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Chemo-biocatalytic regioselective one-pot synthesis of different deprotected monosaccharides

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ABSTRACT

A simple and straightforward chemo-enzymatic approach has been developed to synthesize a small library of different monohydroxy tetraacetylated monosaccharides useful as building blocks for the synthesis of oligosaccharides. The strategy is based on the combination of a regioselective hydrolysis catalyzed by immobilized lipases with a subsequent mild chemical acyl migration. Thus immobilized lipases were highly regioselective towards the hydrolysis in C-6 position allowing, in most cases, yields up to 95%. The hydrolysis was particularly efficient using *Candida rugosa* lipase (CRL) with the alpha anomers of the peracetylated monosaccharides tested. The hydrolysis of the beta anomers resulted much slower.

An acyl chemical migration of C-6 products at pH 8.5–9.5 and 4 °C allowed the preparation of glycopyranosides bearing a free hydroxy group at the C-4 and C-3 position. In the optimal conditions, these products can be obtained in yields from 30 up to 80% depending on the monosaccharides used.

1. Introduction

Carbohydrates play a critical role in many biological processes. Indeed a high number of bioactive compounds are glycosylated and the sugar moiety is essential for their bioactivity [1]. Among these, glycoproteins involved in cell–cell recognition of different pathologies are of special interest [2]. In fact, oligosaccharides contained in glycoproteins are specifically recognized by lectin receptors which are a key element for carbohydrate-mediated recognition events [3]. For example, linear tetrasaccharides of "lacto series" are involved in several structures with high biological interest such as glycolipids (i.e. paraglobosides) and glycoproteins [4] (Fig. 1). Thus, many research groups have focused their effort in synthesizing glycopeptides or oligosaccharides mimicking glycoproteins [5]. The chemical synthesis of such structures is generally achieved by a complex multi-step route, with important limitation especially in the prospective of an industrial development of the synthetic process. In fact, the elaboration of monosaccharidic building blocks usually requires the design of a multi-step sequence of transformations, involving several protecting group manipulations [6], to obtain a sugar acceptor bearing only one free hydroxyl group in a desired position. Recently, has been reported an elegant methodology to synthesize oligosaccharides building blocks, based on the regioselective manipulation of per-*O*-silylated glucopyranosides, involving different protecting groups [7].

O-Acetylated pyranoses bearing only one free hydroxyl group (APs-OH) could be useful building blocks to synthesize glycoconjugates by using only acetyl ester as protecting group, easily removed in only one step at the end of the synthesis. In this context, to overcome the well-known difficulty in the preparation of the APs-OH by the classical chemical synthesis, we have considered the enzymatic regioselective hydrolysis of peracetylated pyranoses followed by a controlled chemical acyl migration [8] as a simple "one-pot" strategy to develop an efficient preparative process for these compounds.

Considering that the deprotection in the anomeric position can be easily performed [9], an amino group is often present in the C-2 position, and that the glycosidic bonds normally occurs at the C-3, C-4 or C-6 positions, we focused our attention on the enzymatic production of APs-OH deprotected in these positions.

The use of lipases could be a good approach because of the high activity of these enzymes towards a broad range of non-natural substrates with high regio and enantioselectivity [10,11]. The

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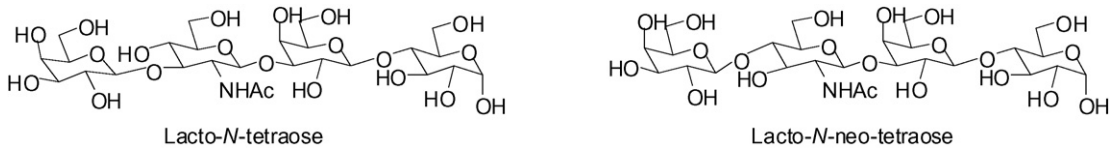


Fig. 1. Linear oligosaccharides from "lacto series".

regioselectivity of most lipases, in biotransformations involving pyranoses, has been described to be mainly towards the anomeric or C-6 positions [12,13]. Furthermore, these monohydroxy molecules can suffer a chemical migration of the acyl moiety; often, this is a problem in the production and management of these compounds [14]. However, if the migration is controlled, may be a useful tool to have other free positions (e.g., it has been used to obtain C-4 hydroxy-tetraacetyl-α-D-pyranose) [8,15].

Herein, we describe the chemo-enzymatic synthesis of a library of different regioisomers of tetraacetylated glycopyranoses bearing only a free hydroxyl group by combining a highly regioselective enzymatic hydrolysis with a temperature and pH-controlled acyl migration.

