Judith Buchmaier

Towards amino group functionalized macromolecules and the assessment of their biocidal activity using LIVE/DEAD Kits.

MASTER THESIS

Masterarbeit

zur Erlangung des akademischen Grades einer

Diplom-Ingenieurin

der Studienrichtung Biotechnologie

erreicht an der Technischen Universität Graz

Mai 2011

Betreuer: Univ.-Doz. Dipl. Ing. Dr. techn. Christian Slugovc

Institut für Chemische Technologie von Materialien

Technische Universität Graz

Deutsche Fassung:

Beschluss der Curricula-Kommission für Bachelor-, Master- und Diplomstudien vom 10.11.2008

Genehmigung des Senates am 1.12.2008

EIDESSTATTLICHE ERKLÄRUNG

Ich erkläre an Eides statt, dass ich die vorliegende Arbeit selbstständig verfasst, andere als die angegebenen Quellen/Hilfsmittel nicht benutzt, und die den benutzten Quellen wörtlich und inhaltlich entnommene Stellen als solche kenntlich gemacht habe.

Graz, am ....

(Unterschrift)

Englische Fassung:

STATUTORY DECLARATION

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

....

Date (Signature)

Index

Acknowledgement

Herein I want to express my gratitude to all those, who have supported me during my studies and while writing my diploma thesis.

First of all I want to thank my supervisor Christian Slugovc for his guidance and motivation. I´m happy, that I could be a part of this working group.

Nadja Noormofidi as well earns my special thanks, as she enabled me to participate in the outstanding program "ErFIndERin - *erfolgreiche frauen in der industrie–entwicklung, research, innovatio*n" where I could acquire various useful skills through workshops, meetings and the opportunity of participating on an international conference.

My sincere gratitude goes to Julia Kienberger, as she always listened to my concerns and responded with helpful explanations and encouraging words – Thanks!!

Furthermore I would like to thank Karl Rametsteiner, KE KELIT Kunststoffwerk GmbH, Martin Koller and his working group, Josefine Hobisch, GPC analysis and everybody who helped me in any way during this work.

Moreover I want to thank my working group – for assistance and advices, joy and fun, and simply for having such a great atmosphere.

Finally I want to express my gratefulness for my family, for supporting me while my studies, for their faith and trust in me. Thanks to all of the special people that I'm allowed to call my friends - particularly to my dear brother Jochen and Noemi my best friend since kinder garden.

Zusammenfassung

Das Interesse an antimikrobiellen Polymeren wächst ständig, da sie ein breites Anwendungsspektrum bieten. Speziell in der Medizintechnik, im Lebensmittelsektor und in Systemen zur Wassereinigung gewinnen diese Polymere an Bedeutung, da sie viele Vorteile gegenüber nieder-molekularen Verbindungen darbieten. Die Rest- Toxizität wird reduziert während Effizienz, Selektivität und Lebensdauer durch die Verwendung dieser Polymere gesteigert werden.

Die allgemeine Zielsetzung dieser Forschungsarbeit war es, durch Einbringen einer Stickstoff Funktionalität in das Polymer, eine biozide Eigenschaft zu erlangen. Dafür wurde zum einem das Monomer zu Beginn funktionalisiert und zum Anderen das Polymer post-funktionalisiert. Die daraus gewonnenen funktionalisierten Polymere, wurden anschließend analysiert und dazu verwendet ein gültiges Protokoll zur Evaluierung des bioziden Charakters der Polymere zu erstellen.

2-Isopropenyl-2-Oxazolin wurde als Monomer einer Funktionalisierung mittels Thiol-en Reaktion unter verschiedenen Bedingungen unterzogen. Die Alternierende Dien Metathese Polykondensation, ALTMET, wurde als weitere Methode eingesetzt, um Polymere mit Doppelbindungen für eine anschließende Thiol-en Funktionalisierung zu synthetisieren. Darüber hinaus wurde eine ROM Polymerisation mit Norbonenen durchgeführt und darauffolgende antimikrobielle Tests mittels LIVE/DEAD® Viability Kit von Invitrogen arrangiert.

Abstract

Interest in antimicrobial polymers is continuously growing as they offer various areas of applications. Especially in medical devices, in the food sector and water purification systems these polymers are steadily gaining in importance as they provide several advantages over low molecular weight antimicrobials. Residual toxicity is reduced, whereas efficiency, selectivity and lifetime can be increased through the use of polymers.

The general aim of this research work was to achieve a biocidal property of a polymer by introducing amino functionalities. Pre- and post-functionalization approaches of a monomer or respectively a polymer have been carried out. The functionalized polymers gained from the synthesis have furthermore been analyzed and used to create a valid protocol for the evaluation of the biocidal character of the polymer.

2-isopropenyl-2-oxazoline was used as monomer for pre-functionalization with various thiols under several conditions. Alternating Diene Metathesis Polycondensation, ALTMET was used as another method to obtain a polymer including double bonds for the subsequent thiol-ene functionalization reaction. Furthermore a ROM polymerization has been carried out with a norbornene, followed by antimicrobial tests using the LIVE/DEAD® viability kit from Invitrogen.

1. Introduction

Microorganisms affect our daily lives, as they can be found in various parts of the biosphere. There are plenty areas of application in medical devices, health care and hygienic applications.[1] Food making processes, such as the production of yogurt, cheese, alcoholic beverages and bakery products are included. Zymomonas mobilis for example plays an important role in bioethanol production which leads to another major field of application – the energy generation. Bioethanol can be derived from renewable agricultural products, and therefore serves as an important alternative energy source.[2] In addition to the already mentioned applications, some bacteria are also used to produce biodegradable polymers, which can be applied in new implant concepts. Further medical applications are antibiotics and insulin. Moreover some bacteria are capable of breaking down solvents, pesticides and various environmental toxins.

Microorganisms have a broad area of application with several positive aspects, but in spite of everything, they can be harmful as well. Pathogen bacteria can cause diseases like tuberculosis or pneumonia, infections like diphtheria or tetanus and food borne illnesses like the recently occurred hemolytic-uremic-syndrome (HUS) which is caused by a pathogenic E. coli strain.[3] Diarrhoeal diseases caused by bacterial infections are still a major health

---

[1] R. Broughton, S.D. Worley, E. Kenawy, *Biomacromolecules*, **2007**, 8, 1359-1384

[2] A.Muthaiyan, A. Limayem, S. C. Ricke, *Progress in Energy and Combustion Science* **2011**, 37, 351-370

[3] D.Werber, K. Stark, G. Krause, M. Wadl, T. Suess, M. Höhle, R. Prager, A. Fruth, A. Gilsdorf, H. Bernard, C. Frank, M.S. Faber, M. Askar, *Euro Surveill.* **2011**, 16 (22)

problem which leads to about 2 million deaths per year.[4] Especially in developing countries the risk of infection is high because of the poor hygienic conditions and insufficiently treated water.

Therefore the interest in developing antibacterial agents grows continuously. A particular concern is dedicated to antimicrobial polymers, which feature some positive effects in comparison to low molecular active ingredients. Advantageous is mainly the reduced residual toxicity and solubility, as well as the increased efficiency and selectivity. Applications for those biocidal polymers include water treatment, medicine and healthcare products, food and textile products. [1]

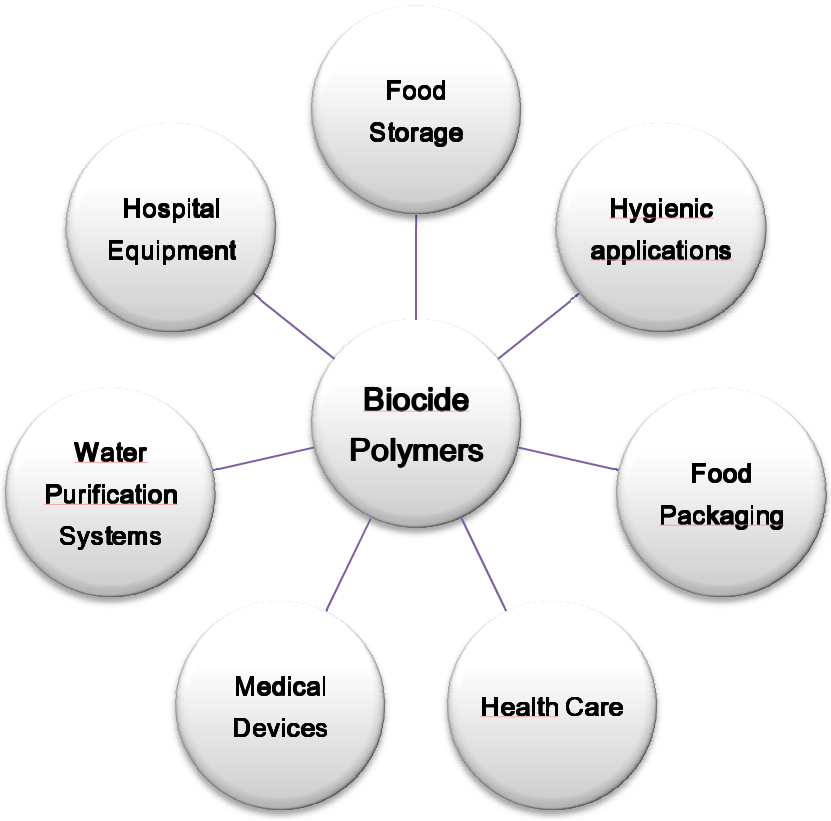


Figure 1: Applications of biocide polymers

[4] http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index.html, 14.06.2011

2. Scope of this Work

The objective of this work was the synthesis and the subsequent characterization of biocide polymers. Therefore a monomer was functionalized with an amino group to achieve an antimicrobial character. After polymerization via cationic ring opening polymerization, ring opening metathesis polymerization and alternating diene metathesis polycondensation the polymers were used to establish an easy way for the evaluation of their capacity to act as a biocide. For this purpose a protocol was tried to be established using a LIVE/DEAD Kit from Invitrogen.

Consequently this thesis is composed of two main parts: synthesis and analysis of the biocidal polymers.

3. General Background

3.1 Bacteria

3.1.1 Structure and Occurrence of Bacteria

Bacteria are omnipresent unicellular prokaryote microorganisms of some micrometers size and various shapes such as rods and spheres. Prokaryotes do not possess a cell nucleus and therefore all the organelles are loose in the cytoplasm. Bacteria cells consist of the cell wall, cytoplasm, plasma membrane, ribosomes, plasmids and a circular DNA as pictured below.[5]

[5] M. T. Madigan, J. M. Martinok, J. Parker, *Brock Mikrobiologie, Spektrum Akademischer Verlag Heidelberg-Berlin, 1,* **2001**

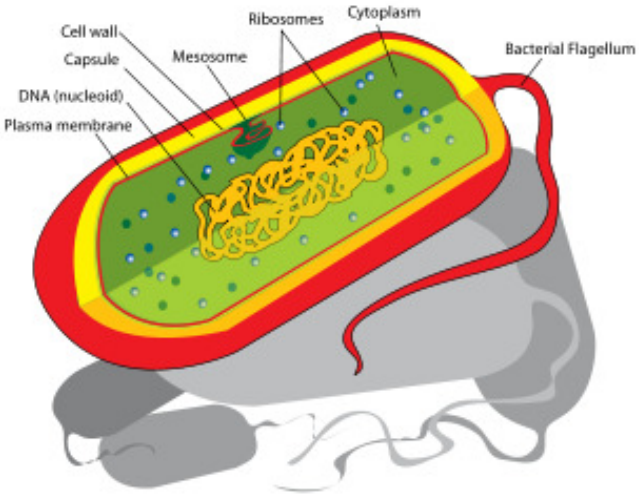


Figure 2: Structure of a bacterial cell[6]

3.1.2 Cell Wall

The cell wall protects the cell from environmental influences and is responsible for the shape of the cell.[7] Bacteria can be classified in two large groups- Gram positive and Gram negative strains. Gram staining is a method for differentiating bacteria due to chemical and physical properties of the cell wall developed by Hans Christian Gram.[5,8] In dependence of the presence of peptidoglucan in the cell wall, gram positive and gram negative bacteria is colored differently.

---

[6] http://www.mysciencebox.org/node/481- 21.07.2011

[7] A. v. d. Wal, W. Norde, B. Bendinger, A. J.B. Zehnder, J. Lyklema, *Journal of Microbiological Methods*, **1997**, 28, 147-157.

[8] R. Scherrer, *TIBS*, **1984**, 9, 242-245.

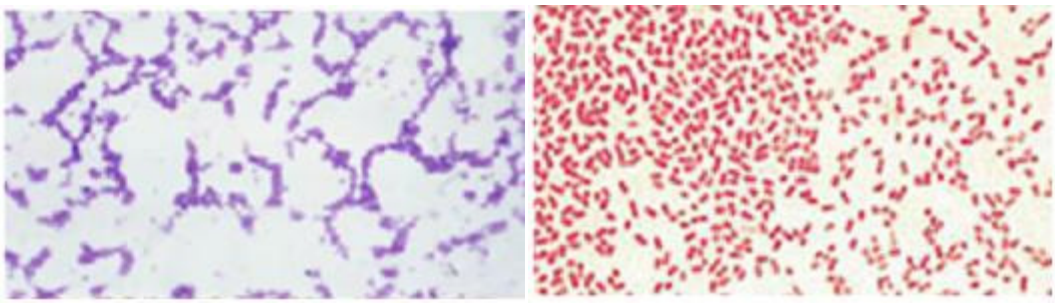


Figure 3: Gram positive (left) and Gram negative cells.[9]

The cell wall of Gram positive bacteria (e.g. *Staphylococcus aureus, Streptococcus mutans*) is thick and consists mainly of peptidoglucan which is covalently linked to teichoic acid. The teichoic acid is among other functions responsible for the supply of the cell with cations, the import and export function and plays a role for rigidity and in the growth regulation of the cell.

In contrast, Gram negative bacteria (e.g. *Escherichia coli, Pseudomonas aeruginosa)* have a thinner intermediate peptidoglucan layer and an additional outer membrane that contains lipopolysaccharides. The membrane accomplishes the selective permeability for small molecules as well as the exclusion of big molecules. It also plays a role in protein export and as a receptor.[10,11]

---

[9] http://www.microbiologylabs.info/introduction-to-microbiology/gram-stain-procedure - 22.7.2011

[10] http://www.hull.ac.uk/php/chsanb/LMWeb/BacterialMembranes.pdf - 22.7.2011

[11] PhD thesis: G. Seyfriedsberger, Kontaktbiozide auf Polymerbasis: Herstellung und Charakterisierung, *TU Graz*, **2007**

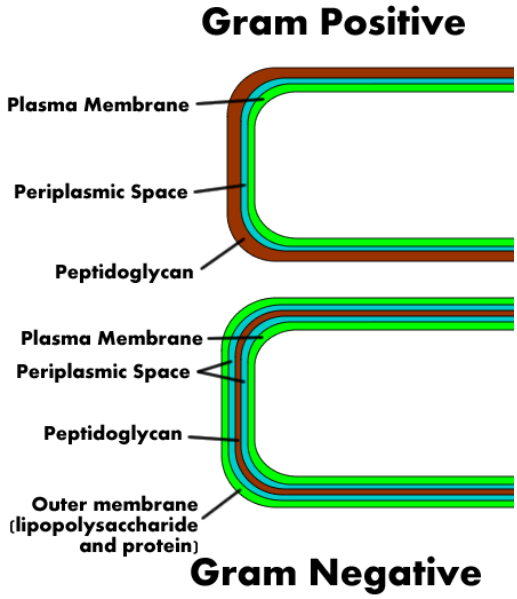


Figure 4: Comparison of the structure of Gram positive and Gram negative bacterial cells.[12]

3.2 Antimicrobial substances

Antimicrobial substances or respectively microbiocides are active agents that kill micro-organisms such as bacteria, yeasts, fungi and algae. Pasteur was a pioneer in the area of antimicrobial treatment when he discovered that microorganisms were killed through a heating process, called pasteurization.[13] He further demonstrated in the germ theory, that microorganisms do not appear spontaneous, but that they reproduce their selves.[14]

There are different types of antimicrobial agents: Chemical and/or physical. Dry and moist heat or ionization radiation rank among the physical sterilization ways, whereas alcohols,

[12] http://en.wikipedia.org/wiki/File:Gram_Pos_and_Neg_Cell_Wall.png – 22.7.2011

[13] W. Paulus, *Microbiocides for the protection of material*, Champman & Hall, **1993**

[14] http://www.creatingtechnology.org/biomed/germs.htm - 25.07.2011

bases and acids, aldehydes such as phenols are some representatives of chemical microbicides.[15]

3.2.1 The Challenge of Antibacterial Systems

Sophisticated strategies are necessary in the fight against microorganisms and their diverse survivability. Microorganisms have high reproduction rates and undergo many evolutionary processes. Therefore they are easily adaptive to their environment and to enemies. The cell wall is capable of compensating pressures up to 2 bar, and protects the cell from heat, cold, dryness and radiation. When forming aggregates – known as biofilms, their resistance is even more favored. So antibacterial systems hold a challenge in combating them.[16]

3.2.2 Mode of Action of Biocides

Generally biocidal substances can be distinguished concerning to their way of interacting with the microorganisms. Chemically reactive agents such as aldehydes bind covalently to proteins or peptidoglycan of the cell wall where electrons are stripped. These substances have electrophilic groups which interact with the nucleophilic groups, such as amines, thiols or amides, of the cell. Thereby important metabolic processes are interrupted and afterwards the biocide itself is deactivated as well. In contrast membrane-active agents, e.g. quaternary ammonium compounds, are not deactivated while physically interrupting the cell membrane causing intracellular compounds to emit. Therefore membrane active substances have the

---

[15] S.S. Block, *Disinfection, Sterilization and Preservation*, Lippincott Williams and Wilkins, Philiadelphia, **2001**

[16] PhD thesis: *Synthese, Charakterisierung und Untersuchung der Wirkungsparameter bakerizider Poly(oxazoline) und damit modifizierter Polymernetzwerke,* C. Waschinski, Albert-Ludwigs-Universität zu Freiburg, **2007**

advantage of a long lasting life. The figure below shows various sites of interaction, also multiple effects can be observed.[17,18]

