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Influencing the regioselectivity of lipase-catalyzed hydrolysis with [bmim]PF_6

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ABSTRACT

An easy preparation of mono-deprotected thioglucopyranosides via a selective *Candica cylindracea* lipase-catalyzed hydrolysis of a commercially available peracetylated precursor is described. Especially, ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranoside and ethyl 2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside were obtained in 100% and 54% isolated yields, respectively. The influence of the ratio of [bmim]PF_6/buffer towards the regioselectivity of the deacetylation step and the acyl migration is discussed.

Non-cellulosic linear and branched β-D-glucans of biological relevance are encountered in many different organisms, including fungi, plants and bacteria. Among them, fungal β-(1,3)- and β-(1,6)-glucans appear to be effective immunomodulators.[1–5] The branching positions and frequencies of fungal β-(1,3),(1,6)-glucans are thought to determine their biological activity.[6] For instance, current studies aim at clarifying whether β-(1,6)-glucans have the ability to induce release of inflammatory mediators as their β-(1,3)-counterparts.[7] The chemical synthesis of such complex biomolecules generally involves many protecting group interconversion. In connection with our ongoing interest in β-D-glucans, we studied the opportunity to introduce biocatalyzed deprotection steps in order to (i) facilitate the introduction of branching units, and (ii) reduce the overall number of reactions.

Fatty acids of carbohydrates are well described to be potentially useful as synthetic intermediates. Interestingly lipase-catalyzed deacylation of fatty acid esters of sugars is known to occur under neutral conditions. The initial work of Wong[8] underlined that the *Candida* lipase is the best biocatalyst for selective deacylation of the primary position from peracylated methyl pyranosides. Moreover most lipases appeared to be highly regioselective towards the hydrolysis of the primary position of peracetylated monosaccharides. For example, *Candida rugosa* lipase (CRL) was shown to be particularly efficient,[9–12] as well as the immobilized *Candida antarctica* lipase (CAL-B). Nevertheless, the same enzyme was also able to hydrolyse the 3,4,6-tri-*O*-acetyl-glucal at the C-3 secondary alcohol group in 99% yield, demonstrating the importance of the nature of the sugar for the regioselectivity of the deprotection.[13] In addition lipases are also well known to work more efficiently in the presence of organic solvent. Recently ionic liquids have been used advantageously to replace added organic co-solvent since they are more respectful of the environment and enzyme-harmless solvents. Their effects on the structure, stability and activity of enzymes has been surveyed by few authors.[14,15] Considering their unconventional properties, brief overviews of the literature would assume that polarity concept is too elusive to serve as a basis to predict solubility behaviour or reaction rate. Nevertheless, [bmim]PF_6 appears to be particularly advantageous in dissolving such hydrophobic substrates because of a modest polarity compared to others ionic liquids according to Reichardt's polarity scale.[16] Moreover its positive influence on the rates and regioselectivity of lipase-catalyzed hydrolysis and alcoholysis of 3,4,6-tri-*O*-acetyl-glucal has also been reported and opposed to the results obtained with the hydrophilic ionic liquid [bmim]BF_4.[17] However, whereas the regioselective enzymatic mono-hydrolysis of different fully acetylated glycopyranoses and glycopyranosides has been described conducting to either secondary or primary free alcohol group, the one of thioglycosides has not been reported yet. In addition, thioglycosides are extremely useful synthetic intermediates since they can act both as acceptors during oligosaccharidic synthesis and, once activated, as powerful donors too.[5,18,19] Herein, we describe our investigation on the regioselective lipase-catalyzed hydrolysis of the peracetylated ethyl thioglucopyranoside **1** in order to readily access to convenient mono-deacylated building blocks (Scheme 1). The influence of [bmim]PF_6/buffer ratio on the regioselectivity of the deacylation was also studied.

