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A chemo-biocatalytic approach in the synthesis of β-*O*-naphtylmethyl-*N*-peracetylated lactosamine

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Abstract

A chemo-enzymatic approach combining an enzymatic regioselective hydrolysis of peracetylated *N*-acetyl-α-D-glucosamine (**1**) with a mild controlled acyl migration led to 2-acetamido-2-deoxy-1,3,6-tri-*O*-acetyl-α-D-glucopyranose, which was further used in a glycosylation reaction in the synthesis of β-*O*-naphtylmethyl-*N*-peracetylated lactosamine.

Candida rugose lipase (CRL) immobilized on octyl-agarose and modified by covering it with polyethyleneimine was the best catalyst in terms of activity, stability and regioselectivity in the hydrolysis of **1**, producing the deacetylation in C-6 in 95% overall yield. Other immobilized lipases were not specific or with a very low activity towards the hydrolysis of **1**.

An acyl chemical migration by incubation of the deacetylated C-6 derivative at pH 8.5, 4 °C, and 10–20% acetonitrile permitted to obtain up to 75% overall yield of the 4-OH derivative product. This molecule was successfully applied in a glycosylation reaction to get the peracetylated α-D-lactosamine and finally, the peracetyl-β-*O*-naphtylmethyl-lactosamine derivative in 20% overall yield.

Keywords: Glycosylation reaction; Regioselectivity; Lipases

1. Introduction

Carbohydrates exist in very different forms in nature playing a very important role in many biological processes [1].

Pure regioisomers of *O*-acetyl-glycopyranosides presenting only one free hydroxyl group may be employed as key intermediates in the preparation of different glycoconjugates [2–5].

Indeed one interesting application of these molecules could be in the synthesis of acetylated β-*O*-naphtylmethyl-lactosamine(LacNAcNM), which, after a *in vivo* deacetylation, act as substrates of glycosyltransferases located in the Golgi apparatus resulting in the blocking of glycan biosynthesis from endogenous glycoconjugates [6,7].

Previously, the synthesis of LacNAcNM has been performed using an elegant chemical approach by Wong and co-workers [6–8] although the development of other synthetic methods could be tackled using per-*O*-acetyl-glycopyranoses as raw material to obtain the monohydroxy peracetylated derivatives. Following this methodology, the target molecule could be obtained directly in its fully acetylated form.

Nevertheless, the preparation of these monohydroxy derivatives is difficult by classical chemical approaches, making it necessary to use many chemically selective protection/deprotection steps, [9] – with a poor final overall yield – because of the low regioselectivity to remove only one acetyl group among different esters with similar reactivity.

Consequently, the use of enzymatic catalysts, especially lipases – due to their high versatility recognizing a broad range of substrates with high regio and enantioselectivity – [10–14] could be an attractive alternative to obtain high specificity and regioselectivity.

Herein, we present a chemo-enzymatic approach in the preparation of 4-hydroxy tetraacetylated-pyranose by a regioselective enzymatic hydrolysis of 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose **1** using immoblized lipases combined with a controlled chemical acyl migration step. This monodeprotected glucopyranose was used as building block in the preparation of β-*O*-naphtylmethyl-*N*-peracetylated lactosamine.

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2. Material and methods

2.1. General

The lipase from *Candida rugosa* (Type VII) (specific activity 875 U/mg solid), Porcine pancreatic lipase (PPL), *p*-nitrophenyl propionate (*p*NPP), peracetylated *N*-acetyl-α-D-glucosamine **1** and peracetylated *N*-acetyl-β-D-glucosamine **2** were from Sigma. Lipase from *Aspergillus niger* (ANL) was purchased from Fluka (Neu Ulm, Germany). The lipase from *Candida antarctica* B (Novozym 525L) (CAL-B) and Lecitase Ultra were purchased by Novozymes. Lipase from *Pseudomonas fluorescens* (PFL) was from AMANO. Octyl-agarose 4BCL was purchased from Pharmacia Biotech (Uppsala, Sweden).

