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Maastricht University

Faculteit Geneeskunde en Levenswetenschappen
School voor Levenswetenschappen
master in de biomedische wetenschappen

Masterthesis

Synthesis of an acrylamide based synthetic amino-acid library

Toon Goris

Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen, afstudeerrichting bio-elektronica en nanotechnologie

PROMOTOR :
Prof. dr. Wouter MAES

PROMOTOR :
Prof. dr. Tanja JUNKERS

BEGELEIDER :
Dhr. Dries WYERS
dr. Yana DE SMET

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www.uhasselt.be
Universiteit Hasselt
Campus Hasselt:
Martelarenlaan 42 | 3500 Hasselt
Campus Diepenbeek:
Agoralaan Gebouw D | 3590 Diepenbeek

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Acknowledgments

I can remember it as if it was yesterday, the final exam of my educational career. The only thing holding me and my colleagues back from getting our final master diploma was a seven-month internship. At that point, this still sounded like ages. But here we are, at the end of the line finally done after a 5-year run in Biomedical Sciences. These years have brought me nothing but joy, friends, and opportunities for which I am very grateful. Even more so, these experiences that I have gathered tickled an interest in pursuing a further career in science. The multiple internships, especially my senior practical training at the *Polymer Reaction Design* (PRD) group, have given me a clear view over my strengths and weaknesses and made me able to re-evaluate my future career choices. The experiences gathered during these internships have given me new insights on how to approach problems and made me more efficient in solving them. For all these accumulated memories, I would like to thank a few people that made this journey possible.

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Content

List of abbreviations

SUMI:	Single Unit Monomer Insertion
MWD:	Molecular weight distribution
IUPAC:	International Union of Pure and Applied Chemistry
FRP:	Free radical polymerization
RDRP:	Reversible-Deactivation Radical Polymerization
LRP:	Living Radical Polymerization
NMP:	Nitroxide-Mediated Radical Polymerization
RAFT:	Reversible Addition-Fragmentation Chain-Transfer
R:	Leaving group
Pm:	new radical species
SPPS:	Solid Phase Peptide Synthesis
ppm:	Parts per million
NMR:	Nuclear magnetic resonance
s:	Singlet
d:	Doublet
t:	Triplet
m:	Multiplet
FCC:	Flash Column Chromatography
I.D.:	Internal diameter
O.D.:	Outer diameter
Vol.:	Volume
eq.:	Equivalent
THF:	Tetrahydrofuran
n.a.:	Not applicable
CDCl3:	Deuterated chloroform
DMSO:	Dimethyl sulfoxide
ATRP:	Atom Transfer Radical Polymerization
DCM:	Dichloromethane
$NaHCO_3$:	Sodium bicarbonate
Na_2SO_4 :	Sodium Sulphate
1H NMR:	Proton nuclear magnetic resonance
HCl:	Hydrochloric acid
Min:	Minutes
mL:	Millilitres
TLC:	Thin layer chromatography
DMF:	*N, N*-dimethylformamide
DI-BOC:	Di-tert-butyl decarbonate
HPLC:	High Pressure Liquid Chromatography

Abstract

The field of precision polymer synthesis has matured to a point where almost any polymer structure can be synthesized along a single chain. Yet, one grand challenge still remains unsolved: the ability to fully control the monomer sequence. Single unit monomer insertion (SUMI) is a promising technique to address this problem. If the feature of perfect sequence control can still be added to the synthetic polymer toolbox, then artificial materials will become available that match the preciseness of natural polymers such as peptides in their accuracy.

Peptides consist of a short sequence of monomers called amino acids. These macromolecules execute important functions in all organisms as hormones, cell attachment molecules or function as building blocks to create bigger proteins. Nature can produce these molecules in a seemingly effortless way, forming a peptide bond stable in the physiological condition of the cell in which it is present. Recreation of peptide structures and thus the creation of sequence defined polymer chains would mean a massive leap forward in protein research. This recreation process would involve creating synthetic amino acid mimics containing similar functional groups. On top of that, being synthetic molecules, these mimics would be more stable and thus be able to withstand more extreme conditions.

This study involves the synthesis of peptide mimics starting from the production of the basic building blocks up to the creation of an amino acid mimic library used for polymerization studies. A flow setup has been developed capable of synthesizing acryloyl chloride as a basic building block, on a daily basis. Optimization studies and upscaling where performed ensuring a high output of 0.5 L (0.5 M) per day. This acryloyl chloride was used in the synthesis of an acrylamide based amino acid mimic library by combining it with the appropriate amines. In this thesis, the synthesis of such synthetic mimics regarding valine, threonine, phenylalanine, glycine, leucine, aspartic acid, and arginine is described.

Nederlandse samenvatting

Het veld van exacte polymeer synthese is gevorderd tot een punt waar het mogelijk is elke polymeer structuur te synthetiseren. Desondanks is één grote uitdaging nog niet opgelost: de mogelijkheid om de monomeer sequentie volledig te controleren. Single unit monomer insertion (SUMI) is een veelbelovende techniek om dit probleem op te lossen. Als de mogelijkheid om perfecte controle over de sequentie te verkrijgen kan toegepast worden op synthetische polymeren, zullen artificiële materialen beschikbaar worden die de precisie van natuurlijke polymeren zoals peptiden kunnen evenaren in hun accuraatheid.

Peptiden bestaan uit een korte sequentie monomeren genaamd aminozuren. Deze macromoleculen voeren belangrijke functies uit in alle organismen. Zo zijn ze aanwezig als hormonen, cel hechting moleculen of dienen ze als bouwsteen voor grotere proteïnen. De natuur is in staat zulke moleculen moeiteloos te produceren door het vormen van een peptide binding die stabiel is in de fysiologische condities van de cel waarin het zich bevindt. Recreatie van peptide structuren en dus de creatie van sequentie gedefinieerde polymeerketens zou een enorme sprong voorwaarts zijn in proteïne research. Dit recreatie proces gaat gepaard met de creatie van synthetische aminozuur mimics die gelijkaardige functionele groepen bevatten. Omdat deze moleculen synthetisch zijn zullen de mimics stabieler zijn en dus in staat zijn meer extreme condities te weerstaan.

Deze studie omvat de synthese van peptide mimics startende van de productie van basis bouwstenen tot de creatie van een aminozuur bibliotheek. Een flow opstelling werd opgesteld die in staat is acryloyl chloride te synthetiseren op dagelijkse basis. Optimalisatie studies en opschaling zorgde voor een uiteindelijke productie van 0.5 L (0.5 M) per dag. Dit acryloyl chloride werd gebruikt in de synthese van een acrylamide gebaseerde aminozuur mimic bibliotheek door het te combineren met de gepaste amines. In deze paper wordt de synthese beschreven van zulke synthetische mimics waaronder: valine, threonine, phenylalanine, glycine, leucine, asparaginezuur en arginine.

1 Introduction

1.1 Polymers

Hearing the word polymer immediately reminds us of plastic materials. It is indeed true that plastic materials consist of polymers, but this represents only the tip of the iceberg. When looking closer at the materials present in the average house one can see that the majority consists of polymers in one way or another. Think about electronics, clothes, bags and even natural products such as wood, rubber and cells, all consist of polymers. Other than being the general building block of a variety of materials, what are these polymers exactly and what do they contribute to modern scientific research?

Polymers or macromolecules are molecules of a high molecular weight consisting of smaller building blocks called monomers.[1-3] These monomers have a low molecular weight and are bound together, in a specific order to form one long chain of covalently bound molecules, called polymers (Figure 1). The use of monomers is not constricted to one kind; different sorts of monomers can be linked together creating more complex structures with their corresponding functionalities. The type of monomer and their distribution along the chain determines the characteristics of the polymer. Macromolecules can exist in all sizes and shapes, for example, star-shaped, linear, grafted, and dendrimers.[1-3] This work will focus on linear polymers.

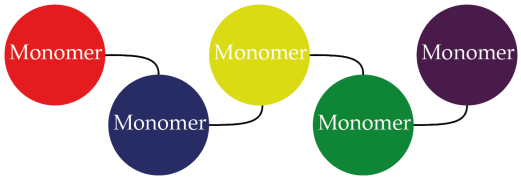


Figure 1: Monomers linked together to form a polymer.

Polymers have been known for quite some time. In the late 20th century, only polymers consisting of relatively ill-defined materials were synthesized made of random copolymers. Now scientists can synthesize very specific compounds with precise microstructures and functionalities using different techniques.[4] Because of this, polymers are used in a variety of research fields ranging from tissue engineering to nanomaterials. Inasmuch as there are different sorts of polymers, there are ways to synthesize them. The most important ones are explained in the following paragraphs.

1.2 Synthesis of polymers

Polymers can be acquired by a variety of methods. These methods can be divided into two major groups, step-growth polymerization, and chain-growth polymerization.[5, 6] Both groups enable the production of polymers with a well-defined structure and function.

Step-growth polymerization is the reaction between molecules containing functional groups.[5, 6] The reaction starts with two functionalized monomers binding, forming a dimer. Linking can occur between any two molecules whether they are of monomeric or polymeric nature. For example, the newly formed dimer can react with a monomer forming a trimer or can react with another dimer forming a tetramer.

To make sure that the reaction propagates onwards, only molecules can be inserted that have at least two functional groups. More functional groups on the built-in monomers will result in branching or grafting of the polymer product. Because of this stepwise addition of varying lengths of monomer chains, there is only a small increase in average molecular weight over time.[6] In other words, chain length and the molecular weight of the polymer are dependent on the conversion and stoichiometry of the reaction.[7] The polymer chains created using step-growth polymerization usually have a broad molecular weight distribution (MWD).[6, 8, 9] Two of the most important step-growth polymerization reactions are condensation and addition polymerization. The first involves a reaction which produces a small molecule as a side product during the polymerization reaction. This formed molecule does not contribute to the polymer chain and is usually removed during purification steps. In the latter, the molecules go through an electron rearrangement upon monomer reaction.[10, 11]

The second major polymerization technique is chain-growth polymerization, defined by the International Union of Pure and Applied Chemistry (IUPAC) as "*A chain reaction in which the growth of a polymer chain proceeds exclusively by reaction(s) between monomer(s) and reactive site(s) on the polymer chain with regeneration of the reactive site(s) at the end of each growth step*."[5, 6, 12] Chain or addition polymerization consists of three major parts: initiation, propagation, and termination.[13] Initiation performed by an initiator molecule generates the active center driving the chemical process. An example of such an initiator molecule is a peroxide. After initiation, propagation occurs in which the polymer chain enlengthens by addition of monomers to the active end region. [13] The monomers used in this process are unsaturated compounds containing a double bond. Polymerization between different sorts of monomers, called copolymerization, can occur. [14] The final step is the chain ending or termination step. This can occur by recombination of the chains e.g. in radical polymerization two chains containing radicals react with each other, thereby removing the active center of both chains and terminating the polymerization process. Opposed to the slowly growing molecular mass of step-growth polymerization, chain-polymerizations have a high molecular weight early in the reaction, at low monomer conversions (Figure 2).[10, 13, 15]

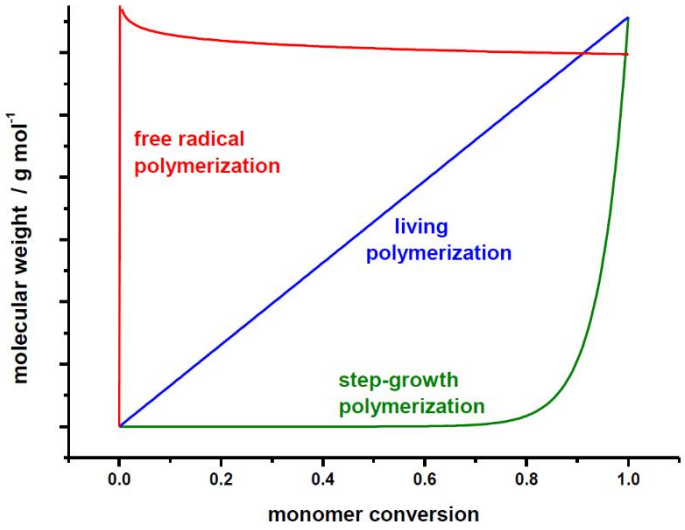


Figure 2: Molecular weight of the polymer plotted against monomer conversion. Step-polymerization has a slow increase in molecular mass and needs a high conversion to create polymers with a high mass as opposed to chain-polymerization.

Chain-polymerization can again be subdivided into different polymerization methods. In the next section, a few of the most popular will be explained in more detail.

Free radical polymerization (FRP) is the first of these chain polymerization methods. FRP follows the same rules as chain-growth polymerization but has a specific way of initiating the entire process. Homolytic dissociation of an initiator species creates radicals which immediately react with the monomers present in solution forming the initiator of the polymerization process.[16] Radical reactions are non-specific, meaning that different sorts of monomers can be used in the reaction.[17] The dissociation process can be induced by a variety of stimuli such as photochemical, thermal, radiation. After the formation of the initiating radical, the next monomer is added to the chain. Each addition creates a new radical at the end of the chain with the same identity as the previous one.[18] Reactions involving radical molecules are the fastest reactions known so far. Hence the fast growth of the polymer chain and subsequently the molecular weight as described in Figure 2.[19] The downside of this reaction speed is that the reaction occurs mostly in an uncontrollable way.

Just as in step-growth polymerization the molecular weight distribution of polymer chains synthesized using FRP is broad. The main reason for this is that the initiation of chain growth occurs over the entire reaction time. Chains initiated at the beginning of the reaction, when almost no monomers are converted yet, produces longer chains than chains initiated near the end of the reaction.[9] Chain propagation is a continuous process until termination occurs, most likely by the reaction of two radical molecules as described earlier. The chances of a propagating chain interfering with another one are higher when most of the monomers are already converted resulting in smaller polymer chains and therefore a broader distribution. Even though FRP produces polymers with a broad MWD, it is still one of the key polymerization techniques producing a variety of polymers used in everyday life.

So far, only techniques capable of producing polymers with a broad MWD have been discussed. The major problem producing this broad MWD is bi-molecular chain termination or, in other words, the production of dead chains during the entire reaction. It is more interesting to produce polymers with narrow MWD since they resemble a more uniform product as seen in nature. Fortunately, techniques exist enabling the control of the aforementioned free radical polymerization in such a way that the radical is protected from termination and continues to grow during the complete reaction time. These techniques are called Reversible-Deactivation Radical Polymerization (RDRP) or Living Radical Polymerisation (LRP). [20]

RDRP controls bimolecular termination by creating an equilibrium between dormant and active polymer chains.[21, 22] Through, in most cases, nearly instantaneous initiation of all the polymer chains and the decrease in chain termination events, the growth of all chains is kept nearly similar.[23] To gain such control over the reaction a reagent is added capable of reversible capping or deactivating of the active center of the polymer chain. Binding of this reagent will put the polymer in a dormant state, unable to attack more monomers. In an RDRP reaction, most of the polymer chains are in this dormant state. This keeps the active/dormant ratio low, decreasing the chance of two radicals finding each other and increases the total reaction time. Once in a while, these dormant chains get reactivated again at which point they behave the same as the active chains in the free radical polymerization. The active chains react with a few monomers, adding them to the polymer chain, before reacting with the control agent transforming them to their dormant state again.[24] Due to a

large number of chains being in a dormant state for most of the time, the chain growth is slow and continuous. All chains are formed early in the reaction, after only a small percentage of conversion, in contrast with radical polymerization where new chains are formed continuously during the reaction.[10] The slower reaction allows more control over the molecular weight of the polymer chains. In RDRP, the molecular weight of the polymer increases linearly with conversion as can be seen in Figure 2. Another important aspect of RDRP is that if a new monomer is added to the solution, the dormant chains can turn active again making further elongation possible hence the livingness of the polymerization. This livingness enables the synthesis of more complex structures as different monomers can be introduced after previous ones have been consumed allowing the formation of block copolymers. Using a sequential addition of monomers in living polymerization makes the synthesis of block copolymer chains more controllable.[10, 25]

How these active chains can be transferred to a dormant state varies according to the applied technique. Two main categories exist which are: reversible termination and reversible chain-transfer. In the first category, deactivation occurs through the termination of an active chain by binding of a certain molecule to the active center. An example of reversible termination is Nitroxide-Mediated Radical Polymerisation (NMP). NMP is a living polymerization technique which uses nitroxide containing molecules to deactivate radical polymer chains transferring them to their dormant form.[19, 26] The reaction of the nitroxide with the polymer radical forms an alkoxyamine, stabilizing the complex in the dormant state.[21, 26, 27] Another example of reversible termination is Atom Transfer Radical Polymerization or ATRP. Instead of using a nitroxide containing molecule to deactivate the active polymer chain as NMP does, ATRP uses a redox reaction to deactivate/activate the polymer chain. The basic method of the reaction relies on a reversible transfer of a halogen atom between the active chain and a transition metal present in the solution.[28] The chain is reversibly terminated by binding of the halogen to the active polymer chain forming an alkyl halide.[19] In the second category, reversible chain-transfer, the dormant state is achieved by transferring a molecule from a dormant chain to an active chain meaning that activation and deactivation occur simultaneously. When the exchange of this deactivating molecule is fast, a small MWD can be achieved. An example of reversible chain transfer is Reversible Addition-Fragmentation Chain-Transfer polymerization (RAFT).

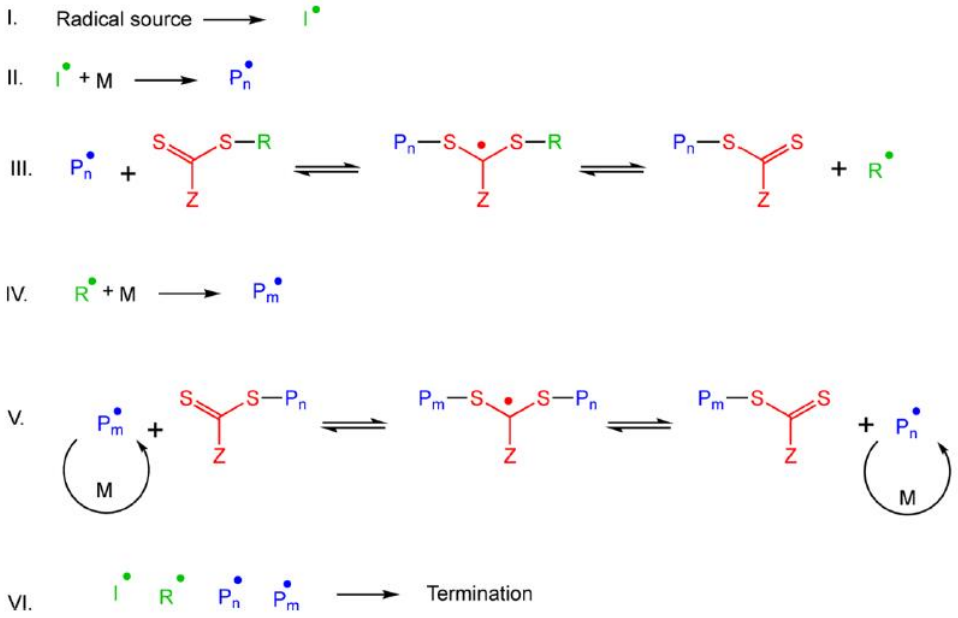
1.2.1 Reversible Addition-Fragmentation Chain-Transfer Polymerization

Reversible Addition-Fragmentation Chain-Transfer polymerization is a living polymerization technique which uses a chain transfer reaction to control the reactiveness of the active polymer chains.[19, 29] As with normal chain-growth reactions, initiation, propagation, and termination still take place. Additional to the monomer and initiator present in the reaction mixture a chain transfer agent is added called a RAFT agent (Scheme 1). This RAFT agent consists of different components. The Z group present in the molecule acts as a radical stabilizer and activates the C=S double bond towards radical addition, enabling the binding of an active chain forming an intermediate state.[29-31] The R-group presents a free radical leaving group, being released from the RAFT agent after binding of the first radical polymer chain.[29] This specific R-group should be a good leaving group compared to the active polymer chain and should be an efficient initiator of new polymer chains.[30, 31] The latter is because after the release of this radical leaving group it can bind to a monomer initiating a new

chain.[9] The RAFT agent is the molecule which shuttles a radical species to a less reactive species. In other words, RAFT agents are the molecules being transferred from dormant chains to active chains activating the dormant and deactivating the active chain in the process. [9] The next section describes the different reaction steps present in a RAFT reaction being: initiation, pre-equilibrium, reinitiation, propagation, main equilibrium, and termination (Scheme 1).[29]

The RAFT reaction starts by cleavage of the initiator molecules into radical species called initiation (step I.). The cleavage can be spontaneous or can require a certain trigger e.g. temperature, pH … The produced radicals can react with monomers present in the reaction solution adding the first monomer to the chain (step II.). Afterwards, these radical species can form the pre-equilibrium by binding of a radical chain to the RAFT agent. This creates an intermediate state in which the radical chain and leaving group (R) are bound to the central RAFT agent (step III.). The intermediate state will reversibly release the leaving group which will attack monomers present in the reaction mixture initiating new chains (step IV.). Initiation of polymer chains by the leaving group is called the reinitiating step.[29]

After the release of the leaving group, a new radical species (P_m) can bind the RAFT agent forming again the intermediate state, this time with two polymer chains bound to the RAFT agent. This complex and the following release of the previously bound polymer chain P_n is called the main equilibrium (step V.). When the intermediate state is too unstable, the reaction will have little control and a broad MWD will be observed. Likewise, the transfer constant, another word for the rate at which the chains are transferred, also has a major effect on the MWD.[30] A high transfer constant means that a rapid exchange between dormant and active species occurs, making the number of added monomers equal for every chain generating a small MWD.[30] The final step in the reaction process is termination in which radical species react with each other eliminating the radicals present and stopping the addition of more monomers (step VI.).[29]



Scheme 1: Mechanism of Reversible Addition-Fragmentation Chain-Transfer Polymerization. Green shows the chain initiating molecules. Blue shows the propagating polymer chains, and red the RAFT agent.[29]This image was reused with permission from ACS.

