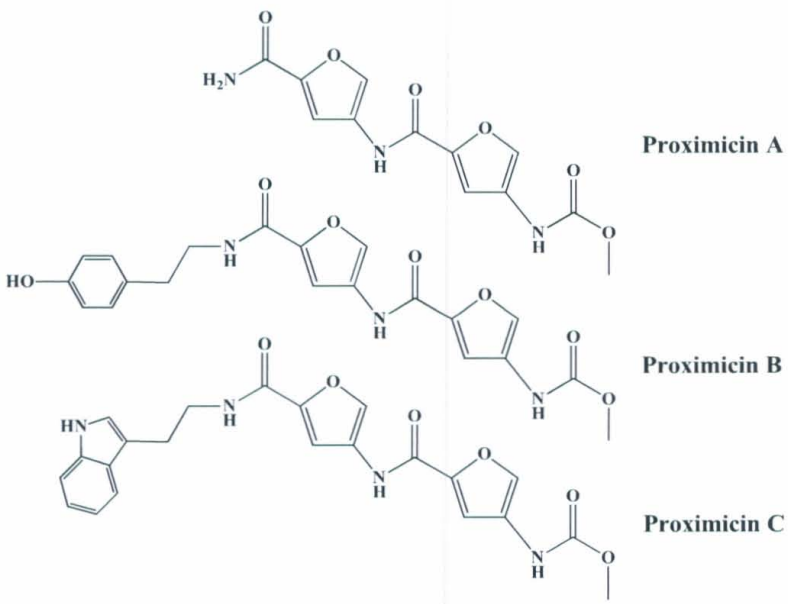


Figure 3-2. Structures of proximicin A, B, and C.

3-2 Results and Discussion

In preliminary tests, chitin was investigated as a renewable feedstock. A reaction of 100 mg chitin and 2 mL DMF was performed without any added catalyst. The reaction was heated using microwave irradiation to 240 °C for 15 min. No product was generated from chitin under these conditions. After adding 0.24 mmol of 11.9M HCl to the previously mentioned mixture, 3A5AF was identified as a product. Quantification using GC-MS indicated that the yield of 3A5AF was 0.3%. A wide range of catalysts (0.24 mmol) were added in an attempt to get a better yield (Figure 3-3). The following base catalysts were not able to produce 3A5AF: NaOH and NH_3. Perchlorates of Fe, Ni, Cu, Cr, and Zn were also not able to hydrolyse chitin and no 3A5AF was produced. $Yb(CF_3SO_3)_3 \cdot xH_2O$ and $Gd(CF_3SO_3)_3 \cdot xH_2O$ were able to produce 0.02 and 0.04% yield of 3A5AF, respectively. Weak acids (formic acid, acetic acid, trichloroacetic acid, and oxalic acid) were tested in this study and produced almost the same yield of 3A5AF (0.11%). Metal chloride compounds ($InCl_3$ and $ZrCl_4$) were not able to produce 3A5AF but $SnCl_4 \cdot 5H_2O$ produced 0.11% yield of 3A5AF. Sulfonic acid based compounds hydrolysed chitin to a somewhat larger, but still marginal extent and produced 3A5AF in a higher yield compared with previous discussed chemicals. For example, methane sulfonic acid, chlorosulfonic acid and aniline-2-sulfonic acid produced 0.15, 0.19, and 0.22% yields of 3A5AF, respectively. It could be concluded that the more acidic the added compound, the more hydrolysis of chitin occurred and greater yields of 3A5AF could be detected. Therefore, more mineral acids were tested. Interestingly, 14.6M H_3PO_4 produced higher yields of 3A5AF than 11.9M HCl, which were 0.46 and 0.28% yield, respectively. The effect of

dilution of mineral acids on the % yield of 3A5AF was investigated. 7.3M H_3PO_4 and 5.9M HCl produced 0.54 and 0.33% yields of 3A5AF, respectively. It was also noted that less acidic phosphate compounds produced less 3A5AF. For example, $NaH_2PO_4 \cdot 2H_2O$ produced 0.23% yield of 3A5AF, which is less than that produced using 7.3M H_3PO_4 (0.54% yield).

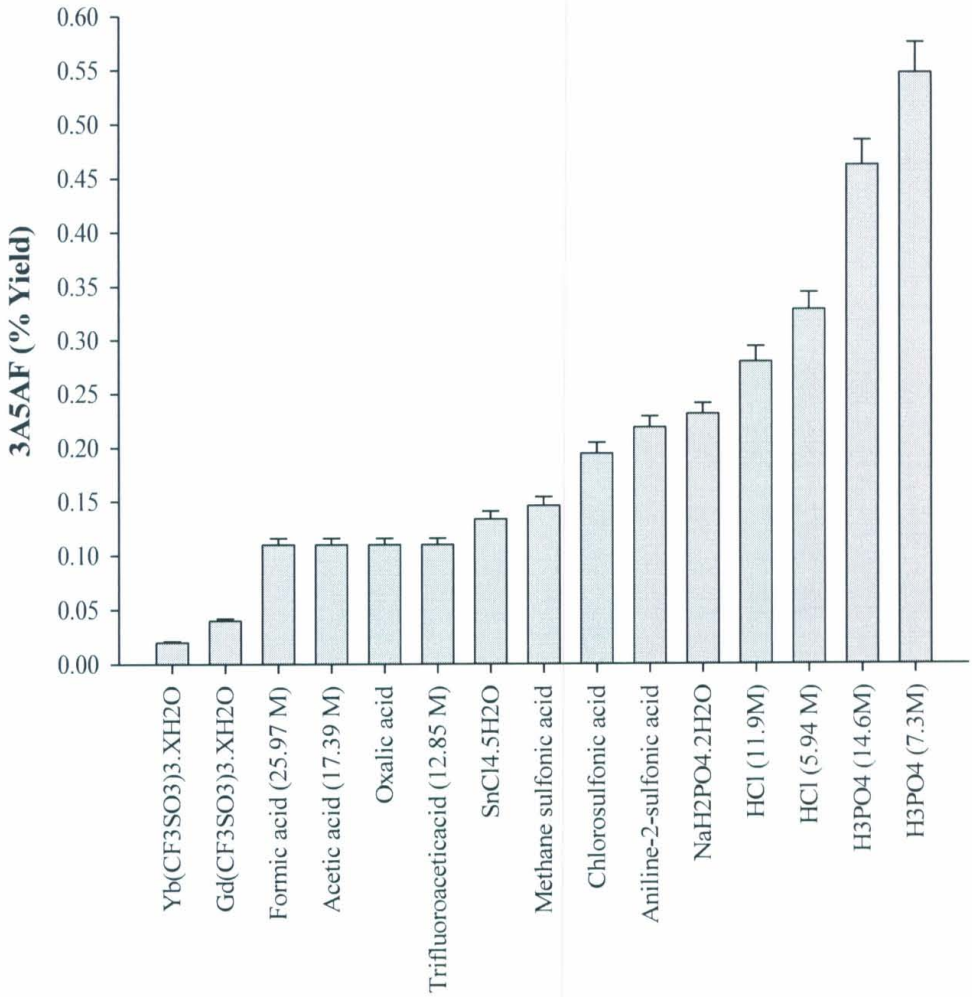


Figure 3-3. Comparison of % yields of 3A5AF produced from chitin for a range of catalysts. Reaction conditions: 100 mg chitin, 2 mL DMF, 0.24 mmol catalyst, MW, 240 °C, 15 min.

Because the hydrolysis process of chitin appeared to be only occurring to only a small extent, NAG was tested to check if the % yield of 3A5AF could be increased. This was

because NAG is the monomer of chitin and therefore, the initial hydrolysis step to break down the biopolymer would not be needed. The first attempt using NAG instead of chitin in the presence of 0.24 mmol of 7.3M H_3PO_4 and 2 mL DMA, MW at 240 over 15 min produced 10.3% yield of 3A5AF, but surprisingly, 15.7% yield of 3A5AF was produced in the absence of H_3PO_4. Therefore, further reactions of NAG were performed in the absence of additive.

3-2-1 Solvent Screening

At the beginning of this study, DMF was used as a solvent in this process. The reaction mixture (50 mg NAG and 2 mL DMF) was microwave heated at 207 °C for 15 min. This temperature was selected based on the maximum safe working temperature for microwave-heating of acetonitrile in the Biotage initiator 2.5 instrument. Acetonitrile was one of the screened solvents. The % yield of 3A5AF that was produced from this reaction using DMF was the best among the solvents studied, Figure 3-4. Different solvents were examined hoping to replace DMF with a greener solvent and produce more 3A5AF. The selected solvents have different "greenness", some of them have few and some have major environmental, health, and safety issues associated with them.[14] TBME, CPME, DEA, and EG did not yield any 3A5AF. Figure 3-4 shows that in ester solvents (TBOAc, IPOAc, and EtOAc) small amounts of 3A5AF were produced (average 1.7%) but in the related solvent EL 6.3% was obtained. Interestingly, 3A5AF did not form in EG, but 8.1% yield 3A5AF was produced in PEG. However, overall with respect to product yield, dipolar aprotic solvents (DMF, NMP, CH_3CN, and DMSO) performed the best with 24.6% yield of 3A5AF produced in DMF. Clearly, to produce a significant quantity of

3A5AF under the conditions studies, a dipolar aprotic solvent is required. DMA is less dangerous than DMF, in terms of physical properties including boiling point, flashpoint, and acute toxicity. Therefore, DMA was used and a yield of 31.3% was obtained. This yield was ca. 15 times greater than the amount produced via pyrolysis reported in the literature. Unfortunately, no correlation between solvent polarities (and other solvent parameters) and the yields obtained in this solvent-screening study were found. However, it should be noted that no catalyst or other additive was used in these initial experiments.

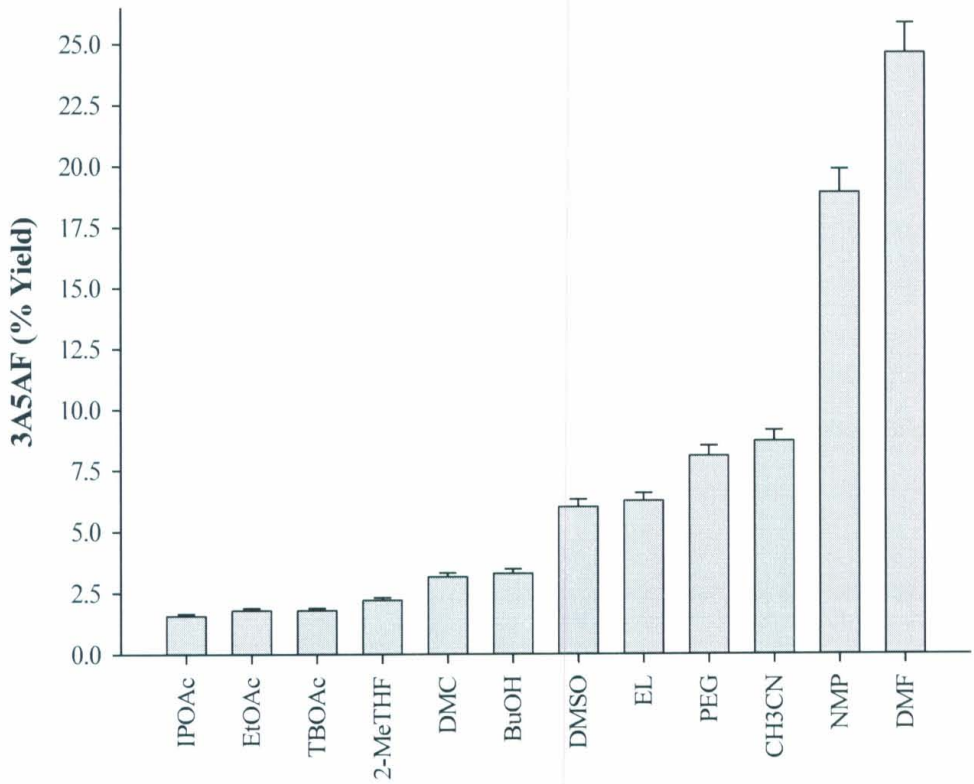


Figure 3-4. Production of 3A5AF in a range of solvents [n-butanol (BuOH), t-butyl acetate (TBOAc), isopropyl acetate (IPOAc), dimethyl carbonate (DMC), ethyl lactate (EL), ethyl acetate (EtOAc), t-butyl methyl ether (TBME), 2-methyltetrahydrofuran (2-MeTHF), cyclopentyl methyl ether (CPME), dimethyl sulfoxide (DMSO), n-metyl pyrrolidone (NMP), acetonitrile (CH_3CN), diethanol amine (DEA), ethylene glycol (EG), and polyethylene glycol (PEG, Mn 300)]. Note: if the solvent does not appear in the graph, 0% yield was obtained. Reaction conditions: 50 mg NAG, 2 mL Solvent, MW, 207 °C, 15 min. 3A5AF was quantified using GC-MS.

3-2-2 Catalyst/Additive Screening

After determining that DMA will be the best solvent to use, addition of a catalyst or other additive was studied to obtain better results, Table 3-1. The reaction mixtures consisted of 10 wt% NAG of the total reaction mass and 6 mol% catalyst. In the case of reactions in the presence of LiBr, it was added at 10 wt% of the total mass. LiBr was added because bromide is a good nucleophile that has been shown to interact with glucose and fructose in DMA to increase the productivity of 5-HMF.[15]

Table 3-1 shows that in presence of LiBr, the % yield of 3A5AF is reduced. This is in direct contrast to the results using glucose and fructose.[15] $CrCl_3$ has been shown to facilitate the isomerization of glucose into fructose to produce a high yield of 5-HMF.[16] $CrCl_3$ has also been used in the conversion of cellulose into levulinic acid.[17] Therefore, it was tested in this study but it failed to significantly increase the yield (entries 3 and 4). The best catalysts were basic ones (entries 6-11). Despite NH_3 not giving the best results in the screening, it was selected to study via factorial design for optimizing the method because it is cheap and relatively benign.

Table 3-1. Catalyst screening and effect of additives on 3A5AF production. Reaction conditions: 4.5 mL DMA in the absence of LiBr and 4 mL in the presence of LiBr, 10 wt% NAG, 6 mol% catalyst (if used), 10 wt% LiBr (if used), 207 °C, MW, 15 min.

#	Catalyst	Additive	3A5AF (% yield)[a]
1	none	LiBr	11.2
2	none		18.3
3	$CrCl_3$	LiBr	15.2
4	$CrCl_3$		20.8
5	HCl(aq)		20.6
6	NH_3(aq)	LiBr	14.5
7	NH_3(aq)		21.6
8	NaOH		19.8
9	$(CH_3CH_2)_3N$		24.8
10	DBU[b]		24.7
11	DABCO[c]		21.1

[a]Determined using GC-MS.

[b]DBU = 1,8-Diazabicycloundec-7-ene.

[c]DABCO = 1,4-diazabicyclo[2.2.2]octane.

3-2-3 2^5 Factorial Design (FD)

Although studying one factor at a time to optimize reaction conditions is a common approach, it has some limitations such as neglecting the interaction between factors. Using FD, a researcher can study not only one factor at a time but also the effects of factors interactions on the result.[18] Therefore, the effects of five factors with each factor at

two levels were studied. These factors were NAG, DMA, and amount of ammonia, temperature, and time. The factors and levels are shown in Table 3-2.

Table 3-2. 2^5 FD of 3A5AF production.

T (°C)	Time (min)	0.26 mmol NH_3				0.13 mmol NH_3			
		0.4711 g NAG		0.2356 g NAG		0.4711 g NAG		0.2356 g NAG	
		DMA (mL)							
		4.5	2.5	4.5	2.5	4.5	2.5	4.5	2.5
		3A5AF (% Yield)[a]							
217	15	27.5, 27.5	20.0, 20.5	42.5, 42.6	24.2, 24.1	20.6, 20.3	14.8, 15.2	19.2, 20.0	21.7, 21.8
	5	25.9, 25.8	22.1, 23.5	29.8, 29.7	23.1, 23.5	20.6, 20.3	14.3, 15.1	21.7, 21.1	22.4, 23.0
180	15	18.3, 19.6	17.1, 16.0	24.4, 25.0	22.3, 21.4	15.6, 16.5	15.6, 15.5	18.1, 18.0	9.0, 9.3
	5	15.2, 13.8	14.2, 13.7	18.1, 18.5	19.0, 19.4	12.4, 12.1	11.5, 12.8	14.1, 14.5	4.3, 4.5

[a]3A5AF was quantified using GC-MS.

The maximum % yield of 3A5AF obtained was 42.6%. This yield was achieved using 0.2356 g NAG, 4.5 mL DMA, 0.26 mmol NH_3, 217 °C, and 15 min. The results in this table were analyzed using Minitab software. The normal plot of the effects (Figure 3-5) shows that all effects are significant. In fact, in this case the individual effects (i.e., each factor alone) are more significant than combined effects/factors.

