Lipase-Catalyzed Synthesis of Methyl 6-*O*-Poly(ϵ-caprolactone)glycopyranosides

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ABSTRACT: An enzymatic approach to combine ring-opening polymerization of ϵ-caprolactone and regioselective acylation of methyl glycopyranosides has been investigated. *Candida antarctica* lipase B (Novozym 435) catalyzed the regiospecific acylation of methyl galacto- and glucopyranoside and the ring-opening polymerization of ϵ-caprolactone to give methyl 6-*O*-poly(ϵ-caprolactone)glycopyranosides. The synthetic strategy led to the synthesis of methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside with a M_w of 3750 and a polydispersity of 1.3. The best results were obtained by drying the system and carrying out the polymerization at 60 °C in bulk, without solvent. The overall conversion from methyl β-D-glucopyranoside was 80%.

Introduction

The ability to attach synthetic polymers onto carbohydrates is a pathway to new applications in the fields of detergents, packaging, and pharmaceuticals, and it increases the biodegradability of the target polymers.[1,2] However, selective functionalization of carbohydrates is complicated, since carbohydrates contain multiple hydroxyl groups. Selective monoacylation of the carbohydrate is difficult without using protective group strategies. Deprotection is then required and the synthetic scheme becomes complex. Enzymes are highly selective and therefore they have been used to regioselectively acylate carbohydrates in organic solvents without the use of protective groups.[1−6] This regioselectivity, in combination with lipase-catalyzed polyester synthesis, would be an attractive alternative to poorly selective chemical catalysts. The lipase-catalyzed ring-opening polymerization of lactones is a special type of transesterification, since no leaving group is released as a separate molecule. This has previously been investigated by Knani et al.,[7] Uyama et al.,[8,9] MacDonald et al.[10] and Hendersson et al.,[11] who reported the polymerization of ϵ-caprolactone to the corresponding polyester with molecular weights of up to 7700. In this paper, we have combined the above synthetic strategies and investigated the lipase-catalyzed ring-opening polymerization of ϵ-caprolactone by using methyl β-D-glucopyranoside as the initiator. Methyl β-D-glucopyranoside was regiospecifically acylated at the primary hydroxyl group. The lipase-catalyzed polymerization was performed in organic solvent as well as in bulk. To characterize the polyesters, we used matrix-assisted laser desorption and ionization time-of-flight mass spectroscopy (MALDI-TOF MS).[12−14] MALDI-TOF MS is a soft ionization process, and therefore the polymer is not fragmented by the ionization process. This method is often used for measuring the true molecular weight of large biomolecules. In polymer chemistry, it is employed for the characterization of polymers. Analysis is very rapid and yields true molecular weights as well as the mass of the repeat unit. The technique has, for example, been used for the characterization of partially hydrolyzed bacterial polyhydroxybutanoate.[15] Recently, Nobes et al.[16] characterized polyesters, obtained by lipase-catalyzed ring-opening polymerization, using MALDI-TOF MS.

Materials and Methods

Materials. *Candida antarctica* lipase B, Novozym 435 (7000 U/g), an immobilized enzyme, was a gift from Novo Nordisk A/S. Lipase from *Pseudomonas cepacia* (30 000 U/g) was obtained from Amano Pharmaceutical Co. ϵ-Caprolactone, was obtained from Aldrich Chemical Co. Methyl β-D-glucopyranoside and methyl α-D-galactopyranoside were obtained from Sigma Chemical Co. Analytical grade acetonitrile was obtained from J. T. Baker and, prior to use, dried by shaking with molecular sieves.