The use of a RAFT agent will have another effect on the created polymer chains except for the ones described above. Because most of the chains are initiated by the leaving group present on the initial RAFT agent, almost all of the chains will have the same functional end groups, being the RAFT agent and the leaving group R. This is a major advantage as it enables the creation of end-functionalized polymer chains in one step.[29] The addition of new monomers after the full conversion of a previously added monomer can reinitiate the polymer chains making it possible to use RAFT polymerization for the creation of block copolymers. Another advantage of RAFT polymerization is its compatibility with a wide range of monomers including functional ones containing, e.g. acid groups.[30] The wide range of monomers that can be used makes it possible to create complex polymers with a narrow dispersity.

The aforementioned techniques enable the synthesis of complex polymer structures as well as block copolymers consisting of different sorts of monomers. Especially RAFT polymerization is a well-manifested technique to produce multiblock polymers with specific functions. Unfortunately, further development in polymer synthesis techniques has stumbled upon two major problems that RAFT polymerization cannot solve solely. These two problems are sequence and stereo control. In other words, the ability to add one monomer at a time to a polymer chain and the ability to control the orientation of the added monomer. Addition of monomers so far only happened in a statistical nature, meaning that the product mixture consists of polymer chains with different lengths making it unable to precisely identify the exact location of the functional groups in these chains. Polymer chains like this are called sequence-controlled multiblock copolymers.[32] Even with the low MWD acquired in RAFT polymerization, this chain length difference still creates a problem when trying to synthesize polymer chains with a precisely defined monomer sequence. To enable the production of these sequence-defined polymers, polymer chains of which the location of all functional groups in the chain is precisely known, a technique should be used that makes the addition of one monomer at a time possible.[32] This would mean the creation of a monodisperse product solution.

1.3 Natural polymers and their mimics

Nature has always been an amazing example for scientists and polymer science is no exception. Nature's capability to synthesize polymer chains with perfect sequence reproducibility is one of the major goals still left to reach. The most well-known natural polymer structures present in every human body are DNA and peptides. A DNA chain acts as an information storage molecule of which the nucleotide sequence codes information by using only four kinds of molecules bound together in a specific order.[33] Proteins are also composed of a long chain of alternating molecules called amino acids. The position of the amino acids in protein synthesis is essential.[33] Functional groups present on the amino acid molecules need to be on their specific position inside the molecule to give the protein its specific function. The primary structure of a protein/peptide describes the linear amino acid sequence and thus provides the basic blueprint.[34] The sequence of these individual monomer units becomes an important aspect. Slight deviations in this sequence can cause mutations with devastating effects as can be seen in a variety of diseases like sickle cell anemia, cystic fibrosis, and even cancer.[35-37]

The past decades, scientists have thrived for the recreation of polymers that resemble the sequence-defined natural polymers. Enabling the recreation of these natural proteins would mean a giant leap forward in polymer chemistry. It would completely open the way for synthetic protein production

making scientists able to make fully artificial protein mimics. However, most of these synthesized polymers are sequence-controlled and the required control over monomer insertions is lacking.[38]

Various attempts have been made to reproduce such naturally occurring peptides. One of the more popular techniques is Solid Phase Peptide Synthesis (SPPS). Consisting of three steps, SPPS enables the synthesis of polypeptides.[34] The first step involves the attachment of a cyclobenzyl N-protected amino acid derivate to the amino group of a resin-bound peptide chain. Next, the newly created N-terminus is deprotected followed by the next coupling step. The reaction ends by a final deprotection step and liberation of the peptide from the resin.[39] Few examples of protein resembling structures that can be synthesized using SPPS are β-peptides and peptoids. β-peptides are peptide mimetics which carry their amino functionality on the β-carbon instead of the normal α-carbon (Figure 3).[40] This shift of amino groups gives these structures higher stability and resistance against degradation.[40] Another class of synthetic peptides is peptoids. They differ from normal peptide structures in the placement of their functional R-group. In peptoids, these functional groups are attached to the nitrogen in the backbone instead of the α-carbon.[41] The shift in binding position results in a protein that is less susceptible to proteolytic degradation. Furthermore, the secondary structure is not dependent on hydrogen bonds decreasing degradation in elevated temperatures. A negative aspect of this lack of hydrogen bonds is that the secondary structure of the molecules is not stabilized resulting in a flexible side chain with low conformational stability.[42]

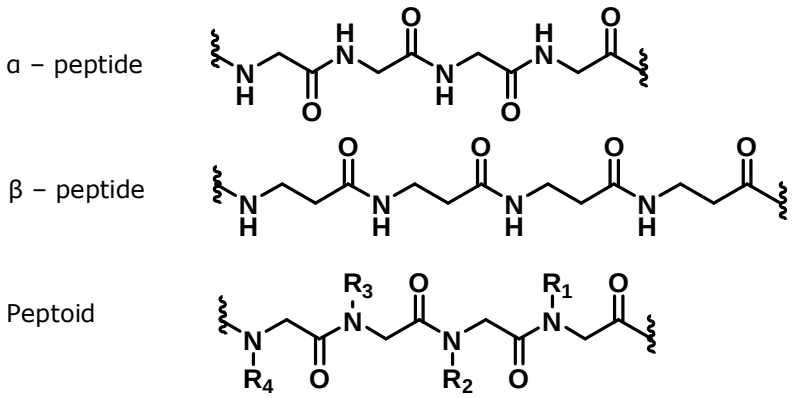


Figure 3: Backbones of α-, β-peptides and Peptoids. α-Peptides have one carbon atom per residue between the amine and the carbonyl functionality whereas β-peptides display two carbon atoms per residue. In peptoids, the functional side chains are attached to the nitrogen of the peptide backbone.

β-peptides and peptoids are fine ways to produce peptide mimics. These structures allow us to produce protein mimics capable of doing the same function as their natural counterpart. The downside of these peptide mimics is that they still are biomolecules meaning that their practical use is limited due to their physical limitations. β-peptides and peptoids are still susceptible to temperature, pH, organic solvents and degradation.[40, 41] Even though production of these molecules is possible, the production process still has some issues that need to be removed before being able to start the synthesis of peptide mimics on a bigger scale. Despite SPPS enabling the production of almost any kind of peptide, the high cost of the reaction process, due to the use of large amounts of coupling reagents and amino acids, makes this technique only affordable for small-scale synthesis.[43]

Another method implemented to synthesize naturally occurring proteins is recombinant DNA technology. In this method, the gene coding for a specific protein is built into cells, transcribing the gene, producing the protein product. This method is commonly used in the production of insulin.[44] Recombinant DNA allows for easy production of peptide mimics once the coding DNA strand is made but still has the same problem as the previous examples as it is still a biomolecule thus possesses a weak peptide bond. This peptide bond makes the molecule unstable in more extreme conditions.

In biomolecules, the amino acids are linked together by peptide bonds as opposed to synthetic proteins, in which the linkage occurs in a different way. The peptide bonds, consisting of a linkage between an amino group and a carboxyl group, result in proteins susceptible to hydrolytic cleavage making them unstable in conditions differing from the normal physiological environment.[8]

A step in the direction of a more synthetic structure is achieved by polymer-peptide conjugates. This protein mimic consists of both natural and synthetic parts. The combination allows the material to benefit from advantages seen in both classes of materials which are the precise chemical structure of natural proteins and the stability and processability of synthetic molecules.[45] The production process of these structures can be divided into two parts. The first one being the synthesis of the protein performed by SPPS. The second is the production of the polymer structure which can be performed by one of the earlier described methods.[46, 47] Even though polymer-peptide conjugates consist of a partially synthetic structure, this is not yet the holy grail. The biological part still makes the molecules more susceptible to degradation. To overcome this degradation problem, peptide mimics should be completely synthetic even up to the functional groups present on the molecule. Unfortunately, no protein mimics consisting fully of synthetic polymer have been reported yet.

1.4 Sequence control in polymer chemistry

Synthesis of synthetic polymers has allowed the production of a variety of materials that can be used in many ways. However, most available synthetic polymers belong to the same class of sequence-controlled polymers. Sequence-controlled polymers are polymers with a defined monomer sequence. Generally, these polymers consist of block copolymers that can be built up out of a broad spectrum of monomers.[32] These blocks can exist of a not specified number of monomers of the same type and functionality. Because the number of monomers present in the blocks is not constant over every chain, the exact location of the functional groups in the polymer chain is not known. This is not a big problem when creating bulk polymer products as their bulk functional properties are an average of the functional properties of the chains they are build up of. However, when working on a smaller scale, these small deviations in the location of the functional groups can have a big impact on the functionality of the product e.g. proteins. This problem is one of the remaining roadblocks in clearing the way to perfectly precise polymerization processes. Enabling the creation of these well-defined functional groups in a polymer chain would mean a big step forward in polymer science. Sequence-defined polymer chains are such chains of which the exact location of the individual monomers is known.[32] Sequence-defined polymers differ from sequence-controlled polymers in terms of purity and functionality.[48] Previously stated techniques can be used to synthesize such sequence-defined biologic structures. The problem with these biological structures is their stability in environments differing from the physiological environment.

To improve protein stability in conditions outside the body, the peptide bond in natural peptides should be exchanged for something more rigid. This can be achieved by using RAFT polymerization. As mentioned earlier, RAFT polymerization allows the cost-effective synthesis of relatively monodisperse products in a controlled reaction. The major downside of RAFT was the statistical nature of the reaction, producing products with different amounts of monomer insertions. To synthesize fully synthetic sequence-defined polymers, a "new" technique should be used that circumvents the statistical nature of radical chain growth. Single Unit Monomer Insertion (SUMI) is such a technique and uses RDRP to link monomers to the growing chain, creating dispersity as a side effect. To counter the synthesis of different chain sizes, oligomer purification is performed after the insertion, creating a monodisperse product. Synthesis of fully synthetic peptides using the SUMI technique would allow the production of peptide mimics that are more stable and are able to withstand more hostile environments than their natural counterparts in a more effective way.

1.5 Single Unit Monomer Insertion

So far, RAFT polymerization came close to producing sequence-defined polymer chains. RAFT polymerization indeed has some benefits which are usable in the further development of synthetic peptide production. A variety of monomers can be chosen independently of their reactivity. The radical chain-growth techniques are also cost-effective, easy to perform and can be upscaled.[29] Use of radicals in the polymerization process makes it fast and enables the addition of more than one monomer at a time. When using RAFT, a chemically stable carbon-carbon backbone is formed in the product, making it more stable than when using iterative strategies.[49] Another advantage is that a lot of functional end groups can be incorporated in one step. A downside of this technique, however, is that purification steps are necessary due to the statistical nature of the monomer addition.[15, 49] This is the case for every technique seen so far in this work. Purification in this way makes the product yield low due to the separation of the monodisperse molecules.[15] This can be made up for by the fact that separation of monodisperse molecules results in the creation of a library so that discarded molecules in one reaction can be reused in another one.[48] This all results in an efficient and economic production process.[48] Single Unit Monomer Insertions or SUMI is not a separate reaction technique, it represents the specific conditions to which a reaction should fulfill to primarily allow the insertion of a single monomer unit to the polymer chain.[15] These reaction conditions can be found in many different techniques but here, RAFT will be used because of the aforementioned reasons (Figure 4). Herein, the use of the abbreviation, SUMI, refers to the conditions used in RAFT polymerization.[15]

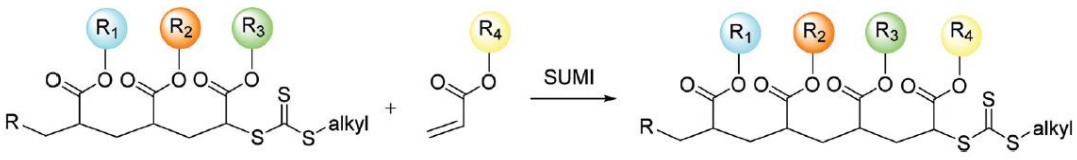


Figure 4: Single Unit Monomer Insertion reaction using the RAFT process.[15]

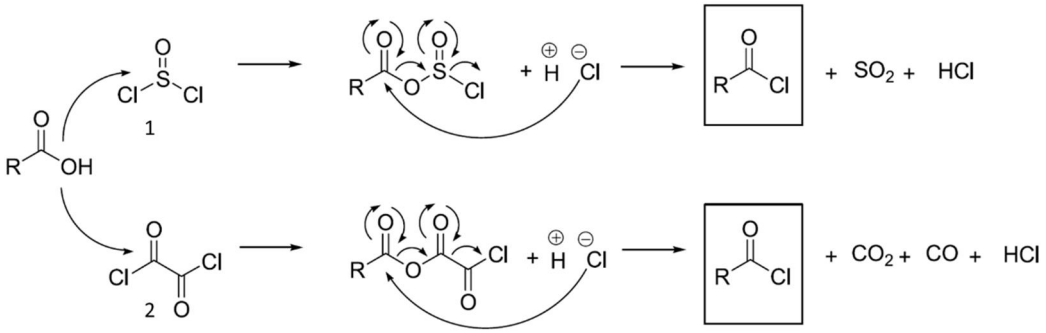
In order to be able to synthesize SUMIs via RAFT polymerization, careful selection of initiator, monomer, RAFT agent and reaction times are required. The RAFT agent should have an adequately high transfer constant so that per activation cycle less than one monomer is added. The rate coefficient for addition and fragmentation is dependent on the degree of polymerization and the type

of propagating chains. The first monomer additions cause huge differences in this rate coefficient causing no longer oligomers to be formed before all RAFT agent is converted to the single monomer adduct. Another important aspect that needs to be kept in mind when choosing the RAFT agent is that it should reinitiate as efficient as possible. Furthermore, a low ratio of monomer to RAFT agent favors single monomer insertions. Extending the reaction time reduces the yield of the wanted product so stopping the reaction before full conversion is advised.[48] SUMI indeed enables the insertion of a single monomer unit inside the polymer chain. Although the statistical nature of monomer insertion is severely lowered, it is still present. Therefore, the purification of the final product is still necessary and can be performed using chromatography separation.[50] The purified product can then be used for its purpose or can be used in another monomer insertion step. So far, the host research group has already shown that the synthesis of oligomers where in up to four different acrylate monomers were built in a trithiocarbonate RAFT agent using SUMI was possible.[38] Optimization of the SUMI method provides the required control and enables the synthesis of polymers mimics with the same sequence, shape, and function as the ones occurring in nature, hereby bringing polymer science to the next level.

1.6 Objectives

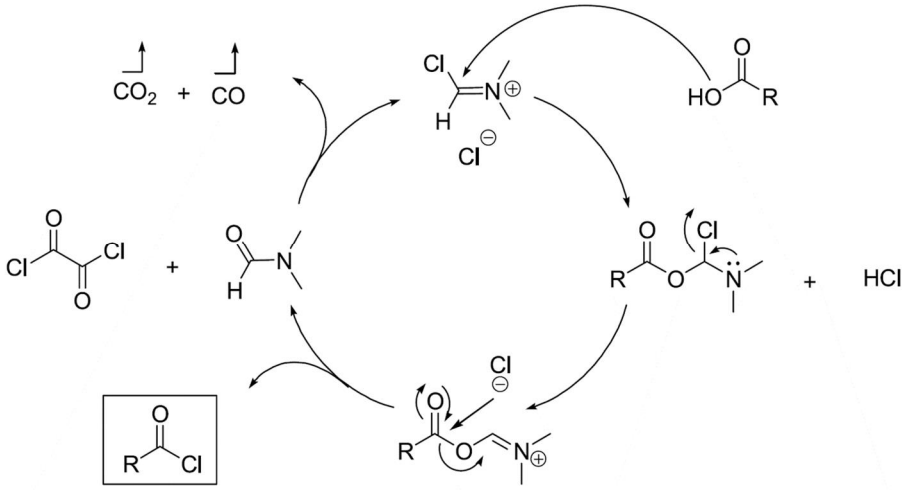
As previously stated, there are a broad variety of methods available for the synthesis of partly synthetic amino acids, with SPPS being the major method of production. Although being a well-established method, the synthesis of synthetic proteins still features some bottlenecks (labor intensive, expensive, ….). In this work, a new approach to design fully synthetic peptides will be explored. Modern techniques allow the synthesis of peptide mimics which are stable in physiological conditions or even in more hostile environments. What does not exist at this moment is a technique capable of producing a variety of protein mimics that are both stable and cost-efficient. In this work, the synthesis of fully synthetic peptides composed out of acrylamide monomers will be explored. Such peptides have several advantages over their natural counterparts as they are relatively easy to polymerize, have a cost-effective production, and increased stability.

As the first goal of this thesis, acryloyl chloride, a crucial component for the synthesis of acrylamide monomers, will be synthesized. There have been various reports on its synthesis already, all based on the reaction of acrylic acid and either an inorganic (thionyl chloride) or an organic (oxalyl chloride) chlorinating agent (Scheme 2).[51] This two-step reaction usually consists of the conversion of the acid into an acyl halide after which the coupling itself takes place. The reaction starts with the nucleophilic attack of the carbonyl group on the chlorinating agent (oxalyl or thionyl chloride) after which the Cl leaving group is removed, forming a chlorosulfite intermediate. This intermediate is a better leaving group. A second nucleophilic attack, this time on the carbonyl of the intermediate, is followed by the removal of the chlorosulfite leaving group. The final step is deprotonation of the acyl chloride, resulting in the formation of the acid chloride and HCl as a side product. Scheme 2 illustrates this acyl chloride formation for both thionyl chloride (1) and oxalyl chloride (2).[52]



Scheme 2: Acyl chloride formation for both oxalyl chloride (2) and thionyl chloride (1).

The acyl chloride formation can be performed with the addition of *N, N*-dimethylformamide (DMF) which acts as a catalyst in the reaction. DMF addition results in a significant increase in reaction speed by the formation of a reactive chloro-iminium intermediate (Vilsmeier–Haack reagent) acting as a halogenating reagent.[51, 53] After the formation of the intermediate reagent, the two nucleophilic attacks, as described above, transform the acid into its acyl chloride (Scheme 3).



Scheme 3: Role of DMF as a catalyst in the formation of acyl chlorides.

Earlier reports describing the synthesis of acryloyl chloride using multiple chlorinating agents such as oxalyl and thionyl chloride showed poor results with maximum yields up to 72%.[54, 55] This was mostly caused by the presence of side reactions due to the reactive nature of the molecule.

