BELSPO POST-DOCTORAL FELLOWSHIP 2014

Post Doc: *Dr. Angel J. Satti*
Director: *Prof. Filip Du Prez*
Host University: *Ghent University (6 months)*

Final Scientific Report

The following report applies for the research stay of the post-doctoral fellow Dr. Angel J. Satti in UGent under direction of Prof. Filip Du Prez from the Department of Organic and Macromolecular Chemistry (Polymer Chemistry Research Group). It comprises the goals achieved of the original proposal, as well as other activities done during the stay, related to the main topic of the original proposal.

Original Proposal: ***Development of a solid-supported thiolactone-based high-throughput synthetic protocol for the preparation of multi-functionalized sequence-defined oligomers***

Introduction

Two decades of progress in the field of living and controlled polymerizations, combined with the elaboration of efficient conjugation reactions, greatly contributed to the elegant preparation of functionalized macromolecular architectures.[1] However, these state-of-the-art methodologies, though providing a high degree of structural and topological control, are inadequate tools for controlling the polymer microstructure. Crucial parameters like primary structure (*i.e.* monomer sequence) and tacticity largely remain unmastered by current man-made approaches.

Expectations for the next generation synthetic polymers include their performance as single chains, being able to fold and self-regulate, sense specific molecules and/or catalyze reactions.[2] These precisely functionalized linear polymers should exhibit sharply defined and tailored structure-activity relationships, analogous to Nature's delicately engineered macromolecules. Therefore, progress towards reliable sequence-controlled polymerization, enabling pre-programmed distribution of multiple functional groups along the backbone, is undoubtedly drawing much attention in a growing number of research groups dealing with organic and polymer chemistry.[3]

This research activity is perfectly fitting into work package 1 of the IAP project (P7/05) 'Functional Supramolecular Systems' in which the research towards sequence controlled polymers is one of the main targets.

Recent pioneering efforts to control the primary structure of functionalized polymers have been based on several approaches like sequential addition of building blocks on a solid[4] or liquid[5] support, leading to sequence control as a result of iterative coupling steps, between others [6-8]. Building blocks on a solid support currently remains the most versatile tool for controlling monomer sequence. Nevertheless, related protocols, typically established for peptide[9] and oligonucleotide[10] synthesis, also have less favorable characteristics. Indeed, they generally require the use of protecting groups and the restricted number of readily available building blocks, i.e. the so-called 'monomer alphabet',

equipped with the appropriate functional handle can further hamper the upscalable preparation of tailor-made functionalized sequences.

These drawbacks justify ongoing developments of new chemical protocols for chain elongation, often on a solid support, resulting in sequence-defined (macro)molecular structures with unique backbones and side chain functionalities. Therefore, the focus in this research project will initially be on the development of new chemistries for chain elongation on a solid support, enabling the direct introduction of chemical functionalities along the backbone and subsequent topological upgrade. New chemical methods automatically lead to novel (macro)molecular structures, potentially exhibiting unprecedented supramolecular behavior.

General Objective, Project Tasks and Methodology

Ongoing worldwide research interest in the field of sequence-controlled polymerization and single chain (nano-)technology requires dedicated and straightforward synthetic efforts, enabling the design and efficient preparation of reactive systems, ultimately leading to the targeted delicately tailored, synthetic polymers.

In this respect, the first aim of this research proposal is the further elaboration of a preliminary research effort within the PCR group, in which sequence-defined multi-functionalized oligomers have been generated on a solid support making use of thiolactone-based chemistry.