Lipase-Catalyzed Polymerization. Polymerization in Acetonitrile. The methyl glycopyranosides were dried in a desiccator over P_2O_5. ϵ-Caprolactone was dried by activated molecular sieves. A mixture (1 mL) of ϵ-caprolactone (438 mM) and methyl glycopyranoside (14.6 mM) in dry acetonitrile was added to capped vials, each containing lipase (10 mg), which had previously been dried in a desiccator over P_2O_5. The vials were shaken at 120 rpm for different periods of time at 60 °C. Samples were withdrawn from the reaction mixtures and immediately analyzed by GC (Hewlett-Packard 5890 chromatograph, equipped with a 25 m × 0.32 mm CP Sil-8 CB column). The consumption of ϵ-caprolactone and the production of dimeric ϵ-caprolactone were analyzed using hexadecane as the internal standard. The consumption of methyl glycopyranoside was separately analyzed by GC after derivatization with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane, also using hexadecane as the internal standard. Samples (10 μL) were withdrawn for MALDI-TOF MS analysis and mixed with an equal volume of matrix (gentisic acid dissolved in a 1:1 mixture of methanol and water). An aliquot (0.5 μL) was applied to the sample probe, the solvent evaporated by vacuum, and the probe inserted into the spectrometer (Hewlett-Packard G20205 A LD- TOF system). The average M_w and polydispersity for methyl glycopyranoside-initiated poly(ϵ-caprolactone) were determined.

Bulk Polymerization. Dried ϵ-caprolactone (4.4 mmol) was added to vials, each containing methyl β-D-glucopyrano-

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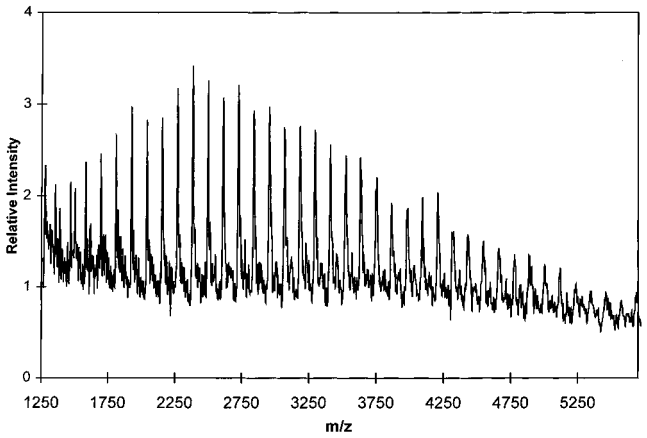


Figure 1. Example of a MALDI-TOF MS spectrum of methyl 6-*O*-poly(ϵ-caprolactone)-*β*-D-glucopyranoside obtained from *C. antarctica* lipase B-catalyzed ring-opening polymerization of ϵ-caprolactone with methyl *β*-D-glucopyranoside. The average M_w is 3168, corresponding to a degree of polymerization of about 26.

side (0.15 mmol) and *C. antarctica* lipase B (10 mg), which had previously been dried in a desiccator over P_2O_5. The polymerization was performed using the same reaction conditions as in the acetonitrile experiment (60 °C, 120 rpm). Either the contents of the incubated vials were, after different periods of time, immediately silylated and methyl *β*-D-glucopyranoside and ϵ-caprolactone consumption was analyzed by GC or the polymer product was dissolved in acetonitrile and the lipase removed by filtration. The average M_w was determined by MALDI-TOF MS.

Structure Determination of Methyl 6-*O*-Poly(ϵ-caprolactone)-*β*-D-glucopyranoside. The crude methyl 6-*O*-poly(ϵ-caprolactone)-*β*-D-glucopyranoside prepared by bulk polymerization was dissolved in acetonitrile and fractionated on a silica gel column. The solvent system was ethyl acetate−methanol−water (100:10:1) v/v. Fractions were screened by MALDI-TOF MS, and a pure fraction of methyl *β*-D-glucopyranoside-initiated poly(ϵ-caprolactone) was obtained (Figure 1). The fraction had an average M_w of 3168 and a monomer repeat mass of 114 Da, thus confirming the structure of the poly(ϵ-caprolactone) chain. Detectable peaks were registered from 1559 Da (corresponding to the 12-mer including the methyl *β*-D-glucopyranoside end group) to 5094 Da (corresponding to 43-mer). The polydispersity (M_w/M_n) of the fraction was 1.1. Both the repeat unit and the masses obtained were consistent with a polydisperse solution of methyl 6-*O*-poly(ϵ-caprolactone)-*β*-D-glucopyranoside.

