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# Biocatalytic Route to Well-Defined Macromers Built around a Sugar Core

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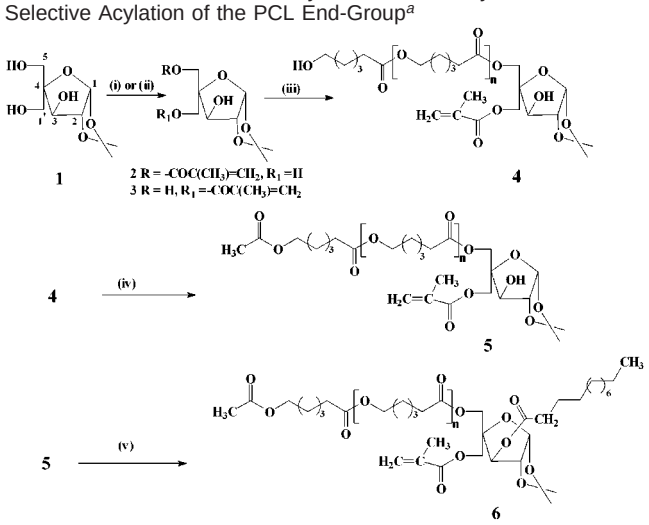
The synthesis and study of polymers that contain carbohydrates has captured the attention of researchers who wish to attain (i) highly functional polymers,[1] (ii) specific biological functions, and (iii) complex systems that fit into the category of "smart" materials.[2−4] For example, such polymers have been studied for their ability to regulate interactions with lectins,[5] as pseudo-glycoproteins,[6] and as carriers for drug delivery systems.[7] Structure−property studies have proven that variations in the macromolecular architecture from linear to multiarm can have dramatic effects on the morphological and physical-mechanical properties of the corresponding materials. Recently, our laboratory [8,9] and others [10,11] have explored the use of in vitro enzyme-catalysis for the preparation of monomers and polymers. Recent reviews and books have been published that document the rapid development of these methods.[12] Early reports that describe the synthesis of linear chains that are attached to a multifunctional initiator have been published.[13,14] The above findings provide incentive to further extend the level of control attainable during the synthesis of these products.

In this work, a general route was demonstrated that permits the efficient placement of selected structures at specific positions around a carbohydrate core. A key synthetic step that makes this possible is lipase-catalyzed diastereoselective acylation. This, and the judicious choice of a carbohydrate substrate, permits a level of control in the construction of hetero-arm star copolymers that was previously nonattainable or extremely difficult to realize by traditional chemical methods where a large number of protection−deprotection steps have impeded progress.[15−17]

The lipase-catalyzed synthesis of the macromer 4′-hydroxymethylmethacryl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-pentofuranose (HMG, **3**, Scheme 1) and its use as a multifunctional initiator to prepare a polyester arm specifically linked to the other (C-5) diastereometric center (**4**, Scheme-1) are described. The 4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-pentofuranose (**1**) was subjected to acryloylation with vinyl methylacrylate in dry THF.[18] The ability of the lipases *Porcine pancreatic* lipase (PPL), *Candida rugosa* lipase (CRL), and *PS*-30 and lipases from *Pseudomonas AK*, *Pseudomonas AY*, and Novozyme-435 to catalyze prochiral asymmetrization of **1** was studied at 30−35 °C for 8 h and results of this work are shown in Figure 1. Novozyme-435 and lipase *PS* resulted in highly diastereoselective monoacryl derivatization with use of 1 equiv of vinyl methacrylate as the acyl donor (Figure 1). Furthermore, for both Novozyme-435 and lipase *PS*, even when a 2-fold excess of vinyl methacrylate was used and the reaction was prolonged to 24 h, the major product was still the monoacryl derivative (∼95−97%). Catalysis by Amano *PS* preferentially placed the acryl moiety at the C-5 hydroxyl, affording **2** as the major product (d.e. *78%*, yield = 72%,). In contrast, Novozyme-435 resulted in acrylation at the C-1′ position (d.e. 93%, yield = 95%) giving **3** as the main product. The diastereomeric excess of the products **2** and **3** was calculated from $^1$H NMR spectra (see Supporting Information) by the difference in the integral values of the anomeric protons of the corresponding epimers at $\delta$ 5.93 and 6.01.[19]

