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Faculteit Industriële ingenieurswetenschappen

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Masterthesis

Synthesis of sequence-defined nucleobase acrylate oligomers via RAFT polymerization

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Scriptie ingediend tot het behalen van de graad van master in de industriële wetenschappen: chemie

Gezamenlijke opleiding UHasselt en KU Leuven

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UHASSELT

KU LEUVEN

Foreword and acknowledgments

As my previous studies in processing technology were very industry-focussed, I was excited that an R&D thesis project in the field of controlled radical polymerization was available at Hasselt University. During this project, I have met quite a few people who had an impact on the successful outcome.

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List of abbreviations

AAM	Adenine Acrylate functionalized Monomer
AcOEt	Ethyl Acetate
AIBN	2,2'-Azobis(2-methylpropionitrile)
ATRP	Atom Transfer Radical Polymerization
BDDA	1,4-Butanediol diacrylate
BHT	Butylated Hydroxytoluene
CAM	Cytosine Acrylate functionalized Monomer
CDTPA	4-Cyano-4-[[(dodecylthio)thoxomethyl]thio]pentanoic acid
$CHCl_3$	Chloroform
CMD-TTC	Cyanomethyl dodecyl trithiocarbonate
CPD-TTC	2-Cyano-2-propyl dodecyl trithiocarbonate
CPE-TTC	2-Cyano-2-propyl ethyl trithiocarbonate
CRP	Controlled Radical Polymerization
CS_2	Carbon Disulfide
CTA	Chain Transfer Agent
DCM	Dichloromethane
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DMSO-*d6*	Deuterated Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
ESI-MS	Electron Spray Ionization Mass Spectroscopy
FRP	Free Radical Polymerization
IUPAC	International Union of Pure and Applied Chemistry
K_2CO_3	Potassium Carbonate
KOH	Potassium Hydroxide
KO*t*Bu	Potassium *tert*-butoxide
LRP	Living Radical Polymerization
MeOH	Methanol
$MgSO_4$	Magnesium Sulphate
NMP	Nitroxide Mediated Polymerization
^{1}H-NMR	Proton Nuclear Magnetic Resonance
O.N.	Overnight
PEG	Poly(ethylene glycol)
PPV	poly(p-phenylene vinylene)
PRD	Polymer Reaction Design
p-TsCl	*p*-Toluenesulfonyl chloride
R.T.	Room Temperature

RAFT	Reversible Addition-Fragmentation chain Transfer
RDRP	Reversible-Deactivation Radical Polymerization
rec-SEC	Recycling Size Exclusion Chromatography
Rf value	Retention value
RNA	Ribonucleic Acid
SC	Sequence-Controlled
SD	Sequence-Defined
SUMI	Single Unit Monomer Insertion
TAM	Thymine Acrylate functionalized Monomer
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet

Abstract

Nucleobases are well known functionalities in nature which have an important role in biomolecules such as DNA and RNA. The potential of sequence-defined (SD) nucleobase acrylate functionalized oligomers lies not only in mimicry of these biomolecules, but also in its application as a molecular sensor, a tag or even as a chemical data unit.

Synthesis and purification of the first single unit monomer insertion (SUMI) with adenine functionalized acrylate monomer (AAM) is the main goal of this project. Firstly, synthesis of adenine as well as cytosine functionalized acrylate monomers was performed with corresponding yields of 55-68% and 42%. Secondly, different reaction parameters such as reaction time, RAFT agent, concentration, solvent and starting product equivalents were varied to find the ideal reaction conditions for the first AAM SUMI synthesis. Thymine as well as cytosine acrylate monomer SUMI synthesis was performed using these ideal conditions as a proof of concept. Thirdly, a flash chromatography purification method was developed for the crude AAM SUMI product. Products were analysed using ^{1}H-NMR spectroscopy and ESI-MS.

Optimization of the reaction parameters and purification method was performed. The molar ratios are monomer:CTA:initiator (5:1:0.05) when using AAM as monomer, CPD-TTC as RAFT agent and AIBN as initiator in DMSO when reacting 1h at 100 °C. A purity of 95% was achieved after purification of the SUMI product with flash chromatography when using MeOH:DCM (7:93) as mobile phase.

Samenvatting

Nucleobasen zijn gekende structuren die in de natuur voorkomen met een belangrijke functie in biomoleculen zoals DNA en RNA. Sequentie gedefinieerde nucleobase acrylaat oligomeren tonen niet alleen potentieel in het nabootsen van deze biomoleculen maar kunnen ook dienen als moleculaire sensor, label of als chemische data eenheid.

Het hoofddoel van deze thesis bestaat uit de synthese en opzuivering van de eerste *single unit monomer insertion (SUMI)* van adenine gefunctionaliseerd acrylaat monomeer (AAM). Eerst is de synthese van adenine- en cytosine gefunctionaliseerde acrylaat monomeren uitgevoerd met opbrengsten van 55-68% en 42%. Vervolgens zijn verschillende parameters als reactietijd, RAFT reagens, concentratie, solvent en equivalenten startproduct gevarieerd om de ideale condities voor de eerste AAM *SUMI* synthese te vinden. Thymine- en cytosine acrylaat monomeer SUMI syntheses zijn uitgevoerd met deze ideale condities als *proof of concept*. Als laatste stap is een *flash* chromatografie opzuiveringsmethode ontwikkeld voor het AAM *SUMI* product. De producten werden geanalyseerd met ^{1}*H-NMR* spectroscopie en *ESI-MS*.

Optimalisatie van zowel reactieparameters als opzuivering werden uitgevoerd. De molaire startverhoudingen zijn monomeer:CTA:initiator (5:1:0.05) wanneer AAM als monomeer, CPD-TTC als RAFT reagens en AIBN als initatior gebruikt worden in DMSO gedurende 1h op 100 °C. Een zuiverheid van 95% werd bekomen na opzuivering van het *SUMI* product met *flash* chromatografie wanneer MeOH:DCM (7:93) als mobiele fase gebruikt werd.

1 Introduction

1.1 Polymerization

Polymers are indispensable in our daily life. Due to their low cost, these materials are commonly used in fabrication of objects as plastic bottles and boxes, but also in car bumpers, casings for electronic devices, spacecraft etc. In industry, polymerization is a common production process for different types of polymers. As shown in Figure 1, different polymerization techniques exist to synthesize these remarkable materials. In step growth polymerization the formed chains will react with other growing chains, while in chain growth polymerization the formed chains only will react with unreacted monomers. Within the chain growth branch, different polymerization techniques exist, such as radical polymerization which uses monomers that are capable to form radicals, cationic and anionic polymerization which use ionisable monomers, and coordinative polymerization which uses metal salt catalysts such as the Ziegler-Natta catalyst.

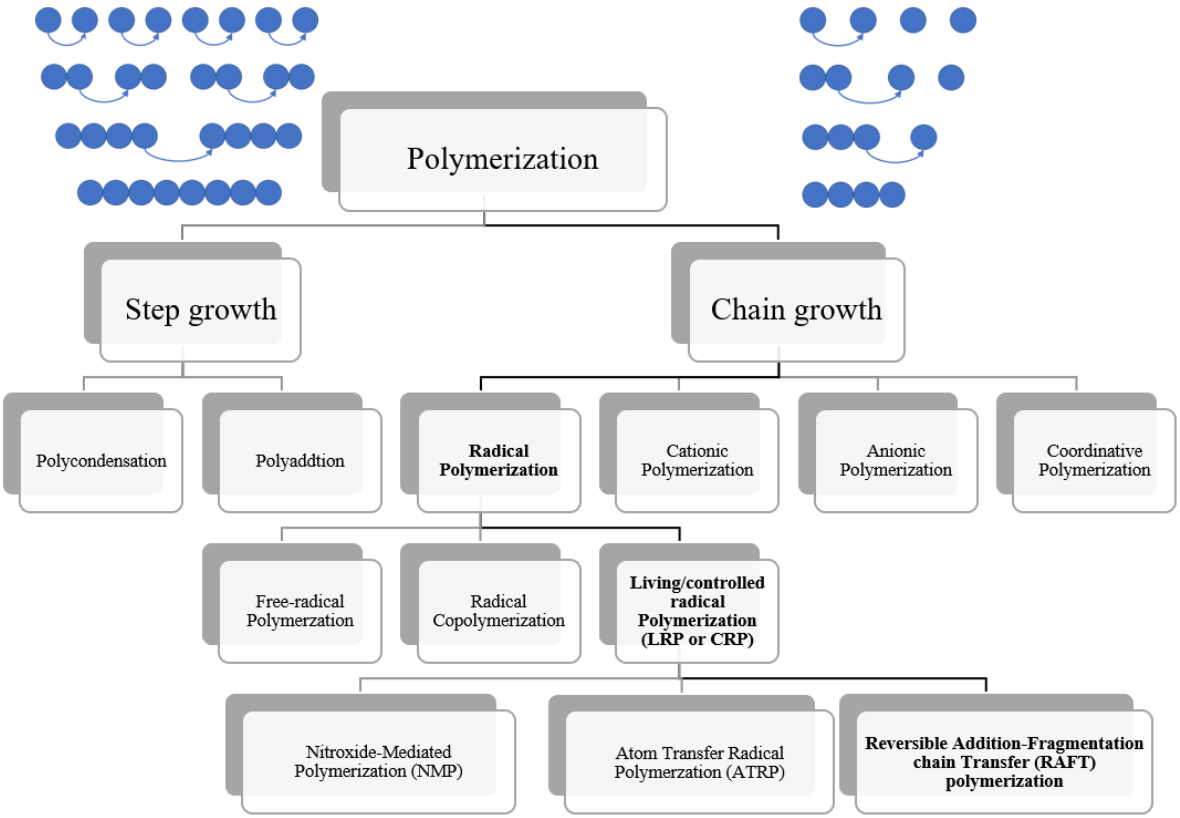


Figure 1: Overview of different polymerization techniques

The IUPAC definition describes radical polymerization as "a chain polymerization in which the kinetic-chain carriers are radicals," with an additional note that "usually the growing chain end bears an unpaired electron [1]." Radical polymerization is therefore an addition of free monomers to an active radical. By repeating this addition process a polymer chain is formed resulting in growth of a polymer. In radical polymerization there is a distinction between free radical polymerization (FRP) and reversible-deactivation radical polymerization (RDRP), also known as living/controlled radical polymerization (LRP/CRP) [2].

1.1.1 Free radical polymerization

FRP is the radical form of chain growth polymerization as shown earlier in Figure 1. Its mechanism consists of three important steps: initiation, propagation and termination. This is visualized in Figure 2 [3].

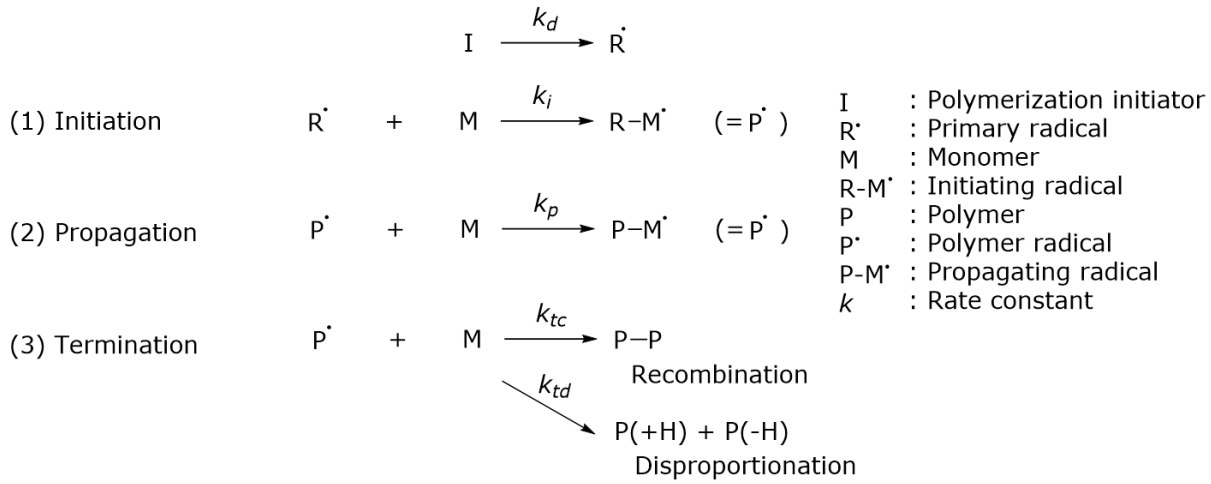


Figure 2: Reaction mechanism of free radical polymerization

First an initiator molecule is added on which an active site is generated with conventional energy sources such as radiation or heat. This initiator molecule then adds on a free monomer molecule and activates a monomer which forms a radical (initiation). A new monomer molecule now adds onto the recently generated radical, hereby generating a radical centre on itself (propagation) and therefore proceeds the reaction. The result is a growing chain by adding monomer after monomer [3]. The problem with FRP is that radicals will terminate whenever they meet (termination) and this reaction is irreversible [4]. When a short chain terminates, it will never extend further. Typically, FRP has a wide chain length distribution, as FRP typically only take a few seconds to propagate before termination occurs. With this technique, high molecular weights are obtained in a short period of time.

In industry this large chain length distribution is not a problem. If an overall more controlled chain length is preferred, the overall lifetime of the propagating chain must be extended. Synthesis of shorter chains with a smaller chain length distribution is also difficult with FRP due to its quick reaction rate and termination. Once a polymer chain is terminated, it is not possible to extend the existing chain with radical polymerization. It is only possible to extend the existing chain by coupling it with other irreversible terminated polymers. Therefore, the synthesis of block polymers is not possible with FRP as all chains will be terminated after the first block. These problems were solved with reversible deactivation radical polymerization (RDRP).

1.1.2 Controlled radical polymerization

RDRP or controlled/living polymerization (CRP/LRP) is a form of polymerization where a controlling agent or specific initiators are used. As FRP has the problem of the shorter lifetime of the radical and uncontrollable chain lengths, CRP typically has a spontaneous initiation and simultaneous propagation. These types of controlling agents have the ability to control the polymerization reaction in such way it keeps its living character, which means that they propagate longer and at the same rate. Hereby the chain length is more controlled and narrow chain length distributions are achieved compared with FRP. In contrary to FRP which has an almost constant molecular weight with conversion, CRP has an increasing chain length with conversion as shown in Figure 3.