NMR Analysis. All analyses were performed on a Bruker AMX 300 WB system at ambient temperature using a $^1H/^{13}C$ dual probe head at a magnetic field strength of 7.04 T (300 MHz ^1H resonance frequency).

The positions of the protons were determined by a 2D $^1H/^1H$ COSY experiment. The 2D $^1H-^1H$ COSY spectra were acquired at 292 (±1) K, using a 90° mixing pulse, 2 dummy scans, and 128 transients for each of the 256 experiments (F1-domain). The digital resolution in the F2 domain was 0.44 Hz/pt. The time domain consisted of 4096 data points, the spectral width was 6.0 ppm, and the delay between transients was 4 s including acquisition time. The acquired data were sine-bell apodized in both dimensions and Fourier transformed, and finally a symmetrized magnitude spectrum was calculated. The resulting spectra were 4096 times 256 data points. The chemical shift scale was calibrated with respect to TMS by assigning a value of 1.94 ppm to the position of the central signal in the quintet from residual protons in acetonitrile.

Polymer chain: ^{13}C NMR (CD_3CN) δ 173.2 (CO), 63.7 (OCH_2), 33.6 (CH_2), 28.1 (CH_2), 25.1 (CH_2), 24.3 (CH_2); ^1H NMR (CD_3CN) δ 1.34 (m, CH_2), 1.53 (m, CH_2), 1.59 (m CH_2), 2.27 (t, CH_2CO), 4.01 (t, CH_2O).

Table 1. Methyl Glycopyranoside-Initiated Polymerization of ϵ-Caprolactone in Acetonitrile and in Bulk at 60 °C (Catalyzed by *C. antarctica* Lipase B and *P. cepacia* Lipase)[a]

solvent	lipase	A/B	time (h)	conv (%) A	conv (%) B1	conv (%) B2	di (%)	pol (%)	M_w
CH_3CN	*C. antarctica*	30:1	56	95	73		60	40	2141
CH_3CN	*C. antarctica*	30:1	56	95		58	61	39	1037
CH_3CN	blank	30:1	120	0	0		0	0	0
CH_3CN	blank	30:1	130	0		0	0	0	0
CH_3CN	*P. cepacia*	30:1	100	20		40	2	98	2363
bulk	*C. antarctica*	30:1	8	93	32		3	97	3101
bulk	*C. antarctica*	60:1[b]	24	95	81		1	99	3170
bulk	*C. antarctica*	60:1	8	45	80		2	98	3757
bulk	blank	30:1	24	0	0		0	0	0

[a] A is ϵ-caprolactone, B1 is methyl *β*-D-glucopyranoside, B2 is methyl-α-D-galactopyranoside, and di is percent dimeric ϵ-caprolactone of the total products, all determined by GC. Pol is the percent polymers of the total products. M_w is the average weight of methyl 6-*O*-poly(ϵ-caprolactone)glycopyranoside determined by MALDI-TOF MS. The ϵ-caprolactone concentration was 0.44 M in acetonitrile (1 mL) and 8.8 M in bulk. Methyl *β*-D-glucopyranoside had a concentration of 15 mM in acetonitrile (1 mL) and an amount of 30 mg (0.15 mmol) in bulk. The amount of lipase was 10 mg. [b] Performed by starting with a mole ratio of 30:1 and then adding ϵ-caprolactone after 4 h, obtaining a ratio of 60:1.

Methyl *β*-D-glucopyranoside end group: ^{13}C NMR (CD_3CN) δ 103.7 (C1), 76.3, 73.6, 73.4, 70.0, 56.1 (OCH_3). The carbohydrate C6 could not be assigned, due to overlapping with end groups of the polymer chains. ^1H NMR (CD_3CN): δ 3.07 (1 H, dd, J = 8.6, 7.7 Hz, H2), 3.29 (1 H, H3), 3.32 (1 H, H4), 3.40 (1 H, H5), 3.42 (3 H, s, OCH_3), 4.13 (1 H, d, J = 7.7 Hz, H1), 4.19 (1 H, dd, J = 11.9, 5.8 Hz, H6), 4.30 (1 H, dd, J = 11.9, 2.2 Hz, H6).