The synthetic scope of thiolactones as reactive precursors for thiols in various polymeric systems was reported by the Polymer Chemistry Research Group since 2011.[11] These cyclic thioesters are susceptible to selective aminolysis, releasing a thiol, and thus providing a functional handle for subsequent efficient conjugation reactions that are generally referred to as thiol-X chemistries.[12]

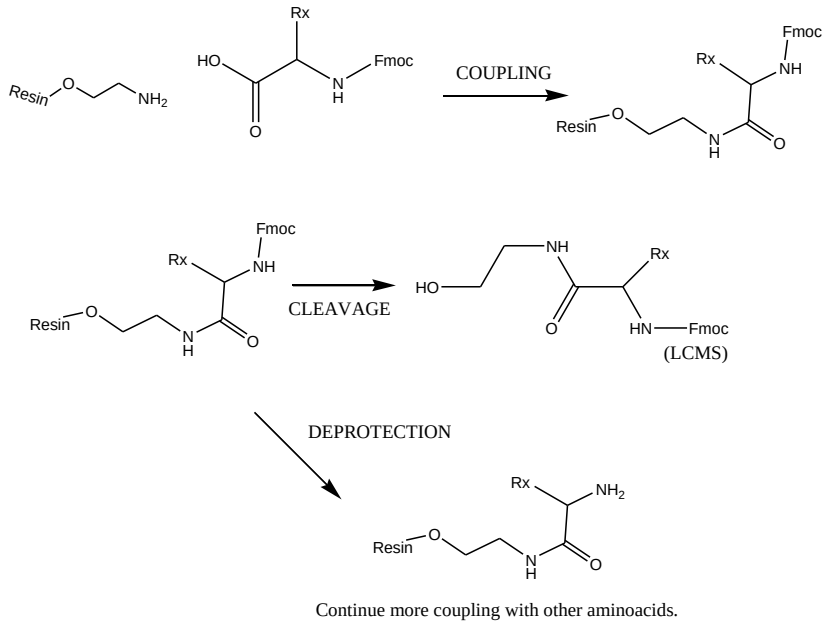
Immobilization of such a thiolactone unit on a solid support enables chain extension after on-resin aminolysis, using a judiciously selected thiolactone building block, to reinstate the thiolactone functionality, which is the start of a next iterative reaction sequence. This two-step aminolysis/chain extension protocol relies on a single thiolactone-containing building block for chain extension. Most importantly, a myriad of functionalities can be introduced *via* readily available amines.

When synthesizing peptides, a multifucntional sequence can also be achieved. Each peptide has an aminoacid (AA) wich can have a different functionality. The synthesis of the peptide can be done on the same solid support, and after the last AA of the sequence, a thiolactone building block can also be coupled along with the aminoacids on the resin. After cleavage from the resin and further functionalization with an acryloyl group, throug thiol-ene chemisrty and aminolysis, more functionalization can be done in each joint of peptides by the amine chosen for the attack to the thiolactone ring during the chain extension that could form a polypeptide (scheme 1).

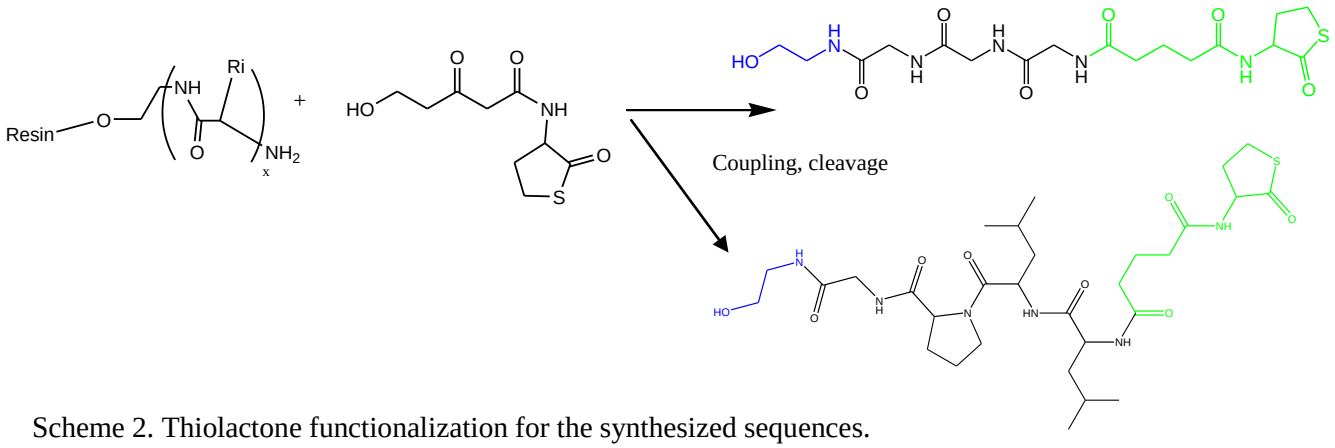
As the repetitive aminolysis and chain extension steps occur in basic medium, an acid-labile linkage will be foreseen for final cleavage from the solid support. Consequently, the sequence would be coupled to a 2-chlorotrityl resin using standard conditions.[13]