2. Experimental

2.1. General

Lipase from *Aspergillus niger* (ANL) and Lecitase ultra (LECI) were purchased from Fluka (Neu Ulm, Germany). The lipase from *Candida rugosa* lipase (CRL) and peracetylated monosaccharides **1**–**7** were from Sigma Chem. Co. Immobilization of lipases on octyl-sepharose was performed according to the procedure reported [16].

Reagents and chemicals were purchased from Fluka, Aldrich, Pharmacia Biotech, were used without further purification. HPLC analyses were performed using a L-7100 Merck-Hitachi. Analyses were run at 25 °C using a Merck-Hitachi L-7300 column oven and a Merck-Hitachi UV detector L-7400 at 210 nm. ^{1}H NMR data were recorded on a Bruker AMX 400 instrument.

2.2. Enzymatic regioselective deprotection of different O-peracetylated monosaccharides

Substrates **1**–**7** (1.84 mmol, 720 mg) were added to 100 mL solution of phosphate buffer 50 mM with 20% acetonitrile at pH 5, 25 °C and the reaction was initialized by adding 3 g of biocatalyst. The reaction was performed at this pH in order to avoid the chemical acyl migration in the per-*O*-acetylated carbohydrates hydrolysis [8]. The hydrolytic reaction was carried out under mechanical stirring and the pH value was controlled by automatic titration. Hydrolysis reactions were followed by HPLC.

2.3. Chemical acyl migration

The aqueous solution (50 mM KH_2PO_4 buffer) of the 6-OH derivatives **8**–**14** was incubated at different pHs (8.5–9.5), 4 °C and 20% acetonitrile as co-solvent. The acyl migration reaction 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.

2.3.1. 1,2,3,4-Tetra-O-acetyl-α-D-glucopyranose (8)

This compound was characterized how previously reported in the literature [8].

2.3.2. 1,2,3,4-Tetra-O-acetyl-β-D-glucopyranose (9)

This compound was characterized how previously reported in the literature [8].

2.3.3. 2-Acetamido-2-deoxy-1,3,4-tri-O-acetyl-α-D-glucopyranose (10)

This compound was synthesized following the general procedure of enzymatic hydrolysis above described 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: 98%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 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 (2s, CH_3, 6H), 1.96 (s, CH_3, 3H). The hydroxyl proton was not observed due to broadening of the corresponding signal.

2.3.4. 2-Acetamido-2-deoxy-1,3,4-tri-O-acetyl-β-D-glucopyranose (11)

This compound was synthesized following the general procedure of enzymatic hydrolysis above described and purified by flash chromatography. Elution of the flash chromatography column was performed with 95:5 dichloromethane–methanol. HPLC analysis: t_R = 8.4 min; yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.80 (d, J = 9 Hz, H-1), 5.50 (d, J = 9 Hz, 1H-NH), 5.30 (t, J = 9.9 Hz, H-3), 5.10 (t, J = 9.6 Hz, H-4), 4.35 (dd, J = 9.8 Hz, J = 6.70 Hz, H-2), 4.28–4.20 (m, 2H, H-6), 4.19–4.10 (m, H-5), 2.21 (s, 9H, 3x CH_3), 1.96 (s, 3H, CH_3).

2.3.5. 1,2,3,4-Tetra-O-acetyl-α-D-galactopyranose (12)

This compound was characterized how previously reported in the literature [8].

2.3.6. 1,2,3,4-Tetra-O-acetyl-β-D-galactopyranose (13)

This compound was characterized how previously reported in the literature [13].

2.3.7. 2-Acetamido-2-deoxy-1,3,4-tri-O-acetyl-α-D-galactopyranose (14)

This compound was synthesized following the general procedure of enzymatic hydrolysis above described 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: 80%.^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.22 (d, J = 3.46 Hz; 1H-1), 5.56 (bd, 1H-NH), 5.41 (bdd, 1H-4), 5.27 (dd, J = 8.7 Hz, J = 3.1 Hz; 1H-3), 4.76 (ddd, J = 11 Hz, J = 9.9 Hz, J = 3.6 Hz; 1H-2), 4.08 (bt, J = 12.7 Hz; 1H-5), 3.48 and 3.62 (2dd, J = 2.2 Hz, J = 4.4 Hz, J = 12.4 Hz; 2H-6a,b), 1.96–2.23 (4s, CH_3, 12H).

2.3.8. 1,2,3,6-Tetra-O-acetyl-α-D-glucopyranose (15)

This compound was characterized how previously reported in the literature [8].