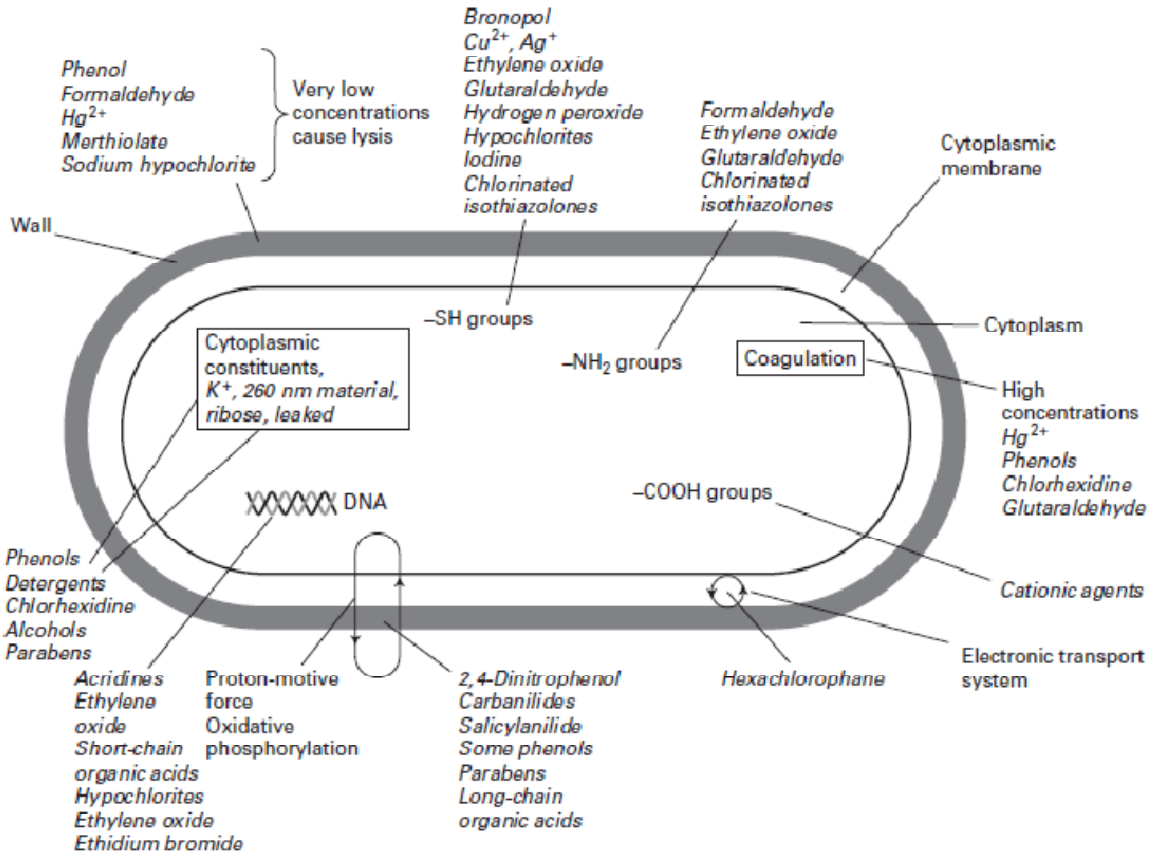


Figure 5: Various sites of action of biocides.[17]

The following figure[18] shows a model of the possible mode of action when a biocide polymer and a microorganism interact at its cytoplasm membrane. Because of the negative charge of the bacterial membrane, the positive charged groups of antimicrobial polymers are attracted. The biocides replace the ions on the surface of the membrane and bind to the negatively

[17] Russell, Hugo & Ayliffe, *Principles and Practice of Disinfection, Sterilization and Preservation*, Blackwell Publishing, **2004**

[18] PhD Thesis: E. Kreutzwiesner, Entwicklung von compoundierbaren Kontaktbioziden auf Basis aminfunktionalisierter Polymere, *TU Graz*, **2010**

charged phospholipids. A phase separation begins within the membrane. The permeability of the membrane changes and leakage of low-molecular weight compounds follows.[11]

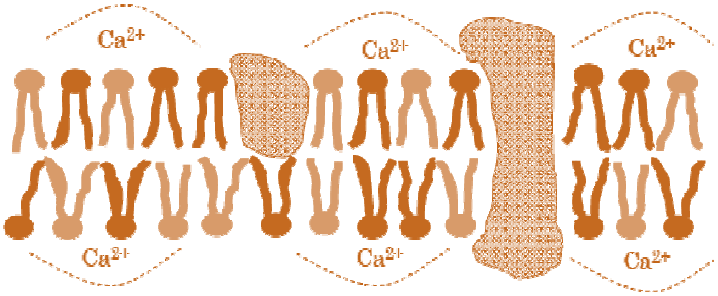


Figure 6: Cytoplasm membrane of bacteria with integral proteins and stabilized through calcium ions and acidic and neutral phospholipids.

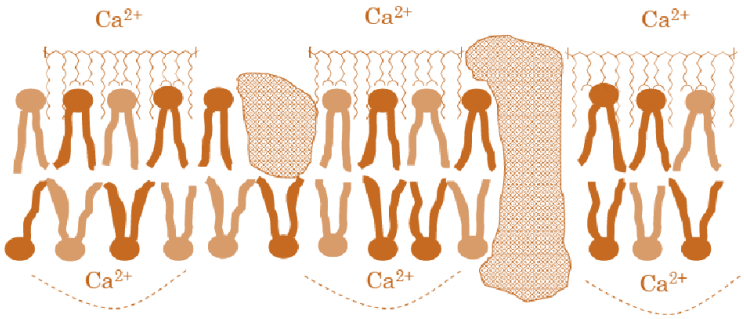


Figure 7: Binding of a biocide polymer and replacement of calcium ions.

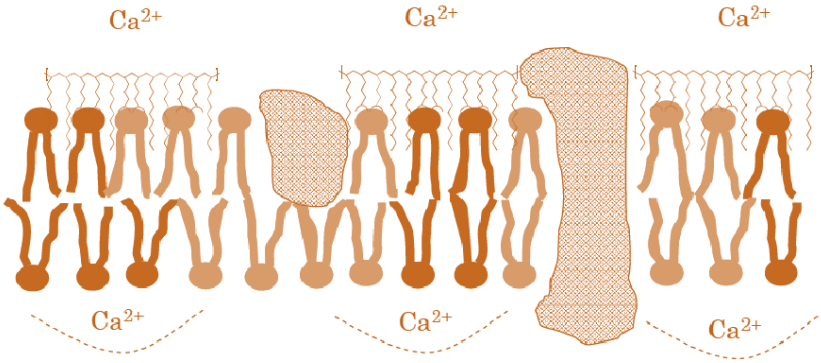


Figure 8: Separation of charged and neutral lipids.

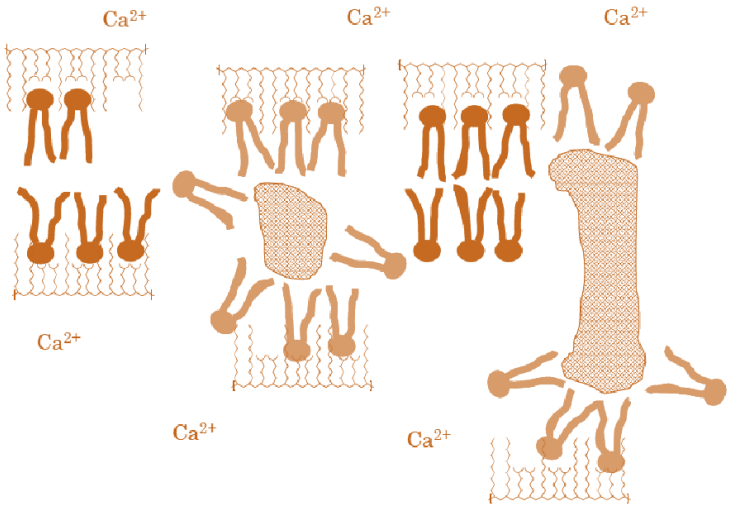


Figure 9: Decomposition of cytoplasm membrane.

3.2.3 Influences on Biocidal Activity

Not all microorganisms are equally sensitive to biocides. Factors influencing the antimicrobial property are listed below:

- concentration of the active agent
- time of incubation
- temperature
- the environment at which it is acting (pH)

3.2.4 Overview of Various Antimicrobial Substance Classes

Table 1: Substance classes of biocidal agents with one representative each.

Substance Class	Representatives
Phenols	• p-Chloro-m-phenol
Organic and inorganic acids	• Propionic acid
Biguanides	• Chlorhexidine
Surface-active agents	• Benzethonium chloride
Aldehydes	• Formaldehyde
Antimicrobial dyes	• Quinones
Halogenes	• Chloroform
Alcohols	• Isopropanol
Heavy metal derivates	• Tin

3.3 Biocidal Polymers

Considering the spread of bacterial infections, antimicrobial substances are steadily becoming more important. Especially biocidal polymers raise interest because of their advantages in comparison to low molecular weight compounds with antimicrobial properties

such as antibiotics. But increasing resistance of microorganisms to antibiotics represents a serious problem.[19] Via gene transfer and subsequent natural selection of surviving organisms with antibiotic resistant genes various resistant traits constantly develop. (E.g. Methicillin – Resistant *Staphylococcus Aureus* - MRSA) MRSA is one of the most common hospital acquired infection which is difficult to treat because of its multi-drug resistance. Not only staphylococcal infections but also other diseases including HIV infections, tuberculosis, influenza, gonorrhea, candida infections, and malaria are therefore so hard to treat. According to the "National Institute of Allergy and Infectious Diseases" the U.S. healthcare costs increase every year of about $5 billion because of hospital patients developing an infection. Approximately 90 000 of them die as a result of it.[20]

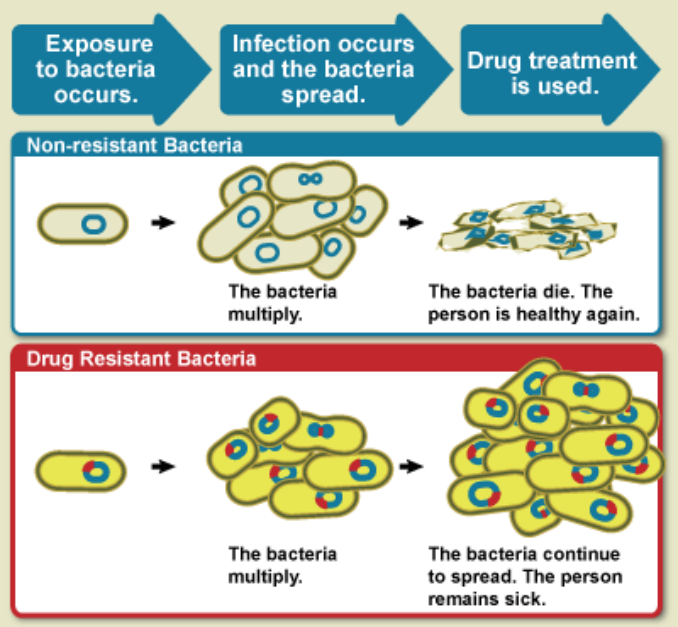


Figure 10: Drug resistant bacteria in comparison to non-resistant bacteria.[20]

[19] U.S. Schubert, N. Adams, *Poly(2-oxazolines) in Biological and Biomedical Application Contexts*, Eindhoven University of Technology, **2007**.

[20] http://www.niaid.nih.gov/Pages/default.aspx - 26.7.2011

Macromolecular systems offer some advantages in comparison to low-molecular weight biocides such as enhanced efficiency, reduced residual toxicity, increased selectivity and prolonged lifetime. Furthermore they are not volatile, do not permeate through skin and are chemically stable. There are several areas of application in health care and medical devices, drugs and hygienic applications, as well as in water purification systems, textiles and in the food packaging/storage sector.

Kenawy et al. describe the basic requirements for polymeric biocides:

- Easy and inexpensive synthesis
- Stability for long term usage
- Same storage and application temperature
- No emission or decomposition of toxic products
- Broad spectrum of microorganisms affected by its biocidal activity
- Regeneration upon loss of activity

For optimization of antimicrobial polymers factors like the molecular weight, hydrophobic-hydrophilic balance, steric effects and the nature of counter ions play an important role.[1]

3.4 Polymerization Methods

3.4.1 Ring Opening Metathesis Polymerization

There are many various examples for olefin metathesis such as the ring-opening metathesis polymerization, ROMP, cross metathesis, CM, acyclic diene metathesis, ADMET and the ring-closing metathesis, RCM. Already 50 years ago researches concerning metathesis

reactions have started. Ever since interest is growing, as new highly active and stable initiators are constantly discovered.[21,22,23,24]

ROMP is a very versatile tool for the synthesis of novel polymers. The high ring strain is the driving force for this reaction. The reaction starts with the initiation step, which is shown in the scheme below. The initiator, usually a transition metal alkylidene complex, reacts with the monomer and results in an active species which in turn propagates and adds new monomers. Many groups have contributed in initiator design, which plays an important role for the popularity of ROMP. R.H. Grubbs, H.H. Schrock and Y. Chauvin were honored with the Nobel Prize for chemistry for their very significant findings in this area. [25]

IUPAC defines “living polymerization” as: ‘chain polymerizations from which chain - transfer and chain - termination reactions are absent ’. ROMP can be carried out in a living manner. There are some aspects which describe a living polymerization: A fast propagation and an even faster initiation are special characteristics which reduce chain transfer or back-biting side reactions. Furthermore a very narrow molecular weight distribution is given through living polymerization.[26]

---

[21] A. Leitgeb, J. Wappel, C. Slugovc, *Polymer*, **2010**, 51, 2927

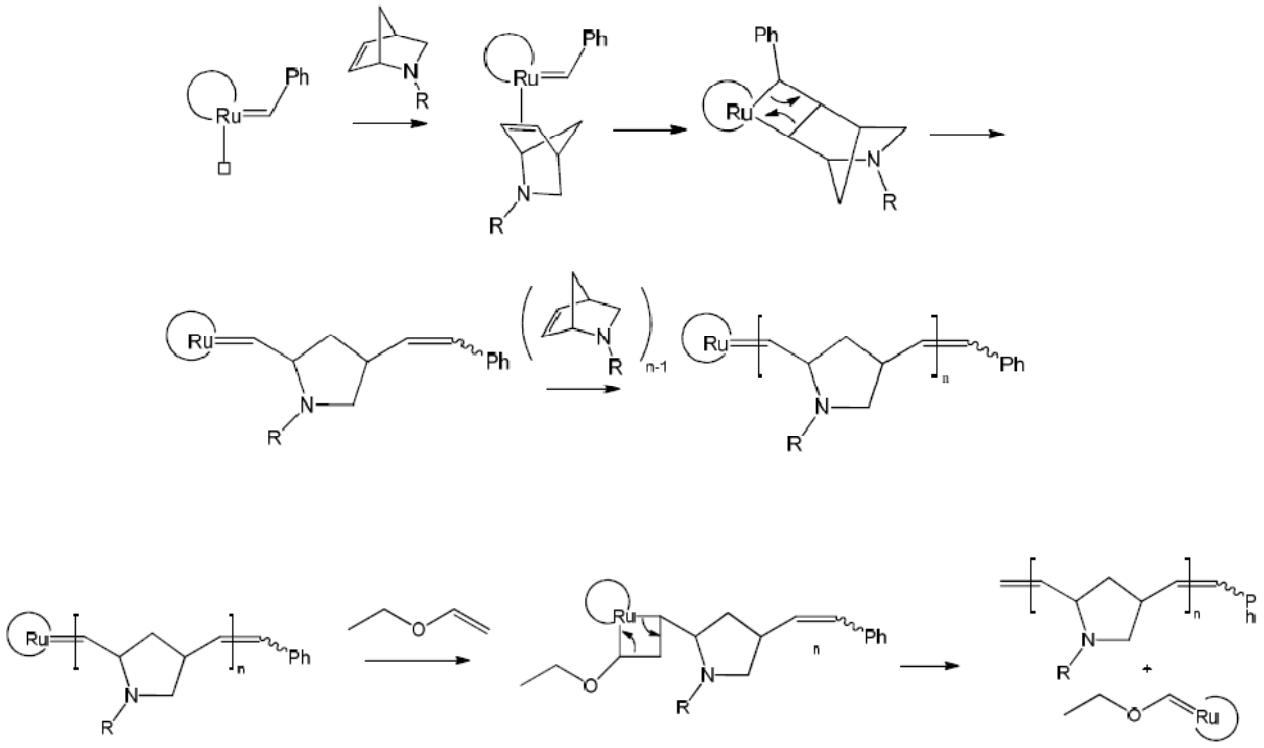
[22] http://nobelprize.org/nobel_prizes/chemistry/laureates/2005/index.html - 27.7.2011

[23] C.W. Bielawski, R.H. Grubbs, *Prog. Polym. Sci.* **2007**, 32, 1

[24] R.H. Grubbs, „Handbook of Metathesis”, WILEY-VCH Verlag GmbH & Co., **2003**

[25] J.M. Raquez, O. Coulembier, P. Dubois, *Handbook of Ring-opening Polymerization*, WILEY-VCH Weinheim, **2009**.

[26] Master thesis: C. Gstrein, *Synthesis and Characterization of Poly(azanorbornene)s via Ring-Opening Metathesis Polymerization using Ruthenium-based Initiators,* TU Graz, **2011**.



Scheme 1: Mechanism of the initiation and termination of a ROM Polymerization.[26]

3.4.2 Cationic Ring Opening Polymerization

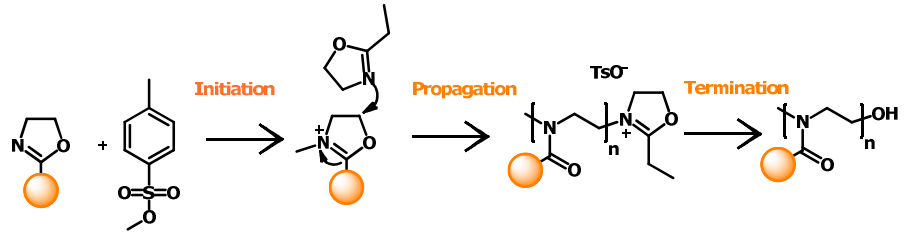
Investigations of the living cationic ring-opening polymerization of 2-oxazolines already began in 1966, when 4 research groups independently worked on this topic.[27,28] There are various applications such as micellar catalysis, drug delivery or hydro gels which retain interest on further attempts.[29] As demonstrated in the figure below, the nitrogen initiates the electrophilic attack whereby an oxazolinium cation is formed and subsequently the

[27] F. Wiesbrock, V. Schenk, L. Ellmaier, T. Bodner, *Polym. Int,* **2011**.

[28] D.A. Tomalia, D.P. Sheetz, *J. Polym. Sci.* **1966**, *4*, 2253

[29] U.S. Schubert, C.H. Abeln, R. Hoogenboom, F. Wiesbrock, *Macrom. Rapid Comm.*, **2004**, 1895-1899.