Methylation Analysis. To confirm the acyl position, a methylation of the hydroxyl groups according to Arnap et al.[17] was performed. Then, hydrolysis with aqueous trifluoroacetic acid, followed by $NaBH_4$ reduction to the corresponding alditol, and finally acetylation were carried out. The only product detected by GC-MS was 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl glucitol.

Results and Discussion

Polymerization in Acetonitrile. Our group has previously investigated the lipase-catalyzed acylation of methyl glycopyranoside mixtures by octanoic acid in acetonitrile.[18] In an enzyme screening experiment for the acylation of methyl *β*-D-glucopyranoside, *C. antarctica* lipase B was shown to be an efficient catalyst with a high production of the 6-*O*-monoester. This regioselectivity, in combination with lipase-catalyzed ring-opening polymerization of ϵ-caprolactone, would be an attractive alternative when methyl 6-*O*-poly(ϵ-caprolactone)-*β*-D-glucopyranoside was prepared.

In Table 1, the results from such reactions in acetonitrile are shown. *C. antarctica* lipase B is an efficient catalyst both when it comes to acylating methyl *β*-D-glucopyranoside and to ring-opening of ϵ-caprolactone. *C. antarctica* lipase B has, after 56 h, converted 95% mol of the ϵ-caprolactone to dimeric ϵ-caprolactone and methyl *β*-D-glucopyranoside- and water-initiated polyester products. The average M_w was 2141 for the methyl 6-*O*-poly(ϵ-caprolactone)-*β*-D-glucopyranoside with a polydispersity of 1.3. The MALDI-TOF MS registered detectable peaks from 570 to 4811 Da (corresponding to the 2-mer and 39-mer, including the methyl *β*-D-glucopyranoside end group), with a monomer repeat mass of 114 Da.

In Figure 2, the conversion of ϵ-caprolactone into dimeric ϵ-caprolactone and methyl 6-*O*-poly(ϵ-caprolac-

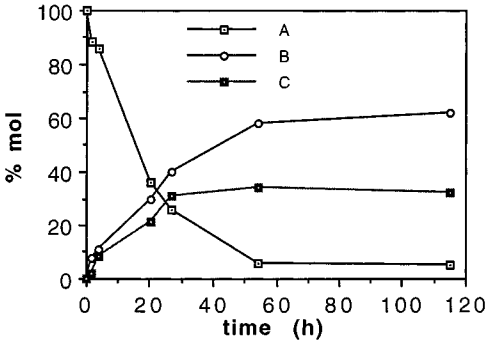


Figure 2. *C. antarctica* lipase B (10 mg)-catalyzed polymerization of ϵ-caprolactone (0.44 M) with methyl β-D-glucopyranoside (15 mM) at 60 °C. Time dependence of ϵ-caprolactone (A) consumption and ϵ-caprolactone incorporation into dimeric ϵ-caprolactone (B) or methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside (C), in acetonitrile (1 mL).

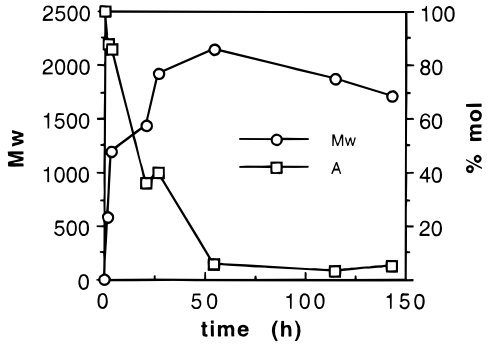


Figure 3. Average M_w of methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside and ϵ-caprolactone (A) consumption as a function of time determined by MALDI-TOF MS and GC, respectively (reaction in acetonitrile).

tone)-β-D-glucopyranoside is shown. Almost 60% of the ϵ-caprolactone had been converted to dimeric ϵ-caprolactone, and only 30% of the ϵ-caprolactone had been converted to the methyl glycopyranoside-initiated poly(ϵ-caprolactone). Of the remaining ϵ-caprolactone, 5% had been included in the water-initiated polyester as well as in a small amount of macrocyclic ϵ-caprolactones. The last 5% had not reacted. Macrocycles of this kind have been reported by Knani et al.[7] In our case there was always a small amount of water-initiated poly(ϵ-caprolactone) present, since water was not totally excluded.

