Highly Anomer- and Regio-selective Transesterification Catalyzed by Alkaline Protease from *Bacillus subtilis* in Organic Media

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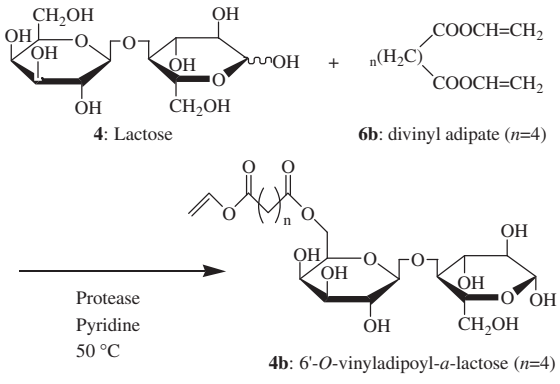
Highly anomer-selective-6(6′)-acylation reactions from anomeric mixtures of several mono- and disaccharides with divinyl dicarboxylates ranging from 4 to 10 carbon atoms were catalyzed by an alkaline protease from *Bacillus subtilis* in pyridine. A series of new pure α-anomeric polymerisable vinyl sugar esters of dibasic acids were synthesized in good selectivity and yields.

Polymerizable acylated α- or β-D sugars are of great interests as surface-active compounds with industrial potential applications, as optically active functional water-soluble monomers for sugar-containing copolymers preparation, and as valuable chiral intermediates in glycoconjugates synthesis.[1] The chemical methods for preparation of acylated α- or β-D sugars at a specific hydroxy group are difficult, and have relied on complex protection–deprotection techniques. Under the usual conditions, the removal of the protective group was drastic so as to cleave the ester, cause migration or lead to anomerization.[2]

Biotransformation using isolated enzymes has been increasingly exploited, as regio- and stereo-selectivity can be achieved. Since Klibanov and co-workers first demonstrated selective acylation of monosaccharide catalyzed by lipases,[3] various studies concerning similar biotransformations have been reported.[4] To the best of our knowledge, there are no reports for the stereoselective preparation of α- or β-D sugar ester isomers from hexoses and disaccharides using enzyme methods. Most sugar esters that have been reported were anomeric mixtures. Degn et al. synthesized some long chain fatty acid esters of glucose having high proportions of the α-anomer in *tert*-butanol using immobilized lipases from *C. antarctica* and *M. miehei.* However, only when the chain lengths of acyl donors up to a certain number (>8), the enzyme shows the selective preference for the α-anomer substrate, but no preparation of pure α-anomeric products were reported.[5]

We report herein a highly anomer-selective transesterification reaction catalyzed by an alkaline protease from *Bacillus subtilis* (Wuxi Enzyme Co. Ltd, Wuxi, P. R. China) in pyridine. A series of new α-anomeric polymerisable vinyl sugar esters were synthesized in good selectivity and yields starting from anomeric mixtures of several monosaccharides (glucose **1**, mannose **2**, galactose **3**) and disaccharides (lactose **4**, maltose **5**). Divinyl dicarboxylates (**6a–c**: $n = 2, 4, 8$) were selected as acyl donors. One ester function of divinyl dicarboxylates was used in transesterification and another vinyl ester function as a polymerisable group. Sugar-containing polymers have recently been a focus of intensive research as functional materials for drug delivery systems, DNA probe or cell fixing material, biocompatible and biodegradable polymers.[6]

We found that the alkaline protease from *B. subtilis* is more active in pyridine than lipase from porcine pancreas (PPL) and other lipases with the optimum at 50 °C. The synthesis of vinyl sugar esters is shown in **Scheme 1** for **4b** as the representative example. A mixture of lactose (3.6 g, 10 mmol, α- and β-mixture), divinyl adipate (7.9 g, 4 equiv.), anhydrous pyridine (100 mL), alkaline protease from *B. subtilis* (2.0 g) was stirred at 50 °C for 5 days. The product was isolated by silica gel chromatography with an eluent consisting of ethyl acetate/methanol/water (100:10:5, v/v) to give 6′-*O*-vinyladipoyl-α-lactose (**4b**) (2.28 g, 46% isolated yield) as a white solid.[7] Various kinds of vinyl esters from sugar **1**–**5** were prepared in good selectivity under similar conditions (**Table 1**).



Scheme 1.

The position of acylation was determined by ^{13}C NMR (Bruker AVANCE DMX 500) according to the general strategy described by Yoshimoto et al.[8] The reactions were highly regioselective towards the specific hydroxy group of sugar substrates. The acylation of monosaccharides (glucose **1**, mannose **2**, galactose **3**) occurred preferentially to the primary hydroxy group, while lactose **4**, maltose **5** were specifically acylated in the primary hydroxy group of the non-reducing-end moiety.