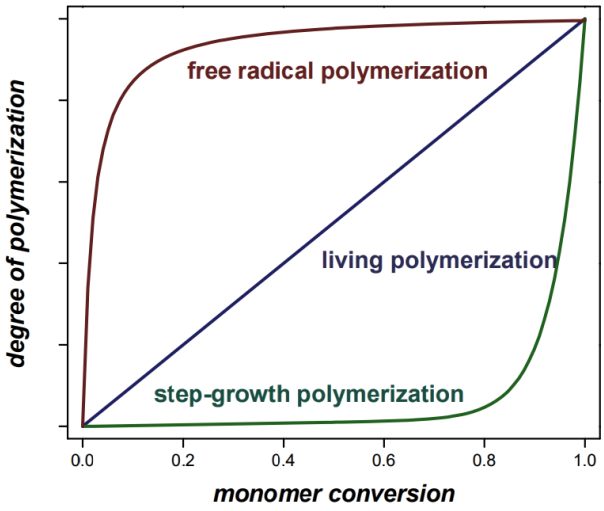


Figure 3: Degree of polymerization in function of monomer conversion for different polymerization systems [5]

Due to this characteristic it is much easier to synthesize polymers with a predetermined chain length, which results in a narrow molecular weight distribution. In Figure 3, the degree of polymerization (DP_n) stands for the number of monomeric units in a polymer. With FRP, long chains are formed in a short time, and therefore a large DP_n is achieved. With step-growth polymerization only longer polymer chains are only formed at high conversions. With FRP also the exponential character is visible as with CRP the controlled character is visualized by the linear relationship between the degree of polymerization and the monomer conversion.

In literature, dispersity (*Đ*) is used to characterize the chain length distribution. This is the mass average molar mass *Mw* of all macromolecules divided by the number average molecular mass *Mn*. Some typical dispersity's of different polymerization mechanisms are shown in Table 1 [6] and Figure 4.

Table 1: Polymer dispersity with different polymerization mechanisms

Polymerization method	**Dispersity (*Đ*)**
Addition (FRP)	> 1.5
Step growth	2
Controlled polymerization	< 1.5

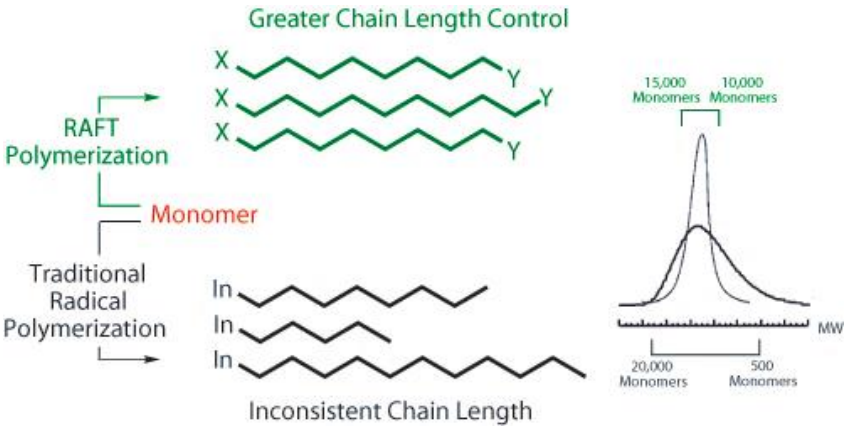


Figure 4: Chain length comparison between CRP and FRP [7]

With FRP the exponential growth in degree of polymerization results in a large difference in chain length and therefore a wide Poisson distribution. This results in a larger dispersity. With CRP, the controlled character corresponds in smaller differences in chain length, which results in a narrower molecular weight distribution. The ultimate goal of CRP is to achieve a molecular dispersity of 1 while with FRP sometimes dispersity's up to 5 or even higher are acquired [8]. The difference between FRP and CRP is visualized in Figure 4.

There are two main mechanisms of CRP. The first type, visualized in Figure 5a, shifts in an equilibrium between its active and sleeping form with the equilibrium leaning towards the sleeping form [9]. The second type, visualized in Figure 5b, functions with a reversible addition fragmentation chain transfer molecule [10].

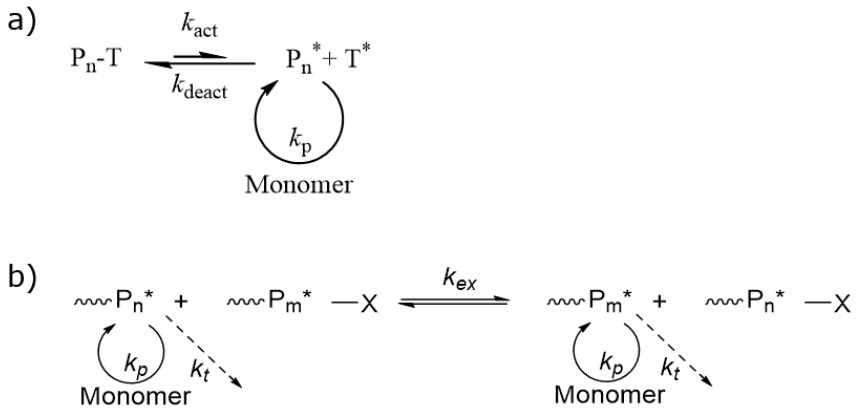


Figure 5: Sleeping-active equilibrium (a) and a chain transfer equilibrium (b)

With the sleeping/active mechanism, polymerization will occur as monomer units react and add on its active site. As the equilibrium leans towards the sleeping side, almost no molecules are in the active form and termination caused by recombination or disproportionation is therefore minimized. Less termination will result in longer polymer chains as the overall lifetime of a growing polymer chain is extended.

With the chain transfer polymerization equilibrium, a degenerative chain transfer occurs. The molecule itself does not react with monomers, it merely transfers the active site from one chain to another, hereby deactivating the first chain and activating the second chain.

Nitroxide-mediated polymerization (NMP) and atom transfer radical polymerization (ATRP) are two well-known polymerization examples which both make use of the mechanism of Figure 5a. NMP uses an alkoxyamine initiator such as TEMPO (pictured as T*), a stable radical. Homolysis of the C-O bond between TEMPO and the polymer chain results in two radicals, the reversed reaction reforms the bond between both. ATRP usually uses an alkyl halide as initiator and a transition metal complex as catalyst. During the activation reaction, the halide gets transferred to the transition metal which increases one oxidation number, resulting in a different complex and a radical alkyl group (P*) which can propagate. In the reversed reaction the halide is returned to the polymer chain. Reversible addition-fragmentation chain transfer (RAFT) polymerization is a well-known example that uses the mechanism of Figure 5b. A RAFT agent typically has the structure as shown in Figure 6.

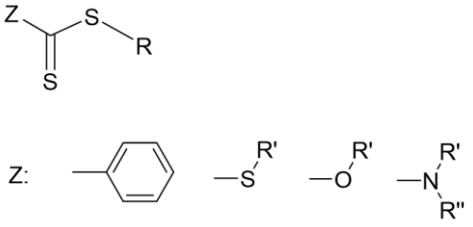


Figure 6: Representation of different RAFT agents

The four conventional chain transfer agent (CTA) classes are dithiobenzoates, trithiocarbonates, xanthates and dithiocarbamates. The Z-group controls the reactivity of the CTA and therefore has an influence on the rate of radical addition and fragmentation [11]. The R-group is the free radical leaving group [12]. As these four classes have different Z-groups, their reactivity is fundamentally different. Each Z-group has a good reactivity whether with more activated monomers (MAM) or less activated monomers (LAM). These Z-groups also not only have a function in reactivity, but also facilitate coupling reactions or the addition of functionalities. For example functionalities could be added with EDC coupling by using an acid leaving group on the RAFT agent [13]. In Figure 7 the reaction scheme of a chain transfer agent is visualized.

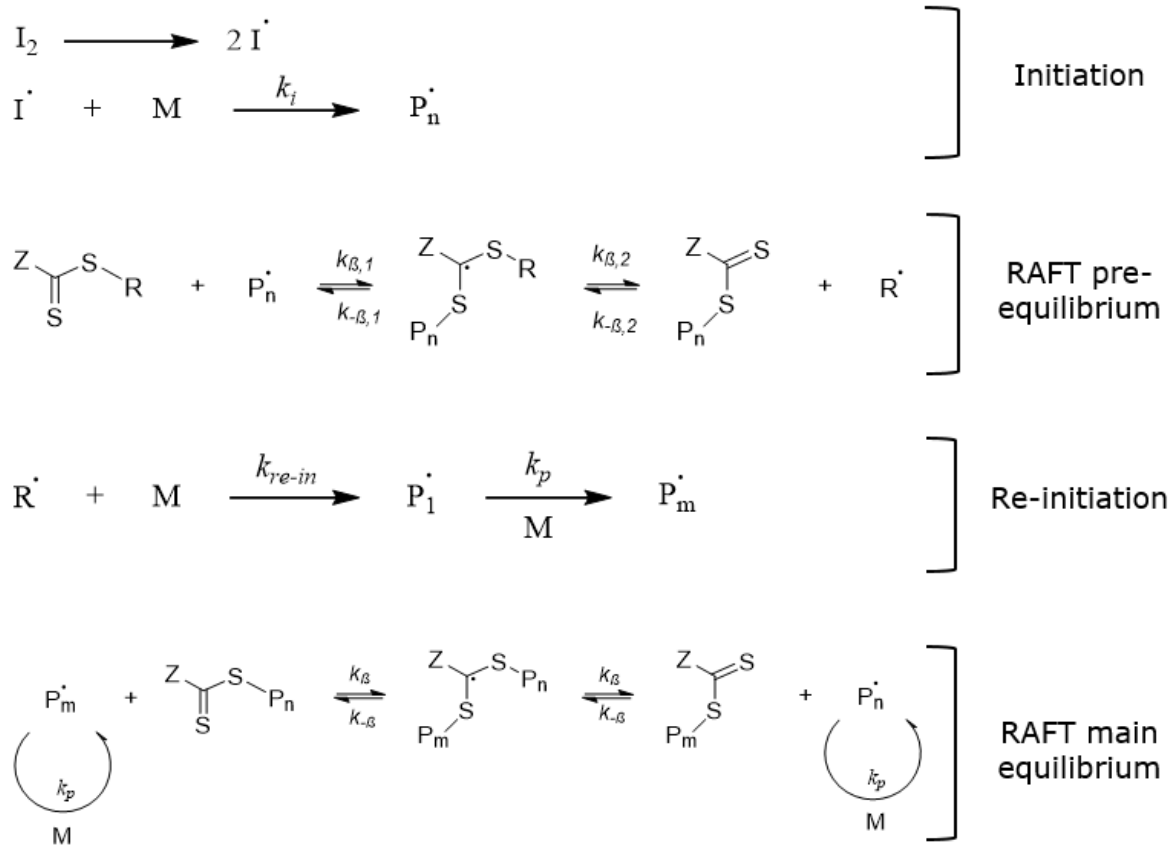


Figure 7: Overview of the RAFT polymerization mechanism

RAFT agents are used in conjunction with conventional initiator molecules which is shown in the initiation step. Heat or light is used to form initiator radicals. As shown in the RAFT pre-equilibrium step, new R* radicals are formed. In the re-initiation step these new radicals also start to form radical chains. The RAFT main equilibrium is the most important part of the mechanism as this ensures the chain length control. Also termination can happen as with FRP as in Figure 2, but this is minimal due to the use of a CTA. Due to competition between FRP and RDRP, the concentration of initiator should be far lower than the concentration of RAFT agent as this facilitates longer preservation of the living character [14]. This also minimizes the formation of side products and dead chains.

Because of the chain length control that the RAFT main equilibrium facilitates and further development of this polymerization technique, new materials were developed with a more specific structure as shown in Figure 8. Now sequence-controlled (SC) polymers consisting of successive blocks of different monomers could be synthesized. One step further was the development of sequence-defined (SD) polymers where an exact sequence of monomers is synthesized.

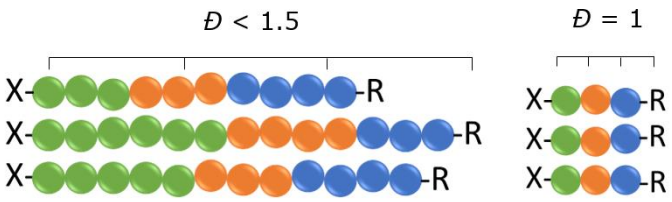


Figure 8: SC block co-polymers and SD polymers

In Figure 8 is clearly the difference is visualized between the polydisperse SC block co-polymer and the monodisperse SD polymer. In the SC block, the blue and orange blocks are added with dispersity 1.02, the green ones with dispersity 1.07. Although these dispersity's are very low, with the monodisperse SD polymers the sequence of monomers is exactly the same in each chain. Therefore they have a dispersity of 1.

1.2 Sequence-defined oligomers with multiple hydrogen bond functionalities

1.2.1 Sequence-defined materials

In nature, biomolecules as DNA and RNA have genetic information fixed in their sequence of nucleotides. With subsequent mechanisms of enzymes this information gets converted for the synthesis of polypeptides, consisting of sequences of amino acids. These peptides fulfil a wide variety of biological functions, but only if they have the correct sequence. The potential of mimicking these biomolecules received a growing interest and pathways were developed for synthetic synthesis procedures.

A milestone in the synthesis of SD was the discovery of solid-phase synthesis by Merrifield. He synthesized polypeptides with the stepwise addition of amino acids to a growing chain attached to a solid resin particle, removing impurities by simply washing these solids [15]. This peptide-synthesizing procedure was further developed and led to the synthesis of functioning hormones and enzymes. Later this solid phase synthesis was used with different kinds of monomers to synthesize macromolecules consisting of up to 100 monomeric units for applications such as information storage or chemical tags [16-18]. The problems that usually arise with this technique is that the starting monomers are difficult to synthesize and it uses devious chemistry with protection and deprotection reactions.

Iterative divergent/convergent approaches of exponential growth polymerization made it possible to synthesize sequence-defined oligopeptides with up to 64 monomeric units [19]. The limitation of this method is that it only capable of

synthesizing alternating or palindromic sequences. Also, with this method protection and deprotection reactions are used which is inefficient for the overall procedure.

