UNIVERSITY OF CRETE
SCHOOL OF SCIENCES AND ENGINEERING
DEPARTMENT OF CHEMISTRY

Department of Materials Science and Technology-
Laboratory of Synthetic Biomaterials

Bachelor Thesis

"Synthesis of perfluorinated biopolymers"

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Contents

Περίληψη

Τα φθοριωμένα πολυμερή συναντώνται ευρύτατα σε πολλούς τομείς καθώς εμφανίζουν αυξημένη χημική αντοχή. Αυτό τους το προτέρημα αποτελεί ταυτόχρονα και μειονέκτημα καθώς η μικρή διαλυτότητα που έχουν στους περισσότερους οργανικούς διαλύτες και στο νερό καθώς και οι ιδιαίτερες ιδιότητές τους, δρουν περιοριστικά για το εύρος των εφαρμογών τους.

Ο σκοπός της συγκεκριμένης διπλωματικής εργασίας είναι η σύνθεση υπερφθοριωμένων βιοπολυμερών μέσω της προσέγγισης ATRP grafting from ώστε να αποδειχθεί η αποτελεσματικότητα της μεθόδου και ως προς τη σύνθεση φθοριωμένων βιοπολυμερών. Πιο συγκεκριμένα, η παρούσα διπλωματική εργασία είχε ως σκοπό την επιβεβαίωση προηγούμενων πειραματικών μελετών, καθώς και τη χρήση νέων μονομερών για την επέκταση της οικογένειας των υπερφθοριωμένων βιοπολυμερών.

Επιπλέον στόχος της διπλωματικής ήταν και η μελέτη της σύνθεσης φθοριωμένων βιοπολυμερών χρησιμοποιώντας άλλες μεθόδους πολυμερισμού όπως είναι η SET LRP και η photo-induced LRP. Αξίζει να σημειωθεί ότι πραγματοποιήθηκε μερικός χαρακτηρισμός των βιοπολυμερών μέσω φασματοσκοπίας ^{19} F- NMR.

Abstract

Fluorinated polymers have a variety of applications in the real life mainly due to their extreme chemical resistance. However, their solvophobicity and their unique properties also pose disadvantages on their use. Recently, a new class protein-fluorinated polymer bioconjugates was synthesized in the laboratory.

The purpose of this bachelor thesis was to create giant amphiphilic bioconjugates consisting of a protein and a fluorinated polymer using the classical ATRP grafting from approach for previously studied and a variety of new fluorinated monomers in order to prove the efficacy of classical ATRP. Our goal was to confirm and optimize previous experimental studies and expand with new monomers in order to fully comprehend the properties of this new category of biopolymers.

We additionally aimed at performing the synthesis of the same bioconjugates using two different types of polymerization SET LRP and photo-induced LRP as it was recently reported that these methods can be performed without deoxygenation, simply by eliminating the reaction headspace. It should be noted that ^{19}F- NMR was also employed for biopolymer characterization.

Acknowledgements

First of all, I would like to thank my supervisor Dr Kelly Velonia who gave me the opportunity for this cooperation and with her kindness, encouragement and patience shared valuable time in order to teach me all these methods and information needed for this thesis, I feel grateful.

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Eventually, I would like to dedicate this thesis to my beloved little sister, Sofia Grafanaki whom I believe empodies all things, enthusiast, beautiful and pure and gives me the strength in my life.

Abbreviations

ATRP	Atom Transfer Living Radical Polymerization
BSA	Bovine Serum Albumin
DFHA	2,2,3,3,4,4,5,5,6,6,7,7- Dodecafluoroheptyl acrylate
DMSO	Dimethyl Sulfoxide
EDTA	Ethylene diamine tetraacetic acid
GPC	Gel Permeation Chromatography
HFIPMA	1,1,1,3,3,3-hexafluoroisopropyl methacrylate
LRP	Living Radical Polymerization
Me_6TREN	Tris[2-(dimethylamino)ethyl]amine
MeCN	Acetonitrile
MWCO	Molecular Weight Cut- Off
NMR	Nuclear Magnetic Resonance
PAGE	Polyacrylamide Gel Electrophoresis
SET- LRP	Single Electron Transfer Living Radical Polymerization
TFEA	2,2,2-trifluoroethyl acrylate
TFEMA	2,2,2-trifluoroethyl methacrylate
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

1. Introduction

Perfluorinated organic compounds include of various fluorine-substituted carbon homologues. The great strength between the C-F bond offers an important resistance to oxidation or reduction reactions as well as to reactions with acids and bases and contributes to the stability of perfluorinated compounds. Because of their unique properties such as, low surface energy, thermal and chemical stability and surface activity, perfluorinated compounds have wide applications in the industrial and consuming area. They can be for example found in surfactants and repellants in food packaging and textile impregnation. [1,2] The prominent stability of these compounds results from their limited chemical activity.

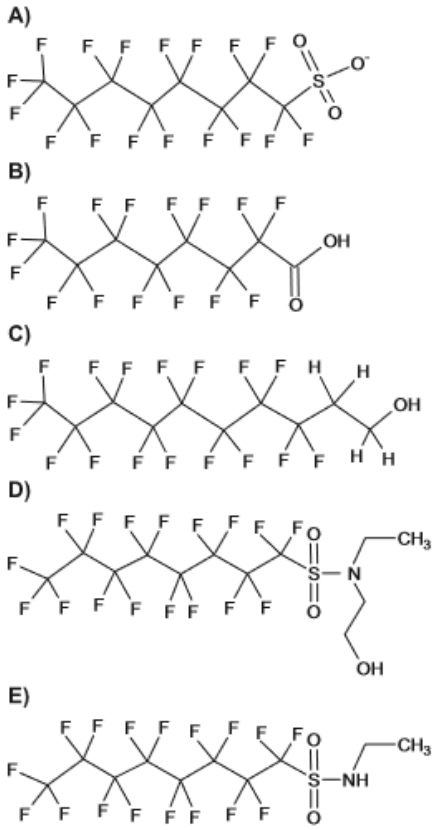


Figure 1. Structures of typical perfluorinated substances: (A): Perfluorooctane sulfonate, (B): perfluorooctanoate, (C): 1-hydroxyethane-2-perfluorooctanol, (D): N-ethyl perfluorooctane sulfonamidothanol, (E): N-ethyl perfluorooctane sulfonamide.[1]

Fluorinated compounds most often found in organisms and the environment are the perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). These molecules, their precursors, and compounds originating from them have a variety of applications ranging from textiles, paper, food packing materials and electronics, while the PFOA and related compounds can be used for the synthesis of other fluoropolymers such as polytetrafluoroethylene (PTFE).[1]

In conclusion, perfluorinated compounds have great scientific research interest as they can contribute in industry as well as in health care. Fluorine-substituted

compounds can be used in order to synthesize perfluorinated polymers and perfluorinated biopolymers and study their behavior.

1.1 Fluorinated polymers

The strongest bond a carbon can form is with a fluorine atom and this is something that makes fluorine substitution appealing for the expansion of pharmaceuticals and a wide range of materials.

Fluorine is the most electronegative atom of the periodic table and this property provides a less covalent and more electrostatic character to the C–F bond. This activates a relatively large dipole which interacts with other dipoles around and thus the preferred conformations of organo-fluorine compounds can often be interpreted by considering these electrostatic interactions. Fluorine possesses three lone pairs which are held tightly due to the high electronegativity of the atom and thus are reluctant to get involved in resonance or interact as hydrogen bonding acceptors. However, when the fluorine in a C–F bond does interact with its environment it is usually through electrostatic-dipole interactions. Because of their strength and character, substances containing atoms of C-F exhibit important properties such as stability, inertness, persistence, potential applications as antithermal, chemical proof, lubricant, water repellency and electric insulation materials.[3,4,6]

Polymers with low surface energy have a great commercial and industrial importance with target products such as non-wettable and nonstick materials, antifouling coatings and breathable textiles.

There are many ways to incorporate a fluorinated monomer within a polymer such as the fluorination of a polymer side chain, the use of fluorinated derivatives in order to reshape the polymer terminals and even the participation of a fluorinated unit in the main chain. Fluorinated polymers have also been synthesized following different approaches in the lab including group transfer polymerization, anionic polymerization, cationic polymerization, and free radical polymerization.[5] In order to handle appropriately the solvophobic fluorinated polymers, it is often necessary to synthesize hybrid compounds with no- fluorinated moieties to increase their solubility in common solvents. [4]

1.2 Amphiphilic molecules

Amphiphile molecules consist of a polar head group and a non-polar tail which makes them hydrophilic and hydrophobic at the same time.[7,8] The hydrophilic head group is water soluble and can be neutral, ionic or zwitterionic, while the hydrophobic tail is usually composed of one or more saturated or unsaturated alkyl chains.[8]

Amphiphiles can form spherical micelles, rodlike or spheroidal micelles, or even bilayer membranes. There a lot of parameters that play an important role

concerning the type and the shape of the superstructures formed by amphiphilic molecules such as the concentration, temperature, pressure, and, more importantly, molecular structure.

Typical amphiphilic molecules, "small" or molecular amphiphiles, have molecular weights on order of 500 Da, while "large" amphiphiles have 10-1000 times bigger size. The large amphiphiles are often created when a block of one type of an homopolymer attaches to another block, forming block copolymers with a variety of useful properties.[10]

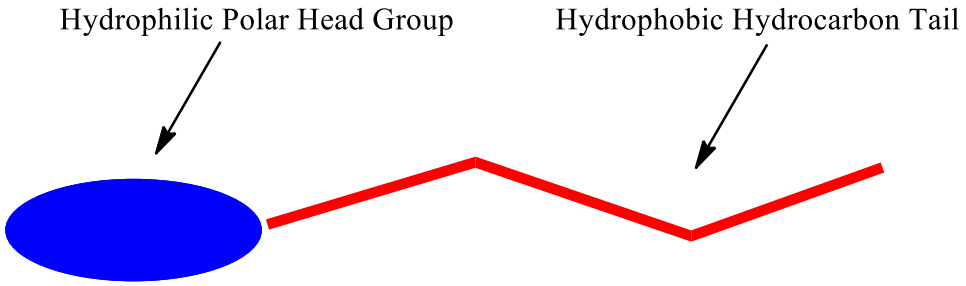


Figure 2. Structure of an amphiphilic molecule

Because of their properties, amphiphiles self-assembled into vesicles can be used as drug delivery systems and artificial cells and contribute to the development of effective delivery systems.[7,9]

1.3 Giant amphiphiles

There are three categories of amphiphile molecules i.e. molecular amphiphiles, super amphiphiles and giant amphiphiles, which differ in their molecular weight as well as their structure (Figure 3). In this thesis giant amphiphiles were the main subject of research.

Giant amphiphiles are molecules consist of a polar head group consisting of an enzyme or a protein connected to a non-polar polymeric tail have higher molecular weights than the other two categories. Usually, the shape of the amphiphilic molecule determines the forming aggregate.[11] These giant molecules can form nanosized assemblies, a property that contributes to their unique properties and thus they have a variety of applications and are a promise to the development of nanosciences.[8,9,10,11]

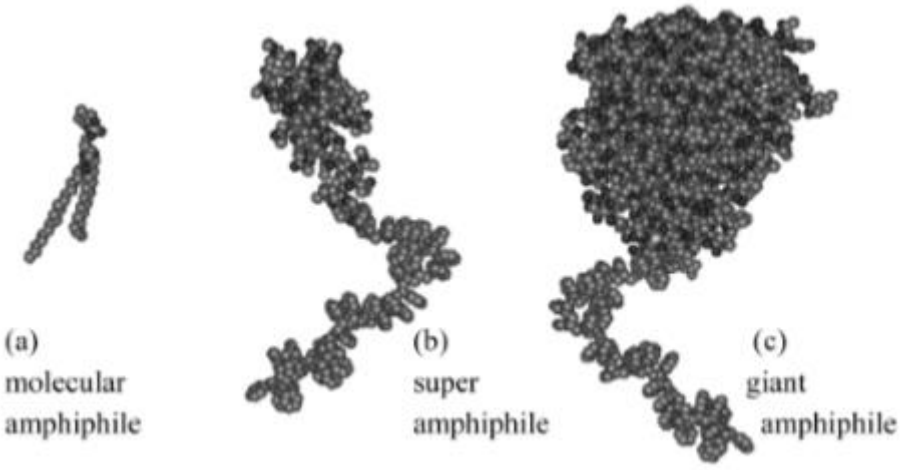


Figure 3. Schematic representation of (a) a molecular amphiphile with the low molecular weight ~ 1kDa, (b) a super amphiphile with medium to high molecular weight ~ 6kDa, (c) a giant amphiphile with the highest molecular weight ~ 40kDa.[(11)]

1.4 Self assembly

Self-assembly is the autonomous organization between two or more subunits into patterns or structures which are common are common throughout mother nature and technology. There various types of self assembly such as the static, dynamic, template and biological.

System	Type	Applications/importance
Atomic, ionic, and molecular crystals	S	Materials, optoelectronics
Phase-separated and ionic layered polymers	S	
Self-assembled monolayers (SAMs)	S, T	Microfabrication, sensors, nanoelectronics
Lipid bilayers and black lipid films	S	Biomembranes, emulsions
Liquid crystals	S	Displays
Colloidal crystals	S	Band gap materials, molecular sieves
Bubble rafts	S	Models of crack propagation
Macro- and mesoscopic structures (MESA)	S or D, T	Electronic circuits
Fluidic self-assembly	S, T	Microfabrication
"Light matter"	D, T	
Oscillating and reaction-diffusion reactions	D	Biological oscillations
Bacterial colonies	D, B	
Swarms (ants) and schools (fish)	D, B	New models for computation/optimization
Weather patterns	D	
Solar systems	D	
Galaxies	D	

Table 1. *Types of self-assembling systems and their applications (S: static, D: dynamic, T: template, B: biological).* [(13)]

In static self assembly (S), the generation of the desirable structure requires energy, but the product is stable while in dynamic the self assembly (D) the components require energy in order for the system to interact and form the ordered structure. In the templated self-assembly (T), interactions between the components and regular features in their surrounding area determine the resulting morphologies. Finally, in biological self assembly (B) the functions that are formed may be complex

and appear with great diversity.[13] Each methodology of self-assembly involves different systems which are depicted in Table 1 shown above.

All amphiphilic molecules undergo self-assembly in morphologies related to the monomeric units. The aggregate morphologies can be predicted using a theoretical model developed by the chemical engineer Jacob N. Israelachvili. This model, asserts that the packing parameter (P), is directly proportional to the volume (V) and inversely proportional to the mean cross-sectional (effective) head group surface area (a) multiplied with the length (l) of the molecule.[8]

$$P = \frac{V}{a * l}$$

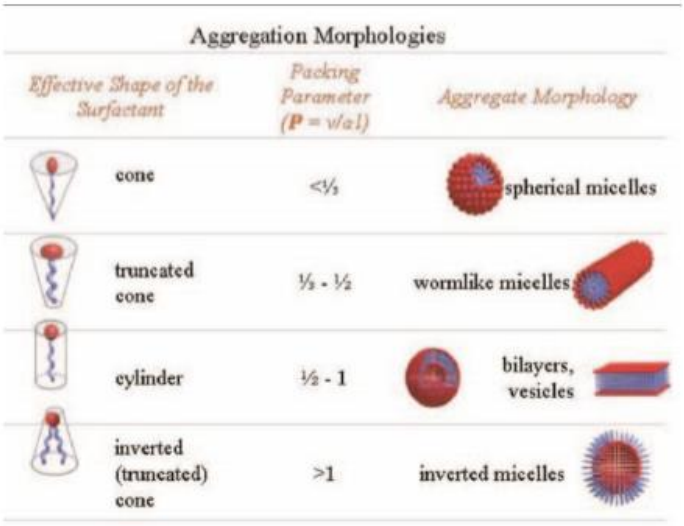


Figure 4. *Various supramolecular structures arising from molecular amphiphiles depending on the value of P factor..*[8]

1.5 ATRP

The creation of well-defined polymers with controlled architecture, composition and range of capabilities is of great importance in polymer chemistry. The evolution of controlled living radical polymerization methods has been a long-standing goal in polymer chemistry, due to the fact that a radical process is more tolerant in impurities and functional groups and consequently nowadays represents the main approach in the polymer synthesis.