The building block for chain extension should be equipped with a functional handle, attached to the thiolactone, allowing reaction with the generated thiol, preferentially in a nucleophilic fashion. As the idea in this specific part of the project is to control the synthesis of peptides and polypeptides, a sequence of amionacids is going to be coupled to the resin and then functionalized with the thiolactone on resin (scheme 2). After cleaving this sequence from the resin, further end-chain functionalization with a nucleophilic acryloyl group would be done to induce the chain extension by

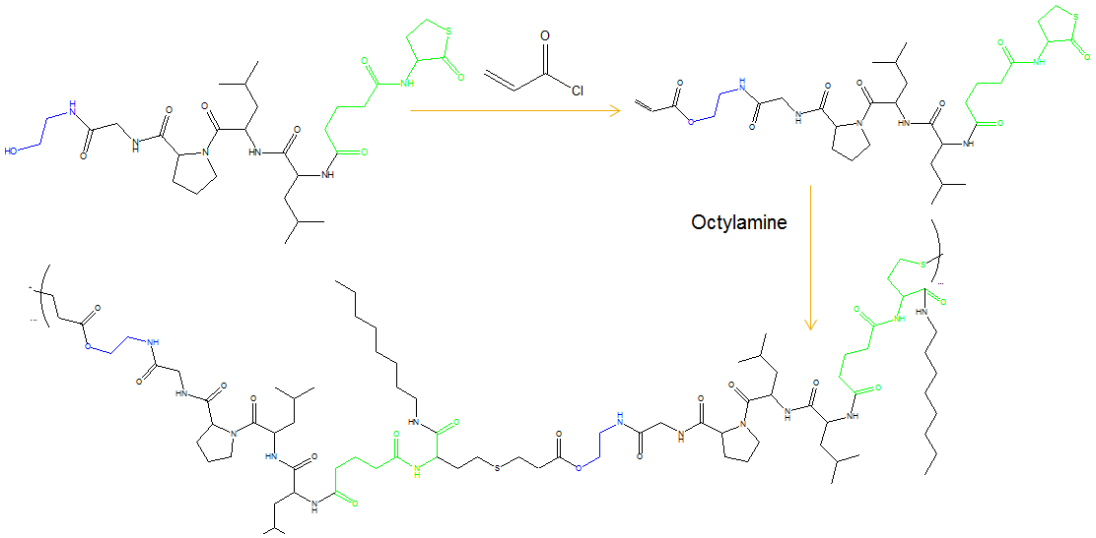
reaction with a primary amine and subsequent thiol-ene reactions (scheme 3). The evaluation of the whole sequence reaction outcome and polymerization relies on LC-MS analysis.



Scheme 1. Synthesis of a multifunctionalized sequence from a solid support.



Scheme 2. Thiolactone functionalization for the synthesized sequences.



Scheme 3. Acrylation and chain extension of multifunctionalized sequence through a functionalized amine.

Research objetives

> ***Task 1****: Large-scale preparation of enantiomerically pure building blocks for loading and chain extension*
> ***Task 2****: Optimization of a thiolactone-based high-throughput synthetic protocol for the preparation of multi-functionalized sequence-defined oligomers*

Accomplished methodologies and objectives:

The initial focus was the development of the solid-supported chemical protocol. Such objective was accomplished by synthesizing different oligoaminoacid sequences using a chlorotrityl resin as solid support. In such sequences, aminoacids with different functionalizations were coupled in order to have a multi-functionalized sequence. Thereafter, such sequences were further functionalized with thiolactone and acryloyl chloride on the extremes in order to polymerize via thiol-ene chemistry after primary amine attack to the thiolactone ring.