A different approach is to apply RDRP in combination with intermediate purification and exploit its characteristic to achieve the insertion of a single monomer. This single unit monomer insertion (SUMI) procedure is described in 1.2.2. With cationic polymerization this first was achieved by the successive insertion of four vinyl ether monomers [20]. Also subsequent insertion of a single functionality followed by chain elongation was achieved with living cationic polymerization [21].

More recent approaches with controlled radical polymerization achieved the synthesis of two oligoacrylates with four consecutive insertions of functionalised acrylate monomers *via* RAFT polymerization [22] and up to five consecutive insertions with photo-induced copper-mediated reactions [23]. Also alternating SUMI and multiple unit monomer insertions were performed for the synthesis of 9- and 10-mers, which were coupled to 18- and 20-mers [24]. Acrylates therefore allow facile integration of functionalities using the SUMI procedure but also the insertion of multiple monomers in one polymerization step is possible.

1.2.2 Single Unit Monomer Insertion procedure

As earlier mentioned, using CRP makes it possible to control the chain length during polymerization. If this chain length is controlled on monomeric level, SD oligomers are formed, as the addition of each monomer on its own is controlled. The radical addition of one monomer unit is called a single unit monomer insertion (SUMI). As shown in Figure 4, there is still a narrow statistical behaviour on the chain length with CRP, although very narrow. This means that when only one monomer addition is desired, there also is the possibility that other chains get an extension of two or even three monomers. This makes the SUMI procedure very time consuming as for every SUMI a purification step is required. In most publications, the purification of SUMI products is performed using recycling size exclusion chromatography (rec-SEC) [22, 24, 25]. This is a very intensive method as only small batches can be purified at once. However, in recent publications, flash chromatography is used to purify these products [24, 26]. As with flash chromatography larger batches could be purified, this method will be aimed for during the project.

An overview of the SUMI procedure in the formation of a sequence defined trimer is shown in Figure 9. In this figure a trimer is synthesized by performing three subsequent SUMI's, each consisting of an RDRP step followed by purification.

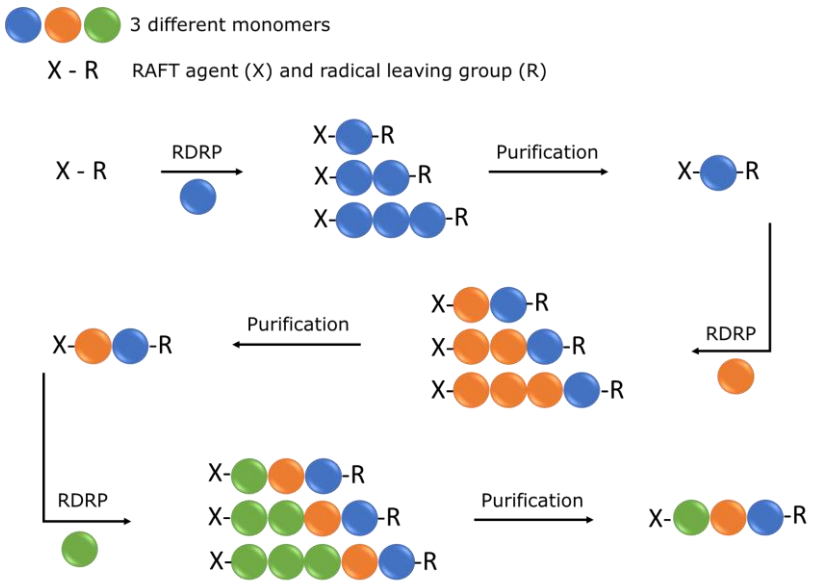


Figure 9: Synthesis of a trimer using the SUMI procedure

1.2.3 Multiple Hydrogen Bond functionalities

As sequence-controlled and sequence-defined oligomers are already being synthesized, an interesting approach can be to combine these techniques with specially functionalized monomers. Nucleobases are well known from biomolecules as DNA and RNA which have very specific hydrogen bonding properties. Both DNA and RNA are sequence-defined molecules. With acrylate functionalized monomers, these sequences could be mimicked or these materials could function as a biosensor, a tag or even as a chemical memory unit. A simple visualization of this concept is shown in Figure 10.

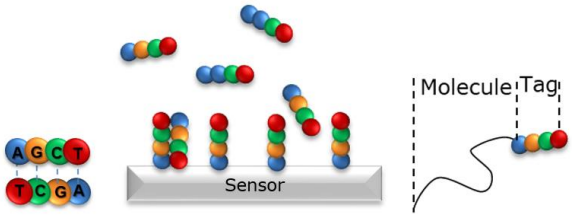


Figure 10: Future applications of nucleobase functionalised acrylate oligomers [27]

SC nucleobase functionalized oligomers are already synthesized and show very interesting properties. An overview of the power of nucleobase functionalized SC polymers is summarized below.

Van Hest *et al*. describe the synthesis of poly(ethylene glycol) (PEG)-poly(nucleobase) block co-polymers using ATRP. The adenine and thymine functionalities were integrated by first synthesizing nucleobase functionalized methacrylate monomers and then performing the ATRP synthesis on PEG. When mixing these molecules in water, these assemble in micellar-like structures [28]. The next step in this research field was the synthesis of nucleobase functionalized methacrylate copolymers [29]. In recent literature it is shown that the solvent has an important function in the formation of the H-bridges between adenine and

thymine. Chloroform, a rather apolar solvent, promotes the formation of H-bonds between adenine and thymine, while dimethylformamide, which is more polar than chloroform, suppresses these interactions. A similar synthesis procedure was developed by Zhang *et al*. but instead of methacrylates here acrylates are used [30]. ABA triblocks of nucleobase functionalized acrylates were synthesized and their supramolecular association possibilities were reported. They described that when a flexible spacer is placed between the different nucleobase functionalities promoted the intermolecular recognition of nucleobases and also improved the solubility of the functionalized polymers as their Tg was lowered. Another publication reports of pH sensitive cytosine- and guanosine-modified hydrogels which showed self-healing properties [31].

The incorporation of nucleobase functionalised monomers can also be extended to the field of SD materials. During this project the first step into this unexplored research field will be the synthesis and optimization of the first insertion of nucleobase SUMI oligomers *via* RAFT polymerization, followed by purification of these products. This overall goal can be divided in three sub-objectives.

Firstly, the synthesis of nucleobase acrylate monomers will be performed. The synthesis procedures of adenine-, and cytosine functionalized acrylate monomers are already known, but further optimization of these procedures as synthesis on a larger scale or easier purification of these functionalized monomers is desirable [32].

Secondly, the synthesis of the first nucleobase SUMI product will be performed and optimized. Hereby different parameters such as solvent and monomer:CTA:initiator ratio's will be tested as a first screening. Also, different functionalized monomers will be tested to check if the optimized procedure still works when the monomer is changed.

Thirdly the purification of the SUMI product will be performed. Recently the separation of monodisperse SUMI products form polydisperse mixtures with flash chromatography was performed [22]. A similar method will be developed for purification of the nucleobase containing product.

Acrylates will be used as monomers because they are known for allowing facile incorporation of functionality's without being significantly affected in their reactivity [22]. Also, acrylates provide a strong covalently bound C-C backbone which is very stable while most counterparts require incorporation of a hetero atom. Because of the radical nature of RAFT polymerization, oligomer growth is not limited to one unit at a the time. Also, as the backbone is built up from vinylic monomers functionalities can be incorporated very close to each other where with other mechanisms a connective spacer is required. Also the radical reaction is a rather fast procedure as it does not require protection and deprotection reactions while solid phase synthesis and exponential growth polymerization these do require. The SUMI procedure requires only one intermediate separation for which a fast automated silica column procedure is available.

In this thesis the synthesis of nucleobase functionalized acrylate SUMI synthesis *via* RAFT polymerization is described for the first time. The developed procedure can be a starting point for the synthesis of nucleobase containing SD oligomers using a fast and straightforward procedure. These oligomers can be applied in fields like DNA mimicry, biosensors or even data storage.

2 Materials and methods

2.1 Materials

1,4-Butanediol diacrylate (BDDA, Aldrich Chemistry, 90%), 1,4-Dioxane (Fisher Chemical, 99.99%), 1-Dodecanthiol (Acros Organics, 98%), 2,6-Di-tert-butyl-4-methyl-phenol (BHT, Alfa Aesar, 99%), 4,4'-azobis(4-cyanovaleric acid) (Aldrich Chemicals, >98.0%), Acetone (Sigma Aldrich, >99%), Adenine (Alfa Aesar, 99%), Carbon disulfide (CS_2, Acros Organics, 99.9%), Chloroform ($CHCl_3$, Fisher Chemical, 99%), Cytosine (TCI, >98.0%), Dichloromethane (DCM, Fisher Chemical, >99%), Dimethyl sulfoxide - d6 (DMSO-*d6*, Eurisotop, 99.90%), Dimethyl sulfoxide (DMSO, Fisher Chemical, 99.99%), Ethyl acetate (EA, Fisher Chemical, >99%), Hexane (Fisher Chemical, fraction from petroleum), Magnesium sulphate ($MgSO_4$, Acros Organics, 97%, anhydrous), Methanol (MeOH, VWR, 98.5%), N,N-Dimethylformamide (DMF, Fisher Chemical, 99.99%), Potassium carbonate (K_2CO_3, Acros Organics, 99+%), Potassium hydroxide (KOH, Acros Organics, 85% pellets), Potassium tert-butoxide (K*t*BuO, Acros organics, 98+%), *p*-toluenesulfonylchloride (*p*-TsCl, TCI, >99.0%), Siliciumdioxide (Davisil chromatographic, 70-200 µm), Tetrahydrofuran (THF, Fisher Chemcial, 99.5%) were used as received.

α,α'-azoisobutyranitrile (AIBN, Glentham Life Sciences) was recrystallized twice from MeOH prior to use.

2-cyano-2-propyl ethyl trithiocarbonate (CPE-TTC)[1], 2-cyano-2-propyl dodecyl trithiocarbonate (CPD-TTC)[1], 2-cyano-2-methyl trithiocarbonate (CMD-TTC)[2] and thymine functionalized acrylate monomer (TAM)[3] were synthesized according to previously reported literature.

2.2 Characterization

2.2.1 NMR

^{1}H-NMR spectra were recorded in DMSO-*d6* with a Varian Inova 300 or 400 spectrometer at 300 or 400 MHz.

^{1}H-NMR spectra were analysed using the MestReNova software package.

Conversions of the polymerization were calculated according to the mean of the three typical acrylate ^{1}H-NMR signals. These signals were normalized with a DMF internal standard. The integrals of the sample before and after the reaction were compared to determine the conversion.

[1] J. Haven, J. A. De Neve, and T. Junkers, "Versatile Approach for the Synthesis of Sequence-Defined Monodisperse 18- and 20-mer Oligoacrylates," *ACS Macro Letters,* vol. 6, no. 7, pp. 743-747, 2017
[2] S. K. J., H. Simon, and K. D. J., "Phosphorus-Containing Gradient (Block) Copolymers via RAFT Polymerization and Postpolymerization Modification," *Macromolecular Chemistry and Physics,* vol. 217, no. 20, pp. 2310-2320, 2016.
[3] S. Cheng, M. Zhang, N. Dixit, R. B. Moore, and T. E. Long, "Nucleobase Self-Assembly in Supramolecular Adhesives," *Macromolecules,* vol. 45, no. 2, pp. 805-812, 2012.

2.2.2 ESI

ESI-MS was performed using an LTQ Orbitrap Velos Pro spectrometer (Thermo-Fischer Scientific) equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electro spray mode. The instrument was calibrated in the m/z range 220-2000 using a standard solution containing caffeine, MRFA and Ultramark 1621. A constant spray voltage of 5 kV was used and nitrogen at a dimensionless auxiliary gas flow-rate of 5 and a dimensionless sheath gas flow-rate of 10 were applied. The S-lens RF level, the gate lens voltage, the front lens voltage and the capillary temperature were set to 50%, -90V,-8.5V and 275°C respectively. A 250 µL aliquot of a polymer solution with concentration of 10 µg mL^{-1} was injected. A mixture of THF and methanol (THF:MeOH = 3:2), all HPLC grade, was used as solvent.

ESI-MS spectra were analysed using the Xcalibur software package.

2.3 Purification

2.3.1 Flash column purification

Flash column chromatography is essentially the same as silica gel chromatography, differing in packing size and elution pressure. Because of the smaller packing, it is more difficult for the mobile phase to pass through. That's why with flash chromatography a pump is used as driving force. A UV photosensor is used to detect the passage of compounds and the sample collector collects each compound in a different test tube. When no UV signal is detected, the solvent contains no compound and thus the solvent will be sent to a waste bin. As with the additional software the test tubes containing the product can be selected while test tubes containing side products can be neglected and the evaporation of the solvent takes less time. Because this type of system is more or less automated, it is a faster but more expensive method of purification.

During the purification of SUMI products, an elution speed of 60 mL/min was used in combination with a manually packed Macherey-Nagel Chromabond® DL 330.

2.4 Synthesis

2.4.1 Functionalized Acrylate monomers

2.4.1.1 Adenine Acrylate Monomer

The synthesis of adenine acrylate monomer (AAM) was performed according to an adapted method of Long [32]. The synthesis reaction is visualized in Figure 11.

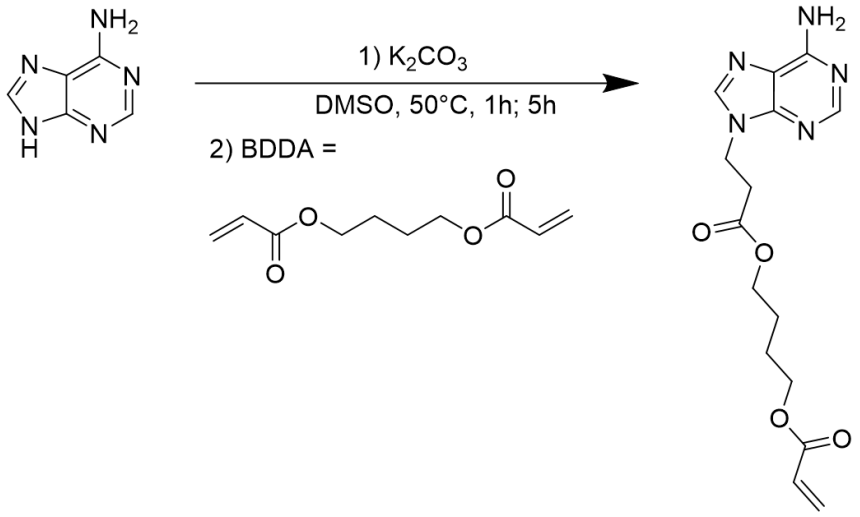


Figure 11: Synthesis of adenine functionalized acrylate monomer

Adenine (6 g, 44.6 mmol), butylhydroxytoluene (360 mg, 1.64 mmol) and potassium carbonate (242 mg, 1.75 mmol) were suspended in DMSO (120 mL). The suspension was stirred at 50 °C for 1h. Then 1,4-butanedioldiacrylate (18 mL, 95 mmol) was added and the mixture was stirred at 50 °C for another 5h.