The pH of the solutions during the enzymatic hydrolysis was kept constant using an automatic titrator 718 Stat Tritino from Metrohm (Herisau, Switzerland). HPLC analyses were performed using an HPLC L-7100 Merck-Hitachi (E. Merck, Darmstadt, Germany). The column was a Gemini-C18 (250 mm × 4.6 mm and 5 μm Ø) from Phenomenex-Chemtek (Chemtek Analitica, Anzola Emilia, Bologna, Italy). Analyses were run at 25 °C using a Merck-Hitachi L-7300 column oven and a Merck-Hitachi UV detector L-7400 at 220 nm. Flash chromatography was performed using silica gel 60 (E. Merck) 40–63 μm. ^{1}H NMR spectra were recorded on a Bruker AMX 400 instrument using tetramethylsilane as the internal standard. The products obtained by enzymatic hydrolysis were characterised by NMR studies in order to assign the exact position of the hydrolysis. Mass spectra were recorded in methanol on a LCQ-DECA Thermo Finnigan.

2.2. Standard enzymatic activity assay

In order to follow the immobilization process, the activities of the soluble lipases and their immobilized preparations were analyzed spectrophotometrically measuring the increase in absorbance at 348 nm ($\epsilon = 5.150\,M^{-1}\,cm^{-1}$) produced by the release of *p*-nitrophenol (pNP) in the hydrolysis of 0.4 mM *p*NPP in 25 mM sodium phosphate buffer at pH 7 and 25 °C. To initialize the reaction, 0.05–0.2 mL of lipase solution (blank or supernatant) or suspension was added to 2.5 mL of substrate solution. Enzymatic activity was determined as mmol of hydrolyzed *p*NPP per minute (IU) per milligram of enzyme under the conditions described above.

2.3. Immobilization of lipases on octyl-agarose

The purification of the lipases was performed using a previously described protocol, based on the interfacial activation of lipases on hydrophobic supports at low ionic strength [15]. 0.32 g of CRL commercial solid powder (60 mg protein), 0.5 g of ANL commercial solid powder (22 mg protein), 11 g of PFL commercial solid powder (60 mg protein), 5 mL CAL-B and 5 mL LECI commercial solution (12 mg protein/mL) [16] were dissolved in 95 mL of 10 mM sodium phosphate buffer at pH 7.0 in each case, respectively. Then, 5 g of octyl-agarose support were added in each enzymatic solution. The activity of supernatant and suspension was periodically checked by the method described above and the immobilization was finished after 5 h by filtration. In all cases, more than 90% of lipase was immobilized. Following this protocol, the SDS–PAGE analysis of the protein adsorbed to the octyl-sepharose [15] only showed a single band with a molecular weight corresponding to that of the different native lipases.

2.4. Adsorption of polyethylenimine on immobilized lipase preparations

The immobilized lipase preparations were incubated in 1% of polyethyleneimine (20 kDa) in 5 mM phosphate buffer pH 7.0. The suspension was gently stirred for 1 h and then washed with 5 mM phosphate buffer. The immobilized preparations were filtered under vacuum and stored at 4 °C.

2.5. Enzymatic hydrolysis of peracetylated monosaccharides

Standard assay was performed as follows: **1** or **2** (0.1 mmol, 40 mg) was added to 40 mL solution of phosphate buffer 50 mM with 10% acetonitrile at pH 5, 25 °C and the reaction was initialized by adding 0.5 g (**1**) or 1 g (**2**) of biocatalyst. The reaction was performed at pH 5 in order to avoid the chemical acyl migration in the per-*O*-acetylated carbohydrates hydrolysis [15a]. The hydrolytic reaction was carried out under mechanical stirring, and the pH value was controlled by automatic titration. Hydrolysis reactions were followed by HPLC. Finally, the optimization of the reaction in each case was performed using 8 g/L substrate and the products were isolated and identified by ^{1}H NMR and 2D-COSY.

2.6. Chemical acyl migration

5 mM of **3** were incubated in a 50 mM KH_2PO_4 buffer solution at different pHs, temperatures and with different percentages of co-solvent. The acyl migration was monitored by HPLC and, when the maximum concentration of the desired product was achieved, the solution was saturated with NaCl and extracted with ethyl acetate. After evaporation of the solvent under reduced pressure, the residue was purified by flash chromatography.