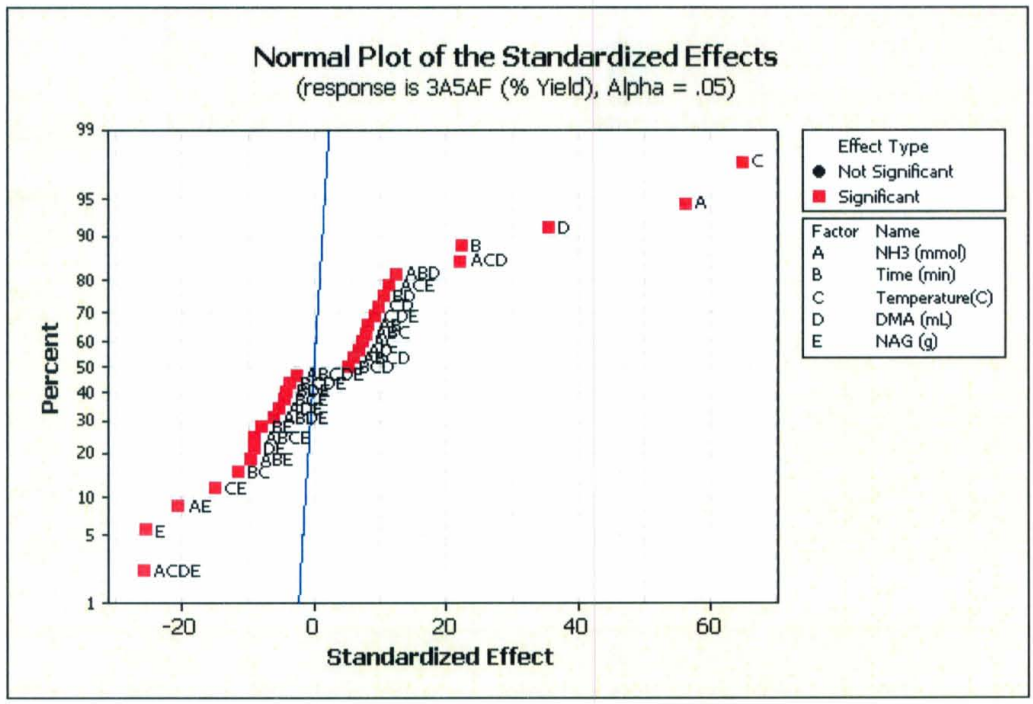


Figure 3-5. Normal plot of the effects for 3A5AF production. This plot was produced using Minitab software. Reaction conditions (See Table 3-2).

The most significant effects at different levels were tested, e.g., increased *T*, Figure 3-6, but none of these improved on the amount of 3A5AF produced (42.6%) during the FD experiments.

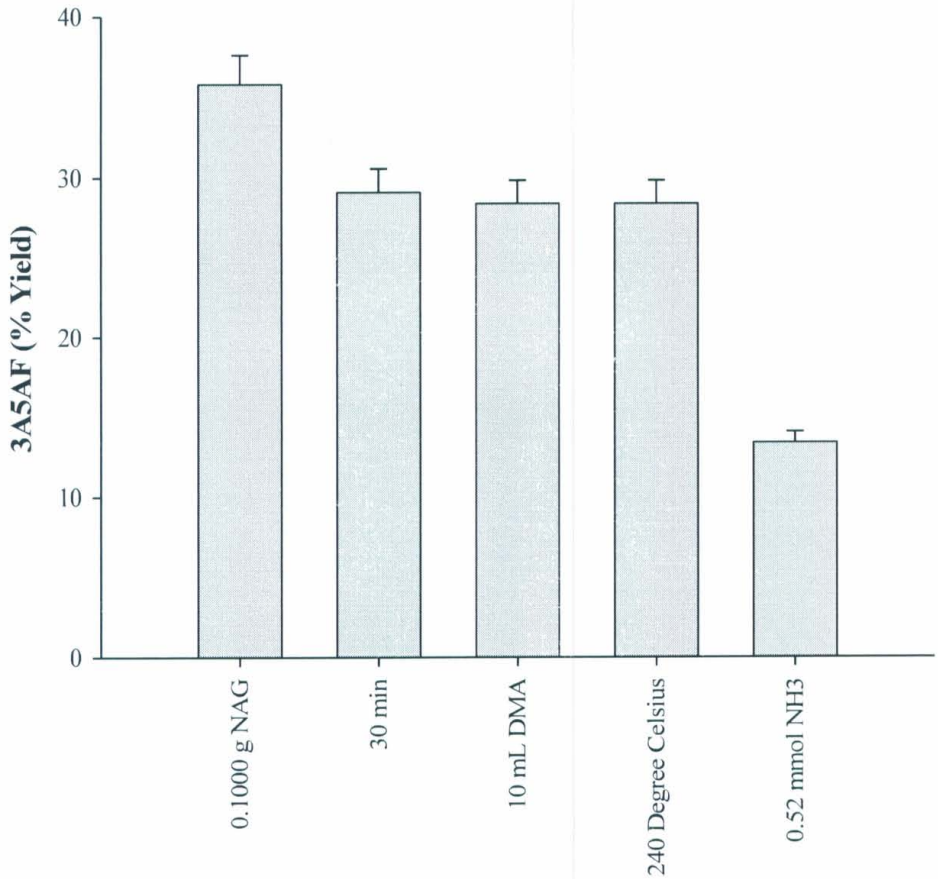


Figure 3-6. Effect of changing a reaction parameter from the following optimum reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 0.26 mmol NH_3, and microwave irradiation for 15 min at 217 °C. Under optimum conditions, Table 3-2, 42.6% yield of 3A5AF was obtained. 3A5AF was quantified using GC-MS.

3-2-4 Testing Different NAG Sources

The results described above were obtained using NAG purchased from Toronto Research Chemicals (TRC). When NAG from AK Scientific or Alfa Aesar was tested, the yields of 14.7% and 6.2%, respectively were obtained. A challenge was posed in the form of this question; why did changing the supplier/source of NAG cause such a dramatic decrease in the yield? After studying the impurity levels in the NAG from each supplier using ICP-MS, boron (B) and chloride (Cl) concentrations stood out as being significantly higher in the NAG from TRC, Table 3-3.

Table 3-3. ICP-MS analyses for B and Cl in NAG from three suppliers.

Chemical Supplier	**B[a]**	**Cl[a]**
TRC	27.9	20681
AK Scientific	4.4	819
Alfa Aesar	0.3	391

[a]mg B or Cl per Kg NAG

Cl^- ions have been shown to have a significant and positive effect on biomass conversions processes[15,17,20] Therefore, different amounts of NaCl were added to reaction mixtures to test the effect of Cl^- concentration on 3A5AF production using NAG from AK Scientific and Alfa Aesar. Figure 3-7 shows that NaCl at 30-50 mol% with respect to NAG produced the highest % yield of 3A5AF.

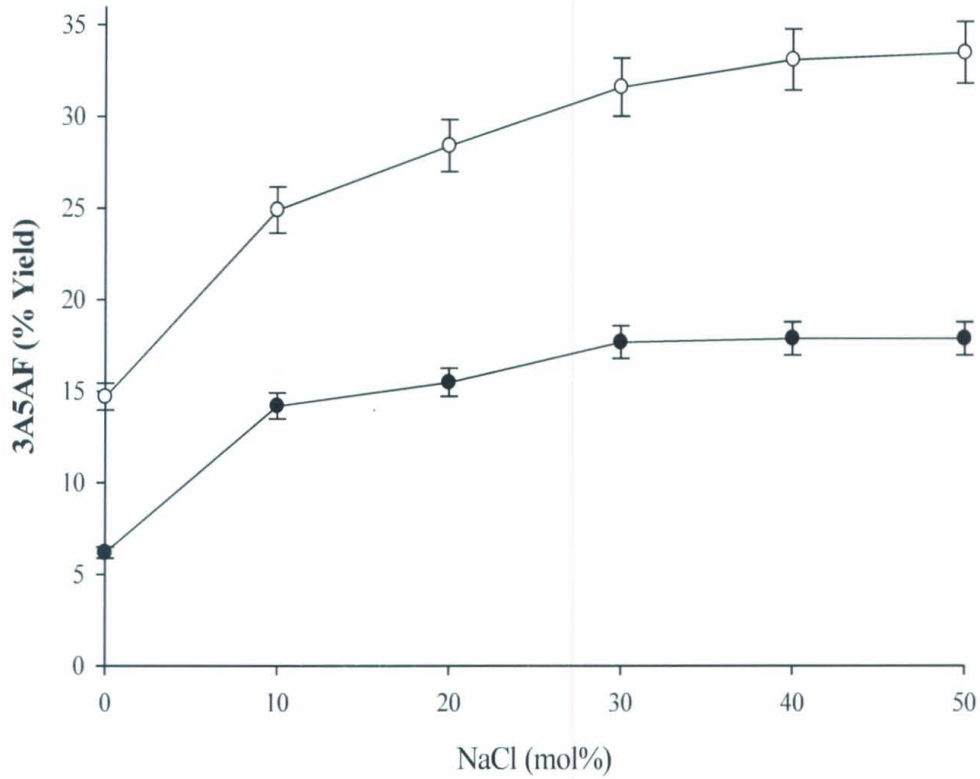


Figure 3-7. Effect of adding NaCl on yield of 3A5AF from NAG supplied by AK Scientific and Alfa Aesar. Solid circles = Alfa Aesar, Hollow circles = AK Scientific. Reaction conditions: 0.2356 g NAG, 0-50 NaCl (mol% of NAG), 4.5 mL DMA, ammonia (24.4 mol% of NAG), MW, 217 °C, 15 min.

NAG from Alfa Aesar consistently produced less 3A5AF compared with AK Scientific because it contains less boron (see Table 3-3). Therefore, boron was added to the reaction mixture in the form of boric acid ($B(OH)_3$). $B(OH)_3$ is a weak acid, non-toxic, inexpensive, and readily available. Boric acid with NaCl has been used previously to

increase 5-HMF production from hexoses.[19,20] Yields of up to 42% from glucose and as much as 66% from sucrose have been reported.

Figure 3-8 shows the effect of adding $B(OH)_3$ at a 2:1 $B(OH)_3$:NAG mol ratio on 3A5AF production in the presence and absence of NaCl (30 mol% with respect to NAG), and the presence and absence of ammonia (24.4 mol% of NAG). In all example reactions, Figure 3-8, the NAG from AK scientific produced more 3A5AF because of its initially higher B and Cl content, Table 3-3. Figure 3-8 emphasizes the significance of adding Cl^- to the reaction mixture.

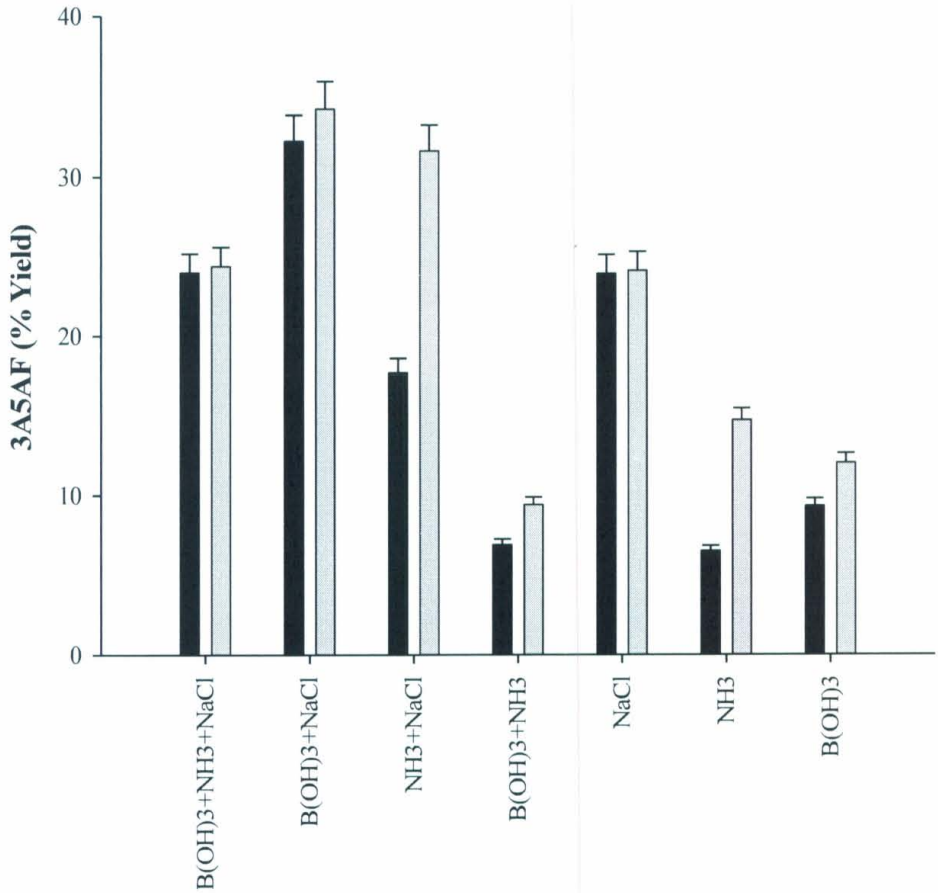


Figure 3-8. Effect of added $B(OH)_3$, NaCl, and NH_3 on 3A5AF production. Black = NAG from Alfa Aesar. Grey = NAG from AK Scientific. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA in the presence and absence of NaCl (30 mol% with respect to NAG), NH_3 (24.4 mol% with respect to NAG), 2:1 $B(OH)_3$:NAG mol ratio, MW, 217 °C, 15 min. Yield determined using GC-MS.

The importance of $B(OH)_3$ and NaCl in dehydrating NAG led us to study 3^2 FD to determine the optimum amounts that should be added. Table 3-4 shows 3^2 FD using NaCl

at three levels 10, 30, and 60 mol% with respect to NAG and $B(OH)_3$ at 0.5:1, 1:1, and 2:1 mole ratios with respect to NAG. The other parameters for the reactions were kept constant at 0.2356 g NAG, 4.5 mL DMA, 220 °C, and 15 min.

Table 3-4. 3^2 FD using 2 factors (NaCl and $B(OH)_3$) at three levels.[a]

mol:mol	**NaCl (mol% of NAG)**		
$B(OH)_3$:NAG	10	30	60
	3A5AF (% Yield)[b]		
0.5:1	27.3, 28.4	29.2, 29.5	32.3, 32.3
1:1	31.0, 31.4	36.2, 35.4	39.4, 40.1
2:1	30.1, 29.6	32.8, 35.5	38.5, 37.4
[a]Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, NaCl (mol% of NAG), 1:1 $B(OH)_3$:NAG mol ratio NaCl and $B(OH)_3$ amounts are as in the table below, MW, 220 °C, 15 min. [b]3A5AF was quantified using GC-MS			

The results were analyzed using Minitab software. The interaction plot for 3A5AF (Figure 3-9) shows that at each level of $B(OH)_3$, the amount of 3A5AF produced increased with increasing NaCl concentration. The interaction plot also clearly shows that a 1:1 mole ratio of $B(OH)_3$:NAG produced the highest yield of 3A5AF.

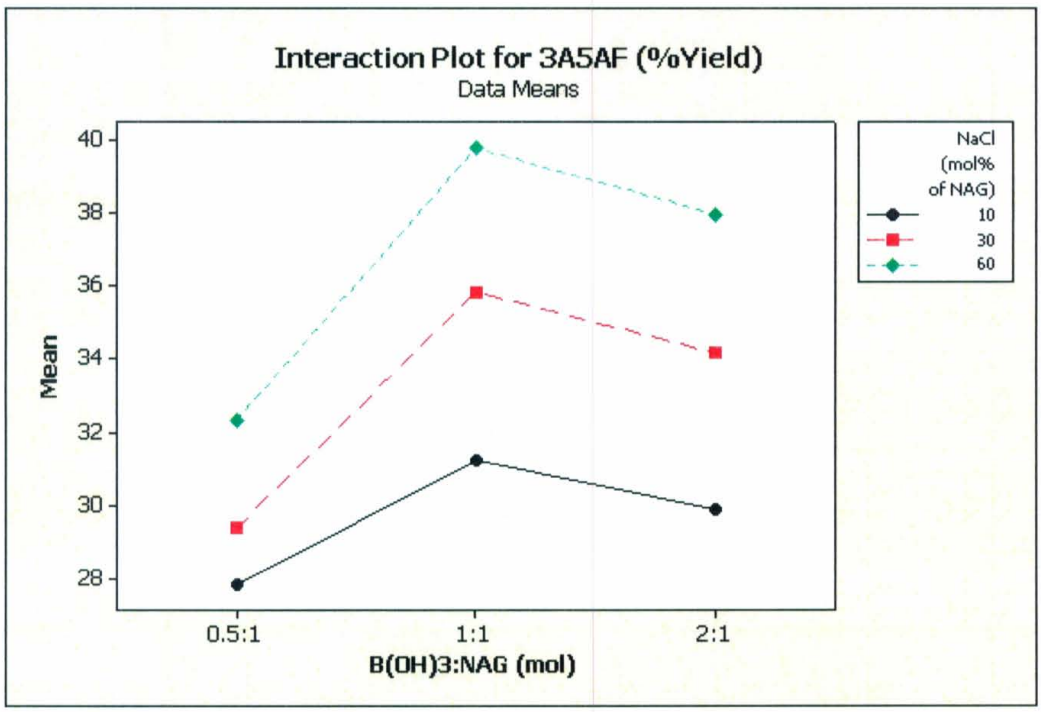


Figure 3-9. The interaction plot of 3A5AF. This plot was generated using Minitab software.