Herein, this reaction will be optimized by using a continuous flow reactor as this allows better control of the reaction conditions, safer handling and a possibility for upscaling. The production process will be based on an already existing setup present at the host research group in Belgium.[51] Acryloyl chloride has a very reactive nature and because of this, distribution of the product in Australia has stopped. Therefore, constructing a setup capable of a steady production of this basic building block is important to sustain the available supply in the lab. A similar study was performed by Movsisyan et al.[51] They used a solvent-free approach using flow chemistry to synthesize acryloyl chloride using acrylic acid, DMF and multiple chlorinating agents later discussed in section 3.2.1.

As a second goal, the acquired acryloyl chloride will be combined with the appropriate amines in an aminolysis reaction to form the desired acrylamide monomers mimicking amino acid structures. These monomers will possess a side chain that mimics the functional R-group of natural amino acids as closely as possible.[56] Usually, a base is present in this reaction to capture the formed HCl and to prevent the formation of non-reactive HCl salts.[52]

After the establishment of the monomer library, polymerization of the monomer units will be performed using the SUMI procedure. SUMI reactions in continuous flow reactors are targeted, as flow chemistry allows for better heat dissipation. This will result in higher yields and a safer working environment when working with acrylates and acrylamides. Furthermore, flow reactions have efficient mixing and allow efficient upscaling and automation. [57, 58]

The SUMI approach will result in the synthesis of a monodisperse product solution. The carbon-carbon backbone will give the protein mimics more stability and the reaction itself is cost-efficient. The easiness and reproducibility of controlled living polymerization is a huge advantage of this technique. Protein mimics can be used in a variety of fields e.g. antibiotics, hormones, vaccines, opioids, anti-cancer drugs, imaging….[43] The large market would benefit from further development in this field.

2 Materials

Oxalyl Chloride (98%), Thionyl Chloride (97%), Anhydrous Tetrahydrofuran (99.9%), 3-Amino-1-Propanol, Anhydrous Dichloromethane (99.8%), phenethylamine (99%), Methylamine solution 2M in THF, Isopentylamine, and Amino-2-propanol were purchased from Sigma-Aldrich and used as acquired. Acrylic Acid (stabilized with hydroquinone monomethyl ether), N,N-Dimethylformamide, Triethylamine, and isobutylamine were purchased from Merck and used as acquired. Ethyl acetate and Tetrahydrofuran were purchased from Thermo fisher scientific and used as acquired. Di-tert-butyl decarbonate (Boc Anhydride) (99%) was bought at AK Scientific. Petroleum ether and Dimethyl sulfoxide (99%) were purchased from Chem-supply and used as acquired.

3 Methods

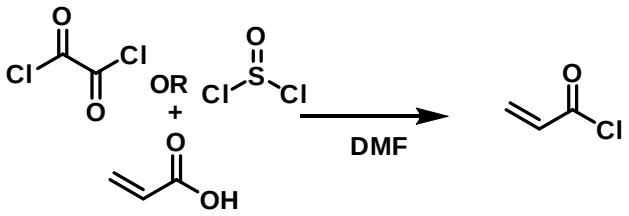
3.1 Characterization and purification

Nuclear Magnetic Resonance (NMR): Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker Avance III (400 MHz / 600 MHz, 9.4 Tesla magnet) with Z-gradients and BACS 60 tube autosampler. The system has variable temperature capabilities. For the ^{1}H NMR spectra, the chemical shifts (δ) are quoted in parts per million (ppm) with residual protons in the deuterated solvent as internal standard ($CDCl_3$ at 7.26 ppm, $(CD_3)_2SO$ at 2.50 ppm). Abbreviations used in the description of resonances are s (singlet), d (doublet), t (triplet), q (quartet) and br (broad). Coupling constants (J) are quoted to the nearest 0.1 Hz. For the ^{13}C NMR spectra, the chemical shifts (δ) are quoted in parts per million (ppm) with $CDCl_3$ at 77.0 ppm as internal standard. The NMR spectra are collected and analyzed in MestReNova software.

Flash Column Chromatography: Purification of monomers and polymers was performed via Flash Column Chromatography (FCC) on a Puriflash® XS420+ (Interchim®) equipped with Puriflash® Intersoft V5.0 software. Separation was monitored via a diode array detector (range 200-400 nm) at lambda 254 nm and 305 nm. The product was embedded on silica and dry loaded on a pre-column cartridge (Interchim puriflash® F0012). The pre-column was subsequently wetted with eluent and attached to the pre-wetted normal phase silica cartridge (Interchim puriflash® F0040) on the integrated column holder. Product separation was performed with an optimized mobile phase (eluent) gradient mixture with a flow rate of 25 mL/min. Fractions were automatically collected by a fraction collector in racks with 18 x 150 mm glass tubes.

3.2 Small molecule synthesis

3.2.1 Acryloyl chloride synthesis



Scheme 4: Reaction scheme for the synthesis of acryloyl chloride.

Two different approaches were used in the synthesis of acryloyl chloride.

The first method involved performing the reaction diluted in anhydrous Tetrahydrofuran (THF). These circumstances were tested using two different chlorinating agents, oxalyl chloride and thionyl chloride. Initiating the procedure, two different stock solutions were prepared. The first containing acrylic acid (0.7206 g, 0.1 Mol, 1eq.) and dimethylformamide (DMF) (0.0064 eq., 0.013 eq., 0.019 eq., 0.025 eq., 0.03 eq.). The second containing the chlorinating agent: oxalyl or thionyl chloride (0.11 Mol, 1.1 eq.). Both solutions were prepared under inert conditions, diluted with anhydrous THF, and transferred into two separate 10 mL gastight syringes (Trajan Scientific and Medical). Next, the syringes were placed in the holder of one syringe pump (Chemyx fusion touch model) which delivered the reagent solutions with correct flowrates using perfluoroalkoxyalkane (PFA) tubing (0.75 mm I.D., 1/16" O.D.). Mixing of both solutions occurred at a static Y-shaped mixer (PEEK, P-512, Upchurch Scientific) out of ethylene tetrafluoroethylene (ETFE) just as the connection parts. The mixed solution passed through the reactor (2 mL or 6 mL Vol.) consisting of PFA tubing with the same diameter. The reactor was placed in an isothermal oil bath with a temperature dependant of the chlorinating agent used (Figure 5). The reaction involving oxalyl chloride was performed at 50°C while the one using thionyl chloride was set to 60 °C. The resulting reaction product was collected in a glass vial and no further purification was done (Scheme 4).

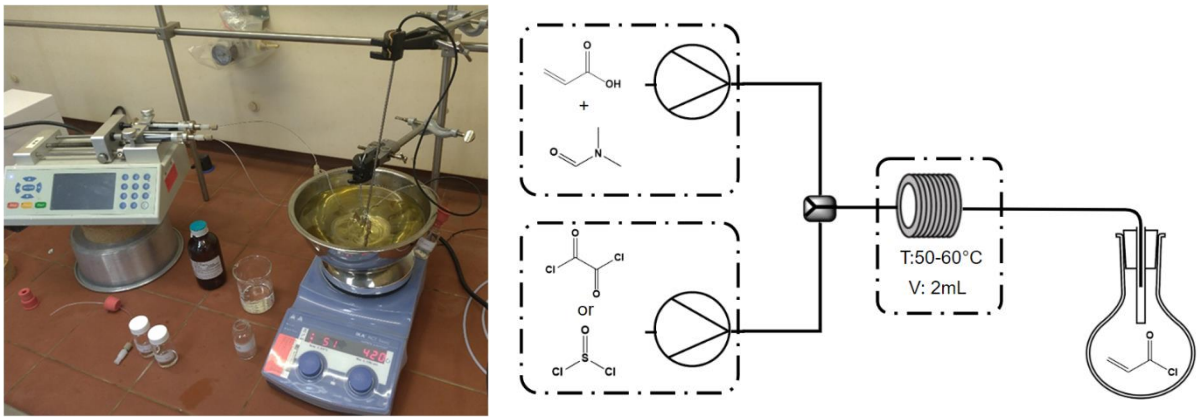
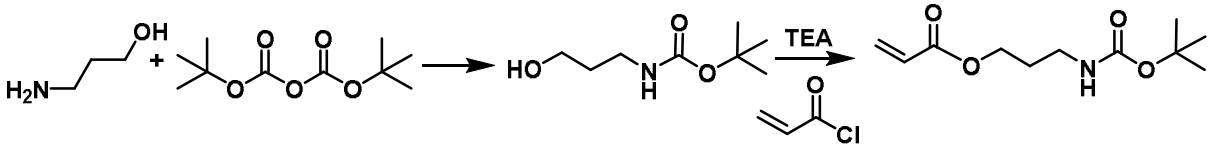


Figure 5: Picture of the real setup (left). Schematic representation (right) of the acryloyl chloride synthesis setup. The bottom syringe contains the chlorinating agent oxalyl chloride (top) or thionyl chloride (bottom).

The second method involved a solvent-free approach. Starting with the preparation of two different stock solutions. The first one containing acrylic acid (3 mL, 0.0438 mol, 1 eq.) and the catalyst dimethylformamide (DMF, 7 mL, 0.0904 mol, 2.07 eq.). The other stock solution contained pure oxalyl chloride (3.7 mL, 0.0438 Mol, 1 eq.). Both solutions were prepared under inert conditions, and afterwards, transferred to their designated 10 mL gastight syringes. The syringes were mounted on two different syringe pumps (chemyx) with a flow rate of respectively 0.2432 mL/min and 0.0901 mL/min to compensate for the difference in concentration. The syringe pumps delivered the reagent solutions using PFA tubing (0.5 mm I.D., 1/16" O.D.) to a static y-shaped mixer (PEEK, P-512, Upchurch Scientific) where the two solutions were merged. Next, the mixed reagents were passed to the reactor (1 mL Vol.) using the same diameter PFA tubing. The reactor was placed in an isothermal oil bath (25°C). The reaction product was collected in a glass vial and was purified via a discontinuous distillation under reduced pressure at room temperature resulting in the pure product (Figure 6, Scheme 4).

Figure 6: Overview of the experimental setup used for the synthesis of acryloyl chloride.

3.2.2 (3-Boc-amino) Propyl acrylate.



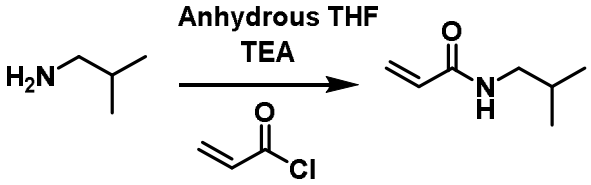
Scheme 5: Reaction scheme for the synthesis of (3-Boc-amino) Propyl acrylate.

All used glassware was dried overnight in an oven of 130°C.
3-Aminopropanol (0.25 g, 0.0033 mol, 1 eq.) was dissolved in dry THF (1.8 mL) in a 100 mL 3-neck round bottom flask equipped with a magnetic stirrer bar. Di-tert-butyl decarbonate (DI-BOC) (0.0808 g, 0.0037 mol, 2 eq.) was added. The solution was allowed to react for 1.5 hours at room temperature. Next, triethylamine (0.678 g, 0.0067 mol, 2 eq.) was added, the flask was placed in an ice bath at 0°C. In the following step, synthesized acryloyl chloride (8.5 mL, 0.0040 mol, 1.2 eq.), as described in 3.2.1, was added dropwise (0.1818 mL/min). The reaction was then left to stir for 1.5 hours under a nitrogen atmosphere. The acquired solution was filtered and purified using column chromatography on silica gel using petroleum ether: ethyl acetate (7/3) as eluent. Finally, the solvent was evaporated under reduced pressure and the product was collected as a yellow oil, Scheme 5 (0.165 g, 21.8% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.41 (dd, *J* = 17.3, 1.5 Hz, 1H), 6.12 (dd, *J* = 17.4, 10.4 Hz, 1H), 5.84 (dd, *J* = 10.4, 1.4 Hz, 1H), 4.69 (s, 1H), 4.23 (t, *J* = 6.2 Hz, 2H), 3.22 (q, *J* = 6.5 Hz, 2H), 1.87 (p, *J* = 6.5 Hz, 2H), 1.44 (s, 10H).

3.3 Acrylamide based monomer synthesis

All acryloyl chloride used in the synthesis of the following monomers was produced as described in 3.2.1.

3.3.1 *N*-isobutylacrylamide



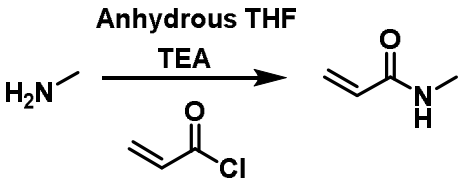
Scheme 6: Reaction scheme for the synthesis of N-isobutylacrylamide.

Triethylamine (1.3834 g, 0.0137 mol, 4 eq.) and isobutylamine (0.250 g, 0.0034 mol, 1 eq.) were dissolved in anhydrous THF (30 mL) in a 100 mL 3-neck round bottom flask equipped with a magnetic stirrer bar. The flask was then placed in an ice bath at 0°C under a nitrogen atmosphere. The mixture was stirred vigorously to avoid salts fixating the stirrer bar. After cooling down of the solution, acryloyl chloride (9.3 mL, 0.0041 mol, 1.2 eq.) was added dropwise directly after synthesis (Figure 7). The reaction was then removed from the ice bath while the reaction solution was again exposed to air. Finally, the salt formed during the reaction was filtered off. The collected fluid was purified using flash column chromatography on silica gel using petroleum ether: ethyl acetate (1/1) as eluent. The product was collected as a yellow oil, Scheme 6 (0.2583 g, 59.41% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1H), 6.24 (dd, *J* = 17.1, 10.1 Hz, 1H), 6.06 (dd, *J* = 17.1, 2.3 Hz, 1H), 5.56

(dd, *J* = 10.1, 2.3 Hz, 1H), 2.95 (t, *J* = 6.4 Hz, 2H), 1.70 (dhept, *J* = 13.2, 6.6 Hz, 1H), 0.86 – 0.82 (m, 7H). ^{13}C NMR (101 MHz, DMSO) δ 164.59, 131.90, 124.71, 46.11, 28.08, 20.12.

Figure 7: Setup used in the synthesis of monomer mimics. Acryloyl chloride is produced and added immediately to the reaction flask.

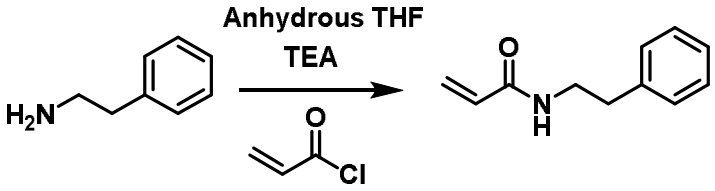
3.3.2 *N*-methylacrylamide



Scheme 7: Reaction scheme for the synthesis of N-methylacrylamide.

Triethylamine (2.277 g, 0.0225 mol, 4 eq.), methylamine (2M solution in THF) (2.8125 mL, 0.0056 mol, 1 eq.), THF (25 mL) and acryloyl chloride (15 mL, 0.0067 mol, 1.2 eq.) were used. The procedure is identical as in 3.3.1. The collected solution was purified using flash column chromatography on silica gel using ethyl acetate: petroleum ether (1/1) as eluent. The product was collected as a yellow liquid, Scheme 7 (0.1981 g, 41.6% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (s, 2H), 6.18 (dd, *J* = 17.1, 10.0 Hz, 1H), 6.05 (dd, *J* = 17.1, 2.4 Hz, 1H), 5.55 (dd, *J* = 10.1, 2.4 Hz, 1H), 3.77 (t, *J* = 6.4 Hz, 0H), 2.65 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 164.58, 139.42, 131.80, 128.60, 128.32, 126.09, 124.93, 40.24, 35.07.

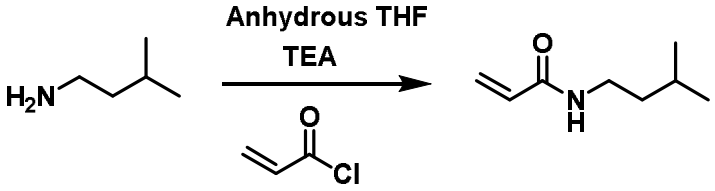
3.3.3 *N*-phenethylacrylamide



Scheme 8: Reaction scheme for the synthesis of N-phenethylacrylamide.

Triethylamine (1.032 g, 0.0102 mol, 3 eq.), phenethylamine (0.497 g, 0.0041 mol, 1.2 eq.), THF (30 mL) and acryloyl chloride (7.6 mL, 0.0034 mol, 1 eq.) were used. The procedure is identical as in 3.3.1. The collected fluid was purified using flash column chromatography on silica gel using diethyl ether: dichloromethane (1/1) as eluent. The product was collected as a colourless oil, Scheme 8 (0.3951 g, 66.34% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.24 (dd, *J* = 29.2, 7.4 Hz, 6H), 6.19 (dd, *J* = 17.1, 10.0 Hz, 1H), 6.06 (dd, *J* = 16.9, 2.3 Hz, 1H), 5.56 (dd, *J* = 10.0, 2.3 Hz, 1H), 2.75 (q, *J* = 9.6, 7.3 Hz, 2H), 2.08 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 164.58, 139.42, 131.80, 128.60, 128.32, 126.09, 124.93, 40.24, 35.07.

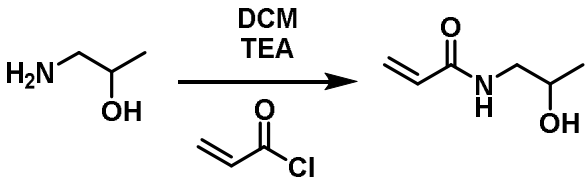
3.3.4 *N*-isopentylacrylamide



Scheme 9: Reaction scheme for the synthesis of N-isopentylacrylamide.

Triethylamine (1.032 g, 0.0102 mol, 3 eq.), isopentylamine (0.296 g, 0.0034 mol, 1 eq.), THF (30 mL) and acryloyl chloride (9.12 mL, 0.0041 mol, 1.2 eq.) were used. The procedure is identical as in 3.3.1. The collected solution was purified using flash column chromatography on silica gel using ethyl acetate: petroleum ether (1/1) as eluent, Scheme 9 (0.2663 g, 55.46% yield). ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.01 (s, 1H), 6.19 (ddd, *J* = 17.2, 10.0, 0.8 Hz, 1H), 6.05 (ddd, *J* = 17.1, 2.4, 0.8 Hz, 1H), 5.55 (ddd, *J* = 10.2, 2.4, 0.8 Hz, 1H), 3.22 – 3.09 (m, 2H), 1.63 – 1.48 (m, *J* = 6.7 Hz, 1H), 1.39 – 1.23 (m, 2H), 0.87 (dd, *J* = 6.7, 0.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 164.40, 131.90, 124.60, 38.05, 36.75, 25.18, 22.31.

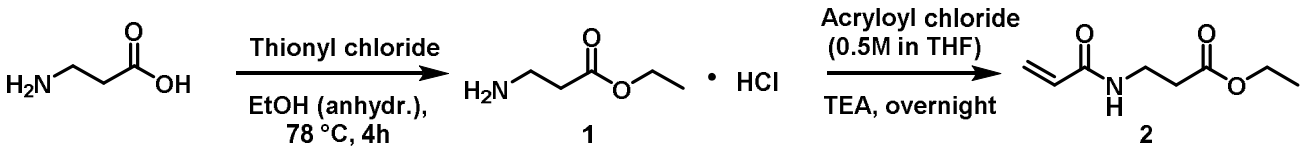
3.3.5 Synthesis of *N*-(2-hydroxypropyl)acrylamide



Scheme 10: Reaction scheme for the synthesis of N-(2-hydroxypropyl)acrylamide.