Figure 3 represents the average M_w of the methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside and consumption of ϵ-caprolactone as a function of time. The average M_w increased until almost all ϵ-caprolactone was consumed (95%, in 56 h). At high consumption of ϵ-caprolactone (above 95%), the average M_w decreased. This may be due to decreased chain propagation rates at high consumption so that competing reactions, such as chain cleavage, become increasingly important. The polydispersity was between 1.3 and 1.4 for all measurements.

In our recent lipase selectivity experiments, we also found that *C. antarctica* lipase B was somewhat lower in selectivity for the acylation by octanoic acid of methyl α-D-galactopyranoside relative to methyl β-D-glucopyranoside, while *Pseudomonas cepacia* lipase was highly selective for methyl α-D-galactopyranoside.[18] A few experiments with methyl α-D-galactopyranoside and *P. cepacia* lipase were therefore included to look at the effect of the change of methyl glycopyranoside and lipase (Table 1). The *C. antarctica* lipase B shows the expected

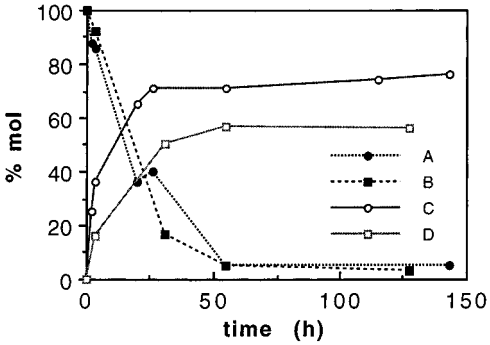


Figure 4. *C. antarctica* lipase B (10 mg)-catalyzed polymerization of ϵ-caprolactone (0.44 M) with methyl β-D-glucopyranoside (15 mM) at 60 °C and the same experiment with methyl α-D-galactopyranoside as initiator. Time dependence of ϵ-caprolactone (A, B) consumption and methyl β-D-glucopyranoside (C) and methyl α-D-galactopyranoside (D) conversion (% mol) in acetonitrile (1 mL).