2.3.9. 1,2,4,6-Tetra-O-acetyl-α-D-glucopyranose (16)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and

purified by silica gel column chromatography. Elution was performed with 50:50 *n*-hexane–ethyl acetate to provide the desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 16.7 min; yield: 30%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.33 (d, J = 3.5 Hz; 1H-1), 5.45 (t, J = 9.8 Hz; 1H-3), 5.18 (dd, J = 10.2 Hz, J = 3.7 Hz; 1H-2), 4.3 (t, J = 9.3 Hz; 1H-4), 4.05 and 4.27 (2dd, J = 4.0 Hz, J = 2.1 Hz, J = 12.6 Hz; 2H-6a,b), 4.15 (m, 1H-5), 2.00–2.20 (4s, CH_3, 12H).

2.3.10. 1,2,3,6-Tetra-O-acetyl-β-D-glucopyranose (17)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 50:50 *n*-hexane–ethyl acetate to provide the desired product. HPLC analysis: 15% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 36.45 min; yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.80 (d, J = 8.90 Hz; 1H-1), 5.28 (t, J = 9.8 Hz; 1H-3), 4.94 (dd, J = 10 Hz, J = 3.7 Hz; 1H-2), 3.90 (m, 2H-6), 3.60 (m, 1H-5), 3.59 (t, J = 9.0 Hz; 1H-4), 1.95–2.10 (4s, CH_3, 12H).

2.3.11. 1,2,4,6-Tetra-O-acetyl-β-D-glucopyranose (18)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 50:50 *n*-hexane–ethyl acetate to provide the desired product. HPLC analysis: 15% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 24.79 min; yield: 18%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.88 (d, J = 9.1 Hz; 1H-1), 5.38 (t, J = 9.8 Hz; 1H-3), 5.10 (dd, J = 10 Hz, J = 3.6 Hz; 1H-2), 4.1 (t, J = 9.5 Hz; 1H-3), 3.80 (m, 2H-6a,b), 3.75 (m, 1H-5), 2.00–2.20 (4s, CH_3, 12H).

2.3.12. 2-Acetamido-2-deoxy-1,3,6-tri-O-acetyl-α-D-glucopyranose (19)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described 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: 75%. ^{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 (2s, CH_3, 6H), 1.95 (s, CH_3, 3H).

2.3.13. 2-Acetamido-2-deoxy-1,4,6-tri-O-acetyl-α-D-glucopyranose (20)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described 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: 30%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.19 (d, J = 3.28 Hz, 1H-1), 5.96 (d, 1H-NH), 5.0 (t, 1H-4), 4.35 (ddd, 1H-2), 4.09–4.30 (2 dd, 2H-6a,b), 3.99 (m, 1H-5), 3.8 (t, 1H-3), 2.00–2.30 (4s, CH_3, 12H).

2.3.14. 2-Acetamido-2-deoxy-1,3,6-tri-O-acetyl-β-D-glucopyranose (21)

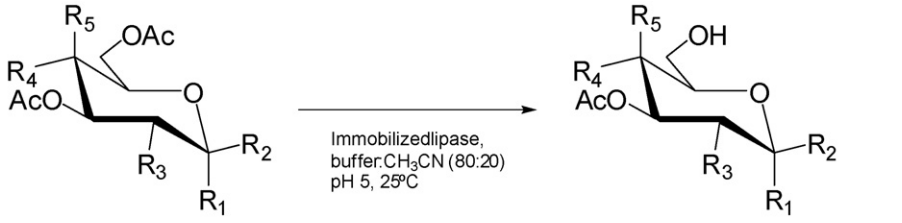
This compound was synthesized following the general procedure of chemo-enzymatic synthesis above described and purified by flash chromatography. Elution of the flash chromatography column was performed with 95:5 dichloromethane–methanol; yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.77 (d, 1H, J = 9.05 Hz, H-1), 5.53 (d, 1H, J = 7.60 Hz, 1H-NH), 5.30 (m, 1H, H-3), 5.14 (t, 1H, J = 9.78 Hz, H-2), 4.32 (m, 1H, H-6), 4.20 (m, 2H, H-5, H-6), 4.14 (m, H-4), 2.10 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.96 (s, 3H, CH_3).

2.3.15. 2-Acetamido-2-deoxy-1,4,6-tri-O-acetyl-β-D-glucopyranose (22)

This compound was synthesized following the general procedure of chemo-enzymatic synthesis above described and purified by flash chromatography. Elution of the flash chromatography column was performed with 95:5 dichloromethane–methanol; yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.78 (d, J = 9.29 Hz, H-1), 5.50 (d, J = 7.50 Hz, 1H-NH), 5.29 (m, 2H, H-4, H-2), 5.15 (m, 1H, 3H), 4.30 (m, 1H, H-6), 4.20 (m, 1H, H-6), 4.18 (m, 1H, H-5), 2.10 (s, 3H, CH_3), 2.03 (s, 6H, 2x CH_3), 1.96 (s, 3H, CH_3).