2.7. Identification of products

2.7.1. 2-Acetamido-2-deoxy-1,3,4-tri-O-acetyl-α-D-glucopyranose (*3*)

This compound was synthesized following the general procedure of enzymatic hydrolysis described above and purified by flash chromatography. Elution of the flash chromatography column was performed with 95:5 dichloromethane-methanol. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) at pH 4, flow rate 1.0 mL/min; $t_R = 5.70$ min. Yield: 95%. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.19$ (d, $J = 3.32$ Hz, 1H-1), 5.61

(d, 1H-NH), 5.30 (t, 1H-3), 5.16 (t, 1H-4), 4.46 (m, 1H-2), 3.81 (m, 1H-5), 3.59 and 3.71 (2 dd, 2H-6a,b), 2.20 (s, CH_3, 3H), 2.05–2.11 (2 s, CH_3, 6H), 1.96 (s, CH_3, 3H). The hydroxyl proton was not observed due to broadening of the corresponding signal.

2.7.2. 2-Acetamido-2-deoxy-1,3,6-tri-O-acetyl-α-D-glucopyranose (6)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis described above in Sections 2.2 and 2.3 and purified by silica gel column chromatography; elution was performed with 95:5 dichloromethane-methanol to provide the desired product. HPLC analysis: 15% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 7.5 min. Yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.15 (d, J = 3.59 Hz, 1H-1), 5.75 (d, 1H-NH), 5.14 (dd, 1H-3), 4.59 (dd, 1H-6b), 4.38 (m, 1H-2), 4.20 (dd, 1H-6a), 3.85 (m, 1H-5), 3.65 (t, 1H-4), 3.16 (bs, 1H-OH), 2.19 (s, CH_3, 3H), 2.13–2.15 (2 s, CH_3, 6H), 1.95 (s, CH_3, 3H).

2.7.3. 2-Acetamido-2-deoxy-1,4,6-tri-O-acetyl-α-D-glucopyranose (7)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis described above in Sections 2.2 and 2.3 and purified by silica gel column chromatography; elution was performed with 95:5 dichloromethane-methanol to provide the desired product. HPLC analysis: 15% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 9.3 min. Yield: 20%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.19 (d, J = 3.28 Hz, 1H-1), 5.96 (d, J = 6.96 Hz, 1H-NH), 5.0 (t, J = 9.6 Hz; 1H-4), 4.35 (ddd, J = 10.6 Hz, J = 6.2 Hz, J = 3.95 Hz; 1H-2), 4.09–4.30 (2 dd, AB part of ABX system, $J_{1,3}$ = 1.59 Hz, $J_{1,3}$ = 4.4 Hz, $J_{1,2}$ = 12.5 Hz; 2H-6a,b), 3.99 (m, 1H-5), 3.8 (t, J = 9.9 Hz, 1H-3), 2.00–2.30 (4s, CH_3, 12H).

2.7.4. α-D-Lactosamine octaacetate (9)

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyltrichloroacetimidate (**8a**) (213 mg, 0.43 mmol) and 2-acetamido-1,3,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose (**6**) (100 mg, 0.28 mmol) were dissolved in dry CH_2Cl_2 (2 mL), and BF_3*OEt_2 (0.053 mL, 0.43 mmol) was added in the presence of activated molecular sieves 4 Å (200 mg) at −20 °C under nitrogen. After stirring at −20 °C for 5 h, the mixture was filtered through celite, diluted with $CHCl_3$, washed with satd. aq. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and evaporated, and the residue purified by silica gel column chromatography (CH_2Cl_2 40: EtOH 1) to provide the product (105 mg, 55%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.07 (d, J = 3.52 Hz, 1H-1), 5.61 (bd, 1H-NH), 5.35 (dd, 1H-4′), 5.22 (bdd, 1H-3), 5.10 (dd, 1H-2′), 4.95 (dd, 1H-3′), 4.51 (d, J = 7.89 Hz, 1H-1′), 4.30–4.43 (m, 2H: H-2, H-6b), 4.03–4.16 (m, 3H: H-6a′, H-6b′, H-6a), 3.79–3.92 (m, 3H: H-4, H-5, H-5′), 1.90–2.10 (8s, 24H-CH_3). ESI-MS: calcd. for $C_{28}H_{39}NO_{18}Na$ (M+Na^+) 700.61, found 700.2 (M+Na^+).