One of the widely used methods, is Atom Transfer Radical Polymerization mediated by copper (or other metal) complexes which can give a successfully control the radical polymerization for the preparation of well-defined polymers with an accurate and desirable architecture. It allows to control, under mild reaction conditions, molecular weight and molecular weight distribution (Mw/Mn) using a wide range of monomers, such as styrenes, acrylates, (meth)acrylates and others.[14,15,16]

In ATRP the equilibrium between the activation process (generation of radicals, k_{act}) and the deactivation process (formation of alkyl halides, k_{deact}) is the key

for controlling polymerization as it determines the concentration of radicals as well as the rates of the polymerization, the termination and, as a result, polydisperity. Ordinarily, a low radical concentration which leads to the termination of the reactions is difficult to maintain because of the very small K_{ATRP}. Nevertheless, control of polymerization is achieved by both k_{act} and k_{dact}. A great control of the polymerization accompanied by a reasonable polymerization rate could be achieved only if both rate constants are large enough.[14]

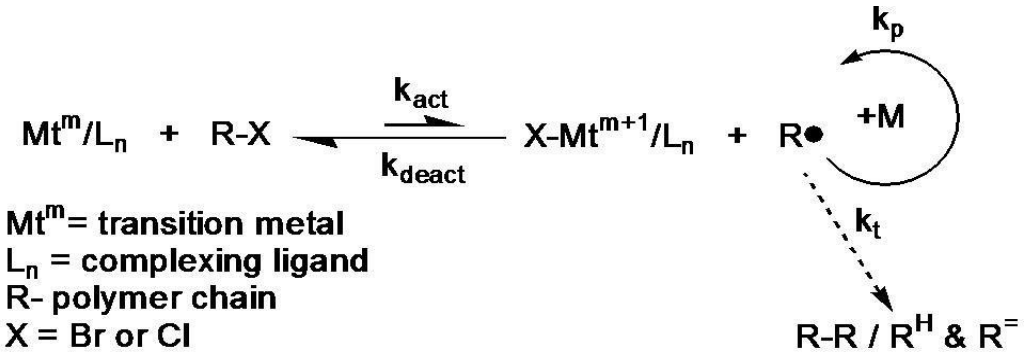


Figure 5. *Proposed mechanism for ATRP.*[14]

The mechanism of ATRP involves the abstraction of a halogen atom from an alkyl halide (R-X) by a transition metal complex (e.x. CuBr) as a catalyst, which undergoes redox (from Cu(I)-X/L to CuII-X(II)/L). The free radical formed after the homolytic cleavage can undergo propagation as in conventional free radical polymerization. Nevertheless, the halogen can be abstracted back from the metal by the free radical, reproducing the dormant species. These processes are very quick, and the equilibrium that is established favors the dormant species. In the first step, all chains can begin growth at the same time, and then, the actual concentration of free radicals is quite low, followed by a reduced amount of irreversible radical-radical termination, mostly when relatively low molecular weights are targeted.

The structure of the initiator which is necessary in ATRP polymerization plays an important role and its activity depends on the degree of initiator substitution (primary<secondary<tertiary), on the leaving atom/group (for methyl 2-halopropionates: Cl<Br<I), as well as on the radical stabilizing groups (-Ph ~ -COOR, -CN).[15,16]

1.6 SET- LRP

Single Electron Transfer - Living Radical Polymerization (SET-LRP) is a method providing constant and controlled polymerization due to its ability to meditate fast polymerization of activated and non-activated vinyl monomers, which is necessary in order to synthesize well defined and functional polymers and copolymers, with high molecular weights and high chain fidelity no matter the complexity of the starting monomers and other reagents or guest molecules present in the reaction mixture. [18]

The proposed mechanism for SET-LRP requires disproportionation of Cu(I) in polar solvents in order to generate the activated Cu(0) and Cu(II) and therefore, conditions such as high solvent polarity and unstable Cu(I) complexes are necessary. Cu(0) acts as an activator allowing for polymerization initiation from macroinitiators (including protein/peptide macroinitiators). Cu(0) is less susceptible to interference from protein/peptides, which contain polar groups, such as amines and acids, etc., that can complex with copper cations.

The fundamental mechanistic aspect involves achieving equilibrium between Pn-X and $Pn^{\bullet}$ (Figure 6) through a heterolytic outer sphere single electron- transfer process, wherein Cu(0) or other electron-donor species such as Cu_2O, Cu_2S, Cu_2Se, and Cu_2Te, donate an electron to Pn/ Pn-X resulting, depending on the structure of the monomer and initiator, in a radical- anion $[Pn/P\text{-}X]^{\bullet -}$, which degrades via a step-wise or a concerted pathway to $Pn^{\bullet}$ and X^-. During the SET event or afterwards, the Cu(I) species becomes associated with a N-containing ligand. Cu(I)X/L is rapidly disproportionated in coordinating solvents such as water, protic, dipolar aprotic, ionic liquids, and other polar solvents in the presence of several N- containing ligands to generate extremely reactive nascent atomic Cu(0) and $Cu(II)X_2/L$ species.[18] The $Cu(II)X_2/L$ generated via disproportionation of Cu(I)X/L is thought to perform the reverse outer sphere oxidation of $P/Pn^{\bullet}$ to P/Pn-X. This methodology facilitates an ultrafast LRP of various activated monomers such as acrylates and methacrylates, and of nonactivated monomers containing electron-withdrawing groups such as vinyl chloride at 25°C and below. The use of raw monomers containing radical inhibitors is also achievable. Because of their high reactivity, commercially available vinyl monomers are stabilized with free radical inhibitors to prevent polymerization during transport, storage, and handling.[17,18]

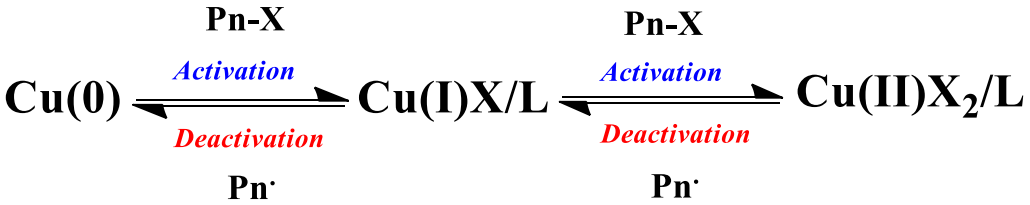


Figure 6. *SET-LRP mechanism*

In conclusion, SET LRP in comparison with other polymerization methods using a transition metal as a catalyst, can succeed a quick reaction, effective monomer conversion, providing polymers with functional chains and, high molecular weights all because of the near-complete suppression of termination reactions.[21]

1.7 Photo-induced Living Radical Polymerization

Polymer chemistry has been transformed in the last few decades as a variety of polymers can be synthesized with accurate control over the architecture as well as the molecular weight because of the contribution of living radical polymerization. A

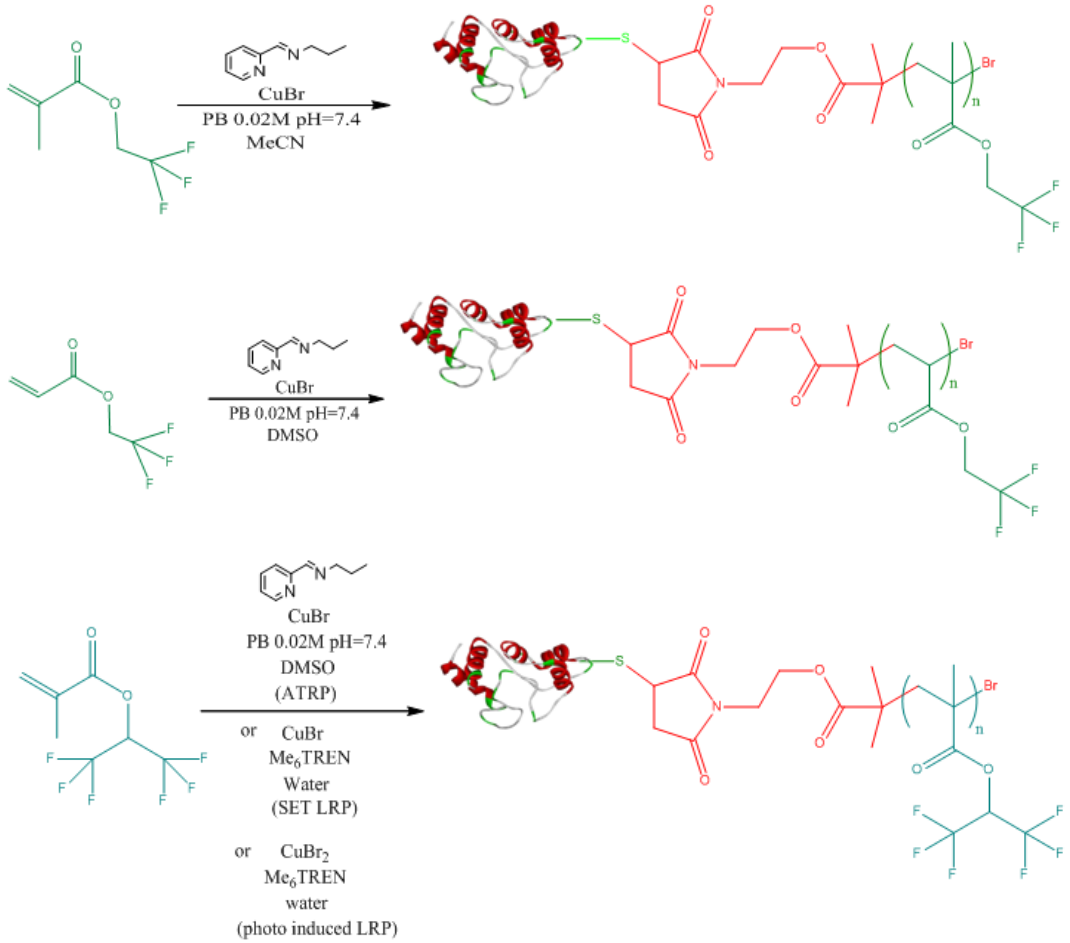
variety of macromolecules has been recently synthesized using rather simple systems has successfully such as the photoinduced living polymerization technique which is able to polymerize a variety of monomers, using ultralow concentrations of a photoredox catalyst and a low energy visible LED, as the light source.

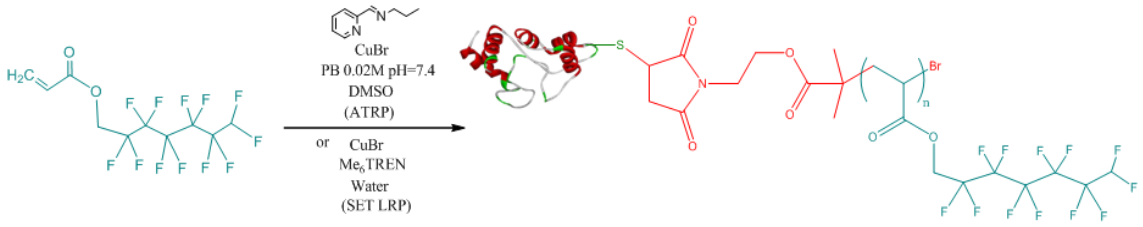
Photo induced polymerization is a new evolving technique with encouraging results in the synthesis of polymers using monomers, such as styrene, methyl acrylate, and methyl methacrylate, vinyl acetate (VAc), N-vinyl-pyrrolidinone, and N-vinylcarbazole.[19] Investigations in order to expand this encouraging method of polymerization, are using acrylates under a UV irradiation (λ_{max} ~ 360 nm), the presence of an aliphatic tertiary amine ligand (Me_6TREN), and low concentrations of $CuBr_2$, to yield poly(acrylates) with high end-group fidelity i.e. barely any change in their end group.[20]

The process of photo induced LRP polymerization method occurs when an aliphatic tertiary amine such as Me_6TREN undergoes photo-stimulation and the mediated $CuBr_2$ uses an outer sphere single electron transfer in order to split the C-X bond of an alkyl halide initiator. This has as a result an initiating radical which starts the polymerization. $Cu(Me_6TREN)X_2$ which is regenerated by the disproportionation of $Cu(Me_6TREN)X$, deactivates the polymerization when necessary.[21]

1.8 Purpose of the thesis

The purpose of the current thesis was to synthesize bioconjugates using perfluorinated protein-polymer conjugates, using Bovine Serum Albumin (BSA) as a model protein. In order to achieve this, the classical ATRP grafting from approach was used. Characterization of the products was performed using PAGE electrophoresis, Gel Permeation Chromatography (GPC) and ^{19}F- NMR. By this approach, we verified previous experimental studies[22] and further achieved the formation of novel bioconjugates using a new monomer containing twelve fluorine atoms. The resulting bioconjugates contained the different monomers 2,2,2-trifluoroethyl methacrylate, 2,2,2-trifluoroethyl acrylate, 1,1,1,3,3,3-hexafluoroisopropyl methacrylate and 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptyl acrylate, which are are depicted below:





***Scheme 1**: Scope of the thesis*

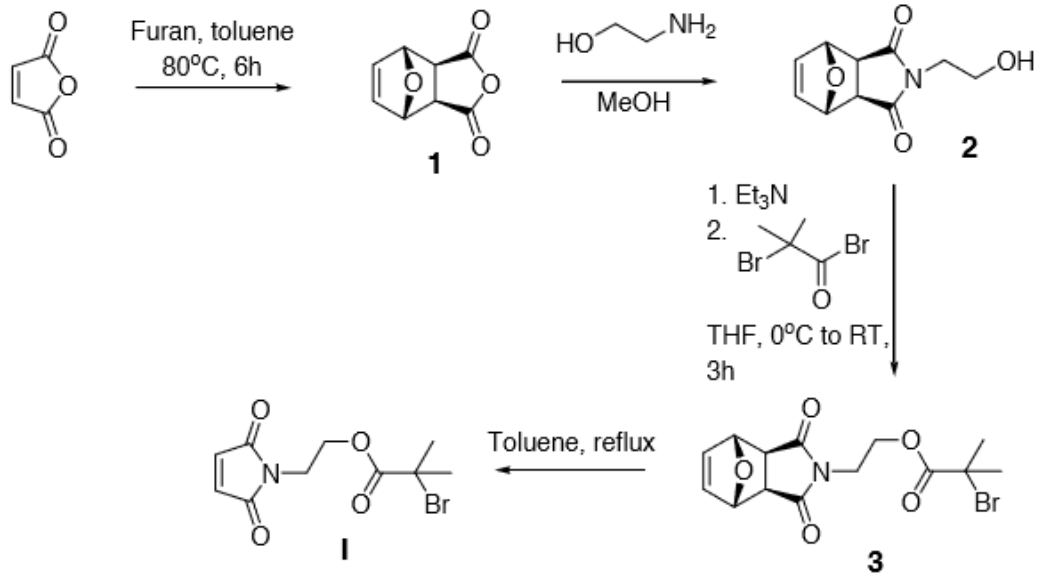
More importantly, two other grafting approaches, namely the SET-LRP and photo induced LRP, we used for the first time for the formation of perfluorinated biopolymers using the appropriate macroinitiator and a fluorinated monomer. We achieved polymerization using the monomers 1,1,1,3,3,3-hexafluoroisopropyl methacrylate and 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptyl acrylate by SET LRP while by the photo induced LRP proved to be unsuccessful in the synthesis of the 1,1,1,3,3,3-hexafluoroisopropyl methacrylate derived bioconjugate. The results appear to be encouraging concerning the SET LRP polymerization and should initiate new studies in the future.

The future goal is to further research methods which can successfully lead to perfluorinated biopolymers using simpler experimental protocols and to characterize them using ^{19}F- NMR a in order to study their behavior and applications.

2 Results and discussion

The purpose of this bachelor thesis is to create giant amphiphilic perfluorinated biopolymers using the classic ATRP, as well as the SET LRP and photo induced LRP grafting from approaches. In order to achieve this purpose, the synthesis of the ATRP initiator **I** which would be used in order to create the BSA macroinitiator **II** was necessary and the steps of its synthesis are listed below.

2.1 Synthesis of the maleimido- ATRP initiator I [23]



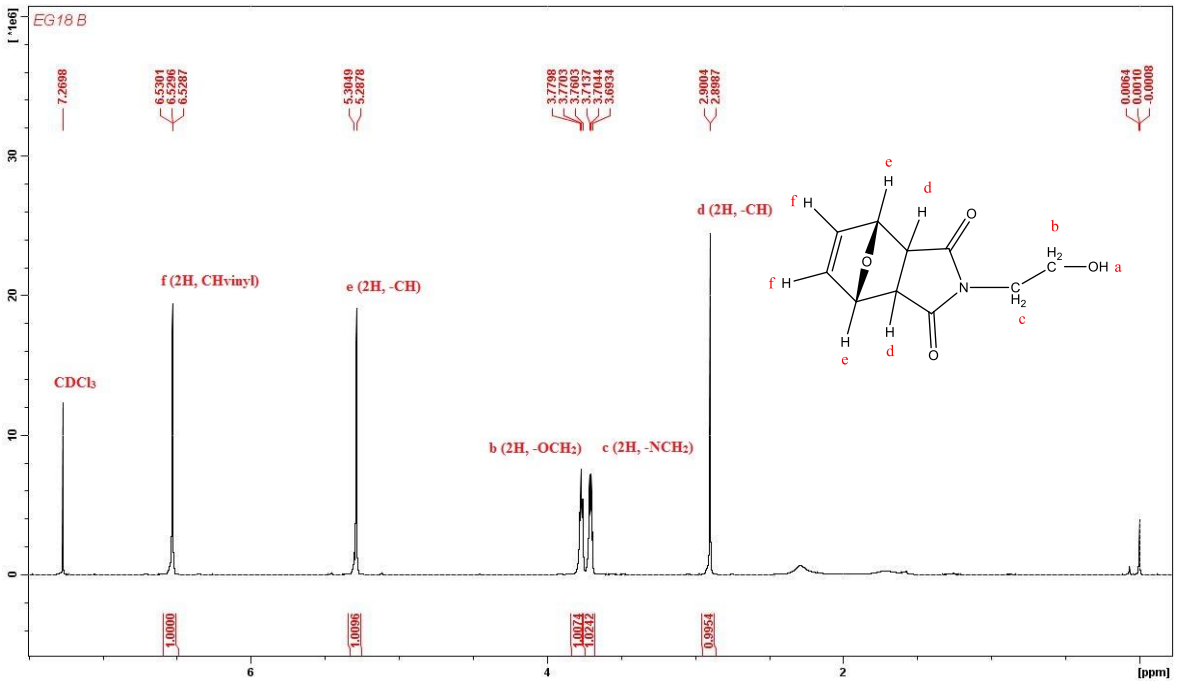
Scheme 2. *Synthesis of the maleimido- initiator I [23]*

In the first step we synthesized the unprotected maleimido-initiator **I** from the maleimido-protected initiator **3**, which was in stock in the lab. However, during this thesis, the synthesis of the alcohol **2**, and the subsequent steps described in *Scheme 2* were necessary and carried out three separate times. The experimental procedure for the typical synthesis of maleimido-initiator I is described in the following paragraphs.