Based on these considerations, initial experiments were performed using a two phase reaction mixture composed of 50% of [bmim]PF_6 and 50% of sodium phosphate buffer (50 mM, pH 7.0),

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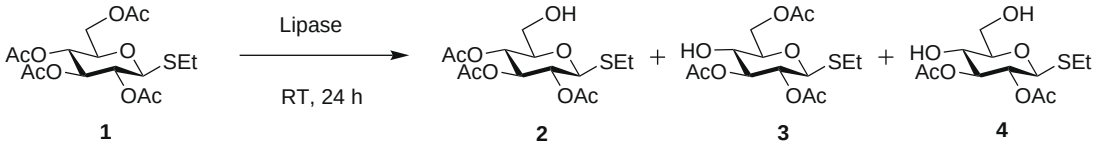

1 2 3 4

Scheme 1. Lipase-catalyzed deprotection of the commercially available ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **1** in [bmim]PF_6 reaction mixtures.

at room temperature. Since the following of the reaction by TLC was difficult due to the presence of a significant proportion of ionic liquid, the experiment time was arbitrary set up to 24 h. A screening using large excess (>500 Units) of readily available enzymes was then carried out (Table 1). Reactions were stopped by extracting all carbohydrates from the reaction mixture. Considering their different retention properties on silica gel, products **1**–**4** were isolated by flash chromatography on silica gel and easily characterized by NMR.[20] For example and as expected for ^{1}H NMR of deacetylated compounds, signals corresponding to H-6, H-6′ and/or H-4 were shifted highfield of about 1 ppm in compounds **2**–**4** when compared to **1**. Interestingly, previous experiments using triolein as substrates demonstrated that all the biocatalysts of this study were active in such media containing half of ionic liquid. Considering our substrate **1**, all of the reactions were completely selective for the preparation of one compound, that is, only one product (**2**, **3** or **4**) was obtained in each separated experiment. The only esterase used in this study (entry 1) gave us thioglucoside **3** owing a free secondary alcohol at the position 4 in a moderate isolated yield of 27%. On the contrary, the lipases, whatever the source, catalyzed the regioselective removal of the acetyl group at the primary position, and thus afforded the mono-deprotected thioglucopyranoside **2** (entries 2–6). It is noteworthy that the use of Novozyme 435, an immobilized *Candida antartica* Lipase B, produced the di-deprotected sugar **4** in 16% isolated yield, but no trace of **2** or **3** (entry 8). However, two enzymes (entries 4 and 5) coming from two different suppliers exhibited high yields according to this process. Especially, the lipase from *Candida cylindracea* (LCC) was able to convert quantitatively **1** into **2** (entry 4). Therefore, we focused our attention only on the utilization of LCC in biocatalyzed reactions using [bmim]PF_6-containing mixtures.

Reactions were then further performed with various concentration of LCC (2.8 Units/mg) in order to determine the optimum amount of enzyme needed (Table 2). As expected, the more we decreased the ratio of substrate over the quantity of enzyme, the more converted product **2** we obtained (entries 1–4). Once again, compounds **3** and **4** were never isolated according to these reactions, even when a reaction time up to 6 days was used. Surprisingly, a relatively high concentration of LCC, corresponding to a ratio of four molecules of substrate per molecule of lipase, was needed to catalyze the reaction quantitatively (entry 4). This does not represent a limitation to this method since LCC is a quite cheap enzyme. Furthermore, this enzymatic procedure constitutes an easy one step access to the interesting synthon **2** obtained in quantitative yield (entry 4).

Finally, using the optimized amount of LCC, we decided to investigate the influence of the proportion of the ionic liquid [bmim]PF_6 towards the enzymatic reaction. So, a set of experiments was designed with media containing from 0% to 100% of ionic liquid (Table 3). In pure phosphate buffer (entry 1), the bioconversion was incomplete, probably because of the low solubility of **1** in aqueous phase, and led to a mixture of the three possible compounds **2**–**4**. This result was not a surprise as it has already been reported in the literature on methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, notably by the team of Wong.[8] Then, when increasing the amount of [bmim]PF_6 up to 50% (entries 2 and 3), the conversions observed were better and the isolated proportion of **2** raised to 100%. After that critical concentration, the enzyme seemed to be less and less potent and up to nearly half of the starting material remained unreacted (entry 4). More surprisingly, the yield of **2** decreased too and we were able to isolate only the compound **3**, owing a free secondary alcohol at the position 4, when using pure ionic liquid as media (entry 5). These two different experimental conditions, ie 50% and 100% of [bmim]PF6, were used in scale-up procedures starting from 500 mg of compound **1**, allowing us to unambiguously characterized the deprotected sugars **2**–**4**.