Figure 3-10 shows the % yields of 3A5AF at different NaCl levels using a fixed amount of 1:1 $B(OH)_3$:NAG (mol ratio). 57.7% yield 3A5AF was produced at 2:1 in 15 min while the maximum % yield was 62.3 at 4:1 NaCl:NAG mol ratio. This compares well with studies using fructose and glucose, which produce 5-HMF. Using NaCl and $B(OH)_3$, fructose has been shown to produce 60% yield 5-HMF and glucose produced 14% yield 5-HMF.[20]

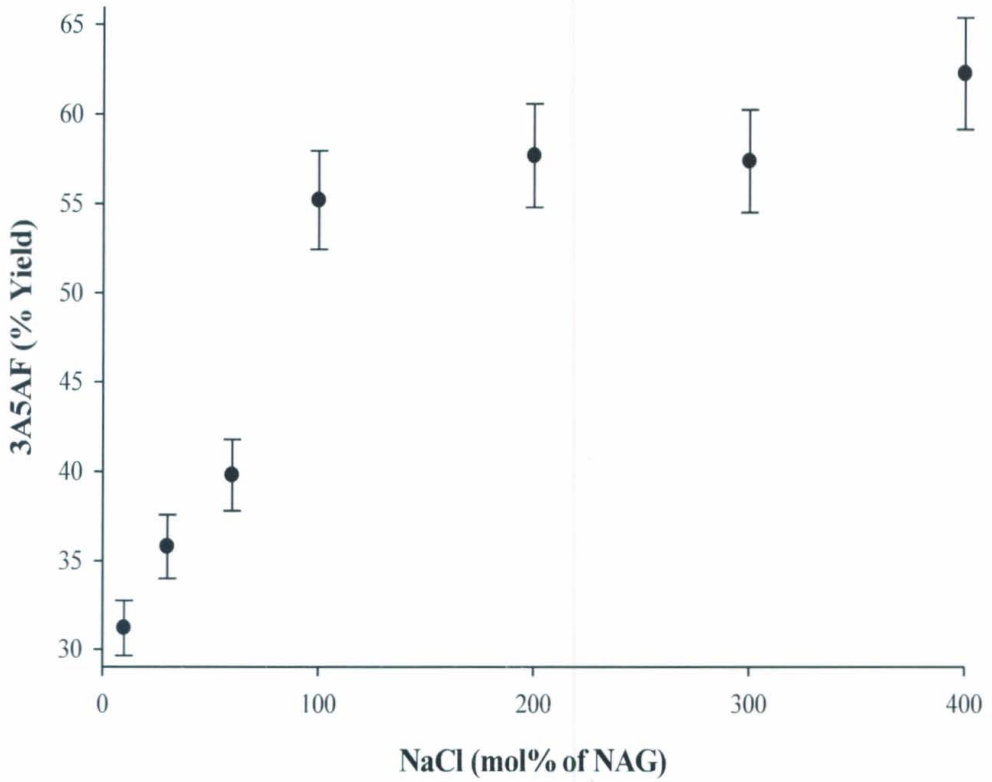


Figure 3-10. Effect of adding different levels of NaCl at 1:1 $B(OH)_3$:NAG (mol ratio). Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 10-400 NaCl (mol% of NAG), 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 15 min. 3A5AF was quantified using GC-MS.

Different Cl^- sources were tested ($SnCl_4 \cdot 5H_2O$, $MnCl_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$, KCl, $CaCl_2 \cdot 2H_2O$, $CaCl_2$, NaCl, and LiCl) Figure 3-11. NaCl is the most benign and the cheapest chemical among them. Compared with NaCl (57.7% yield), every chemical produced less 3A5AF except LiCl, which produced 59.2% yield. DMA-LiCl (10%) as a solvent in the presence of CuCl and 1-ethyl-3-methylimidazolium chloride ([EMIm]Cl)

has been shown to produce 83% yield 5-HMF from fructose.[15] $CaCl_2$ produced 54.5% yield 3A5AF and $CaCl_2 \cdot 2H_2O$ 50.7% yield, which shows that unfortunately water inhibits the production of 3A5AF in this system.

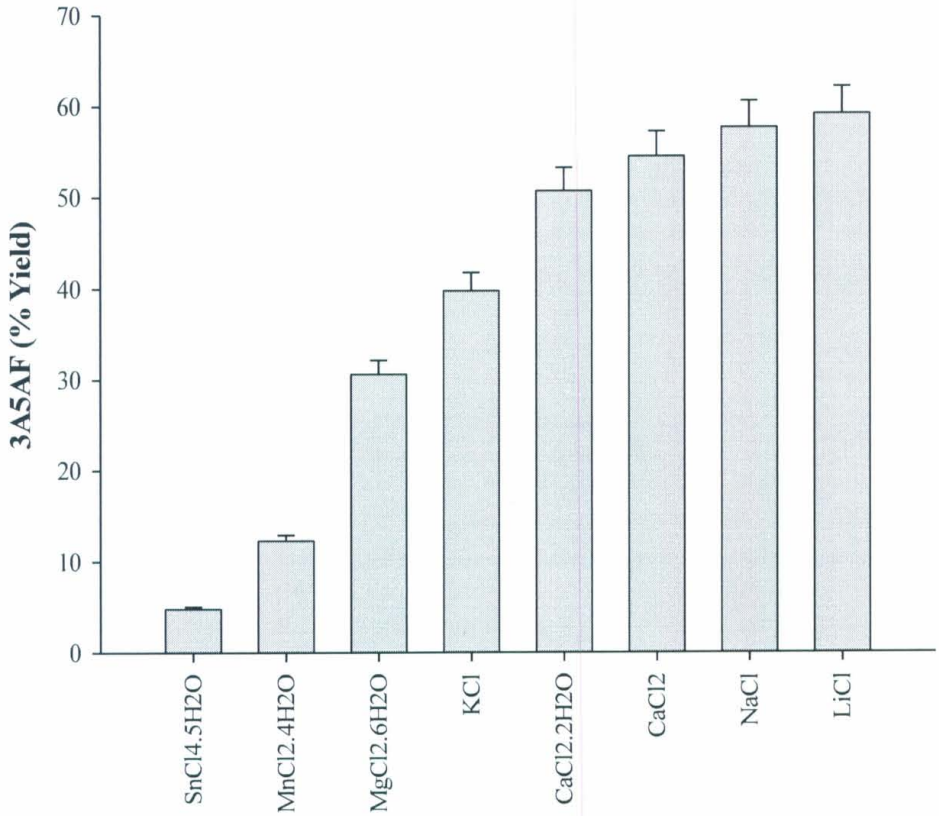


Figure 3-11. Effect of chloride sources on 3A5AF production. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 2:1 Cl:NAG mol ratio, 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 15 min. 3A5AF was quantified using GC-MS.

To further study the effect of adding water to the reaction, several amounts of water were added (1, 2, 4, 6, 8, 10, 15, and 20 %v/v in 4.5 mL DMA). Figure 3-12 shows that the %

yield of 3A5AF produced decreases as the amount of added water increases, proving water inhibits this process. If dry, distilled DMA was used, no significant increase in 3A5AF yield was observed.

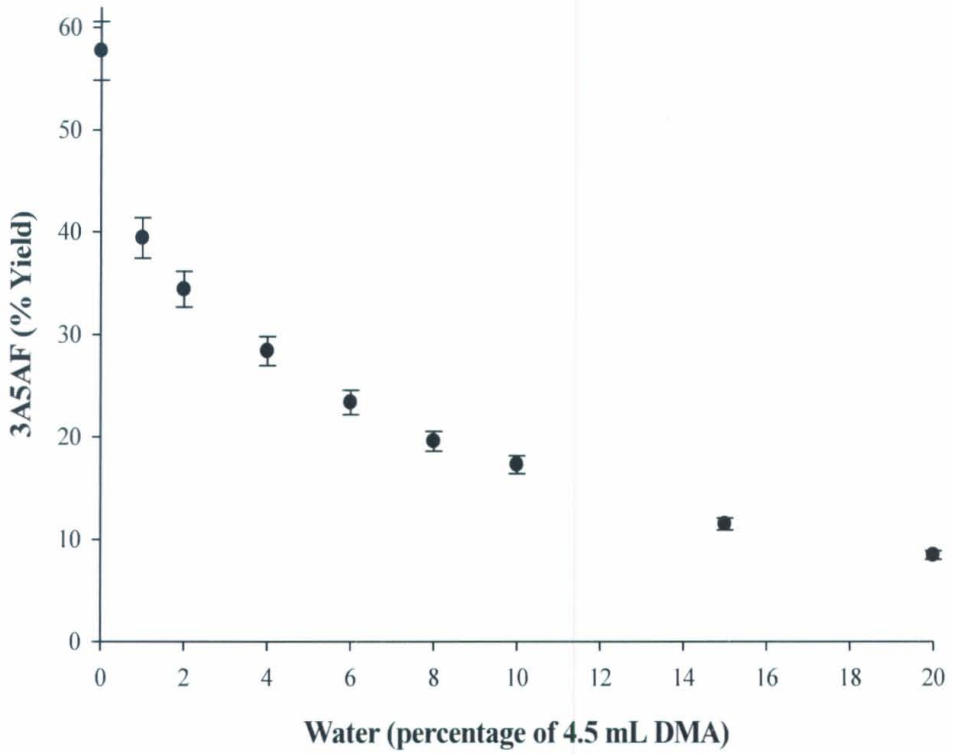


Figure 3-12. Effect of water addition to the system on 3A5AF productivity. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA (0-20 %v/v H_2O), 2:1 NaCl:NAG mol ratio, 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 15 min. 3A5AF was quantified using GC-MS.

The effect of time on the reaction was studied at 0, 5, 10, 15, 30, 45, 60, 75, 90 min. Figure 3-13 shows an approximately linear increase in 3A5AF production between 0 and 15 min but the amount of 3A5AF does not change significantly between 15 and 45 min.

However, the % yield starts to decrease after 45 min, which is probably due to thermal decomposition of 3A5AF.

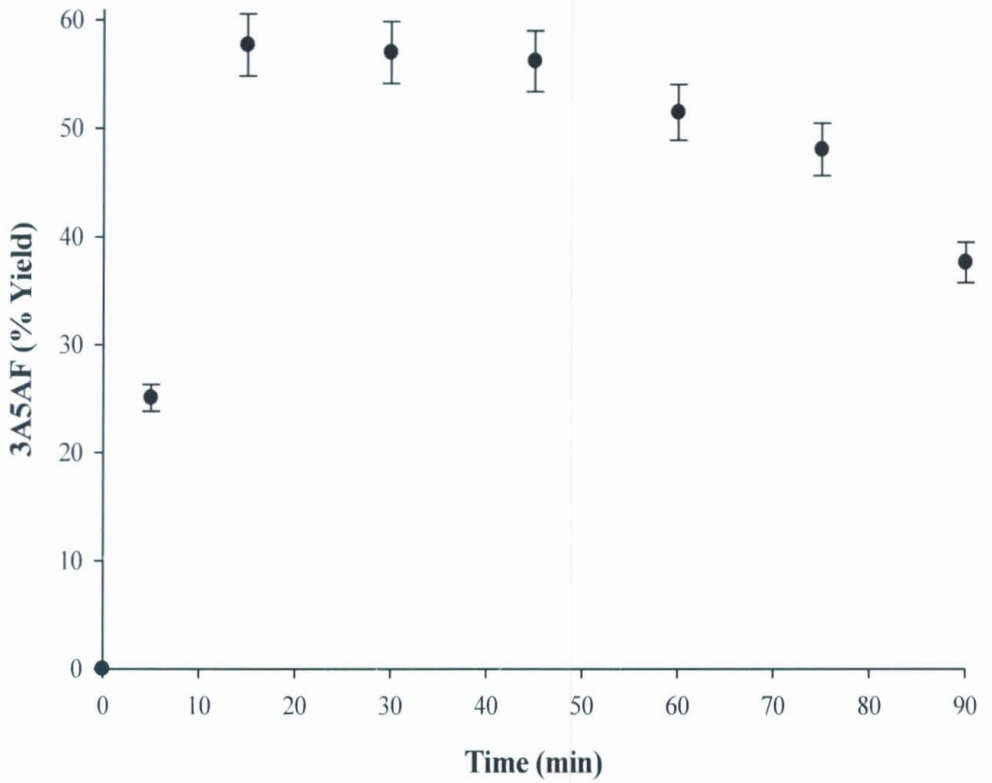


Figure 3-13. Effect of time on 3A5AF production. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 2:1 NaCl:NAG mol ratio, 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 0-90 min. 3A5AF was quantified using GC-MS.

Our system was also tested at different temperatures 160, 180, 200, 220, 240, and 250 °C for 15 min, Figure 3-14. The amount of 3A5AF increased linearly between 160 and 220 °C. Above 220 °C, 3A5AF % yield decreased presumably due to product decomposition.

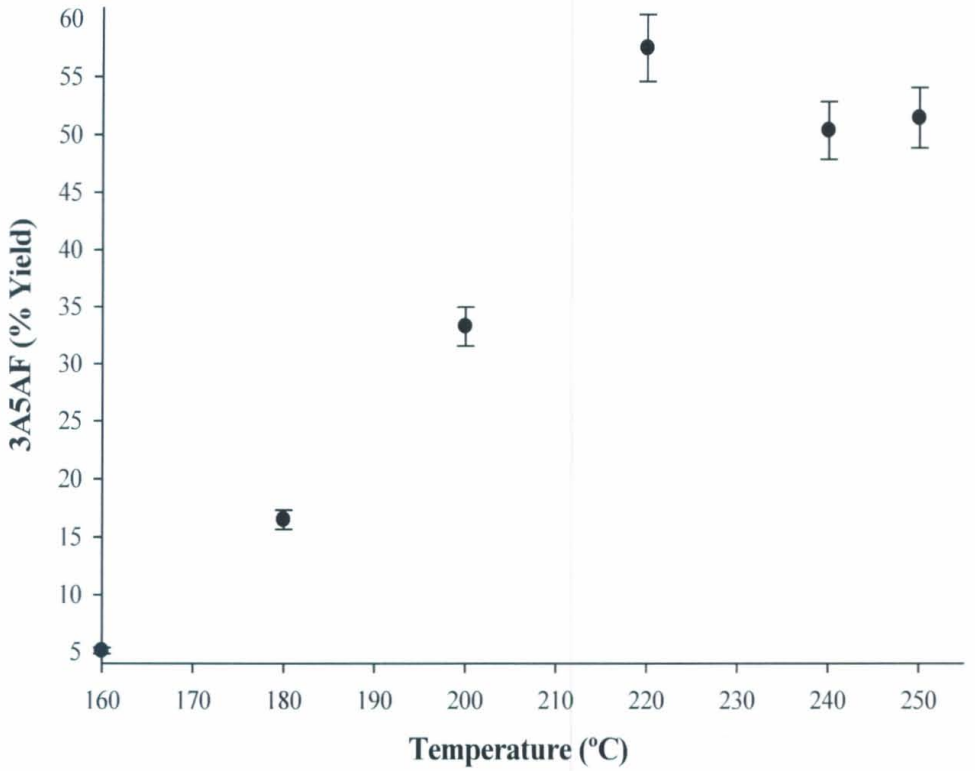


Figure 3-14. Effect of temperature on 3A5AF production. Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 2:1 NaCl:NAG mol ratio, 1:1 B(OH)3:NAG mol ratio, MW, 160-250 °C, 15 min. Yield was determined using GC-MS.

However, decomposition products were not observed in the chromatograms from these reactions and are likely insoluble in ethyl acetate (Figure 3-15).

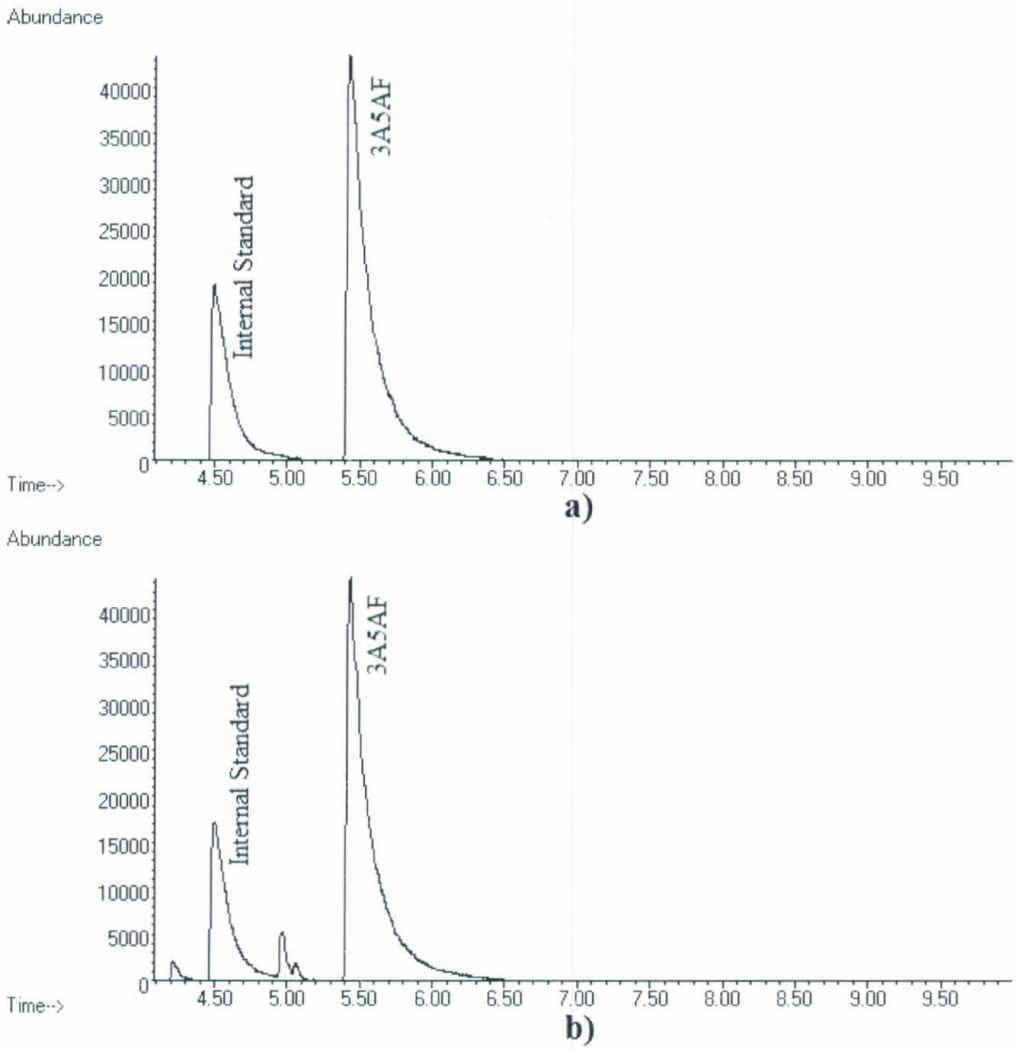


Figure 3-15. GC chromatograms at a) 250 °C and b) 220 °C.

Figure 3-16 shows the optimum reaction conditions that produced 57.7% yield 3A5AF from NAG. This process produces 30 times more 3A5AF than previously reported pyrolysis methods.