2.1.1 Synthesis of 4-(2-Hydroxyethyl)-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2):[23]

The first step was to suspend the anhydride 1 (2.00 g, 12.00 mmol) in MeOH (50 mL) and the mixture cooled at 0 °C. A solution of ethanolamine (0.72 mL, 12.4 mmol) in 20 mL of MeOH was added dropwise (over ~30 min) and the resulting solution was stirred for 5 min at 0 °C. The mixture was left at ambient temperature for 30 minutes, and then refluxed for 4 h. After cooling the mixture to ambient temperature, the solvent was removed under reduced pressure and the white residue was dissolved in 140 mL of CH_2Cl_2 and washed 3 times with 90, 80 and finally 90 ml

of water. The organic layer was dried over $MgSO_4$ and filtered. After removal of the solvent under reduced pressure, a white-yellow residue was isolated (0.228 gr, 0.785 mmol, 22.8% yield). The yield of the reaction was very low in comparison to bibliography and so the aqueous phase was extracted again to recover the desirable quantity of the product.



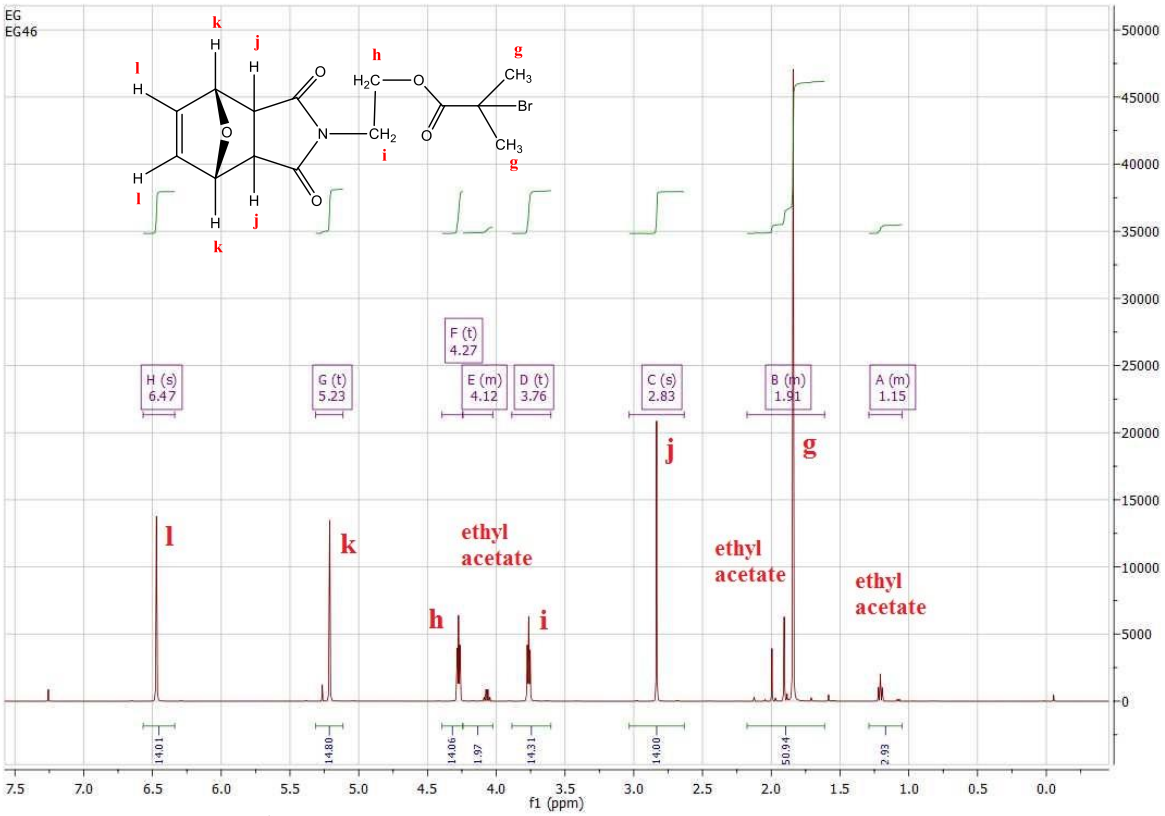
NMR Spectrum 1: *^1H NMR spectrum of 4-(2-Hydroxyethyl)-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione* ***2*** *(500 MHz, $CDCl_3$, 298 K): δ = ~1.90 (s, 1H, OH), 2.90 (s, 2H, CH), 3.69-3.71 (m, 2H, NCH_2), 3.76-3.78 (m, 2H, OCH_2), 5.29-5.30 (d, 2H, CH), 6.53 (d, 2H, CH_{vinyl}).*

2.1.2 Synthesis of 2-Bromo-2-methyl-propionic acid 2-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8en-4-yl)-ethyl ester (3):[23]

The alcohol **2** and Et_3N were suspended in THF and the mixture, that remained slightly turbid, was cooled to 0 °C. A solution of 2-bromo-isobutyryl-bromide in THF was added dropwise (~30 min) and the white suspension was stirred for 3 h at 0 °C and then subsequently overnight at ambient temperature. TLC *(Image 1)* revealed the appearance of the desired product. The ammonium salt was filtered off and the solvent removed under reduced pressure to give a pale-yellow residue.

In order to isolate the desirable maleimido-protected initiator **3**, a column chromatography (CC, SiO_2, petroleum ether/ ethyl acetate 1:5) was performed. The pale-yellow residue was dissolved in a 1:1 mixture of petroleum ether/ ethyl acetate. Although the initial residue was not completely dissolved in the selected solvent mixture, the maleimido- protected initiator 3 was recovered in pure form as a pale yellow solid.

Image 1: *TLC plates of the fractions eluted during the chromatographic separation of the reaction mixture revealing that the maleimido-protected initiator 3 was eluted in fractions 23-44 which were combined, placed in a round bottom flask and, upon removal of the solvent under reduced pressure, yielded the final product.*

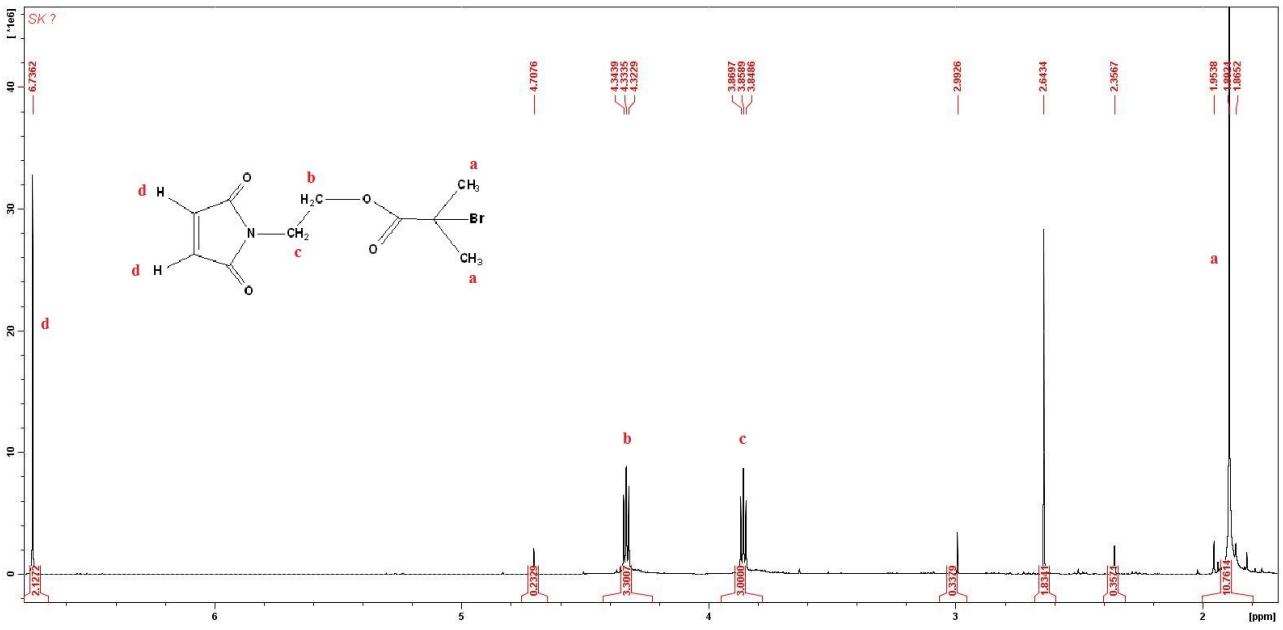


NMR Spectrum 2: *^{1}H NMR spectrum of 2-bromo-2-methyl-propionic acid 2-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8en-4-yl)-ethyl ester* ***3*** *(500 MHz, $CDCl_3$, 298 K): δ = 1.91 (m, 6H, CH_3), 2.83 (s, 2H, CH), 3.76 (t, 2H, NCH_2), 4.27 (t, 2H, OCH_2), 5.23 (t, 2H, CHO), 6.47 (t, 2H, CH_{vinyl}).*

2.1.3 Synthesis of 2-Bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester (I):[23]

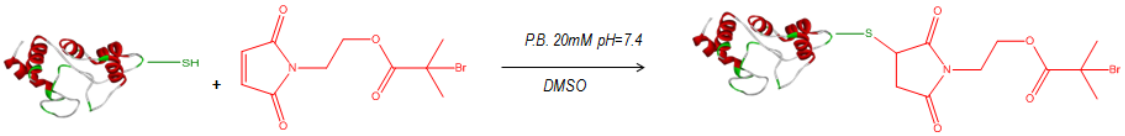
A solution of the maleimido-protected initiator **3** was dissolved in toluene and refluxed under nitrogen atmosphere overnight. The solvent was then removed under reduced pressure to give a pale-yellow residue which was characterized by ^{1}H NMR.

The analysis of the spectrum revealed the formation of the final product together with the presence of by-products in low concentrations.



NMR Spectrum 3: *^{1}H NMR spectrum of 2-bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester* ***I*** *(500 MHz, $CDCl_3$, 298 K): δ = 1.86-1.95 (s, 6H, CH_3), 3.84-3.87 (t, 2H, NCH_2), 4.32-4.34 (t, 2H, OCH_2), 6.74 (d, 2H, CH_{vinyl}).*

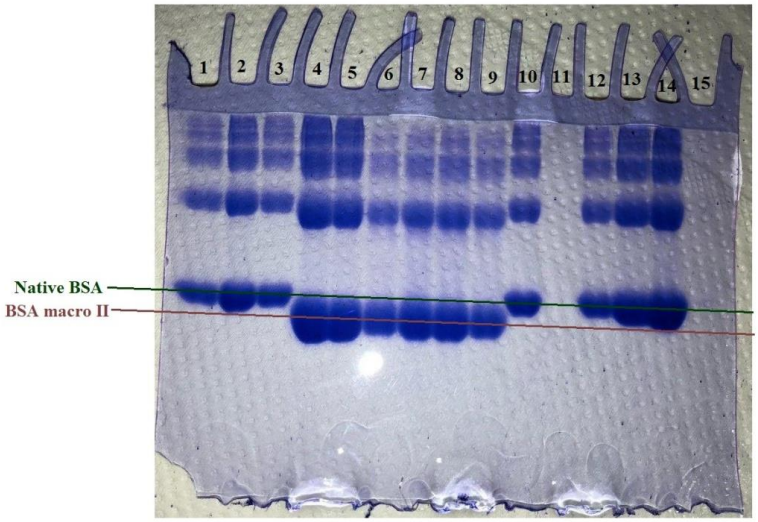
2.2 Synthesis of BSA- macroiniator II[23]



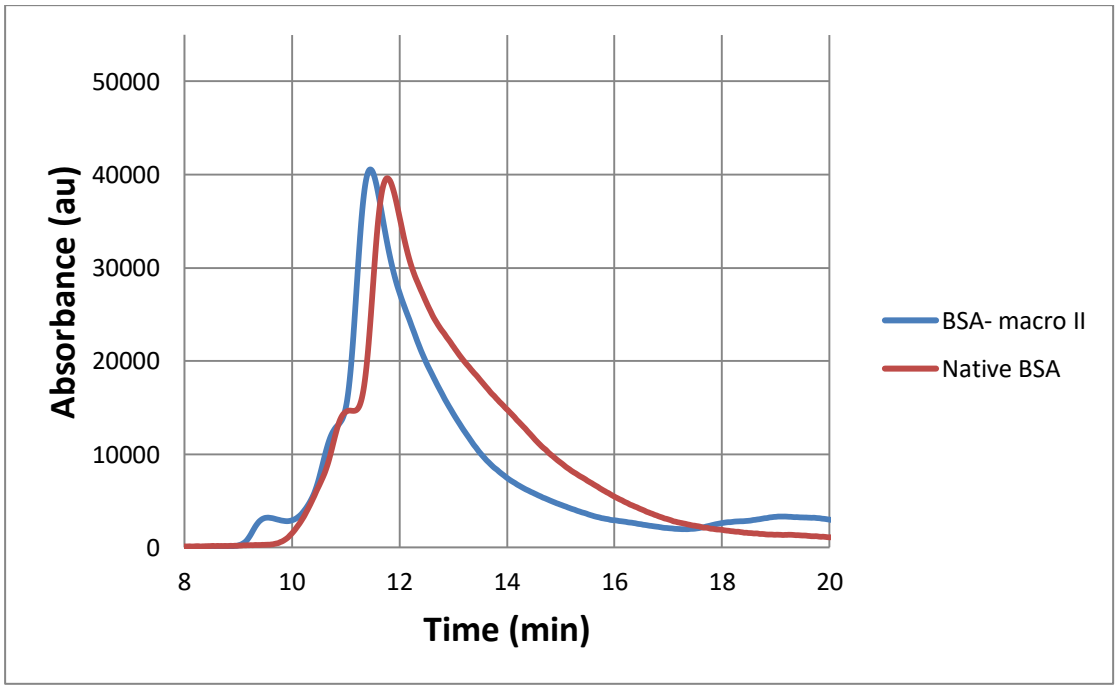
Sceme 3*. Synthesis of the BSA-macroinitiator II*

A 2 mL solution of the maleimido-initiator **I** in DMSO (150 mM, 40 eq.) was prepared and slowly added to a 21 ml solution BSA (0.36 mM, 1 eq.) in 20 mM phosphate buffer (pH 7.4). The reaction mixture was gently shaken for about two days at ambient temperature. In order to remove the excess of the ATRP initiator **I** and enrich the product, the mixture was extensively dialyzed against 20 mM phosphate buffer pH 7.4 using regenerated cellulose dialysis membranes with a MWCO of 25 kDa. The solution of the BSA macroinitiator **II** was consequently freeze dried and stored at -20 °C. The same experimental procedure was repeated three times during this thesis in order to synthesize the required amounts of BSA macroinitiator **II**. The products were stored either in solution or in solid form.

PAGE-electrophoresis under native conditions showed quantitative reaction (*Electrophoresis 1*), as the product, was observed to have the characteristic band lower than that band of native BSA. The formation of the bioconjugate was further supported by GPC (*GPC chromatogram 1*) which showed a small difference in the retention time of the BSA macroinitiator **II** compared to that of native BSA, as expected for a bio molecule with higher molecular weight.



Electrophoresis 1*: Electrophoretic behavior of BSA- macroinitiator II. Lane 10: Native BSA, Lane 7: BSA- macroinitiator II (8 µl), Lane 6: BSA- macroinitiator II (5 µl) All samples were visualized with Coomassie Brilliant Blue staining.*



GPC chromatogram 1*: GPC chromatogram of the BSA-macroinitiator **II** (blue line) and native BSA (red line).*

2.3 ATRP grafting of 2,2,2-trifluoroethyl methacrylate from BSA-macroinitiator II –Synthesis of BSA-poly(TFEMA):[23]

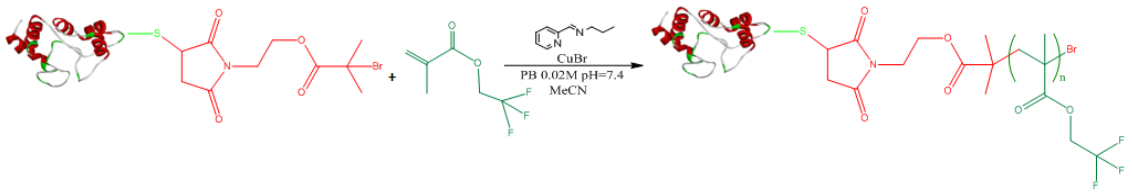


***Figure 1**. Synthesis of BSA-poly(TFEMA)*

2,2,2-trifluoroethyl methacrylate (1000 equiv.) and N-(Propyl)-2-pyridylmethanimine (70 equiv.) were placed in a Schlenk tube and dissolved in solvent (10.25 mL, 10% MeCN in 20 mM phosphate buffer pH 7.4). The mixture was deoxygenated by 5 freeze-pump-thaw cycles and finally sonicated for 5 minutes to emulsify the monomer. The polymerization was triggered by the cannulation of the monomer solution under nitrogen atmosphere in a second deoxygenated Schlenk tube containing the crystalline BSA-macroinitiator **II** (1 equiv.) and CuBr (41 equiv.) under N_2 atmosphere. A dark brown colour was immediately observed. The reaction mixture was stirred under N_2 atmosphere over night and the day after it was exposed to oxygen atmosphere in order to oxidize Cu(I) to Cu(II), and stirred for approximately another hour.