Triethylamine (0.997 g, 0.00985 mol, 1.11 eq.), amino-2-propanol (2 g, 0.02663 mol, 3 eq.) were dissolved in DCM (5.266 mL) in a 100 mL 3-neck round bottom flask equipped with a magnetic stirrer bar. The flask was then placed in an ice bath at 0°C under nitrogen atmosphere. The mixture was stirred vigorously to avoid salts fixating the stirrer bar. After cooling down of the solution, acryloyl chloride (18.89 mL, 0.00888 mol, 1 eq.) was added dropwise directly after synthesis. The reaction was then removed from the ice bath and left stirring at room temperature for another hour. Afterwards, the reaction was exposed to air and the salt formed during the reaction was filtered off. The filtrate was purified using flash column chromatography on silica gel using on first instance petroleum ether: ethyl acetate (1/1) as eluent. After the first fraction was collected, the product was obtained with 100% acetonitrile as eluent. The product was concentrated and collected as a clear oil, Scheme 10 (0.485 g, 42.3% yield). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.47 (s, 1H), 6.27 (d, *J* = 17.0, 1.4 Hz, 1H), 6.14 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.65 (d, *J* = 10.3, 1.4 Hz, 1H), 3.94 (dqd, *J* = 7.8, 6.3, 3.1 Hz, 1H), 3.50 (ddd, *J* = 14.0, 6.5, 3.1 Hz, 1H), 3.17 (ddd, *J* = 14.0, 7.7, 5.2 Hz, 1H), 1.20 (d, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.79, 130.73, 126.87, 67.22, 47.20, 20.94.

3.3.6 Synthesis of Ethyl 3-acrylamidopropanoate



Scheme 11: Reaction scheme for the synthesis of Ethyl-3-acrylamidopropanoate.

Synthesis of β-alanine ethyl ester hydrochloride (1)

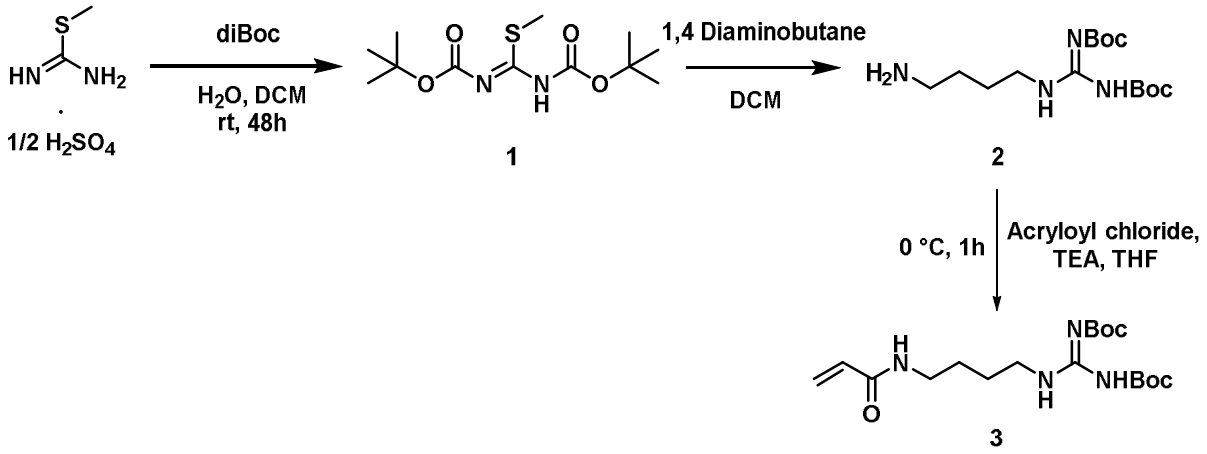
To a suspension of β-alanine (1.04 g, 0.0112 mol, 1 eq.) in anhydrous EtOH (33.7 mL), $SOCl_2$ (4.00 g, 0.0337 mol, 3 eq.) was added dropwise at 0 °C. The reaction mixture was refluxed at 78 °C for 4 h. The solvent was removed under vacuum to obtain the pure product **1** quantitatively as a white solid (1.77 g), Scheme 11. ^{1}H NMR (600 MHz, DMSO-d6) δ 8.13 (s, 2H), 4.10 (q, J = 7.1 Hz, 2H), 2.99 (t, J = 7.1 Hz, 2H), 2.69 (t, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H).

Synthesis of Ethyl 3-acrylamidopropanoate (2)

Triethylamine (2.635 g, 0.026 mol, 4 eq.), **1** (1.05 g, 0.0065 mol, 1 eq.), anhydrous DCM (13.5 mL) and acryloyl chloride (0.5 M solution in THF, 16.5 mL, 0.0078 mol, 1.2 eq.) were used. After addition of acryloyl chloride, the mixture was stirred at 0 °C for 2h, and then at room temperature overnight. The reaction mixture was washed with 5% $NaHCO_3$, brine, and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the crude was purified using flash column chromatography on

silica gel using ethyl acetate: petroleum ether (1/1) as eluent. The product was collected as a yellowish oil, Scheme 11 (0.4133 g, 37%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.31 (s, 1H), 6.25 (dd, *J* = 17.0, 1.4 Hz, 1H), 6.07 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.62 (dd, *J* = 10.3, 1.4 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.58 (q, *J* = 6.1 Hz, 2H), 2.56 (t, 2H), 1.25 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, CDCl3) δ 165.68, 163.35, 156.37, 153.21, 131.26, 125.85, 83.23, 79.39, 40.16, 39.15, 28.26, 28.04, 27.05, 25.71.

3.3.7 Synthesis of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide



Scheme 12: Reaction scheme for the synthesis of2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide .

Synthesis of 1,3-Bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea (1)

S-methylthiourea hemisulfate (5 g, 0.036 mol, 1 eq.) was dissolved and stirred in a biphasic mixture of saturated $NaHCO_3$ solution (39.5 mL) and DCM (84 mL). Next, di-tert-butyl dicarbonate (15.63 g, 0.072 mol, 2 eq.) dissolved in DCM (60 mL) was added. The mixture was allowed to react for 48h at room temperature. Subsequently, the organic phase was isolated and the aqueous layer was washed with DCM (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The crude solid was dissolved in a 1:9 mixture of EtOH and water and stirred for 1h. Subsequently, the mixture was cooled to 0 °C. The precipitate was filtered via vacuum filtration and washed with water. Purification via column chromatography using Petroleum ether: $CHCl_3$ (15:85) as eluent yield the pure product **1** as a white solid, Scheme 12 (8.2 g, 78.44%). 1H NMR (600 MHz, Chloroform-d) δ 2.40 (s, 3H), 1.52 (s, 18H) (S1.7.).

Synthesis of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylamine (2)

1,4-Diaminobutane (0.322 g, 0.3672 mL, 0.00365 mol, 1 eq.) was dissolved in 3.122 mL DCM. Next, **1** (0.3713 g, 0.00128 mol, 0.35 eq.) was dissolved in 2.53 mL DCM and added dropwise to the solution. After addition, the reaction mixture was allowed to react for 4h at room temperature. After reaction, the mixture was washed with water (3 x 10 mL) and brine (2 x 10 mL), dried over $MgSO_4$ and concentrated in vacuo to yield the pure product **2** as a cloudy oil, Scheme 12 (0.361 g, 85.35%). ^{1}H NMR (600 MHz, Chloroform-d) δ 11.47 (s, 1H), 8.33 (s, 1H), 3.45 – 3.37 (m, 2H), 2.72 (t, J = 7.0 Hz, 1H), 1.73 (s, 2H), 1.61 (dt, J = 15.0, 7.4 Hz, 2H), 1.47 (s, 20H) (S1.8.).

Synthesis of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide (3)

Triethylamine (1.41 g, 0.0139 mol, 3 eq.), **2** (1.5313 g, 0.00463 mol, 1 eq.), DCM (15 mL) and acryloyl chloride (0.5 M solution in THF 11.71 mL, 0.0056 mol, 1.2 eq.) were used. After addition of acryloyl chloride, the mixture was stirred at 0 °C for 2h, and then at room temperature overnight. Saturated $NaHCO_3$ (30 mL) was added and the aqueous layer was extracted with DCM (3 x 25 mL). The organic layer was dried over $MgSO_4$. After removal of the solvent under reduced pressure the crude product was purified using flash column chromatography on silica gel using ethyl acetate: petroleum ether (2/8 to 8/2) as eluent. The product was collected as a white solid (0.5 g, 28% yield), Scheme 12. ^{1}H NMR (600 MHz, Chloroform-d) δ 11.44 (s, 1H), 8.36 (t, J = 5.3 Hz, 1H), 6.81 (s, 1H), 6.24 (dd, J = 17.0, 1.9 Hz, 1H), 6.17 (dd, J = 17.0, 10.0 Hz, 1H), 5.56 (dd, J = 10.0, 1.9 Hz, 1H), 3.39 – 3.32 (m, 4H), 1.57 (dq, J = 23.1, 6.6 Hz, 4H), 1.45 (d, J = 5.2 Hz, 19H).

4 Results and Discussion

As was mentioned earlier, the development of sequence-controlled and defined polymeric structures opened ways to a broad array of novel applications of these polymeric materials. The use of these materials in biological applications becomes notably attractive as high precision structures become readily available.

One of the main points of interest would be to evaluate how far sequence-defined oligomers as derived from acrylate or acrylamide Single Unit Insertion reactions can be used in biological applications. Therefore, peptide structures could be copied to oligoacrylate structures and subsequently analyzed for their activity in comparison to their natural counterparts. In order to achieve this all, a library that contains acrylate or acrylamide mimics of all-natural amino acids will have to be established. One should note that not all naturally occurring amino acids can be copied exactly as the synthetic mimics are envisioned unstable. Two examples stand out in this case: glycine and proline. Proline is an amino acid which displays a side group that is directly attached to the amino functionality. A feature which will not be present in its synthetic form. For this, an open pyrrolidine side group is envisaged. Glycine, in the other case, is accompanied by the problem that it does not contain any functional R-group. The formal equivalent in an acrylamide would be *N*-methyl acrylamide.

In this project, the first foundations of this monomer library are established. Figure 7 displays the acrylamide monomers that match all 21 naturally occurring amino acids as closely as possible. Initially, the use of acrylate monomers was proposed as the use of these molecules will result in an easier purification both after synthesis of the monomers and definitely after polymerization reactions. However, via this approach, not all functional side chains are available. Therefore, a quick shift to acrylamides was taken. The benefit of using acrylamides over acrylates is that virtually all functional groups are available through the decarboxylation of natural amino acids. By removing the carboxylic acid functionality of natural amino acids, their amine functionalities become readily available. As can be seen in Figure 8, one structure is marked with a blue star. *N*-(2-hydroxyethyl)acrylamide, which acts as the mimic of the natural amino acid Serine and is the only compound of the library which is commercially available. The synthetic monomers as depicted in Figure 8, all have one additional carbon atom in comparison with the natural amino acids. This additional carbon atom is added to form a more uniform library since the decarboxylation of natural amino acids will result in the addition of one carbon atom. Therefore, by starting to design these molecules with one carbon atom extra, all molecules present in this library will have this feature and not only the ones derived from the decarboxylated amino acids.

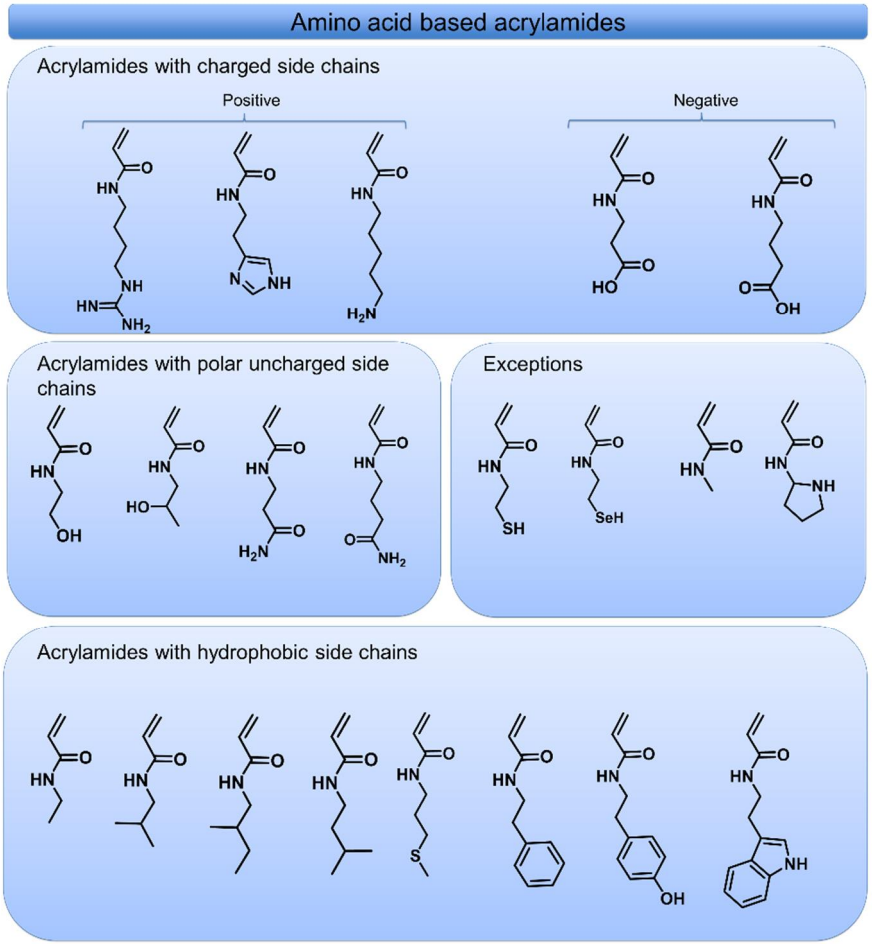


Figure 8: Targeted library of amino acid based acrylamides. The molecule marked with a star is commercially available.

The general synthesis strategy of these amino acid mimics is based on the combination of acryloyl chloride with its appropriate amine forming an acrylamide. However, being an unstable and highly reactive molecule, acryloyl chloride is not commercially available in Australia. Unfortunately, it is an essential compound in the synthesis of acrylamide monomers. As a result, acryloyl chloride had to be synthesized first.

4.1 Acryloyl chloride synthesis

Various reports have already been published on the synthesis of acryloyl chloride based on the transformation of acrylic acid into an acyl chloride.[59, 60] Even though being a well-described reaction, the synthesis is not straightforward and requires specific conditions. The process involves the addition of a chlorinating agent able to chlorinate the acrylic acid present in solution. In literature various organic and inorganic chlorinating agents have already been studied and reported for their use in the synthesis of acryloyl chloride.[59, 60] In this work, two different chlorinating agents were used: oxalyl chloride, an organic molecule, and thionyl chloride, an inorganic compound. Both were tested and compared. Whereas most reports on the synthesis of acryloyl chloride focus on the use of conventional batch reactors, a continuous flow setup is employed in this work. The use of flow chemistry can greatly benefit the reaction as it is fast and highly exothermic. Flow reactors display a high surface-to-volume ratio which allows fast mass and heat transfer, so no hot spots are created.

This results in better control over the reaction parameters, high efficiency, and high selectivity. [57] Moreover, it enables fast and efficient mixing through added or built-in mixing units and due to their smaller volumes, the safety of operation is increased.[61] Finally, the use of flow reactors allows the ability to scale up the production relatively easy.

The setup used in this work was based on an already existing setup developed by the host group at Hasselt University, Belgium. This setup consists of a syringe pump attached to a reactor made out of PFA tubing and allows a safe, reliable and reproducible method to synthesize the acryloyl chloride (Figure 9).

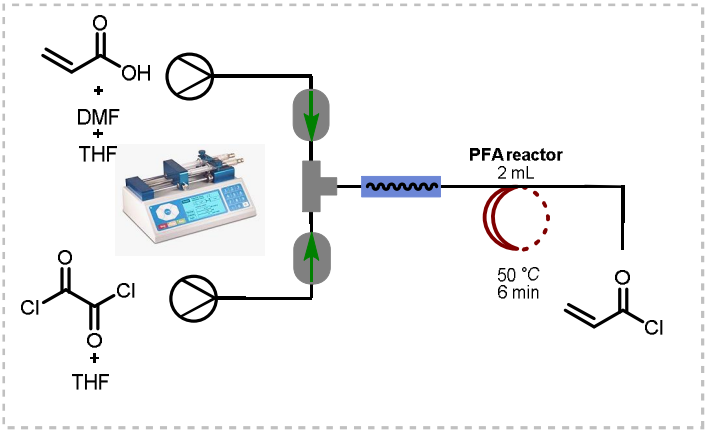


Figure 9: Schematic representation of the continuous flow setup used for the synthesis of acryloyl chloride. Oxalyl chloride was used as a chlorinating agent. One syringe pump containing 2 syringes was attached to a 2 mL reactor in an oil bath of 50°C.

In order to start up the flow setup for the synthesis of acryloyl chloride, optimal reaction conditions had to be determined. Oxalyl chloride was used as a primary chlorinating agent and various reaction times were screened in order to reach optimal reagents to product conversion. For these reactions, a PFA reactor with a volume of 2 mL was used. Two stock solutions were prepared. One containing acrylic acid and DMF, the other containing oxalyl chloride. Both are diluted in anhydrous THF. These reaction conditions were based on previous results from the setup present in our group. They showed that high conversion (>90%) was achieved after 4 min reaction time using a catalyst concentration of 0.0032 M, so these conditions were used as a starting point. Four different reaction times (1, 2, 3 and 4 minutes) were screened and conversion percentages were determined via ^{1}H NMR. An increasing conversion with longer reaction times could be noted. As the setup was composed of two 10 mL syringes, only 4 reaction times could be screened per stock solution. Figure 10 shows the conversion to reaction time relationship with a stabilization of the conversion around 4 min of reaction time using 3.2 mM DMF.

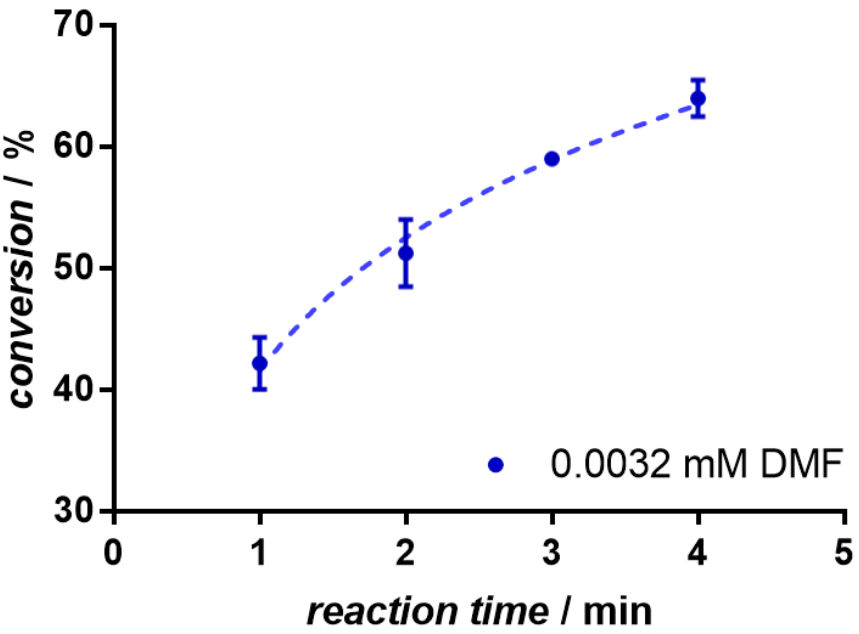


Figure 10: Conversion of acryloyl chloride synthesis using oxalyl chloride as a chlorinating agent. The conversion was measured over different residence times in minutes. The dotted line represents the logarithmical regression line of reaction time over conversion. The data point present at 3 min. reaction time shows no error bars since the error value was too low.

A maximum conversion measured over these time screenings was 63.95% which is not high enough for further use. Therefore, the amount of catalyst used in the reaction was increased. Gradual increases in catalyst concentration (0.00654 M – 0.01615 M) while screening the same reaction times of 4 - 7 minutes shows increasing values of conversion as seen in Table 1. The stabilization at 4 minutes is confirmed when looking at the measurements using a higher concentration of catalyst. Starting measurements at 4 minutes up to a residence time of 7 minutes showed only slight deviations (2%) in conversion when a catalyst concentration of 16.15 mM is used.

All experiments were performed in duplo and the displayed conversion values are average values. As no precise quenching step was employed, the reaction still continued after collection. To ensure valid ^{1}H NMR measurements, samples were taken directly into the solvent in which was measured and were quenched by freezing them with liquid nitrogen. A reaction time of 6 minutes and a catalyst concentration of 0.01615 M proved to be the optimal conditions as product conversions of 96.41% were achieved (Table 1). The proton NMR measurement showing a conversion above 95% can be seen in Figure 11. In the Proton NMR spectrum, the presence of a side product is seen. This molecule is identified as chlorinated acryloyl chloride, 3-chloropropionyl chloride. Integration of these peaks show a value of 0 meaning that the amount present in the product solution is neglectable.