For the workup the mixture was diluted with H_2O (900 mL) and washed with hexane (200 mL). The water phase then was extracted with DCM (3x 120 mL). Because of the salt an emulsion was formed during the extraction. Therefore brine solution was used to partially break the emulsion. Also mixing with a spatula in the separation funnel helped breaking the emulsion. Afterwards the organic phase was washed with brine solution (100 mL). Then the organic phased was dried with $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The crude mixture was purified using silica column chromatography with $CHCl_3$:MeOH (9:1). The final fraction contained the product. After evaporation of the solvent the pure product was obtained (8.07 g; 55% yield). The purity was confirmed with ^{1}H-NMR spectroscopy. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 – 1.66 (m, 4H), 2.96 (t, J = 6.8 Hz, 2H), 3.93 – 4.16 (m, 4H), 4.38 (t, J = 6.8 Hz, 2H), 5.93 (dd, J = 1.7, 10.3 Hz, 1H), 6.16 (dd, J = 10.3, 17.3 Hz, 1H), 6.31 (dd, J = 1.7, 17.3 Hz, 1H), 7.21 (s, 2H), 8.10 (s, 1H), 8.13 (s, 1H).

2.4.1.2 Cytosine Acrylate Monomer

The synthesis of cytosine acrylate monomer (CAM) was performed according to an adapted from Long [33]. The synthesis reaction is visualized in Figure 12.

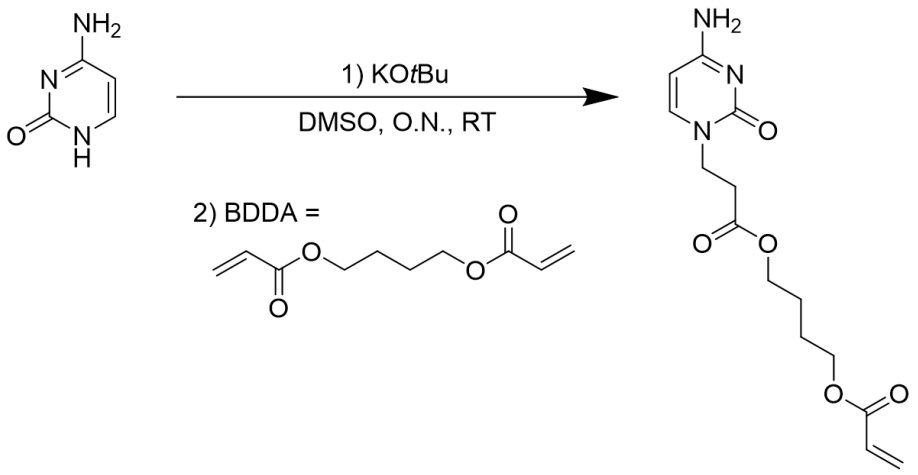


Figure 12: Synthesis of cytosine functionalized acrylate monomer

Cytosine (3.05 g, 27.5 mmol), butylhydroxytoluene (0.238g, 0.59 mmol) and potassium *tert*-butoxide (0.121g, 1.08 mmol) (KO*t*Bu) were suspended in DMSO (60 mL) and stirred 45 min. at room temperature. A cloudy white solution was formed. Then 1,4-butanedioldiacrylate (10.2 mL, 54 mmol) was added in one go and after stirring for 10 minutes at room temperature, the solution turned opaque yellow. After stirring for 10 more minutes, the solution turned clear yellow. The mixture was stirred overnight at room temperature.

For the workup, the mixture was poured in 450 mL of water and washed with 100 mL of hexane. Then the water phase was extracted with DCM (3x 60 mL) and hereafter the organic phase was dried with $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure. The crude was purified on a silica column using $CHCl_3$:MeOH 9:1 as the mobile phase. The final fraction contained the product which was obtained after evaporation of the solvent (3.56 g, 42% yield). The purity was confirmed with ^{1}H-NMR spectroscopy. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.63 (dd, *J* = 2.4, 3.9 Hz, 4H), 2.67 (t, *J* = 6.7 Hz, 2H), 3.82 (t, *J* = 6.7 Hz, 2H), 4.00 – 4.13 (m, 4H), 5.60 (d, *J* = 7.2 Hz, 1H), 5.94 (dd, *J* = 1.7, 10.3 Hz, 1H), 6.17 (dd, *J* = 10.3, 17.3 Hz, 1H), 6.32 (dd, *J* = 1.6, 17.3 Hz, 1H), 6.96 (s, 1H), 7.04 (s, 1H), 7.53 (d, *J* = 7.2 Hz, 1H).

2.4.2 RAFT agent

2.4.2.1 4-Cyano-4-[[(dodecylthio)thoxomethyl]thio]pentanoic acid

The synthesis of 4-Cyano-4-[[(dodecylthio)thoxomethyl]thio]pentanoic acid (CDTPA) consists of two synthesis steps which both have been previously performed by Yan [34]. First, the synthesis of the bisdodecyl-bistrithiocarbonate is performed. Second, the further reaction to CDTPA is performed. The synthesis reaction of the first step is visualized in Figure 13.

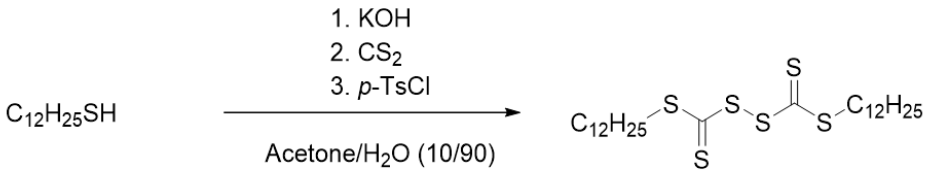


Figure 13: Synthesis of bisdodecyl-bistrithiocarbonate

Dodecanthiol (10.120 g, 50 mmol) was dissolved in water/acetone (32/8; 40 mL) and placed in an ice bath. Potassium hydroxide (3.41 g, 61 mmol) of was dissolved in 6.7 ml of water and added dropwise to the dodecanthiol solution over a period of 15 minutes. Colours went from mushy white into bright white solution. Then carbon disulfide (3 mL, 50 mmol) was added in one portion and the mixture was stirred for 30 min at room temperature. Colours went from bright yellow to transparent yellow. Afterwards the mixture was cooled in an ice bath. While the mixture being stirred, *p*-Toluenesulfonylchloride (4.78 g, 25 mmol) was added in portions over 5 minutes and thereafter the mixture was stirred for 1h at room temperature. The solution turned pumpkin orange. Afterwards the temperature was raised to 45 degrees for 10 min.

The mixture was dissolved in DCM (200 mL) and washed 2 times with water (70 mL; 50 mL), if necessary brine was added in small portions to facilitate phase separation. Afterwards the organic layer was dried with $MgSO_4$ and filtered. The filtrate was evaporated and resolved in a minimal amount of DCM. This was brought up a flash column (2 columns) and separated with an hexane/DCM (85/15) mixture. The product was obtained as an orange oil (8.94 g; 64%) after evaporation of the first fraction.

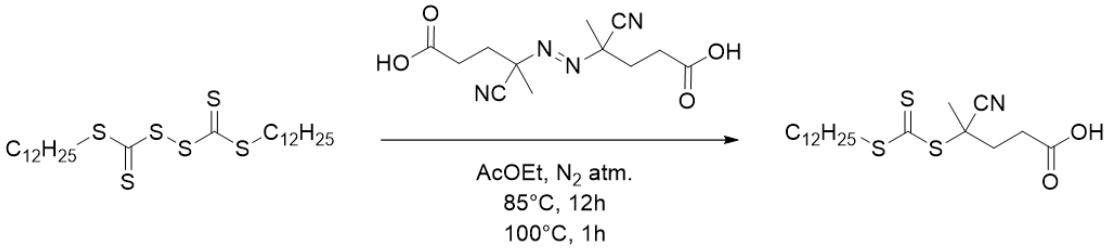


Figure 14: Synthesis of CDTPA

Bis(dodecylsulfanylthiocarbonyl) disulfide (8.94 g, 16.2 mmol) was added together with ethyl acetate (72 mL) and 4,4'-azobis(4-cyanovaleric acid) (6.942 g, 24.8 mmol) and stirred for 12h at 85 °C under N_2 atmosphere. Afterwards again 4,4'-azobis(4-cyanovaleric acid) (2.21 g, 7.9 mmol) was added together with ethyl acetate (30 mL) and the mixture was stirred at 100 °C for 1h.

After evaporation, crystals were formed in de crude mixture. The mixture was diluted with ethyl acetate and then filtered to remove the excessive solids. The filtrate was evaporated and purified by silica column chromatography hexane: ethyl acetate (gradient 100:0 - 20:80). The first fraction was unreacted Bis(dodecylsulfanylthiocarbonyl) disulfide, the second fraction is invisible with UV

lamps, the third fraction was the product. After evaporation of the third fraction an orange oil (5.96 g; 46%) was obtained which crystallized at room temperature.

2.4.3 SUMI products

2.4.3.1 SUMI of AAM on CPE-TTC

The synthesis of CPE-TTC AAM SUMI as shown in Figure 15 was carried out multiple times with different conditions. The starting conditions were roughly based on the starting conditions of other acrylate SUMI reactions performed by Vandenbergh [22]. Down here a typical procedure is described for the synthesis of CPE-TTC AAM SUMI. Different conditions, equivalents and quantities are described in Results and discussion.

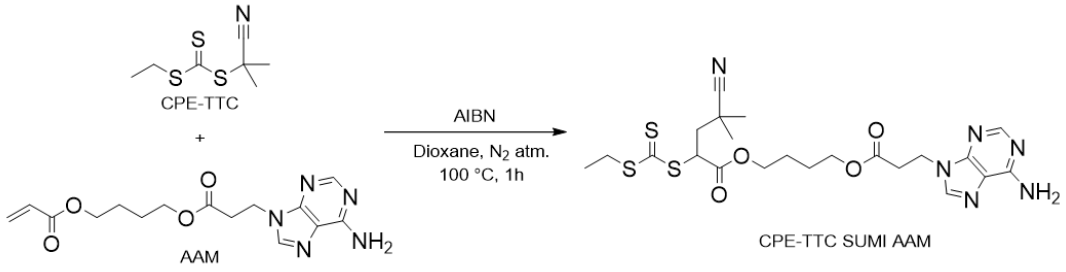


Figure 15: Synthesis of CPE-TTC AAM SUMI

2-cyano-2-propyl ethyl trithiocarbonate (104.3 mg, 0.509 mmol) and adenine acrylate monomer (163.5 mg, 0.491 mmol) were added to a small vial. Then 1mL of azobisisobutyronitrile in dioxane stock solution (0.024 M, 4 mg/mL) was added. 1 mL of pure dioxane was added to help dissolve all reagents. A DMF standard (38 µL) was added for ^{1}H-NMR spectroscopy analysis. A stirring bar was added and the vial was closed with a septum and purged with N_2 gas for 5 minutes. Then the vial was put in a thermostatically stabilized heat block at 100 °C for 1h under N_2 atmosphere. The conversion of the reaction was followed by taking small samples of 0.05 mL with a needle and syringe. These were taken before the reaction and every 10 minutes during the reaction. These samples were dissolved in DMSO-*d6* and analysed with ^{1}H-NMR spectroscopy.

2.4.3.2 SUMI of CAM and TAM on CPE-TTC

After obtaining the ideal conditions for the synthesis of CPE-TTC AAM SUMI, these conditions were used to perform the same experiment as in 2.4.3.1, now exchanging AAM with CAM and TAM. The results are reported in Results and discussion.

2.4.3.3 SUMI of AAM on other RAFT agents

After having optimized the reaction with CPE-TTC, other RAFT agents were tested in the same conditions to compare their reactivity. SUMI reactions were performed with AAM on CPD-TTC, CMD-TTC and CDTPA.

SUMI of AAM on CPD-TTC

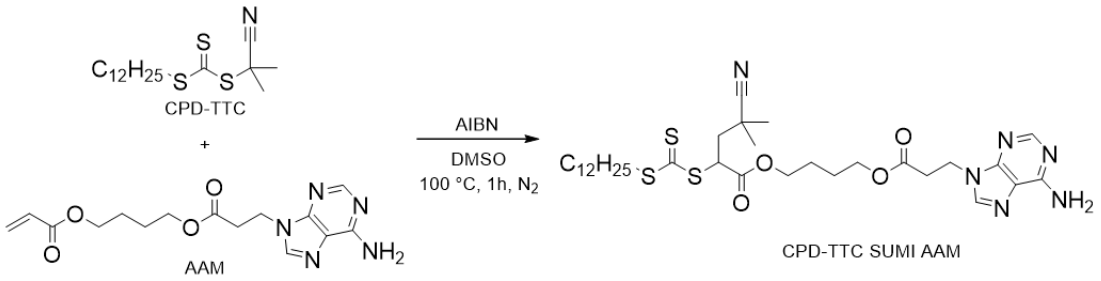


Figure 16: Synthesis of CPD-TTC AAM SUMI

2-cyano-2-propyl dodecyl trithiocarbonate was added as 0.5 mL from a stock solution (0.2 mM, 69.2 mg/mL) in DMSO and adenine acrylate monomer (167 mg, 0.50 mmol) were added to a small vial. Then 0.5 mL of azobisisobutyronitrile in DMSO stock solution (0.01 M, 1.6 mg/mL) was added. A DMF standard (38 µL) was added for ^{1}H-NMR spectroscopy analysis. A stirring bar was added and the vial was closed with a septum and purged with N_2 gas for 5 minutes. Then the vial was put in a thermostatically stabilized heat block at 100 °C for 1h under N_2 atmosphere. The conversion of the reaction was followed with by taking small samples of 0.05 mL with a needle and syringe. These were taken before the reaction and every 10 minutes during the reaction. Thereafter they were dissolved in DMSO-*d6* and analysed with ^{1}H-NMR spectroscopy.