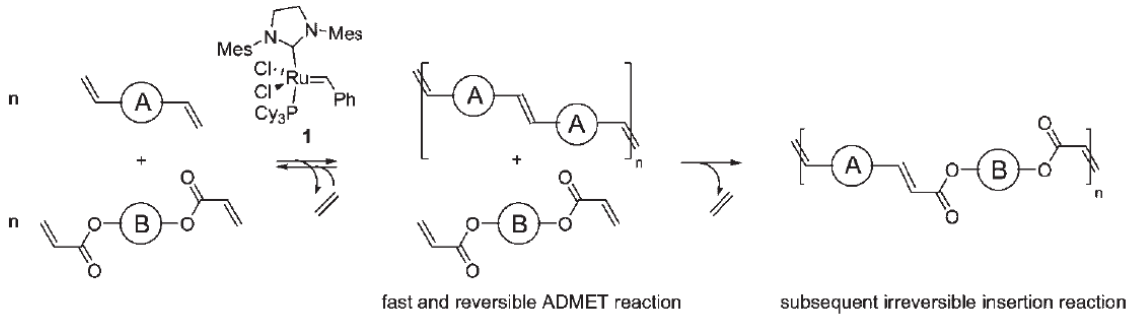
nucleophilic attack of the 2-oxazoline monomer takes place. For initiation various catalysts have been used, such as methyl and benzyl tosylates or methyl triflates.[27,25]



Scheme 2: Mechanism of the cationic ring opening polymerization of 2-oxazolines.[27]

3.4.3 Alternating Diene Polycondensation

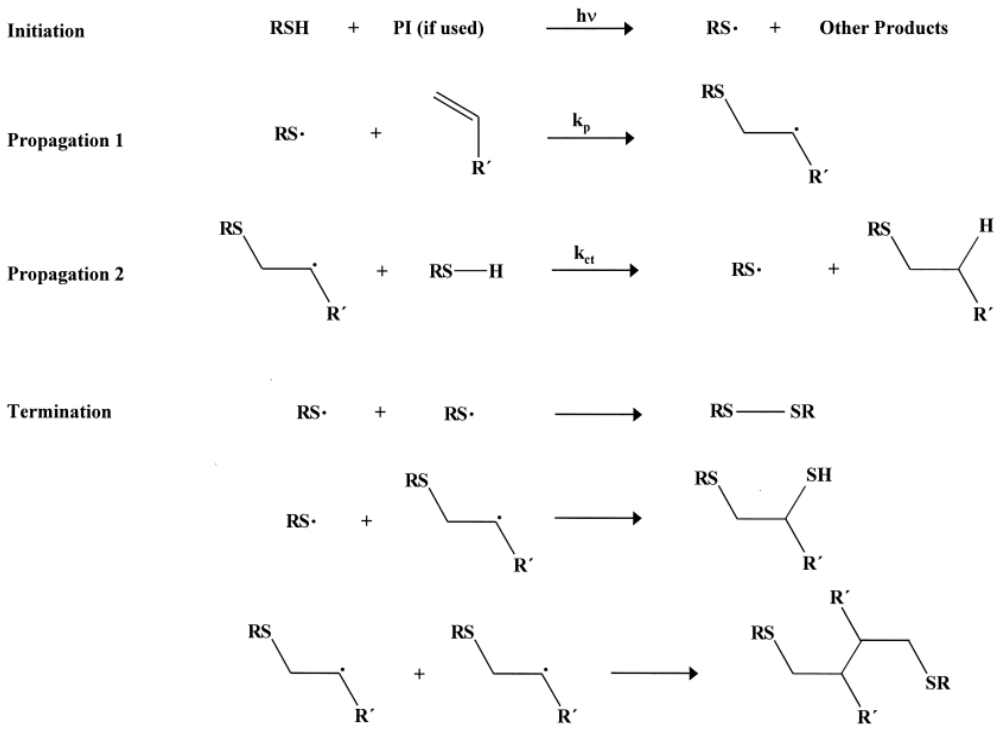
For preparing perfectly alternating AB copolymers, the Alternating Diene Polycondensation, ALTMET is a wide-ranging tool. The method is based on Acyclic Diene Metathesis Polymerization, which is generally similar to ROMP but differs in the step-like propagation. The condensation reaction yields less active and less defined polymers, but opens a broader range of architectures and compositions. To obtain mild conditions and simplicity, ALTMET was developed. As shown on the scheme below a diacrylate and a diene react with each other and result in an alternating AB copolymer.[41]



Scheme 3: Insertion polymerization of a diene and a diacrylate.[41]

3.5 Functionalization

Thiol-ene reaction shows a very important tool for pre- or post functionalization of polymers. It has already been discovered in 1905 by Posner. Thiol-ene reactions follow either a free radical or ionic mechanism whereby a thiol reacts with a double or triple bond. Radicals are generated during the initiation steps. The Propagation step combines the initial stochiometric mixture of the double bond – species and the thiol. The mechanism of the termination is thought to be a reaction of two radicals as seen in the scheme below. Anyway the reaction of a radical with the initiating species might as well be a possible termination mechanism.[30]



Scheme 4: Initiation, Propagation and Termination steps of a thiol and a double bond.[31]

[30] C.N. Bowman, N. B. Cramer, *Journal of Polymer Science; Part A: Polymer Chemistry*, **2001**, *39*, 3311-3319.

[31] C. E. Hoyle, T. Y Lee, T. Roper, *J. Polym. Sci. Part A: Polym. Chem.* **2004**, *42*, 5301-5338.

4. Results and Discussion

The aim of this work was to generate amino functionalized polymers with biocidal activity and to subsequently determine their antimicrobial potential. The functionalization was carried out via thiol-ene reaction and the polymerization mechanisms included Cationic Ring Opening Polymerization, Ring Opening Metathesis Polymerization and Alternating Diene Metathesis Polycondensation.

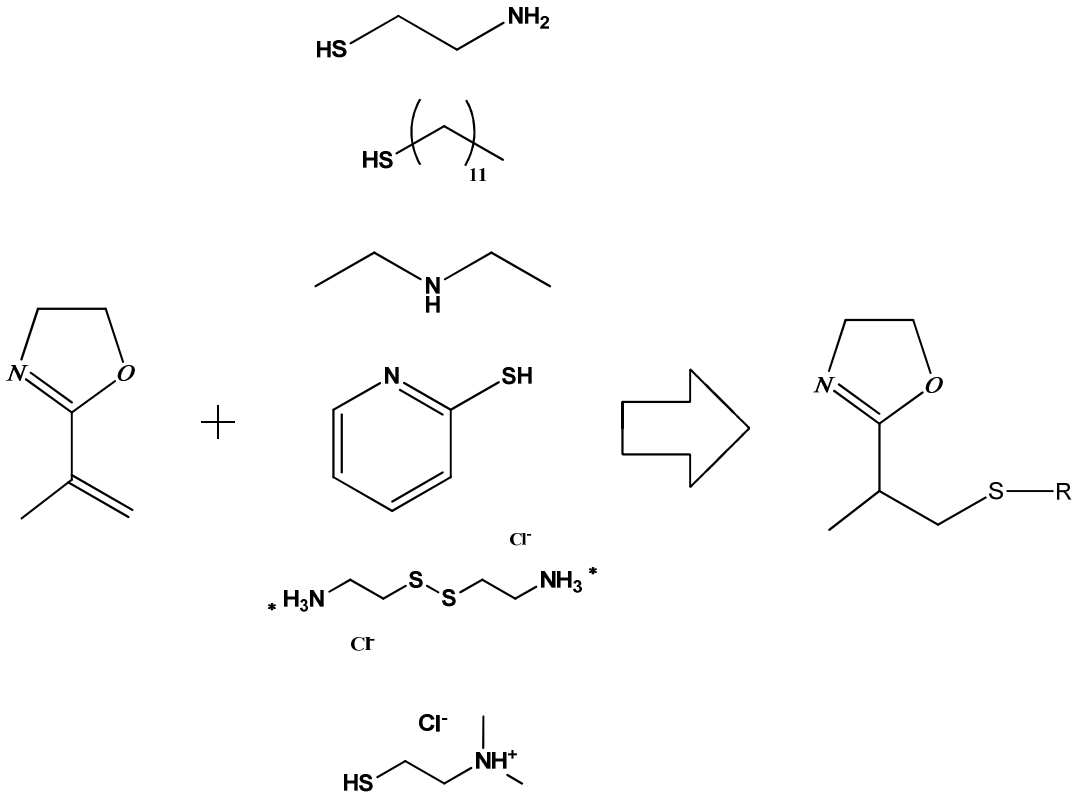
Herein the synthesis of the polymers and some unexpected difficulties are discussed. For characterization of the polymers NMR spectroscopy was used as the main method.

The second part of this work deals with the development of an adequate method for determination of the biocidal activity. The general objective was to create an alternative to the micro-biocidal activity testing via JIS standard tests Z2801:2000 using LIVE/DEAD Kit instead which was purchased from Invitrogen. This was expected to lead to cheaper and quicker test results which were thought to be done in the working group.

4.1 Functionalization of 2-Isopropenyl-2-Oxazoline

4.1.1 Introduction

Like described above, several different thiols were tested in regard to their ability of binding via thiol-ene reaction to the 2-isopropenyl-2-oxazoline.



Scheme 1: Various reactants for the functionalization of the 2-isopropenyl-2-oxazoline with cysteamine, dodecanethiol, diethyl amine, mercaptopyridin, MEDA and cystamine.

Basically the reactions were performed under argon atmosphere in tetrahydrofuran at 65 °C. The thiol was provided in the Schlenk tube and when it was completely dissolved the 2-isopropenyl-2-oxazoline was added. With the addition of a catalytic amount of *azo-*

bis(isobutyronitril) AIBN the reaction was initiated. This procedure was carried out in style of known protocols.[32,33,34]

In case of cysteamine a mixture of THF and methanol (2:1) had to be used as solvent due to bad solubility of the reactant in non-polar solvents. As the methanol was assumed to hinder the reaction progress, also a solid phase reaction was carried out, where the undissolved cysteamine was processed. In the beginning reactions with dodecanethiol were executed, as we wanted to check if it was indeed the thiol reacting with the double bond of the 2-isopropenyl-2-oxazoline. NMR characterization showed that the thiol reacted with the 2-isopropenyl-2-oxazoline. Still this does not necessarily mean that if cysteamine is the educt, the thiol reacts as well. A possible method to find it out would be the analysis via bicinchoninic acid assay, BCA in which primary amines chelate with a Cu^{+1} ion and build a violet complex that absorbs at 562 nm.[35] Anyway it could be proved that the 2-isopropenyl-2-oxazoline ring did not open and that the educt has bound in all cases. Our aim was to introduce a terminal amino group, but actually it is not an absolute must for having a biocidal activity. Therefore no further approaches concerning the position of the amine were realized.

4.1.2 Characterization

The following spectrum shows the result of one of the functionalization reactions executed during this work- **JB 16**. The green line shows the cysteamine, which was recorded in MeOH-d_4, in contrast to the 2-isopropenyl-2-oxazoline (red) and the product (yellow) spectrum,

---

[32] M. A. Cortez, S. M. Grayson, *Macromolecules* **2010**, *43*, 4081-4090.

[33] A. Gress, A. Völkel, H. Schlaad, *Macromolecules* **2007**, *40*, 7928-7933.

[34] A. B. Lowe, M. A. Harvison, *Aust. J. Chem.* **2010**, *63*, 1251–1266.

[35] http://www.piercenet.com/files/1296as8.pdf - 11.7.2011

which were recorded in $CDCl_3$ as usual. It is clearly observable, that the reaction worked to some extent, the double bonds reacted completely and the product peaks arose as expected. Nevertheless the peaks are not in perfect shapes and ratios to each other, what shows that the product was not obtained pure. Various attempts were carried out to achieve pure functionalized 2-isopropenyl-2-oxazoline without byproducts. But because of the similar solubilities of educt and product precipitation did not work, because either none or both of them dissolved in the solvent. Also column chromatography did not adduce an improvement. It seemed that the 2-isopropenyl-2-oxazoline ring opened because the silica column was too acidic. We considered the possibility to simply use another column material such as aluminium oxide since it is available with pH= 9.5. Distillation would have been a further possible option for purification. However this additional purification step made the whole procedure even more time-consuming and complex. For this reason it was not really profitable for us to continue to prospect these further opportunities. Instead we tried to achieve a pure product by varying the reaction conditions.

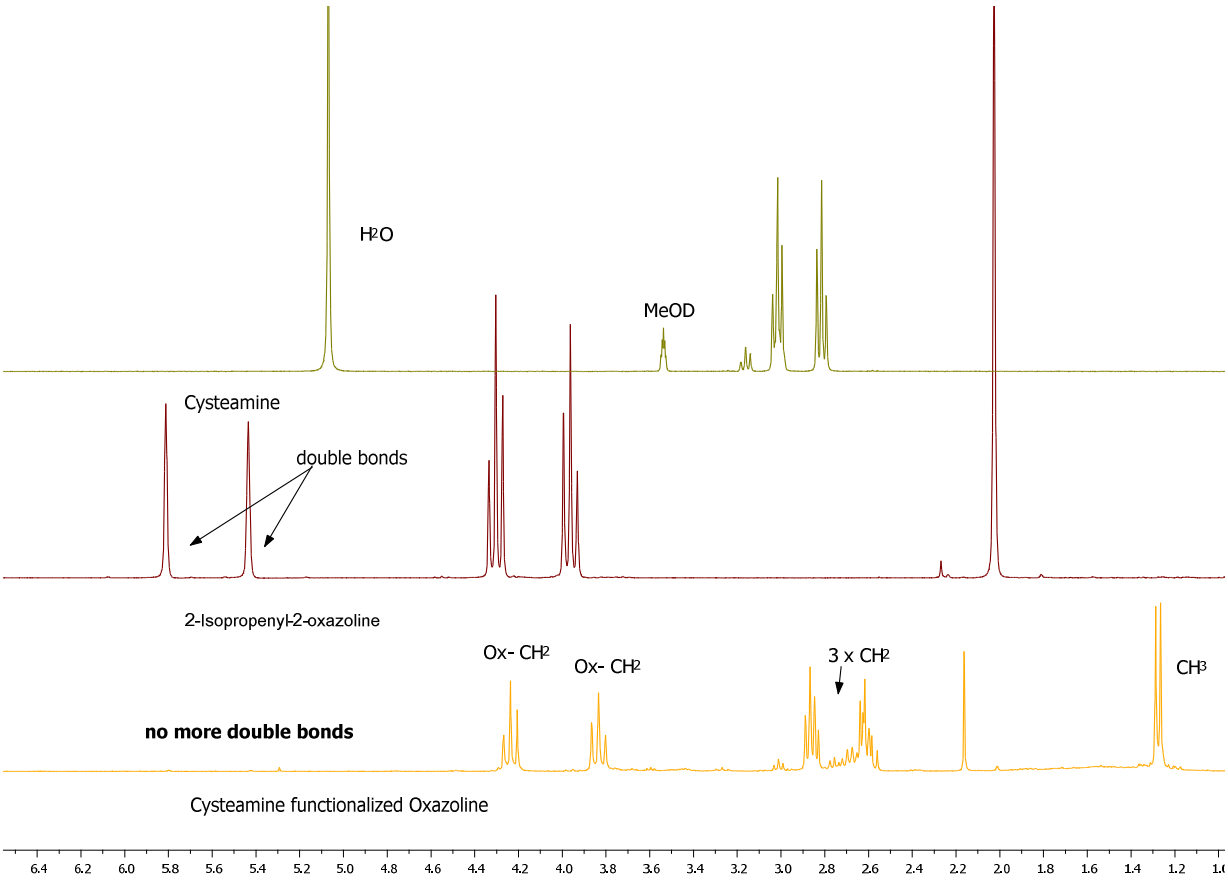


Figure 11: Spectrum of the thiol-ene functionalized cysteamine and its educts.

4.1.3 Methylation of Cystamine Dihydrochloride and Reduction of the Disulfide Bond.

Due to imperfect purity, it was thought to perform the reaction with the dye dansyl chloride, to arrange a better way of tracking the process of the reaction. According to Rose et al.[36] dansyl chloride and cystamine dihydrochloride reacted for 2 hours at room temperature. Afterwards the disulfide bond of the cystamine was reduced to cysteamine using zinc in acetic acid and ethanol. After purification the product was characterized via NMR spectroscopy, but already at that point the product was not pure due to the fact that the conversion was not completed.

[36] S.D. Rose, R.F. Hartman, C. Robinson, *Bioorg. Chem.*, **2008**, *36*, 265-270.

So a further try was executed, but instead of using dansyl chloride, the dimer cystamine dihydrochloride was methylated, in compliance with reports of Wood et al.[37] and other protocols[38]. The objective behind this step was to get a pure methylated cysteamine in the end that could be used for further thiol-ene reaction. An Eschweiler-Clarke methylation was performed in the microwave with formic acid and formaldehyde in DMSO.[37] The advantage of the use of the microwave was focused, as the reaction time is drastically reduced in such. Nevertheless the more time consuming conventional way in a Schlenk tube resulted in better product yields, so it was chosen as method of choice. The methylated cystamine was subsequently reduced to cysteamine as described above.[38] Zinc had to be added in a big excess and the reaction time was up to 24 hours. The organic phase was washed with water, NaOH and brine. Afterwards it was dried with sodium sulfate. But ^{1}H-NMR spectrum revealed a proper conversion.

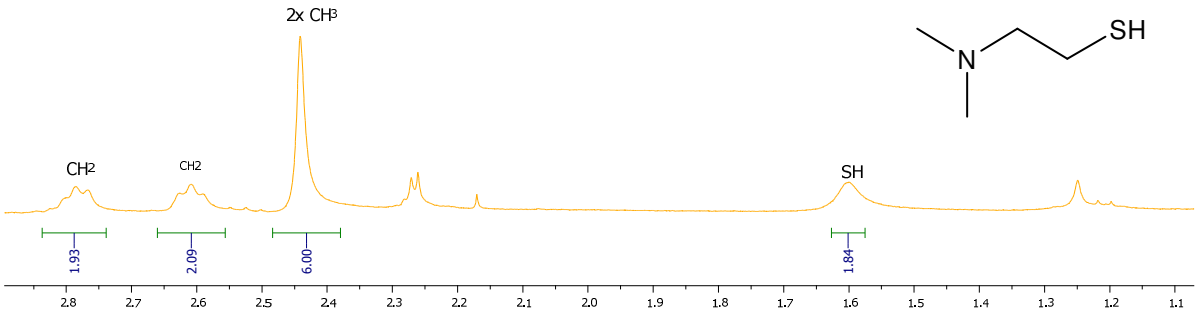


Figure 12: JB 24.a - Methylated cysteamine after disulfide reduction from cystamine.

[37] R. Wood, S. Lu, J.R. Jones, J.R. Harding, *Tetrahedron Letters*, **2002**, *43*, 9487-9488.

[38] L.W. Bieber, I.H.S. Estevam, R.A. da Silva, *Science Direct*, **2007**, *48*, 7680-7682.

This product was further processed in a thiol-ene reaction under the same conditions as already described, but still no progress concerning the purity in comparison to other trials could be achieved.

4.1.4 Acylation of Cysteamine

Another approach to get a pure monomer, which could be used for the subsequent polymerization without purification deals with the protection of the nitrogen of the cysteamine prior to the thiol-ene reaction.

Based on various protocols[39,40] the acylation of cysteamine was realized. The protection mechanism follows the Polonovski rearrangement and the reaction can be performed under eco friendly conditions, without any solvent.

Cysteamine and acetic anhydride were weighted out in equimolar ratios. The reaction was persecuted via TLC. After 30 minutes still some unreacted educt was detected via TLC, so the reaction was allowed to stir over night. Sodium hydrogen sulfate and dichloromethane were thereafter added to bind the rest of the acetic anhydride. It was purified via phase separation. The organic phase was evaporated and the acetylated cysteamine was characterized via ^{1}H-NMR and further processed for the thiol-ene reaction with 2-isopropenyl-2-oxazoline.

---

[39] B.K. Patel, V.R. Kavala, G. Bhattacharjya, S. Naik, *ARKIVOC*, **2004**, 55-63.

[40] F.K. Behbahani, M.M. Heravi, M.S. Abaee, M.M. Mojtahedi, *Chem. Monthly*, **2007**, 138, 95-99.