Table 1: Conversion of oxalyl chloride based acryloyl chloride at residence times up to 7 minutes and increasing concentration of DMF in millimolar. The largest conversion is observed at a residence time of 6 minutes and a DMF concentration of 16.15 mM.

Reaction time (min)	3.20 mM	6.54 mM	9.50 mM	12.50 mM	16.15 mM
1	42.16%	n.a.	n.a.	n.a.	n.a.
2	51.23%	n.a.	n.a.	n.a.	n.a.
3	58.99%	n.a.	n.a.	n.a.	n.a.
4	63.95%	74.07%	80.04%	86.34%	94.23%
5	55.38%	n.a.	82.69%	84.42%	94.73%
6	59.70%	80.64%	83.54%	91.19%	96.41%
7	51.15%	n.a.	79.58%	87.24%	n.a.

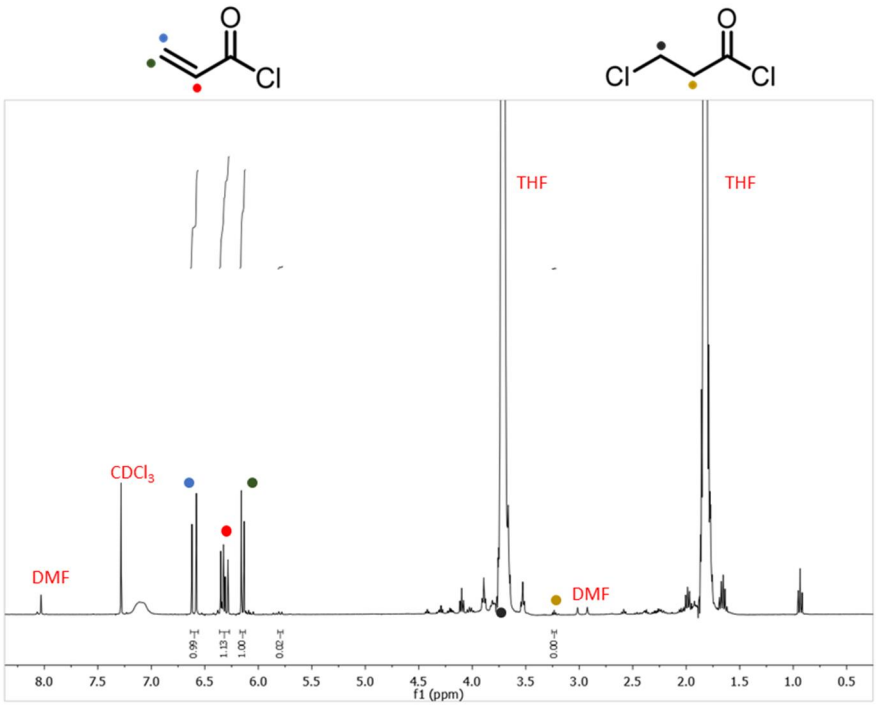


Figure 11: Proton NMR spectrum of oxalyl chloride based acryloyl chloride product in THF. All peaks describing acryloyl chloride are present. A smaller peak attributable to the presence of 3-chloropropionyl chloride is seen. However, the integrated value is zero meaning that its presence in solution is neglectable.

One should note that the exact reaction times may differ slightly from the targeted residence times due to gas build up in the tubular reactor. The synthesis of acryloyl chloride is accompanied by the generation of HCl, CO_2 and CO gas. This gas formation makes an accurate determination of exact reaction times tedious. Because of this, the mentioned reaction times are overestimates of the actual values.

Thionyl chloride was used as an alternative chlorinating agent. Thionyl chloride is, opposed to oxalyl chloride, an inorganic chlorinating agent which is slightly cheaper than oxalyl chloride. Just as in the experiments involving oxalyl chloride, the first step was to determine the optimal reaction conditions to reach a high conversion. Therefore, various reaction times were screened. As a starting point, the

optimal reaction conditions of the reaction using oxalyl chloride were taken. A reaction time of 6 minutes at 50° C with a catalyst concentration of 0.01615 M resulted in a maximum conversion of around 50% in case of thionyl chloride, whereas 96% with oxalyl chloride. This decrease in conversion seems counter-intuitive since in general thionyl chloride is more reactive than oxalyl chloride.[52] This decrease in reactivity was not further investigated in this work. Residence times varying from 2 to 11 minutes were screened at an increased temperature of 60°C (Figure 12). Compared to the reactions using oxalyl chloride as a chlorinating agent, the residence times of these reactions could be determined more precise as the excessive gas formation was not observed when thionyl chloride was used.

Analysis of the samples via ^{1}H NMR showed again a plateau formation, this time with a starting time around 11 minutes, reaching a maximum conversion around 72%. In order to increase the product conversion, the catalyst concentration was gradually increased up to 0.05 M DMF. As a result, a maximal conversion of 96% was reached at a residence time of 11 minutes as seen in Figure 12.

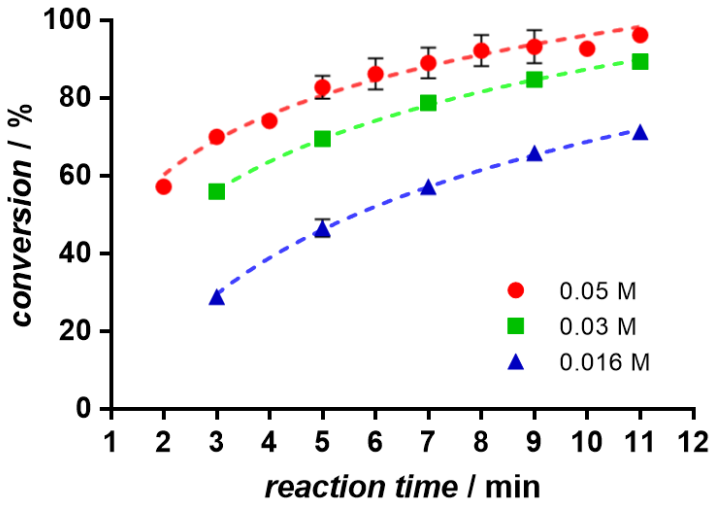


Figure 12: Conversion measurements in acryloyl chloride synthesis using thionyl chloride as a chlorinating agent. The graphs show a logarithmic increase over time and appear to have a plateau phase starting at 11 minutes. Increase in catalyst concentration increased the conversion drastically until a maximum value of 94% was reached at DMF concentration of 0.05 M after 11 minutes.

Table 2: Conversion of thionyl chloride based acryloyl chloride at residence times up to 11 min and increasing concentration of DMF. The largest conversion is observed at a residence time of 11 min and a DMF concentration of 50 mM.

Reaction time (min)	16.15 mM	30 mM	50 mM
2	n.a.	n.a.	57.14%
3	28.81%	55.86%	69.93%
4	n.a.	n.a.	74.07%
5	44.84%	69.44%	81.96%
6	n.a.	n.a.	82.64%
7	57.14%	78.74%	89.28%
8	n.a.	n.a.	88.49%
9	64.93%	84.74%	93.45%
10	n.a.	n.a.	92.59%
11	71.94%	89.28%	96.15%

All experiments were performed in duplo and the displayed values are averages, displaying the reproducibility of the setup. Again, the samples used in these screening reactions were frozen in liquid nitrogen before NMR measurements to prevent further reaction in the NMR tube. In depth analysis of the ^{1}H NMR spectra indicated the formation of side products during the reaction. One specific side product, 3-chloropropionyl chloride, was clearly visible (Figure 13) (3%). The double bond of acryloyl chloride is prone to HCl addition. As no clear gas formation was visible during the reaction, a possible explanation would be that HCl remains in solution. This would also explain the larger amount of 3-chloropropionyl chloride formation in the reaction with thionyl chloride compared with the reaction employing oxalyl chloride as a chlorinating agent. The difference in gas formation in both reactions is not yet clear. Longer exposure to the acid causes more degradation and a lower yield of the desired product. Storing while the HCl is still present in the solution is impossible due to the rapid degradation overnight, decreasing the product yield significantly. Immediate use of the synthesized 0.5 M solution of acryloyl chloride bypasses this problem by reducing the exposure time to the acid.

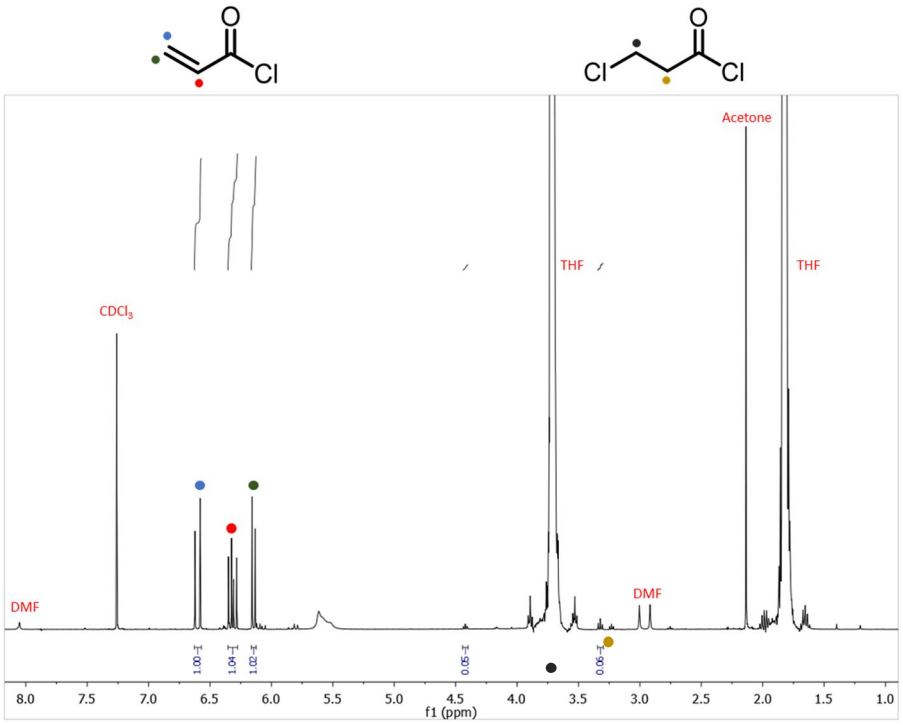


Figure 13: Proton NMR spectrum of acryloyl chloride product in THF. All peaks describing acryloyl chloride are present. A smaller peak attributable to the presence of 3-chloropropionyl chloride is seen (yellow) showing a presence of 3%.

4.1.1 Upscaling acryloyl chloride synthesis

After determination of the ideal reaction parameters, the ability to upscale the reaction was studied. The scale up of the procedure would allow the immediate production of large quantities of product per reaction. Upscaling itself can be achieved using multiple approaches such as increasing the concentration or increasing the flow speed by adapting the reactor.

The upscaling of the acryloyl chloride production process was performed through various approaches. The first approach was inspired by the work reported by Movsisyan et al.[51] In their work they

established a continuous flow procedure for the synthesis of acryloyl chloride out of acrylic acid, via a solvent-free approach. Different chlorinating agents were tested (thionyl chloride, oxalyl chloride, benzoyl chloride), each time screening the reaction time, temperature and variable concentrations of the chlorinating agents. Their results showed a selective conversion of 97% at room temperature, after a reaction time of 1 min. with 8.6 M of DMF, 4.3 M acrylic acid and 11.8 M of oxalyl chloride as a chlorinating agent. These results were used as a starting point but resulted in low conversion values. As a result, adaptations to the process were necessary.

In this report, different reaction conditions were investigated by screening different reaction times and temperatures. The first chlorinating agent of choice was oxalyl chloride and a smaller reactor with a volume of 1 mL was used. Two separate syringe pumps were used, one associated with a syringe containing chlorinating agent and the other associated with a syringe containing acrylic acid and DMF. During the mixing phase, in which the acrylic acid and oxalyl chloride were reacted in equimolar concentrations (4.3 M) in the presence of an excess of DMF (8.6 M), an intense reaction took place with large amounts of gas formation. As a result, the residence times were overestimated. Product conversion was measured after reaction times of 1, 3 and 6 minutes at a reaction temperature of 50° C. After a reaction time of 6 min at 50°C, proton NMR measurements showed a conversion of 95% into acryloyl chloride. Fractional distillation at room temperature was used to purify the synthesized product to make it less susceptible to degradation. Since acryloyl chloride is a reactive molecule it's better to isolate it as much as possible when trying to store it. Therefore, distillation seemed the best solution to purify the product even though the use of the impurified solution is possible in further reactions. This was proven to be successful as purified acryloyl chloride was confirmed by both ^{1}H and ^{13}C NMR (Figure 14). Unfortunately, the distillation step was not able to entirely separate the product from the solvent since acryloyl chloride, oxalyl chloride and THF only have small differences in boiling point. Furthermore, the distillation of the product resulted in very small yields lower than 10%.

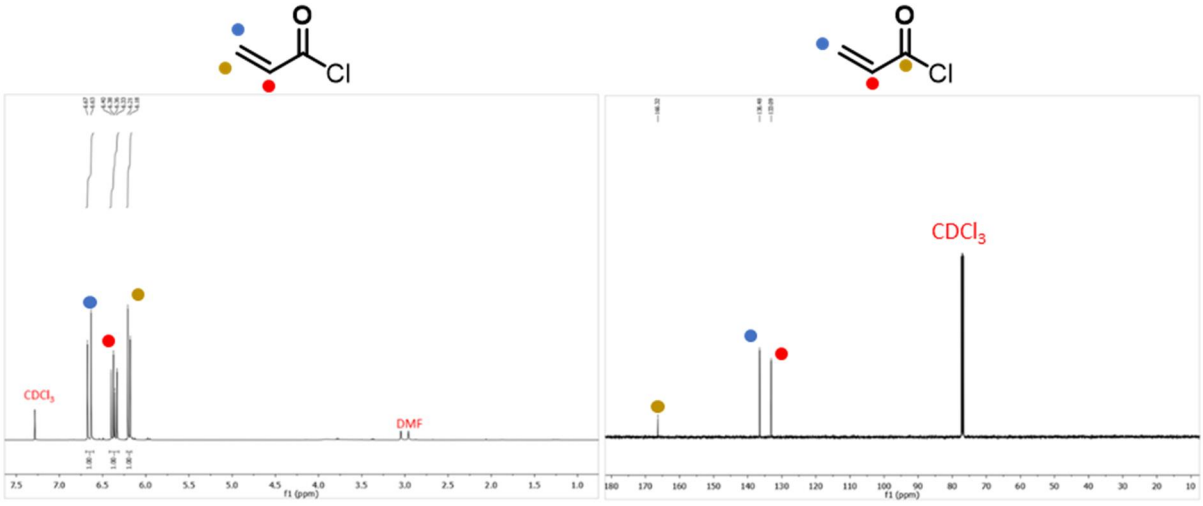


Figure 14: ^{1}H NMR (left) and ^{13}C NMR spectra of acryloyl chloride prepared in a continuous flow reactor after purification via vacuum distillation.

A second attempt to upscale the reaction was performed by doubling the reactant concentration. By doing this, theoretically, a product mixture with a concentration of 1M could be achieved. The catalyst concentration was kept constant in this reaction. A reaction time of 13 minutes showed promising results with proton NMR showing a product conversion of 99%, however, proton NMR also showed a significantly increased amount of 3-chloropropionyl chloride formation of 15% compared to the 8% formed during the reaction with stock solutions of 1M. This increase in side product formation can be attributed to the increased amount of HCl formation. As this gas remains in solution and is present in double the concentration, the chance of side product formation increases tremendously. As a result, this approach was not used to upscale the procedure.

The third attempt to increase the production was adapting the reactor in an attempt to increase the output. A copy of the reactor was created so that two reactions could be performed in parallel. A second syringe pump and two glass 20 mL syringes were connected to the other two mL reactor (Figure 15). The second reactor showed the same conversion values as the first on proton NMR. With the two reactors running in parallel, a total amount of 90 mL per day can be produced. This translates to 4.5 gram of acryloyl chloride per day.

Figure 15: Flow setup for the upscaling of acryloyl chloride production. A replica of the existing reactor was made attached to a second syringe pump. This enables the simultaneous reaction in both reactors doubling the produced amount.

Upscaling of the output by tweaking the reactor itself was also achieved. A reactor was built with three times the volume of the initial two mL reactor. The flow speed was simultaneously increased to three times its original value. Both changes made sure that the residence time was still kept constant at 11 minutes, but the pump speed increased by a factor three creating a larger flow rate. A downside of this larger volume was that in the initial stabilization step 6 mL of stock solution is needed to fill the reactor before collection could take place. This problem can be circumvented by refilling the syringes after the addition of the full volume to the reactor. Refilling the syringes enables this method to produce a continuous flow of acryloyl chloride (0.5 M) with a speed of 0.5454 mL/min.

Since both reactor adaptations resulted in an increased output, a combination of both was tested. A second reactor with a volume of 6 mL was built and the reaction was performed in both 6 mL reactors in parallel. This resulted in an increased output of 1.09 mL/min. This output would result in a total production of 20 g acryloyl chloride per day. Converted to hourly production, this is 2.5 g per hour which is almost 4 times lower than the output described in the paper of Movsisyan et al. They describe an hourly production of 8.5 g acryloyl chloride. Even though our production is lower, we have the advantage of being able to use our product immediately after production while the solvent-free

method requires purification using distillation. While at this point an output of 2.5 g seems sufficient to obtain the needs of acryloyl chloride in the laboratory, the easy scalability by increasing the reactor volume combined with the use of HPLC pumps can increase this output with ease depending on the need.

4.2 Acrylate monomer synthesis

As was previously stated, the initial strategy was to establish an acrylate monomer library. This is possible through esterification reactions of acryloyl chloride with the appropriate alcohols. The first target was 3-((tert-butoxycarbonyl)amino)propyl acrylate, a mimic of the natural amino acid lysine. the formation of a lysine mimic via a two-step reaction. The first being a protection reaction using tert-butyl dicarbonate, protecting the amine present on the molecule. The second involves the esterification of the molecule using acryloyl chloride.

Esterification using the previously synthesized acryloyl chloride showed successful results. Proton NMR of the product showed a conversion of 72.9%. A follow-up proton NMR measurement of the purified product showed the clear presence of the lysine mimic in a pure form as seen in Figure 16. However, further calculations only showed a purified yield of 21.8%. The loss of product can be attributed to several factors. First, the protection reaction was not fully completed. Since this was only a proof of concept reaction, the next step was performed with a low yield of protected molecules. A second reason is that due to the presence of acryloyl chloride in a THF solution, the reaction was performed in THF. THF and water mix well with each other making the extraction step using water tedious. Not using this separation procedure resulted in less efficient purification of the product and a lower yield.

The reaction has proven that the use of the synthesized acryloyl chloride is efficient and that formation of amino acid mimics is possible. With further adaptations in the reaction mechanisms and workup procedure, it should be possible to increase the yield. Further reactions will focus on the synthesis of amino acid mimics based on acrylamides, so no further research was performed on acrylates.