In order to eliminate the excess of monomer and other low molecular weight reagents (such as the ligand and copper), the mixture was extensively dialyzed against 20 mM phosphate buffer pH 7.4, ~2 mM EDTA, using regenerated cellulose dialysis membrane with a MWCO of 25 kDa. After enrichment, the product was characterized with PAGE-electrophoresis, GPC and ^{19}F-NMR

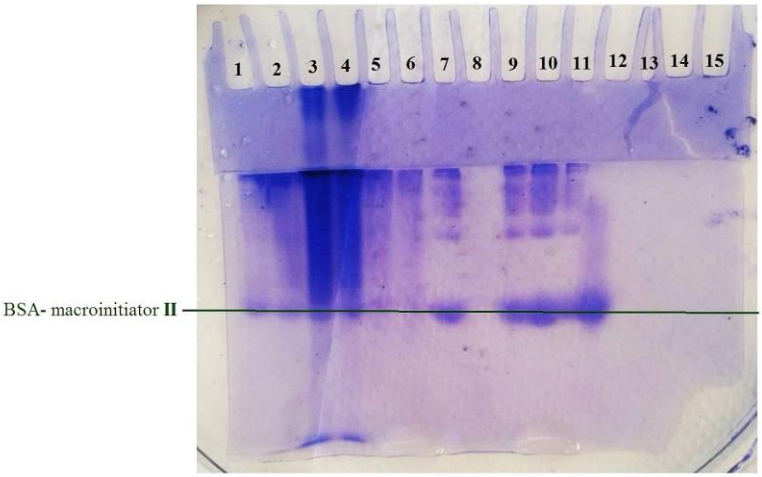
A first electrophoresis showed that the enriched bioconjugate sample had low concentration (*no visible bands*), so a second electrophoresis was performed after freeze drying the sample and creating a higher concentration solution- the results appear in *Electrophoresis 2*. The dim new dark blue band in the stacking gel well, migrating slowly throughout the stacking gel and the frontline of the resolving gel, indicated the formation of a new product.

The formation of the bioconjugate was further studied by GPC (GPC spectrum 2). The GPC chromatogram run with elution rate of 0.5 ml/min showed a peak with very short difference in the retention time as compared to that of native BSA. For this reason, a second GPC was acquired using 1 ml/min elution rate (GPC spectrum 3). However, once again the chromatogram revealed elution time the same as the elution time of native BSA. Nevertheless, these results, combined with electrophoresis that shows almost complete disappearance of the biomacroinitiator **II**, support the formation of the bioconjugate.

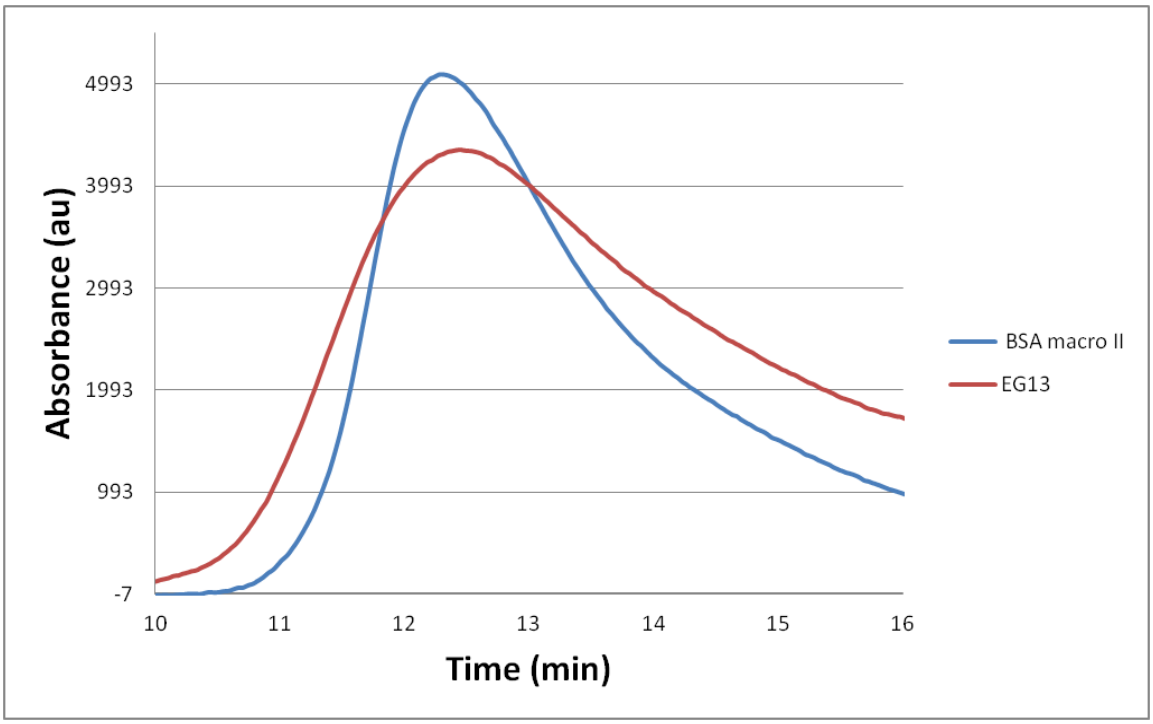
The product was also analyzed with ^{19}F-NMR. For the NMR studies, the solvent was removed under vacuum and the solid was resuspended in D_2O. In the ^{19}F

NMR spectrum (*^{19}F NMR Spectrum 1*) a new peak appeared in about -75 ppm which indicates the formation of the BSA-poly(TFEMA).

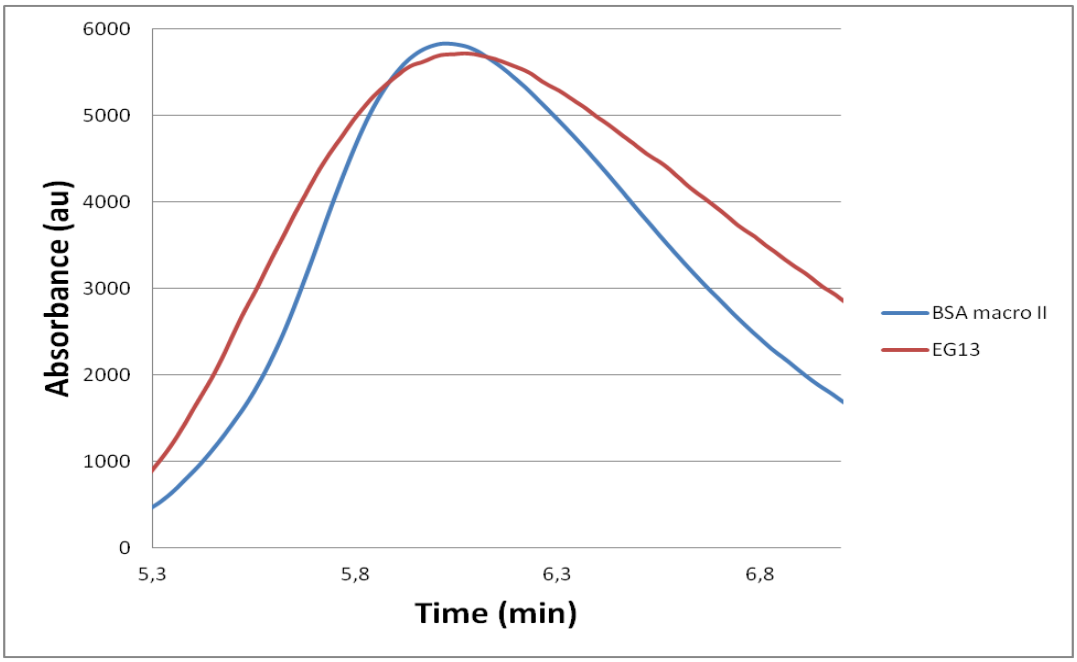
Since the synthesis of BSA-poly(TFEMA) was previously reported in the laboratory,[22] we placed no further effort to characterize this product.



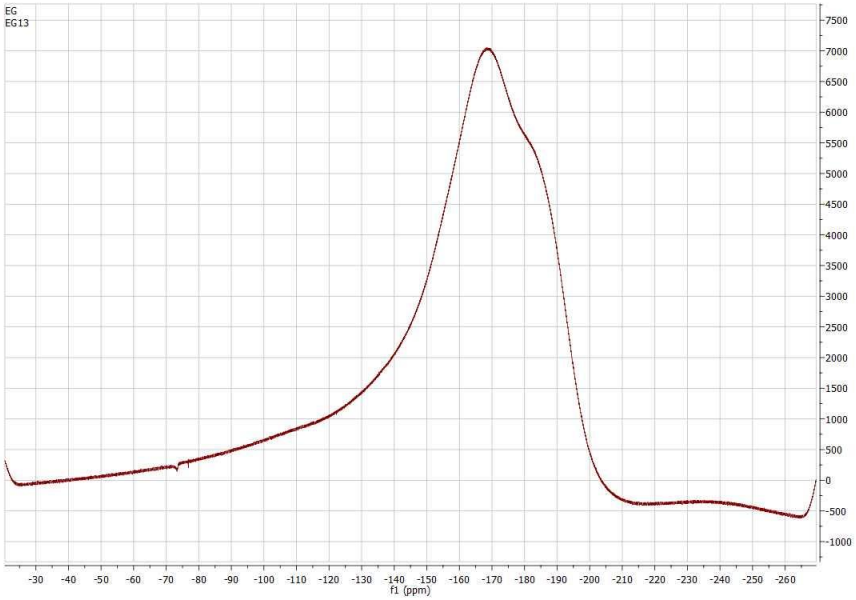
Electrophoresis 2*. Electrophoretic behavior of BSA-poly(TFEMA) under native conditions: Lane 5: BSA-poly(TFEMA) concentrated sample, Lane 6: BSA-poly(TFEMA) initial sample, Lane 7: BSA-macroinitiator* ***II****.*



GPC chromatogram 2*: GPC chromatogram of BSA macroinitiator* ***II*** *(blue line), bioconjugate BSA-poly(TFEMA) (red line) with elution rate 0.5ml/min.*



***GPC chromatogram 3**: GPC chromatogram of BSA macroinitiator **II** (blue line), bioconjugate BSA-poly(TFEMA) (red line) with elution rate 1ml/min.*



***^{19}F NMR Spectrum 1**: BSA-poly(TFEMA) ^{19}F NMR (500 MHz, D_2O, 298 K) δ=~ -75ppm.*

2.3.1 ATRP grafting of 2,2,2-trifluoroethyl acrylate (TFEA) from BSA-macroinitiator II - Synthesis of BSA-poly(TFEA): [23]

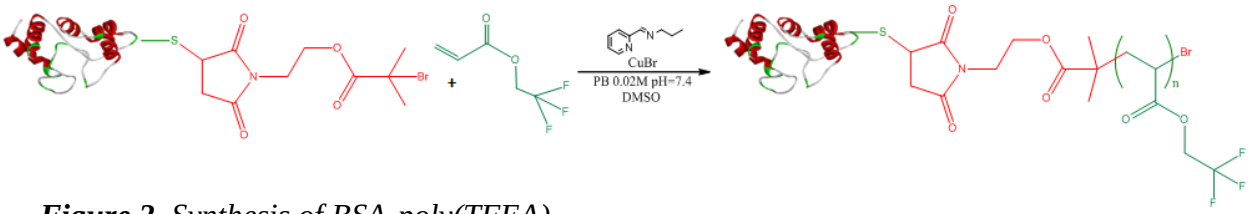
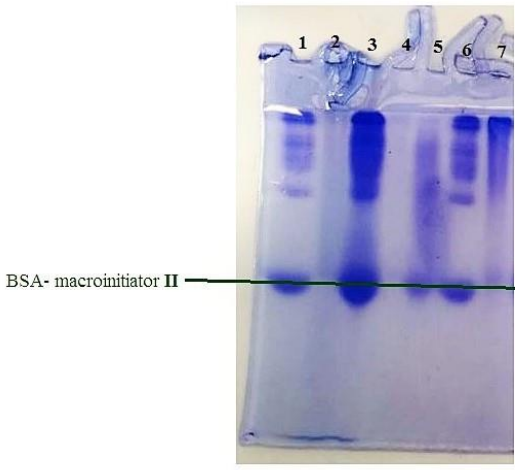


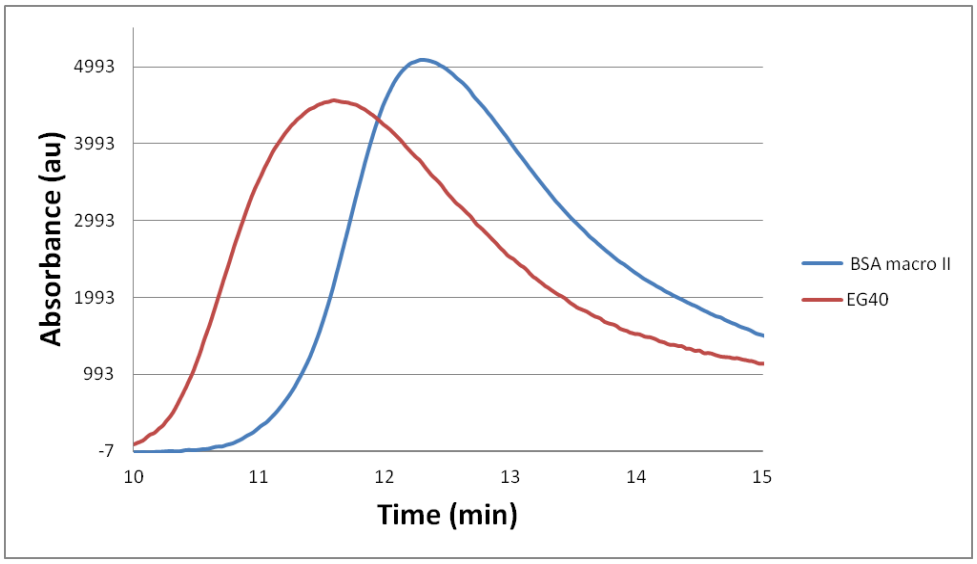
Figure 2. *Synthesis of BSA-poly(TFEA).*

The synthesis and isolation of BSA-poly(TFEA) was performed by using 2,2,2-trifluoroethyl acrylate (1000 equiv.) under the classical ATRP conditions analyzed in the previous paragraph. After enrichment with dialysis, the product was characterized with PAGE-electrophoresis (*Electrophoresis* 3). The new dark blue bands appearing in the stacking gel well and migrating slowly throughout the stacking gel and the frontline of the resolving gel, indicated the formation of a new product.

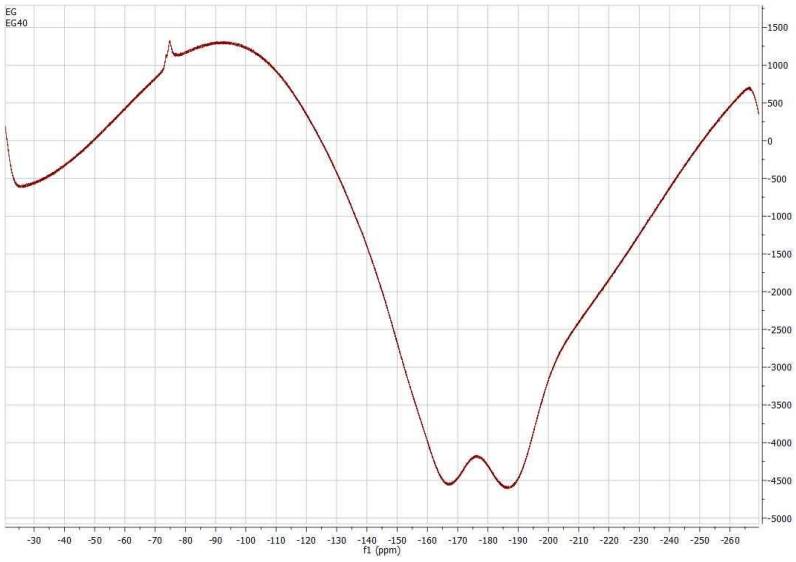
The formation of the bioconjugate was further supported by GPC (*GPC spectrum 2*). The GPC chromatogram revealed faster elution of the bioconjugate as compared to the elution of the native BSA. The new product was also analyzed with ^{19}F NMR, aftter removal of the solvent under vacuum and addition of D_2O. In the ^{19}F NMR spectrum (*^{19}F NMR Spectrum 2*) a new peak appearing at about -76 ppm indicated the formation of the BSA-poly (TFEA).