***Scheme 1.*** Stereoselective Acrylation Followed by ROP and Selective Acylation of the PCL End-Group[a]




[a] Conditions: (i) vinyl methacrylate, THF, 30 °C, 8 h, Amano*PS*; (ii) vinyl methacrylate, THF, 30 °C, 8 h, Novozyme-435; (iii) e-CL, toluene, 60 °C, 8 h, Novozyme-435; (iv) vinyl acetate, toluene, 6 h, 30 °C; (v) lauric acid, DMF, DCC, DMAP, 60 °C.

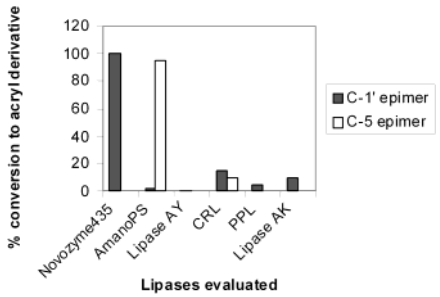



***Figure 1.*** Ability of lipases to carry out the selective acrylation of 4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-pentofuranose.

The sugar acryl derivative **3** was studied as a multifunctional initiator for $\epsilon$-CL polymerization. On the basis of previous work in our laboratory[8,9] and elsewhere,[10,11] Novozyme-435 was chosen as the selective catalyst for this ring-opening polymerization (Scheme-1). Recent work by us[20] showed that Novozyme-435 catalysis of $\epsilon$-CL polymerizations is accelerated when it is performed in low-polarity organic media. Thus, the acryl sugar **3** initiated ring-opening polymerization of $\epsilon$-CL was performed in toluene. The $^1$H NMR spectrum (see Supporting Information) of product **4** from an 8 h ring-opening polymerization of $\epsilon$-CL initiated by **3** and catalyzed by Novozyme-435 indicates that **4** consists of the acryl sugar moiety that is linked to the carboxyl terminal of a



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PCL chain ($M_n$ = 11 300, $M_w/M_n$ = 1.36). Product **4** was separated from nonreacted acryl sugar by precipitating the polymer into methanol. Analysis of product **4** by $^1$H NMR and $^{13}$C NMR revealed that the reaction was highly regioselective at the C-5 hydroxyl. Derivatization of **4** (after purification by precipitation in methanol) with oxalyl chloride (see Supporting Information for the detailed procedure) revealed that <5% of the consumed $\epsilon$-CL formed chains with a carboxyl terminus. The homopolymer PCL results from competitive transacylation and/or initiation by water instead of by **3**.[9] The remaining 95% of the consumed $\epsilon$-CL was incorporated into chains that are exclusively attached by an ester group to the C-5 primary hydroxyl moiety of the acryl sugar **3**.

For the attachment of a different substituent (e.g. bioactive, photolytically reactive) at another chosen site of the sugar core, it was first necessary to end-cap the terminal $\omega$-hydroxyl group of the PCL chain of **4**. Reaction of the PCL hydroxyl chain-end group was performed by using Novozyme-435 as the catalyst and vinyl acetate as an irreversible acylating group, at 30 °C, in toluene as the solvent. The reaction progress was monitored by $^1$H NMR (see Supporting Information). At a 5:1 molar ratio of vinyl acetate to **4**, selective acetylation of the $\epsilon$-hydroxyloligo($\epsilon$-CL) terminal group was observed within 6 h. Analysis of the product by $^1$H NMR showed <2% acylation at the secondary hydroxyl group of the sugar. The triplet due to the $\omega$-hydroxyl methylene protons ($-CH_2$-OH, at 3.66 ppm) of PCL chains disappeared, and a new singlet at 2.05 ppm due to the corresponding acetyl ($-COCH_3$) chain end group protons was observed. The $^{13}$C NMR spectrum of product **5**, when compared to that for **4**, showed a downfield shift of 2.30 ppm for the signal due to the $\omega$-hydroxymethylene carbon of the PCL segment. Also, a resonance due to the acetyl group carbon was seen at 21.06 ppm. All other carbon resonances assigned to **4** showed no substantial change after acetylation, which excludes transesterification between the C-3 secondary hydroxyl of **4** and ester groups of the PCL main chain. Molecular weight analysis of **5** ($M_n$ = 10 900 g/mol, $M_w/M_n$ = 1.36) showed no chain degradation during the end-capping reaction. Chemical esterification of **5** with lauric acid to form **6** (see Scheme 1) further confirmed that acetylation at the C-3 position results in a signal at $\delta$ 5.2 due to the corresponding C*H*-OAc proton. Study of the $^1$H NMR spectrum shows that, for **5**, a signal at 5.2 ppm was not observed.