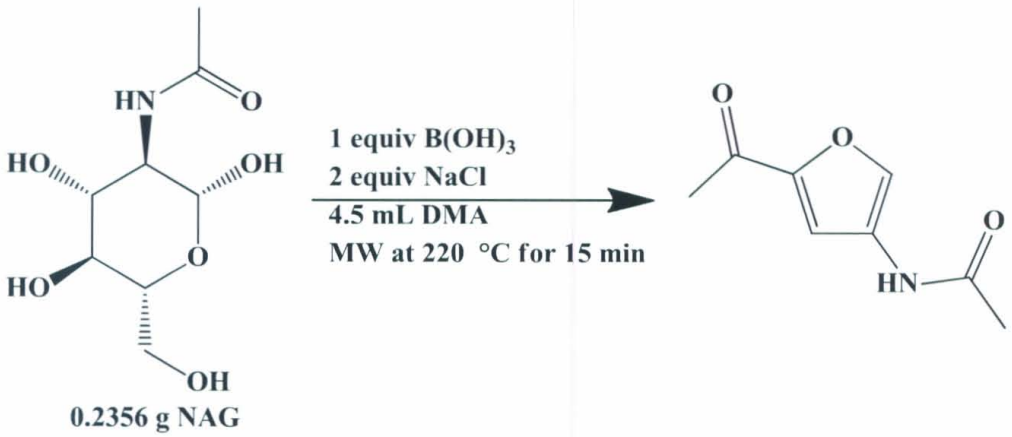


Figure 3-16. The optimum reaction conditions for dehydrating NAG to form 3A5AF.

Preliminary studies towards scaling up the reaction were performed in a 300 mL autoclave (Parr® reactor 5500 series). The following conditions were used: 5.00 g NAG, 2.67 g NaCl, 1.40 g $B(OH)_3$, 100 mL DMA, 220 °C, 15 min. It should be noted that it took 30 min to achieve the desired reaction temperature and that the pressure at 220 °C was approximately 6 bar. Under these reaction conditions, 42.8% yield of 3A5AF was obtained. Further studies are needed to optimize scale-up of this reaction.

Chitin and chitosan were tested using the optimum conditions as in Figure 3-16. No 3A5AF was produced from either of these polysaccharides. Therefore, it can be concluded that these biopolymers need relatively harsh reaction condition to produce chemicals at interest via hydrolysis.

3-2-5 Literature Comparison

In 1984, Franich et al. developed a pyrolysis method using an oven at 400 °C to produce a 2% yield of 3A5AF. The NAG solution (4:1 water:methanol) was prepared in a glass apparatus, which was placed in the middle of a platinum coil in the oven. The formed tar was eluted over a silica gel column using chloroform and methanol mixtures.[9] Another study was performed by Ho et al. who carried out the pyrolysis of NAG mixed with anhydrous disodium hydrogen phosphate and quartz sand in an oil bath at 200 °C over 30 min.[10] The extraction process was accomplished using many steps including 150 mL of 0.1M HCl, 1M NaOH to increase the solution pH to 11.0, and 90 mL dichloromethane. Then the organic solvent was dried over anhydrous sodium sulfate (0.04% yield of 3A5AF was generated). Based on this study (Chapter 3), a 57.7% yield of 3A5AF was recorded which is ca. 30 times more than Franich et al.. In Chapter 4 of this thesis, a method for producing 60.0% yield of 3A5AF in an IL is described. In these last two studies, the reaction mixtures were heated using microwave irradiation and NaCl and $B(OH)_3$ were used as additives. To compare the results shown here with the cellulose and other renewable feedstocks including glucose and fructose that were used to produce 5-HMF, see Table 1-4.

Table 3-5. Comparison between this study and the literature for 3A5AF production using NAG.

Reference number	Reaction condition	3A5AF (% yield)
9	Oven pyrolysis (400 °C), heating rate 75 °C.ms^{-1} for 20 s	2
10	Oil bath pyrolysis (200 °C over 30 min), in presence of Na_2HPO_4 and quartz sand	0.04
Chapter 3	MW (220 °C, 15 min), in presence of NaCl, $B(OH)_3$ and DMA	57.7
Chapter 4	MW (180 °C, 3 min) or conventional heating (180 °C, 1 h), in presence of $B(OH)_3$ and [BMIm]Cl	60.0

3-3 Conclusions

The optimised reaction produced the highest % yield of 3A5AF to date and comparable to results obtained in an IL reaction medium (Chapter 4). The productivity of 3A5AF is approximately thirty times more than the Franich et al. pyrolysis method. The run time needed was reasonably low (15 min). NaCl and $B(OH)_3$ were very important additives in this reaction and markedly increase 3A5AF production. Indeed, the importance of B and Cl in the reaction was discovered by analysis of starting materials for impurities that seemed to be affecting the reaction. This study will hopefully allow the chemistry of 3A5AF, a carbohydrate-derived amide, to be studied further and it may find use either as a platform chemical, a source of renewable amines, or as a high-value precursor to proximicins and other biologically active compounds.

3-4 Experimental

3-4-1 Materials

NAG was purchased from Toronto Research Chemicals (TRC), AK Scientific and Alfa Aesar. 3A5AF was prepared in GCCG labs and purified using flash chromatography (Biotage, Isolera One) and used as an analytical standard. All other catalysts and additives tested were purchased in 98% purity or greater from Fisher Scientific, Alfa Aesar, Aldrich or Strem Chemicals. Benzamide (minimum 98%) was purchased from Alfa Aesar and used as an internal standard in GC-MS experiments. Ethyl acetate (HPLC grade, 99.8%) was purchased from EMD Chemicals Inc. DMA was purchased from Caledon Laboratory Chemicals. All other solvents were purchased from Aldrich and Fluka. Deionized water was obtained using a Nanopure II system (manufactured by Barnstead/Thermolyne, USA) with distilled water as the source for the inlet feed.

3-4-2 General Procedure for 3A5AF Production from NAG

NAG (50-500 mg) was mixed with a specific volume of solvent, a known amount of catalyst and/or additive. The mixture was heated to the desired temperature under microwave irradiation using a Biotage Initiator 2.5 for a specific period of time. Figure 3-17 shows the temperature, pressure, and power graphs of the optimum reaction using microwave heating instrument. After this time, an aliquot of the reaction mixture was mixed with 100 μL of 2 mg benzamide/mL ethyl acetate and 2 mL deionized water. In the case of dipolar aprotic solvents except acetonitrile, the aliquot was diluted with a pure solvent up to 2 mL. Extraction was performed with 3 × 2 mL ethyl acetate. After each

addition of ethyl acetate, the mixture was vortex-mixed at high speed for 30 seconds and then centrifuged at 2500 rpm for 2 min. The combined ethyl acetate layers were transferred to a vial and evaporated to dryness at 50 °C using a Radleys Greenhouse Blowdown Evaporator under a stream of nitrogen. The contents of the dried tube were reconstituted in 500 µL ethyl acetate for GC-MS analysis.

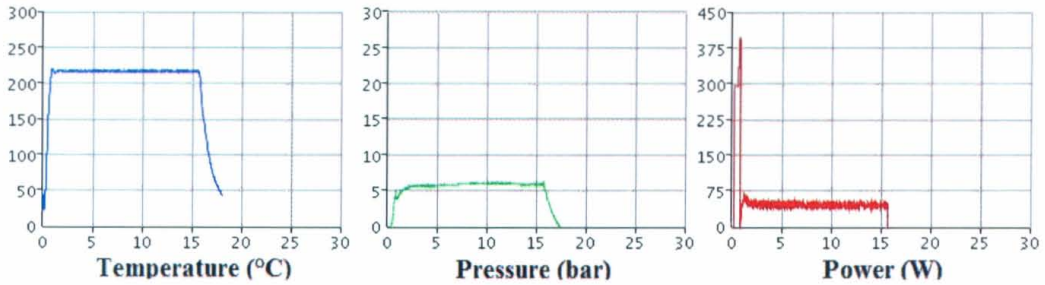
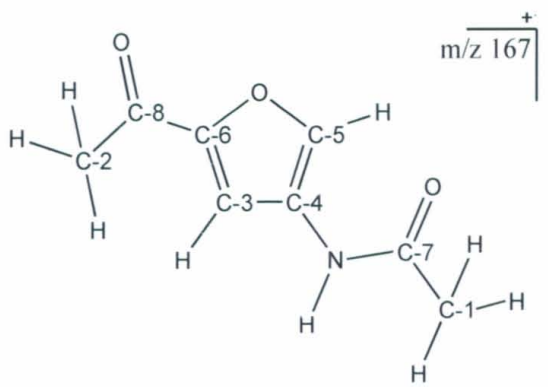


Figure 3-17. Temperature, pressure, and power graphs obtained using the optimum reaction conditions from the microwave instrument (Biotage Initiator 2.5). Reaction conditions: 0.2356 g NAG, 4.5 mL DMA, 2:1 NaCl:NAG mol ratio, 1:1 $B(OH)_3$:NAG mol ratio, MW, 220 °C, 15 min.

3-4-3 3A5AF Identification

After the reaction and extraction were carried out using ethyl acetate, the solvent was evaporated at 50 °C using Radleys Greenhouse Blowdown Evaporator under a stream of nitrogen until ca 500 µL was remained. 3A5AF was purified using flash chromatography (FC) Biotage Isolera One. FC was run on SiO_2 column (SNAP cartridge, silica, 10 g) using variable 200-400 nm wavelength detector at 254 and 293 nm. The sample was previously analyzed on SiO_2 thin layer chromatography (TLC) plates using a 50% ethyl acetate/hexanes mixture and the obtained retention factor (R_f) was used to build up a

method of separation using the FC instrument. The R_f was 0.13. Samples of the product were combined. After evaporating the solvent using the evaporator at 50 °C until dryness, the dried residue was placed under vacuum using a standard Schlenk line overnight. Then the residue was dissolved in CD_3CN. ^{1}H-NMR (Figure 3-18) and ^{13}C-NMR (Figure 3-19) spectra were obtained using Bruker AVANCE 500MHz and Bruker AVANCE III 300MHz spectrometers, respectively. IR was conducted using Bruker ALPHA FTIR instrument with ALPHA-T sample compartment. High-resolution mass spectrometry (HRMS) was performed using Waters GCT Premier TOF mass spectrometer (electron ionization (EI)). GC-MS was also used for identification and quantification of 3A5AF (Agilent Technologies 7890 GC with 5975 MSD), see below for more details on GC-MS method.



^{1}H-NMR δ_H (298 K, 500MHz; CD_3CN) 2.04 (3H, s, C(7)Me), 2.39 (3H, s, C(8)Me), 7.12 (1H, s, C(3)H), 8.13 (1H, s, C(5)H) and 8.45 (1H, br s, NH).

^{13}C-NMR δ_C (298 K, 300 MHz; CD_3CN) 23.3 (C-1), 26.3 (C-2), 111.4 (C-3), 128.1 (C-4), 136.5 (C-5), 151.5 (C-6), 169.1 (C-7) and 187.4 (C-8).

Selected IR ν_{max}/cm^{-1} 1668 br, s (C=O stretch in amide I band and C=O stretch in ketone).

HRMS, calculated exact mass for 3A5AF ($C_8H_9NO_3$) = 167.0582, found = 167.0584, accuracy = 1.2 ppm.

MS *m/z* 167 (53%), 125 (91), 110 (100), 96 (15), 83 (17), 69 (6), 54 (11).

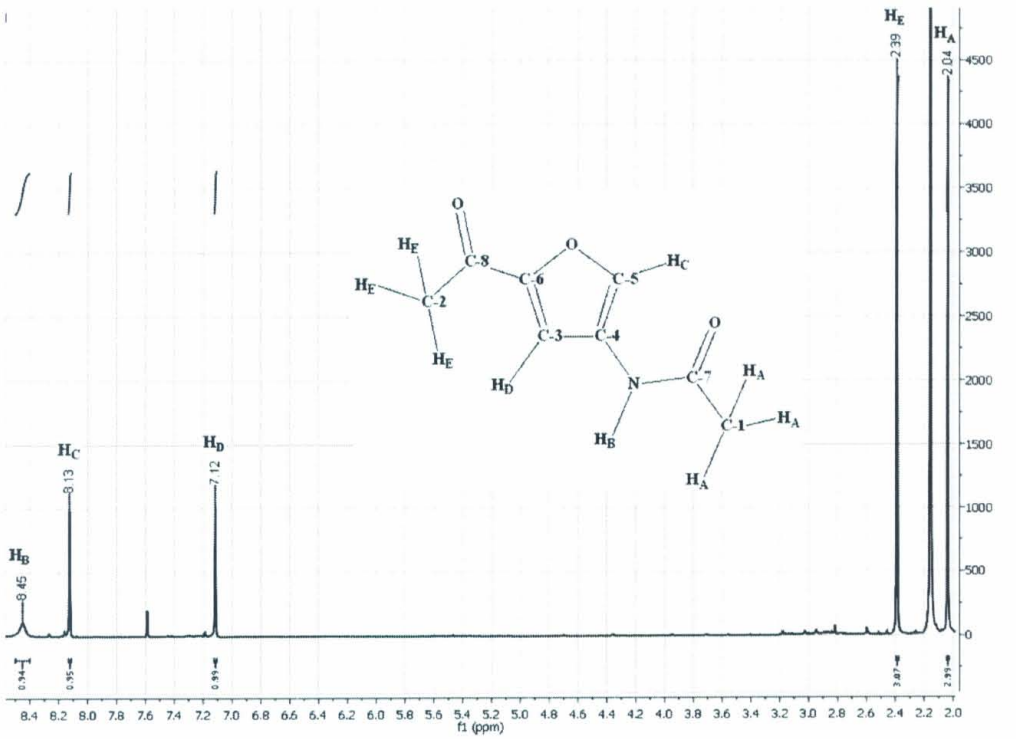


Figure 3-18. ^{1}H-NMR spectrum of 3A5AF in CD_3CN.

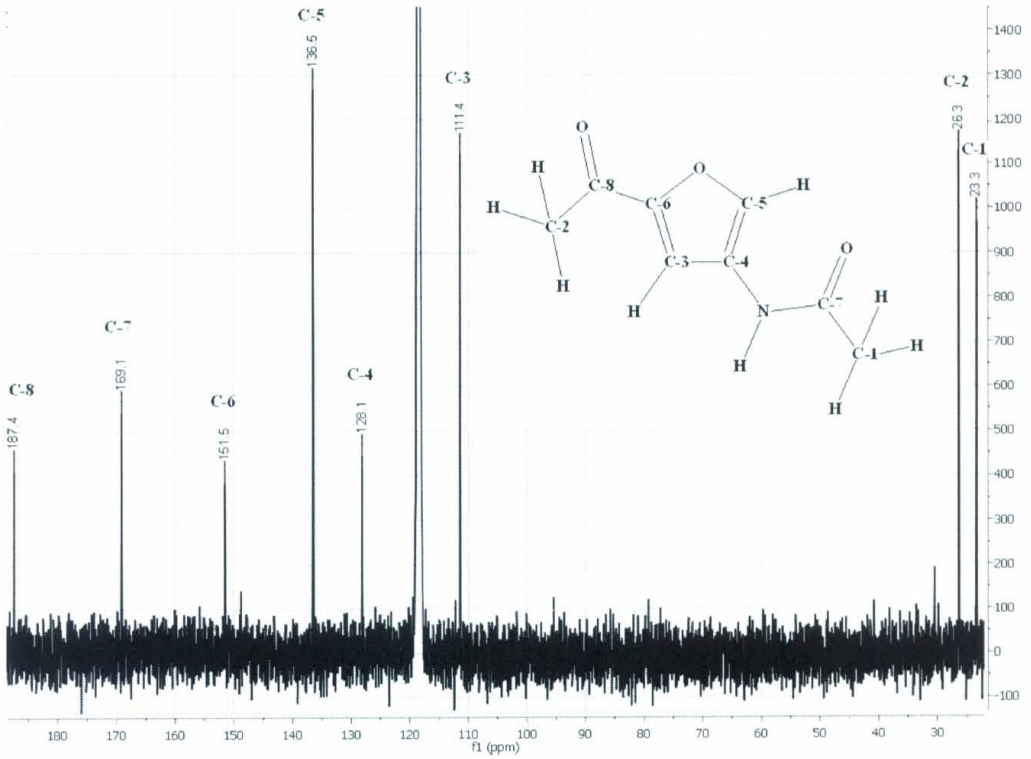


Figure 3-19. ^{13}C-NMR spectrum of 3A5AF in CD_3CN.

3-4-4 Gas Chromatography-Mass Spectrometry (GC-MS) Determination of 3A5AF

3A5AF was analyzed using an Agilent Technologies 7890 GC with 5975 MSD. 1 μL of reconstituted sample was injected through a 7683B Series Injector using a split mode of 50%. The GC separation was done using a DB5 column at a flow rate of 1 mL/min He 99.999%. The oven temperature was programmed as follows: 50 °C (hold 1 min), 65 °C/min to 215 °C, 2.5 °C/min to 225 °C, and 20 °C/min to 250 °C for 1.212 min. (The total run time was 10 min). Products were detected at *m/z* 50-175 scan range. Under these conditions, the retention times of 3A5AF and benzamide were 5.42 and 4.49 min,

respectively. Figure 3-20 shows the mass spectra of the internal standard (benzamide) and 3A5AF, respectively. R^2 of 3A5AF calibration curve was 0.9991, Figure 3-21. Accuracy was greater than 92% and RSD was less than 5.0%.