SUMI of AAM on CMD-TTC

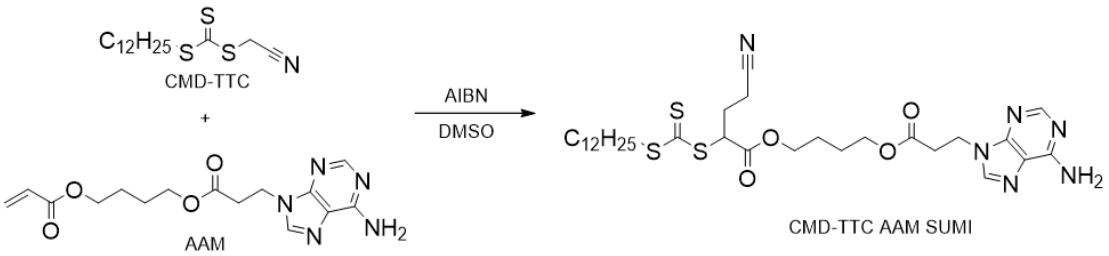


Figure 17: Synthesis of CMD-TTC AAM SUMI

2-cyano-2-methyl dodecyl trithiocarbonate was added as 0.5 mL from a stock solution (0.2 mM, 63.6 mg/mL) in DMSO and adenine acrylate monomer (167 mg, 0.50 mmol) were added to a small vial. Then 0.5 mL of azobisisobutyronitrile in DMSO stock solution (0.01 M, 1.6 mg/mL) was added. A DMF standard (38 µL) was added for ^{1}H-NMR spectroscopy analysis. A stirring bar was added and the vial was closed with a septum and purged with N_2 gas for 5 minutes. Then the vial was put in a thermostatically stabilized heat block at 100 °C for 1h under N_2 atmosphere. The conversion of the reaction was followed with by taking small samples of 0.05 mL with a needle and syringe. These were taken before the reaction, at 30 minutes and after the reaction. Thereafter, they were dissolved in DMSO-*d6* and analysed with ^{1}H-NMR spectroscopy analysis.

SUMI of AAM on CDTPA

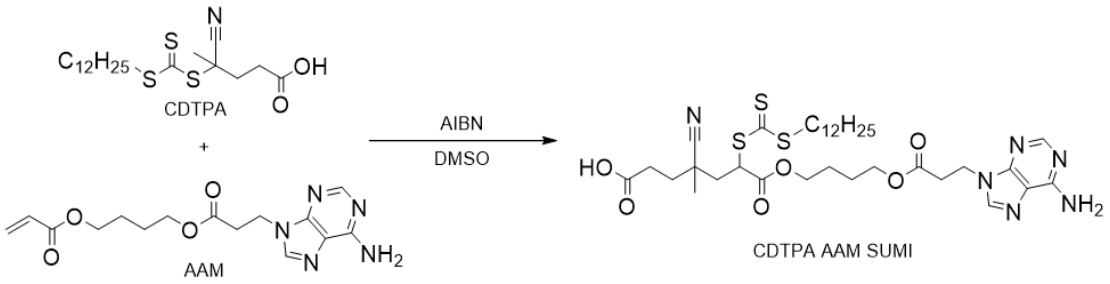


Figure 18: Synthesis of CDTPA AAM SUMI

4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA) was added as 0.5 mL from a stock solution (0.2 mM, 80.8 mg/mL) in DMSO and adenine acrylate monomer (167 mg, 0.50 mmol) were added to a small vial. Then 0.5 mL of azobisisobutyronitrile in DMSO stock solution (0.01 M, 1.6 mg/mL) was added. A DMF standard (38 µL) was added for ^{1}H-NMR spectroscopy analysis. A stirring bar was added and the vial was closed with a septum and purged with N_2 gas for 5 minutes. Then the vial was put in a thermostatically stabilized heat block at 100 °C for 1h under N_2 atmosphere. The conversion of the reaction was followed by taking small samples of 0.05 mL with a needle and syringe. These were taken before the reaction, at 30 minutes and after the reaction. Thereafter they were dissolved in DMSO-*d6* and analysed with ^{1}H-NMR spectroscopy analysis.

2.4.4 Purification of chosen RAFT AAM SUMI product crudes

The crude RAFT AAM SUMI solution was diluted with water (water:DMSO 5:1) and extracted two times with ±50 mL DCM. 2 mL of brine was added to the water phase and this mixture was extracted again with ±50 mL DCM. All the extracts were combined and evaporated to yield a yellow powder.

A small amount of this powder was dissolved in DCM to test if separation with TLC was possible. Different solvent combinations are tested as described in 3.3. Afterwards, it was decided to only work further with the CPD-TTC extract as only this product showed an acceptable separation on TLC with an Rf-value of 0.62 with the AAM monomer having an Rf-value of 0.49 when using DCM:MeOH 90:10 as mobile phase. As flash chromatography is not exactly the same as TLC, after finetuning the used solvent mixture was DCM:MeOH 93:7. Purity was confirmed with ^{1}H-NMR spectroscopy and ESI-MS. Yields are not reported as different tests were performed with the product batch and this was not the focus of the investigation. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 – 0.96 (m, 3H), 1.22 (s, 16H), 1.33 (s, 3H), 1.38 (s, 3H), 1.57 (s, 4H), 1.58 – 1.67 (m, 2H), 2.09 (dd, *J* = 5.7, 14.6 Hz, 1H), 2.37 (dd, *J* = 8.3, 14.6 Hz, 1H), 2.44 – 2.55 (m, 2H), 2.95 (t, *J* = 6.8 Hz, 2H), 3.35 (s, 2H), 3.93 – 4.02 (m, 2H), 4.05 – 4.12 (m, 2H), 4.37 (t, *J* = 6.8 Hz, 2H), 4.87 (dd, *J* = 5.6, 8.3 Hz, 1H), 7.20 (s, 2H), 8.08 (s, 1H), 8.12 (s, 1H).

3 Results and discussion

3.1 Synthesis of adenine and cytosine containing acrylate monomers

Adenine as well as cytosine acrylate functionalized monomer were synthesized according to in literature described procedures [32, 33]. These describe a Michael addition of a dicrylate on adenine (or cytosine). The reaction mechanism of the Michael addition is visualised in Figure 19.

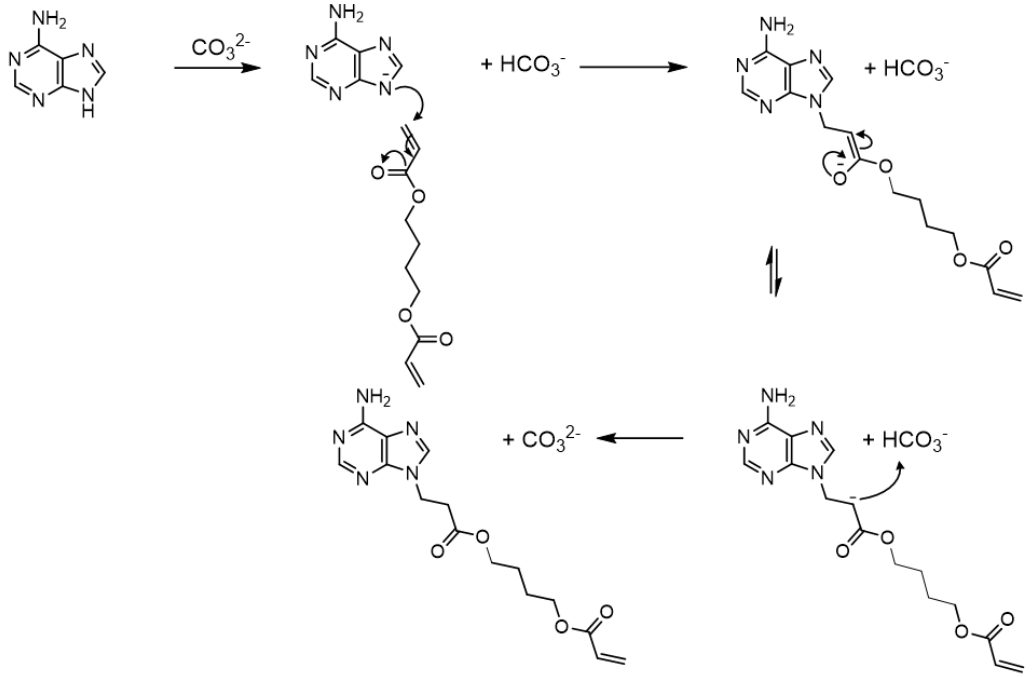


Figure 19: Michael addition of a diacrylate molecule on adenine

The adenine first gets deprotonated by a base. As the pK_a value of adenine is 9.80 [35], potassium carbonate (pK_a=10.3 [36]) is sufficient for deprotonation. The negatively charged nitrogen thereafter does a Michael addition reaction on a vinyl group of 1,4-butanediol diacrylate (BDDA). The addition on the vinyl end-groups is preferred as in this way the electrons are stabilized by the electronegative oxygen. The Polymer Reaction Design (PRD) research group previously also tested the method of Van Hest [37] for synthesis of these monomers but the Michael addition reaction was preferred over the substitution reaction. This was because acrylates are less stabilized than methacrylates which showed only minimal reaction [38]. Excess of adenine or cytosine must be avoided to minimalize the chance of a double addition to BDDA. BHT is added as a polymerization inhibitor, it scavenges radicals and therefore prevents auto-polymerization of the monomer or BDDA during the synthesis [39].

The synthesis was performed three times, gradually increasing the starting product quantities but still using the same equivalents. This was done to get an idea to what extend a scale-up of the procedure was possible. The different starting quantities and their corresponding yields are summarized in Table 2.

Table 2: Different adenine starting quantities with AAM synthesis

No	Adenine starting quantity (g)	Amount AAM (g)	Yield AAM (%)
1	3.05	5.14	68
2	4.06	5.60	56
3	6.02	8.07	55

In comparison with literature, all experiments achieved a higher yield. Reaction 1 has an exceptional yield when compared with reaction 2, reaction 3 and the reaction by Long which yielded 53% [32]. This could be due to residual chloroform solvent from the reaction as there is still some present as shown in the spectrum. Nevertheless the ^{1}H-NMR spectroscopy analysis confirmed the structure and purity of the product and is visualized in Figure 20.

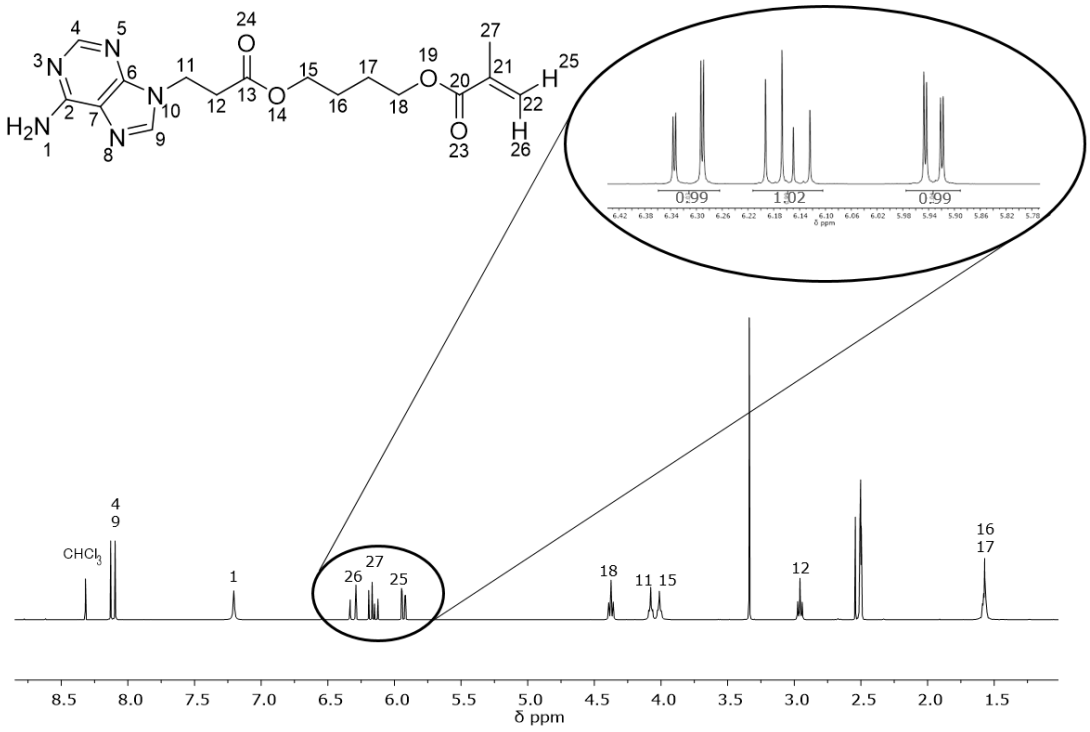


Figure 20: ^{1}H-NMR spectrum analysis of AAM

The residual DMSO and water peaks come from the NMR solvent. As shown in the enlarged spectrum, there is no residue of BDDA present. This is absolutely necessary for the further SUMI reactions as crosslinking could happen during the reaction. This is also the reason why an extraction is not sufficient for purification and why a subsequent chromatographic purification is performed.

The synthesis of the cytosine functionalized acrylate monomer also consists of a Michael addition reaction as visualised in Figure 19, but a different base than K_2CO_3 was used as catalyst. Literature described this reaction using triethylamine (TEA) as base [33]. As triethylamine has a pK_a of 10.75 in water [40], it is theoretically not basic enough to deprotonate cytosine which has a pK_a value of 12.2 in water [35]. As literature describes TEA has pK_a of 9 for TEA in DMSO [41], it is also possible the pK_a of cytosine is lower in DMSO and thus still has some functionality. Nevertheless KO*t*Bu (pK_a = ±17) was used instead of TEA.

A yield of 42% was achieved which is very close to the 40% reported in literature [33]. The ^{1}H-NMR spectroscopy analysis is visualized in Figure 21 and confirms the structure and purity of the product.