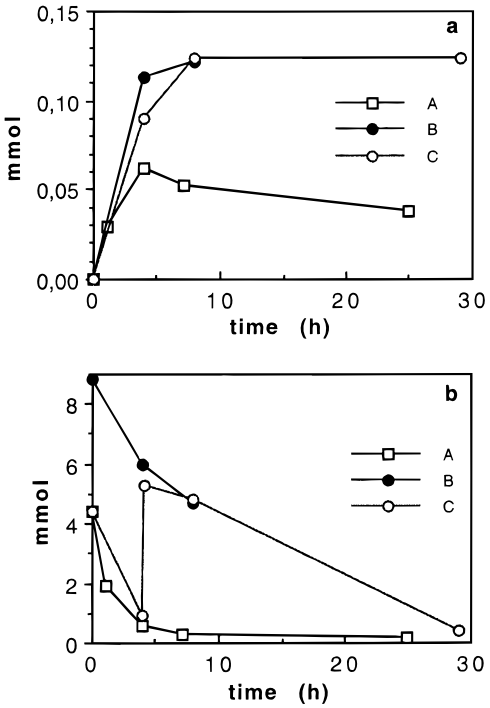
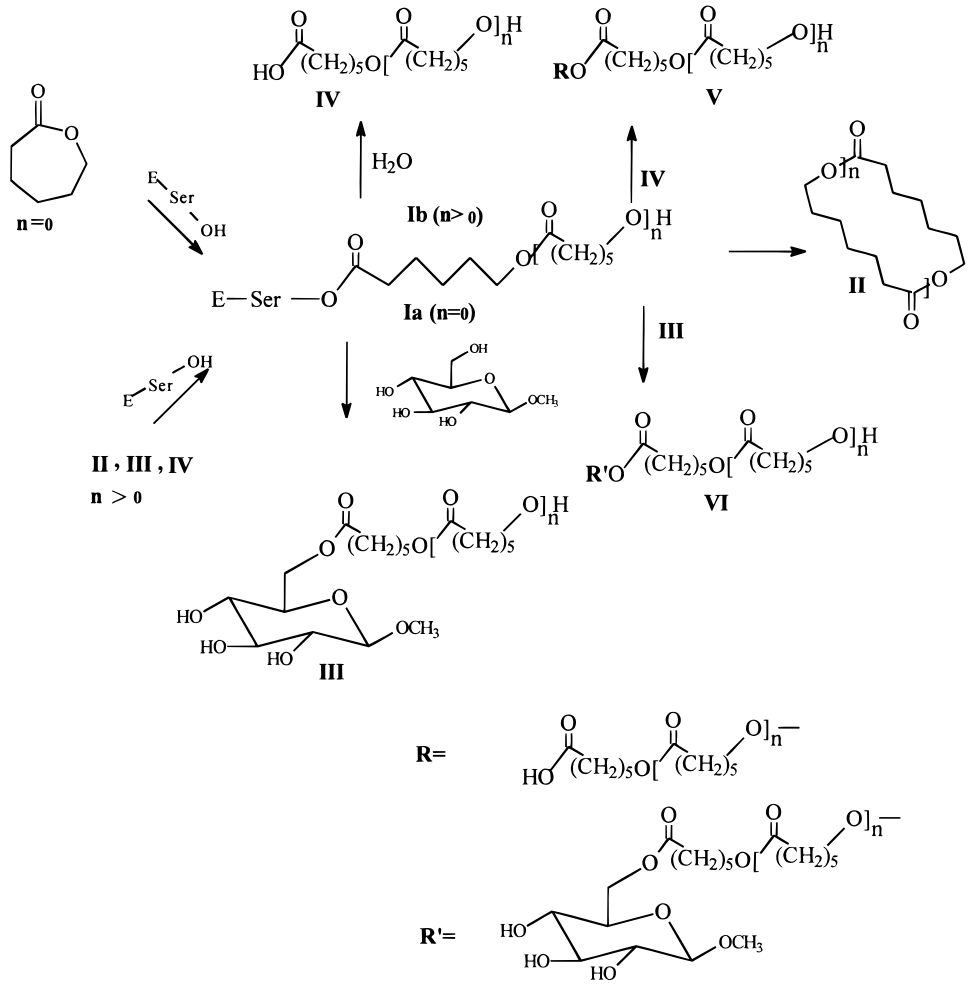


Figure 5. *C. antarctica* lipase B-catalyzed polymerization of ϵ-caprolactone with methyl β-D-glucopyranoside at 60 °C in bulk, with the ratio of 30:1 (A) or 60:1 (B). (a) Time dependence of methyl β-D-glucopyranoside (A−C, all 155 μmol) conversion (mmol). (C) is the methyl β-D-glucopyranoside conversion, starting with 4.4 mmol of ϵ-caprolactone and, after 4 h, adding the same amount of ϵ-caprolactone again. (b) Time dependence of ϵ-caprolactone (A, 4.4 mmol; B, 8.8 mmol; C) consumption (mmol). (C) is the ϵ-caprolactone consumption, starting with 4.4 mmol of ϵ-caprolactone and, after 4 h, adding the same amount of ϵ-caprolactone again.

lower conversion of the methyl α-D-galactopyranoside compared to the methyl β-D-glucopyranoside (Figure 4).[18] The rate of ϵ-caprolactone consumption was the same, whether it was methyl β-D-glucopyranoside or methyl α-D-galactopyranoside being acylated.

P. cepacia lipase was able to acylate methyl α-D-galactopyranoside, but the conversion of methyl α-D-galactopyranoside was lower than in the case of *C. antarctica* lipase B (Table 1). The formation of a cyclic byproduct, 2% dimeric ϵ-caprolactone, was, however, low, indicating that the cyclic mechanism was suppressed when *P. cepacia* lipase was used.