Electrophoresis 3**:** Electrophoretic behavior of BSA-poly(TFEA) under native conditions: *Lane 1*: BSA- macroinitiator **II**, *Lane 7*: BSA-poly(TFEA).



***GPC chromatogram 4**: GPC chromatogram of BSA macroinitiator **II** (blue line), bioconjugate BSA-poly(TFEA) (red line).*



***^{19}F NMR Spectrum 2**: BSA- poly(TFEA) ^{19}F NMR (500 MHz, D_2O, 298 K) δ=~ (-76 - 80)ppm.*

2.3.2 ATRP grafting of 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) from BSA-macroinitiator II – Synthesis of BSA-poly(HFIPMA): [23]

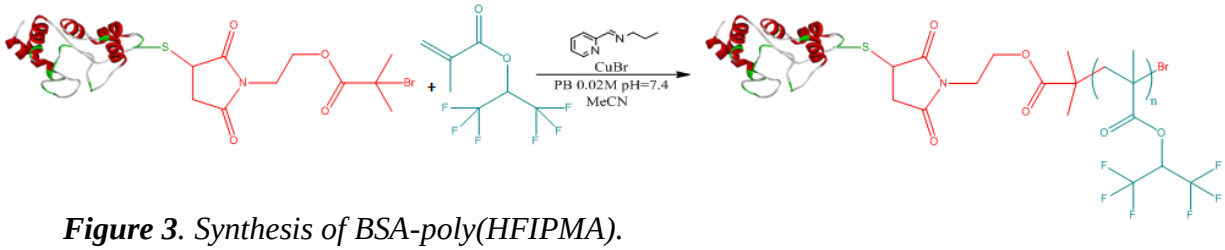
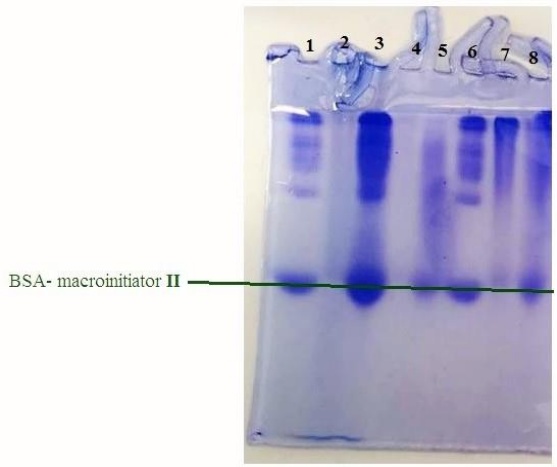
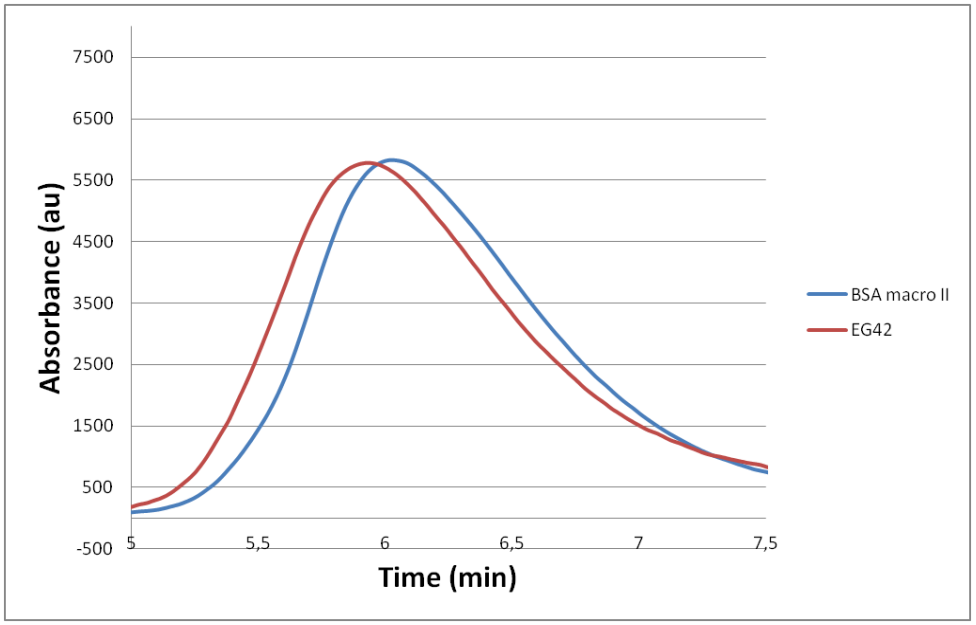


***Figure 3**. Synthesis of BSA-poly(HFIPMA).*

The synthesis and isolation of BSA-poly(HFIPMA) was performed by using 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (1000 equiv.) under the classical ATRP conditions analyzed in a previous paragraph. After enrichment with dialysis, the product was characterized with PAGE-electrophoresis (*Electrophoresis 4*). A new dark blue band in the stacking gel well migrating slowly throughout the stacking gel and the resolving gel, indicated the formation of a new product although the presence of the band corresponding to the BSA-macroinitiator indicated a non-quantitative reaction.



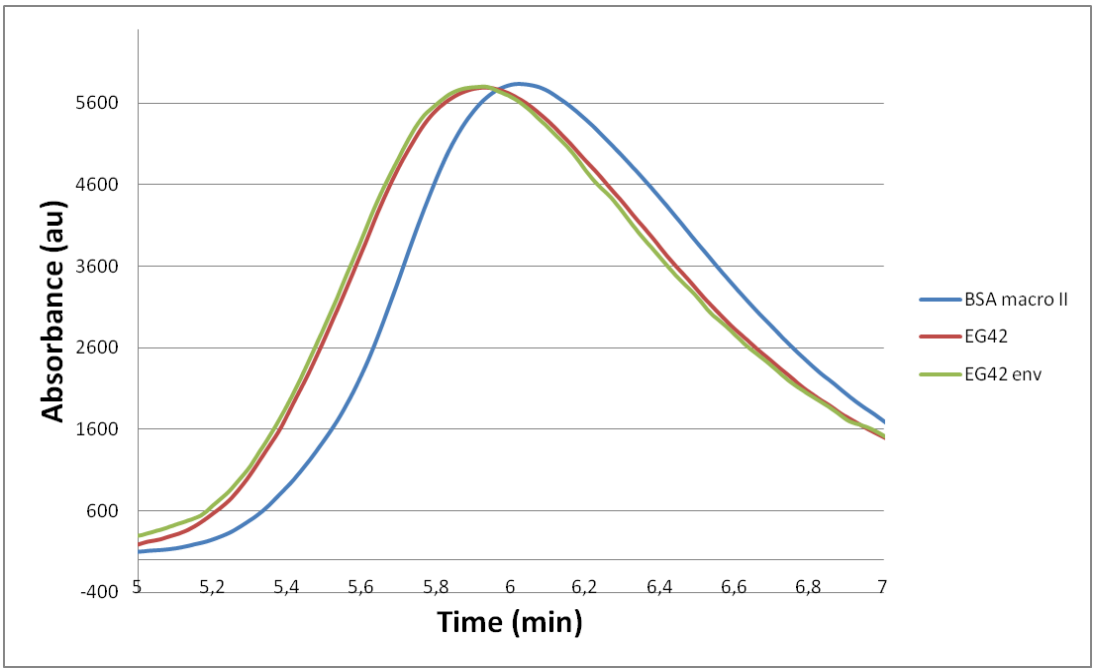
Electrophoresis 4: Electrophoretic behavior of BSA-poly(HFIPMA) synthesized under native conditions: *Lane 1*: BSA- macroinitiator **II**, *Lane 8*: BSA-poly(HFIPMA)



GPC chromatogram 5*: GPC chromatogram of BSA macroinitiator* ***II*** *(blue line), bioconjugate BSA-poly(HFIPMA) (red line), at 1 ml/min.*

The formation of the bioconjugate was further supported by GPC (*GPC chromatogram 5*). The GPC chromatogram revealed faster elution of the bioconjugate as compared to BSA- macroinitiator II and the presence of unreacted bioinitiator supporting the findings of electrophoresis.

Since perfluorinated poly-methacrylates have been reported to degrade through time-dependent hydrolysis, we stored this sample at environmental conditions (25 °C and sunlight) for over a week and restudied its chromatographic behavior. The GPC chromatograms revealed that the bioconjugate remained intact in comparison with a sample stored in the dark at 4 °C (*GPC chromatogram 6*).



GPC chromatogram 6: *GPC chromatogram of BSA macroinitiator II (blue line), bioconjugate BSA-poly(HFIPMA) (red line), bioconjugate BSA-poly(HFIPMA) after being at ambient temperature for over a week (green line), 1 ml/min.*

2.3.3 ATRP grafting of 2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptyl acrylate (DFHA) from BSA- macroinitiator II – Synthesis of BSA-poly(DFHA):[23]

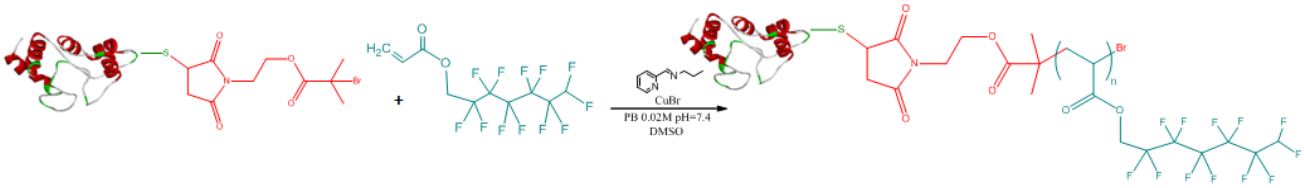
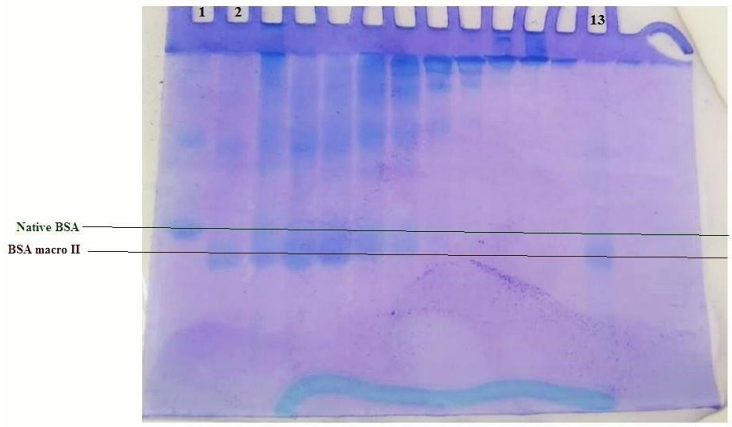


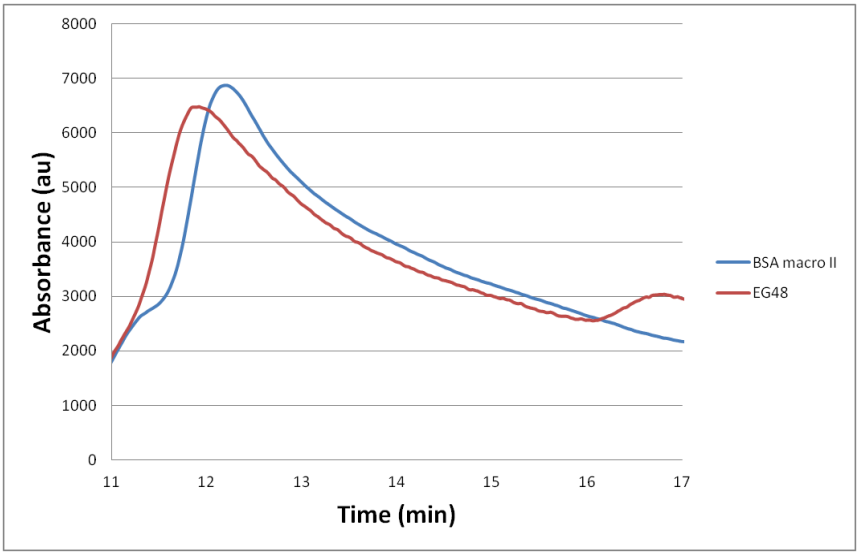
Figure 4. *Synthesis of BSA-poly(DFHA).*

The synthesis and isolation of BSA-poly(DFHA) was performed by using 2,2,3,3,4,4,5,5,6,6,7,7-δodecafluoroheptyl acrylate (1000 equiv.) under the classical ATRP conditions analyzed in a previous paragraph. After enrichment with dialysis, the product solution was opaque, a fact pointing to the formation of a colloidal solution that was not previously observed. The product was characterized with PAGE-electrophoresis (*Electrophoresis 5*). Avery dim blue band in the stacking gel migrating slowly throughout the stacking gel and the resolving gel, indicated the formation of a new product. The presence of the band corresponding to the BSA macroinitiator **II** indicated a non-quantitative reaction.



Electrophoresis 5: Electrophoretic behavior of BSA-poly(DFHA) under native conditions: *Lane 1*: native BSA, *Lane 2: BSA-macroinitiator II, Lane 13*: BSA-poly(DFHA).

The formation of the bioconjugate was further supported by GPC (*GPC chromatogram 7*). The GPC chromatogram revealed a faster elution of the bioconjugate as compared to the elution of the native BSA. The wide peak of the bioconjugate reveals the presence of unreacted biomacroinitiator, also shown by electrophoresis.



GPC chromatogram 7*: GPC chromatogram of BSA macroinitiator* ***II*** *(blue line), bioconjugate BSA-poly(DFHA) (red line).*

All characterization data from electrophoresis, GPC and ^{19}F NMR showed partial formation of the bioconjugates indicating that ATRP method didn't proceed as intended. A possible scenario for this effect can be drawn when taking into account possible hydrolysis of the ester group of the side chains of the poly(methacrylate) leading to water soluble bioconjugate mixtures.[23] The tendency of the ester bond to hydrolyze in aqueous solutions is strengthened by the presence of substituent fluorine atoms.

In order to successfully polymerize the fluorinated molecules and expand our studies we also studied a SET and photo induced living polymerization approach (LRP), which according to literature are efficient alternatives to controlled polymerization using simpler systems than the demanding and time consuming process of classical ATRP.

2.4 SET-LRP grafting of 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) from BSA-macroinitiator II – Synthesis of BSA-poly(HFIPMA):[20]

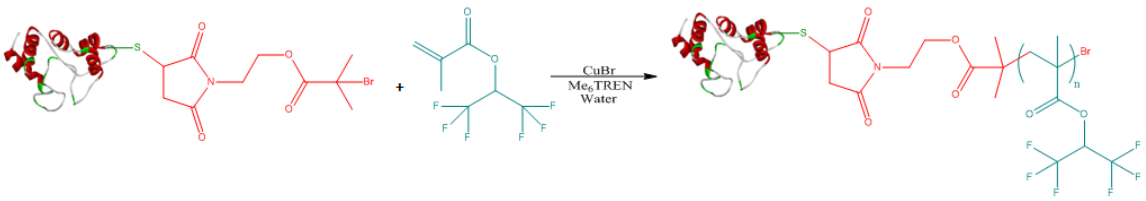
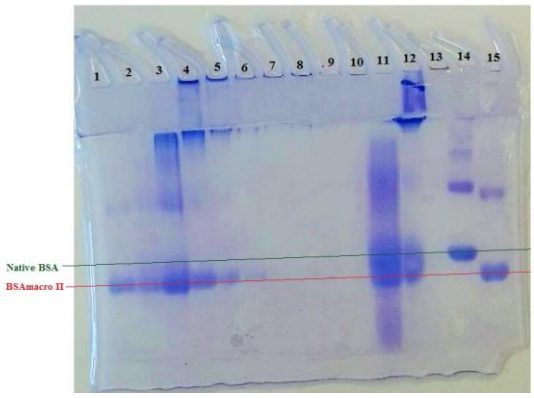


Figure 5. *Synthesis of BSA-poly(HFIPMA).*

Deionized water, a solution of CuBr in DMSO (40 equiv.) and a solution of Me_6TREN in DMSO (40 equiv.) were placed in a syringe and the mixture was cooled to 0^{o}C under gentle stirring for a few minutes in order to achieve disproportionation. The polymerization was triggered by the addition of the monomer 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (4000 equiv.) and BSA-macroinitiator **II** (1 equiv.).

A light blue colour was immediately observed. The reaction mixture was allowed to react for ~20 h under gentle stirring at ambient temperature. During this time, minute samples were withdrawn from the reaction mixture in order to monitor the progress of the reaction. The crude polymerization mixture was extensively dialyzed against 20 mM phosphate buffer 7.4, and 2% EDTA, using regenerated cellulose dialysis membrane with a MWCO of 10 kDa.

After enrichment, the product was characterized with PAGE-electrophoresis (*Electrophoresis 6*). A new dark blue band in the stacking gel well migrating slowly throughout the stacking gel and the resolving gel, indicated the formation of a new product at 40-minute reaction time. The presence of a band corresponding to the reactant BSA-macroinitiator **II** revealed partial formation of the bioconjugate at that time. From the electrophoresis it is obvious that no macroinitiator is left after ~120 minutes and all samples after this verify the formation and stability of the product.