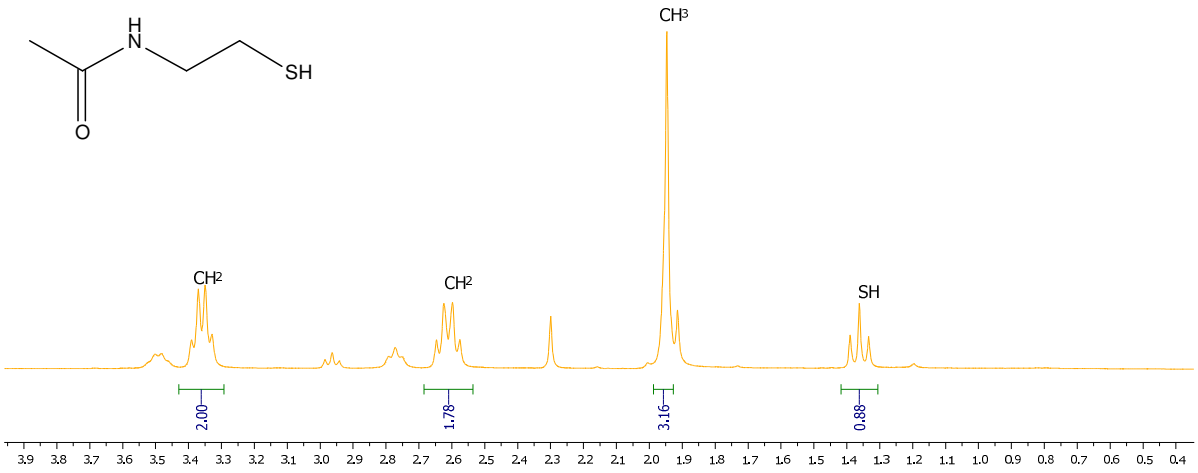


Figure 13: ^{1}H-NMR spectrum of the acetyl-protected cysteamine.

The left peaks next to the both CH_2 peaks correspond to remaining educt. It could not be easily removed. However the functionalization was carried out at that stage. The reaction resulted in a yield 86% by weight.

4.1.5 Functionalization with Acetylated Cysteamine

2-Isopropenyl-2-oxazoline was used in a 1.2 times excess to the acetylated cysteamine dissolved in THF and initiated with 1 mol% of AIBN. The reaction was stirred over night and for characterization an ^{1}H-NMR was made. As the product was hardly soluble in $CDCl_3$, D_2O and $(CD_3)_2CO$, the NMR was recorded in DMSO-d_6. It behaves strongly hygroscopic, and therefore a large peak derived from H_2O is visible at 3.35 ppm.

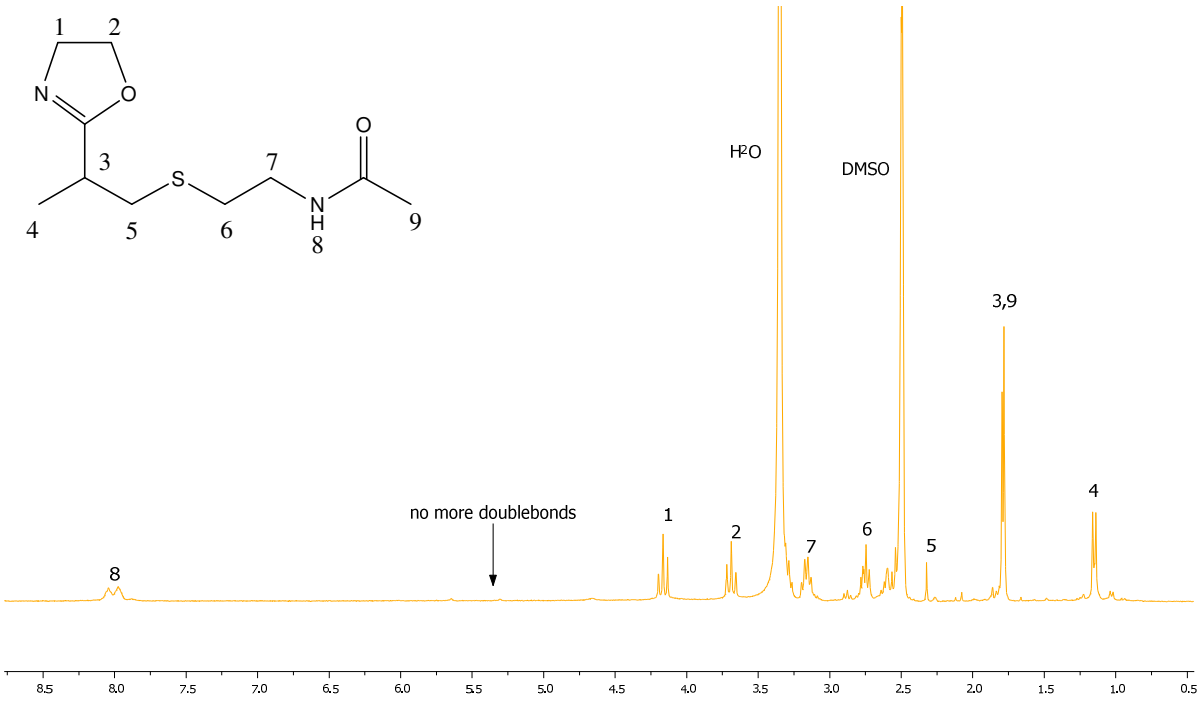


Figure 14: Spectrum of the functionalization of 2-isopropenyl-2-oxazoline with the protected cysteamine

Through the application of the acylation reaction, the possibility that the nitrogen could have reacted instead of the thiol could be excluded. The NMR shows that the double bond peaks of the 2-isopropenyl-2-oxazoline, around 5 ppm, disappeared after the reaction. So the reaction worked, but still some impurities maintained. As it was not meaningful to continue with the polymerization reaction of an impure monomer and as far as all tries of purification were not successful, this experiment was not further pursued. Instead another approach was started with the aim to achieve a water-soluble polymer via ROMP with biocidal character that can be produced in an easy profitable way, which could be realized in large scales as well.

4.1.6 ROM Polymerization of (exo,endo-Bicyclo[2.2.1]hept-5-ene-2-carbonicacid(2-dimethylamino-ethyl)ester

The monomer (*exo,endo*-Bicyclo[2.2.1]hept-5-ene-2-carbonicacid(2-dimethylamino-ethyl)ester was provided in a Schlenk tube and diluted in CH_2Cl_2:MeOH (1+1), the Grubbs initiator M31, was thereafter added at once. The reaction stirred for two hours and was stopped by adding 1 mL ethyl vinyl ether and stirring for further 15 minutes.

For purification the product was precipitated in cold *n*-pentane. The success of the polymerization was controlled by ^{1}H-NMR spectroscopy and GPC analysis.

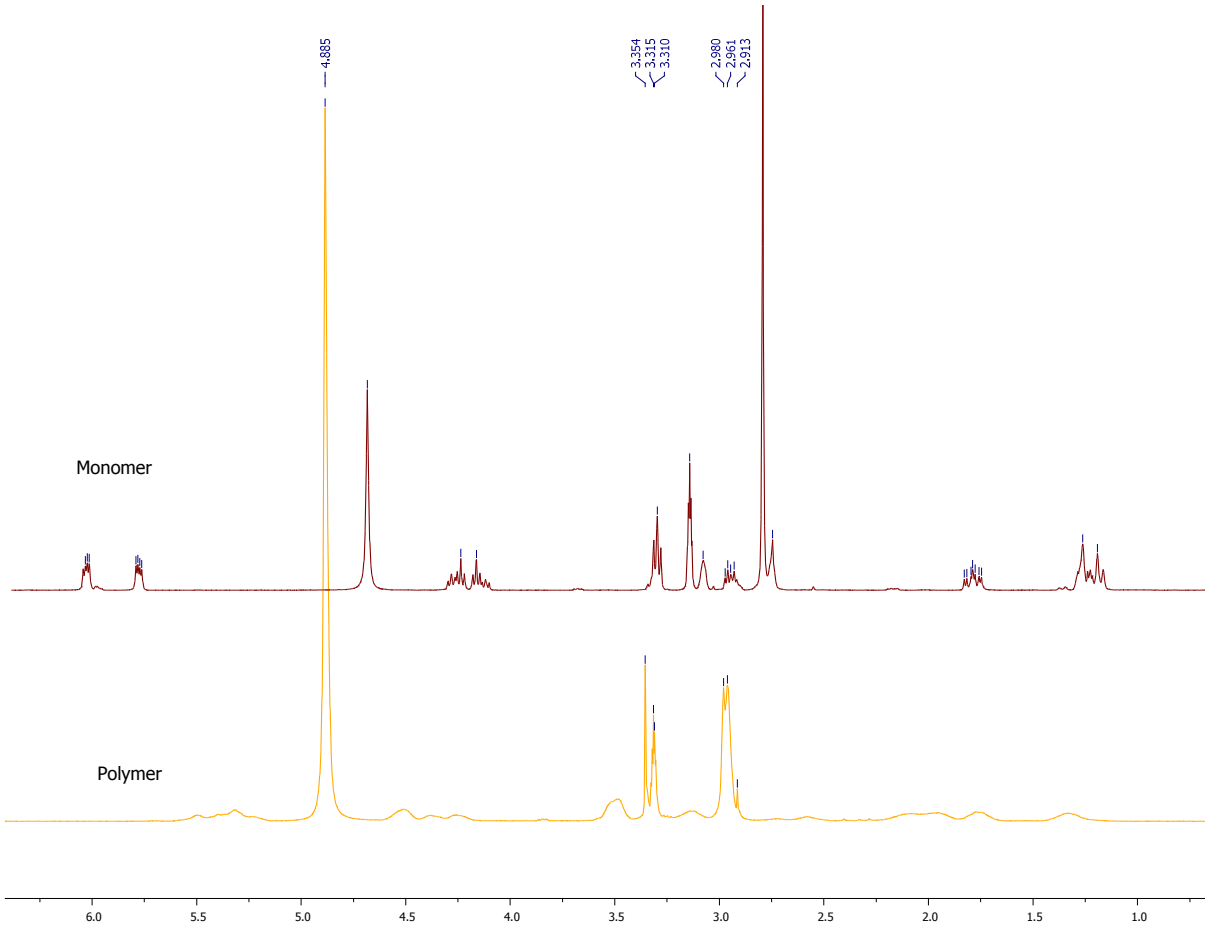


Figure 15: 1H-NMR spectra of (*exo,endo* Bicyclo[2.2.1]hept-5-ene-2-carbonicacid(2-dimethylamino-ethyl)ester and the corresponding polymer JB 30

Concerning the NMR spectra it can be assumed, that the polymerization worked well, because the characteristic broad polymer peaks are clearly recognizable. The GPC analysis showed an acceptable value for the polydispersity of PDI= 1.3. (M_n= 37000 g/mol) against a poly(styrene) standard in $CHCl_3$/Et_3N/isopropanol = 94:4:2. Because of the presumed biocidal activity and the fast synthesis the polymer JB 30 was produced, to perform further viability tests with it. The polymer was thought to have biocidal properties because of the already in the monomer existent quaternary amine. So no additional protonation had to be executed. **JB 30** was used for LIVE/DEAD tests as described in the next chapter. Additionally a new non-water-soluble polymer was synthesized via alternating diene polycondensation. The aim thereof was to avoid the problems of impurity that arose with 2-isopropenyl-2-oxazoline and to achieve a quick, cheap and efficient protocol of synthesizing a biocidal polymer.

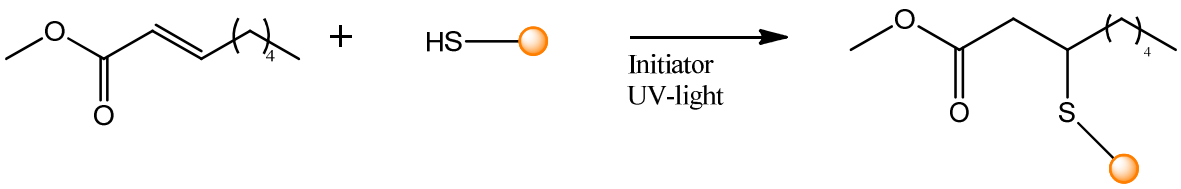
4.1.7 Alternating Diene Metathesis Polycondensation with 1,4-Butandiol diacrylate and 1,9-Decadiene.

The alternating diene metathesis polycondensation reaction was performed as an alternative to the already carried out functionalization and ROMP reaction. Some possible benefits were expected through a possible post-functionalization of the polymer. Because in the case of the 2-2-isopropenyl-2-oxazolinewe could not even get to the point of polymerization, as the monomer could not be obtained in a pure manner. In the style of known protocols of Slugovc

et al. the polymerization was implemented.[41] In order to find the best reaction conditions for the post functionalization some model reactions were carried out.

4.1.8 Model Reaction with Methyl-trans-2-octanoate

The molecule chosen for the model reaction has similar features as those ascribed to the polymer and was therefore used to figure out the favored reaction conditions for introducing amino functionalities via thiol-ene coupling.



Scheme 2: General reaction scheme of the model reaction with Methyl-trans-2-octanoate and various thiols.

The reaction scheme above shows the general conditions. Reactants, amount of initiator and exposure time to UV-light were varied in order to get products with high yields.

Table 2: Register of diverse conditions.

Reactants	**MEDA**	**Dodecanethiol**
Initiator	Lucerin TPO	DMPA
Exposure Time	5, 15,30 min	

The findings resulting from these initial tests showed that as well variations of the exposure time as the different ratios of reactants did not have an influencing factor on the product

[41] G.Galli, K.Fodor-Csorba, F.Stelzer, C.Slugovc, S.Demel, *Macromol. Rapid Communication*, **2003**, *24*, 636-641.

yield. However, the amount of used initiator is crucial. It turned out that more initiator supported the recovery. So for the subsequent functionalization of the polymer 1 weight percent of the initiator 2,2-dimethoxy-2-phenylacetophenone, DMPA was used. In-between the two tested initiators no big difference could be seen, so DMPA was preferred over Lucerin, as it was easier to handle. The reaction was performed with dodecanethiol equally well as with cysteamine.

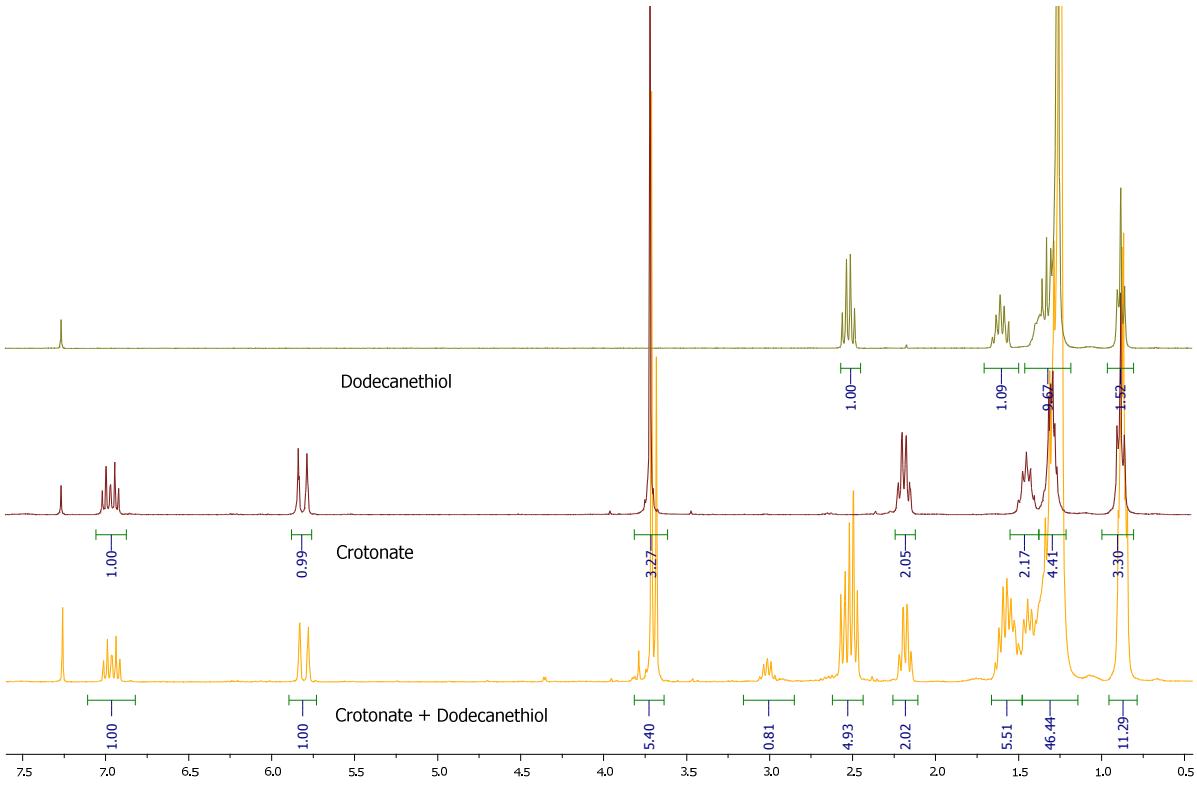


Figure 16: ^{1}H-NMR spectra of the model reaction with methyl-trans-2-octanoat and dodecanethiol.

By means of the spectrum a conversion of the 55 % can be measured.

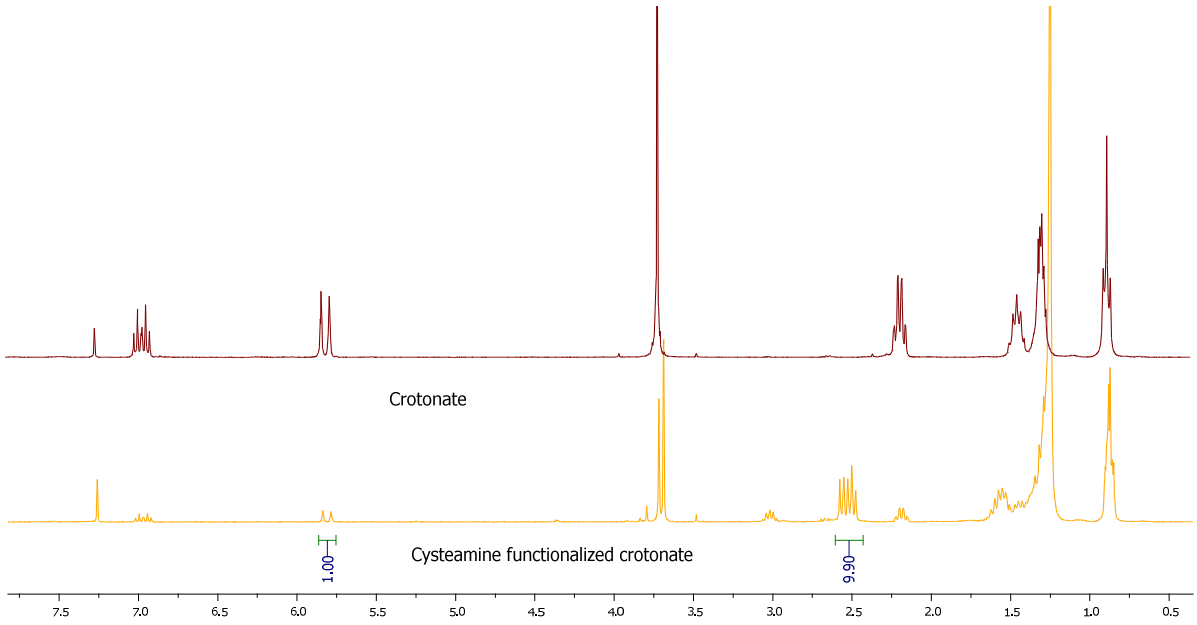
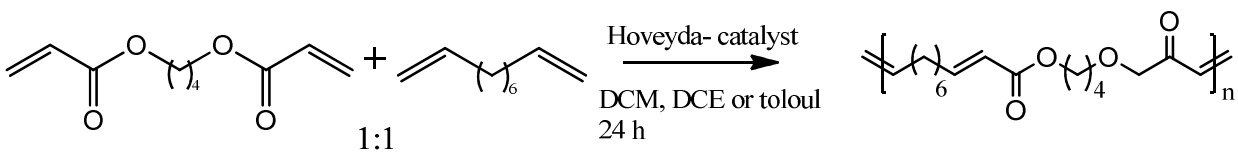


Figure 17: ^{1}H-NMR spectra of the model reaction with methyl-trans-2-octanoat and cysteamine.