Scheme 1



Bulk Polymerization. To investigate if the amount of cyclic byproducts could be decreased, polymerizations were performed without solvent. The initial experiment in bulk was made with the same mole ratio of ϵ-caprolactone and methyl β-D-glucopyranoside as in acetonitrile, that is, 30:1 (Figure 5a,b). The ϵ-caprolactone was rapidly consumed, and after 4 h, the methyl β-D-glucopyranoside was not able to further initiate the ring-opening polymerization. To increase the yield of methyl β-D-glucopyranoside, the mole ratio was doubled to 60:1. This was carried out in two ways: either by starting with the doubled ratio or by starting with a mole ratio of 30:1 and then, after 4 h, adding ϵ-caprolactone. The results are presented in Figure 5a,b (curves B and C). The methyl β-D-glucopyranoside conversion was increased, and after 8 h, a plateau was reached. The average M_w of the methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside was at that point 3757, with a polydispersity of 1.3 and constituting 90% of the polyester products. MALDI-TOF MS registered peaks from 654 to 6358 Da (corresponding to the 4-mer and 54-mer, including the methyl β-D-glucopyranoside end group), with a repeat monomer mass of 114 Da. The conversion of methyl β-D-glucopyranoside into methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside was 80%.

In Figure 6, the average M_w of the methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside and consumption of ϵ-caprolactone as a function of time are shown. The chain propagation rate increased compared to the polymerization in acetonitrile (Figure 3). So did the

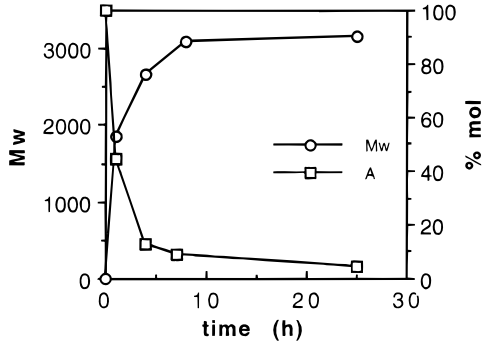


Figure 6. Average M_w of methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside (M_w) and ϵ-caprolactone (A) consumption as a function of time determined by MALDI-TOF MS and GC, respectively (bulk polymerization).

average M_w, which increased by 1000. The average M_w after 25 h was 3168 and almost all ϵ-caprolactone (95%) was consumed. However, this average M_w was almost reached within 8 h. The amount of dimeric ϵ-caprolactone never exceeded 3%, and no discernible amounts of macrocycles could be observed in the bulk experiment.

Determination of Acylation Position. NMR analysis and methylation analysis were made to confirm the acylation position since MALDI-TOF MS only gives information about the molecular weight. Due to the high average M_w of the methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside, the ^1H and ^{13}C NMR spectra were characterized by low signal intensities for the carbohy-

drate end group. The methyl β-D-glucopyranoside end group of the polyester gave the following shifts: ^{13}C NMR (CD_3CN) δ 103.7 (C1), 76.3, 73.6, 73.4, 70.0, 56.1 (OCH_3). The carbohydrate C6 could not be assigned, due to overlap with the signal cluster from end groups of the polymer chains at δ 63.7 ppm. As established by Yoshimoto et al.,[19] acylation of a hydroxyl group at the C6 position of glucose results in a downfield shift (1−3 ppm) of the signal corresponding to the O-acylated carbon and an upfield shift (1−3 ppm) of the signal corresponding to the neighboring carbon. In the ^{13}C spectrum of methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside, one signal shifted upfield to the 73 ppm region as compared to methyl β-D-glucopyranoside.[20] No downfield shift was observed, and this indicates acylation at the hidden C6 position.

The positions of the protons of the methyl β-D-glucopyranoside end group could be determined by a $^1H/^1H$ COSY experiment. The observed unshielding of the H6 protons (δ 4.19 and δ 4.30) as compared to methyl β-D-glucopyranoside (δ 3.58 and δ 3.74)[20] is in agreement with the presence of an acyl substituent at the primary alcohol function.[21]

Carbohydrates that are labile to bases or that possess alkali-labile substituents such as *O*-acyl groups cannot be methylated under strongly alkaline conditions without the loss of some or all of those groups. The application of methyl trifluoromethanesulfonate as an alkylation agent together with 2,6-di-*tert*-butylpyridine as a proton scavenger in nonpolar solvents has been reported.[17, 22] In these reactions, *O*-acetyl and *O*-benzoyl are essentially stable and the methylated products are obtained in nearly quantitative yields. The methylation analysis of methyl-6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside using these mild conditions gave 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl glucitol as the only product. Together with the NMR analysis, this confirms that the primary hydroxyl group of methyl β-D-glucopyranoside had been preferentially acylated.