Each AA sequence synthesis was followed step by step by LCMS, meaning each step as the addition of a different aminoacid, on a one pot reaction. This reaction consisted in adding the first AA of the sequence with a promotor of reaction, HBTU, and the resin to the reactor. Then DIPEA as base and DMF as solvent were added. The reaction mixture was gently shaken and then a sample of the coupled product was cleaved with TFA solution and analysed throguh LCMS to confirm if it corresponded to the respective AA. After that, the second AA was added to follow the sequence and the methodology was repeated with the following AA so on, until obtaining the desired sequence. The procedure is shown in schemes 1-3.

Two different sequences were designed. The first one consisted simply on a triglycine, and the second one was a more complex peptide designed with different functionalities in order to twist the

sequence mainbone with proline units and functionalize it with leucine (scheme 2). Both of them could be synthesized, so the second one was used to be end-functionalized for chain extension.
The functionalization with thiolactone was achieved through the thiolactone building block shown in scheme 2, in a similar one pot reaction pathway to the AA coupling. After cleaving the thiolactone functionalized peptide from the resin, the other end group of the sequences had an hydroxyl group. This end group comes from the resin, which was another reason for chosing this chlorotrityl. However, the hydroxyl group needed to be converted to an acryloyl functionality in order to succeed in the chain extension reaction. After testing in different reaction conditions, the acrylation shown in scheme 3 was fullfilled up to an 85 %. Thus, the double functionalized AA sequences were prepared for chain extension.

After purification and precipitation with TFA and ether respectively, chain extension was done via aminolysis of the thiolactone group, by reacting octylamine with the functionalized peptide in different concentrations and in different solvents (dioxane and THF), with stirring overnight. The reaction should procceed via thiol-ene mechanism. After primary amine attach to the thiolactone group, a thiol group is formed that reacts with the double bond of the acryloyl group at room temperature. Anyway, in every experimental condition tested, the LCMS and also GPC results on molecular weight of the obtained product, seemed to indicate that no more than a dimeric species was obtained. That could happen because of the incomplete acryloyl functionalization of the peptide. However, all the whole process was repeated in a larger scale obtaining hundreds of mg in a bigger reactor, by using ten times of each reagent. Although the upscale worked as good, the acrylation didn´t go furhter even with more material and thereafter, ease of reaction. Further work need to be accomlpished to fullfill the inital objetives of this project, meaning obtaining a pure product for further chain extension.

An important development in order to accelerate the overall process, allowing for high-troughput preparation of multiple sequences, would be the development of a (semi-automated) microwave-assisted protocol using microwave equipment, available in our department.

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Added proposal during the stay: ***Enhanced thermosensitive chromatographic columns through grafting of thiolactone functionalized PNIPAAm onto aminopropyl-silica***

As the original proposal depended solely in the LCMS equipment to follow up the synthesis reactions, and it also had been out of service due to unpredictable technical problems for a significant lapse of time, the fellow also joined a new project started during the stay, which had good results and made the basis for ongoing work. ***This research activity also consists in thiol-ene and amine chemistry as the other one , and it perfectly fits into work package 1 of the IAP project (P7/05) 'Functional Supramolecular Systems'.***

Introduction

There is a constant focus on methods that lead to less harmful procedures and waste, which in the last years, gained the name of green chemistry. In the separation area, work is on progress to develop chromatographic systems for separation of different organic species with less harmful solvents, with water as an ideal. For such systems, the silica particles of the columns should have organic groups in order to retain the organic compounds that elute in the polar mobile phase. In such way, special attention was given to water-soluble polymers that are also thermosensitive, like the poly(n-isopropylacrylamide) (PNIPAAm). The possibility to use it somehow in the stationary phase of chromatographic columns is being studied within the last two decades[1-11].