2.7.5. 2-Methyl-{3,6-di-O-acetyl-1,2-dideoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano}-[2,1-d]-oxazoline (10)

TMSOTf (45.71 μL, 0.25 mmol) was added to a solution of α-D-lactosamine octaacetate (**9**) (150 mg, 0.22 mmol) in dry 1,2-dichloroethane (3 mL) and the mixture was stirred at 50 °C. After 5 h an extra amount of TMSOTf (11 μL, 0.06 mmol) was added. After a further 1.5 h, the reaction was stopped with triethylamine (100 μL). Solvent evaporation, followed by LC (toluene-ethyl acetate-triethylamine 100:150:1) gave the desired product **10** as a glassy foam (102.36 mg, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (d, 1H-1), 5.62 (br d, 1H-3), 5.34 (dd, 1H-4′), 5.15 (dd, 1H-2′), 4.98 (dd, 1H-3′), 4.62 (d, J = 7.89 Hz, 1H-1′), 4.18 (dd, 1H-6b), 4.01–4.15 (m, 4H: H-6a′, H-6b′, H-6a, H-2), 3.94 (ddd, 1H-5′), 3.63 (m, 1H-5), 3.44–3.49 (m, 1H-4), 1.92–2.17 (7s, $COCH_3$ and CH_3). ESI-MS: calcd. for $C_{26}H_{35}NO_{16}Na$ (M+Na^+) 640.55, found 640.5 (M+Na^+).

2.7.6. 2–Naphthylmethyl-2,3,4,6 –tetra-O-acetyl-β-D-galactopyranosyl-(1 → 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (11)

A solution of the oxazoline **10** (74 mg, 0.12 mmol) in dichloroethane (2 mL) at 80 °C was treated sequentially with camphorsulfonic acid (13.8 mg, 0.06 mmol) followed by 2-naphthylmethanol (94.78 mg, 0.6 mmol), and the resulting solution was maintained at 80 °C for 3 h. The reaction mixture was diluted with CH_2Cl_2 (4 mL), and the organic phase was washed with water (4 mL), followed by brine (4 mL), then dried (Na_2SO_4). The organic phase was concentrated in vacuo and the residue was purified by flash chromatography (hexane:ethyl acetate 1:9) to afford the target compound **11** (50.7 mg, 55%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.84–7.39 (m, 7 H, ArH), 5.44 (d, J = 9.35, 1H-NH), 5.35 (d, 1H-3′), 5.12–5.08 (dd, 1H-2′), 5.03–4.95 (m, 3H: H-3, H-3′ and CH_2), 4.74 (dd, 1H-CH_2), 4.56 (d, 1H-6a/b), 4.50 (d, J = 8.75 Hz, 1H-1′), 4.47 (d, J = 7.25 Hz, 1H-1), 4.19–4.13 (m, 2H: H-2 and H-6a/b), 4.12–4.09 (m, 2H: H-6′a and H-6′b), 3.88 (dd, 1H-5′), 3.83 (dd, 1H-4), 3.62–3.59 (m, 1H-5), 2.14–1.94 (7s, 21 H, CH_3CO). ESI-MS: calcd. for $C_{37}H_{45}NO_{17}Na$ (M+Na^+) 798.75, found 798.5 (M+Na^+). The NMR-data are in agreement with the reported values [7].

3. Results and discussion

3.1. Specific and regioselective hydrolysis of 1 and 2 by immobilized lipases

Different lipases from different sources, immobilized on octyl-agarose were studied as catalysts in the hydrolysis of peracetylated *N*-acetyl-α-D-glucosamine **1** and peracetylated *N*-acetyl-β-D-glucosamine **2** (Table 1, Scheme 1).

In the hydrolysis of **1**, the most active catalyst was the octyl-CRL preparation. This enzyme displayed 200 times higher activity compared to the octyl-PFL preparation and more than 1000-fold to the immobilized CAL-B or ANL (Table 1). No

Table 1
Regioselective hydrolysis of different anomers of peracetylated glucosamine (**1**, **2**) by different lipases immobilized on octyl-agarose at pH 5 and 25 °C

Enzyme	Substrate	Time (h)	Activity[a]	c[b] (%)	c_m[c] (%)	3 (%)	4 (%)	5 (%)
PPL	**1**	168	nd	<5	–	–		–
CAL-B	**1**	144	0.025	19	12	8		4
PFL	**1**	168	0.150	56	50	32		18
CRL	**1**	5	30	100	100	100		
ANL	**1**	144	0.003	12	12	9		3
LECI	**1**	168	nd	<5		–		–
PPL	**2**	168	nd	<5	–	–		–
CAL-B	**2**	168	0.011	38	32		22	10
PFL	**2**	168	0.030	76	73		42	31
CRL	**2**	168	0.003	28	20		13	7
ANL	**2**	168	0.140	100	95		63	31
LECI	**2**	168	0.010	34	19		13	6

nd: Not determined.
[a] Enzymatic activity was defined as μmol × g_{cat}^{-1} × h^{-1}. It was calculated at 10–15% conversion.
[b] c: Conversion.
[c] c_m: Conversion of the monohydroxy peracetylated products.

reaction was observed with PPL and LECI. Furthermore, the octyl-CRL preparation was the most specific and regioselective catalyst, producing only the monohydroxy **3** (Scheme 1) in more than 99% product conversion. Using immobilized PFL, a mixture of hydroxy products was achieved in a relation of isomers of 1.78:1 (**3**:**5**) (Table 1).