Reaction Mechanism. Scheme 1 proposes the different types of reactions that compete during the polymerization of ϵ-caprolactone, catalyzed by *C. antarctica* lipase B. In the initial step, the serine 105 of the lipase[23] will make a nucleophilic attack on ϵ-caprolactone and formation of acyl complex Ia is achieved. Acyl complex Ia can then be deacylated by methyl β-D-glucopyranoside or water to form III ($n = 0$) or IV ($n = 0$), respectively.[24] III and IV will then deacylate Ia to produce V and VI. V and VI will be one monomer unit larger than III and IV. Deacylation of Ia by V and VI will lead to further propagation with one monomer unit added each time. The major chain propagation will follow this mechanism. The system is, however, more complex, since *C. antarctica* lipase B can form acyl complexes Ib ($n > 1$) with oligomer units. This is necessary for formation of cyclic products, II, which is a significant mechanism in acetonitrile (Figure 2). When the oligomeric acyl complex Ib is formed, from III, it will be the acyl groups of the poly(ϵ-caprolactone) chain and not the ester bond to the methyl β-D-glucopyranoside that are exposed to a nucleophilic attack by serine 105, since the polyester chain is a better substrate for *C. antarctica* lipase B. This is explained by Figures 3 and 4, where the average M_w of methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside decreased at long reaction times (Figure 3), while the reacted methyl β-D-glucopyranoside did not decrease (Figure 4).

Table 2. Initial Rates of Consumption of the Three Substrates (ϵ-Caprolactone (A), Methyl β-D-Glucopyranoside (B), and Methyl α-D-Galactopyranoside (C)) in Acetonitrile and in Bulk

solvent	V_A [nmol/(min·mg)][a]	V_B [nmol/(min·mg)][a]	V_C [nmol/(min·mg)][a]	V_A/V_B
CH_3CN	50	3	1	20
bulk	1200	50	not done	24

[a] The initial rates were calculated by using the linear parts of the curves (Figures 4 5a,b) and dividing by the total weight of the Novozym 435 (10 mg) preparation.

Initial Rates and Selectivity of *C. antarctica* Lipase B. In Table 2, the initial reaction rates of *C. antarctica* Lipase B are shown. *C. antarctica* lipase B has an initial reaction rate for ϵ-caprolactone that is 24 times higher in bulk than in acetonitrile. This can be explained by a high K_m of the lipase for ϵ-caprolactone and a better saturation of the enzyme under the bulk conditions, where the substrate concentration is 20 times higher than in the acetonitrile solution (8.77 M in bulk and 0.44 M in acetonitrile). The initial rate ratios of consumption of the ϵ-caprolactone to methyl β-D-glucopyranoside are in the same order ($V_{\epsilon-\text{caprolactone}}/V_{\text{methyl }\beta-\text{D}-\text{glucopyranoside}} \approx 20$) both in acetonitrile and in bulk. The ratios substantiate the fact that *C. antarctica* lipase B preferably acylates the polymer hydroxyl group relative to the acylation of the primary hydroxyl group of methyl β-D-glucopyranoside. Of the total deacylation rate of the lipase-ϵ-caprolactone acyl complex (Ia), only 5% (1/20) is contributed by methyl β-D-glucopyranoside.

Conclusions

C. antarctica lipase B is an excellent lipase combining the regiospecific acylation of the primary hydroxyl group of methyl β-D-glucopyranoside with the ring-opening polymerization of ϵ-caprolactone to give methyl 6-*O*-poly(ϵ-caprolactone)-β-D-glucopyranoside. To avoid substantial amounts of cyclic byproducts, the reaction should be conducted as a bulk polymerization.

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