The change in hydrophilicity of this polymer by just increasing temperature, allows the control of the retention times of some analytes using mainly water as mobile phase. That is because above the lower critical solution temperature (LCST) of the system, similar elution profiles are obtained as in conventional reversed phase liquid chromatography.

Although there are many works regarding the use of PNIPAAm grafted silica, up to now the procedure is based on direct polymerization to the silica or multiple steps procedures[1-11]. Also, retention times can be controlled with temperature. In that way, better retention can be found at higher temperatures, and sometimes good retention times are achieved in the grafted systems but at

temperatures above the LCST of the PNIPAAm, that is 35 ºC. So, it is implicit to heat the system to achieve a good separation.

General Objective, Project Tasks and Methodology

Mainly, the idea is to prepare a grafted PNIPAAm-*g*-Silica functionalized system with improved retention times through an easier grafting pathway than what is reported up to now. The principle of this lies in previous experience in the PCR group, where functionalized PNIPAAm with a thiolactone moiety, specific amines and acrylates can tune the LCST behaviour of the polymer, to lower values[12]. With this functionalized thermosensitive polymer, there should be possible to synthesize a grafted material with improved retention times, at least, at room temperatures.

Also, the added value of this procedure is that it would imply an easier path of grafting, since the PNIPAAm is functionalized with thiolactones that can be attacked by the amine groups present in aminopropyl silica. The grafting reaction could be simply done by mixing the thiolactone functionalized PNIPAAm and the amine functionalized silica. Then, further functionality with an specific acrylate can be done when reacting with the latent thiol group released after the amine attack to the thiolactone during the grafting procedure. This thiol-ene reaction should involve not the functionalization of the grafted system, but also the protection of the thiol group. The overall reaction shouldn´t be so different than the reactions previously studied, and can be done as a one pot reaction. The only difference is that the amine groups here are retained in the solid phase. The obtained product, would be tested on a stainless steel column on a liquid chromatography equipment with different organic analytes.

Research objetives

> ***Task 1****:* Achieve a filler for a column with enhanced retention times for different organic compounds, through thiolactone functionalized thermosensitive polymer.
> ***Task 2****:* Present an easier procedure for obtaining an applicable thermosensitive stationary phase.

Accomplished methodologies and objetives:

A thiolactone functionalized PNIPAAm (TL-PN) was succesfully synthesized by Reversible Addition-Fragmentation chain Transfer polymerization (RAFT) under control over the polymerization. Then, an amount of this polymer was stirred along with the aminopropylsilica overnight in THF or dioxane. This is a one pot procedure where a functionalized acrylate is also present, and via thiol-ene reaction, provides with a desired functionalization and protected the thiol

group. This type of grafting innovates also in the sense that more than one similar column could be obtained from the same batches of silica and polymer, just by changing the functionalized acrylate.

The separation behaviour of the columns obtained from this procedure were analyzed in pure water at different temperatures, using different mixes of standards analytes as parabens, steroids and peptides with a separation module equiped with a dual λ absorbance detector.

First, a mixture of parabens was tested to analyze the retention behaviour of the column, while increasing the hydrophobicity of the analytes. This mixture included methyl-, ethyl-, propyl- and n-butyl- paraben. It seemed that the expected separation behaviour for reversed phase chromatography was observed (figure 1), as the more hydrophobic analaytes were eluted last. Moreover, as the temperature was increased, the retention times became higher as a response of the thermoresponsitivity of the system. As temperature increases, the grafted polymer has a higher hydrophobic behaviour since it goes further from the LCST. As it can be clearly seen, this improved hydrophobicity in the stationary phase is more important for the more hydrophobic analytes since they present a higher difference between retention times while compared at different temperatures.