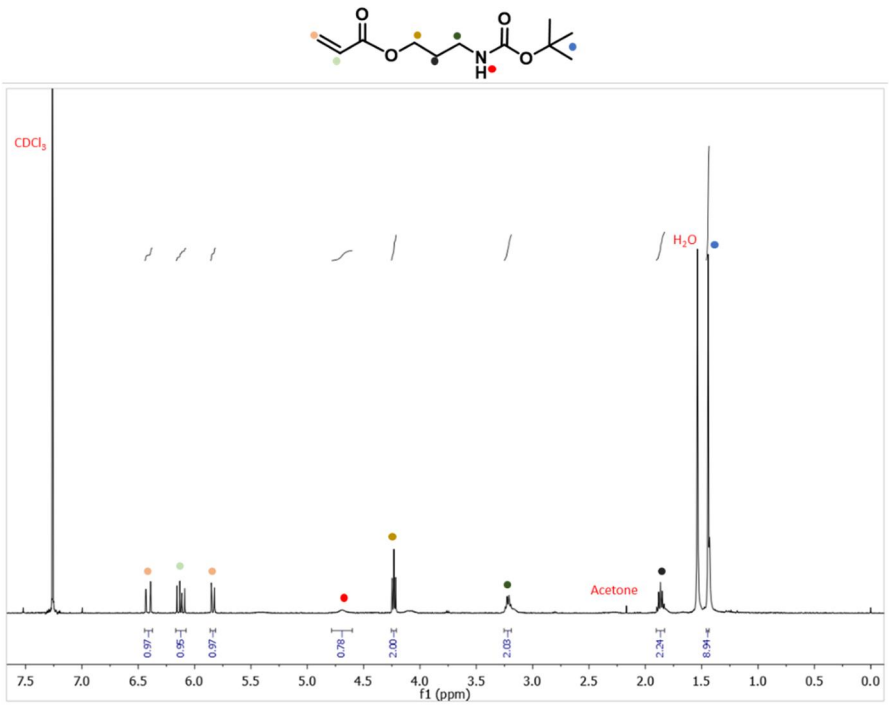


Figure 16: ^{1}H NMR spectra of 3-((tert-butoxycarbonyl)amino)propyl acrylate. All signature peaks are present in the spectrum.

4.3 Acrylamide monomer synthesis

As mentioned earlier, not all functional side chains are available to create an acrylate based monomer library. A quick solution to this problem is a shift towards acrylamide based molecules. Using acrylamides makes all functional side chains available as they can be achieved via the decarboxylation of the natural amino acids.

The main strategy for the monomer synthesis involved the addition of acryloyl chloride, straight from production, into a solution containing the appropriate amine together with triethylamine (TEA) (Figure 17). The mixture was diluted in THF and placed in an ice bath to prevent heating of the product solution as a result of the highly exothermic character of the reaction. Different amines are described in this paper showing successful results in synthesizing a monomer library consisting out of acrylamides. All further mentions of acryloyl chloride refer to the product synthesized as described in 4.1.

Figure 17: Flow setup for the synthesis of monomer mimics. The produced acryloyl chloride was added dropwise to a vial containing the appropriate amine and TEA in THF.

4.3.1 *N*-isobutylacrylamide

The first produced monomer was *N*-isobutylacrylamide by reacting acryloyl chloride with isobutylamine. *N*-isobutylacrylamide is the acrylamide mimic of the natural amino acid valine. The reaction was straightforward since the only functional group available on the molecule was an amine. During the reaction, there was a large amount of salt formation as a result of the presence of HCl in the synthesized acryloyl chloride and the additional formation of it during the formation of the acrylamide. Because of this, a bigger stirrer bar needed to be used at a faster rotation speed to prevent clogging of the magnet. By doing this the formation of salt particles can be reduced to small particles present in suspension making this method transferrable to a flow setup. After the reaction, the reaction solution containing the monomer was filtered and the remaining salt was washed three times using THF to make sure all product is removed. Sufficient washing off the salt resulted in an

increase in purified product yield up to 54.50% which is a 10% increase in yield in comparison to the previous method, in which the salt was not washed, yielding 44.69%.

Small scale synthesis targeted the formation of 0.0034 mol of acrylamide using a slight excess of acryloyl chloride. After purification 0.2583 grams of product was collected translating to a 54.50% purified yield. The isolated product is pure as seen in the proton NMR in Figure 18. The only impurity present is a small amount (5%) chlorinated side product (3-chloro-N-isobutylpropanamide) also pictured in Figure 18. A small fraction of chlorinated molecules seems to be inseparable from the targeted molecule. However, the presence of the small amount of side product is acceptable as the chlorinated molecule lacks a double bond and as a result will not interfere with following polymerization reactions. Because of this, the unwanted molecules can be removed in a later reaction step.

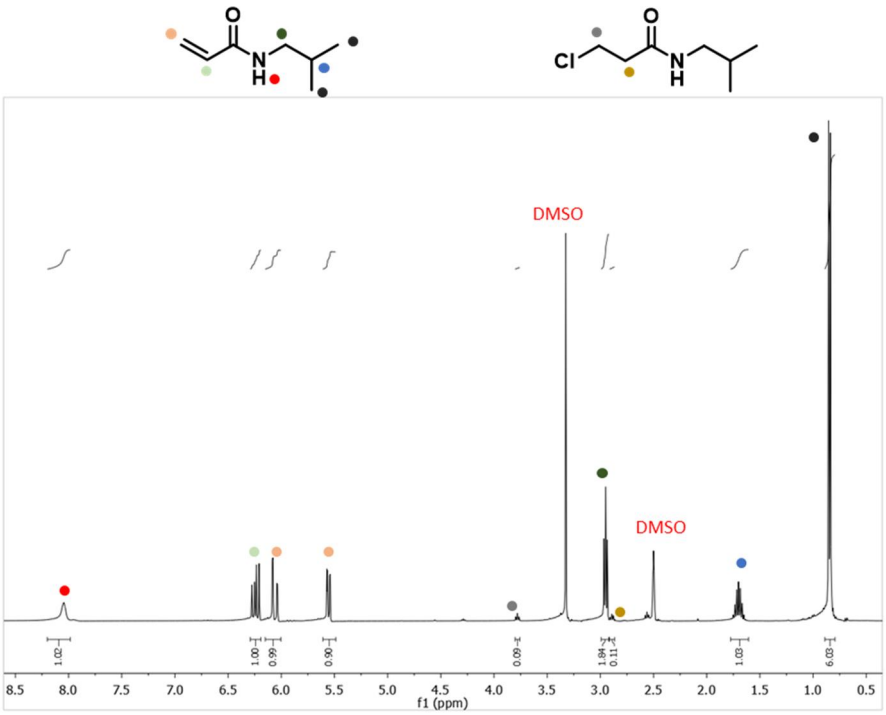


Figure 18: ^{1}H NMR spectrum of N-isobutylacrylamide with all signature peaks present in the spectrum. A small amount of 3-chloro-N-isobutylpropanamide (5%) is still present after purification but does not influence the further use of the molecules.

As the synthesis of *N*-isobutylacrylamide is a very straightforward procedure, it is an ideal synthesis protocol to test the ability to scale up the reaction. Ideally, the monomers will have to be synthesized in multiple gram scale. As stated before, the use of flow reactors allows a relatively easy scale-up of reactions. In an attempt to scale up the reaction, a total amount of 24 g of *N*-isobutylacrylamide was targeted. The upscaling itself was approached by using two different earlier described upscaling techniques, namely doubling the number of reactors and increasing the reactor volume to increase the flow speed. Two identical reactors were put together, both with a volume of 6 mL instead of the previous 2 mL. These changes to the reactor enabled a continuous output of 1 mL, 0.5 M product solution. Refilling of the syringes loaded on the syringe pumps for the synthesis of acryloyl chloride allowed continuous addition over an entire day (8 hours), good for a total production of 20g. The reaction conditions and equivalents were the same as the one used in the small-scale synthesis. The

collected product after filtration was purified using column chromatography just as described for the small scale. After purification, 11.489 g of pure product, confirmed by proton and C^{13} NMR (S1.1.), was collected, resulting in a total purified yield of 47.12%. This yield, although low, is within limits described by Zang et al.[62] In their paper they tested the influence of different acyl chlorides on amide formation by varying the solvent used in the reaction as well as the base used to capture the formed HCl. When using the same conditions applied in our reaction (THF and TEA) the resulted yield varied between 30 and 88 percent depending on the acyl chloride used. Important to mention is that by changing the used salt from TEA to tripotassium phosphate they were able to increase the yield of all reported couplings on primary amines up to 94% or more. As a result, the use of tripotassium phosphate instead of TEA can be beneficial in increasing the purified yield of the synthesis procedures involving a primary amine.

Another paper published by Agüero et al reported the synthesis of monomers based on amines using a nucleophilic substitution reaction.[63] Here, yields over 90% were reported which is almost double the amount acquired by our process. One of the reasons for this difference could be that in their work commercially bought acryloyl chloride was used in its pure form (one-step reaction) while we work with a diluted form (two-step reaction) resulting in less efficiency and more side product formation.

4.3.2 *N*-methylacrylamide

The addition of acryloyl chloride to methylamine produced *N*-methylacrylamide. This molecule represents the acrylamide mimic of the natural amino acid glycine which is the simplest of all amino acids. Glycine is an amino acid that does not display a specific side chain and the formal equivalent in an acrylamide would be methylacrylamide. As methylamine is a gas, a solution of 2 M in THF was employed. Small scale synthesis targeted the production of 0.0056 Mol of product. Again, the reaction involved a rapid salt formation due to the high presence of HCl in solution. Purification of the product resulted in a pure liquid, confirmed by the C^{13} (S1.2.) and proton NMR (Figure 19), weighing 0.1981 g. This translates into a purified yield of 41.6%.

The proton NMR spectrum taken from the product solution again shows the presence of a small fraction (4%) of chlorinated side product. 3-chloro-*N*-methylpropanamide is visible in the spectrum seen in Figure 19. Just as in the previous molecule the lack of a vinyl group results in this molecule being non-reactive in further polymerization reactions. This makes it able to remove this waste molecule in later steps.

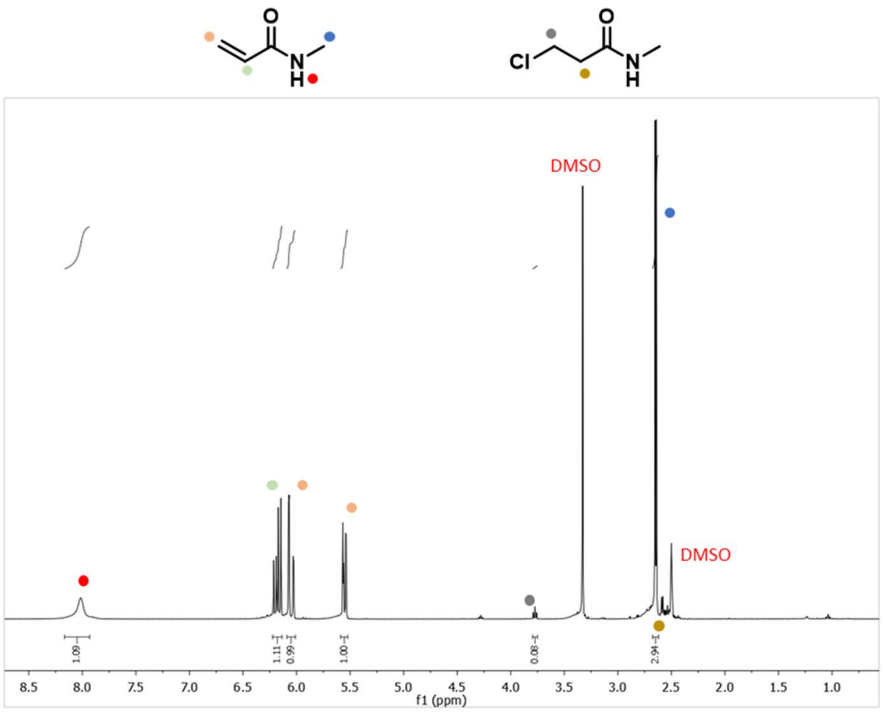


Figure 19: [1]H NMR spectra of N-methylacrylamide with all signature peaks present in the spectrum. A small fraction of 3-chloro-N-methylpropanamide is still present. One of the CH2 molecules (yellow) is present underneath the methyl group peak (blue).

Upscaling of this procedure, targeting 0.0155 mol of product, showed similar results. Purification of the product solution delivered 0.412 g of purified product. This again only is a purified yield of 42.1%. After revaluation of the reaction procedure, the problem was discovered in the filtration step. Simple rinsing of the salts was not enough to flush all product out, thus, multiple washing steps using THF were used to wash as much product out as possible. Another upscaling, attempt targeting 0.023 Mol of product, showed that the rinsing of the salt increased the purified yield significantly. 1.3289 g of the purified product was collected, indicating a purified yield of 67%. An increase of 25 percent compared to the method without washing. This major increase in yield indicates that a lot of product remains in the salt formed during the reaction. Therefore, a change in base to tripotassium phosphate instead of TEA, as described in the paper by Zhang et al, could potentially lower the amount of product stuck in this salt and as a result, increase the yield.[62]

4.3.3 *N*-phenethylacrylamide

The third monomer synthesized was *N*-phenethylacrylamide by combining acryloyl chloride with 2-phenylethan-1-amine. The resulting product is a synthetic mimic of the natural amino acid phenylalanine. Just as in the previously described monomers the synthesis involves the dropwise addition of acryloyl chloride immediately after synthesis to a THF diluted amine solution. Small scale synthesis targeted the formation of 0.0034 mol monomer mimic. After filtration and purification, 0.3822 g of product was collected resulting in a purified yield of 66.32% confirmed by proton NMR (Figure 20) and C^{13} NMR (S1.3.). This relatively high yield can be explained by the easiness of the purification process of *N*-phenethylacrylamide. The presence of a benzene ring in the molecule also attributes to the high purified yield. The benzene ring makes the molecule more apolar and so decreases the interaction with the column. This apolarity makes the product flow through the column more easily and could have a small influence in the purification process. Even though purification was simple with a clear detection, some chlorinated side product, 3-chloro-*N*-phenethylpropanamide, was still present (4%).

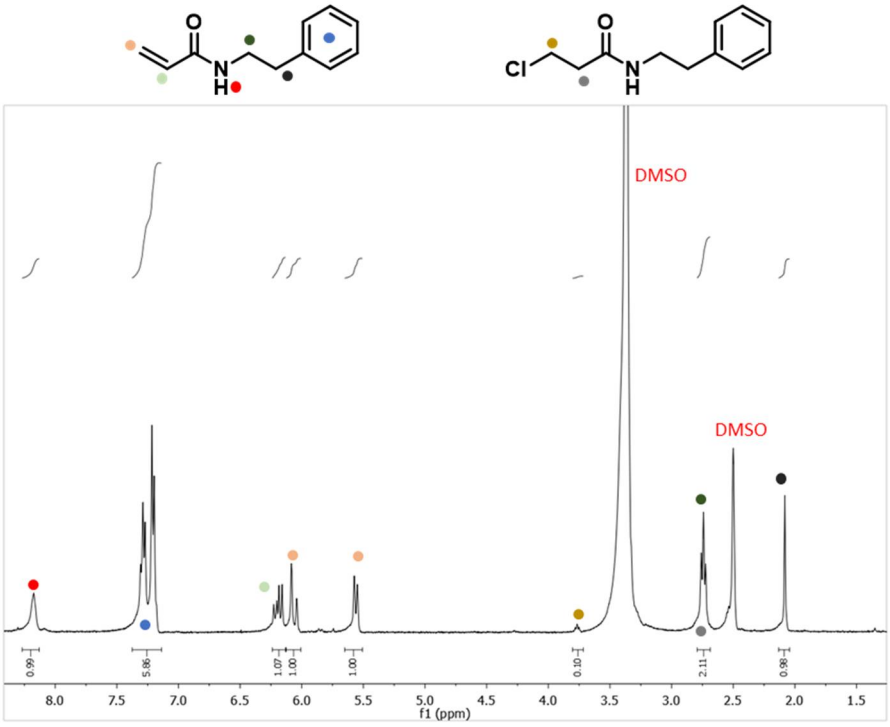


Figure 20: ^{1}H NMR spectra of N-phenethylacrylamide with all signature peaks present in the spectrum. A small fraction (4%) of the chlorinated side product (3-chloro-N-phenethylpropanamide) is detected. One of the CH_2 peaks of the side product (grey) is present underneath one of the CH_2 peaks of the product (green).

4.3.4 *N*-isopentylacrylamide

N-isopentylacrylamide was synthesized by combining 3-methylbutan-1-amine with acryloyl chloride. Resembling the natural amino acid leucine, the product is very similar to the valine mimic, *N*-isobutylacrylamide, differing in only one carbon atom. Being similar molecules, the method of production again involved the dropwise addition of acryloyl chloride straight from production. Small scale synthesis targeted 0.0034 mol monomer mimic. Filtration and purification of the product solution yielded 0.2663 g of product with a purified yield of 55.46%. Proton NMR measurement, as seen in Figure 21 and C^{13} NMR measurement (S1.4.), showed a clear spectrum. However, again a small amount of side product was present (6%). Just as in the previously described monomers this is the chlorinated form of the targeted molecule, 3-methylbutan-1-amine. Again, this side reaction molecule, although present in the product solution, does not influence further polymerization reactions due to the lack of a vinyl group.

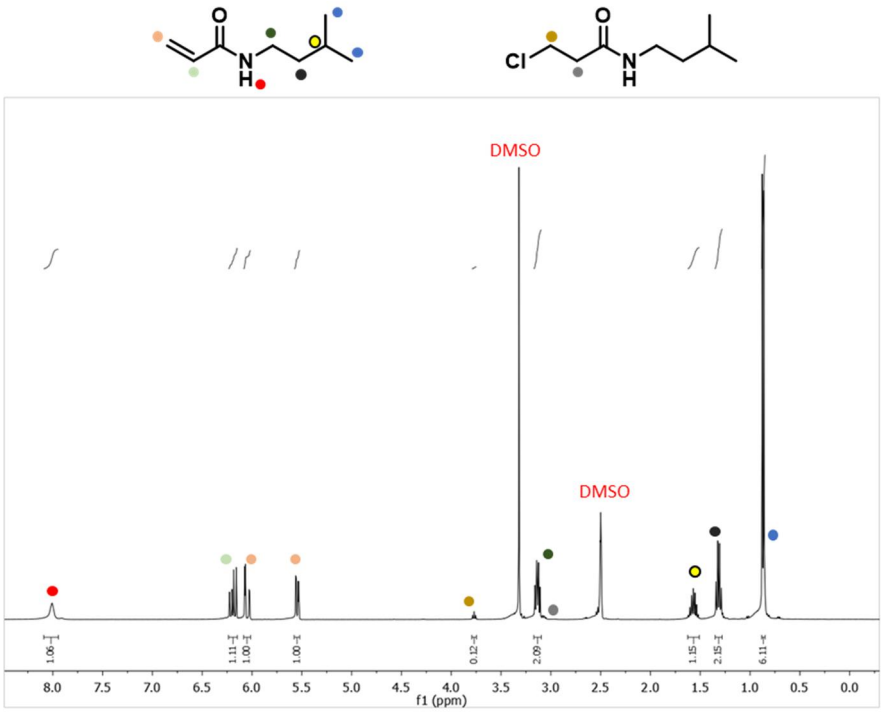


Figure 21: ^{1}H NMR spectra of N-isopentylacrylamide with all signature peaks present in the spectrum. A small fraction (6%) of the chlorinated side product (3-methylbutan-1-amine) is detected. One of the CH_2 peaks of the side product (grey) is present underneath one of the CH_2 peaks of the product (green).

4.3.5 *N*-(2-hydroxypropyl)acrylamide

The synthesis of *N*-(2-hydroxypropyl)acrylamide, also known as the threonine mimic, was performed in a similar way as the previous four acrylamides by the dropwise addition of acryloyl chloride to a diluted amine solution. However, the reaction conditions needed to be slightly adapted due to the presence of the alcohol functionality. As shown in the synthesis of the acrylate lysine mimic, alcohol functionalities also react with the acryloyl chloride solution. If the same reaction conditions would be used as the previous acrylamide monomers, a bifunctional acrylamide would be created and the yield would drop drastically. In order to avoid this from happening, the acryloyl chloride was added to an excess of amine. Because amines are better nucleophiles than alcohols their reaction with acyl

derivates, such as acryloyl chloride, is faster.[64] This would favor the formation of the desired acrylamide. The amount of amine used was three times as much as the acryloyl chloride equivalent to ensure that the addition to the amine was the main reaction and was diluted in DCM instead of THF. The synthesis targeted the formation of 0.00888 mol of monomer. After filtration and purification, 0.485 g of pure product, confirmed by proton NMR (Figure 22) and C^{13} NMR (S1.5.), was collected, which resulted in a purified yield of 42.3%. Furthermore, a total of 6% chlorinated by-product (3-(3-chloropropanamido)propanoic acid) was observed. The presence of this chlorinated molecule can again be attributed to the presence of HCl in solution.