The ketal group of **5** was successfully hydrolyzed to give **7** by using a trifluoroacetic acid/water mixture (80:20 v/v) for 5 min (see Supporting Information for further details). The product obtained provides two additional hydroxyl groups at defined positions around the sugar core. These groups offer further options for the development of strict control of the three-dimensional arrangement of substituents around the carbohydrate core.

The homopolymerization of **7** was conducted by using AIBN as the initiator in dry DMF (See Supporting Information).[21] Polymerization was continued for 24 h at 60 °C and the reaction was terminated by the precipitation of the product by acetone to yield the corresponding homopolymer (**8**) in 70% yield. The $^1$H NMR spectrum of the homopolymer did not show resonances due to the acryloyl protons (see Supporting Information). Furthermore, study of integral values of NMR signals as well as peak positions confirmed that the expected product was formed. The $^{13}$C NMR (see Supporting Information) spectrum of the polymer further supported that the sugar moiety was not disturbed and that the [$-(-CH-CH_2-)-$] carbon backbone was formed ($M_n$ = 23 000).

A summary of the chemo-enzymatic route communicated herein is as follows: (1) selective acrylation along with prochiral selection of **1** was achieved by lipase catalysis; (2) selective ring-opening of $\epsilon$-CL from the remaining primary hydroxyl group created an acryl-sugar capped macromer; (3) selective end-capping of the terminal hydroxyl of oligo(caprolactone) chains was achieved by lipase-catalysis with high selectivity and percent conversion; (4) the ketal group of **5** was hydrolyzed without disturbing the structure of the remaining macromolecule; and (5) homopolymerization of macromer **7** was successfully demonstrated. Furthermore, the chemo-enzymatic strategy, outlined in Scheme 1, allows at least four different groups to be placed at desired locations around the sugar core with only one protection−deprotection step (ketal removal). Without the introduction of biocatalytic methods, access to such structures would be extremely difficult or possibly nonattainable. In principle, the method developed is flexible so that it may be used to generate a wide array of analogues to **6**. We plan to further exploit this approach for the construction of well-defined macromers and hetero-arm stars. Of particular interest to us are those that self-organize based on specific placement in three-dimensions of low or moderate molecular weight substituents around the core sugar.

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**Supporting Information Available:** Experimental details of the product synthesis and characterization (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) It was observed that the protons of both C-1′and C-5 methylene groups of **1** appeared as a multiplet, one of the corresponding methylene protons shifted downfield on acrylation, i.e., in compound **3** the C-1′ methylene protons appeared as a double doublet at $\delta$ 4.32 ($J$ = 11.76 and 11.47 Hz) while in compound **2** the C-5 methylene protons appeared as a double doublet at $\delta$ 4.34 ($J$ = 11.7 Hz). Furthermore, the anomeric proton C-1 of **2** for major diastereomer appeared at higher $\delta$ values (in $CDCl_3$, 300 MHz) at $\delta$ 6.01 ($J$ = 4.12 Hz) and for the minor diastereomer at $\delta$ 5.90 compared to the corresponding protons in **3** where the major diastereomer appeared at $\delta$ 5.93 ($J$ = 4.12 Hz). This clearly indicates that there is reversal of selectivity during enzyme-catalyzed acrylation. Also the position of the acryl group was unequivocally established by the significant NOE effect observed in H-3 on irradiation of H-1′ in compound **3**.
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