With the help of the NMR spectrum the conversion of the functionalization was calculated to a value of 91%.

4.1.9 Polymerization

The ALTMET reaction was performed with 1,4-Butanediol diacrylate, 90% and equimolar amounts of 1,9-Decadiene, 96%. The monomers were provided in a Schlenk tube and 1 mol% Hoveyda initiator was dissolved in dichloromethane (45°C), dichloroethane (80°C) or respectively toluene (100°C). The reaction was allowed to stir overnight and was thereafter stopped with ethylvinylether.



Scheme 3: General reaction scheme of the ALTMET polymerization with 1,4-Butanediol diacrylate and of 1,9-Decadiene

The polymerization worked well with dichloromethane, dichloroethane and toluene,- but the reaction product of the polymerization in DCE, **JB 53**, was used for subsequent functionalization, as it marks a good compromise between the solubility of the used monomer and the reaction temperature of 80°C. The reaction was monitored via thin layer chromatography. After 24 hours still some product was visible, but nevertheless the reaction was stopped by adding 1 mL ethyl vinyl ether. After 15 minutes of further reaction time the solvent was evaporated and the product precipitated in methanol and in *n*-pentane. Purification of the polymer by precipitation turned out to be difficult, as solubility of the functionalization agent and the polymer were too similar. Nevertheless the functionalization was carried out as approved by ^{1}H-NMR.

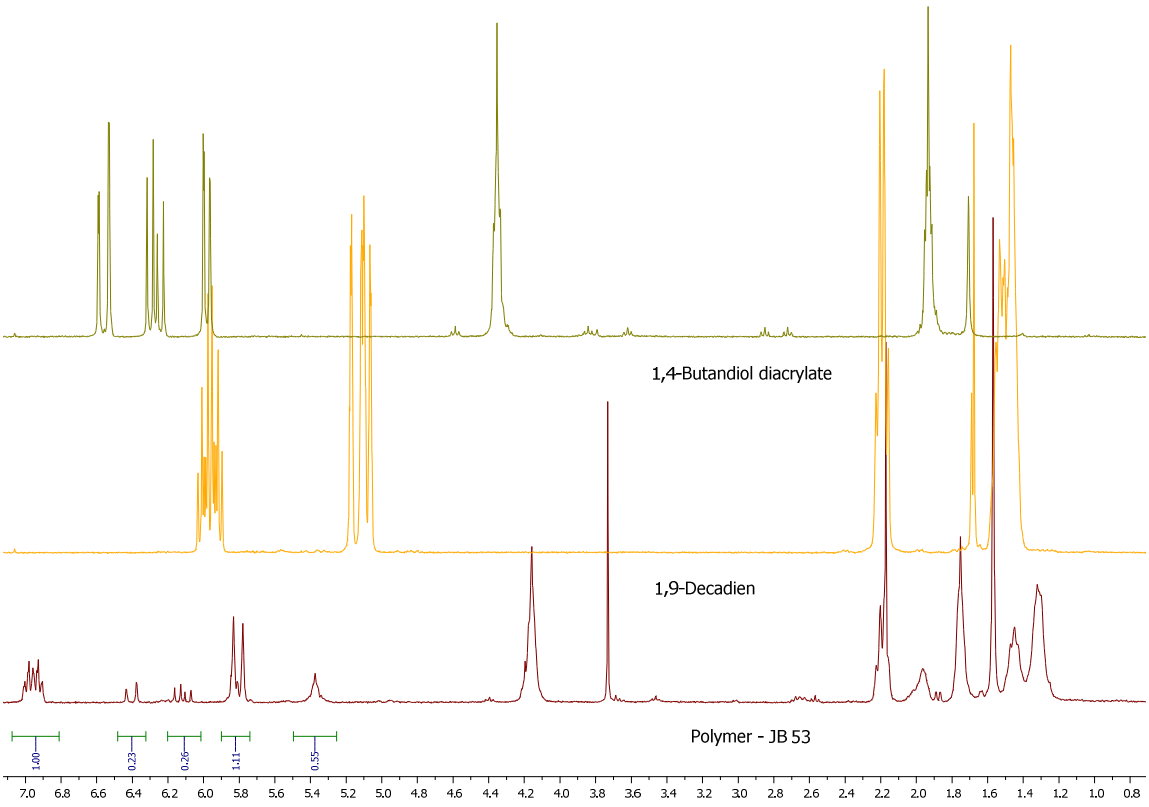
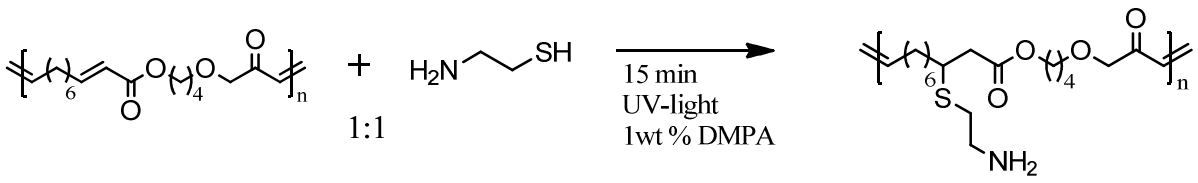


Figure 18: ^{1}H-NMR spectra of the polymerization product and its educts.

Characterization via ^{1}H-NMR spectroscopy shows the polymer (red) and the two educts, 1,9-decadiene (yellow) and 1,4-butandiol diacrylate (green). As visible on TLC still some 1,4-butandiol diacrylate was present, this result is affirmed by the NMR. Based on the ratio between the product and educt peaks of the polymer spectrum the conversion of 81% was calculated. The singlet peak at 5.31 ppm indicates a still remaining internal double bond, which might be derived through the combination of two 1,9-decadiene.

4.1.10 Functionalization

The obtained results from the reaction model with methyl-trans-octanoate were applied to the actual polymer at this point. The polymer **JB 53** was dissolved in THF and the equimolar amount of cysteamine was dissolved in methanol and subjoined, positioned 5 cm under the UV-lamp and when the DMPA initiator was added, the UV-light was turned on for 15 minutes at 9639 mWatt. The functionalized polymer was labeled **JB 55**.



Scheme 4: Functionalization of the polymer JB 53 with cysteamine, initiated by DMPA.

Various attempts were carried out to purify the polymer, but no clean product could be obtained so far. Characterization via ^{1}H-NMR spectroscopy showed that the functionalization worked well. Furthermore a solubility change was recognizable. However cysteamine was expected to be still present, so subsequent quaternization and viability tests were not meaningful up to now.

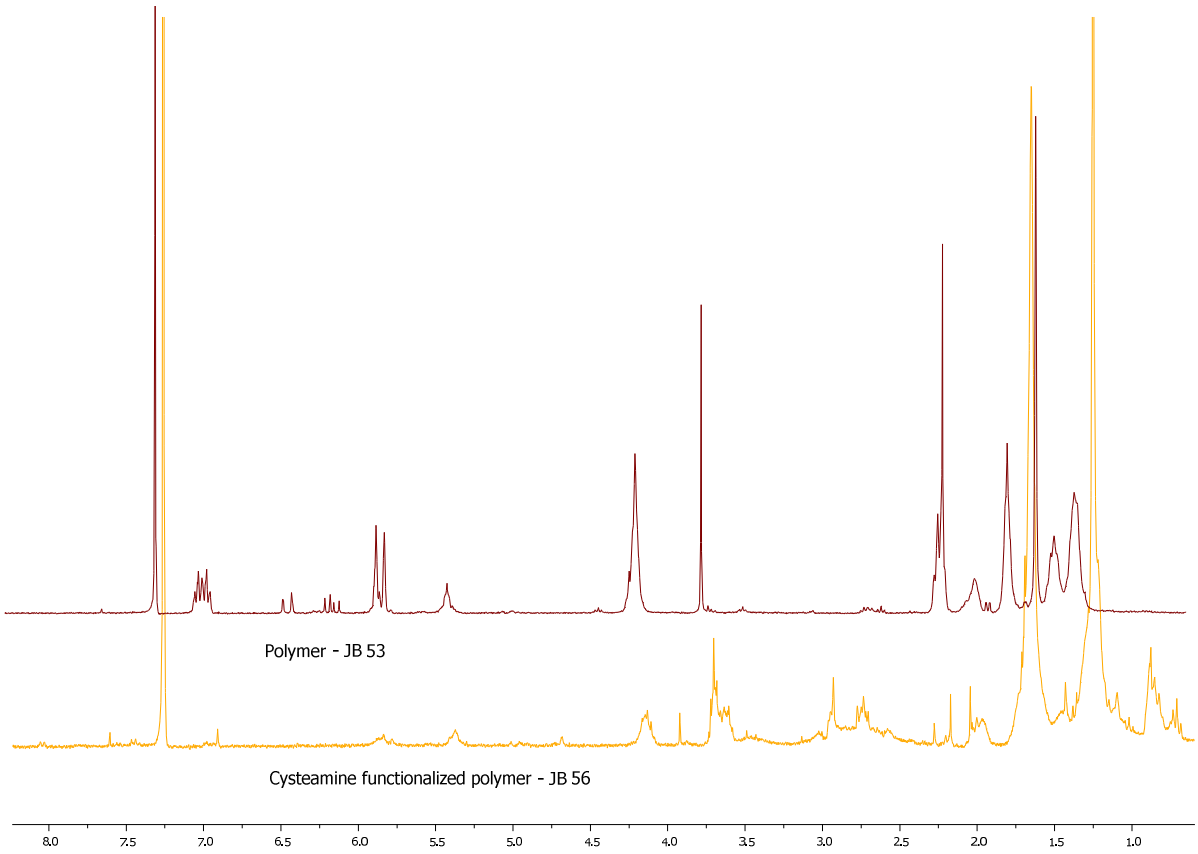


Figure 19: **^{1}H-NMR spectra of the functionalization with cysteamine.**

The spectrum above shows the decrease of the product peaks at 5.8 ppm and respectively 6.9 ppm, as well as the decrease of the educt peaks which were still present after the polymerization. The double bond peak at 5.31 ppm did not significantly decrease through the functionalization and is therefore obviously not reactive. But at around 3 ppm the broad cysteamine peak is visible.

4.2 Analysis of the Biocidal Activity via LIVE/DEAD Tests

4.2.1 Introduction

The so-called LIVE/DEAD® BacLight™ Bacterial Viability Kit from Invitrogen is a two color fluorescent assay, which is designed to analyze bacteria whether it is alive or dead.[42] This method is based on the different mode of actions of the two provided stains. On the one hand SYTO® 9 labels all bacteria green, no matter whether they are alive or dead. On the other hand the red-fluorescent stain, propidium iodide, labels only dead bacteria, as it is able to intercalate with the DNA after penetrating through damaged membranes. In order to this, a ratio between the measured fluorescence intensities can be calculated and therefore the amount of live and dead bacteria can be determined. The ambition of this work was the development of an adequate method for the determination of anti-microbial properties of the polymers synthesized beforehand. Until now the polymers produced in our group were analyzed via JIS Standard tests, what means that the samples had to be sent away. Time and cost were factors that we tried to minimize in using a method which could be realized at our university.

The excitation/emission maxima for the SYTO 9 stain is at 480/500 nm and for propidium iodide at 490/635 nm. So the excitation was executed at 485 nm for both stains. Because of the only available filter the emissions were measured at 520 nm and respectively 620 nm. But as the diagram below shows it is still in the range of possible analysis. (72% emission at 520 nm, 96% emission at 620 nm)

---

[42] http://tools.invitrogen.com/content/sfs/manuals/mp07007.pdf - 05.07.2011

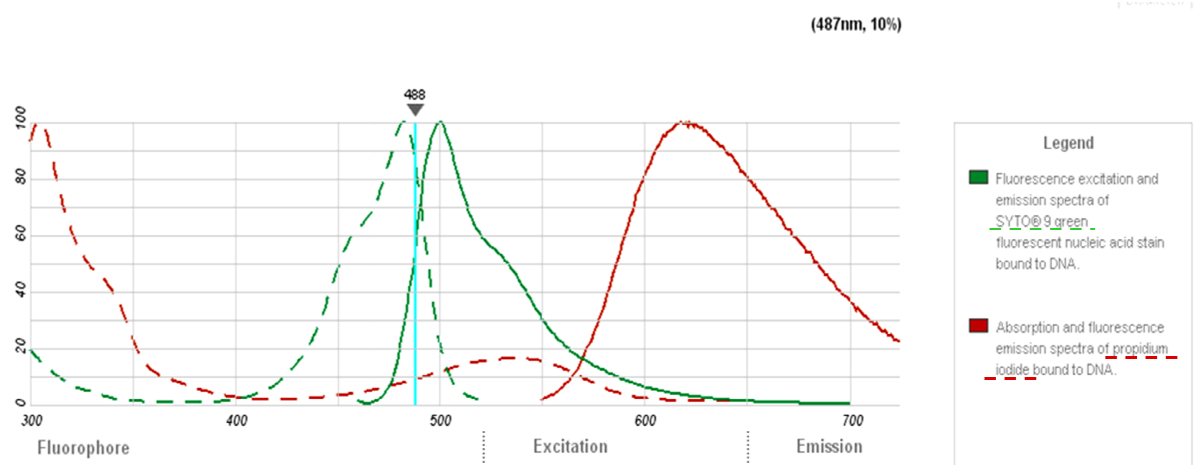


Figure 20: Fluorescence spectra of SYTO 9 and propidium iodide.[43]

4.2.2 Preparation of a Calibration Curve

First of all a calibration curve had to be made, whereas an E. coli culture was grown to late log phase in an usual LB nutrient broth. The bacteria was twice concentrated by centrifugation and washed via resuspension in 0.9% NaCl. After that one part was incubated with 0.9% NaCl and the other part of bacteria was killed by incubating with 70% isopropyl alcohol for 1 hour. The suspensions again were centrifuged and resuspended in NaCl to remove any residues of the alcohol. An OD_{670}= 0.06 was adjusted. Live and dead bacteria were then mixed together in various ratios between 0 and 100% live bacteria. The mixtures were incubated further 15 minutes with the stains and were measured then. For the calibration graph the percentage of live bacteria was applied against the ratio of green to red fluorescence.

[43] http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html - 06.07.2011

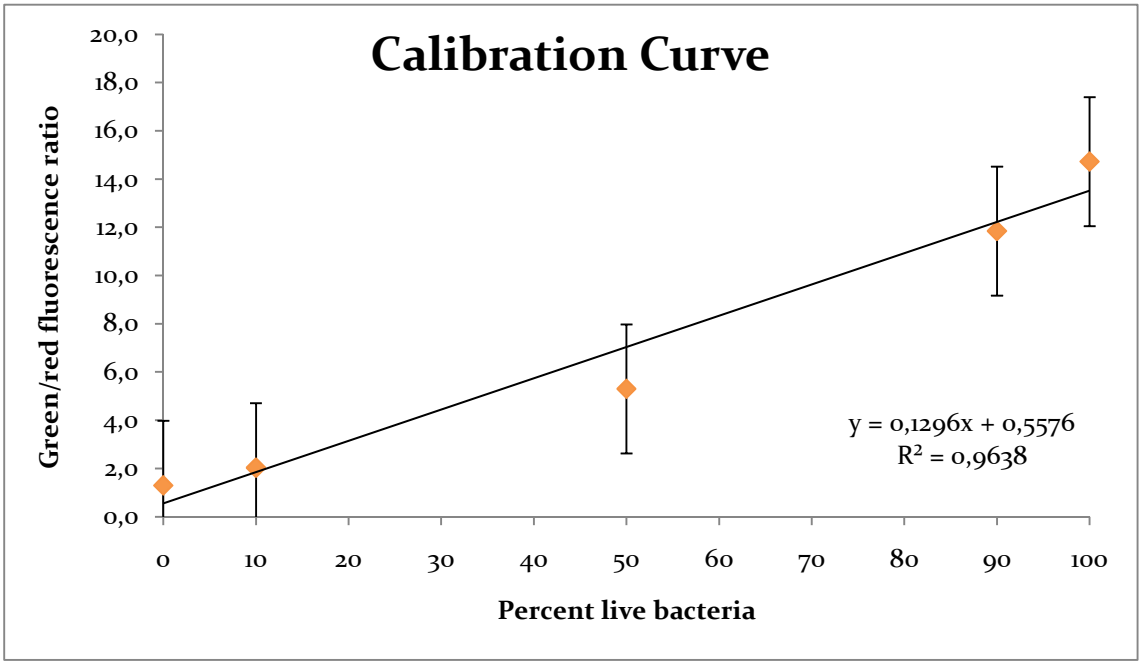


Figure 21: Percentage of live bacteria vs. the green to red fluorescence ratio

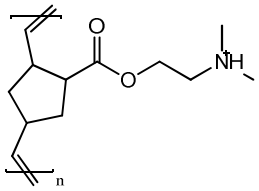
The calibration was done to compare the unknown sample concentrations with the well known concentration ratios of live and dead bacteria. The measured fluorescence ratios of the samples were later inserted into the linear equation, resulting in the demanded percentage of live bacteria. Unfortunately the calibration curve already points out the low accuracy of this method, the correlation coefficient of R^2= 0.9638 shows the acceptance for the linearity of the curve. The error, as visible in the graph above, exceeds 2 units and more, which has to be taken in account in further measurements. However the curve was used for further experiments.

The first trial of the preparations of the calibration curve had to be repeated because the gain was not adjusted well, what means that the instrument detected values which were not in the range of fluorescence intensity acceptable for the detector. The error was not realized immediately because the values achieved have been blank correct already, so it was not visible at the first sight, that the values were far too low. For the right gain adjustment two

samples were prepared - one positive and one negative sample. For P= 99% only living bacteria in staining solution was measured, to receive the highest values in-between the range of 30 000 - 250 000 counts. For the lowest values in-between this range 100% dead bacteria (incubated with isopropyl alcohol) were used. (P= 1%)

4.2.3 Sample **JB 28**, Water-soluble Polymer

The objective of working with a water-soluble polymer was to get to know the mode of operation with the LIVE/DEAD kit, as it is primarily designed for soluble samples.