We were delighted to observe that products **1b–c**, **3a–c** and **4b–c** were the pure α-pyranose derivatives as determined by ^{1}H and ^{13}C NMR. Furthermore α- and β-anomeric ratios of mannose ester products **2a–c** were also higher than that of starting mannose itself (78:22). To the best of our knowledge, it's the first report on anomer-selective-6(6′)-acylation from anomeric mixture of carbohydrate substrates to prepare polymerisable vinyl α-sugar esters using protease method.

The stereo-selectivity of enzymatic reaction has been discovered to markedly depend on the solvent, temperature, enzyme resource, the mode of enzyme preparation and the water content of enzymes.[9] In this work, we found the ratios of α- and β-anomers of products were dependent on the chain length from C-4 to C-10. For example, the ratio of α- and β-anomers in **1a–c** increased from 50/50 (**1a**) and 100/0 (**1b** and **1c**) with in-

Table 1. Highly regio- and stereo-selective enzymatic synthesis of vinyl fatty acid sugar esters

Entry	Sugar substrate	Acyl donor	Product	Acylation site[a]	Yield[b]/%	Anomer ratio[c]/(α/β)
1	Glucose	**6a**	**1a**	6	**80**[d]	50:50
2	Glucose	**6b**	**1b**	6	**53**[e]	100:0
3	Glucose	**6c**	**1c**	6	**38**[e]	100:0
4	Mannose	**6a**	**2a**	6	**85**[d]	82:18
5	Mannose	**6b**	**2b**	6	**69**[f]	90:10
6	Mannose	**6c**	**2c**	6	**43**[g]	91:9
7	Galactose	**6a**	**3a**	6	**70**[d]	100:0
8	Galactose	**6b**	**3b**	6	**47**[e]	100:0
9	Galactose	**6c**	**3c**	6	**35**[g]	100:0
10	Lactose	**6a**	**4a**	6′	**62**[g]	49:51
11	Lactose	**6b**	**4b**	6′	**46**[g]	100:0
12	Lactose	**6c**	**4c**	6′	**31**[g]	100:0
13	Maltose	**6a**	**5a**	6′	**53**[g]	49:51
14	Maltose	**6b**	**5b**	6′	**42**[g]	48:52
15	Maltose	**6c**	**5c**	6′	**34**[g]	50:50

[a]Determined by ^{13}C NMR.
[b]Based on weight of crude samples and relative to the initial loading.
[c]Determined by NMR and GC or HPLC.
[d-g]Reaction time is 0.5 day[d], 3 days[e], 4 days[f], 5 days[g], respectively.

creasing chain length of the acyl donors.

It was interested that the enzyme catalysis for acylation of non-anomeric 6(6′) primary hydroxy groups was involved for the stereoselectivity at anomeric 1-positions of sugars. Although enzymatic acylation occurred at non-anomeric 6(6′) positions, steric or conformational restriction of anomeric position in enzyme binding sites probably caused the anomeric control of enzymatic reaction. We observed that the reactivity of α- and β-anomers of carbohydrates in the transesterification was different. Similar reactivity differences of α- and β-anomers of carbohydrates and related compounds in hydrolysis, oxidation, acylation, and glycosylation reactions which occurred at anomeric 1-position or other carbohydrate carbons have been reported.[10] In this study we also observed that the reactivity of α- and β-anomer was affected by acyl donors and the difference of reactivity was magnified with increasing chain length of the acyl donor similarly to Degn's results.[5] Products with higher α-anomeric proportions were prepared owing to the different reactivity of α- and β-anomers.

However an important question raised along with the enzymatic anomer-selective was what roles the kinetic anomeric effect played in the enzymatic process. There is a kinetic equilibrium between α- and β-anomers of sugars and sugar esters, for example equilibrium α/β ratios of D-glucose (**1**) and vinyl glucose esters (**1a–c**) in pyridine was around 44/56, 50/50, 58/42, and 60/40 respectively. The distribution of α-anomers increased with increasing chain length of the acyl part due to the kinetic anomeric effect. But only the reactivity difference of α- and β-anomers can cause synthesis of products with higher α-anomeric proportions than equilibrium condition. Apparently the mutarotation of products must be slow, otherwise a distribution approximately equal to the equilibrium anomeric ratio would be expected and no products with pure anomers can be prepared. A slow mutarotation was observed in pyridine before or after silylation or in the DMSO solvent conditions used for NMR. Storing the samples for long time at room temperature did not affect the distribution of anomers.

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