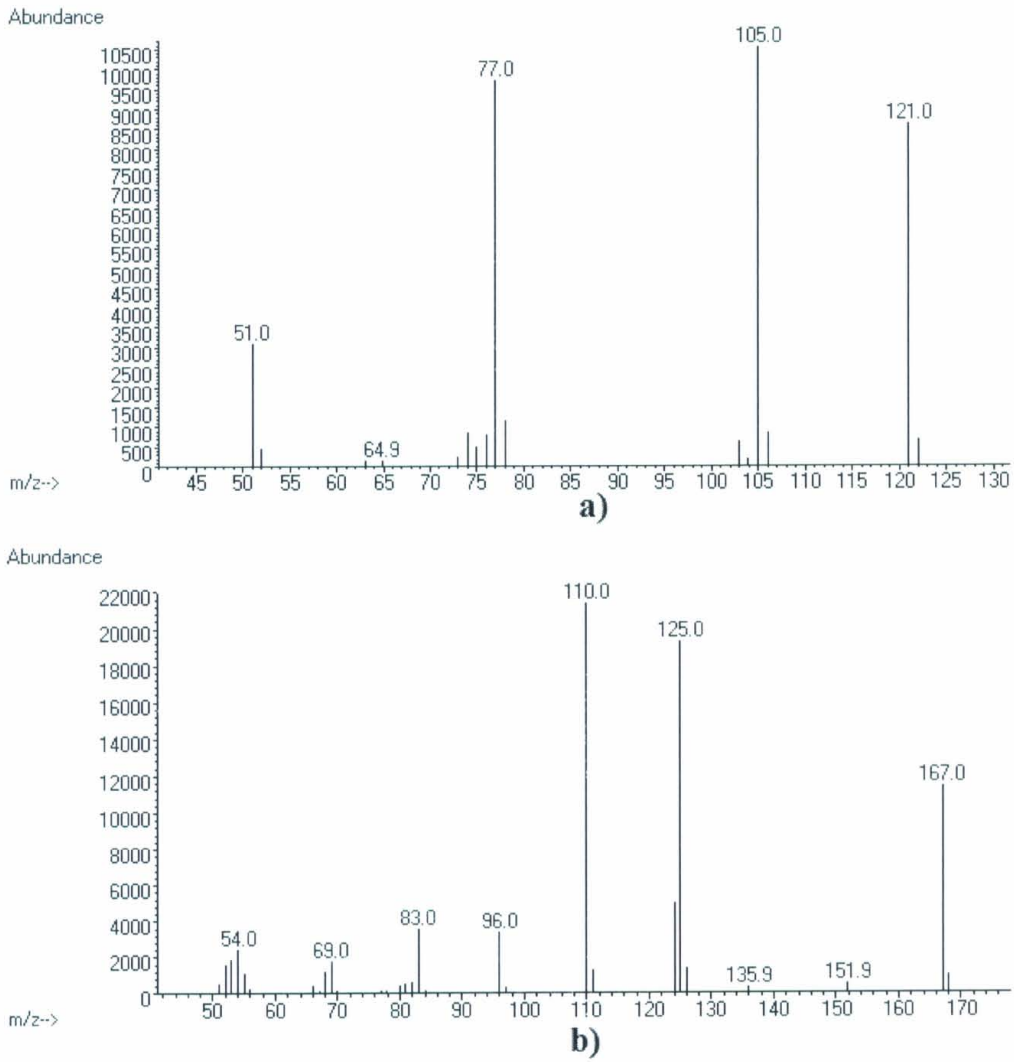


Figure 3-20. Mass spectra of a) Internal Standard (benzamide) and b) 3A5AF.

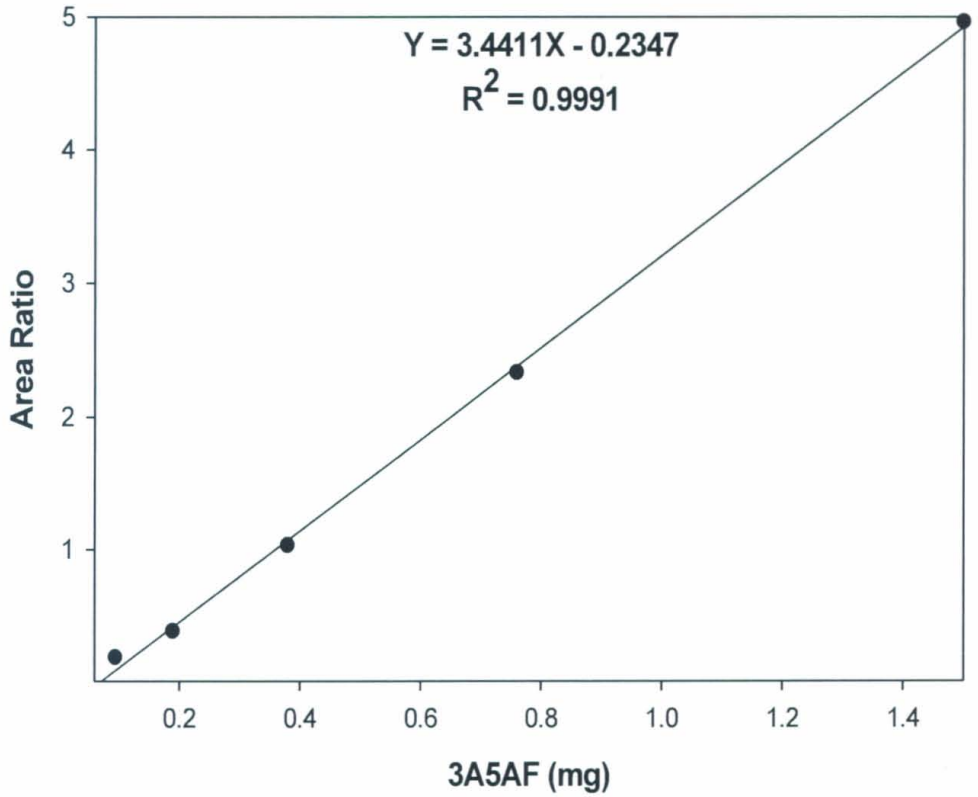


Figure 3-21. 3A5AF Calibration curve using benzamide as an internal standard.

3-4-5 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

50 mg of each sample was dissolved in 1 mL of 8M Nitric acid. The solutions were then diluted to 30 g with nanopure water. 5 g of each sample solution was diluted to 10 g with 0.2M Nitric acid. The samples were analyzed using a ELAN DRC II ICP-MS instrument.

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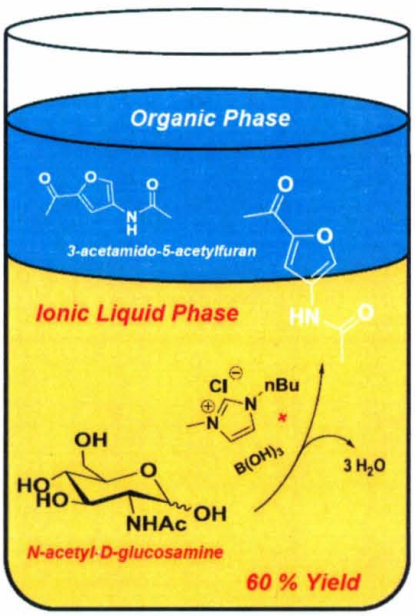
Chapter Four

Formation of a Renewable Amide, 3-acetamido-5-acetylfuran, via Direct Conversion of *N*-acetyl-D-glucosamine



A version of this chapter has been published.

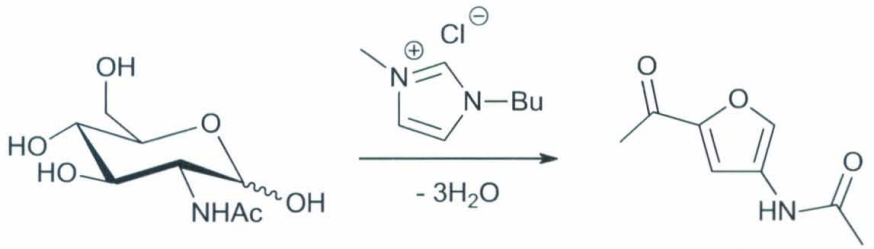
Marcus W. Drover, Khaled W. Omari, Jennifer N. Murphy and Francesca M. Kerton*, Formation of a Renewable Amide, 3-acetamido-5-acetylfuran, via Direct Conversion of *N*-acetyl-D-glucosamine, *RSC Adv.*, 2012, **2**, 4642-4644.

Some modifications were made to the original paper for inclusion as a chapter in this thesis. For example, the supporting information was incorporated in this chapter and some figures have been added. Also, it should be noted that there are similarities between the introduction to this chapter and the introduction to Chapter 3.

4-1 Introduction

The transformation of biomass into useful chemicals has become an important area of research, as a way to reduce global dependence on fossil fuel resources (see section 1-1).[1] Conversion of carbohydrates and their derivatives into useful materials is one such area (e.g., production of 5-hydroxymethylfurfural (5-HMF) from glucose and fructose) (see section 1-14-1-1). Of course, as carbohydrates typically contain C, H, and O only, products from these processes are typically small molecule oxygenated species. Therefore, attempts described herein were made to transform amino-sugars in order to produce N-containing molecules. NAG is the monomer unit of the polysaccharide chitin from which it can be obtained. Chitin is naturally abundant and can be found in the shells of crustaceans (e.g., waste from the fishing industry, and exoskeletons of insects) (see section 1-2). The recently formed American Chemical Society Green Chemistry Institute Formulator's Roundtable has highlighted greener small amines, including those sources from renewable feedstocks, as highly desirable for the consumer products industries.[2] Ionic liquids (ILs) have been used quite widely in the dehydration of carbohydrates.[3] ILs can be considered 'green' solvents under certain conditions, as they are normally non-volatile, non-flammable, and potentially re-usable reaction media (see section 1-11-3).[4] They can also act as catalysts in reactions. Several are known to dissolve cellulose and other sugars, which makes them ideal reaction media for studying the reactivity of hexoses. Previously, 3A5AF has been obtained as one of the major products from the thermal degradation of NAG, albeit in only 2% yield (see section 1-14-3).[5,6] The work reported herein represents a feasible ionic liquid/solution phase method for the direct

conversion of NAG to 3A5AF, Figure 4-1. From this foundation, the chemistry and transformations of 3A5AF can be studied and potentially lead to new renewable amines in the future. Recent research by others on the formation of "renewable" amines has employed ammonia as the source of nitrogen.[7] The work presented here represents a source of biologically-fixed nitrogen in the product and this is the first report of its kind. In Chapter 2, the reaction conditions and the solvent medium deaminated the glucosamine. As a result, neither of the products (LA or 5-HMF) is a N-containing compound (see section 2-2-5). Retaining nitrogen in the production process is therefore a challenge. In Chapter 3, a process in which the nitrogen atom was retained in the final product (3A5AF) was described. The reaction medium used was DMA and this is a toxic solvent with some carcinogenicity issues. In the current chapter, ILs were used as the reaction media and 3A5AF was successfully produced. ILs are good alternatives to organic solvents because they have low vapour pressures and are non-flammable. In terms of price, DMA is cheaper than ILs. However, the invention of a second route to produce 3A5AF shows that there is versatility and a number of ways to make this compound (see section 4-3).



N-acetylglucosamine (NAG) 3-acetamido-5-acetylfuran (3A5AF)

Figure 4-1. Direct conversion of NAG to 3A5AF using a combination of an imidazolium ionic liquid and microwave heating.

4-2 Results and Discussion

In a typical reaction, 100 mg of NAG and 750 mg of IL were mixed and warmed for 1 min in a warm water bath until a homogeneous solution formed. The reaction mixture was then microwave-heated for the appropriate amount of time. An aliquot was extracted with ethyl acetate and analyzed via GC-MS using acetophenone as the internal standard. The dehydration process was first studied at 120 °C under additive-free conditions, Table 4-1. Six different ILs were used with various alkyl chains and anions.

These included 1-ethyl-3-methylimidazolium bromide ([EMIm]Br) and acetate ([EMIm]OAc), and 1-butyl-3-methylimidazolium chloride ([BMIm]Cl), bromide ([BMIm]Br), and acetate ([BMIm]OAc), and 1-butyl-2,3-dimethylimidazolium chloride [BMMIm]Cl, Figure 4-2. NAG was found to be readily soluble in all the ionic liquids studied under the experimental conditions employed.

Table 4-1. Dehydration of NAG using varying solvents.[a]

Entry	Solvent	*T* (°C)	Yield 3A5AF (%)[b]
1	[BMIm]Cl	120	14.1
2	[BMIm]Cl	180	25.5
3	[BMIm]Br	120	4.7
4	[BMIm]OAc	120	trace
5	[EMIm]Br	120	trace
6	[EMIm]OAc	120	trace
7	[BMMIm]Cl	180	25.3
8	20 mol % [BMIm]Cl in DMSO-d_6	180	trace [c]

[a]Reaction Conditions : solvent (750 mg), NAG (100 mg, 0.452 mmol), 3 min.

[b]Determined by GC-MS. [c] Analyzed by ^{1}H NMR.

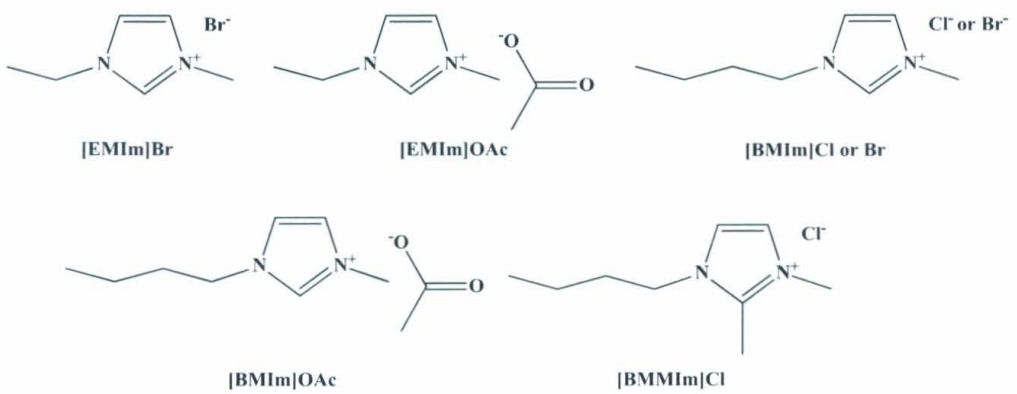


Figure 4-2. Structures of ILs that studied in this chapter ([EMIm]Br, ([EMIm]OAc, [BMIm]Cl, [BMIm]Br, [BMIm]OAc, and [BMMIm]Cl.

The reaction of NAG in [BMIm]Cl at 120 °C gave 14.1% yield of 3A5AF following 3 minutes of microwave (MW) heating. A 25.5% yield was obtained at 180 °C under the same conditions, but prolonged heating (longer than 3 min) or higher temperatures were found to decrease the yield of product, most likely through decomposition of 3A5AF via accelerated side reactions.

The anion within the ionic liquid was found to have a profound effect on reactivity. The incorporation of a chloride counterion (within the IL) was found necessary to form significant quantities of 3A5AF with trace or low yields obtained when bromide or acetate ILs were used (Table 4-1, entries 1, 3-5). This has been observed in the dehydration of glucose/fructose to 5-HMF using imidazolium ILs, where high conversions were obtained in the presence of a loosely bound chloride ion.[8] Chloride ion concentration has also been shown to increase conversions in aqueous transformations of cellulose and hexoses.[9,10] Alkyl chain length on the cation was found to slightly affect yield. In the case of entries 3 and 6, it was found that [BMIm]Br was more able to facilitate the dehydration than the ethyl equivalent. [BMIm]Cl and [BMMIm]Cl (entries 2 and 8) showed equal activity towards 3A5AF formation. In previous research, using fructose as the feedstock, 0% yield of 5-HMF was obtained in [BMMIm]Cl whereas 63% yield was obtained in [BMIm]Cl.[11] It has been proposed that the acidic protons on the imidazolium ring help to catalyze the dehydration reaction. In these studies using [BMMIm]Cl, substitution of a methyl group at the C2 position removes the most acidic proton of the imidazolium cation. Therefore, the protons at the C4 and C5 positions must play a larger role in this conversion process for NAG compared with fructose. This

difference may be due to the basic nitrogen atom within NAG and its absence in fructose. As ionic liquids are expensive and can be toxic, the reaction in [BMIm]Cl was carried out with only catalytic amounts of the IL partnered with a co-solvent, entry 8 in Table 4-1, but unfortunately, only a trace amount of product was detected.

Table 4-2. Dehydration of NAG in [BMIm]Cl with different additives.[a]

Entry	Additive	T (°C)	Yield of 3A5AF (%)[b]
1	none	180	25.5
2	water	120	28.7
3	1-methylimidazole	120	2.9
4	$B(OH)_3$	180	60.0[c, d]
5	NH_4OH	180	30.9
6	NH_4Cl	180	25.3
7	HCl	180	24.1
8	ZrO_2/SO_4^{2-}	180	10.3
9	DBU[e]	180	16.5
10	DABCO[f]	180	16.6
11	$K2CO3 \cdot 1.5H_2O$	180	24.3
12	NaOH	180	11.5
13	$CrCl_2$	120	12.1
14	$CrCl_3$	120	12.4
15	$SnCl_4 \cdot 5H_2O$	180	17.8
16	NaCl	180	38.3 [d]

[a]Reaction Conditions : [BMIm]Cl (750 mg, 0.573 mmol) , NAG (100 mg, 0.456 mmol), 10 mol% additive, 3 min (MW). [b]Determined by GC-MS.

[c]Using 2:1 $B(OH)_3$:NAG [d] Heated by oil-bath at 180 °C for 1 h.

[e]1,8-Diazabicycloundec-7-ene. [f]1,4-diazabicyclo[2.2.2]octane.

To further the study, additives were screened in hopes of increasing product yield, Table 4-2. The addition of water (entry 2) did not affect the yield of product. This is important for biomass transformations where feedstocks are unlikely to be 100% dry. GC-traces of the EtOAc extracts from the reactions showed the presence of 1-methylimidazole and 1-butylimidazole, presumably from decomposition of the IL under reaction conditions. If additional 1-methylimidazole is added at the beginning of the reaction, the yield of 3A5AF is dramatically reduced (entry 3). Use of a more inert reaction medium would therefore be highly desirable, as the presence of IL decomposition products are likely inhibiting the reaction. Future research will focus on using more thermally stable or supported ionic liquids in this reaction. However, it should be noted that 3A5AF could be isolated in an analytically pure form using flash chromatography (section 4-5-2). A wide range of basic and acidic additives were studied (entries 4-12), and with the exception of boric acid, yields of 3A5AF of between 10 and 30% were obtained. Metal salts proved ineffective at increasing the yield of 3A5AF under the reaction conditions employed. This result was surprising given the high catalytic activity of chromium (II)/ (III) chlorides in the dehydration of fructose.[12] This difference might be due to the presence of nitrogen in the substrate, which would coordinate strongly with the transition metal and inhibit turnover within the catalytic cycle.