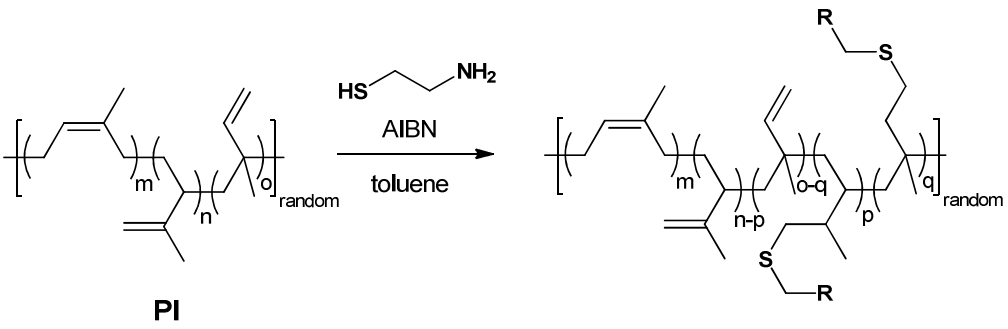
The *E. coli* culture was centrifuged and resuspended in 0.9% NaCl same as in the calibration step before. The OD was adjusted to 0.06 and the washed culture was afterwards incubated with **JB 28** and with polyethylene glycol PEG as a non-biocidal reference. After 1, 12 and 24 hours the samples were measured. But the results were not completely satisfactory, as even in repeated tries negative values for the fluorescence of the blank and the PEG sample were obtained. This is biologically not meaningful. However the fluorescence of the biocidal polymer at 620 nm was considerably lower than the fluorescence of the living bacteria at 520 nm. What shows that at least half of the bacteria were dead at that point.

Table 3: Comparison of the results of fluorescence measurement of JB 28

Wavelength (nm)	„Blank"(without any polymer)	Biocidal polymer	Polymer PEG
520	12352	41473	12355
620	-6859	**23037**	-10213
Ratio 520/620	-1,80	1,80	-1,21

A possible reason for the bad correlation between the values of the blank and those with the polymer could be that already the polymer on its own has a greenish color and therefore the blank does not really subtract the zero value, only a part of it.

4.2.4 Sample **PI 3**, Non-water-soluble Polymer



PI

R: **PI 1**= CH_2NH_2
PI 2= $CH_2NH_3^+ Cl^-$
PI 3= $CH_2N^+(C_3H_7)_3 Br^-$

Scheme 5: Reaction scheme of the functionalization of the polymer PI.

The scheme above shows the functionalization conditions under which poly(isoprene) was equipped biocidic as a part of Julia Kienbergers dissertation work. The polymer has been

synthesized under inert conditions with isoprene and n-BuLi in THF. After three hours reaction time, the mixture was precipitated in ethanol and dried under vacuum. To introduce a nitrogen functionality, the poly(isoprene) reacted over night with cysteamine at 80°C. Toluene was used as solvent and the reaction was initiated with AIBN. For purification the functionalized product was added to cold methanol and thereafter dried under vacuum. Biocide activity was introduced via quaternization of the amine by protonation and alkylation, respectively.

The antimicrobial activity was primary determined via the Japanese Industrial Standard JIS Z 2801:2000, performed by the "Technologie Transfer Zentrum TTZ Bremerhaven". The polymer was spin coated onto a glass plate (5x5 cm) in a concentration of 10mg/mL- and tests with various microorganisms, including *E. coli*, *S. aureus*, and *L. monocytogenes* were carried out. The surviving cells after 24 hours were quantified in comparison to the bacterial population in the beginning. **PI 3** showed a 100% reduction of living bacteria after 24 hours.

So **PI 3** was used for tests with the LIVE/DEAD kit with the knowledge that it has an antibacterial activity.

The polymer was dissolved in a 10 mg/mL CH_2Cl_2/MeOH – solution. Aliquots thereof were transferred to test vials and dried. The OD-adjusted bacterial suspension was added and incubated for 24 hours. After 1, 2, 4 and 24 hours samples were taken and measured.

Table 4: Fluorescence values of PI 3 after 1 hour incubation time.

Concentration of Biocidal Polymer- 1 hour						
Wavelength (nm)	**0 µl**	**100 µl**	**200 µl**	**300 µl**	**400 µl**	**1000 µl**
Ratio 520/620 nm	14,46	13,22	13,8	16,31	14,1	15,01
Ratio 520/620 nm	14,88	14,75	15,57	17,23	15,21	16,15
Ratio 520/620 nm	16,96	15,78	16,25	16,61	16,26	16,75

Table 5: Fluorescence values of PI 3 after 24 hour incubation time.

Concentration of Biocidal Polymer- 24 hours						
Wavelength (nm)	**0 µl**	**100 µl**	**200 µl**	**300 µl**	**400 µl**	**1000 µl**
Ratio 520/620 nm	16,7	13,62	13,09	12,66	11,12	**10,22**
Ratio 520/620 nm	17,09	13,14	12,83	12,85	11,64	**9,61**
Ratio 520/620 nm	17,88	13,5	13,24	13,5	11,82	**10,54**

On basis of these results it can be seen, that after one hour no significant change in the values was achieved. But after 24 hours a trend is visible: The higher the polymer concentration, the more bacteria cells are dead in comparison with the zero value without polymer used. The average of the values with a concentration of 10 mg/mL was inserted to the linear equation. (y= 10, 12)

y = 0,1296x + 0, 5576 → x= 74 % Live bacteria is still existing. That means that we achieved a decrease of 58 % compared to the bacteria still alive after 24 hours.

So in this case we proofed that the method works partly. We have a certain antimicrobial activity of the polymer, which can be verified with this method. The point is that the polymer we are dealing with is a contact biocide. So probably the film of bacteria which was right on the surface of the polymer was killed. For the future a well defined protocol has to be figured out and also a non biocidal polymer with similar properties as **PI 3** should be used as a blank.

5. Summary and Outlook

The general aim of this research work was to achieve a biocidal polymer by introducing amino functionalities. Various polymerization methods have been carried out and subsequently the biocidal property of the polymers has been characterized using LIVE/DEAD® viability kit from Invitrogen.

2-isopropenyl-2-oxazoline was used as monomer for pre-functionalization with various thiols such as dodecanethiol, cysteamine, cystamine, diethylamine and mercapto-pyridin. The functionalized monomer could never be achieved in quantitative yields and without any byproducts. Therefore it was not commercially attractive and the cationic ring opening polymerization experiments have not been continued so far.

Alternating Diene Metathesis Polycondension, ALTMET was used as another method to obtain a polymer including double bonds for the subsequent thiol-ene functionalization reaction. Previously a model reaction has been carried out, where the best reaction conditions have been evaluated. The polymerization of 1,4-butandiol diacrylate and 1,9-decadiene did not result in a completely pure product, as still some educt was present. Nevertheless the functionalization has been carried out but viability tests were not meaningful at that point as far as still some unreacted cysteamine might have been present.

Furthermore a ROM polymerization has been carried out with a norbornene. As a result a water-soluble polymer could be easily achieved and later on characterized via LIVE/DEAD kit.

Apart from the polymerization of biocidal polymers, it was an objective to create a valid method for determining the biocidal property of these polymers. Using the LIVE/DEAD kit form Invitrogen has not yet led to completely satisfactory results but trends could be recognized already.

In future work the protocol for the LIVE/DEAD kit should be improved and proofed for its reproducibility. This means that the optimal polymer concentration needs to be evaluated as well as the time of incubation. Furthermore various bacteria could be used for the tests. As an alternative to the LIVE/DEAD kit also Kirby-Bauer tests[44] could be carried out. In the process bacteria is allowed to grow on agar and after incubating a small polymer disc the minimum inhibitory concentration zone can be evaluated.

6. Experimental

6.1 Materials

All chemicals used for this work were purchased from commercial sources (Fluka, Sigma Aldrich, Lancaster, Merck or ABCR) and if not mentioned otherwise applied without further purification. The catalyst M 31 was obtained from UMICORE AG.

For thin layer chromatography aluminium sheets with silica gel 60 F_{254} from Merck were used. Via UV light irradiation at 365 nm and a 0.5 % dip solution of $KMnO_4$ the visualization was performed.

Argon was used for reactions conducted under inert gas atmosphere.

---

[44] B.Kreter, P.Axelrod, V.Satischandran, A.Truant, J.Woodwell, H. Tumah, T.Fekete, *Diagnostic Microbiology and Infectious Disease,* **1994**, *18*, 251.258

6.2 Instruments

Nuclear Magnetic Resonance Spectroscopy (NMR)

A Burker Avance III 300 MHz spectrometer was used for ^1H spectra characterization. Respective deuterated solvents such as $CDCl_3$, MeOD, DMSO-d_6 and D_2O, purchased from Cambridge Isotope Laboratories, Inc., were used according to different solubilities of the probes. The following abbreviations were used to indicate different peak shapes: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), b (broad), bs (broad singlet).

Gel Permeation Chromatography (GPC)

For determination of polydispersity index (PDI) and the weight average of molecular mass (M_W) gel permeation chromatography was performed. As eluent either tetrahydrofuran (THF) or $CHCl_3$/Et_3N/*iso*-Propanol (94/4/2) was used. Measurements were taken with a Merck Hitachi L-6000A pump and detected via a refractive index detector Waters 410. For calibration a polystyrene standard with separation columns consisting of PSS 5 mm with 106 Å, 104 Å and 103 Å was used.

Microwave Reactor

Initiator ™ Eight Microwave Synthesis System from Biotage was used for microwave reactions. (Temperature range of 40-250 °C, Pressure range of 0-20 bar, Power range to 300 W.)

Centrifuge

Either a Sorvell® RC-5B Refrigerated Superspeed Centrifuge from DuPont Instruments or for smaller amounts a HERAEUS Sepatech, Megafuge 1.0R was used.

Micro Plate Reader – FLUOstar Omega

Reader type: FLUO star Omega

Serial number: 415-0577

Filmware version: 1.13

Control version: Omega 1.20

The following settings were used:

Basic settings:

Measurement type: Fluorescence (FI), multichromatic

Microplate name: COSTAR 96

Endpoint settings:

No. of flashes per well: 50

Optic settings:

Table 6: General excitation/ emission settings for the plate reader.

No.	Excitation	Emission	Gain
1	485	em 520	30000-250000
2	485	620-10	30000-250000

Shake settings:

Shaking frequency [rpm]: 100

Shaking mode: double orbital

Additional shaking time: 5 s before plate reading

General settings:

-Bottom optic used

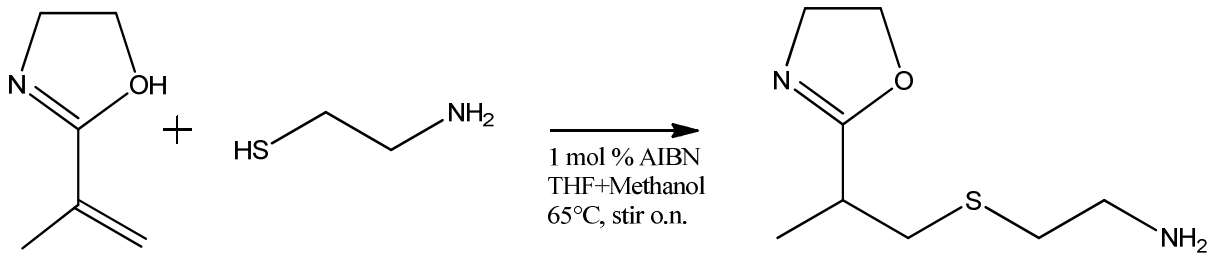
-Positioning delay [s]: 0.2

-Reading direction ⇓⇓⇓

-Room temperature

6.3 Synthesis

6.3.1 Cysteamine functionalized 2- Isopropenyl-2-Oxazoline (**JB 1**)



Scheme 6: Reaction scheme of the performed functionalization

Based on the approach of Grayson et al.[32] a Schlenk tube was evacuated and filled with argon 3 times, 2mL THF and cysteamine (87mg, $9.88*10^{-4}$ mol, 1 eq) were added. For better solubility of cysteamine 0.5 mL methanol were added and the system was kept under argon atmosphere while 2-Isopropenyl-2-oxazoline (100mg, $8.99*10^{-4}$ mol, 1.1 eq) was added. The reaction was initiated with AIBN (1.5 mg, $8.99*10^{-6}$ mol, 1 mol %) and stirred overnight at 65°C. The progress of the reaction was monitored via TLC. The product was dried in vacuum and characterized via NMR. Yield: 72.3 %, TLC: (silica, CH_2Cl_2/MeOH 10:1, R_f= 0.68)

^{1}H- NMR (δ, 20°C, $CDCl_3$, 300 MHz) 5.23 (s, NH_2, H-8), 4.18 (t, $CH_{2\text{-}Ox}$, H-1), 3.78 (t, $CH_{2\text{-}O}$, H-2), 2.95 (t, CH_2, H-7), 2.81 (q, CH_2, H-6), 2.65 (q, CH_2, H-5), 1.22 (d, CH_3, H-3)

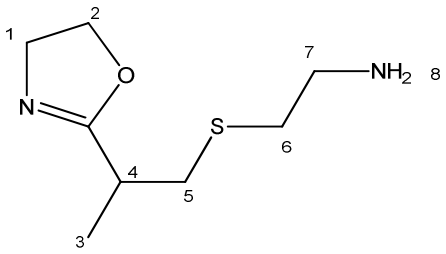
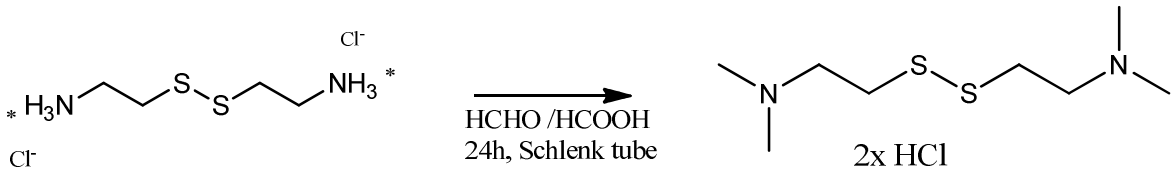


Figure 22: JB1- Cysteamine - functionalized 2 isopropenyl-2-oxazoline

Chemical Formula: $C_8H_{16}N_2OS$

Molecular Weight: 188.29 g/mol

6.3.2 Methylation of Cystamine Dihydrochloride (**JB 24**)



Scheme 7: Reaction scheme of the methylation of cysteamine dihydrochloride

Based on Sanchez et al.[45] cystamine dihydrochloride (100 mg, $4.44*10^{-4}$ mol, 1 eq) was put into an ice cooled round-bottom flask and 98% formic acid (125 mg, $2.66*10^{-3}$ mol, 6 eq) was slowly added. Subsequently an excess of 37% formaldehyde (187 mg, $2.31*10^{-3}$ mol, 2.6 eq) was put into the reaction flask. At a temperature of 80 °C the mixture was stirred for 24 hours.

After cooling, 10 mL of 6 M HCl were added and washed three times with dichloromethane. The aqueous phase was made basic with $NaHCO_3$ and the product was extracted three

[32] B.L. Sanchez, S. Pine, *J. Org. Chem.*, **1971,** 36, 829-832.

times into the organic dichloromethane phase which finally was washed with water and dried with sodium sulfate, filtered and dichloromethane was removed under vacuum. Yield: 88 mg, 69.7%.

^{1}H-NMR (δ, 20 °C, $CDCl_3$, 300 MHz): 2.84 (q, CH_2, H-1), 2.62 (q, CH_2, H-2), 2.28 (d, CH_3, H-3)

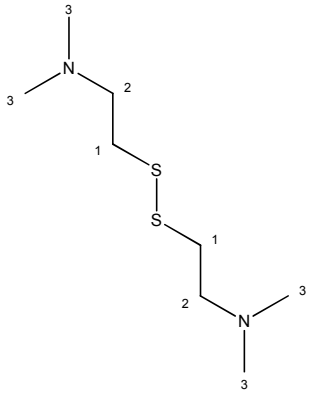
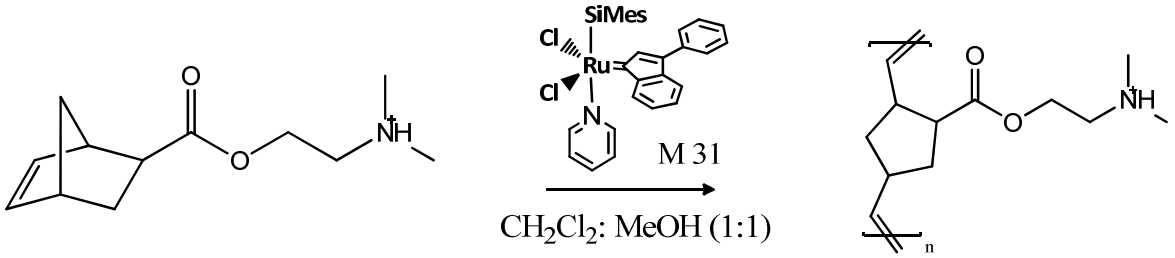


Figure 23: **JB 24 – Methylated Cystamine**

Chemical Formula: $C_8H_{20}N_2S_2$

Molecular Weight: 285.2 g/mol

6.3.3 Polymerization of 2-(((1R, 4R)-bicyclo[2.2.1]hept-5-ene-2-carbonyl)oxy)-N,N-dimethylethanaminium (**JB 28**)



Scheme 8: **Polymerization of (*exo,endo*- bicycle [2.2.1] hept- 5-en -2- carbon acid (2- dimethylamino- ethyl) ester**

A Schlenk tube was evacuated and 1g of the monomer ($4.1*10^{-3}$ mol, 100 eq) was provided. 50 mL of a 1 M solution of CH_2Cl_2: MeOH (1:1) were added. The initiator M 31 (0.030g, $4.1*10^{-5}$ mol, 1 eq) was dissolved in 1 mL solvent mixture and added at once to the Schlenk tube.

After 2 hours reaction time, it was stopped with 2mL ethylvinylether. 15 minutes after addition the mixture was evaporated and precipitated in cold n-pentane. The n-pentane was decanted and the product dissolved again in CH_2Cl_2: MeOH (1:1) and dried under vacuum atmosphere. Yield: 1.12 g

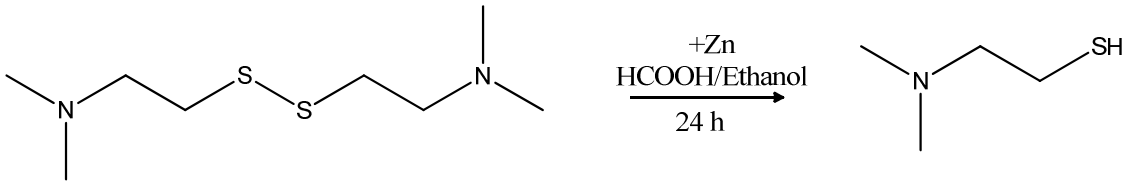
^{1}H-NMR (δ, 20 °C, $CDCl_3$, 300 MHz): 4.51(b), 3.49(b), 2.98(d), 2.91(s)

GPC

Polydispersity Index: 1.29

Mn: 37221 g/mol

6.3.4 Disulfidreduction of 2,2-disulfanediylbis(N,N-dimethylethanamine) (**JB 31**)



Scheme 9: Reduction of the disulfide bond of methylated cystamine

The methylated cysteamine (0.5075g, $2.44*10^{-3}$ mol, 1 eq) **JB 29** was provided in a round bottom flask and dissolved in 100 mL ethanol. Powdered zinc (2.89 g, 5.7 eq) and 10 mL acetic acid were added in portions[38]. The disulfide reduction reacted over night before the zinc was filtered and the solvent was evaporated. Via TLC the reaction progress was

checked. Still some educt was present, so two more equivalents of zinc, ethanol and acetic acid were added and the mixture again reacted over night.