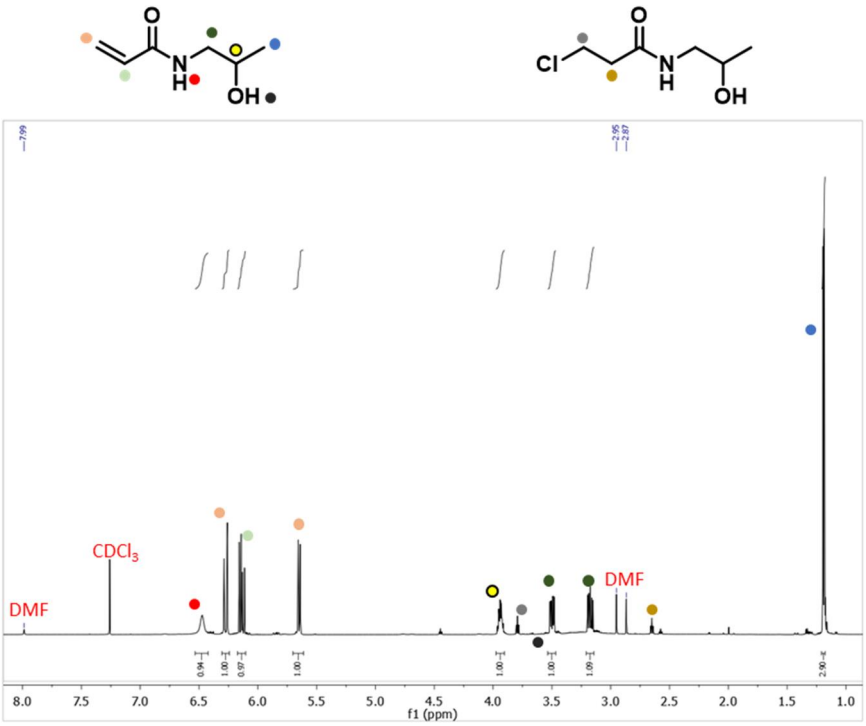


Figure 22: ^{1}H NMR spectra of N-(2-hydroxypropyl)acrylamide with all signature peaks present in the spectrum. A small fraction (6%) of the chlorinated side product (3-(3-chloropropanamido)propanoic acid) is detected. The peak representing the alcohol group (black) is hidden underneath one of the CH_2 peaks (green).

4.3.6 Ethyl 3-acrylamidopropanoate

Ethyl 3-acrylamidopropanoate was targeted as a mimic for the natural amino acid Aspartic acid. Aspartic acid is one of the five charged amino acids. Aspartic acid and glutamic acid are anionic amino acids as they have a carboxylic acid functional side chain which is deprotonated under normal physiological conditions.

In order to synthesize aspartic acid, an amine which holds a carboxylic acid functionality was necessary. β-Alanine, or 3-aminopropionic acid, is a naturally occurring beta amino acid and is the ideal starting component for the synthesis of a mimic of Aspartic acid. The downside of using β-Alanine is, however, that it is insoluble in all organic solvents. It is only soluble in water. As a reaction with acryloyl chloride is envisaged, doing the reaction in water is not ideal as acryloyl chloride will also react with it to form acrylic acid. Therefore, an alternative pathway was studied. In order to make β-alanine soluble in an organic solvent, the carboxylic acid functional group had to be

protected. Starting from this concept, a method which relied on the reaction of alanine with a chlorinating agent in the presence of a large excess of alcohol was used. β-Alanine was reacted with thionyl chloride, in order to form an acid chloride, in anhydrous ethanol. The formed acid chloride would then react with ethanol forming an ester (S1.6.). By protecting the carboxylic acid, the amino acid becomes soluble in organic solvents. An additional benefit of this protection step is the purification afterwards. Purification of compounds that contain carboxylic acid functionalities can be a quite tedious procedure. The reason for this is that, due to the acidic nature of silica, the carboxylic acid interacts with silica. Some of the acid is protonated whereas another part is not. This can cause trailing on the column and makes the separation harder. By protecting the acid functionality, this problem is circumvented. Deprotection of the carboxylic acid can afterwards be done by treating the ester with a strong base in the presence of methanol.

The aminoester was then reacted with acryloyl chloride in order to form the required acrylamide (Scheme 11). Again, a similar procedure was used. Figure 23 shows the proton NMR spectrum of the product after column chromatography. An isolated yield of 38% was obtained. This initial yield is quite low but gave the first indication that the reaction was successful. However, further optimization of the reaction in order to increase the final yield is necessary. The spectrum in Figure 23 also indicates the presence of other chemical compounds even after purification with column chromatography. One chemical which is still present is the chlorinated side product that arises from the addition reaction of the acrylamide and the formed HCl gas. Another product which may still be present is 3-(acryloyloxy)propanoic acid, a product that arises from the dimerization of acrylic acid. The dimerization reaction of acrylic acid is a well-known spontaneous reaction that happens through a Michael addition. The reaction happens when an acrylic acid molecule undergoes spontaneous dissociation and forms an anion. This anion is prone to Michael addition reaction with other acrylic acid molecules and so, forms dimers.[65] The presence of this product, however, has still to be confirmed and further optimization of the synthesis and workup procedures are ongoing to limit the presence of these products.
With this first indication of a successful reaction, the same principle can and will be used for the synthesis of ethyl 4-acrylamidobutanoate, the mimic of Glutamic acid as both Aspartic and Glutamic acid have a very similar structure.

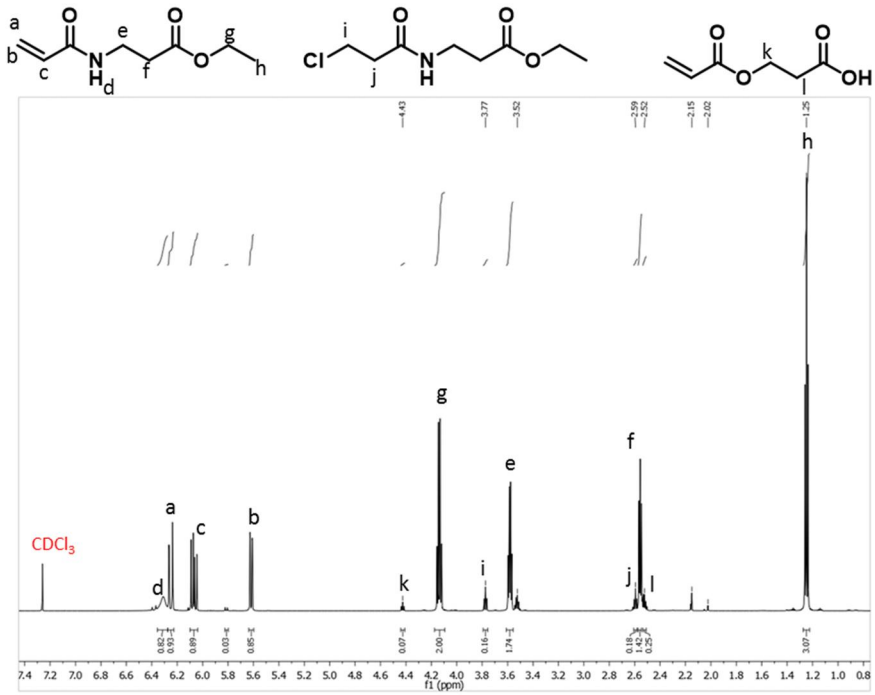


Figure 23: ^{1}H NMR spectra of Ethyl 3-acrylamidopropanoate with all signature peaks present in the spectrum. A small fraction (8%) of the chlorinated side product is detected. A side product of the acryloyl chloride synthesis is present and identified as 3-(acryloyloxy)propanoic acid.

4.3.7 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide

As a mimic of the naturally occurring amino acid Arginine, 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide, was synthesized. The use of arginine mimics has already been reported in literature. Generally, the main focus in the design of this monomer is the incorporation of the guanidine functionality and not much attention is given to the length of the carbon chain between the guanidine and the acrylamide functionalities. As structures, that mimic the natural form as closely as possible, are targeted in this work, the length of the spacer is an important feature. Moreover, two possible synthesis strategies exist: (i) the synthesis of an acrylamide displaying a protected amine terminal group, which can be functionalized post-polymerization, or (ii) the synthesis of an acrylamide displaying a protected guanidine functional group. As the monomers will be used in SUMI reactions via RAFT polymerization in a later phase, the latter strategy was chosen. The use of a protected amine functional monomer would require the post-polymerization modification from amine to guanidine. This reaction involves the modification of free amines under elevated temperatures in the presence of RAFT endgroups. This will result in unwanted side reactions like the aminolysis of RAFT endgroups.

As a first step of the synthesis procedure, S-methylthiourea hemisulfate was protected with di-Boc. As this is a straightforward reaction, it could be performed on a gram scale (8.2 g purified product) and resulted in high purified yields (78%). Subsequently, 1,4-Diaminobutane was modified with the protected salt to form the desired side group. As only one amine group needed to be protected, a large excess of diamine compared to salt, was used. This reaction resulted in purified yields of 85% after 4h reaction times. For the final step, an identical procedure as was described previously, was used. The synthesis involves the dropwise addition of acryloyl chloride immediately after synthesis

to a THF diluted amine solution. After filtration and purification, the product was obtained as a white solid. Proton NMR (Figure 24) also indicated the presence of chlorinated side product. This amount is limited to 8%. The ^{13}C NMR spectrum of the product can be seen in S1.9.

After purification, 0.5 g of product was obtained. This can be translated to a purified yield of 28%, which is quite low. Analysis of the crude ^{1}H NMR indicated a conversion of almost 90%. The most probable explanation for this striking difference in conversion and purified yield is that product is lost during the workup procedure. It is possible that the amines and amides in the structure can form hydrogen bonds with water molecules, causing part of the product to remain in the water phase instead of moving to the organic phase. This, however, has still to be confirmed but due to time limitations, could not be completed in this work.

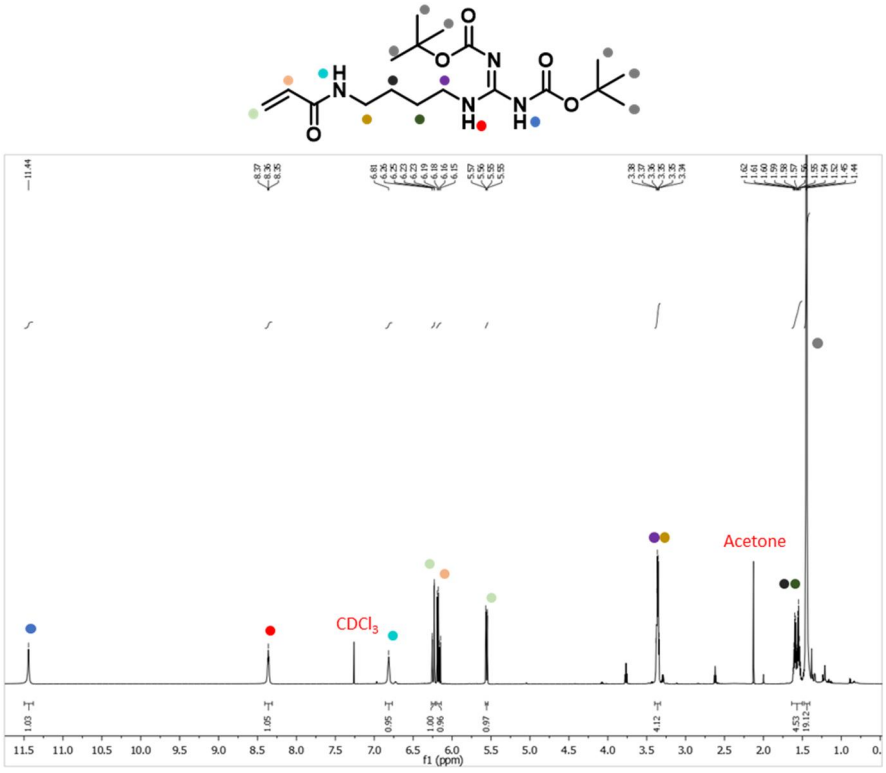


Figure 24: ^{1}H NMR spectra of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide with all signature peaks present in the spectrum. A small fraction (8%) of the chlorinated side product is detected.

5 Conclusion

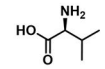
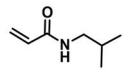
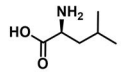
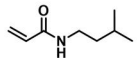
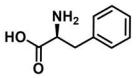
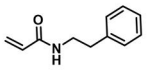
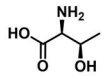
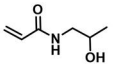
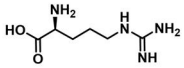
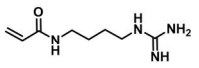
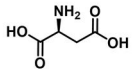
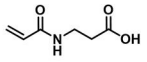
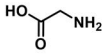
In this thesis, a continuous flow setup was developed for the production of acryloyl chloride by combining acrylic acid with an appropriate chlorinating agent. The ideal reaction conditions for this setup were tested, using a syringe pump attached to a 2 mL PFA tubular reactor. Different chlorinating agents, as well as changes in reaction time and temperature, were screened. The setup was first constructed based on a setup present in the guest research group in Belgium. Development of this setup has proven to be a tedious procedure as the first results showed a low product conversion. Using thionyl chloride as a chlorinating agent and gradually increasing the temperature, reaction time and amount of catalyst, a conversion of 96% was achieved after a residence time of 11 minutes.

When a general procedure for the production of acryloyl chloride was determined, the possibility to produce larger amounts was examined. Upscaling attempts using an increased concentration of both stock solutions was unsuccessful. The higher concentration resulted in increased formation of chlorinated side product (15%) because of the presence of more HCl. Performing the reaction without solvent by adding pure chlorinating agent to a mixture of acrylic acid and DMF showed promising results. High conversion and a low amount of chlorinated side product were measured. However, the product solution was not separable from the used DMF by distillation. Only small fractions of purified product could be collected and as a result, could not be used in further reactions. Upscaling by adapting the characteristics of the reactor was successful. Increasing the reactor volume to 6 mL resulted in an increase in flow by a factor of three decreasing the reaction time by that same factor. Construction of a second 6 mL reactor with the same characteristics allowed the use of both simultaneously doubling the product output. These adaptations increased the total product output to 500 mL of 0.5 M solution or 22.6 g per day. In total, these adaptations to the flow setup resulted in a setup capable of on-demand synthesis of acryloyl chloride needed for the synthesis of multiple molecules.

In the second part of this thesis, the produced acryloyl chloride was used in the synthesis of monomers mimicking natural amino acids. These monomers consist of acrylamides with a functional group resembling their natural counterparts. Acryloyl chloride was added dropwise to a vial containing the appropriate amine diluted in THF together with TEA. The reaction of acryloyl chloride with the amine forms the preferred acrylamide amino acid mimic with as goal the formation of a library consisting of synthetic mimics of all naturally occurring amino acids. Small scale synthesis, targeting only a few 100 mg of product, was successful for seven synthetic monomers out of the total of 21 present in nature. The synthesized molecules include the mimics of valine, glycine, leucine, phenylalanine, arginine, threonine, and aspartic acid. Purification of the products using column chromatography resulted in purified yields ranging from 28-35% for the multi-step synthesis of the arginine and aspartic acid mimics and up to values between 40 and 66% for the single step synthesized remaining monomers. These results indicate promising synthesis pathways; however, further optimization is still necessary. The collected product of all synthesized monomers contained some degree of impurity. This was attributed to the presence of chlorinated side product in the collected product after purification. The presence of this chlorinated molecule causes no harm to the further reaction as it cannot influence polymerizations due to the lack of a vinyl group. Therefore,

this unwanted molecule can be purified easily in future steps. The produced monomers and their yields are depicted in Table 3.

Table 3: Table with an overview of all produced monomers and their yields.

Amino acid	Acrylamide mimic	Amount	Purified yield
 *L*-valine	 *N*-isobutylacrylamide	11.489 g	47.12 %
 *L*-leucine	 *N*-isopentylacrylamide	0.263 g	55.46 %
 *L*-phenylalanine	 *N*-phenethylacrylamide	0.395 g	66.34 %
 *L*-threonine	 *N*-(2-hydroxypropyl)acrylamide	0.485 g	42.30 %
 *L*-arginine	 *N*-(4-guanidinobutyl)acrylamide	0.500 g	28.00 %
 *L*-aspartic acid	 3-acrylamidopropanoic acid	0.413 g	37.00 %
 glycine	 *N*-methyl acrylamide	1.329 g	67.00 %

After determining the optimal reaction conditions for the produced monomers, the possibility for upscaling was examined. Gram scale synthesis was the first step and was performed on the glycine mimic as well as on the valine mimic. This resulted in the production of 1.329 g of *N*-methylacrylamide (glycine) with a yield of 67% and the production of 1.33 g of *N*-isobutylacrylamide (valine) with a yield of 44.69%. These reactions were relatively easy to perform, and the product could be synthesized and purified in the same day. Upscaling of the monomer synthesis relied on the amount of acryloyl chloride produced per minute so upscaling of monomer and acryloyl chloride was dependant on each other. Since the maximal output of acryloyl chloride was 500 mL per day the monomer reaction, with as target *N*-isobutylacrylamide, was scaled to this. The acryloyl chloride was continuously added for 8 hours straight and after purification, the product was collected weighing 11.489 g with a purified yield of 47.12%. Although the knowledge about these reactions is not new, they were optimized to create standard procedures for the synthesis of these monomer mimics so that they could be used in a later stage for on-demand production. Since the monomers will be used in polymerization studies, a reproducible method is necessary for the continued use of the different monomers.

In conclusion, producing a flow setup for a reactive molecule such as acryloyl chloride is a tedious procedure. However, the setup was constructed with a final product output of 500 mL (0.5M) per day. Monomer synthesis based on the addition of acryloyl chloride with the appropriate amine has proven to be successful. The basic foundations for a synthetic amino acid mimic library are established with the synthesis of the first 7 acrylamides. Although the yields are relatively low, further research in the purification of these monomers should be able to increase this value significantly.

6 Future outlook

This thesis reports the first steps towards the synthesis of a synthetic amino acid library composed of acrylamide mimics. The classical approach to synthesize acrylamide monomers is based on the addition of acryloyl chloride to a specific amine creating the acrylamide.

As the first step in this process, a flow setup capable of synthesizing acryloyl chloride was created. Acryloyl chloride is an important building block in the synthesis of the targeted amino acid mimics but, is due to its reactivity, commercially not available in Australia. The created setup, which was capable of producing 500 mL of 0.5 M product solution per day, relied on the use of syringe pumps equipped with gastight syringes of 10 mL. In order to achieve a large scale continuous production of acryloyl chloride, the syringes needed to be refilled every 30 min for the reaction to keep on going. Although the use of this type of setup proved to be successful, future adaptations to the setup could circumvent this slight inconvenience by using, for example, HPLC or peristaltic pumps. The use of these types of pumps would allow the use of larger volumes of stock solution. This would mean that the reaction could run for a full day without the need for human interference. Using HPLC pumps would also allow the use of a bigger reactor volume speeding up the reaction even more so that bigger amounts of product are readily available.

With the synthesis of 7 out of 21 amino acid mimics, the first fundaments of a complete library of amino acid mimics have been established. The synthesis of these mimics relies on the basic reaction of acryloyl chloride with an amine functionality of a molecule resembling the functional group of the natural amino acid it mimics. Basic synthesis pathways were developed resulting in acceptable yields. However, further optimization of these procedures is still required. Especially the workup procedure of these monomers can still be further optimized. With further optimization of these steps, more products should be able to be isolated, thus leading to an increased yield. The transfer of the synthesis reactions from batch to a flow setup are also envisioned. As of now, the synthesis of acryloyl chloride is done in a continuous flow process but the synthesis of the acrylamide monomers is done in a conventional batch process. By translating the second reaction step from batch to flow, a continuous process from acrylic acid to acrylamide monomer could be designed. Translation of monomer synthesis reaction to a flow process would greatly benefit the reaction. The synthesis of acrylamides is a very exothermic reaction. Using a flow setup would allow better temperature control and thus a more efficient reaction.

Further development of the library by the synthesis of the missing monomers is also an important topic for future research. The 7 monomers that were synthesized in this work, each started from amines that were commercially available. For the other 14, this is not the case. However, the amines can be acquired relatively simple through a decarboxylation reaction of natural amino acids in order to yield the required amine. These can then be used further in the development of the synthetic library. Decarboxylation of amino acids has already been studied and reaction pathways have already been established.[66] In this way, all required amines will become available and the remaining monomers can be synthesized.

