Research Networking Programmes

Short Visit Grant ☐ or Exchange Visit Grant ☒

(please tick the relevant box)

Scientific Report

Scientific report (one single document in WORD or PDF file) should be submitted online within one month of the event. It should not exceed eight A4 pages.

Proposal Title: Drug/gene-Carrier via RAFT-Polymerization

Application Reference N°: 3858

1) Purpose of the visit

2) Description of the work carried out during the visit

3) Description of the main results obtained

4) Future collaboration with host institution (if applicable)

5) Projected publications / articles resulting or to result from the grant *(ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)*

6) Other comments (if any)

1 Purpose of the visit

In recent years there is an ever increasing interest in therapeutic polymers due to their advantages in comparison to conventional drug carriers. One possibility to use polymers as drug carrier systems is to create nanostructures based on amphiphilic block copolymers which possess the ability to self-assemble into various formations (e.g. micelles).

Reversible addition-fragmentation chain transfer polymerization (RAFT) which is one of the controlled/living radical polymerization methods is a highly efficient tool to prepare the demanded well-defined block copolymers.

So far, N-acryloyl morpholine was used to synthesize the hydrophilic part of the polymer and lauryl methacrylate was the monomer for the hydrophobic part. Based on the aforementioned monomers well-defined block copolymers were obtained which have the possibility to form micelles in aqueous media.

Unfortunately these polymers give toxic degradation products whereof the effort arises to exchange the polymerizable group of the monomers to vinylesters as their polymers give polyvinylalcohol as degradation product which is a FPA approved low toxic polymer. For further improvement of the biocompatibility of the block copolymers the hydrophilic part should be built from a sugar-based monomer. Usually for the synthesis of such glycopolymers protecting groups are required. In order to avoid the additional steps of protecting and unprotecting the hydroxyl groups of the sugar the desired products can also be synthesized by meaning of enzymatic routes.

The host institute at the University of Groningen focuses on the enzymatic modification of sugars. Hence, the overall purpose of the exchange stay was to get to know how to work with enzymes and to synthesize some desired monomers.

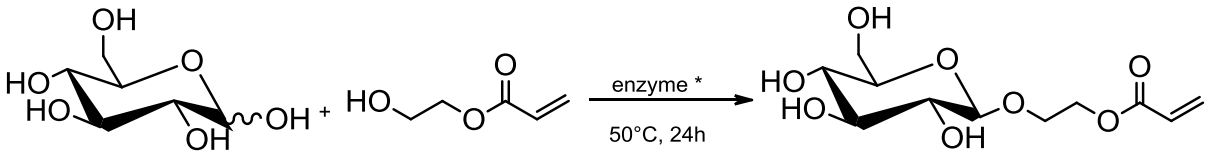
2 Description of the work carried out during the visit

During the exchange stay polymerizable groups were attached to glucose via an enzymatic way. At the beginning reactions developed by the host instituted were carried out to become acquainted with the procedures which are required for working with enzymes. Hence, 2-(β-glucosyloxy)-ethyl acrylate and 2-(β-glucosyloxy)-ethyl methacrylate were synthesized based on the knowledge of the host institute. The used enzyme gives the possibility to perform the reaction selectively between the polymerizeable alcohol and the OH-group on the C_1-carbon of the glucose.

Furthermore two other glucose based monomers were synthesized according to literature. By using a different enzyme than in the other reactions it was possible to add a polymerizable group to the C_6-carbon of the glucose via transesterification.