Zinc and solvent were removed as described above and the residue was dissolved in methylenchloride. It was necessary to work under basic conditions to get the product into the organic phase. So some NaOH was added. Subsequently it was washed three times with distilled water, once with saturated $NaHCO_3$ and once with brine. After the washing steps it was dried with sodium sulfate, filtered and evaporated. An NMR was taken for characterization.

^{1}H-NMR (δ, 20 °C, $CDCl_3$, 300 MHz): 2.78 (t, CH_2, H-1), 2.60 (t, CH_2, H-2), 2.43 (s, 2x CH_3, H-3)

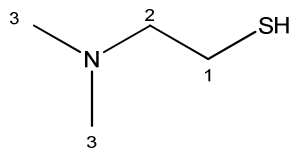


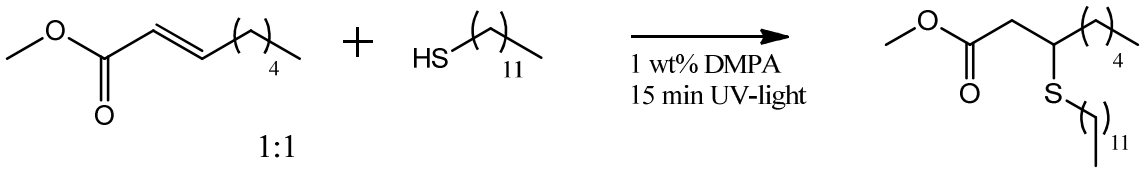
Figure 24: Methylated cysteamine

2-(dimethylamino) ethanethiol

Chemical Formula: $C_4H_{11}NS$

Molecular Weight: 105,20 g/mol

6.3.5 Functionalization of Methyl-trans-2-octanoat with Dodecanethiol (**JB 46**)



Scheme 10: Reaction scheme of the functionalization of methyl-trans-2-octanoat with dodecanethiol

Dodecanethiol (230 µl, $9.60*10^{-4}$ mol, 1 eq) and methyl-trans-2-octanoat (150 µl, $9.60*10^{-4}$ mol, 1 eq) were put together into a vial. It was placed ca. 7 cm under the UV-lamp and the reaction was initiated with 1 wt% 2,2 -dimethoxy-2-phenylacetophenone, DMPA. After 15 minutes exposure to UV- light with 9639 mWatt a DC and an NMR was taken.

^{1}H-NMR (δ, 20 °C, $CDCl_3$, 300 MHz): 0.88 (CH_3, 1), 1.26-1.42 (CH_2, 2), 2.60 (S-CH_2- CH_2, 3), 2.71 (O=CH- CH_2- CH_2-S, 4), 3.61 (S- CH_2- $(CH_3)_2$, 5), 3.68 (CH_3=O, 6)

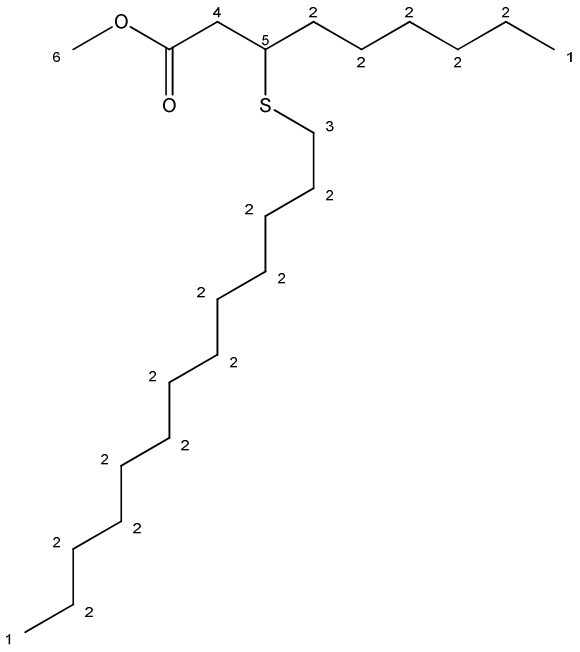


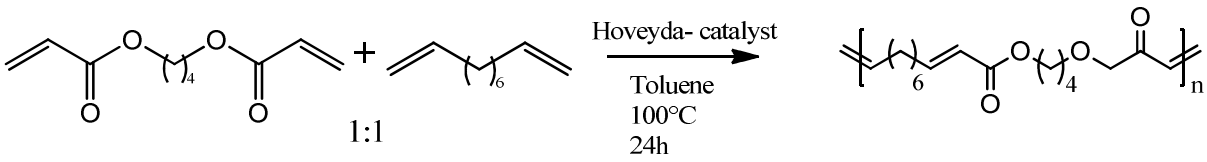
Figure 25: JB 46 – Dodecanethiol - functionalized methyl-trans-2-octanoat

Methyl 3-(tridecylthio)nonanoate

Chemical Formula: C23H46O2S

Molecular Weight: 386,68 g/mol

6.3.6 ALTMET Polymerization of 1,4-Butanediol diacrylate and 1,9-Decadien (**JB 47**)



Scheme 11: ALTMET reaction scheme of butandiol and decadien

1,4-Butanediol diacrylate (1.98 g, 0.01 mol, 1 eq) and 1,9-Decadien (1.38 g, 0.01 mol, 1 eq) were provided in a Schlenk tube and based on the studies of Galli et al.[46] the educts were diluted in 10 mL toluene and the reaction was initiated with 1 mol% Grubbs Hoveyda catalyst. The reaction mixture is allowed to stir on reflux at 45°C for 24 hours. After the progress was checked via TLC the reaction was stopped by adding 1 mL vinylethylether and further 15 minutes of reaction time. The solvent was evaporated and for purification the product was precipitated in methanol and dried under vacuum atmosphere. For characterization an NMR was taken.

^{1}H-NMR (δ, 20 °C, $CDCl_3$, 300 MHz): 6.38 (CH, 1), 6.11 (CH, 2), 4.16, 1.75 (CH_2, 3), 5.86 (CH, 4), 6.96 (CH, 5), 2.18 (CH_2, 6), 5.83 (CH_2, 7)

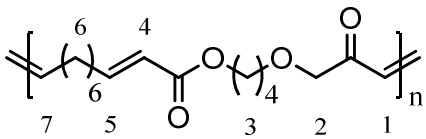
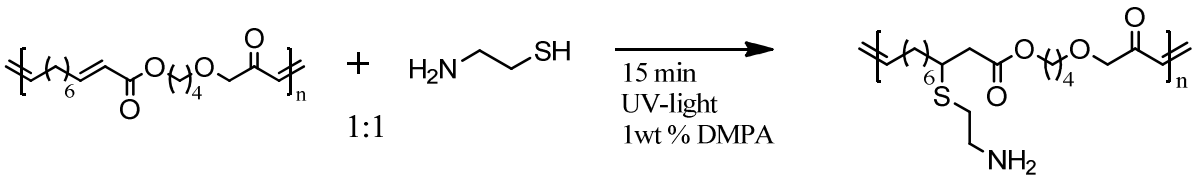


Figure 26: JB 47 – ALTMET Polymer, Poly (ethyl but-2-enoate)

Poly (ethyl but-2-enoate)

[46] G. Galli, K. Fodor-Csorba, F. Stelzer, C. Slugovc, S. Demel, *Macromol. Rapid Commun.* **2003**, 24, 636-641.

6.3.7 Functionalization of Poly(ethyl but-2-enoate) with MEDA (**JB 49.1**)



Scheme 12: Functionalization of the ALTMET polymer JB 47 with MEDA.

The Poly(ethyl but-2-enoate) (0.05 g, $1.68*10^{-4}$ mol, 1 eq) derived from reaction **JB 49.1** and MEDA (0.024 g, $1.68*10^{-4}$ mol, 1 eq) are weighted into a vial. 50 µl MeOH + 50 µl H_2O were used to dissolve the MEDA and then it was mixed with the polymer which was dissolved in 200 µl THF. The reaction was stirred, when the photo initiator DMPA ($7.34*10^{-4}$, 1 wt %) was added. After 15 minutes exposure to UV light, the product was characterized via NMR spectroscopy.

^{1}H-NMR (δ, 20 °C, $CDCl_3$, 300 MHz): 6.11 (CH, 2), 4.16, 1.75 (CH_2, 3), (CH, 4), 2.18 (CH_2, 6), 2.70 (CH_2, 8), 3.00 (CH_2, 9)

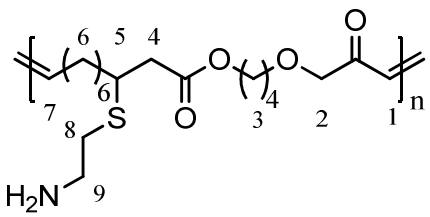


Figure 27: JB 49.1 – Functionalized ALTMET polymer

6.4 General Overview over all Synthesis

The following overview serves as summarization of all the conducted reactions done in this work.

6.4.1 Synthesis of an Amino-functionalized 2-Isopropyl-2-oxazoline

The monomer functionalization was expected to lead to effective products, as the double bond of the 2-isopropenyl-2-oxazolinewas thought to react via thiol-ene reaction with the cysteamine. In order to check whether the reacting group was indeed the thiol, the reaction was carried out as well with dodecanethiol, mercapto-pyridine and diethyl amine. Furthermore the amine of the cysteamine was protected via acylation or methylation to exclude any possibility for the nitrogen to react with the double bond. NMR characterization showed, that the nitrogen group did not react, so there was no Michael Addition observed.

Concerning the protocols of Grayson et al.[32] the thiol-ene click functionalization was also carried out with an additional amino source. The nucleophile for the catalysis of the reaction was prepared using a Merrifield`s peptide resin which was converted with a butyl amine. In comparison to the butyl amine resin also diethyl amine was used to catalyze the reaction, but none of these reactions showed advantages. As in all the other cases the functionalization worked, but it was never fully converted.

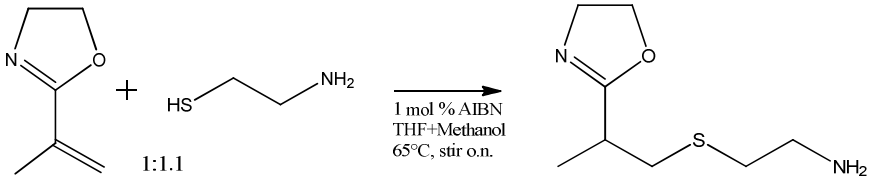
The remaining cysteamine was tried to be removed via column chromatography purification on silica with CH_2Cl_2/MeOH (10:1) but after the purification step the product was even more impure.

This problem could not be bypassed under any of the conditions tried in this work.

6.4.2 Functionalization of 2-Isopropenyl-2-oxazoline

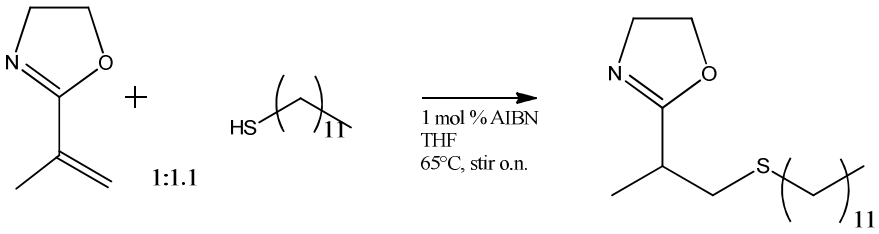
6.4.2.1 Thiol-ene Reaction

JB 1



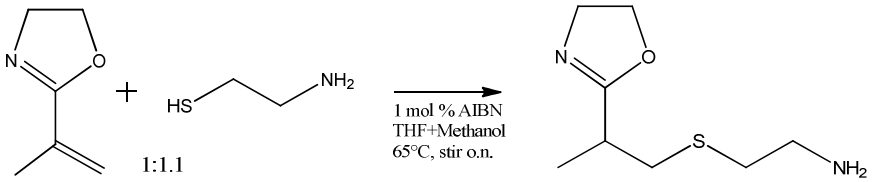
6.4.2.2 2-(1-(dodecylthio)propan-2-yl)-4,5-dihydrooxazole

JB 1.1



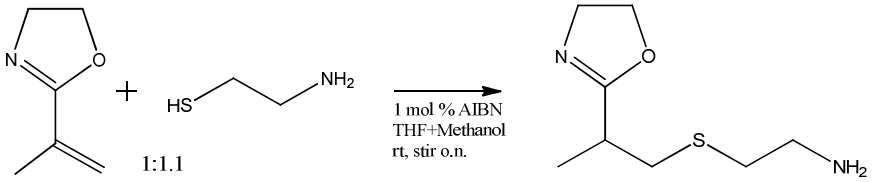
6.4.2.3 2-((2-(4,5-dihydrooxazol-2-yl)propyl)thio)ethanamine

JB 2



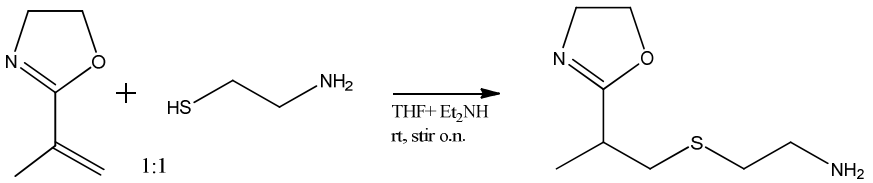
6.4.2.4 2-((2-(4,5-dihydrooxazol-2-yl)propyl)thio)ethanamine

JB 3



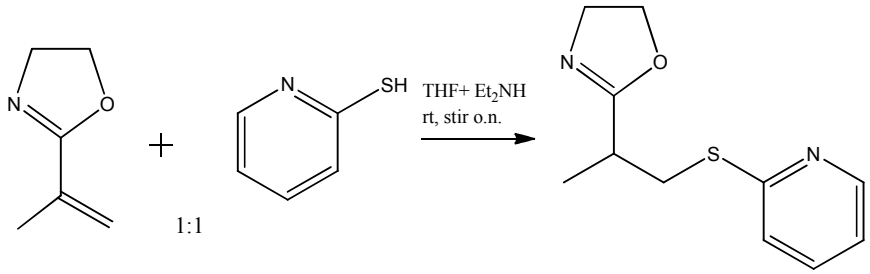
6.4.2.5 2-((2-(4,5-dihydrooxazol-2-yl)propyl)thio)ethanamine

JB 4.1



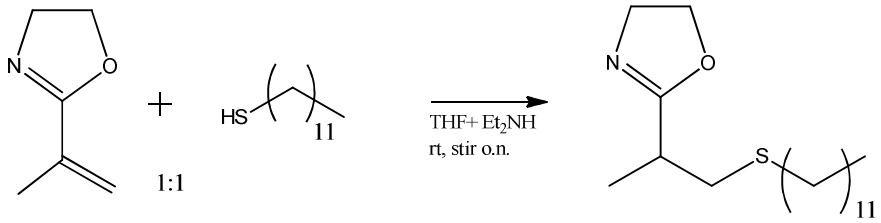
6.4.5 2-(1-(pyridin-2-ylthio)propan-2-yl)-4,5-dihydrooxazole

JB 4.2



6.4.2.6 2-(1-(dodecylthio)propan-2-yl)-4,5-dihydrooxazole

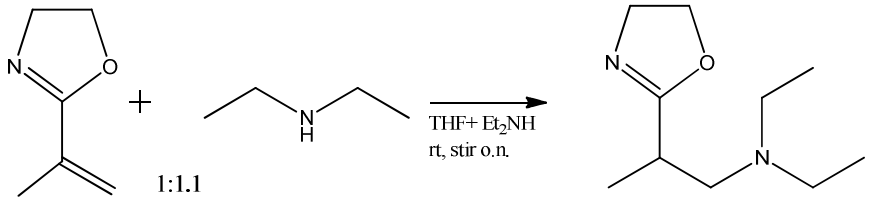
JB 4.3



6.4.2.7 2-(4,5-dihydrooxazol-2-yl)-N,N-diethylpropan-1

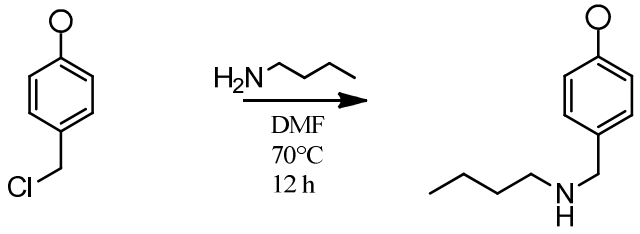
Amine

JB 5



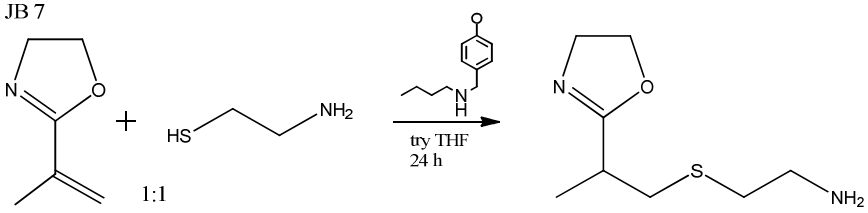
6.4.2.8 Butylamin resin

JB 6/10



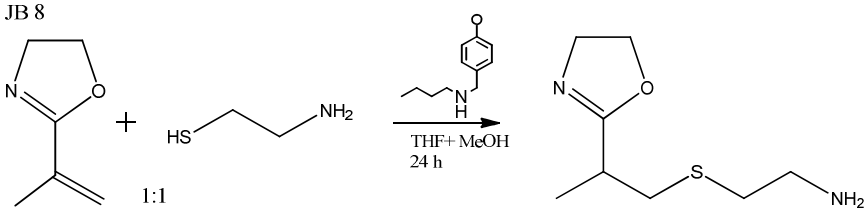
6.4.2.9 2-(4,5-dihydrooxazol-2-yl)-N,N-diethylpropan-1-amine

JB 7



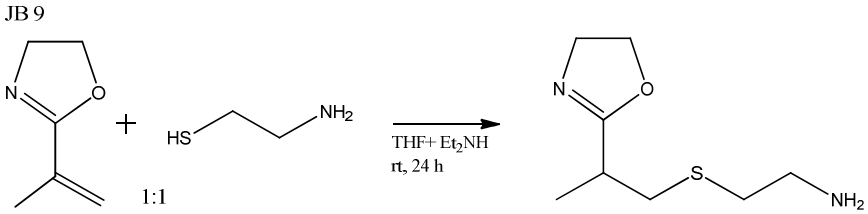
6.4.2.10 2-(4,5-dihydrooxazol-2-yl)-N,N-diethylpropan-1-amine