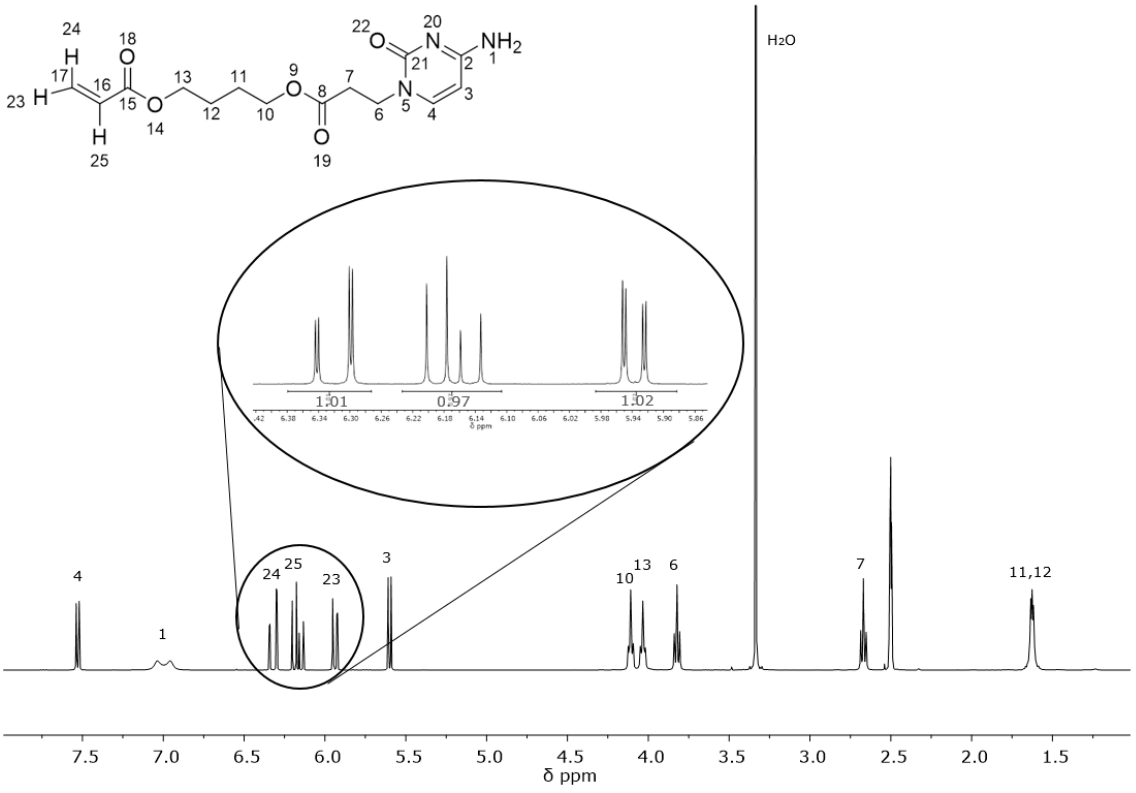


Figure 21: ^{1}H-NMR spectrum analysis of CAM

Some residual H_2O was present as shown in the spectrum. The same as with AAM, here the enlarged spectrum shows that there is no BDDA residue. This is necessary for the same reasons as explained under Figure 20. To conclude, pure AAM and CAM were synthesized and can be used for further polymerizations.

3.2 Fast screening for optimal reaction parameters for SUMI reaction

The goal of these synthesis reactions was to acquire data of the influence of monomer:RAFT-agent equivalents and find optimal conditions which favour only one monomer insertion. For these tests AAM and CPE-TTC was used. Also, the influence of the solvent was tested with reactions in dioxane and DMSO.

3.2.1 CTA:monomer ratio

3.2.1.1 Reaction in dioxane

The first tests were performed in dioxane as it has been previously used for sequence-controlled reactions with nucleobases [29]. As dioxane has a boiling point of 101 °C. It facilitates an easy removal of the solvent compared with DMSO (189 °C) or DMF (153 °C) which have a higher boiling point.

The major idea was to define the equivalents of AAM:RAFT-agent that was needed to get as much RAFT-agent as possible with exactly one SUMI. Due to the limited solubility of AAM in dioxane, the equivalents were kept the same but the concentrations were lowered.

The results are shown in Table 3 and performed following the procedure explained in Materials and Methods with the conditions summarized in the table.

Table 3: Summary of reagent equivalents of different tests in dioxane for 1h at 100 °C

CPE-TTC Eq.	AAM Eq.	AIBN Eq.	CPE-TTC Conc. (M)	AAM Conc. (M)	AIBN Conc. (mM)	Conversion (%)	#insertions
1	1	0.05	0.25	0.25	0.012	23	0.23
1	2	0.05	0.26	0.48	0.014	24	0.45
1	5	0.05	0.12	0.61	0.006	11	0.55
1	1	0.05	0.49	0.49	0.024	29	0.29

When comparing the reactions with monomer:CTA ratio 1:1 and 2:1, the number of insertions per SUMI was doubled when the concentration of AAM was doubled. This is an expected result as the number of possible encounters between CPE-TTC and AAM was also doubled. When comparing the reactions with ratio 1:1 and 5:1, only 2.4 times the amount insertions were observed. This was due the overall lower concentrations in the reaction with the 5:1 ratio. Because of this there are less encounters than there would be if only the AAM concentration was changed. A final comparison of both reaction with 1:1 ratio's, shows that when the concentration was doubled approximately the same percentage of CTA's with SUMI were synthesized.

Increasing the monomer equivalents therefore has a linear correlation on the amount of insertions, for the first insertion. Increasing the overall concentration also increases the amount of insertions.

3.2.1.2 Reaction in DMSO

As the reaction in dioxane showed that the solubility of the monomer was a limiting factor, from now on the experiments were performed in DMSO. Higher equivalents could be tested due to better solubility of AAM in the solvent. The results of these tests are shown in Table 4.

Table 4: Optimization of reagentia equivalents of CPE-TTC AAM SUMI in DMSO for 1h at 100 °C

CPE-TTC Eq.	AAM Eq.	AIBN Eq.	CPE-TTC Conc. (M)	AAM Conc. (M)	AIBN Conc. (mM)	Conversion (%)	#insertions
1	1	0.05	0.1	0.1	5	7	0.07
1	2	0.05	0.1	0.2	5	5	0.11
1	5	0.05	0.1	0.5	5	21	1.03
1	10	0.05	0.1	1.0	5	50	5.01
1	20	0.05	0.1	2.0	5	82	16.03

In the experiments in Table 4 the overall trend is that the amount of insertions increase with an increasing monomer:CTA ratio. An exception to this trend are the reactions with the 1:1 and 2:1 monomer:CTA ratio, where the 1:1 ratio reaction has a higher conversion than the 2:1 ratio reaction. This unexpected result could be caused by the analysis method. ^{1}H-NMR in general has a 5% error on integrals due to the necessary baseline and phase corrections. As only a small amount of DMF was added as internal standard and the DMSO was not removed before determining the conversion, the S/N ratio of the response of the monomer could be too small for a correct analysis. As visualized earlier in Figure 4, RAFT polymerization product chain lengths show a Poisson distribution. Therefore to optimize the crude yield, the amount of insertions should be as close as possible to one insertion. The most interesting result in Table 4 is therefore that of the 5:1 ratio, having an average of 1.03 insertions for each RAFT-agent as this is wat was aimed for.

With higher AAM equivalents a higher conversion is observed which results in a longer oligomer chain. This is logical as there is more monomer available and due to the higher AAM concentration also more encounters are possible between AAM and the macro-RAFT agent. At the 20:1 monomer:CTA ratio even 16 monomers were built in. Due to this length there could be a limited solubility of the macro-RAFT molecule. A visualization of the results in Table 4 is made in Figure 22.

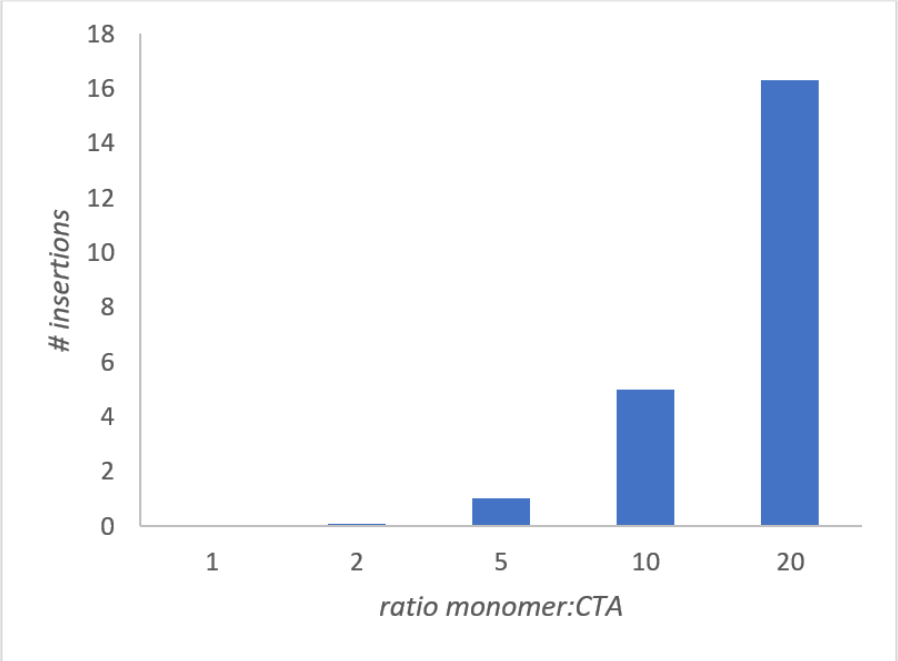


Figure 22: Influence of monomer:CTA ratio on the number of insertions

In Figure 22, the trend of increasing insertions with increasing monomer ratio is visualized. As with the 5:1 ratio the first insertion was achieved, it is clearly visible that when comparing this with the 10:1 ratio, the amount of insertions was 5 times more when only 2 times as much monomer was available. The same result is also seen comparing the 10:1 and 20:1 ratio reactions, where more than three times as much insertions were achieved when only two times as much were expected if this was based on the linear correlation of the number of insertion and available monomers.

3.2.1.3 Optimal conditions

As in future experiments probably a second SUMI will be performed, the reaction in dioxane would be unpractical due to the limited solubility of reagents and product, and thus was chosen to use DMSO as a solvent despite the extra extraction step that is necessary during workup stages. This is because evaporation of DMSO, even under reduced pressure and elevated temperature, would be unpractical due to its boiling point of 189 °C.

As the goal of the project was to have exactly one insertion, and as with the 5:1:0.05 AAM:CPE-TTC:AIBN ratio, in DMSO for 1h at 100 °C, an average insertion of 1.03 monomers for each RAFT-agent was observed, these conditions were chosen for further experiments.

Later experiments with these conditions but in larger batches showed a conversion between 14-21%. This could be due to other heat transmission, different N_2 purging times but also due to the earlier mentioned uncertainties in the ^{1}H-NMR analysis. As no higher conversions were encountered, these conditions are still very practical. This is because the addition rate of the first monomer to the radical leaving group of the RAFT agent is lower than the propagation rate of monomer

on a propagating chain [25, 42, 43]. This simply means the first insertion is the most difficult, all following insertions are quicker. When the 20% conversion limit is exceeded, all RAFT agents start to propgagate faster. When 40% conversion is surpassed, no usable product would be remaining as all macro-RAFT agents would have a second insertion. With the 14% conversion still 70% of the RAFT-agents has one insertion.

Only two factors, the monomer:CTA ratio and the solvent, were optimized. Further optimization of this reaction can be performed. The maximum solubility of the monomer should be used when varying the RAFT-agent ratio. Also, a further optimization of the temperature and reaction time still needs to be explored.

3.2.2 Expanding the scope to different nucleobase functionalized monomers

After the reaction with CPE-TTC and AAM was optimized as described in 3.2.1.3, these conditions were used for the SUMI of cytosine and thymine functionalized acrylate monomers (CAM, TAM). The results of these experiments are summarized in Table 5.

Table 5: Comparing conversions of different nucleobase functionalized monomers

Monomer	Conversion (%)
AAM	14
CAM	14
TAM	14

As all three monomers have the same acrylate functionality, approximately the same conversions were expected. As mentioned in 3.2.1.3, the experiment with AAM was redone in a larger batch with the same stock solutions as the CAM and TAM monomers, with the given result as shown in the table. For all three monomers the same conversion of 14% was achieved. Therefore, can be concluded that they have the same reactivity. Further experiments were performed with AAM as all monomers show a similar reactivity.

3.2.3 Compatibility with different RAFT agents

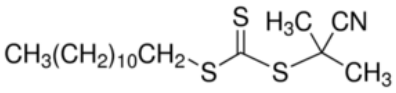
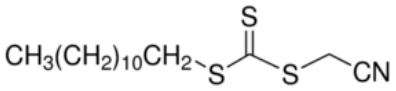
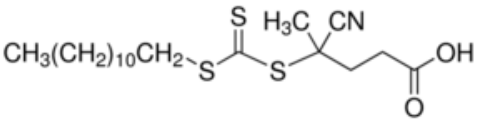
Using different RAFT agents expands the scope of the SUMI reaction. These RAFT agents were not only chosen based on their reactivity with acrylates, but also because of their different functional groups and future applications.

Earlier tests were all performed with CPE-TTC. Most recent tests were performed with CPD-TTC, which has the same leaving group but a different Z-group. CPD-TTC has a long apolar tail, which could influence the separation of the SUMI product. Also, CMD-TTC was tested. CPD-TTC and CMD-TTC have a different leaving group which could not only have a function in reactivity but also on

separative capabilities. Also, reactions were performed with CDTPA. None of the other RAFT-agents has a carboxylic acid group and this could have a function in purification and reactivity. Also, CDTPA is already known as an photoiniferter and therefore also photoinduced reactions would be interesting to investigate in the future. For this project the most important factors were the reactivity of the CTA and retention on silica during purification.

There is a chart available from Sigma Aldrich which compares the reactivity of different RAFT agents [7]. A summary of the used RAFT agents is found in Table 6.

Table 6: Reactivity of tested RAFT agents according to Sigma Aldrich [7]

Name	Structure	Reactivity with acrylates
CPD-TTC		++
CMD-TTC		+++
CDTPA		++

Focussing on reaction time, an agent that reacts too fast has a higher chance of having multiple insertions when only one insertion is desired. An agent that reacts too slow would mean the reaction takes more time.