$B(OH)_3$ afforded the highest yield of 3A5AF (Entry 4). Of particular relevance to this work, Riisager et al. reported $B(OH)_3$ mediated dehydration of glucose to 5-HMF using ionic liquids.[10,13] A yield of up to 42% from glucose and as much as 66% from sucrose was obtained. $B(OH)_3$ has also been used as a selectivity inducer in glycerol

hydrogenolysis via formation of an intermediate borate ester.[14] $B(OH)_3$ acts as a Lewis acid in aqueous solution, resulting in the formation of a tetrahydroxyborate complex, which, upon the addition of a hexose, e.g., glucose or NAG, forms a doubly coordinated borate-hexose complex.[13] The formation of this complex helps to shift the hexose/aldose equilibrium towards the right, resulting in the release of acidic protons which aid in the dehydration process.

To study the effect of $B(OH)_3$ loading, three reactions were screened using 10, 100, and 200 mol% boric acid, yielding 33.5, 44.5, and 60.0% yield of 3A5AF respectively. For comparative purposes, the reaction using 200 mol% $B(OH)_3$ was repeated using conventional heating (180 °C for 1 h) and 60.0% 3A5AF was obtained. After purification, isolated yields of 57-58% could be obtained. Overall, as the amount of boric acid was increased, the yield of 3A5AF increased, presumably due to both formation of a borate-hexose complex and also increased acidity of the reaction mixture. It is also interesting that a larger quantity of boric acid is optimum for this reaction compared with glucose. Again, this is likely due to the presence of the basic nitrogen atom in the substrate.

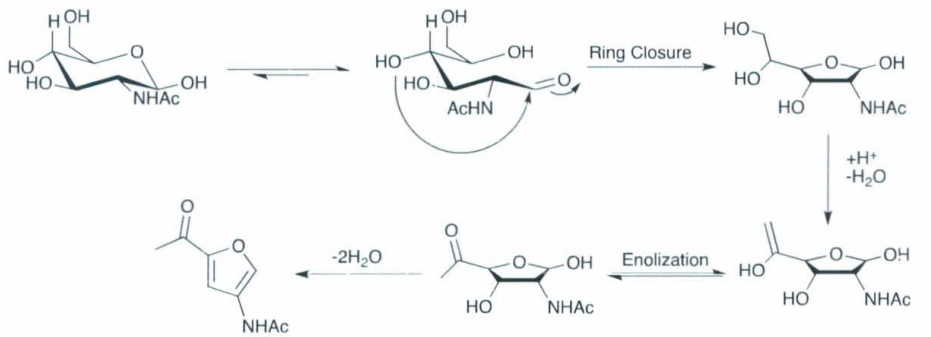


Figure 4-3. Possible mechanism for the formation of 3A5AF from NAG.

The mechanism for this reaction likely has much in common with previously studied fructose and glucose dehydration processes. For example, dissolution of NAG in an IL leads to a disruption of the hydrogen bonds between sugar molecules. Chloride ions are thought to be important in this process and addition of NaCl to the reaction mixture led to a moderate increase in yield of 3A5AF (entry 16). Acidic protons present on the imidazolium ring (or the added $B(OH)_3$) are proposed to interact with the hydroxyl oxygen of the sugar to give a complex, which increases the concentration of the open chain aldose form of the sugar. Next, nucleophilic attack by a hydroxyl group affords the 5-membered heterocycle, which undergoes subsequent dehydration and keto-enol tautomerization to give the product. To help probe the mechanism and the sugar dissolution, the reaction was followed by ^{1}H NMR and the shifts of the three imidazolium protons studied. In the case of H2, H4, and H5 a gradual shift to higher frequency was indeed noted as the reaction progressed. This observation is linked to increased hydrogen bonding with H-bond acceptors (sugar hydroxyl groups) resulting in the deshielding of the acidic imidazolium protons. However, it could also be due to H-bonding with the water released during the reaction. Kinetic studies were performed to assess the activation energy and pre-exponential factor associated with the decomposition of NAG in the absence of additive. As such, reactions were performed at 140, 160, 180, and 200 °C and the concentration of NAG monitored using ^{1}H NMR. In a typical reaction, [BMIm]Cl (1.00 g, 5.75 mmol) and 33 wt % NAG (424 mg, 1.92 mmol) were mixed. The sample was heated using an oil bath and an aliquot taken (20-50 mg) at the desired time. To this sample, was added 3.00 μL acetophenone (internal standard) and 600 μL DMSO-d_6. ^{1}H NMR spectra were obtained and the amount of NAG measured using the added internal

standard (section 4-5-6). The decomposition reaction data at 140, 160, 180, and 200 °C were fitted to first order rate plots, yielding linear correlation coefficients (R^2) close to unity (section 4-5-8). Table 4-3 shows the kinetics data including $\ln(k_{obs})$ and 1/T at the four temperature set shown above.

Table 4-3. Kinetics data for the Arrhenius plot.

Entry	Temperature (Kelvin)	1/T (Kelvin^{-1})	K_{obs}	$\ln(K_{obs})$
1	413	0.00242	0.004274	-5.4552
2	433	0.00230	0.01612	-4.1277
3	453	0.00220	0.03675	-3.3036
4	473	0.00211	0.09528	-2.3509

Through a plot of ln (k_{obs}) vs 1/*T*, the energy of activation and pre-exponential factor were determined to be 82.8 kJ/mol and 1.34 x 10^8 min^{-1}, respectively.

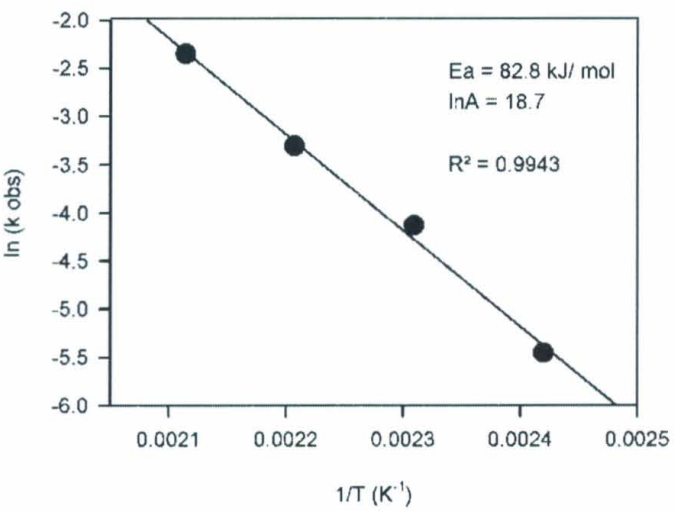


Figure 4-4. The Arrhenius plot for the conversion of NAG into 3A5AF in [BMIm]Cl.

Z. Qi and M. Watanabe et al. calculated the activation energy and pre-exponential factor to be 114.6 kJ/mol and 3.54 x 10^{14} min^{-1}, respectively, for the conversion of glucose to 5-HMF using $CrCl_3$ in [BMIm]Cl under microwave irradiation.[15] Although the two processes differ it is important that the activation energy values are of roughly equal magnitude, as they both involve the dehydration of a hexose molecule. However, the pre-exponential factor determined from this work is six orders of magnitude lower than the value reported for glucose. This is to be expected, as a microwave-heated reaction should have a larger pre-exponential factor compared with a conventionally heated one because of an increased number of collisions among reactant molecules.[16] For the comparison of the % yield of 3A5AF with the literature, see section 3-2-5.

4-3 3A5AF Production Comparison: Chapter Three vs Chapter Four

In Chapters 3 and 4, two different media were studied to prepare 3A5AF, DMA and [BMIm]Cl, respectively. Generally, two routes of production are better than one, from an industrial point of view, to overcome any future shortages in chemicals. For example, in 2008, the shortages in acetonitrile production affected industrial and academic work especially in the LC analytical field. At that time, the price of the acetonitrile jumped from hundreds to thousands dollars per 16 L.[17]

In DMA, the optimum reaction time was relatively short (15 min). In [BMIm]Cl, the reaction time was even shorter (3 min). In general ILs are not cheap. For example, 50 g of 98% and 25 g of 99% [BMIm]Cl[18] cost ca. $ 110 and $ 430 CAD, respectively. Whereas, the cost of 2 L of 99.9% DMA[19] is ca. $ 95 CAD, which is a lot cheaper

compared to [BMIm]Cl . In terms of toxicity, DMA has acute and chronic effects on human health and it has a negative environmental impact.[20] Therefore, this solvent should be substituted wherever possible. However, dipolar aprotic solvents including DMA are difficult to replace with an alternative solvent[21] (see sections 1-12 and 3-2-1). ILs have less environmental impact on air because they possess low vapor pressures (are less volatile). On the other hand, ILs have toxicity issues related to water contamination and some of them are non-biodegradable (see section 1-11-3). The relationship between the toxicity and biodegradability of ILs are conflicting in terms of the length of an alkyl chain i.e. by increasing the alkyl chain length on the cation counterpart, the toxicity and biodegradability increase simultaneously. In both methods, 3A5AF can easily be extracted using ethyl acetate and purified using flash chromatography. In Chapter 3, when the reaction was run at 250 °C, the chromatogram (Figure 3-15a) showed purer product compared with the chromatogram at 220 °C (Figure 3-15b). In that case, potentially, there would be less need for purification. This would save a lot of solvent use regardless of the inferior productivity at 250 °C (Figure 3-14) but the increase in temperature (from 220 °C to 250 °C) could increase energy consumption.

4-4 Conclusions

It has been shown that the N-substituted furan, 3A5AF, can be obtained in good yield from the dehydration of NAG in an imidazolium based ionic liquid. These data contrast with those reported in Chapter 2 where levulinic acid was obtained as the primary product through transformations of aminocarbohydrates (glucosamine, chitosan, and chitin) in aqueous media.[22] Although, some clues concerning the mechanism have been obtained, more detailed studies are needed. Initial studies suggest that there are both similarities and significant differences between this process and previously reported reactions using fructose and glucose. In the future, 3A5AF should be investigated as a source of renewable amines, and as a high-value precursor to proximicins (biologically active compound) of which it is a structural motif.[23]

4-5 Experimental

4-5-1 Materials

NAG (95%) was purchased from Toronto Research Chemicals. 1-butyl-3-methylimidazolium chloride ([BMIm]Cl), 1-butyl-2,3-dimethylimidazolium chloride ([BMMIm]Cl), chlorobutane, bromoethane, 1-methylimidazole, and silver acetate were purchased from Alfa-Aesar. All solvents and chemicals were used as obtained from commercial suppliers, unless otherwise indicated. Centrifugation was performed on an Eppendorf centrifuge 5430 (2500 rpm, 1 min). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 and 300 MHz spectrometers. Gas chromatography-mass spectrometry (GC-MS) was performed on an Agilent 7890A GC system coupled with an Agilent 5975C MS detector that was equipped with a capillary column DB-5 (column length: 30.0 m and column internal diameter: 0.25 mm). Microwave reactions were performed using a Biotage Initiator 2.5 microwave reactor (0.5 – 2.0 mL reaction volume vials. The 'very high' absorption level setting was used each time to ensure controlled heating of the reaction mixture. IR Spectra were recorded using a Bruker Alpha FTIR spectrometer (4 cm^{-1} resolution) using a diamond ATR single reflectance module (24 scans).

4-5-2 General Procedure

In a typical reaction: NAG (0.100 g, 0.426 mmol) and [BMIm]Cl (0.750 g) were placed in a MW vial. This mixture was warmed to 100 °C for 1 min using a water bath to melt the ionic liquid. The reaction mixture was subsequently loaded into a Biotage Initiator MW reactor and heated for an appropriate amount of time. HPLC grade EtOAc was used

to extract the mixtures and prepare samples for analysis, as described below (section 4-5-3). The given extracts ranged from pale yellow to golden brown. For isolated yields: 3A5AF was purified using flash chromatography (FC) Biotage Isolera One. A silica column (SNAP cartridge, silica, 10 g) and a variable 200-400 nm wavelength detector at 254 and 293 nm were used. The sample was previously analyzed on SiO_2 TLC plates using a 50% ethyl acetate/hexanes mixture and the obtained retention factor (R_f) was used to build up a separation method using the FC instrument. The R_f was 0.13. Samples of the product were combined. After evaporating the solvent using the evaporator at 50 °C until dryness, the dried residue was placed vacuum using a standard Schlenk line overnight. CHN microanalytical data (Canadian Microanalytical Service, Delta, BC) were in agreement with the formulation $C_8H_9NO_3$. For three reactions performed under identical conditions (Table 4-2, entry 4), isolated yields were 57-58%.

4-5-3 Gas Chromatography-Mass Spectrometric (GC-MS) Determination of 3A5AF Yields

A typical reaction mixture was unsealed and an aliquot taken (ca. 5-10% w/w). 100 µL of (2.00 mg/mL) acetophenone (internal standard) was added along with 500 µL of deionized water and 3.00 mL HPLC grade EtOAc. The reaction was centrifuged at 2,500 rpm for 1 min, and the organic layer decanted and kept. Two further extractions with 3.00 mL EtOAc were performed yielding a total of ca. 9.00 mL of extract, which was concentrated on a Buchi Rotavap yielding pale yellow oil. This was diluted with 500 µL of EtOAc and analyzed via injecting 1 µL in GC-MS (Agilent Technologies 5975C VL MSD). The retention times for acetophenone and 3A5AF were 5.49 and 7.95 min,

respectively, Figure 4-5. For quantification purposes, the calibration curve shown in Figure 4-6 was used.

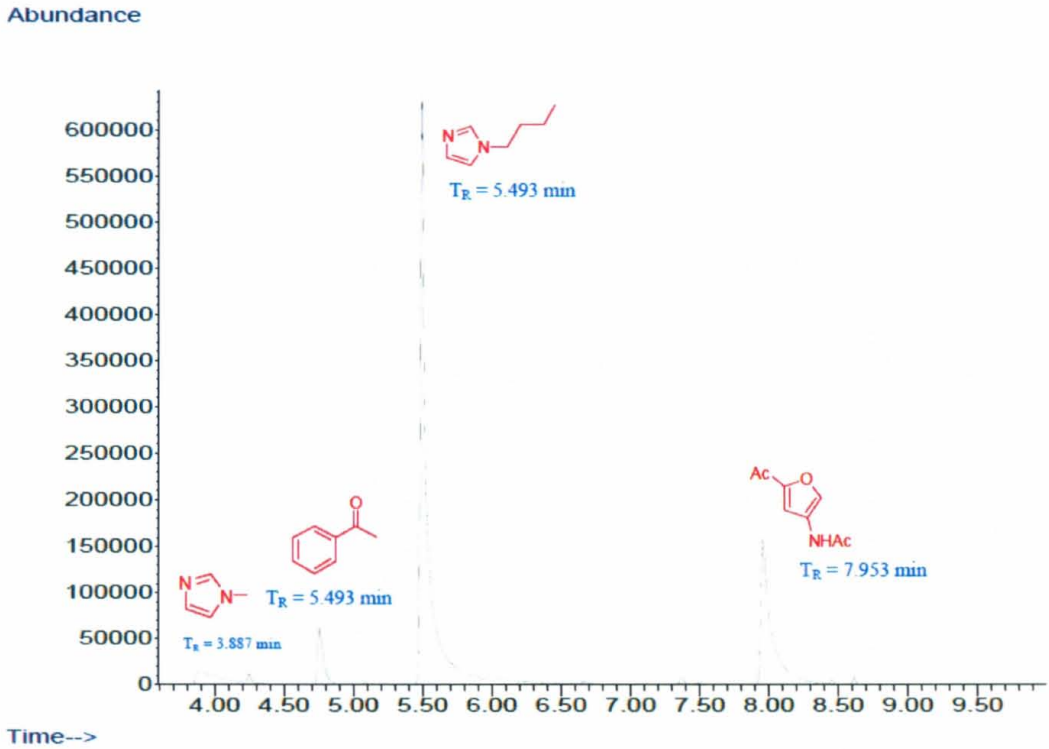


Figure 4-5. Sample GC-MS chromatogram of a representative reaction mixture producing 3A5AF from NAG and 1-butyl-3-methylimidazolium chloride.

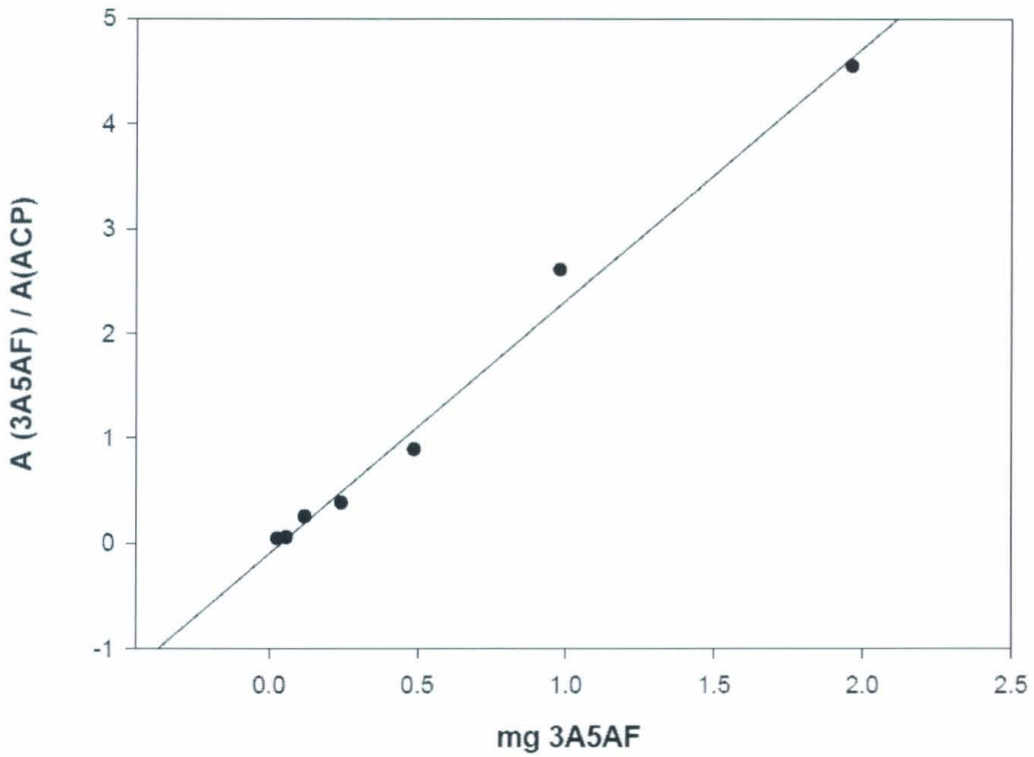


Figure 4-6. Calibration curve for 3A5AF in EtOAc (R^2 = 0.9899), where ACP = acetophenone.