Electrophoresis 6: Electrophoretic behavior of BSA-poly(HFIPMA). Samples from the reaction mixture were withdrawn during the reaction in order to follow the formation of the bioconjugate with time. Lane 1: The reaction mixture after the first 5 min, Lane 2: 20 min, Lane 3: 40 min, Lane 4: 60 min, Lane 5: 80 min, Lane 6: 100 min, Lane 7: 120 min, Lane 8: 140 min, Lane 9: 180 min, Lane 10: ~20 h, Lane 14: Native BSA, Lane 15: BSA macroinitiator **II**.

2.4.1 SET- LRP grafting of 2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptyl acrylate (DFHA) from BSA- macroinitiator II – Synthesis of BSA-poly(DFHA):[20]

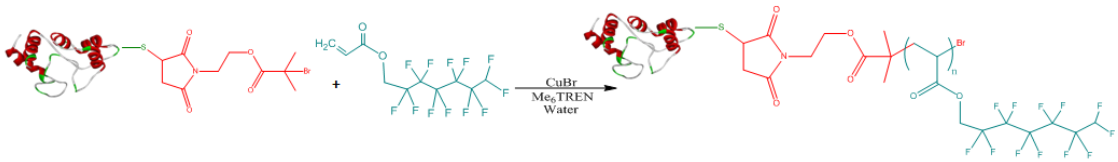
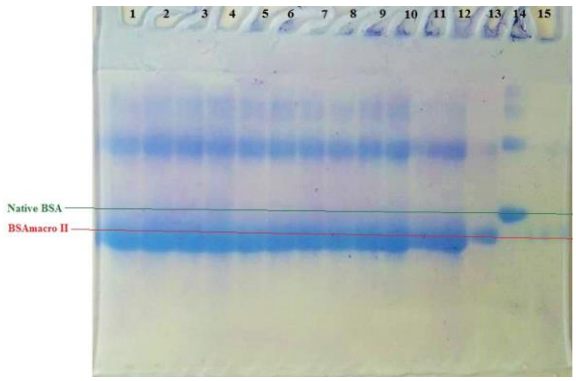


Figure 6. *Synthesis of BSA- poly(DFHA).*

Under the experimental conditions described above, 2,2,3,3,4,4,5,5,6,6,7,7-dodecafluoroheptyl acrylate (4000 equiv.) and the BSA-macroinitiator **II** (4000 equiv.), were utilized to synthesize BSA- poly(DFHA). The reaction was again followed with time. After enrichment, the product was characterized with PAGE-electrophoresis (*Electrophoresis 7*).



Electrophoresis 7: Electrophoretic behavior of BSA-poly(DFHA). Minute samples were withdrawn during the reaction in order to follow the formation of the bioconjugate with time. Lane 1: The reaction mixture after the first 40 min, Lane 2: 60 min, Lane 3: 80 min, Lane 4:

100 min, Lane 5: 120 min, Lane 6: 160 min, Lane 7: 3h, Lane 8: 4h, Lane 9: 5h, Lane 10: 6h, Lane 11: ~20 h, Lane 13: BSA macroinitiator **II**, Lane 14: native BSA.

The new dark blue bands in the stacking gel well indicated the formation of a new product after approximately five hours. Nevertheless, the presence of the band corresponding to the reactant BSA-macroinitiator **II** revealed partial formation of the bioconjugate after even 20 hours of reaction.

2.5 Photo-induced LRP grafting of 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) from BSA-macroinitiator II – Synthesis of BSA-poly(HFIPMA):[20]

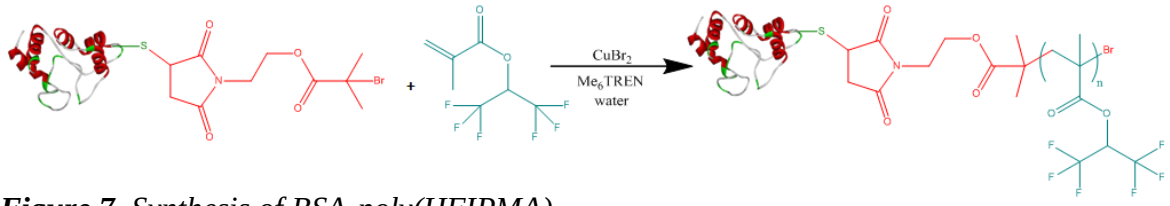
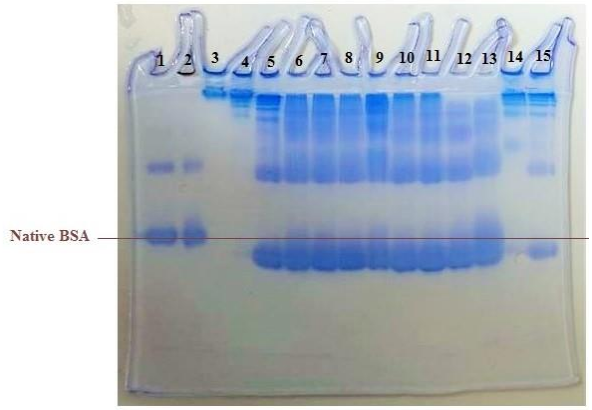


Figure 7*. Synthesis of BSA-poly(HFIPMA).*

A solution of BSA-macroinitiator **II** (1 eq.) and a stirrer were placed in a milliliter syringe. A solution of the monomer 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) (5082 eq.) in nanopure water (0.65 ml) was cooled in ice. A solution of Me_6TREN (2 eq.) and $CuBr_2$ (0.4 eq.) in 10 ml of nanopure water was also prepared. Firstly, the monomer solution was added to the initiator solution using a Gilson pipette. Finally, 100 μl of the aqueous solution containing $CuBr_2$ and Me_6TREN were quickly added in the syringe and air was removed. A light blue color was immediately observed and great care was given in order to avoid air bubbles and eliminate the reaction headspace as much as possible. The syringe was fitted with a rubber septum, placed under a UV nail lamp (365 nm) and the reaction mixture was left under stirring, for about 8 hours. The crude polymerization mixture was extensively dialyzed against 20 mM phosphate buffer 7.4, 2 mM EDTA using regenerated cellulose dialysis membrane with a MWCO of 10 kDa.



Electrophoresis 8: Electrophoretic behavior of the reaction aiming to synthesize BSA-poly(HFIPMA). *Lane 1*: Native BSA, *Lane 12*: BSA-poly(HFIPMA).

After the purification, the product was studied with PAGE-electrophoresis (*Electrophoresis 8*), which revealed absence of any bioconjugate. The presence of the band corresponding to the reactant BSA-macroinitiator **II** revealed that the bioinitiator was intact.

2.6 Conclusions

The purpose of this bachelor thesis was the synthesis of fluorinated biopolymers using the ATRP grafting from approach in order to reproduce previous experiments[21]as well as to expand the family of perfluorinated bioconjugates by using new fluorinated monomers.

During these experiments, the use other approaches of living radical polymerization was necessary in order to avoid the time consuming and demanding process of the classicak ATRP polymerization. For this reason, the newly developed SET LRP and photo induced LRP, where deoxygenation is not necessary were employed.

The first experiments aimed at synthesizing all of the necessary precursors necessary for the synthesis of the BSA-macroinitiator **II**. Previous experiments using the classical ATRP grafting from approach led to successfully synthesizing several of the previously reported bioconjugates, as well as a new bioconjugate containing twelve fluorine atoms on the repeating unit. These results clearly indicate that the classical ATRP approach under deoxygenated conditions, provides a secure way for the synthesis of fluorinated biopolymers.

However, in order to avoid the demanding process of the classical ATRP and improve the yields, we evaluated two novel approaches, namely the SET- LRP and the photo-induced LRP. The reactions in both cases were performed without any deoxygenation, via headspace elimination.

SET-LRP resulted in quantitative synthesis of the BSA-poly(HFIPMA) and showed lower yields, nevertheless encouraging results in the synthesis of other bioconjugates. Interestingly, the photo-induced LRP method, did not prove to be successful in the synthesis of any of the targeted fluorinated biopolymers.

It is necessary therefore, to fully study these approaches and optimize the conditions for fluorinated biopolymers. Furthermore, it is necessary to characterize the bioconjugates using ^{19} F- NMR and study their self assembly in order to obtain more information about their broad properties.

3. Experimental

3.1 Materials

All the reagents and materials used, originated from the companies Sigma- Aldrich, Fluka Chemica, Acros Chemicals, Fischer Scientific and Alfa Aezar and were used without further purification. The Cellulose Dialysis Membranes used for the purification of the bioconjugates had a MWCO of 10 kDa or 25kDa and were purchased by Spectrum Labs.

3.2 Analytical Techniques

Polyacrylamide gel Electrophoresis

Page Electrophoresis under standard non-denaturing conditions, was held in 4% stacking gel and 10% resolving gel, while the system that used were the Mini-PROTEANT Tetra Cell of BIO-RAD. The samples were dissolved in Premixed Sample Buffer for Native Page of BIO-RAD and were visualized with Coomasie Briliant Blue.

Gel Permeation Chromatography (GPC)

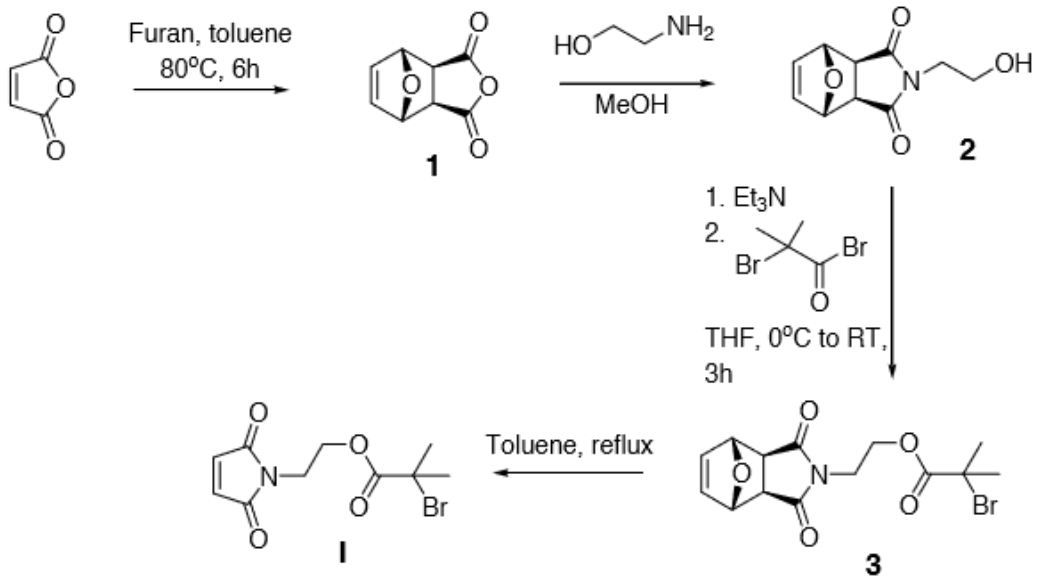
Gel permeation chromatography was used in order to characterize the bioconjugates using the Shimadzu VP HPLC system, which consist of a DGU-14A solvent degasser, an LC-10AD pump, a CTO-10A column oven, a SIL-10AD auto-injector, a RID-10A refractive index detector and a SPD-10A Shimadzu UV- Vis spectrometer. In the course of chromatography a 0.005 M phosphate buffer (pH 7.4) with 10% MeCN was used as the mobile phase A 0.35 mM BSA in 20mM phosphate buffer solution was used as a standard to whom all the other samples were compared.

Nuclear Magnetic Resonance (NMR)

For the characterization of the products NMR spectra (^{1}H- NMR, ^{13}C- NMR ^{19}F-NMR) were recorded on a Bruker 500 MHz spectrometer system. In order to describe the multiplicity of the peaks the following abbreviations were used: s: singlet, d: doublet, t: triplet, m: multiplet.

3.3. Synthetic Procedures

3.3.1 Synthesis of the maleimido- ATRP initiator I



Scheme 1. *Synthesis of the maleimido- initiator I.*

Synthesis of 4-(2-Hydroxyethyl)-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8-ene-3,5-dione (2):

The first step was to suspend the anhydride 1 (2.00 g, 12.00 mmol) in MeOH (50 mL) and the mixture cooled to 0 °C. A solution of ethanolamine (0.72 mL, 12.4 mmol) in 20 mL of MeOH was added dropwise (over ~ 30 min) and the resulting solution was stirred for 5 min at 0 °C. The mixture left at ambient temperature for 30 minutes, and then refluxed for 4 h. After cooling the mixture to ambient temperature, the solvent was removed under reduced pressure and the white residue was dissolved in 140 mL of CH_2Cl_2 and washed 3 times with 90 x 80 x 90 ml of water. The organic layer was dried over $MgSO_4$ and filtered. By the removal of the solvent under reduced pressure, the white-yellow residue appeared. (0.228 gr, 0.785 mmol, 22.8% yield). The yield of the reaction was very low in comparison with the bibliography and so the elaboration of aqueous phase took place in order to maintain the desirable quantity of the product.

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = ~1.90 (s, 1H, OH), 2.90 (s, 2H, CH), 3.69-3.71 (m, 2H, NCH_2), 3.76-3.78 (m, 2H, OCH_2), 5.29-5.30 (d, 2H, CH), 6.53 (d, 2H, CH_{vinyl}).

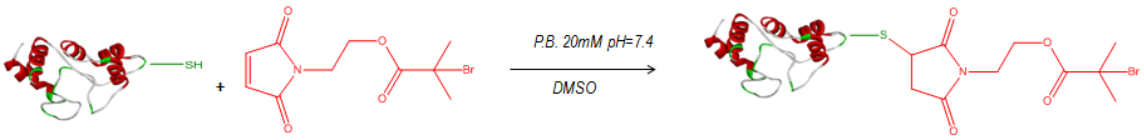
3.3.2 Synthesis of 2-Bromo-2-methyl-propionic acid 2-(3,5-dioxo-10-oxa-4-aza-tricyclo[5.2.1.02,6]dec-8en-4-yl)-ethyl ester (3)

The alcohol 2 (1.055 gr, 3.63 mmol) and Et_3N (0.55 ml, 4 mmol) were suspended in THF (57 ml) and the mixture, that remained slightly turbid, was cooled to 0 °C. A solution of 2-bromo-isobutyryl-bromide (0.47 ml, 3.81 mmol) in THF (13 ml) was added dropwise (~ 30 min) and the white suspension was stirred for 3 h at 0 °C and then subsequently overnight at ambient temperature. TLC revealed the appearance of the desired product. The ammonium salt was filtered off and the solvent removed under reduced pressure to give a pale-yellow residue.
In order to separate the desirable maleimido-protected initiator 3, a column chromatography (CC, SiO_2, petroleum ether/ ethyl acetate 1:5) was prepared and the pale- yellow residue was dialysed with a mixture of solvents petroleum ether/ ethyl acetate 1:1. Although the residue was not totally dialysed, was purified by that column to give the desirable product, maleimido- protected initiator 3 as a pale yellow solid.
^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 1.91 (m, 6H, CH_3), 2.83 (s, 2H, CH), 3.76 (t, 2H, NCH_2), 4.27 (t, 2H, OCH_2), 5.23 (t, 2H, CHO), 6.47 (t, 2H, CH_{vinyl}).

3.3.3 Synthesis of 2-Bromo-2-methyl-propionic acid 2-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-ethyl ester (I)

A solution of the maleimido-protected initiator 3 was suspended in toluene and heated to reflux under nitrogen atmosphere over night. Finally, the solvent was removed under reduced pressure to give the ATRP Initiator I (0.26 gr, 0.9 mmol).
^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ = 1.86-1.95 (s, 6H, CH_3), 3.84-3.87 (t, 2H, NCH_2), 4.32-4.34 (t, 2H, OCH_2), 6.74 (d, 2H, CH_{vinyl}).

3.3.4 Synthesis of the BSA- macroiniator II



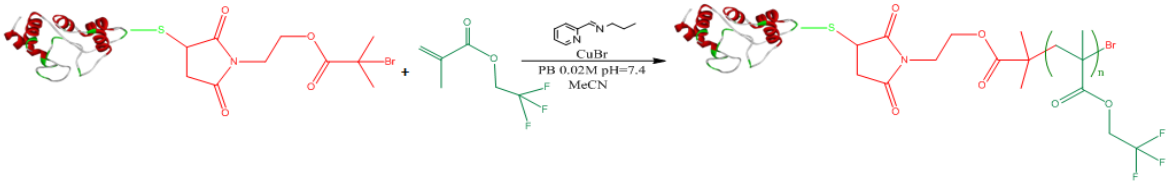
Scheme 2. *Synthesis of BSA- macroinitiator* ***II***

A solution of the maleimido-ATRP initiator **I** in DMSO (150 mM, 2 mL, 40 eq.) was prepared and slowly added in a 21 ml solution of BSA (0.36 mM, 1 eq.) in 20 mM phosphate buffer (pH 7.4). The reaction mixture was gently shaken for about two days at ambient temperature. In order to eliminate the excess of the ATRP initiator I, the mixture was extensively dialyzed against 20 mM phosphate buffer pH 7.4 using

regenerated cellulose dialysis membranes with a MWCO of 25 kDa. After that, the solution of the BSA macroinitiator **II**, was freeze dried. Samples of freeze-dried bioconjugate **II** were stored at -20 °C.