3 Description of the main results obtained

3.1 Synthesis of 2-(β-glucosyloxy)-ethylacrylate

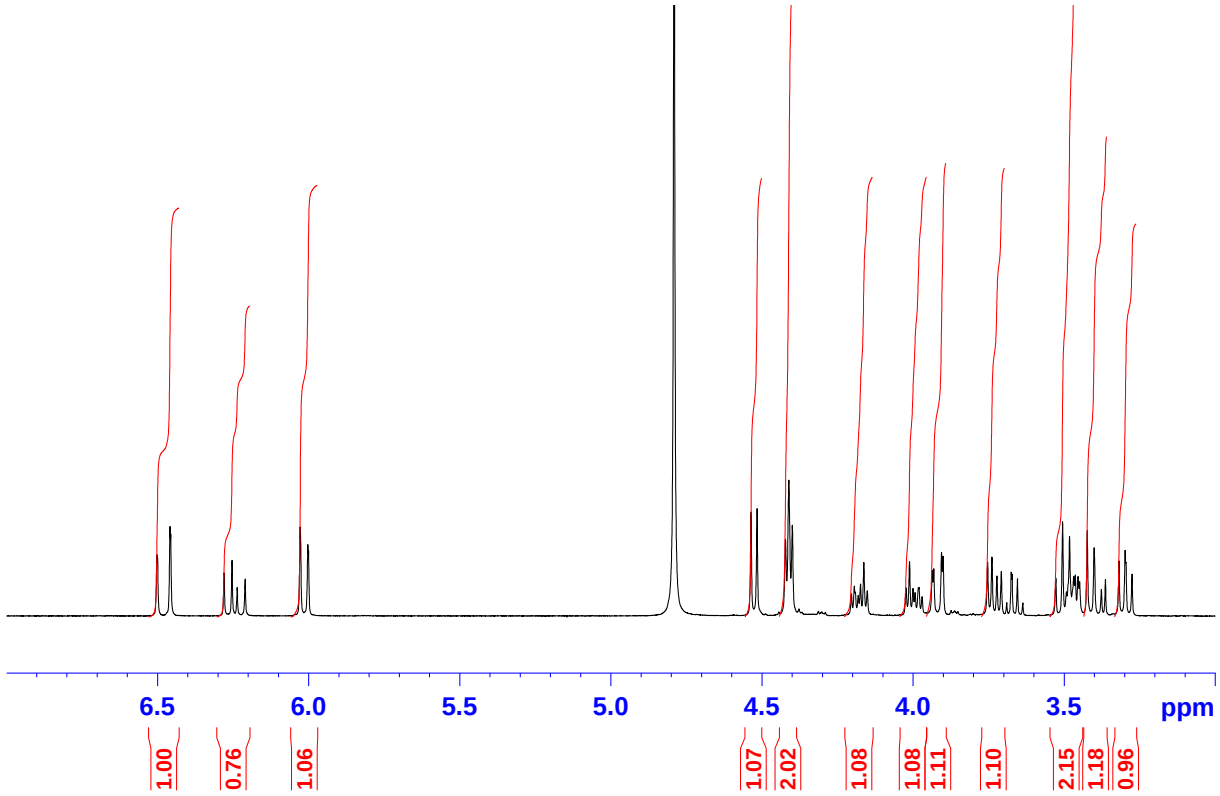


* ß-glucosidase from almonds

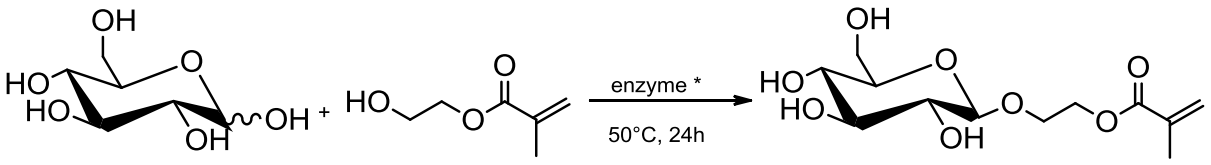
2-(β-Glucosyloxy)-ethylacrylate was synthesized according to W. Kloosterman[1]. D(+) – glucose was dissolved in water and 2-hydroxyethylacrylate, which acted as solvent and reactant, was added. Furthermore, a small amount of 1,4-dioxane was added as co-solvent. This mixture was immersed in to a preheated water bath and the reaction was then started by adding a solution of β-glucosidase from almonds in water. After 24 hours at 50°C the reaction was stopped by filtering off the enzyme. After column chromatography the desired product was obtained as a sticky and colorless product (30% of theory).