In the hydrolysis of the β-anomer **2**, the activity of the immobilized CRL was extremely reduced up to 10,000-fold compared to the obtained result in the hydrolysis of **1**. The best catalyst was the immobilized ANL, 46 times more active than the octyl-CRL preparation (Table 1). In terms of specificity; the immobilized lipases were partially regioselective in the hydrolysis of **2** producing a mixture of isomers **4** and **5** (Table 1).

3.2. Improvement of the biocatalyst in the hydrolysis of *1*

In order to improve the catalytic properties of the most active immobilized lipase, we performed a subsequent treatment with PEI to recover the enzyme (octyl-CRL-PEI) [17].

Fig. 1 shows the stability against 40% acetonitrile as co-solvent of the biocatalyst without and with the treatment with PEI. The immobilized CRL covered with PEI maintained more than 80% of its initial activity whereas the immobilized catalyst without PEI treatment was completely inactive after 17 h of incubation. The non-modified catalyst lost more than 80% activity in 1 h of incubation.

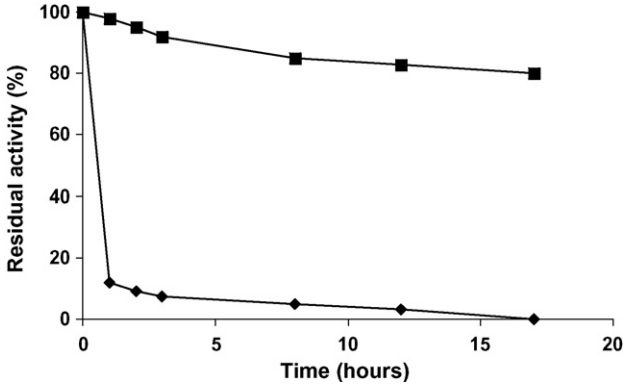
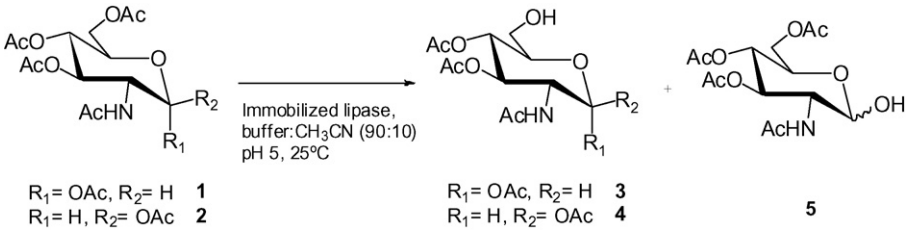


Fig. 1. Stability of immobilized preparations of CRL in 40% acetonitrile at pH 7 and 25 °C. Octyl-CRL-PEI (squares), octyl-CRL (rhombus).

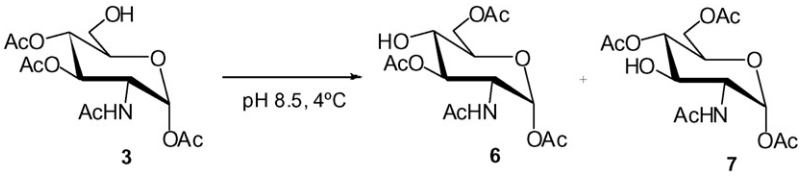
The different CRL immobilized preparations were used in the hydrolysis of **1** in a higher concentration (20 mM) and 20% acetonitrile.

Thus, the catalytic activity of the CRL immobilized on octyl-agarose after the treatment with PEI increased in 10% with respect to the activity value achieved with the catalyst without treatment and the course of both hydrolysis is shown in Fig. 2.

The octyl-CRL immobilized preparation covered with PEI maintained the specificity and regioselectivity in the production of **3** (95% overall yield). Therefore, the octyl-CRL-PEI immobilized preparation was selected as the optimal catalyst for the regioselective hydrolytic reaction.