The same procedure was repeated three times during the thesis in order to synthesize the required BSA macroinitiator **II.** The initiator was either freeze-dried or stored in solution at 4 °C.

3.3.5 ATRP grafting of 2,2,2-trifluoroethyl methacrylate from BSA-macroinitiator II –Synthesis of BSA-poly(TFEMA)



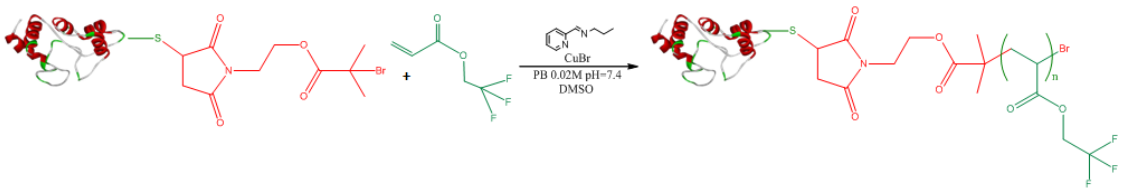
Scheme 3. *Synthesis of BSA-poly(TFEMA).*

2,2,2-trifluoroethyl methacrylate (0.128 mL, 0.0009 mmol, 1000 equiv.) and N-(Propyl)-2-pyridylmethanimine (0.0095 mL, 0.064 mmol, 70 equiv.) were placed in a Schlenk tube and dissolved in 10.25 mL 20 mM phosphate buffer pH 7.4, 10% MeCN. The mixture was deoxygenated with 5 freeze-pump-thaw cycles and sonicated for 5 minutes to emulsify the monomer prior to use. The polymerization was triggered by cannulation of the monomer solution under nitrogen atmosphere in a second deoxygenated Schlenk tube containing the crystalline BSA-macroinitiator **II** (~60 mg, 0.0009 mmol, 1 equiv.) and CuBr (5.3 mg, 0.037 mmol, 41 equiv.) under N_2 atmosphere. A dark brown colour was immediately observed. The reaction mixture was stirred under N_2 atmosphere over night (~15 hours) after which it was exposed to oxygen atmosphere, in order to oxidize Cu(I) to Cu(II), and stirred for approximately another 1 hour.

The crude polymerization mixture was purified by 6 dialysis. Initially 2 dialysis against 5 mM phosphate buffer 7.4 and EDTA for 2 hours, then against 20 mM phosphate buffer 7.4 and EDTA over night. The next day 2 dialysis against 20 mM phosphate buffer 7.4 and EDTA for 2 hours and the last dialysis against 20 mM phosphate buffer 7.4 for 2 hours, carried out. For dialysis, regenerated cellulose dialysis membranes with MWCO 25 kDa were used. After the enrichment, the product was characterized with PAGE-electrophoresis, GPC. Fraction of the product was freeze-dried, dissolved in D_2O and studied with ^{19}F NMR.

^{19}F NMR (500 MHz, D_2O, 298 K): δ = -75 ppm (s, 3F, CF_3).

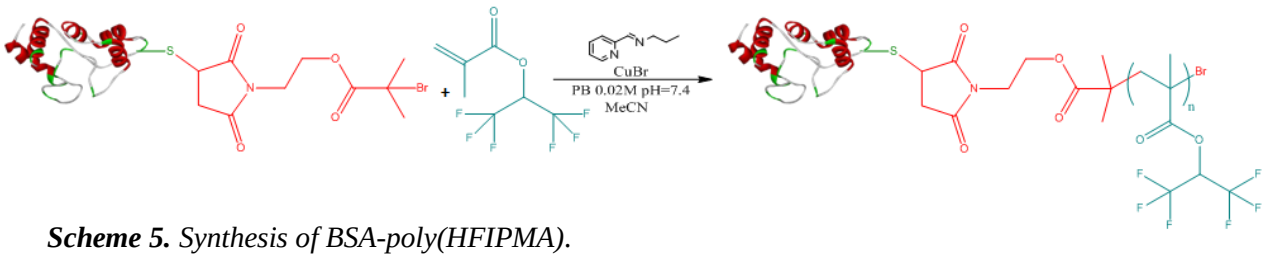
3.3.6 ATRP grafting of 2,2,2-trifluoroethyl acrylate (TFEA) from BSA-macroinitiator II - BSA-poly(TFEA) – Synthesis of BSA-poly(TFEA)



Scheme 4. *Synthesis of BSA-poly(TFEA).*

2,2,2-trifluoroethyl acrylate (0.114 mL, 0.0009 mmol, 1000 equiv.) and N(Propyl)-2-pyridylmethanimine (0.0093 mL, 0.063 mmol, 70 equiv.) were placed in a Schlenk tube and dissolved in 10.25 mL 20 mM phosphate buffer pH 7.4, 10% DMSO. The mixture was deoxygenated by 5 freeze-pump-thaw cycles and sonicated for 5 minutes to emulsify the monomer prior to use. The polymerization was triggered by cannulation of the monomer solution under nitrogen atmosphere in a second deoxygenated Schlenk tube containing the crystalline BSA-macroinitiator **II** (~60 mg, 0.0009 mmol, 1 equiv.) and CuBr (5.3 mg, 0.037 mmol, 41 equiv.) under N_2 atmosphere.A dark brown colour was immediately observed. The reaction mixture was stirred under N_2 atmosphere over night (~15 hours) after which it was exposed to oxygen atmosphere, in order to oxidize Cu(I) to Cu(II), and stirred for approximately another 1 hour. The crude polymerization mixture was purified by 4 dialysis. Initially 2 dialysis against 5 mM phosphate buffer 7.4 and EDTA for 2 hours, then against 20 mM phosphate buffer 7.4 and EDTA over night. The next day 1 dialysis against 20 mM phosphate buffer 7.4 for 2 hours carried out. Regenerated cellulose dialysis membranes with MWCO 25 kDa were used for dialysis. After the purification, the product was characterized with PAGE-electrophoresis, GPC and ^{19}F NMR.
^{19}F NMR (500 MHz, D_2O, 298 K): δ= ~ -76 ppm

3.3.7 ATRP grafting of 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) from BSA-macroinitiator II – Synthesis of BSA-poly(HFIPMA)



Scheme 5. *Synthesis of BSA-poly(HFIPMA).*

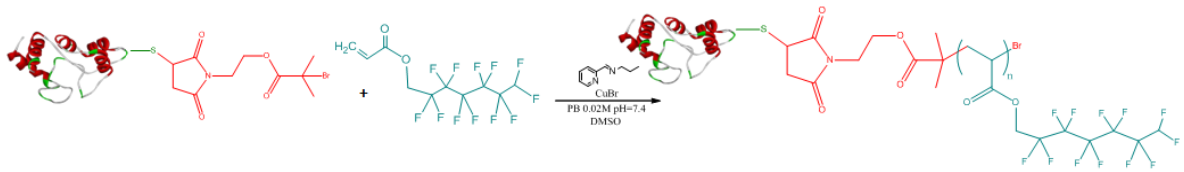
1,1,1,3,3,3-hexafluoroisopropyl methacrylate (0.128 mL, 0.0009 mmol, 1000 equiv.) and N(Propyl)-2-pyridylmethanimine (0.0095 mL, 0.064 mmol, 70 equiv.) were placed in a Schlenk tube and dissolved in 10.25 mL in 20 mM phosphate buffer pH

7.4, 10% MeCN. The mixture was deoxygenated by 5 freeze-pump-thaw cycles and sonicated for 5 minutes to emulsify the monomer prior to use. the polymerization was triggered by cannulation of the monomer solution under nitrogen atmosphere in a second deoxygenated Schlenk tube containing the crystalline BSA-macroinitiator **II** (~60 mg, 0.0009 mmol, 1 equiv.) and CuBr (5.3 mg, 0.037 mmol, 41 equiv.) under N_2 atmosphere.

A light brown colour was then observed and he reaction mixture was stirred under N_2 atmosphere over night, after it was exposed to oxygen atmosphere in order to oxidize Cu(I) to Cu(II), and stirred for approximately another 1 hour.

The crude polymerization mixture was purified by 3 dialysis. Initially 1 dialysis against 5 mM phosphate buffer 7.4 and EDTA for 2 hours, another one dialysis against 5 mM phosphate buffer 7.4 for 2 hours and finally against 20 mM phosphate buffer 7.4 which left over night. For dialysis, a regenerated cellulose membrane with MWCO 25 kDa was used. After the purification, the product was characterized with PAGE-electrophoresis and GPC.

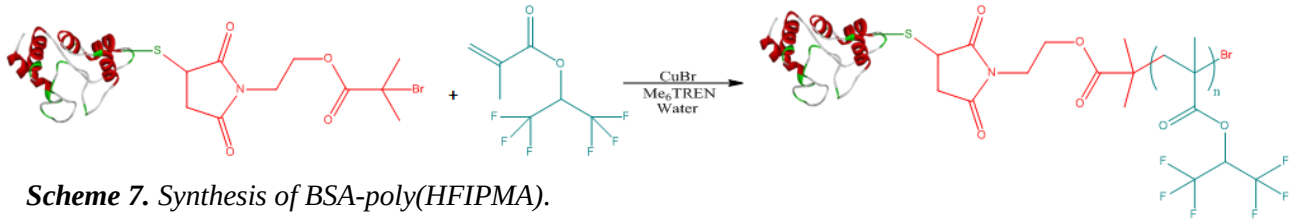
3.3.8 ATRP grafting of 2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptyl acrylate (DFHA) from BSA- macroinitiator II – Synthesis of BSA-poly(DFHA)



Scheme 6. *Synthesis of BSA-poly(DFHA).*

2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptyl acrylate (0.22 mL, 0.0009 mmol, 1000 equiv.) and N(Propyl)-2-pyridylmethanimine (0.0093 mL, 0.063 mmol, 70 equiv.) were placed in a Schlenk tube and dissolved in 10.25 mL 20 mM phosphate buffer pH 7.4, 10% DMSO. The mixture was deoxygenated by 5 freeze-pump-thaw cycles and sonicated for 5 minutes to emulsify the monomer, prior to use. The polymerization was triggered by cannulation of the monomer solution under nitrogen atmosphere in a second deoxygenated Schlenk tube containing the crystalline BSA-macroinitiator **II** (~60 mg, 0.0009 mmol, 1 equiv.) and CuBr (5.3 mg, 0.037 mmol, 41 equiv.) under N_2 atmosphere. A light brown colour was immediately observed. The reaction mixture was stirred under N_2 atmosphere over night (~15 hours) after which it was exposed to oxygen atmosphere, in order to oxidize Cu(I) to Cu(II), and stirred for approximately another 1 hour. The crude polymerization mixture was purified by dialysis. Initially 1 dialysis against 5 mM phosphate buffer 7.4 and EDTA for 2 hours, another one dialysis against 5 mM phosphate buffer 7.4 for 2 hours and finally against 20 mM phosphate buffer 7.4 which left over night. After the purification, the product was characterized with PAGE-electrophoresis and GPC.

3.3.9 SET- LRP grafting of 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) from BSA-macroinitiator II – Synthesis of BSA-poly(HFIPMA)

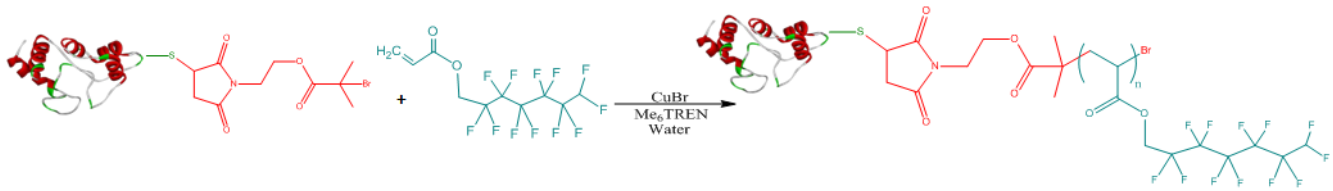


Scheme 7. *Synthesis of BSA-poly(HFIPMA).*

Deionized water (0.37 ml), a solution of CuBr in DMSO (0.0008gr, 0.00588 mmol, 40 equiv.) and a solution of Me_6TREN in DMSO (0.00157 ml, 0.00588 mmol, 40 equiv.) were placed in a syringe and the mixture was cooled to 0 ^oC under gentle stirring for a few minutes in order to achieve disproportionation.

The polymerization was triggered by the addition of the monomer 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (0.1 ml, 0.588 mmol, 4000 equiv.) and BSA-macroinitiator **II** (0.42 ml, 0.000147mmol, 1 equiv.). A light blue colour was immediately observed. The reaction mixture was left for about 20 h under gentle stirring at ambient temperature. During this time, minute samples were withdrawn from the reaction mixture in order to monitor the progress of the reaction. The crude polymerization mixture was extensively dialyzed initially against 5 mM phosphate buffer 7.4 and EDTA for 2 hours, another one dialysis against 5 mM phosphate buffer 7.4 for 2 hours and finally against 20 mM phosphate buffer 7.4 which left over night. After the enrichment, the product was characterized with PAGE-electrophoresis.

3.3.10 SET- LRP grafting of 2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptyl acrylate (DFHA) from BSA- macroinitiator II – Synthesis of BSA-poly(DFHA)



Scheme 8. *Synthesis of BSA-poly(DFHA).*

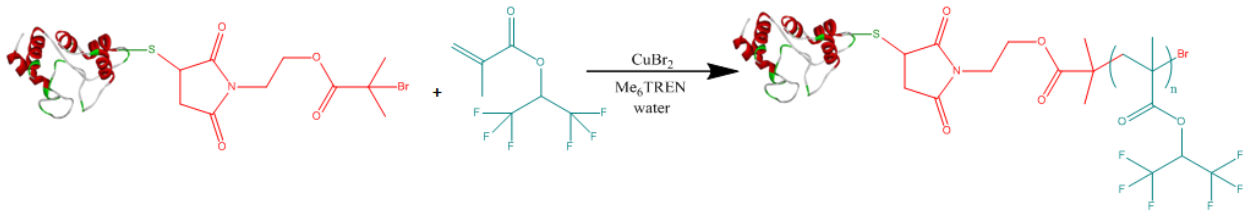
Deionized water (0.37 ml), a solution of CuBr in DMSO (0.0008 gr, 0.00588 mmol, 40 equiv.) and a solution of Me_6TREN in DMSO (0.00157 ml, 0.00588 mmol, 40 equiv.) were placed in a syringe and the mixture was cooled to 0^oC under gentle stirring for a few minutes in order to achieve disproportionation.

The polymerization was triggered by the addition of the monomer 2,2,3,3,4,4,5,5,6,6,7,7-Dodecafluoroheptyl acrylate (0.14 ml, 0.588 mmol, 4000 equiv.) and the BSA-macroinitiator **II** (0.42 ml, 0.000147mmol, 1 equiv.). A light blue color was immediately observed and the headspace eliminated. The reaction

mixture in the syringe was left for about 20 h under gentle stirring at ambient temperature.

The crude polymerization mixture was extensively dialyzed initially against 5 mM phosphate buffer 7.4 and EDTA for 2 hours, another one dialysis against 5 mM phosphate buffer 7.4 for 2 hours and finally against 20 mM phosphate buffer 7.4 which left over night. After the purification, the product was characterized with PAGE-electrophoresis.

3.3.11 Photo-induced LRP grafting of 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) from BSA-macroinitiator II – Synthesis of BSA-poly(HFIPMA)



Scheme 9. *Synthesis of BSA-poly(HFIPMA).*

A solution of BSA-macroinitiator **II** (0.625 ml, 0.0002185 mmol, 1 equiv.) and a stirrer were placed in a milliliter syringe.A solution of the monomer 1,1,1,3,3,3-hexafluoroisopropyl methacrylate (HFIPMA) (0.2 ml, 1.1104 mmol, 5082 equiv.) in nanopure water (0.65 ml) was cooled in ice. A solution of Me_6TREN (12 μl, 0.000437 mmol, 2 equiv.) and $CuBr_2$ (2 mg, 0.0000874 mmol, 0.4 equiv.) in 10 ml of nanopure water was also prepared. Firstly, the monomer solution was added to the initiator solution using a Gilson pipette. Finally, 100 μl of the aqueous solution containing $CuBr_2$ and Me_6TREN were quickly added and air was removed. A light blue color was immediately observed and great care was given in order to avoid air bubbles and eliminate the reaction headspace. The syringe was fitted with a rubber septum, placed under a UV nail lamp (365 nm), and the reaction mixture was left under stirring, for about 8 hours.

The crude polymerization mixture was extensively dialyzed initially against 5 mM phosphate buffer 7.4 and 2 mM EDTA for 2 hours, another one dialysis against 5 mM phosphate buffer 7.4 for 2 hours and finally against 20 mM phosphate buffer 7.4 which left over night. After the purification, the product was studied with PAGE-electrophoresis.