After completion of the full monomer library, the polymerization characteristics of these molecules will be studied. A thorough investigation of the polymerization characteristics and kinetics of the

different monomers will give valuable insights into their behavior. These insights can then be used to establish procedures to create well-defined polymeric structures in an efficient and controlled manner. Appropriate knowledge of the polymerization kinetics will also allow the determination of the ideal reaction parameters that result in sequence defined materials and thus allow the precise placement of each monomer in a polymer sequence. The ultimate goal here is the ability to precisely insert one monomer at a time using RAFT polymerization. This technique called single unit monomer insertion would use alternating purification and polymerization steps to ultimately end up with a polymer chain in which the location of every functional group is precisely known. This will result in the synthesis of polymers that mimic certain oligopeptide sequences that are found in nature. These artificial sequences will then be used for cell tests and their action will be compared to that of their natural forms.

7 References

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8 Supporting information

S1. Small molecule synthesis

1. ^{13}C NMR spectrum of *N*-isobutylacrylamide.

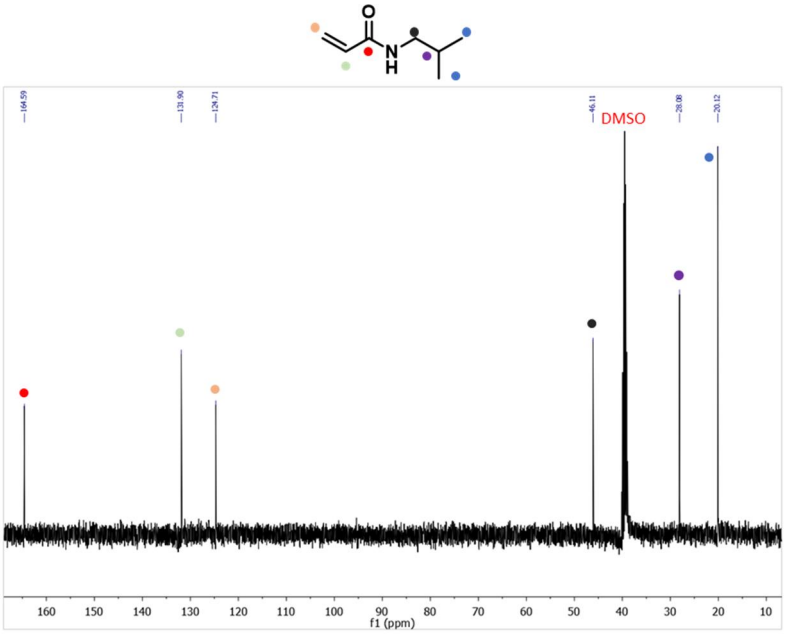

Figure S1.1.: ^{13}C NMR spectrum of N-isobutylacrylamide.

2. NMR spectrum of *N*-methyl acrylamide.

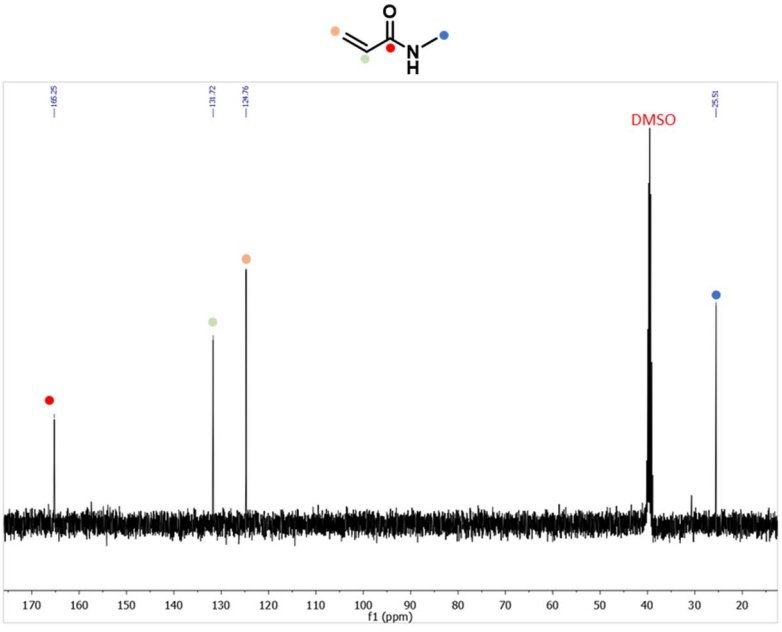

Figure S1.2.: ^{13}C NMR spectrum of N-methylacrylamide.

3. NMR spectrum of *N*-phenethylacrylamide.

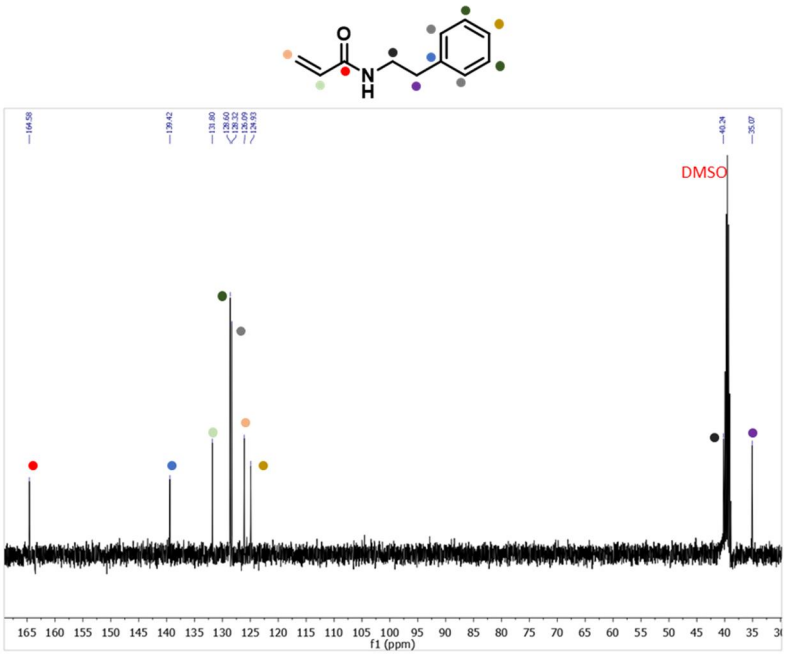

Figure S1.3.: ^{13}C NMR spectrum of N-phenethylacrylamide.

4. NMR spectrum of *N*-isopentylacrylamide.

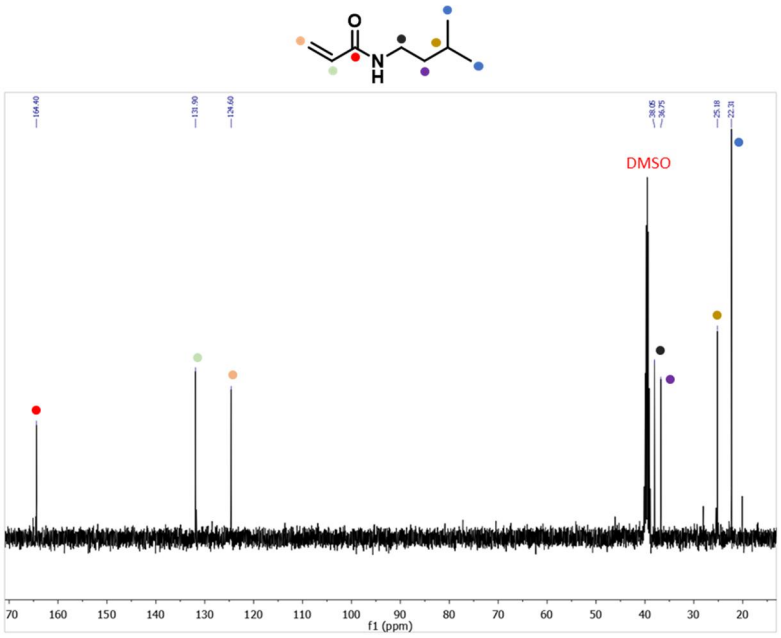

Figure S1.4.: ^{13}C NMR spectrum of N-isopentylacrylamide.

5. NMR spectrum of *N*-(2-hydroxypropyl)acrylamide.

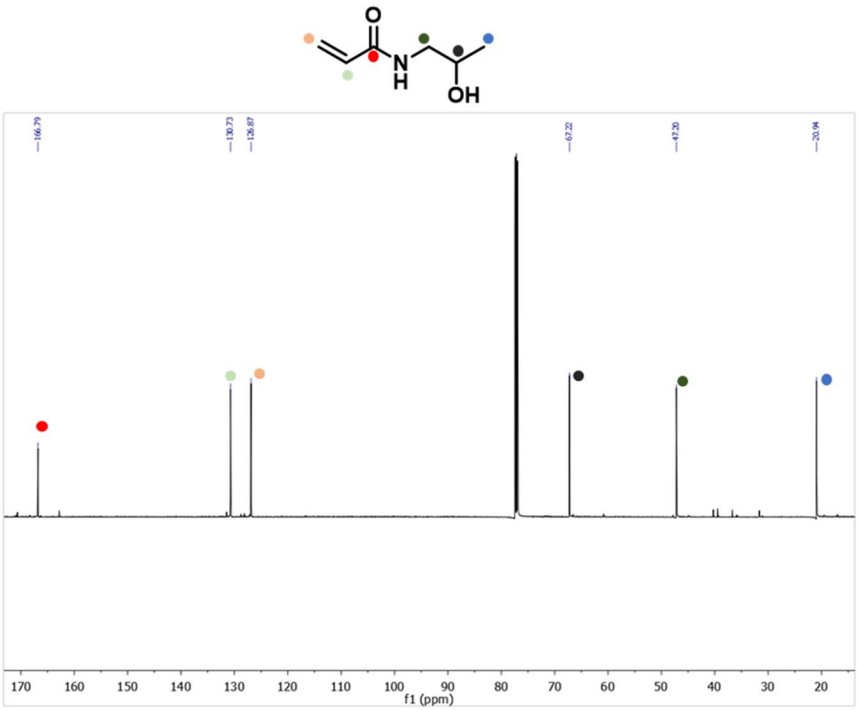

Figure S1.5.: ^{13}C NMR spectrum of N-(2-hydroxypropyl)acrylamide.

6. NMR spectrum of β-alanine ethyl ester hydrochloride.

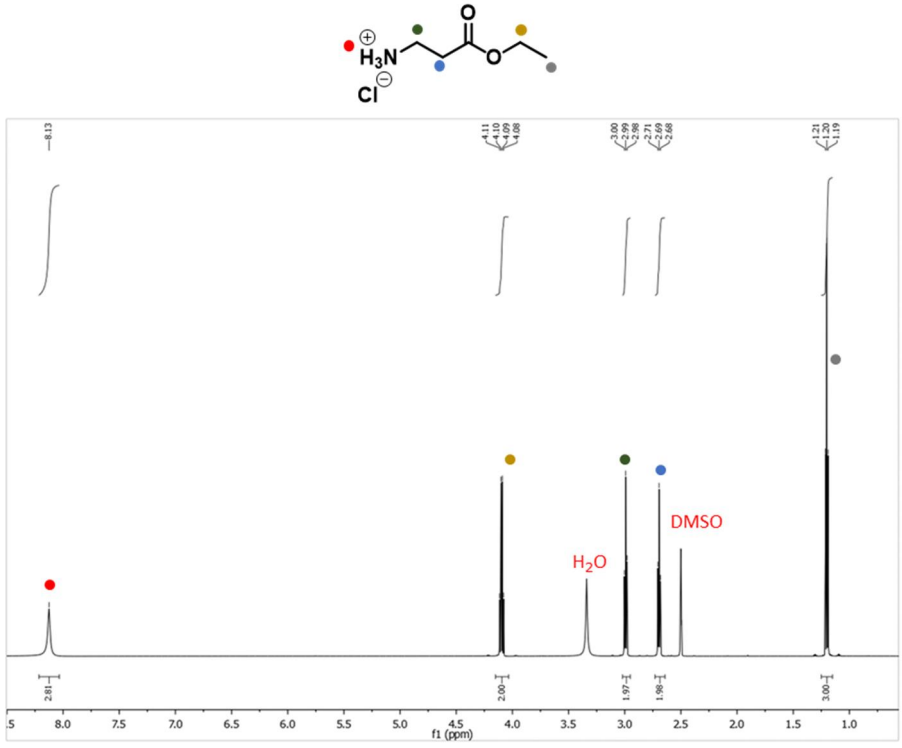

Figure S1.6.: Proton NMR spectrum of β-alanine ethyl ester hydrochloride.

7. NMR spectrum of 1,3-Bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea.

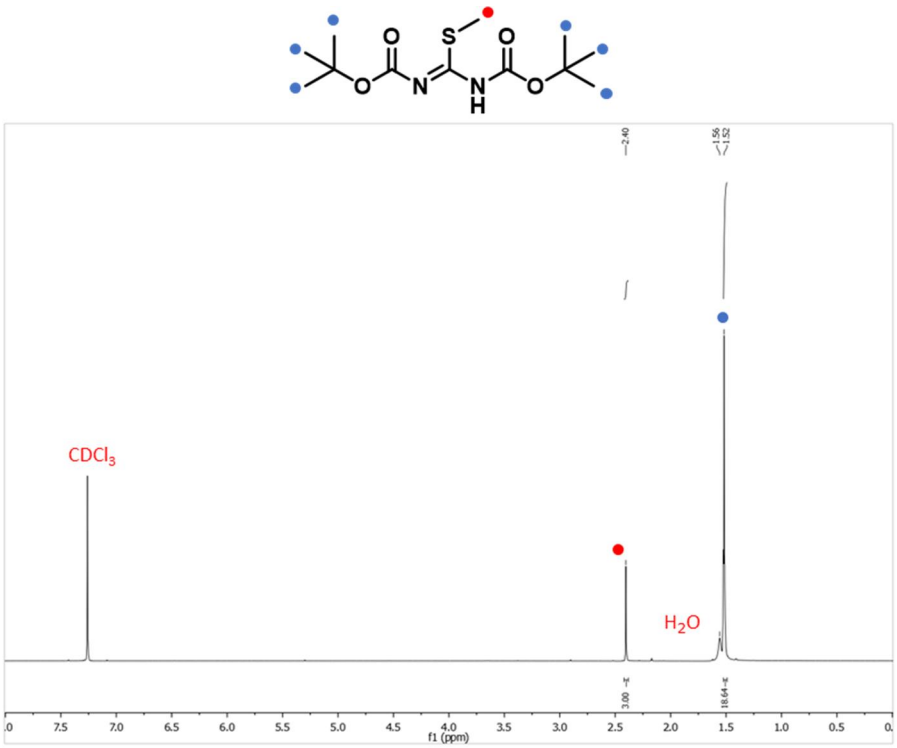

Figure S1.7.: Proton NMR spectrum of 1,3-Bis(tert-butoxycarbonyl)-2-methyl-2-thiopseudourea.

8. NMR spectrum of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylamine.

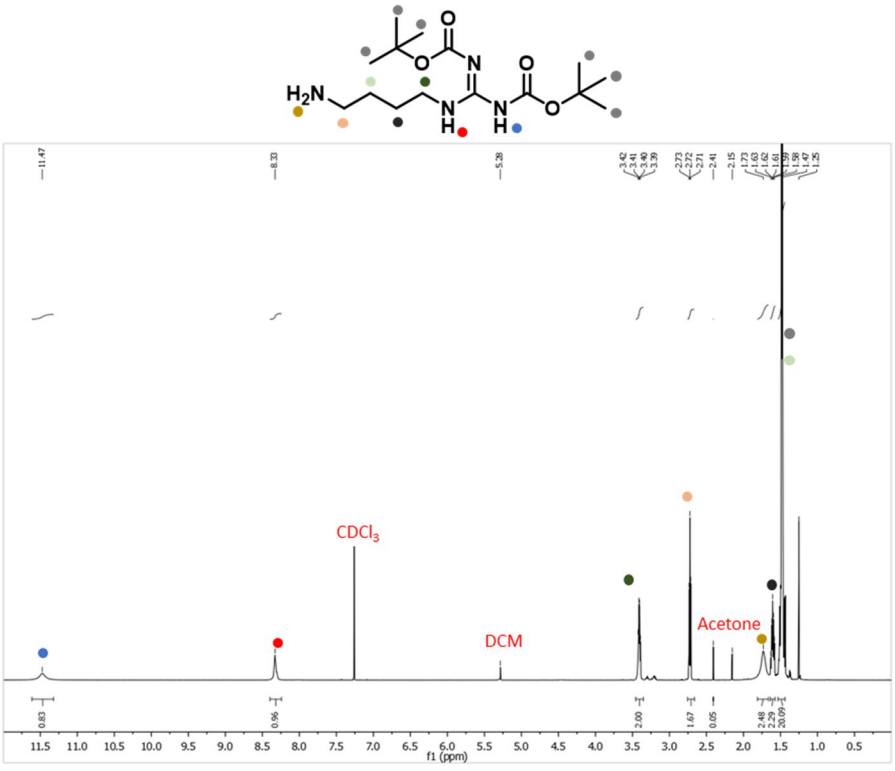

Figure S1.8.: Proton NMR spectrum of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylamine.

9. ^{13}C NMR spectrum of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide.

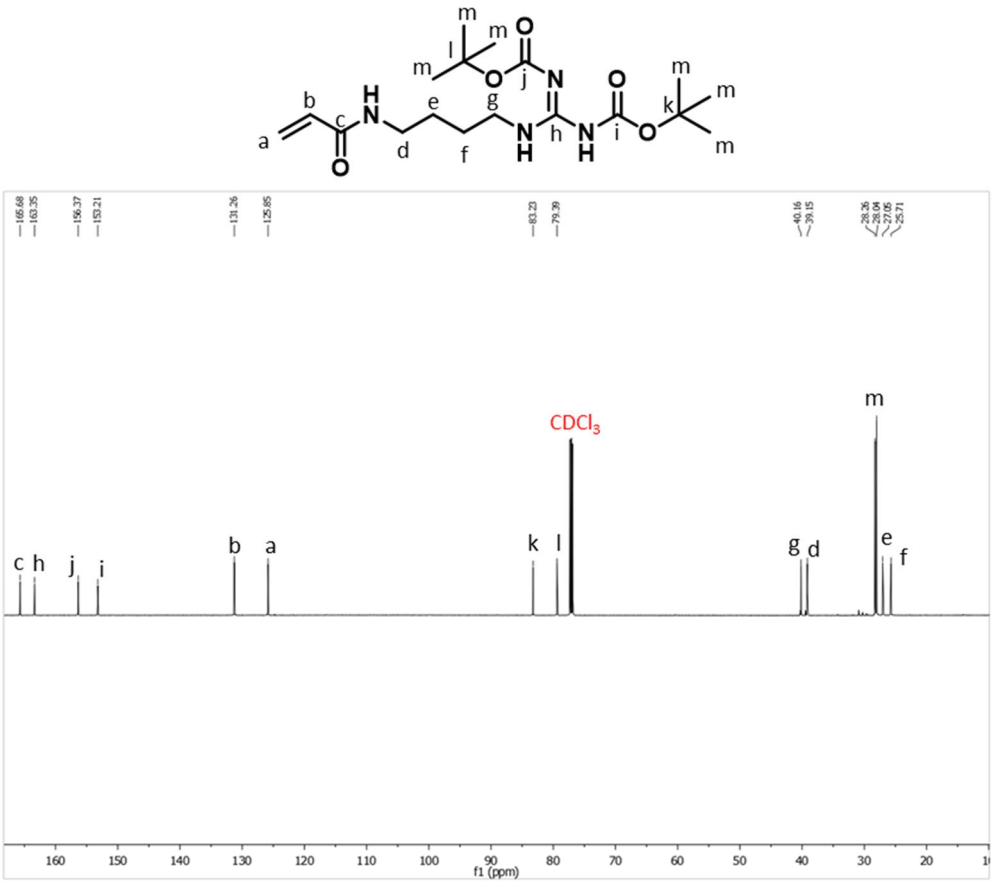

Figure S1.9.: ^{13}C NMR spectrum of 2-[2,3-Bis(tert-butoxycarbonyl)guanidino]butylacrylamide.