These RAFT agents were tested with the same conditions that were optimal for the CPE-TTC RAFT agent (as explained in 3.2.1.3) to compare their reactivity and expand the scope of the project. The different RAFT agents and their conversion rates are shown in Table 7.

Table 7: Tests on the influence of different RAFT agents on conversion

RAFT-agent	AAM Eq.	RAFT Eq.	AIBN Eq.	Conversion (%)	#insertions
CPE-TTC	5	1	0.05	21	1.03
CPD-TTC	5	1	0.05	20	1.02
CMD-TTC	5	1	0.05	81	4.03
CDTPA	5	1	0.05	24	1.21

As in all reactions 5 equivalents of monomer were used with 1 equivalent of RAFT agent, the desired conversion was 20% as this would mean that every RAFT agent has one SUMI.

When comparing the reaction with CPE-TTC and CPD-TTC, a similar conversion percentage is acquired. This is expected because of similar reactivities. This means that both RAFT agents are useful for the chosen SUMI reaction. Due the fact that these products are difficult to dissolve, purification of these products could form a major problem in the future. The difference in length of the ethyl and dodecyl tail of both RAFT-agents can give different retention- and soluble properties leading to an optimal separation of the SUMI product, starting materials and side products.

When comparing the reaction with CPE-TTC and with CMD-TTC, CMD-TTC shows a much higher reactivity as 4 times the amount of conversion was reached. This makes CMD-TTC not applicable in the 5:1:0.05 equivalents combination but may be useful in a for example 1:1:0.05 equivalents combination. This was not tested due to limited time.

Comparing the reaction with CPE-TTC and CDTPA, CDTPA shows a similar conversion rate in the given conditions. Also in this test the CDTPA was activated by thermally initiated radicals. CDTPA on itself is a photo-iniferter. This means the molecule on itself is an initiator and a chain transfer agent which forms radicals when irradiated with UV light. This expands the range of applications as this makes it possible to perform this reaction in batch and flow experiments with an optimized illumination time. The synthesis of SD acrylates with CDTPA has been performed before but with different functionalities on the acrylates [44]. A different separative technique would be necessary as CDTPA already showed difficult separation during synthesis.

3.3 Purification of RAFT AAM SUMI product

As described earlier in 3.2.3, CPE-TTC and CPD-TTC had similar conversion percentages in the same reaction conditions, but due to their different Z-groups, they have different solubility properties. As a first test thin-layer chromatography

(TLC) was performed to determine the ideal solvent mixture. A small amount of the AAM SUMI was dissolved in chloroform and then TLC was performed with different mobile phases. The results of these tests are summarized in Table 8.

Table 8: TLC-solvent analysis of the crude CPE-TTC AAM SUMI and CPD-TTC AAM SUMI

	Mobile phase		Rf value		
	Solvents	Ratio	SUMI product	AAM	Difference
CPE-TTC	$CHCl_3$:MeOH	90:10	0.472	0.472	0.000
	DCM:MeOH	95:5	0.325	0.300	0.025
	DCM:MeOH	90:10	0.488	0.439	0.049
	DCM:MeOH	85:15	0.649	0.622	0.027
	DCM:MeOH	80:20	0.859	0.846	0.013
CPD-TTC	$CHCl_3$:MeOH	90:10	0.51	0.43	0.08
	$CHCl_3$:MeOH	85:15	0.81	0.75	0.06
	DCM:MeOH	95:5	0.35	0.29	0.06
	DCM:MeOH	90:10	0.62	0.49	0.13
	DCM:MeOH	85:15	0.86	0.81	0.05

The results show that for both CPE-TTC AAM SUMI and CPD-TTC AAM SUMI the solvent combination of DCM:MeOH 90:10 has the best separation capabilities on TLC. Only with CPD-TTC AAM SUMI an acceptable separation from AAM was achieved. This was probably because the long apolar dodecyl group improves the solubility properties of the SUMI product in DCM. CPE-TTC AAM SUMI does only have an ethyl tail which is too small to improve the solubility. Therefore only purification of the CPD-TTC AAM SUMI crude was tested with flash chromatography.

First, rough tests were performed to investigate how the product responds to the tight prepacked silica cartridges. From experience from colleagues, flash chromatography usually needs a slightly different solvent mixture than TLC. Due to the higher pressure and better packing of the solid phase, the polarity percentage of the mobile should be slightly lower.

100 mg of crude was dissolved in 2 mL of chloroform. When using a single Interchim puriFlash silica STD 50 µm F0040 flash column with DCM:MeOH 93:7 as the mobile phase, only 1 mL of product crude could be brought on the column. When starting the elution of the solvent, the pressure increased over the limit of the flash apparatus as too much product was brought on the column, followed by blocking and shut down of the elution process. This happened even at very low flow rates (10 mL/min). When repeating this test but with only 0.5 mL of this crude mixture brought on the column, the product did elute at a flow rate of 30 mL/min but no separation was achieved.

When combining two of these cartridges and applying 0.5 mL of the crude mixture, using DCM:MeOH 93:7 as mobile phase, with an elution speed of 30 mL/min, separation of the product was achieved with minor impurities. After evaporation of the test tubes containing the product, a pure product was achieved. This was confirmed with ^{1}H-NMR spectroscopy analysis. Also, mobile phases of DCM:MeOH

95:5 and 97:3 were tested but both showed no separation. The ideal solvent ratio therefore was found to be DCM:MeOH 93:7.

As with this method barely enough product was yielded to perform ^{1}H-NMR analysis, a scale-up was necessary. As the amount of product that could be brought up was dependent from the surface size of the column, the largest manually filled column available in the lab was used as described under Materials and Methods. 400 mg of crude was dissolved in 7 mL of chloroform and all was brought upon the column. After elution at a speed of 60 mL/min with a mobile phase of DCM:MeOH 93:7, separation of the product was achieved with minor impurities. After evaporation of the solvents under reduced pressure, the product was obtained. This was confirmed by ^{1}H-NMR spectroscopy analysis as shown in Figure 23 and ESI-MS as shown Figure 24.

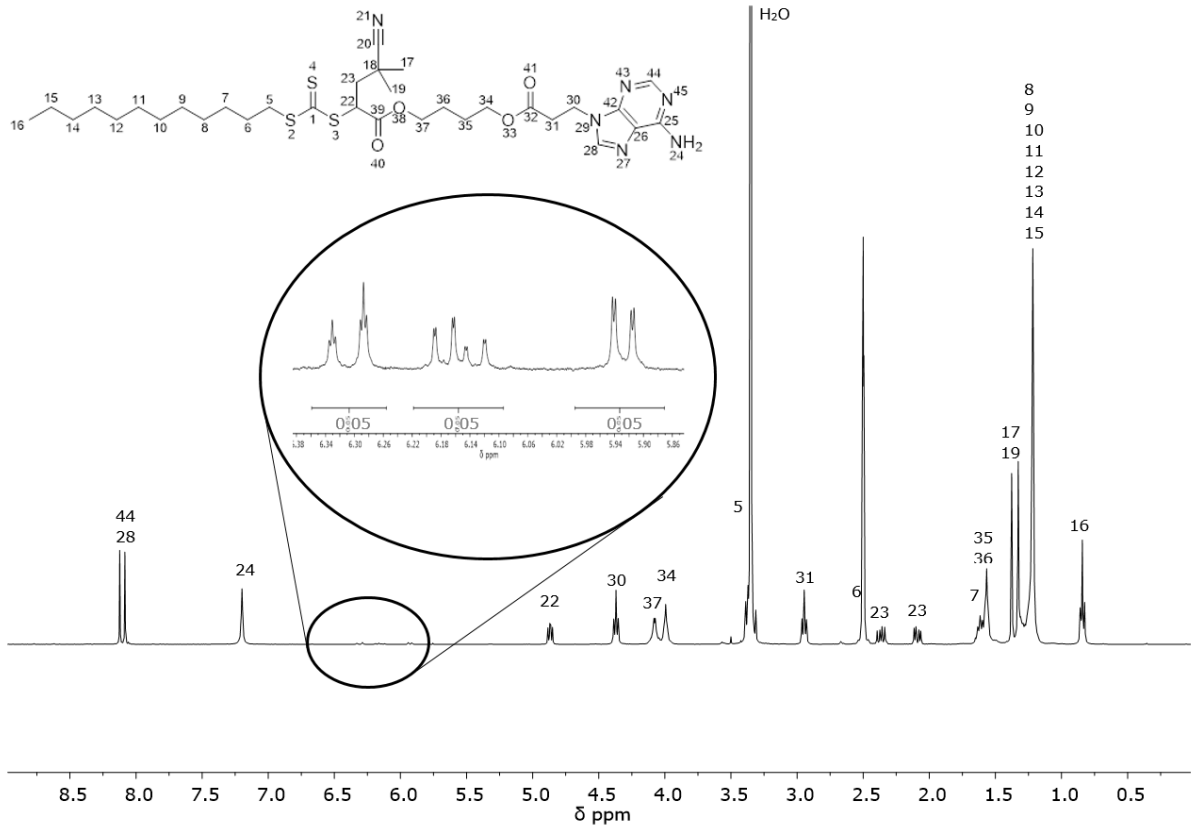


Figure 23: ^{1}H-NMR spectroscopy analysis of purified CPD-TTC AAM SUMI

As shown an almost pure product is obtained apart from the H_2O and DMSO peaks from the NMR solvent and the impurity highlighted in Figure 23. When looking at the zoomed-in area where in an ideal case no peaks should be visible, minor impurities are still remaining. A purity of 95% CPD-TTC AAM SUMI product was achieved. When looking at the enlarged impurities, a mixture of acrylate peaks is visible. The first thought was that these were coming from some unreacted monomer but the ESI-MS in Figure 24 and the analysis below proves this is untrue. It must be further explored if these impurities are coming from degradation of the SUMI product on the column and whether or not these impurities have an influence in future reactions.

The purified AAM SUMI product was also analysed with ESI-MS as is visualized in Figure 24. The annotated exact masses are explained in Table 9.

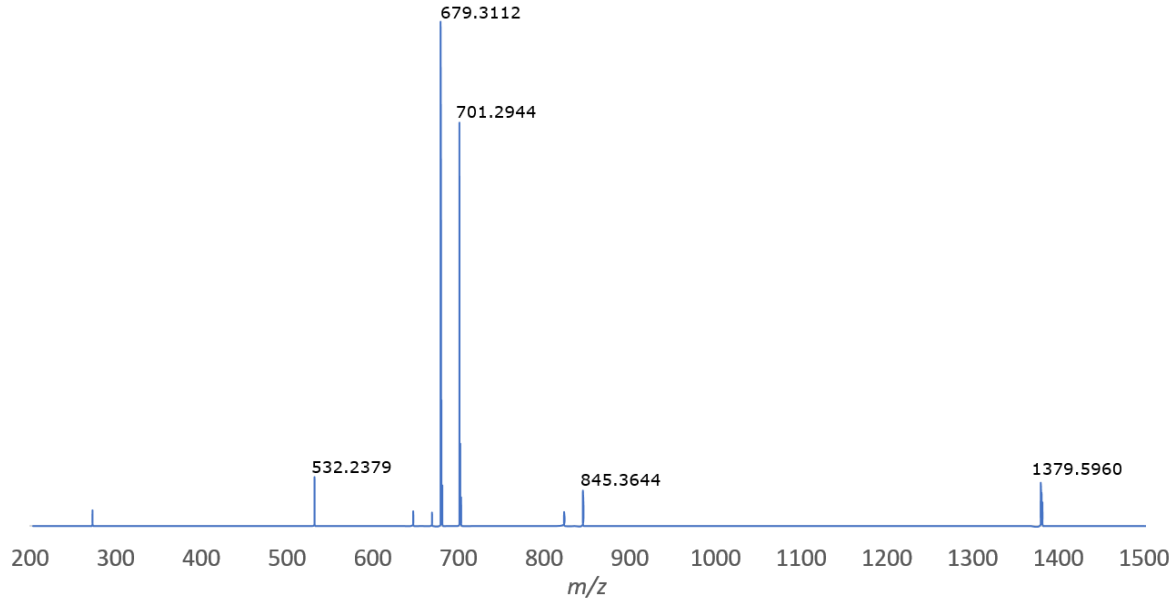


Figure 24: ESI-MS analysis of purified CPD-TTC AAM SUMI

Table 9: ESI-MS peak assignment of CPD-TTC AAM SUMI

Peak	Structure + ion	Exact mass
532.2379	Background signal	
679.3112	CPD-TTC AAM SUMI + H^+	678.31 + 1.0078 = 679.32
701.2944	CPD-TTC AAM SUMI + Na^+	678.31 + 22.9898 = 701.30
845.3644	CPD-TTC AAM SUMI + Na^+ + 2x THF (?)	678.31 + 22.9898 + 2x 72.06 = 845.42
1379.5960	2x CPD-TTC AAM SUMI + Na^+	2x 678.31 + 22.9898 = 1379.61

The peak around 532.2379 is remaining background from previous measurements.

The peak around 679.3112 is the CPD-TTC AAM SUMI product ionized with a proton, 701.2944 is ionized with a sodium ion. The peak around 845.3644 is questionable. The proposed complex, a cohesion of two THF molecules and the SUMI product ionized with a sodium ion, does have a mass difference of 0.06 with the measured mass. As the difference between the calculated and measured masses of the other analyses is only 0.1-0.2, the difference of 0.06 is too big to draw conclusions. Further research is required whether or not this is the actual structure of this peak. The peak around 1379.5960 is a cohesion of 2 SUMI products ionized with a sodium ion.

It can be concluded that the product is pure apart from the acrylate impurities that were visible in the ^{1}H-NMR analysis and the questionable assigned peak in the ES-

MS anaylsis. Further research is required whether or not these impurities are separable and/or these form a problem when performing a second insertion.

When analysing the CPD-TTC AAM SUMI product crude with TLC, different spots with visibly larger retention than the monomer and the SUMI product were present. These spots seemed to be coming from macroRAFT agents with two and three insertions of AAM. If this is the case, a second insertion may be the solution to separate the macroRAFT agent from the acrylate impurities.