^{1}H-NMR (D_2O) δ [ppm]:6.48 (dd, 1H, $\mathbf{H_{cis}}$HC=CHR, J_1=0.98 Hz, J_2=17.3 Hz), 6.24 (m, 1H, CH_2=C**H**R), 6.02 (dd, 1H, $\mathbf{H_{trans}}$H=CHR, J_1=0.99 Hz, J_2=10.5 Hz), 4.52 (d, 1H, Gluc-$\mathbf{H_1}$, J=7.9 Hz), 4.41 (t, 2H, Gluc-OCH_2**CH_2**R, J=4.6 Hz), 4.18 (m, 1H, Gluc-$\mathbf{H_6}$), 3.99 (m, 1H, Gluc-$\mathbf{H_5}$), 3.92 (m, 1H, Gluc-$\mathbf{H_2}$), 3.73 (m, 1H, Gluc-$\mathbf{H_6}$), 3.49 (m, 2H, Gluc-O**CH_2**CH_2R), 3.39 (m, 1H, Gluc-$\mathbf{H_3}$), 3.30 (m, 1H, Gluc-$\mathbf{H_4}$)

Although D(+)-glucose was used as starting material, NMR-analysis showed that only the β-isomer was formed.



3.2 Synthesis of 2-(β-glucosyloxy)-ethylmethacrylate



* ß-glucosidase from almonds

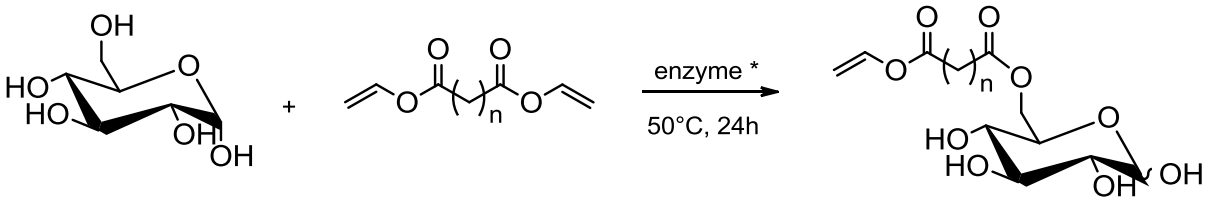
The desired product was synthesized as described by W. Kloosterman[1] 2-Hydroxyethylmethacrylate, which acted as reactant and solvent, and the co-solvent 1,4-dioxane were added to an aqueous solution of D(+) – glucose. Afterwards the reaction mixture was put into a tempered water bath and a solution of β-glucosidase

from almonds in water was added to start the reaction. After stirring the mixture at 50°C for 24 h the enzyme was filtered of to stop the reaction. Column chromatography was used to purify the colorless, sticky product. (32%of theory)

^{1}H-NMR (D_2O) δ [ppm]:6.19 (s, 1H, **H_{cis}**HC=CCH_3R), 5.75 (s, 1H, **H_{trans}**H=CCH_3R), 4.53 (d, 1H, Gluc-**H_1**, J=7.9), 4.40 (m, 2H, Gluc-OC**H_2**CH_2R), 4.18 (m, 1H, Gluc-**H_6**), 4.01 (m, 1H, Gluc-**H_5**), 3.92 (m, 1H, Gluc-**H_2**), 3.73 (m, 2H, Gluc-O**CH_2**CH_2R), 3.48 (m, 2H, Gluc-O**CH_2**CH_2R), 3.30 (m, 1H, Gluc-**H_1**), 1.96 (s, 1H, CH_2=C**CH_3**R)

^{1}H-NMR showed that only the β-isomer was formed although D(+) – glucose was used as starting material for this synthesis.