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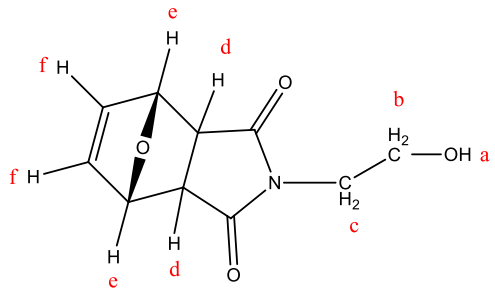
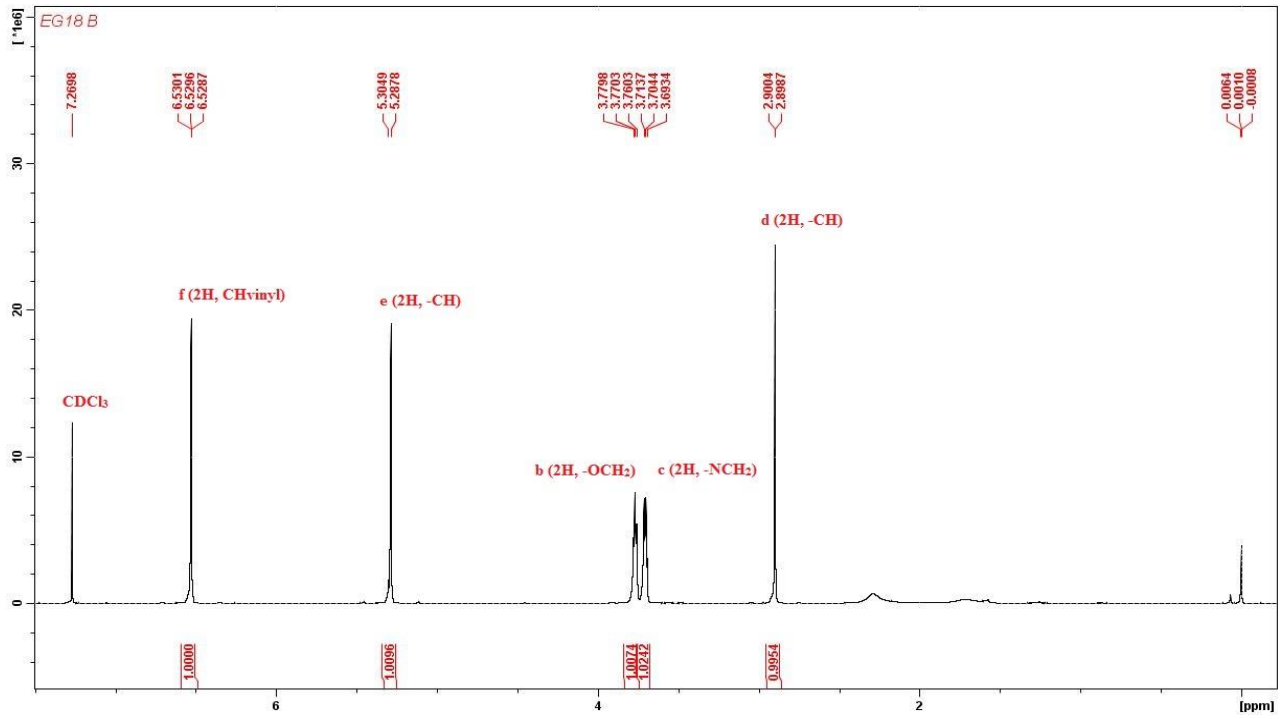
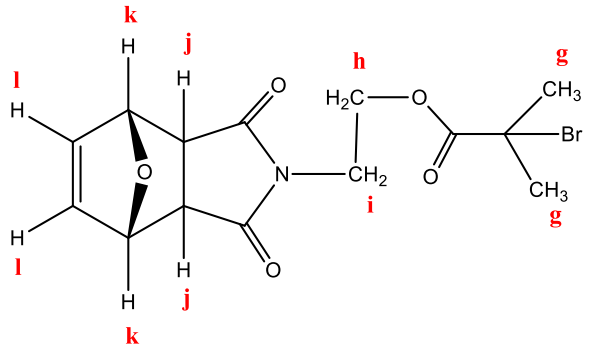
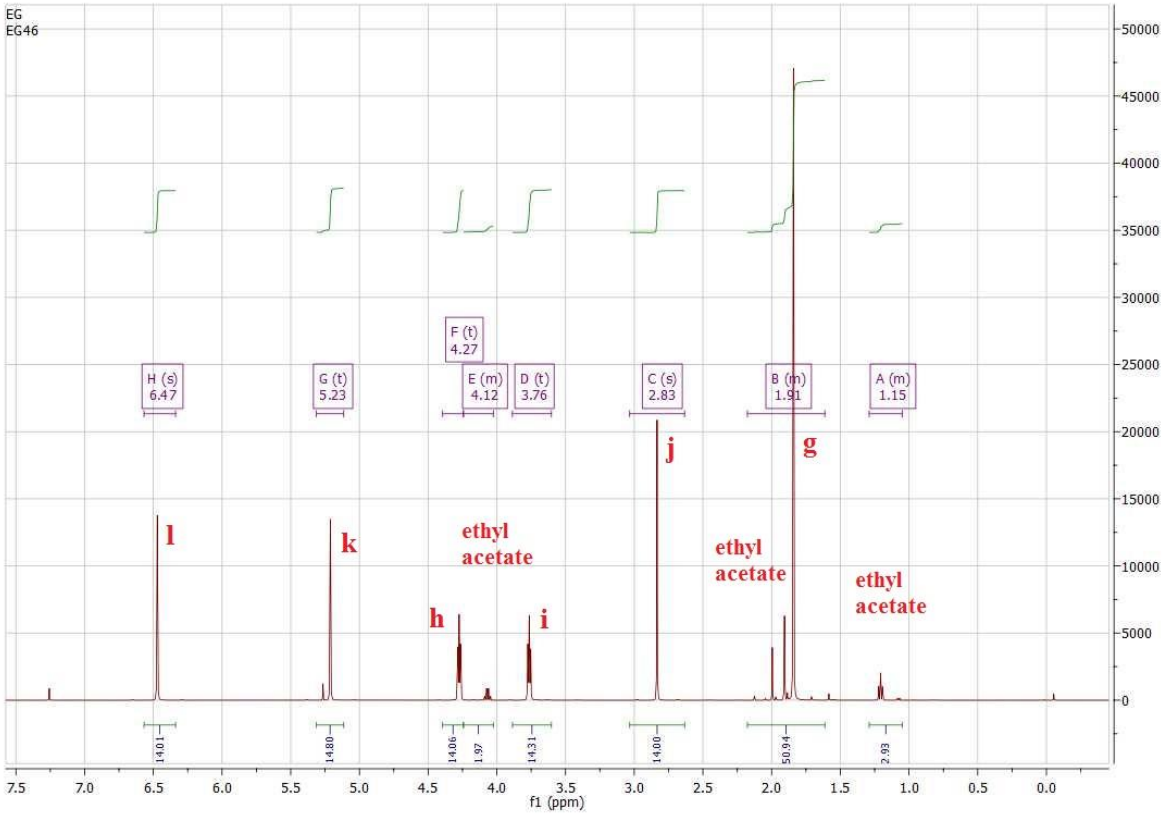
DOI:10.1002/anie.200801007.

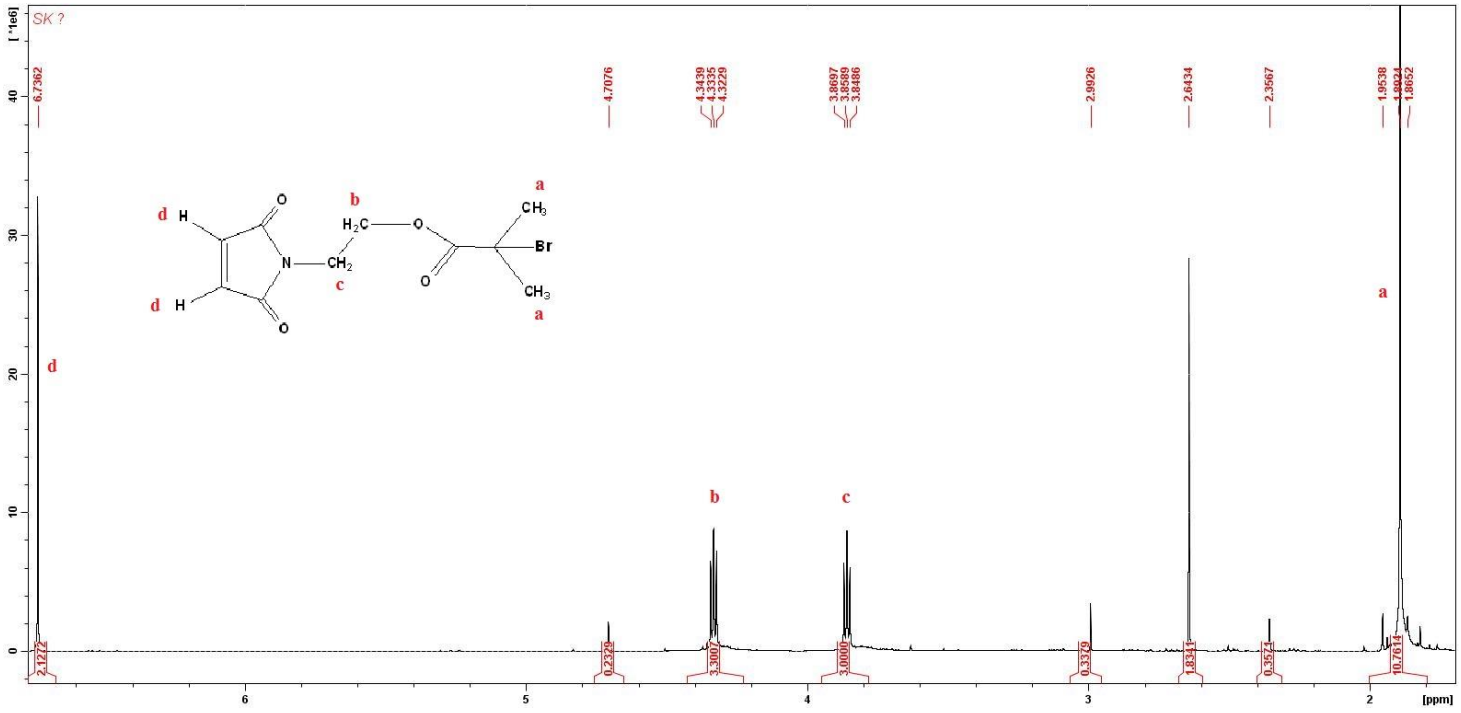
Appendix

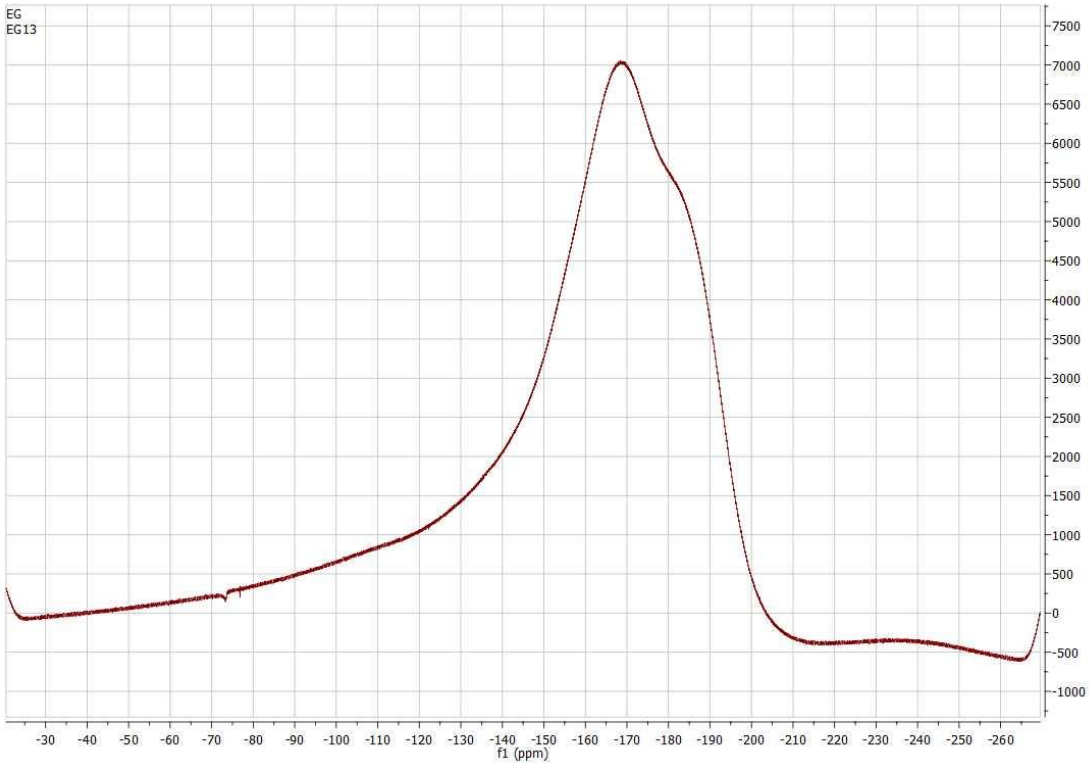

e
H
d
f H
H
O
b
H2
C
OH a
f H
O
N
C
H2
c
H
H
d
e
O

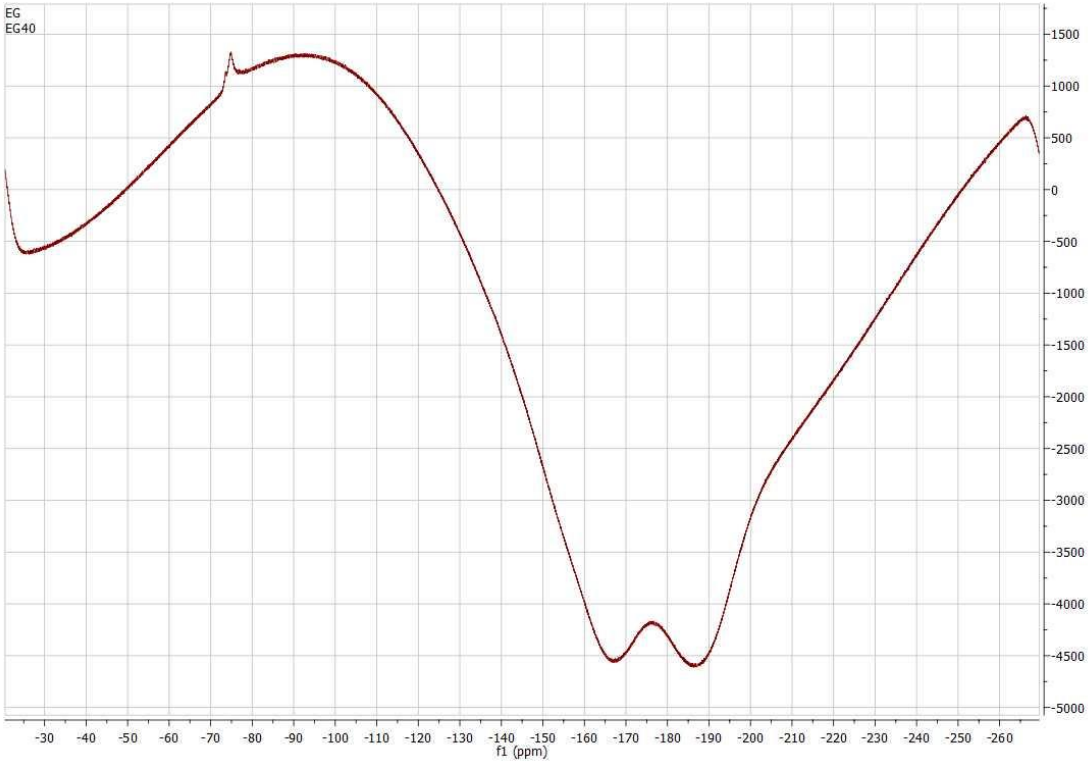

EG18 B
[*1e6]
7.2698
6.5301
6.5296
6.5287
5.3049
5.2878
3.7798
3.7703
3.7603
3.7137
3.7044
3.6934
2.9004
2.8987
0.0064
0.0010
-0.0008
d (2H, -CH)
f (2H, CHvinyl)
e (2H, -CH)
CDCl3
b (2H, -OCH2)
c (2H, -NCH2)
1.0000
1.0096
1.0074
1.0232
0.9954
30
20
10
0
6
4
2
[ppm]


k
H
j
H
l
H
O
O
N
h
H2C
O
g
CH3
Br
CH3
CH2
i
O
O
H
l
H
j
k


EG
EG46
H (s) 6.47
G (t) 5.23
F (t) 4.27
E (m) 4.12
D (t) 3.76
C (s) 2.83
B (m) 1.91
A (m) 1.15
l
k
ethyl acetate
h
i
j
g
ethyl acetate
ethyl acetate
14.01
14.80
14.06
1.97
14.31
14.00
50.94
2.93
f1 (ppm)


SK ?
[*1e6]
6.7362
4.7076
4.3439
4.3335
4.3229
3.8697
3.8589
3.8486
2.9926
2.6434
2.3567
1.9538
1.8924
1.8652
d
b
c
a
2.1212
0.2329
3.3007
3.0000
0.3329
18.3417
0.3572
10.7614
[ppm]


EG
EG13
f1 (ppm)


EG
EG40
1500
1000
500
0
-500
-1000
-1500
-2000
-2500
-3000
-3500
-4000
-4500
-5000
-30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260
f1 (ppm)