4-5-4 Identification of 3A5AF

MS, *m/z* 167 (49%), 125 (88), 110 (100), 96 (15), 83 (17), 69(9), 54(13), Figure 4-7.

^{1}H NMR δ_H (298K, 500 MHz, DMSO-d_6) 2.02 (s, 3H), 2.40 (s, 3H) 7.18 (d, J = 0.8 Hz, 1H), 8.18 (d, J = 0.8 Hz, 1H), 10.23 (s, 1H), Figure 4-8.

^{13}C NMR δ_C (298K, 75 MHz, DMSO-d_6) 22.81, 25.85, 110.99, 127.03, 135.25, 149.75, 167.88, 186.04, Figure 4-9.

HRMS, calculated exact mass for 3A5AF ($C_8H_9NO_3$) = 167.0582, found = 167.0584.

Selected IR ν_{max}/cm^{-1} 1668 br, s (C=O stretch in amide I band and C=O stretch in ketone).

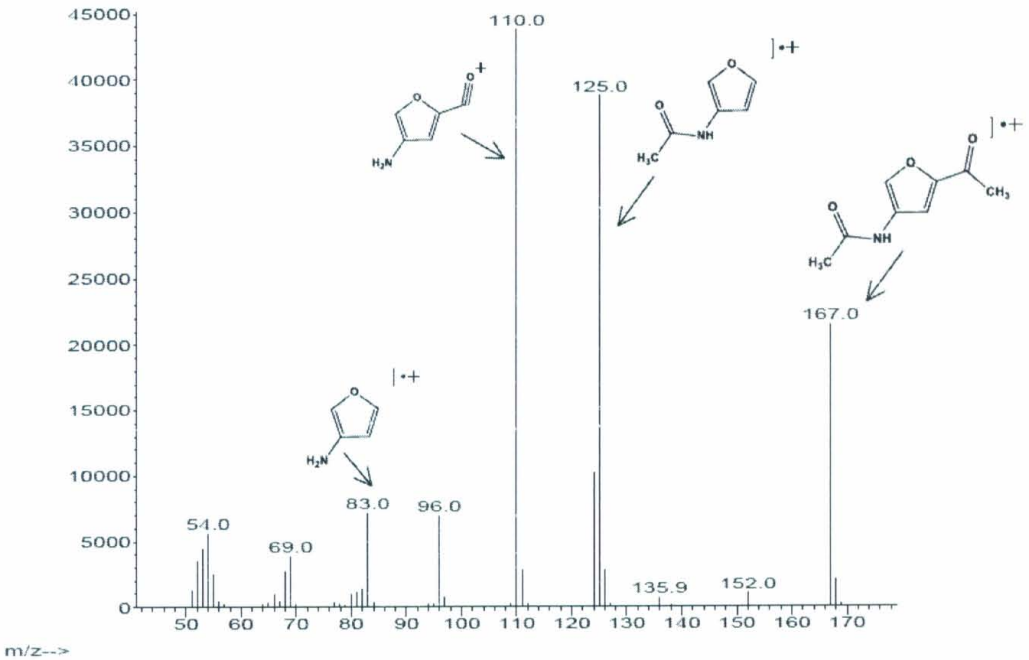


Figure 4-7. Mass spectrum of 3A5AF showing both the base peak and molecular ion peak, along with other peaks of interest.

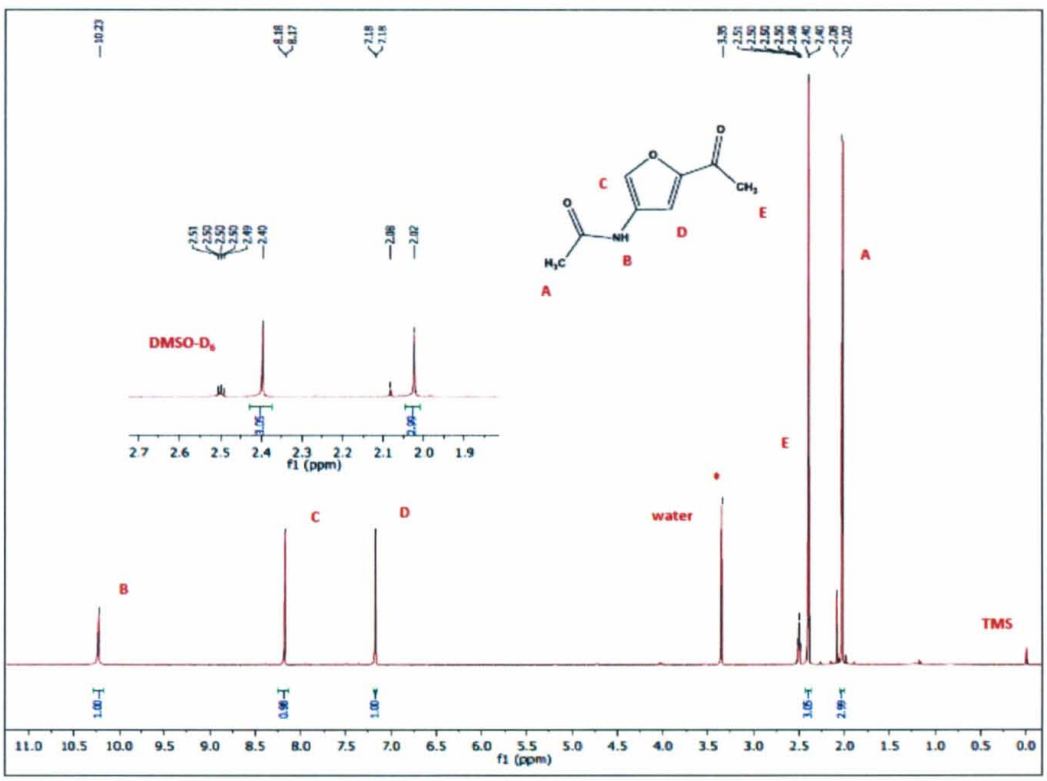


Figure 4-8. ^{1}H NMR spectrum showing the peaks of 3A5AF in DMSO-d_6.

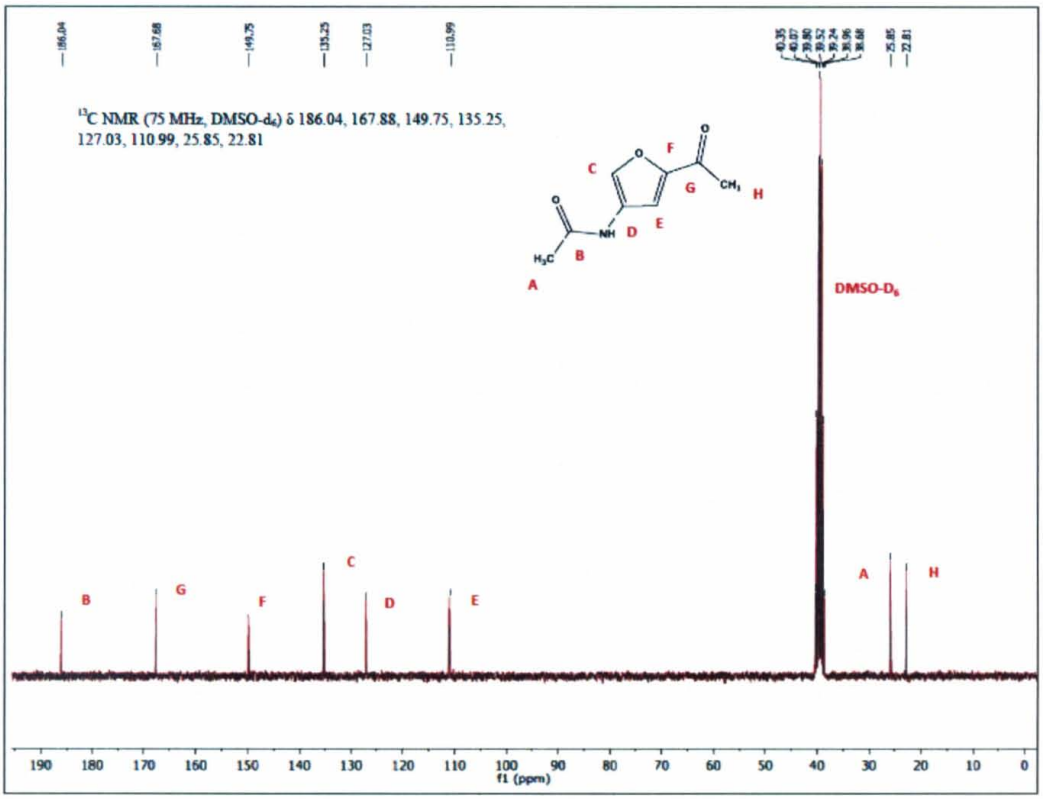


Figure 4-9. ^{13}C NMR spectrum showing the peaks of 3A5AF in DMSO-d_6.

4-5-5 Timed Reactions at 200 °C

In Figure 4-10, strong peaks found between ca. 0.25 – 2.25, 4.25, 7.50, and 9.25 ppm are assigned to [BMIm]Cl; mid-field peaks found in the 4.25 – 7.25 ppm region are characteristic peaks attributable to NAG. The peaks at ca. 2.00, 2.40, 7.25, 8.25, and11.00 ppm are typical signals of 3A5AF. As reaction time is increased, the peaks in mid-field decrease in intensity, while those of the product begin to increase and those of the ionic liquid remain the same. The spectra recorded after 24 min shows the peaks of NAG to be nearly gone. No other products were identifiable during this process. Likewise,

resonances due to acetophenone are also present at ca. 2.51 and 7.50-7.75 ppm (used as an internal standard). Also note the above reaction consisted of 33 wt% NAG (ca. 425 mg) in 1.00 g [BMIm]Cl at 200 °C. * - Indicates product formation.

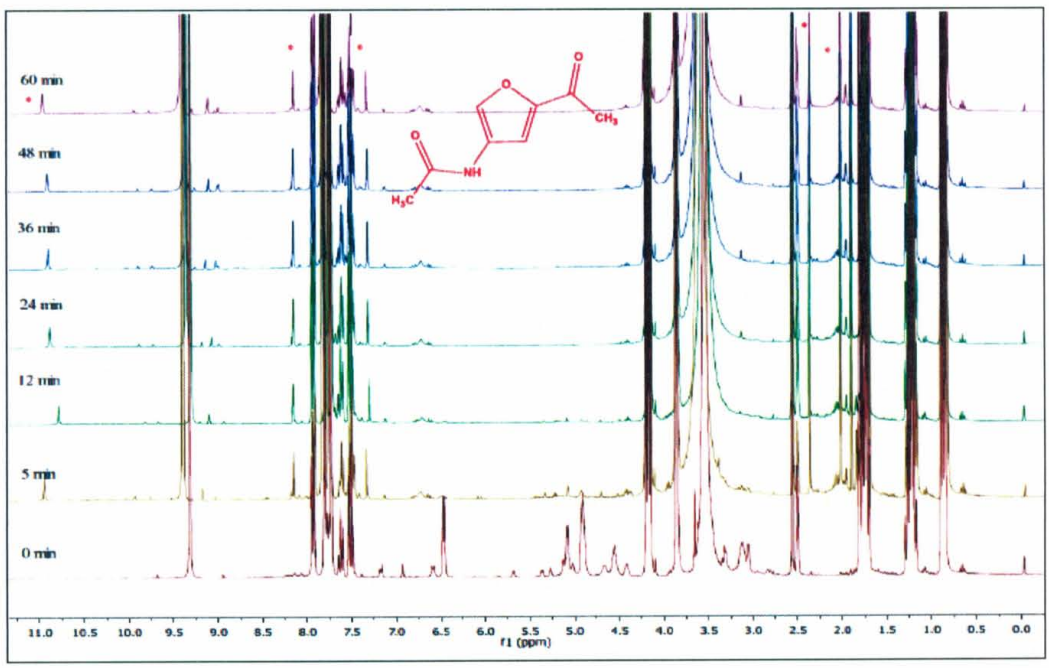
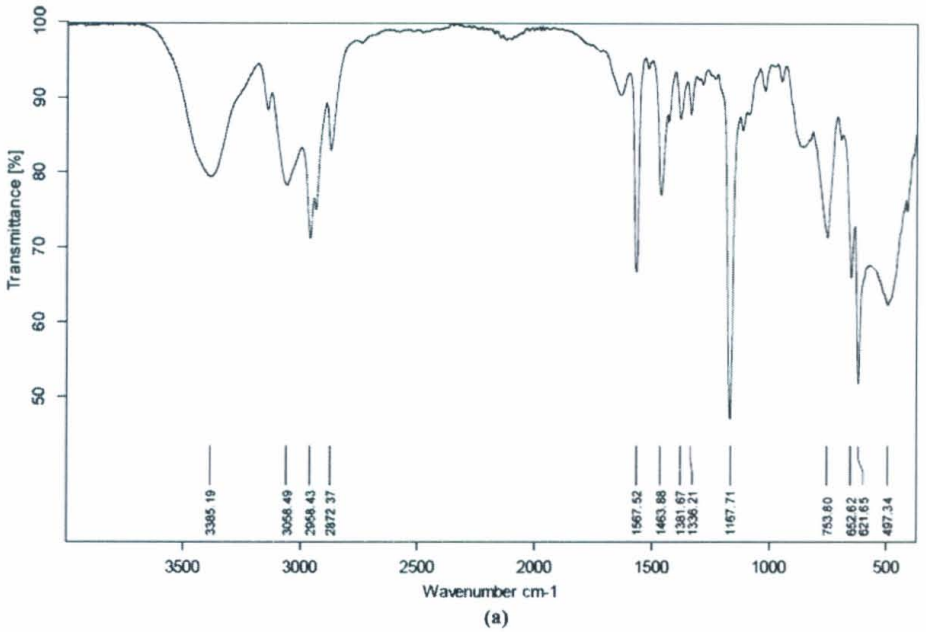


Figure 4-10. Sample stacked ^{1}H NMR spectra showing peaks for [BMIm]Cl between ca. (0.25 – 2.25, 4.25, 7.50, and 9.25) ppm, NAG (4.25 – 7.25) ppm and 3A5AF (2.00, 2.40, 7.25, 8.25, and11.00) ppm.

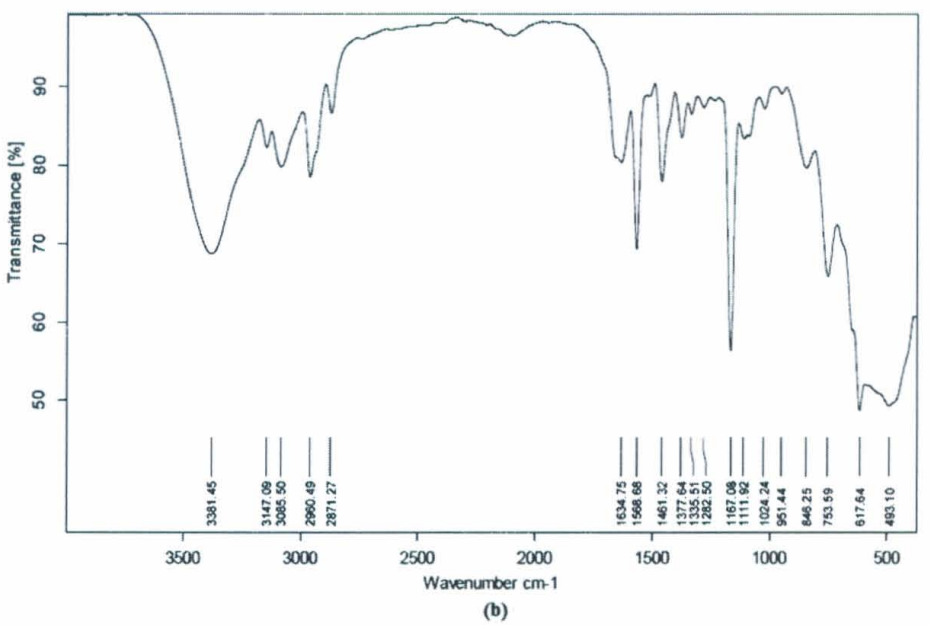
4-5-6 NMR Sample Preparation Procedure for Kinetic Data

33 wt% of NAG (425 mg) was added to 1.00 g of [BMIm]Cl in a vial, and the reaction was heated using an oil bath at the given temperature for 2 h. After a given amount of time, an aliquot of the reaction mixture was removed (20-60 mg) and to it was added 3.00 μL (3.0-4.5 mg) of acetophenone in a small vial. A 500 μL aliquot of DMSO-d_6 was then added and the appropriate NMR experiment was run. With an accurately measured amount of reference material, the sugar (or material of interest) could be integrated to 1 and the methyl group of acetophenone accordingly integrated. Bearing this in mind, the amount of sugar could be calculated at any given time, t, allowing for the plotting of first order rate plots, thereby affording a method to identify the activation energy for the decomposition of NAG.