As mentioned earlier, the first insertion is the hardest and the slowest and the following insertions go faster. This way less monomer will be necessary to achieve the next insertion and thus less monomer will have to be removed afterwards. The impurities could therefore only be present with the first insertion. As mentioned earlier the solubility of SUMI products is limited so again different purification methods should be tested. Manual column chromatography could be a solution for a higher product output as much bigger columns can be prepared with therefore larger separative capabilities. Another possibility is the untested rec-SEC, knowing this has the limitation that only small amounts of crude can be brought upon this system but higher purity could be achieved. Further research is therefore required.

4 Conclusion and outlook

Successful synthesis of AAM (yield = 55- 68%) and CAM (yield 42%) was performed after adapting the in literature described reaction pathways. Also, larger batches were synthesized and thus an upscaling was performed. Furthermore, the synthesis of CDTPA was performed according to literature.

The SUMI of AAM on CPE-TTC was optimized. The ideal reaction equivalents are monomer:CTA:initiator (5:1:0.05) when using AAM as monomer, CPE-TTC as CTA and AIBN as initiator in DMSO when reacting 1h. at 100 °C. A conversion of 21% was achieved and thus an average of 1.03 monomers is added on each CPE-TTC molecule.

The SUMI of CAM and TAM were also performed. For both reactions a conversion of 14% was achieved. After repeating the SUMI of AAM also a conversion of 14% was achieved. All monomers therefore showed the same reactivity. This supports the fact that the nucleobase functional groups are exchangeable and that all experiments performed with AAM probably would have the same results when using CAM or TAM as monomer.

Different RAFT agents were tested with the optimized conditions. The SUMI reaction of AAM with CPE-TTC and CPD-TTC resulted in a similar conversion of 20% for both reactions. The SUMI reaction with CMD-TTC resulted in a higher conversion of 81%. The SUMI reaction with CDTPA resulted in a conversion of 24%. With this result was proven that the SUMI of AAM is possible on different RAFT agents but it is necessary to optimize the reaction parameters for each RAFT agent. For practical reasons was only worked further with CPE-TTC and CPD-TTC SUMI products.

Different solvents were tested for the separation of CPE-TTC and CPD-TTC SUMI products. Only the CPD-TTC AAM SUMI product showed an acceptable separation from AAM when using $CHCl_3$:MeOH 90:10 with TLC. This means only for this product a separation method was developed. The final CPD-TTC AAM SUMI product was purified using flash chromatography with $CHCl_3$:MeOH 93:7 as solvent. A purity of 95% was achieved.

Using 5 equivalents of monomer during synthesis of the SUMI products may impede the purification of the product. Therefore, optimization of the reaction with CMD-TTC, with for example 1 equivalent of monomer, may be a step forward. This way after the SUMI reaction less impurities must be removed.

Also improving the solubility of the products may be a solution that could improve the purification. Performing the first insertion with a regular acrylate and then adding the nucleobase as second monomer may help improve the solubility. When adding more nucleobases, this regular acrylate could then also function as a spacer to promote hydrogen bond formation with other nucleobases and not with adjacent nucleobases. As for now, the solubility of the products in chloroform is limited. If this can be improved, separation with recycling size exclusion chromatography could be tested as in the lab only SEC columns for chloroform and chlorobenzene were available. For now, it is impractical to separate the SUMI products this way as only small amounts could be separated each time and this process takes a lot of time.

To conclude, a strong foundation is laid for the synthesis of monodisperse nucleobase functionalised oligomers. As development of multiple insertions is only a matter of time, can already be thought of applications as molecular structure recognition using the specific hydrogen bonding patterns between nucleobase pairs, molecular tags or as a chemical data unit.

5 References

[1] A. D. Jenkins, P. Kratochvil, R. F. T. Stepto, and U. W. Suter, "Glossary of basic terms in polymer science," *International Union of Pure & Applied Chemistry,* vol. 68, no. 12, pp. pp. 2287-2377, 1996.

[2] IUPAC, "Compendium of Chemical Terminology," A. D. McNaught and A. Wilkinson, Eds., 2nd ed. Oxford: Blackwell Scientific Publication, 1997, p. 2308.

[3] I. M. Ward, "Polymers: Chemistry and Physics of Modern Materials, 3rd edition, by J.M.G. Cowie and V. Arrighi," *Contemporary Physics,* vol. 50, no. 6, pp. 670-670, 2009.

[4] F. A. Bovey and I. M. Kolthoff, "Inhibition and Retardation of Vinyl Polymerization," *Chemical Reviews,* vol. 42, no. 3, pp. 491-525, 1948.

[5] T. Junckers, "FRP kinetics and product control," ed. Diepenbeek: UHasselt, 2010.

[6] W.-F. Su, "Radical Chain Polymerization," in *Principles of Polymer Design and Synthesis*, W.-F. Su, Ed. Berlin, Heidelberg: Springer Berlin Heidelberg, 2013, pp. 137-183.

[7] Sigma-Aldrich. (7/03/2018). *RAFT: Choosing the Right Agent to Achieve Controlled Polymerization*. Available: https://www.sigmaaldrich.com/technical-documents/articles/materials-science/polymer-science/raft-polymerization.html

[8] N. A. Dotson, R. Galvan, R. L. Laurence, and M. Tirrell, *Polymerization Process Modeling*. VCH, 1996.

[9] A. S. Dunn, "The chemistry of free radical polymerization " *Polymer International,* vol. 42, no. 3, pp. 346-346, 1997.

[10] W. A. Braunecker and K. Matyjaszewski, "Controlled/living radical polymerization: Features, developments, and perspectives," *Progress in Polymer Science,* vol. 32, no. 1, pp. 93-146, 2007/01/01/ 2007.

[11] I. Rodríguez-Sanchez, D. Glossman-Mitnik, and E. A. Zaragoza-Contreras, "Theoretical evaluation of the order of reactivity of transfer agents utilized in RAFT polymerization: group Z," *Journal of Molecular Modeling,* journal article vol. 15, no. 9, pp. 1133-1143, 2009.

[12] I. Rodríguez-Sanchez, E. A. Zaragoza-Contreras, and D. Glossman-Mitnik, "Theoretical evaluation of the order of reactivity of transfer agents utilized in RAFT polymerization," *Journal of Molecular Modeling,* journal article vol. 16, no. 1, pp. 95-105, 2010.

[13] J. Chen *et al.*, "Synthesis of linear amphiphilic tetrablock quaterpolymers with dual stimulus response through the combination of ATRP and RAFT by a click chemistry site transformation approach," *Polymer Chemistry,* 10.1039/C2PY20946B vol. 4, no. 6, pp. 1815-1825, 2013.

[14] J. Vandenbergh and T. Junkers, "Alpha and Omega: Importance of the Nonliving Chain End in RAFT Multiblock Copolymerization," *Macromolecules,* vol. 47, no. 15, pp. 5051-5059, 2014.

[15] R. B. Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide," *Journal of the American Chemical Society,* vol. 85, no. 14, pp. 2149-2154, 1963/07/01 1963.

[16] K. Denise, P. B. Eric, B. Michel, C. Laurence, and L. Jean-François, "Identification-Tagging of Methacrylate-Based Intraocular Implants Using Sequence Defined Polyurethane Barcodes," *Advanced Functional Materials,* vol. 27, no. 3, p. 1604595, 2017.

[17] G. Cavallo, A. Al Ouahabi, L. Oswald, L. Charles, and J.-F. Lutz, "Orthogonal Synthesis of "Easy-to-Read" Information-Containing Polymers Using Phosphoramidite and Radical Coupling Steps," *Journal of the American Chemical Society,* vol. 138, no. 30, pp. 9417-9420, 2016/08/03 2016.

[18] A. Al Ouahabi, M. Kotera, L. Charles, and J.-F. Lutz, "Synthesis of Monodisperse Sequence-Coded Polymers with Chain Lengths above DP100," *ACS Macro Letters,* vol. 4, no. 10, pp. 1077-1080, 2015/10/20 2015.

[19] Y. Jiang *et al.*, "Iterative Exponential Growth Synthesis and Assembly of Uniform Diblock Copolymers," *Journal of the American Chemical Society,* vol. 138, no. 30, pp. 9369-9372, 2016/08/03 2016.

[20] M. Minoda, M. Sawamoto, and T. Higashimura, "Sequence-regulated oligomers and polymers by living cationic polymerization," *Polymer Bulletin,* journal article vol. 23, no. 2, pp. 133-139, February 01 1990.

[21] I. Shohei, T. Takaya, O. Makoto, and S. Mitsuo, "Selective single monomer addition in living cationic polymerization: Sequential double end-functionalization in combination with capping agent," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 48, no. 15, pp. 3375-3381, 2010.

[22] J. Vandenbergh, G. Reekmans, P. Adriaensens, and T. Junkers, "Synthesis of sequence controlled acrylate oligomers via consecutive RAFT monomer additions," *Chemical Communications,* 10.1039/C3CC45994B vol. 49, no. 88, pp. 10358-10360, 2013.

[23] J. Vandenbergh, G. Reekmans, P. Adriaensens, and T. Junkers, "Synthesis of sequence-defined acrylate oligomers via photo-induced copper-mediated radical monomer insertions," *Chemical Science,* 10.1039/C5SC02035B vol. 6, no. 10, pp. 5753-5761, 2015.

[24] J. J. Haven, J. A. De Neve, and T. Junkers, "Versatile Approach for the Synthesis of Sequence-Defined Monodisperse 18- and 20-mer Oligoacrylates," *ACS Macro Letters,* vol. 6, no. 7, pp. 743-747, 2017.

[25] E. T. A. v. d. Dungen, H. Matahwa, J. B. McLeary, R. D. Sanderson, and B. Klumperman, "Initialization behavior at various target molecular weight RAFT-mediated methyl acrylate polymerizations," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 46, no. 7, pp. 2500-2509, 2008.

[26] J. Lawrence *et al.*, "A Versatile and Efficient Strategy to Discrete Conjugated Oligomers," *Journal of the American Chemical Society,* vol. 139, no. 39, pp. 13735-13739, 2017/10/04 2017.

[27] L. Maes, "PhD Project overview," 2017.

[28] H. J. Spijker, A. J. Dirks, and J. C. M. van Hest, "Synthesis and assembly behavior of nucleobase-functionalized block copolymers," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 44, no. 13, pp. 4242-4250, 2006.

[29] Y. Kang, A. Lu, A. Ellington, M. C. Jewett, and R. K. O'Reilly, "Effect of Complementary Nucleobase Interactions on the Copolymer Composition of RAFT Copolymerizations," *ACS Macro Letters,* vol. 2, no. 7, pp. 581-586, 2013/07/16 2013.

[30] K. Zhang *et al.*, "Nucleobase-functionalized acrylic ABA triblock copolymers and supramolecular blends," *Polymer Chemistry,* 10.1039/C4PY01798F vol. 6, no. 13, pp. 2434-2444, 2015.

[31] X. Ye, X. Li, Y. Shen, G. Chang, J. Yang, and Z. Gu, "Self-healing pH-sensitive cytosine- and guanosine-modified hyaluronic acid hydrogels via hydrogen bonding," *Polymer,* vol. 108, pp. 348-360, 2017/01/13/ 2017.

[32] S. Cheng, M. Zhang, N. Dixit, R. B. Moore, and T. E. Long, "Nucleobase Self-Assembly in Supramolecular Adhesives," *Macromolecules,* vol. 45, no. 2, pp. 805-812, 2012.

[33] K. Zhang *et al.*, "Ureido cytosine and cytosine-containing acrylic copolymers," *Polymer Chemistry,* 10.1039/C6PY01519K vol. 7, no. 43, pp. 6671-6681, 2016.

[34] L. Liu, L. Fu, T. Jing, Z. Ruan, and L. Yan, "pH-Triggered Polypeptides Nanoparticles for Efficient BODIPY Imaging-Guided Near Infrared Photodynamic Therapy," *ACS Applied Materials & Interfaces,* vol. 8, no. 14, pp. 8980-8990, 2016/04/13 2016.

[35] I. Asimov, "Data for Biochemical Research (Dawson, R. M. C.; Elliott, Daphne C.; Elliott, W. H.; Jones, K. M.; eds.)," *Journal of Chemical Education,* vol. 37, no. 8, p. A490, 1960/08/01 1960.

[36] B. Myers. (2016, 10/05/2018). *pKa Table*. Available: https://www2.onu.edu/~b-myers/organic/2511_Files/Chapter3-pKa%20table.pdf

[37] H. J. Spijker, F. L. van Delft, and J. C. M. van Hest, "Atom Transfer Radical Polymerization of Adenine, Thymine, Cytosine, and Guanine Nucleobase Monomers," *Macromolecules,* vol. 40, no. 1, pp. 12-18, 2007/01/01 2007.

[38] G. Quintens, "Synthesis of nucleobase functionalized acrylate monomers for the design of mono-disperse sequence defined multiple hydrogen bond oligomers and polymers," Chemistry, Hasselt University, Diepenbeek, 2015.

[39] S. Fujisawa, Y. Kadoma, and I. Yokoe, "Radical-scavenging activity of butylated hydroxytoluene (BHT) and its metabolites," *Chemistry and Physics of Lipids,* vol. 130, no. 2, pp. 189-195, 2004/07/01/ 2004.

[40] D. H. Ripin and D. A. Evans. (2015, 10/05/2018). *pKa Table*. Available: http://evans.rc.fas.harvard.edu/pdf/evans_pKa_table.pdf

[41] M. R. Crampton and I. A. Robotham, "Acidities of Some Substituted Ammonium Ions in Dimethyl Sulfoxide," *Journal of Chemical Research, Synopses,* 10.1039/A606020J no. 1, pp. 22-23, 1997.

[42] G. Moad, Y. K. Chong, R. Mulder, E. Rizzardo, and S. H. Thang, "New Features of the Mechanism of RAFT Polymerization," in *Controlled/Living Radical Polymerization: Progress in RAFT, DT, NMP & OMRP*, vol. 1024(ACS Symposium Series, no. 1024): American Chemical Society, 2009, pp. 3-18.

[43] J. Thomas and B. K. Christopher, "The role of mid-chain radicals in acrylate free radical polymerization: Branching and scission," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 46, no. 23, pp. 7585-7605, 2008.

[44] C. Fu, Z. Huang, C. J. Hawker, G. Moad, J. Xu, and C. Boyer, "RAFT-mediated, visible light-initiated single unit monomer insertion and its application in the synthesis of sequence-defined polymers," *Polymer Chemistry,* 10.1039/C7PY00713B vol. 8, no. 32, pp. 4637-4643, 2017.

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