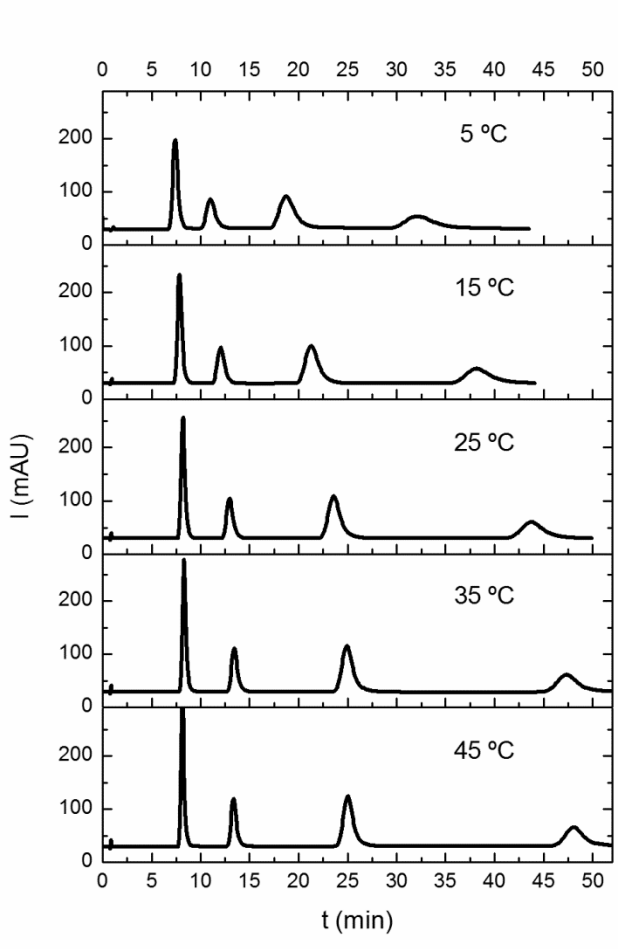

Figure 1: Separation of parabens ran at different temperatures and detected at 254 nm. The order of elution for each of the four peaks in every chromatogram is as follows: Me-, Et-, Pr-, Bu-paraben.

This results clearly indicate that the grafting procedure followed to finally produce this column is succesfull, showing that this one-pot way to develop thermoresponsive columns with functionalization via thiol-ene reaction is possible. Furthermore, from the separation point of view, the retention times were enhanced, if this colum is compared to the ones reported in the literature[1-8,13]. Compared to what is know up to now, the separation is even good at 5 ºC. It also seems that increasing temperature after 35 ºC didn´t change too much the retention times, although the quality of the peaks continued increasing meaning that they got narrower. The former can be explained taking into account a low LCST in the stationary phase, which should be located even below 5 ºC. This LCST was probed to be shifted to other values when using the second column with another functionalized acrylate.

With the steroids, the first column also showed enhanced results at room temperature when compared to similar PNIPAAm-silica stationary phases built up from more complex methodologies[2,3,11], and it developed similar or even better than columns with other types of thermoresponsive polymers[13]. The third set of samples anaylzed was a mixture of three peptides. These compounds are commonly run in mixed mobile phase with an organic solvent, or adding salt or acid to the water[4,5,10,11]. In this case, we test this column with pure water and also in an aqueous solution of 0,1 % formic acid, having practically the same results. They were all retained within 20 minutes in the 10 cm column. At room temperature, even the peaks of two diasteroisomers could also start to be differentiated, in both mobile phases. A set of xanthines was also run with improvements in the peaks performance along with temperature, although retention times didn´t increase with temperature.

The second column behave as well as the first one in terms of performance, but with half the retention times. Anyway, the goals of this project were succesfully reached. A single one pot procedure gave different columns from the same batches of silica and polymer, but just changing an acrylate function. Both columns showed to have good separation of different analytes, and with enhanced retention times. Specially the first one, that reached a really significant decrease in the LCST behaviour, enabling good separation at room temperature without the need of jeopardizing the column at higher temperatures. All runs could be done with pure water, which open the field of application widely to green chromatography.

These results are prone to be presented to the high impact journal “Analytical Chemistry” soon.

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