4-5-7 Ionic Liquid Recyclability Study ([BMIm]Cl before (a) and after (b) reaction)

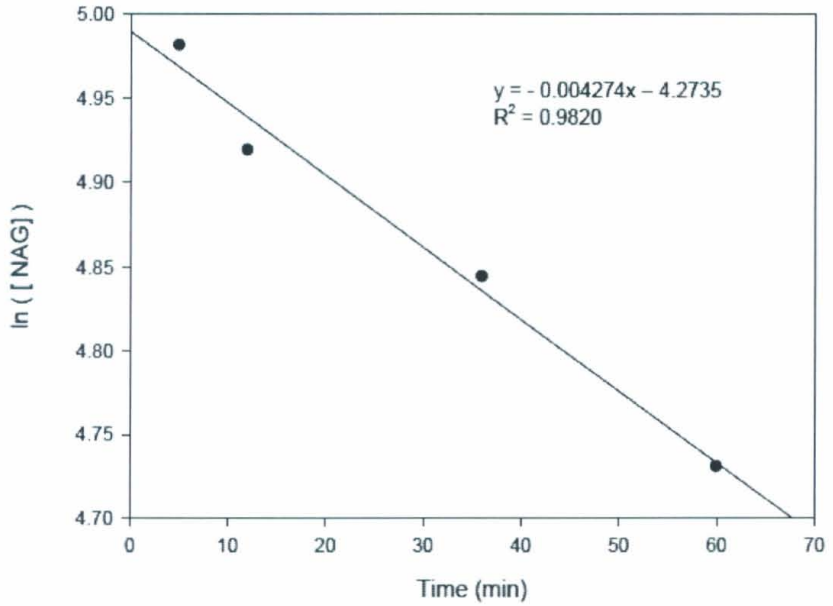


(a)

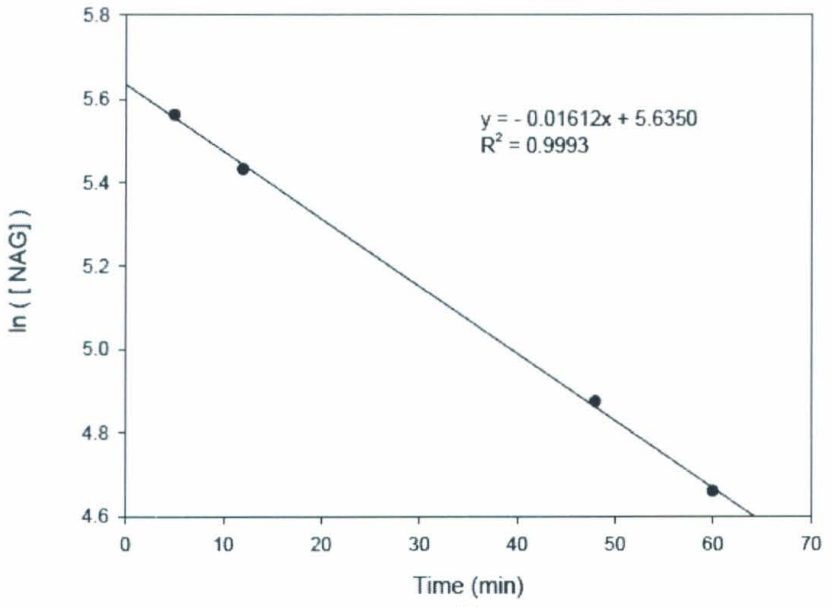


(b)

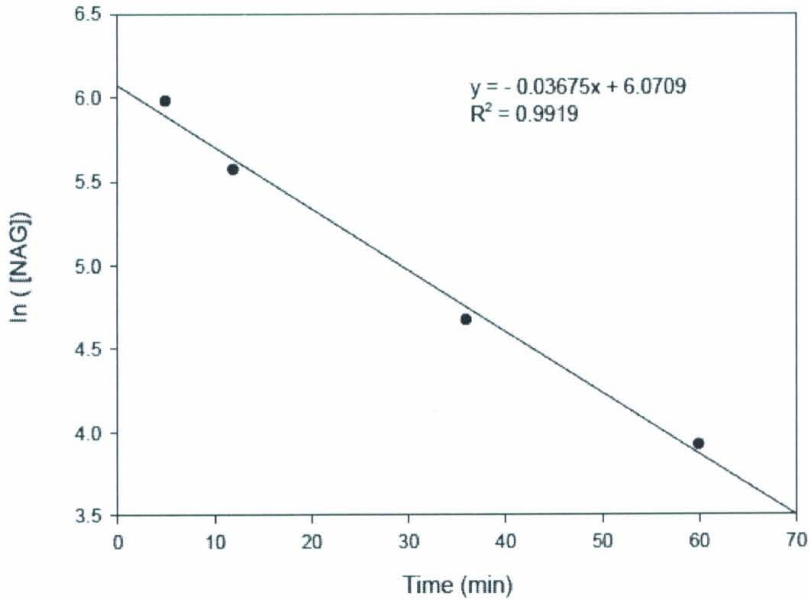
4-5-8 First-Order Rate Plots at (a) 140, (b) 160, (c) 180, and (d) 200 °C



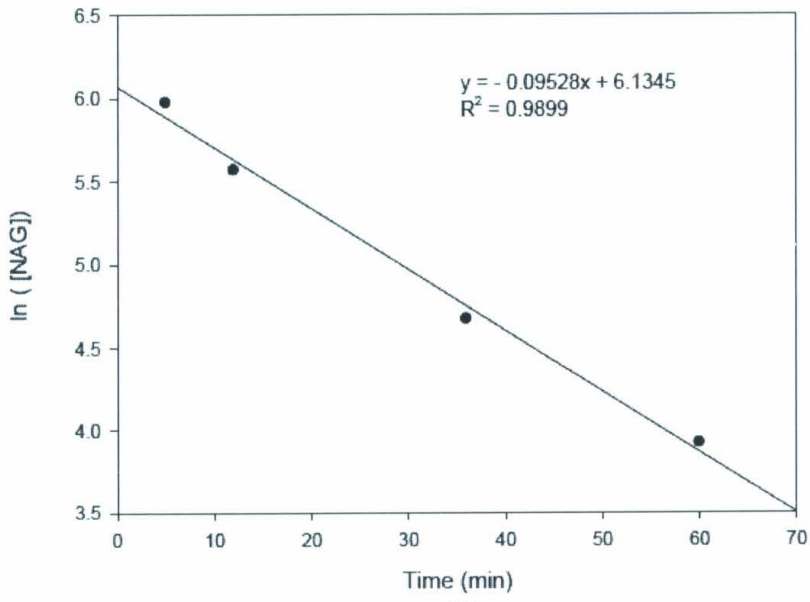
(a)



(b)


y = - 0.03675x + 6.0709
R² = 0.9919
ln ([NAG])
Time (min)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
0
10
20
30
40
50
60
70

(c)


y = - 0.09528x + 6.1345
R² = 0.9899
ln ([NAG])
Time (min)
6.5
6.0
5.5
5.0
4.5
4.0
3.5
0
10
20
30
40
50
60
70

(d)

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Chapter Five

Conclusions and Future Research

5-1 Conclusions

5-1-1 Chapter One

This chapter provided an introduction to chitin, chitosan, and their monomers. Some of the green aspects of the research conducted during this PhD were discussed including catalysts, solvents, and renewable feedstocks. Microwave heating as an alternative to conventional heating was introduced, and its role in relation to the use of alternative solvent media described. The production and applications (both potential and realised) of several useful chemicals from renewable feedstocks (i.e. LA, 5-HMF, and 3A5AF) were described. Several methods for the production of small organic molecules from carbohydrates described in the literature, including their mechanism of formation, were debated in terms of producing useful chemicals from renewable feedstocks. Gas chromatography-mass spectrometry (GC-MS) was introduced as a qualitative and quantitative technique for wt% and % yield determination of products.

5-1-2 Chapter Two

The title of this chapter is "Hydrolysis of Chitosan to Yield Levulinic Acid and 5-Hydroxymethylfurfural in Water Under Microwave Irradiation".

This study demonstrated that chitosan could be used to produce LA and 5-HMF in water under microwave conditions. This work is a proof-of-principle that N-containing biopolymers can be degraded, using green chemistry principles, to give useful chemical building blocks in a similar way to cellulosic feedstocks. The volume of water used and the loading of $SnCl_4 \cdot 5H_2O$ can be varied to produce either LA or 5-HMF with good

selectivity. Factorial design was successfully employed to optimize the reaction conditions for this process. Microwave irradiation proved to be a more effective heating method for the generation of these small molecules compared to conventional heating, as 5-HMF could not be generated effectively under the conditions studied using the latter condition. A mechanism for this process was proposed based on the known chemistry of cellulose and glucose, and some studies using glucosamine. Furthermore, evidence for oligosaccharide intermediates was obtained using MALDI-TOF MS. The last step in the mechanism was performed under microwave irradiation to convert 5-HMF to LA in high yields in water. The method developed for chitosan could be extended to the more robust parent carbohydrate chitin from which LA could also be obtained, albeit in smaller quantities. The results of this study will hopefully allow the possibility of chemical and thermochemical transformations of the non-toxic and cheap biopolymers, chitosan and chitin, to yield useful, sustainable chemicals with possible industrial applications. Studies will continue in the Kerton research group using chitosan, chitin, and amino-sugars as feedstocks in different solvents (e.g., ionic liquids and employing a wide range of catalysts).

5-1-3 Chapter Three

The title of this chapter is "A Simple One-Pot Dehydration Process to Convert *N*-acetyl-D-glucosamine into a Nitrogen-Containing Compound, 3-acetamido-5-acetylfuran".

Up to date, this study produced the highest % yield of 3A5AF (57.7) in the literature, in a reasonable low (15 min) run time. The productivity of 3A5AF was thirty times more than the pyrolysis method employed by Franich et al. (1984) Both NaCl and $B(OH)_3$ were

found to be very important additives in this reaction and markedly increased 3A5AF production. These findings will allow the chemistry of 3A5AF, a carbohydrate-derived amide, to be further studied. Accordingly, 3A5AF may find use either as a platform chemical, a source of renewable amines, or as a high-value precursor to proximicins and other biologically active compounds.

5-1-4 Chapter Four

The title of this chapter is "Formation of a Renewable Amide, 3-acetamido-5-acetylfuran, via Direct Conversion of *N*-acetyl-D-glucosamine".

It has been shown that the N-substituted furan, 3A5AF, can be obtained in high yield from the dehydration of NAG in an imidazolium based ionic liquid. These data contrast with recently published work from GCCG where levulinic acid was obtained as the primary product through transformations of aminocarbohydrates (glucosamine, chitosan, and chitin) in aqueous media.[16] Although some clues concerning the mechanism have been obtained, more detailed studies are needed. Initial studies suggest that there are both similarities and significant differences between this process and previously reported reactions using fructose and glucose. 3A5AF could perhaps be used as a source of renewable amine, and as a high-value precursor to proximicins, which are biologically active compounds.

5-2 Future Work

Producing useful chemicals from renewable resources is a highly topical area of research. Some ideas for extending this research are outlined briefly in the sections above. Biocatalysis is a successful union of chemistry and biology. Biocatalysts can be more efficient than heterogeneous or homogeneous catalysts (see section 1-13) in transforming biological feedstocks. Most biocatalysts are enzymes, which can be modified to function at certain pH and *T*.[1] There are specific microorganisms (e.g., *Escherichia coli, Saccharomyces cerevisiae, and Zymomonas mobilis*) that are popular in industrial biocatalysis. In particular, they are promising in biofuels production technologies.[2] Therefore, enzymes and microorganisms need to be explored in conjunction with chitosan and chitin for production of the molecules described in this thesis.

Chitinases and chitosanases are enzymes that are produced by fungi and bacteria. They are able to hydrolyze chitin and chitosan. As a result, the hydrolysis process, at the glycosidic bond, produces oligomers from chitin and chitosan. The hydrolysis can occur via "exo" or "endo" attack, wherein the breakage takes place at the end of the chain or at any other point in the chain.[3] Figure 5-1 illustrates both exo- and endo-attack of an enzyme on the chitin backbone.

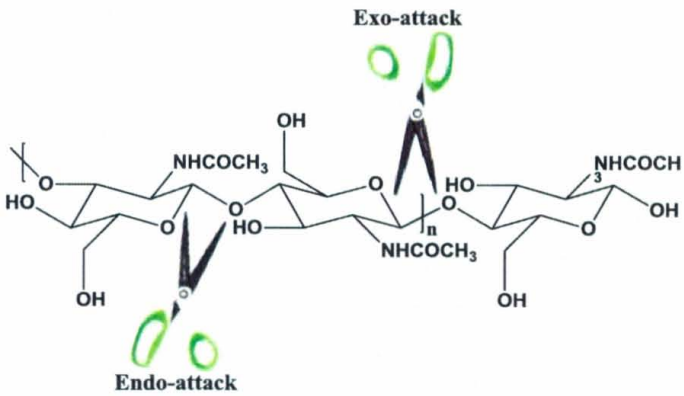

Figure 5-1. Exo- and endo-attacks of enzymes on chitin polysaccharide.

In light of this research, chitinase and chitosanase are worthy of investigation in terms of converting chitin and chitosan into higher value organic molecules. For example, they could be used in a two-step procedure to produce LA or 5-HMF from chitosan. They would produce monomeric sugars in the first step via a biocatalytic digestion and then in a second step, dehydration of glucosamine could be performed using a tin catalyst as described in Chapter 2.[4]

Another option for hydrolysis would be to use scH_2O as the solvent medium. A reaction can take place in scH_2O at a high temperature ca. 400 °C and over a density range of 0.1-0.6 g/mL.[5,6] scH_2O can act as an either acid or a base in the supercritical fluid state (see section 1-11-2). This reaction could be studied without additives to observe the effect of scH_2O on the polysaccharide, as scH_2O could function as a catalyst in this reaction. The effect of additives could also be tested. All the substrates used in this thesis (chitin, glucosamine, and NAG) could be investigated.

In Chapters 3 and 4, $B(OH)_3$ and NaCl were found to be the best additives to produce 3A5AF.[7,8] Previously, pyrolysis had been studied twice and was found to produce 3A5AF.[9,10] In principle, pyrolysis is a method in which a sample, e.g., biomass, is exposed to a high temperature for a short period of time (ca. 2 s) in an oven or other pyrolysis device.[11] The products can be in the form of oil and/or gases. In general, the oil can easily be handled as it remains in the pyrolysis vessel. Catalysts and/or additives can be added to the sample before pyrolysis is performed and reactions can also be performed in a microwave-assisted pyrolysis (MAP) reactor.[12] MAP has several advantages over conventional pyrolysis (e.g., high range of products, fewer side reactions, and higher yields can be obtained). A MAP instrument has a well-controlled temperature over all points in the reactor. Typically, pyrolysis does not consume any solvent (i.e., it is solvent-free). Therefore, it can be considered a benign and environmentally friendly technique. Additives could be added to a feedstock to increase the % yield or enhance the quality of a product (e.g, production of a bio-oil of low-acidity via pyrolysis of lignin in the presence of a zeolite additive).[13] The pyrolysis of NAG could be investigated in both the presence and absence of NaCl and $B(OH)_3$. The parameters under conventional pyrolysis and MAP processes could be optimized (e.g., over a range of temperatures and time).

Pressurised CO_2 has been shown to have a beneficial effect on some reactions including the conversion of biomolecules.[14] Pressurised CO_2 might allow a more environmentally benign solvent to replace DMA in 3A5AF production pathways.

Also in Chapter 3, $B(OH)_3$ was used as an additive in the reaction mixture. A solid form of boron additive could be used in such reactions, such as silica-supported boron

trifluoride,[15] instead of $B(OH)_3$. After the reaction, the additive could be filtered out and reactivated if needed.

Related to the kinetic data for 3A5AF production in ILs, similar could be obtained for the reactions in DMA. Extraction of 3A5AF from the reactions could be performed and quantification of 3A5AF performed via GC-MS. This could be equivalent to the NMR procedure described in Chapter 4. Aliquots of the reaction mixture can be removed by syringe through the septum of the microwave vials after specific times, i.e., Aliquots can be removed from the same vial several times and the reaction continued in the microwave. Furthermore, computational investigations could be performed in order to obtain theoretical data on the thermodynamics of this process. This data would inspire more ideas for further optimization of the reaction.

Scaling the reactions up is important if the process is to become industrially applicable. Each reaction that was studied herein should be investigated on a larger level, especially the 3A5AF production method. Methods which can be applied include performing the reactions in an autoclave or in a continuous reactor. For the time being, 3A5AF can be prepared and purified in the Centre for Green Chemistry and Catalysis (CGCC) on a scale of 0.1 g per reaction in a microwave or 2.0 g in an autoclave.

The processes described in this thesis used ethyl acetate as an extraction solvent. Extraction could be performed using $scCO_2$ (see section 1-12) instead. In this case, there will be no need to expose the solvent and the products to elevated temperatures for evaporation. Reducing the pressure is enough to release CO_2, completely, from the

system. LA and 5-HMF are soluble in $scCO_2$,[16] and 3A5AF, given its structural similarity to 5-HMF, will likely also be soluble in this green solvent.

The research presented in this thesis has proven that chitin, chitosan, and their monomers are potential feedstocks to produce chemicals. In Chapter 2, LA and 5-HMF were the chemicals produced and these can potentially be used in the manufacture of biofuels and other useful chemicals (see section 1-14-1-3 and 1-14-2-3). In Chapters 3 and 4, 3A5AF was the product formed and can be produced in a high yield (ca. 60%) compared with the previously reported pyrolysis methods. This compound could be a key building block in the manufacture of value added chemicals such as proximicins. The vision for future work building on that presented here is to produce such chemicals using greener processes in higher yields, less time, and at lower cost. As a result, this will let the products become commercially viable.

5-3 References

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