Scheme 1. Enzymatic regioselective hydrolysis of **1** and **2**.



Scheme 2. Chemical acyl migration of **3**.

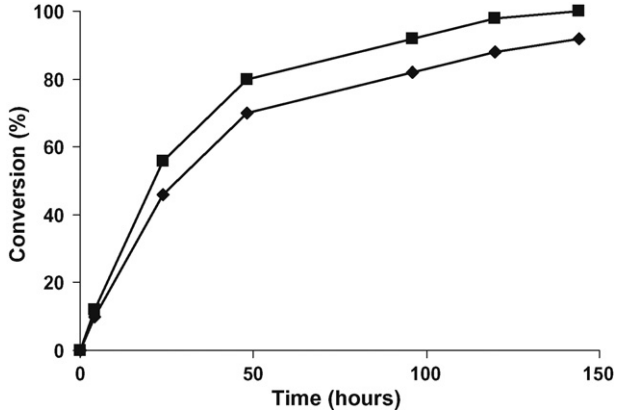


Fig. 2. Conversion profile of octyl-CRL versus octyl-CRL-PEI in the hydrolysis of **1**. The experimental conditions were 20% acetonitrile as co-solvent in phosphate buffer at pH 7 and 25 °C, 20 mM substrate.

3.3. Chemical acyl migration of *3* to prepare *6*

The chemical acyl migration in **3** from the 4- to the 6-position to obtain 2-acetamido-2-deoxy-1,3,6-tri-*O*-acetyl-α-D-glucopyranose **6** was studied (Scheme 2). The analysis of the chemical migration at different experimental conditions is shown in Table 2.

The incubation of the substrate **3** at pH 8.5 and 4 °C without solvent during 1 h permitted to obtain the 4-hydroxy product **6** in 77% yield and also 20% of the 3-OH derivative **7** was formed (entry 1, Table 2). The concentration of these compounds remains constant after the maximum value was achieved. The increase in the pH up to 9.5 caused a slight decrease in the yield of **6** (entry 2, Table 2).

Addition of 10% of solvent in the buffer solution provoked an improvement in the final yield of **6** up to 80% when the substrate incubation was performed at pH 8.5 (entry 3, Table 2). Again the more basic conditions caused a decrease in the yield of **6** (entry 4, Table 2).

Also, the increase of the temperature at 22 °C led to lower yields of **6** instead of shorter times to achieve the migration equilibrium (entries 5–6, Table 2).

The addition of more acetonitrile (entry 7, Table 2) or the use other co-solvents (ethanol, DMSO or acetone) (data not shown) did not produce any significant change in the conversion value of **6**.

Therefore, combining the biocatalytic step – using the octyl-CRL-PEI immobilized preparation – with a mild controlled acyl migration (at pH 8.5, 4 °C, 10% acetonitrile, Table 2), it was possible to get the 4-hydroxy derivative **6** in 75% overall yield after purification by flash chromatography (Scheme 3).

Table 3
Glycosylation studies for the synthesis of **9** from different carbohydrate donors (**8a–d**)

Donor (anomer)	R	Promoter	Yield (%)
8a (α)	$OCNHCCl_3$	BF_3*$O(Et)_2$	55
8b (α)	Br	AgOTf	<5
8c (β)	SPh	NIS/TfOH	<5
8d (β)	SPh	ICl/AgOTf	<5