3.3 Synthesis of 6-O-vinyladipoyl-D-glucopyranose

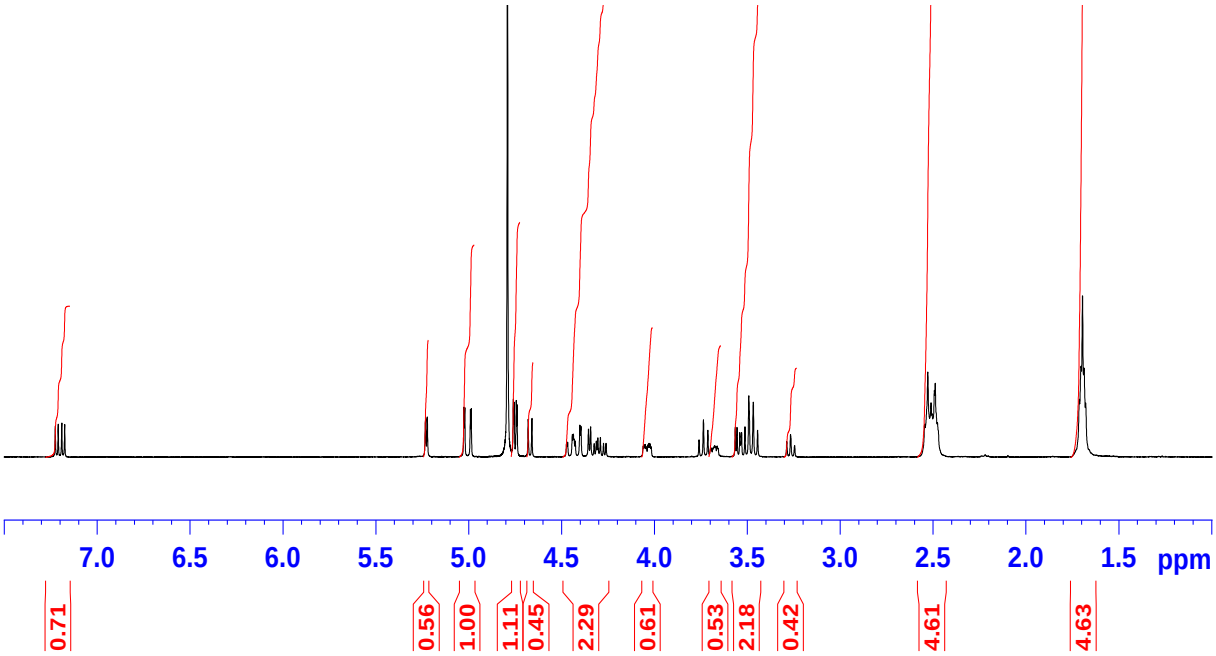


* Lipase acrylic resin from Candida antarctica (Novozyme 435)

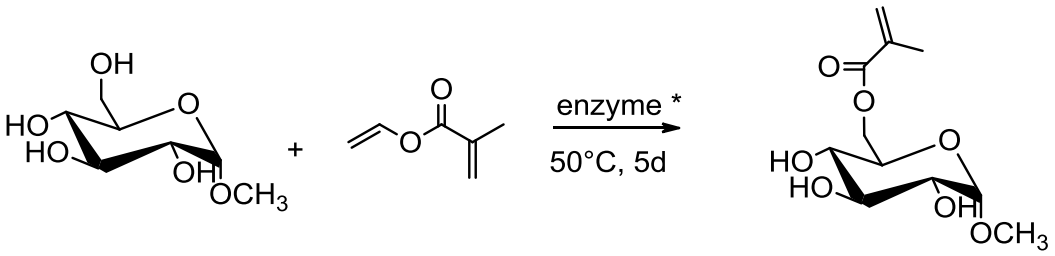
The product was synthesized according to L. Albertin et al[2]. α-D-glucose and 1.5 equivalents divinyl adipate were suspended in acetonitrile and the mixture was immersed into a preheated water bath. The reaction was started by adding lipase from Candida Antarctica, which is an enzyme that can be used for trans-esterifications. After stirring the reaction mixture at 50°c for 24 hours it was stopped by filtering of the enzyme and the solvent was evaporated. Afterwards the crude product was purified by column chromatography. The product was obtained as a white powder at a yield of 48% related to α-D-glucose.

^{1}H-NMR (D_2O) δ [ppm]: 7.20 (dd, 1H, H_2C=**CH**R, J_1=6.2 Hz, J_2=13.9 Hz), 5.23 (d, 0.55H, Gluc-**$H_{1\alpha}$**, J=3.8), 5.00 (dd, 1H, **H_{cis}**H=CHR, J_1=1.9 Hz, J_2=15.2 Hz), 4.75 (dd, 1H, **H_{trans}**H=CHR, J_1=1.9 Hz, J_2=7.6 Hz), 4.67 (d, 1H, Gluc-**$H_{1\beta}$**, J=7.9 Hz), 4.38 (m, 2.55H, Gluc-**H_6**, **Gluc-$H_{5\alpha}$**), 4.04 (m, 0.55H, Gluc-**$H_{3\alpha}$**), 3.68 (m, 0.45H, Gluc-**$H_{5\beta}$**), 3.51 (m, 2H, Gluc-**H_4**, Gluc-**$H_{3\beta}$**, Gluc-**$H_{2\alpha}$**) , 3.27 (m, 0.45H, Gluc-**$H_{2\beta}$**), 2.51 (m, 4H), 1.70 (m, 4H)

Although α-D-glucose was used as starting material the product was obtained as a mixture of the α and β-form of the monomer. This fact can be seen from the NMR-spectra. The integrals show that about 55% are the α-isomer and 45% are the β-isomer.