From these results, two simple observations concerning the influence of [bmim]PF_6 can be made: (i) the ionic liquid improves the substrate solubility and thus, the isolated yields when used up to 50%; and (ii) it is also able to modify completely the regioselectivity of the biocatalyzed reaction, leading either to the formation of **2** or **3** simply depending on the conditions used. Still, one question

Table 1
Screening of enzymatic activity in a reaction mixture containing 50% of [bmim]PF_6 and 50% of phosphate buffer

Entry	Enzyme	**1**[a] (%)	**2**[b] (%)	**3**[b] (%)	**4**[b] (%)
1	Pig liver esterase[c]	73	—	27	—
2	Lipase PS Amano[d]	>95	<5	—	—
3	Lipase from *Pseudomonas fluorescens*[e]	>95	<5	—	—
4	Lipase from *Candida cylindracea*[e]	—	100	—	—
5	Lipase from *Candida rugosa*[e]	5	95	—	—
6	Pig pancreas lipase[c]	77	23	—	—
7	Lipozyme[c]	100	—	—	—
8	Novozyme 435[e]	84	—	—	16

[a] Remaining quantity determined by subtraction of all isolated compounds.
[b] Isolated yields.
[c] Purchased from Sigma.
[d] Purchased from Amano.
[e] Purchased from Fluka.

Table 2
Influence of the quantity of LCC in the reaction mixture

Entry	[*S*]/[*E*]	**1**[a] (%)	**2**[b] (%)	**3**	**4**
1	1710	67	33	—	—
2	855	49	51	—	—
3	34	18	82	—	—
4	4	—	100	—	—

[a] Remaining quantity determined by subtraction of all isolated compounds.
[b] Isolated yields.

Table 3
Influence of the proportion of [bmim]PF_6 in the reaction mixture

Entry	Phosphate buffer (%)	[bmim]PF_6 (%)	**1**[a] (%)	**2**[b] (%)	**3**[b] (%)	**4**[b] (%)
1	100	0	20	39	3	37
2	75	25	—	51	27	22
3	50	50	—	100	—	—
4	25	75	32	56	12	—
5	0	100	46	—	54	—

[a] Remaining quantity determined by subtraction of all isolated compounds.
[b] Isolated yields.

remains unclear: does the amount of ionic liquid change the enzyme's selectivity or does it prevent the acyl migration? Generally, lipases catalyse preferentially the regioselective hydrolysis of the ester group at the O-6 position when the anomeric position is functionalized by something else than an ester group. Then, acyl group migration from the position O-4 to O-6 can occur, even under neutral conditions, and is likely to give **3**.[21,22] This latter further reacts with LCC to produce the di-deprotected sugar **4**. However, and in order to answer this question, we finally dissolved compound **2** bearing a free primary alcohol in both media containing either 50% [bmim]PF_6/50% phosphate buffer or 100% ionic liquid, but lacking the enzyme. After 24 h at room temperature, both reactions led exclusively to the formation of **3**, and proved unambiguously that the ionic liquid did not prevent the acyl migration. Moreover, results were identical with previously inactivated lipase, ruling out the effect of the protein. Furthermore, as the mechanism of catalysis of LCC implies dramatic conformational changes of the enzyme molecules between a 'closed' and an 'open' form,[23] and because lipases are well-known to work better at interfaces,[24,25] it is not surprising that the amount of a hydrophobic ionic liquid in the reaction media is able to modulate the regioselectivity of LCC.

In conclusion, this work demonstrates, to our knowledge for the first time, that the regioselectivity of the lipase LCC can be totally influenced thanks to the proportion of the ionic liquid [bmim]PF_6. This unusual solvent used with water in a biphasic media is therefore thought to have an impact on the surface of this enzyme. Moreover, these biocatalyzed reactions led us to the simple and efficient preparation, in one step, of two important building blocks **2** and **3** which were isolated in 100% and 54% yields, respectively. These latter, bearing either a primary or a secondary free alcohol, can be easily obtained in hundreds mg scale and now served both as acceptors and potent glycosyl donors in more complex oligosaccharide synthesis.