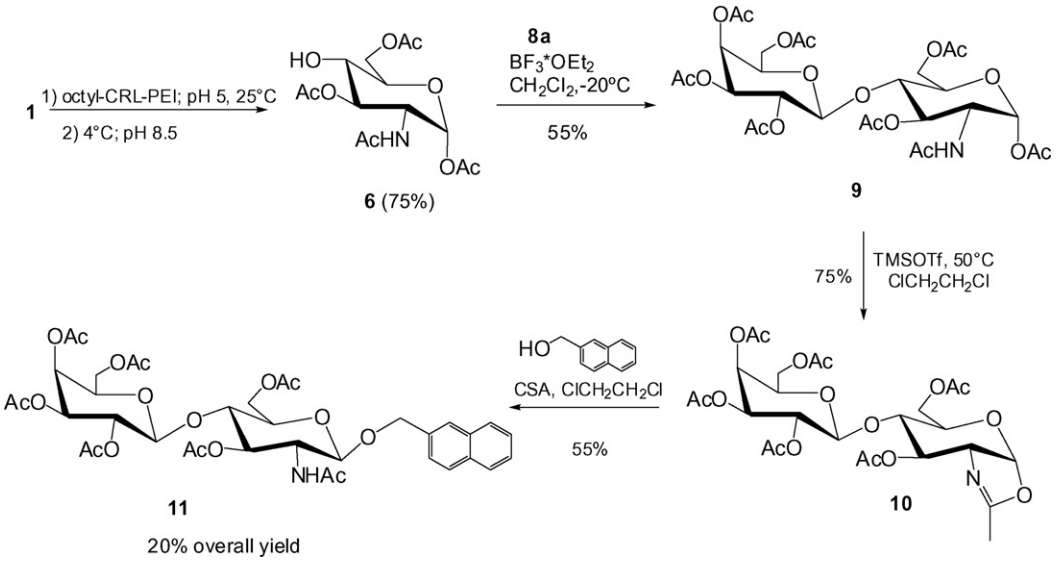
3.4. Glycosylation reaction using *6* as carbohydrate acceptor for the final preparation of β-O-naphtylmethyl-N-peracetylated lactosamine

Once purified, **6** was used as starting material in the glycosylation reaction for the preparation of the disaccharide α-D-lactosamine octaacetate **9** (Scheme 3). Thus, several standard glycosylation procedures (Table 3), using peracetylated galactose **8** activated at the anomeric position in different forms, were tested. Only with tetra-*O*-acetyl-α-D-galactopyranosyl

Table 2
Study of the chemical acyl migration of **3**

Entry	pH	*T* (°C)	CH_3CN (%)	*t* (min)	c (%)	Yield[a] 6 (%)	Yield[a] 7 (%)
1	8.5	4	0	60	96	77	24
2	9.5	4	0	60	>98[b]	64	32
3	8.5	4	10	90	93	80	13
4	9.5	4	10	90	>98[b]	64	33
5	8.5	22	10	10	98	70	28
6	9.5	22	10	15	>98[b]	62	32
7	8.5	4	20	120	97	79	18
8	9.5	4	20	180	98[c]	60	32

[a] The yields have been evaluated by the HPLC method previously described.
[b] In these cases chemical hydrolysis of the products was observed as side reaction.



Scheme 3. Preparation of β-*O*-napthylmethyl-lactosamine peracetate **11** from acetylated monosaccharide **6**.

trichloroacetimidate **8a** as carbohydrate donor and boron trifluoride diethyl etherate as promoter, was possible to achieve the disaccharide **9** in 55% yield after purification (Table 3, Scheme 3).

From the peracetylated lactosamine **9**, the oxazoline derivative **10** (75% of yield in 4 h) was prepared and, after purification by flash chromatography, successively used in the acid-catalyzed reaction with 2-napthylmethanol to synthesize the target compound **11** (yield 55%) (Scheme 3).

4. Conclusion

The immobilized CRL was the best catalyst in terms of activity towards the hydrolysis process and also highly specific and regioselective in the hydrolysis of **1**, producing the monohydroxy product **3** in 95% overall yield. However, in the hydrolysis of the β anomer **2**, this biocatalyst presented very low activity and regioselectivity. These results demonstrated the important effect of the conformational structure of the substrate in the regioselectivity of the same immobilized preparation.

Then, it was found that a modification of the octyl-CRL preparation by covering with PEI permitted to get a better biocatalyst in term of activity and stability against co-solvents and maintaining the regioselectivity.

Thus, a successful chemo-biocatalytic process throughout the enzymatic regioselective hydrolysis of the peracetylated glucosamine **1** with the best catalyst combined with an acyl migration to the desired position, was performed to prepare 2-acetamido-2-deoxy-1,3,6-tri-*O*-acetyl-α-D-glucopyranose **6** in 75% overall yield. The application of **6** as carbohydrate acceptor in a novel glycosylation reaction permitted to synthesize α-D-lactosamine octaacetate in 55% yield. This peracetylated disaccharide was subsequently modified in two steps obtaining the target molecule β-*O*-naphtylmethyl-*N*-peracetylated lactosamine **11** in 20% overall yield. By the synthetic strategy reported in this work, compound **11** is obtained in few steps, directly in fully acetylated form, avoiding several protection and deprotection steps as required by the classical approaches previously reported.

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