3.4 Synthesis of methyl 6-O-methacryloyl-α-D-glucoside



* Lipase acrylic resin from Candida antarctica (Novozyme 435)

Methyl 6-O-Methacryloyl–α–D-glucoside was synthesized as described in literatue [3]. Methyl-α-D-glucoside, an equimolar amount of vinylmethacrylate, and lipase from Candida Antarctica were suspended in acetonitrile. The mixture was then immersed into a preheated water bath at 50°C and was stirred at 200rpm for 5 days. After filtering of the enzyme and washing the filter cake with methanol, the collected organic phases were evaporated to dryness to yield a viscous yellow raw product.

After purification via column chromatography the product was obtained as a colorless and viscous fluid (30% of theory).

^{1}H-NMR (D_2O) δ [ppm]:6.18 (s, 1H, $\mathbf{H_{cis}}$HC=CHR), 5.77 (s, 1H, $\mathbf{H_{trans}}$H=CHR), 4.82 (d, 1H, Gluc-$\mathbf{H_1}$, J=3.7 Hz), 4.53 (dd, 1H, Gluc-$\mathbf{H_6}$, J_1=2.2 Hz, J_2=10.1 Hz), 4.37 (m, 1H, Gluc-$\mathbf{H_5}$), 3.93 (m, 1H, Gluc-$\mathbf{H_2}$), 3.71 (m, 1H, Gluc-$\mathbf{H_6}$), 3.60 (m, 1H, Gluc-$\mathbf{H_3}$), 3.50 (m, 1H, Gluc-$\mathbf{H_4}$), 3.43 (s, 3H, Gluc-O$\mathbf{CH_3}$), 1.96 (s, 3H, CH_2=C$\mathbf{CH_3}$R)

3.5 Conclusion and Outlook

During the exchange stay at the University of Groningen four glucose-based monomers have been successfully synthesized by an enzymatic way. The next step will be the controlled polymerization of the synthesized monomers by means of RAFT polymerization. This will be done by varying the reaction parameters like temperature, initiator concentration, solvent and reaction time. After successful homo polymerization of the monomers, experiments will be started to generate amphiphilic block copolymers. In the case of the acrylates/methacrylates lauryl methacrylate will be used as the hydrophobic part. The vinylester functionalized glucose will be block copolymerized with fatty acid vinyl esters. Since micelles should be generated from these block copolymers, the relation between hydrophilic and hydrophobic will be varied to find out the right value.

As the RAFT group is supposed to be toxic it will be removed from the block copolymer by using an excess of initiator. Afterwards the critical micelle concentration (CMC) will be determined by fluorescence spectroscopy using pyrene as standard. Additionally the diameter of the micelles will be measured with dynamic light scattering.

The overall goal of the project is to generate polymer micelles which can be used as potential drug carrier systems. Therefore the cellular uptake of the micelles will be tested in experiments carried out by the Vienna Medical University.

Summarized it can be said that the exchange stay at the University in Groningen was an important step for the ongoing work of the project. Especially it was important to transfer the knowledge on enzymatic synthesis of carbohydrate monomers without protecting groups to the Vienna University of Technology.

4 Future collaboration with host institute

In the future there will be an exchange of monomers new as well as of knowledge about RAFT polymerization.

5 Projected publications/articles

At the moment the writing of a joint paper about the comparison between RAFT polymerization of (meth-) acralyte based monomers and vinylesters is in progress.

6 Literature

1 Kloosterman W.M.J. “Synthesis of novel saccharide-acrylate monomers using biocatalytic approaches”, 2013

2 Albertin L. et al, “Chemoenzymatic Synthesis of Narrow-Polydispersity Glycopolymers: Poly(6-0-vinyladipoyl-D-glucopyranose)”, Biomacromolecules, 2004, 5 (2), pp255-260

3 Albertin L. et al, Well-Defined Glycopolymers from RAFT Polymerization: “Poly(methyl 6-O-methacryloyl-α-d-glucoside) and Its Block Copolymer with 2-Hydroxyethyl Methacrylate”, Macromolecules, 2004, 37 (20), pp 7530–7537