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20. *Typical procedure for the enzymatic reaction containing 50% of ionic liquid*: To the commercially available **1** (5 mg) dissolved in 50 μL of phosphate buffer (50 mM, pH 7.0) were added 50 μL of [bmim]PF_6. After adding the enzyme, the mixture was vigorously stirred at rt for 24 h and further extracted three times with dichloromethane. After concentration of the organic layer, the residue was purified by flash chromatography (light petroleum/EtOAc, 2/1:v/v) to afford **2**, **3** and/or **4**. Best conditions were extended to 500 mg of **1**. Data for **2**: R_f = 0.16 (light petroleum/EtOAc, 3/2:v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.20 (dd, 1H, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 4.96 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 4.95 (dd, 1H, $J_{1,2}$ = 10.1 Hz, H-2), 4.45 (d, 1H, H-1), 3.68 (dd, 1H, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 3.52 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, H-6′), 3.47 (ddd, 1H, H-5), 2.67 (dq, 1H, ^3J = 7.5 Hz, ^2J = 12.5 Hz, S–CH_2–CH_3), 2.62 (dq, 1H, ^3J = 7.5 Hz, S–CH_2–CH_3), 2.00, 1.99, 1.95 (3s, 9H, 3 CH_3CO), 1.20 (t, 3H, S–CH_2–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.2, 170.1, 169.4 (C=O), 85.5 (C-1), 78.3 (C-5), 73.7 (C-3), 69.9 (C-2), 68.6 (C-4), 61.5 (C-6), 24.0 (S–CH_2–CH_3), 20.8, 20.7, 20.6 (CH_3–CO), 14.8 (S–CH_2–CH_3). Data for **3**: R_f = 0.15 (light petroleum/EtOAc, 3/2:v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.01 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 8.8 Hz, H-3), 4.89 (dd, 1H, $J_{1,2}$ = 9.9 Hz, H-2), 4.43 (d, 1H, H-1), 4.34 (dd, 1H, $J_{5,6}$ = 3.7 Hz, $J_{6,6'}$ = 12.6 Hz, H-6), 4.28 (dd, 1H, $J_{5,6'}$ = 1.8 Hz, H-6′), 3.50 (m, 2H, H-4, H-5), 2.67 (dq, 1H, ^3J = 7.5 Hz, ^2J = 12.5 Hz, S–CH_2–CH_3), 2.62 (dq, 1H, ^3J = 7.5 Hz, S–CH_2–CH_3), 2.04, 2.02, 2.00 (3s, 9H, 3 CH_3CO), 1.20 (t, 3H, S–CH_2–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 171.7, 171.3, 169.6 (C=O), 83.5 (C-1), 78.2 (C-5), 73.7 (C-3), 69.8 (C-2), 68.9 (C-4), 63.0 (C-6), 24.3 (S–CH_2–CH_3), 20.8, 20.7, 20.6 (CH_3–CO), 14.8 (S–CH_2–CH_3). Data for **4**: R_f = 0.10 (light petroleum/EtOAc, 2/3:v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.20 (dd, 1H, $J_{2,3}$ = 9.2 Hz, $J_{3,4}$ = 9.4 Hz, H-3), 4.96 (dd, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 4.95 (dd, 1H, $J_{1,2}$ = 10.1 Hz, H-2), 4.45 (d, 1H, H-1), 3.68 (dd, 1H, $J_{5,6}$ = 2.0 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 3.52 (dd, 1H, $J_{5,6'}$ = 4.8 Hz, H-6′), 3.47 (ddd, 1H, H-5), 2.67 (dq, 1H, ^3J = 7.5 Hz, ^2J = 12.5 Hz, S–CH_2–CH_3), 2.62 (dq, 1H, ^3J = 7.5 Hz, S–CH_2–CH_3), 1.20 (t, 3H, S–CH_2–CH_3).
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