JB 8



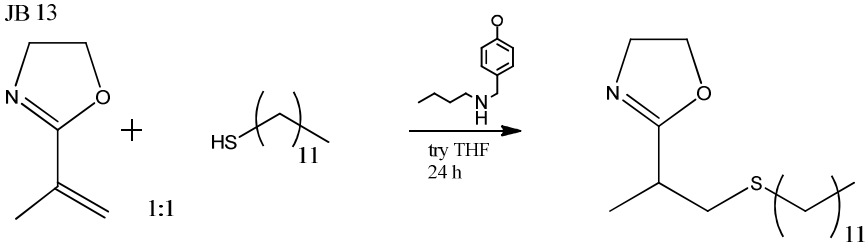
6.4.2.11 2-(4,5-dihydrooxazol-2-yl)-N,N-diethylpropan-1-amine

JB 9



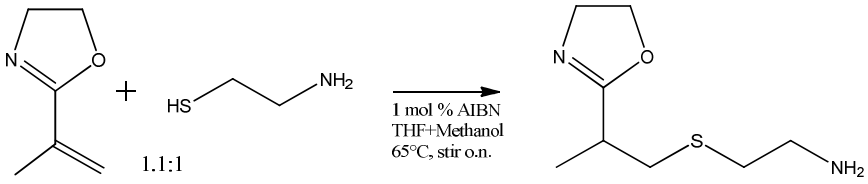
6.4.2.12 2-(1-(dodecylthio)propan-2-yl)-4,5-dihydrooxazole

JB 13



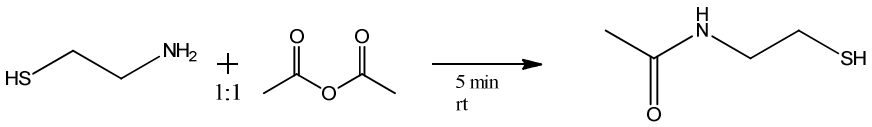
6.4.2.13 2-(4,5-dihydrooxazol-2-yl)-N,N-diethylpropan-1-amine

JB 16



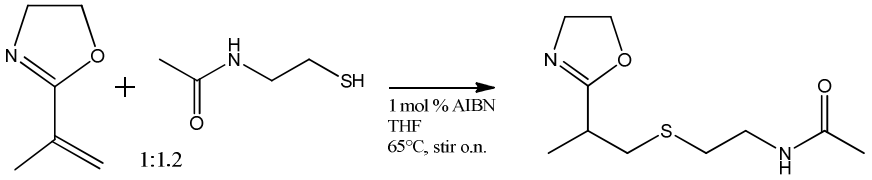
6.4.2.14 N-(2-mercaptoethyl)acetamide

JB 17/20



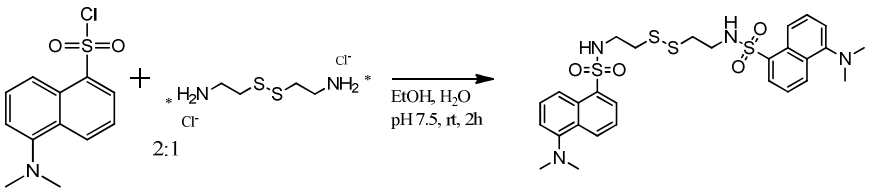
6.4.2.15 N-(2-((2-(4,5-dihydrooxazol-2-yl)propyl)thio)ethyl)acetamide

JB 19



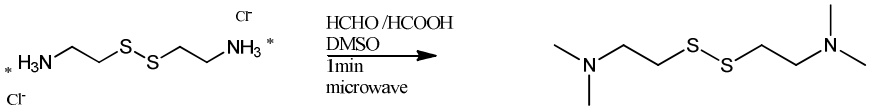
6.4.2.16 N,N'-(disulfanediylbis(ethane-2,1-diyl))bis(5 (dimethylamino)naphthalene-1-sulfonamide)

JB 21



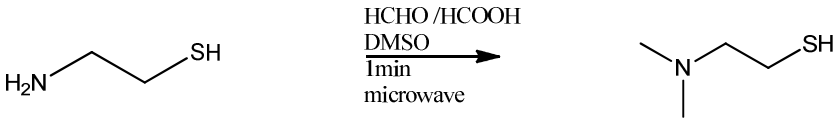
6.4.2.17 2,2'-disulfanediylbis(N,N-dimethylethanamine)

JB 22



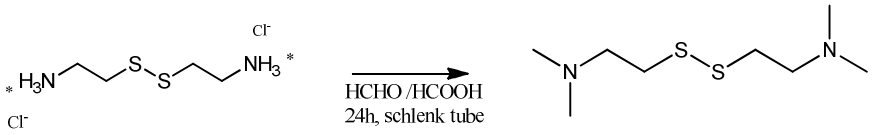
6.4.2.18 2-(dimethylamino)ethanethiol

JB 23



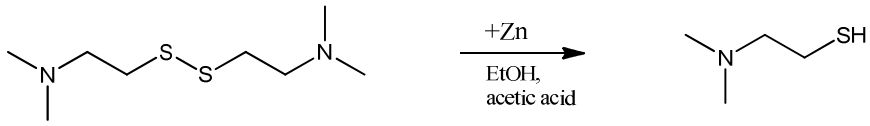
6.4.2.19 2,2'-disulfanediylbis(N,N-dimethylethanamine)

JB 24



6.4.2.20 2-(dimethylamino)ethanethiol

JB 24.a

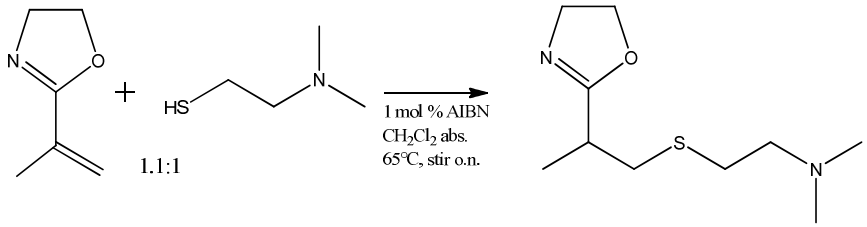


6.4.2.21 2-(dimethylamino)ethanethiol

JB 25

6.4.2.22 2-(dimethylamino)ethanethiol

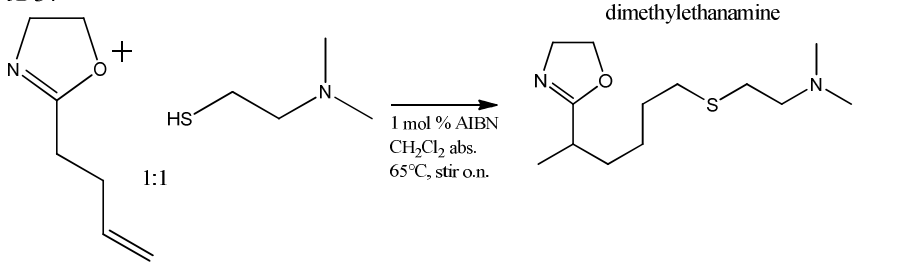
JB 33



6.4.2.23 2-((5-(4,5-dihydrooxazol-2-yl)hexyl)thio)-N,N-dimethylethanamine

JB 34

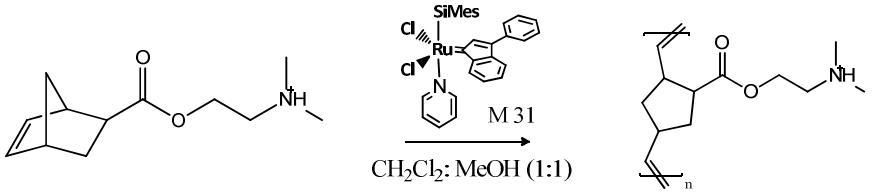
2-((5-(4,5-dihydrooxazol-2-yl)hexyl)thio)-*N*,*N*-dimethylethanamine



6.5 Preparation of a Water-soluble Polymer

6.5.1 Water-soluble Polymer **JB 28**

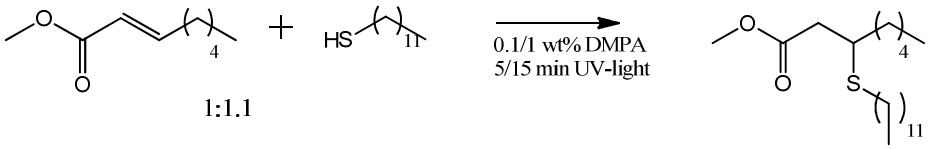
JB 28



6.6 Model Reaction for Subsequent ALTMET Polymerization

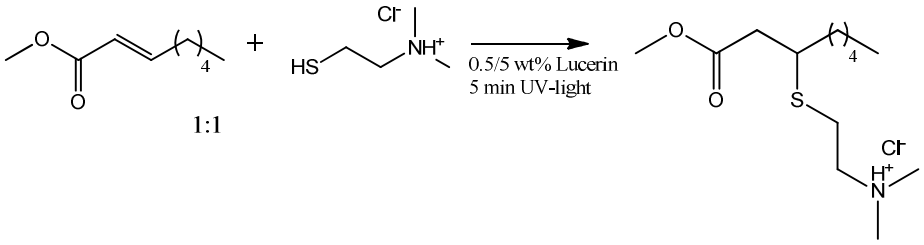
6.6.1 Dodecanethiol- functionalized Methyl-trans-2-octanoat

JB 45/46



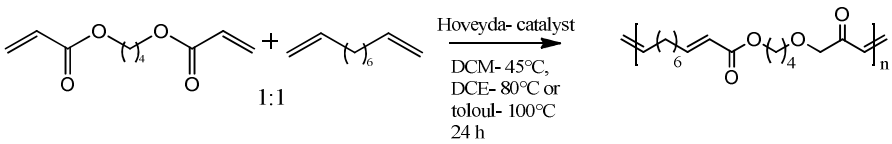
6.6.2 MEDA- functionalized Methyl-trans-2-octanoat (Lucerin initiated)

JB 37/38



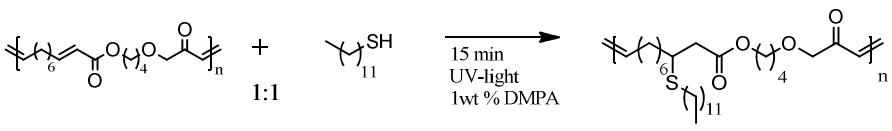
6.7 ALTMET Polymerization

6.7.1 ALTMET Polymerization of Butanediol- diacrylate and Decadiene, **JB 47**, **JB 53**

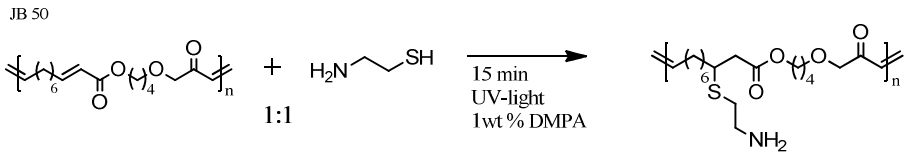


6.7.2 Functionalization of the ALTMET Polymer with Dodecanethiol

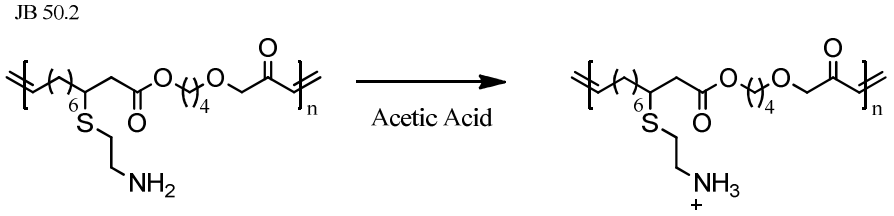
JB 48



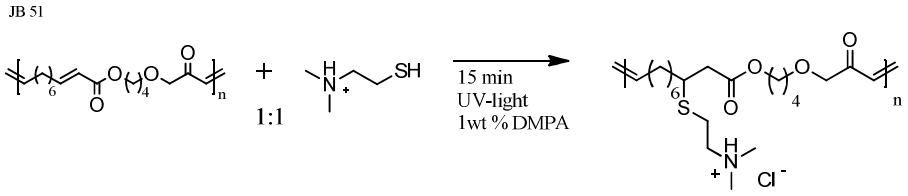
6.7.3 Functionalization of the ALTMET Polymer with Cysteamine



6.7.4 Protonated ALTMET Polymer



6.7.5 Functionalization of the ALTMET Polymer with MEDA



6.5 LIVE/DEAD Bacterial Viability Tests

L7012 LIVE/DEAD® BacLight™ Bacterial Viability Kit for microscopy and quantitative assays was purchased from Invitrogen. This kit was used to develop a simple and rapid method for the evaluation of the biocidal character of the synthesized polymers.

First of all a calibration curve had to be prepared. In support of the protocol which was provided by Invitrogen the following steps were carried out:

6.5.1 LB Media and Culture Preparation

Initially a LB Media was prepared. Therefore 15g/l trypton, 5 g/l yeast extract and 5 g/l NaCl were diluted in 1 l H_2O dest. and the pH was adjusted with a 10 % NaCl solution to a value of 7.0. Under sterile conditions the cooled LB Media was inoculated in a laminar flow cabinet with a single colony derived from a provided strain and kept on a shaker at 37°C.

After two days of growth phase, 1 mL each of the preculture was used to inoculate 100 mL fresh LB Media for the main culture. At all times two inoculated cultures and one sterile control were prepared to check the success of the bacterial growth and respectively if the autoclaving was working in order. The main cultures were allowed to grow to late log phase in the incubating room for two days again.

6.5.2 Preparation of a Calibration Curve

2 x 10 mL of the main culture were centrifuged at 5.000x g for 15 min. and the supernatant was removed. Afterwards the pellets were resuspended in 1 mL 0.9% NaCl each. One tube was incubated with 10 mL 0.9% NaCl to keep the bacteria alive and the other tube was incubated with 10 mL isopropyl alcohol to kill the bacteria, each for one hour at 37 °C in a

shaker. After that the bacteria was again centrifuged at 5000x g, 15 min. and resuspended in 0.9% NaCl. To insure that all residues were removed the centrifugation and resuspension step were repeated was more. Thereafter the optical density, OD was determined and adjusted to a value of OD_{670}= 0.06, which correlates to a number of $2x10^8$ bacteria/mL when working with *E. coli*. 0.9% NaCl was used for dilution.

These two samples were mixed together in different proportions.

Table 7: Various volumes of live and dead cell suspensions mixed together

Ratio of Live:Dead Cells	mL Live-Cell Suspension	mL Dead-Cell Suspension
0:100	0	2.0
10:90	0.2	1.8
50:50	1.0	1.0
90:10	1.8	0.2
100:0	2.0	0

6 µl of each dye (SYTO 9 and propidium iodide) were put together in a micro-centrifuge tube and filled with 2 mL H_2O dest.. The fluorescent staining solution was mixed properly and 100 µl of it were added to 100 µl of the bacterial suspension into 96 well plates. Again it was mixed thoroughly and incubated at room temperature in the dark. One blank (only NaCl + Dye) underwent the same steps as the samples and was automatically subtracted from the micro plate reader. All samples were made in duplicates at least.

Before the measurements could be performed, the gain of the instrument had to be adjusted. Therefore a positive (live bacteria + dye) and a negative (dead bacteria + dye) sample were prepared and for both wavelengths (530nm, 630nm) and certain values were obtained at 1% and 99%. The gain had to be in between 30000-250000 to get reliable results.

At an excitation wavelength of 485nm the fluorescence intensity for the two emissions was measured at 530nm (green emission, 1, SYTO 9, live bacteria) and respectively at 630nm (red emission, 2, propidium iodide, dead bacteria). The ratio between the emissions 1 and 2 was plotted versus the percentage of live bacteria (see table above).

6.5.3 Measurement of the Samples – Water-soluble Polymer

For all measurements the *E. coli* cultures have been grown the same way. 4x10mL were centrifuged and resuspended in 2mL 0.9% NaCl. At this point the OD was adjusted to a value 1.2, to achieve a final OD of 0.06, after a 1:10 dilution, for the measurement. 200 mg of a biocide polymer and 200 mg of polyethylene glycol, which does not act as a biocide, were diluted in 9 mL of 0.9% NaCl and each 1 mL of the bacterial suspension was added. All samples including blank (without polymer) and duplicates incubated for 1, 12 and 24 hours at 37°C in the incubating room. Thereafter 1 mL was taken out, centrifuged at 4.000x g for 15 minutes and resuspended in 1mL 0.9% NaCl. As from now the OD had a value of 0.06, with a concentration of the polymer 10mg/mL bacterial suspension.

Again each 100 mL of the bacterial suspension and 100 mL of the staining solution (6 µl SYTO 9 and 6 µl propidium iodide) were mixed in the micro plates and incubated for 15 minutes in the dark.

6.5.4 Measurement of the Samples – Water-insoluble Polymer

The sample preparation for the not water soluble polymer differed a bit to the already discussed procedure. 40 mg of the biocide polymer were dissolved in 4mL CH_2Cl_2+ MeOH (1:1) to achieve a 10mg/mL polymer suspension. Afterwards different volumes, 100 µl, 200

µl, 300 µl, 400 µl and 1000 µl, were put into fresh vials and the solvent was allowed to evaporate in the hood. To achieve completely solvent – free conditions, the vials were stored 4 more days in the vacuum drying cabinet. On the dry polymer films in the vials, 2 mL bacterial suspension, with OD 0.06, were added. The bacterial suspension was allowed to incubate at 37°C in the incubating room, while shaking for 1, 2, 4 and 24 hours.

The staining process was executed as described above for the water soluble polymer.

7. List of Figures

8. List of Schemes

9. List of Tables

10. Appendix

10.1. List of Abbreviations

AIBN	Azo*bis*isobutyronitrile
CD_3OD	Methanol (deuterated)
$CDCl_3$	Chloroform (deuterated)
$CHCl_3$	Chloroform
Cy	Cyclohexane
DE	Degree of overall elimination
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamid
DMSO	Dimethylsulfoxid
DMSO-d_6	Dimethylsulfoxide (deuterated)
DQ	Degree of overall quaternization
DS	Degree of overall substitution
DSC	Differential scanning calorimetry
E. coli	Escherichia coli
FT-IR	Fourier-transform infrared
Mn	Average-number molecular weight
LB	Lysogeny Broth
EE	Ethyl acetate
eq	Equivalent
Et_2O	Diethyl ether
FT-IR	Fourier Transform Infra Red
GPC	Gel Permeation Chromatography
HCl	Hydrochloric Acid

Mn	Number Average Molecular Weight
MW	Molecular Weight
NMR	Nuclear Magnetic Resonance
NaH	Sodium Hydride
OD	Optical Density
OFET	Organic Field-Effect Transistor
OLED	Organic Light Emitting Device
PDI	Poly Dispersity Index
Ph	Phenyl
ppm	Parts per million
ROMP	Ring Opening Metathesis Polymerization
RT	Room Temperature
sec	Secondary
THF	Tetrahydrofuran
TLC	Thin layer chromatography