2.3.16. 1,2,3,6-Tetra-O-acetyl-α-D-galactopyranose (23)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 50:50 *n*-hexane–ethyl acetate to provide the



R_1= OAc, R_2= H, R_3=OAc, R_4=OAc, R_5=H	1	R_1= OAc, R_2= H, R_3=OAc, R_4=OAc, R_5=H	8
R_1= H, R_2= OAc, R_3=OAc, R_4=OAc, R_5=H	2	R_1= H, R_2= OAc, R_3=OAc, R_4=OAc, R_5=H	9
R_1= OAc, R_2= H, R_3=NHAc,R_4=OAc, R_5=H	3	R_1= OAc, R_2= H, R_3=NHAc, R_4=OAc, R_5=H	10
R_1= H, R_2= OAc, R_3=NHAc, R_4=OAc, R_5=H	4	R_1= H, R_2= OAc, R_3=NHAc, R_4=OAc, R_5=H	11
R_1= OAc, R_2= H, R_3=OAc, R_4= H, R_5=OAc	5	R_1= OAc, R_2= H, R_3=OAc, R_4= H, R_5=OAc	12
R_1= H, R_2= OAc, R_3=OAc, R_4=H, R_5=OAc	6	R_1= H, R_2= OAc, R_3=OAc, R_4=H, R_5=OAc	13
R_1= OAc, R_2= H, R_3=NHAc, R_4=H, R_5=OAc	7	R_1= OAc, R_2= H, R_3=NHAc, R_4=H, R_5=OAc	14

Scheme 1. Regioselective enzymatic hydrolysis of peracetylated monosaccharides **1**–**7**.

desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 16.7 min; yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.37 (d, J = 3.8 Hz; 1H-1), 5.45 (dd, J = 7.1, J = 3.5 Hz; 1H-3), 5.31 (dd, J = 11.1 Hz, J = 3 Hz, 1H-2), 4.39 (m, 1H-5), 4.09–4.23 (m, 1H-4; 2H-6a,b), 2.45 (bs, 1H-OH), 2.02–2.18 (4s, CH_3, 12H).

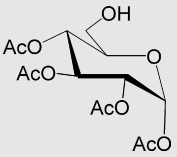
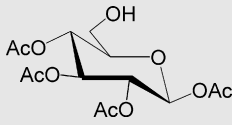
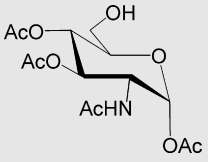
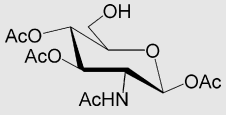
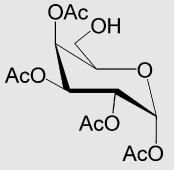
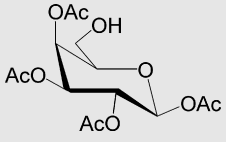
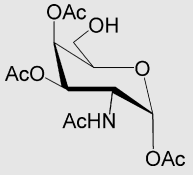
2.3.17. 1,2,4,6-Tetra-O-acetyl-α-D-galactopyranose (24)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 50:50 *n*-hexane–ethyl acetate to provide the desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 18.8 min; yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.33 (d, J = 3.68 Hz; 1H-1), 5.45 (d, 1H-4), 5.18 (dd, J = 3.71 Hz; 1H-2), 4.30 (t, 1H-3), 4.05 and 4.27 (2dd, 2H-6a,b), 4.15 (m, 1H-5), 2.00–2.20 (4s, CH_3, 12H).

2.3.18. 1,2,3,6-Tetra-O-acetyl-β-D-galactopyranose (25)

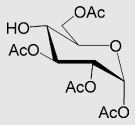
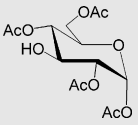
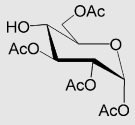
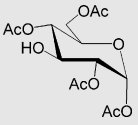
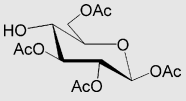
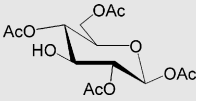
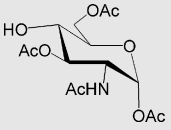
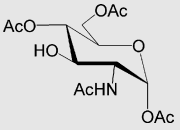
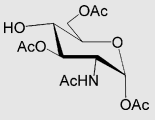
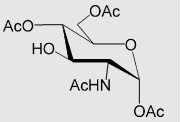
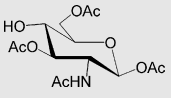
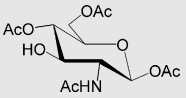
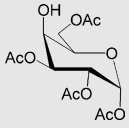
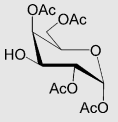
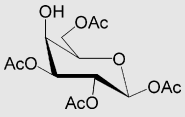
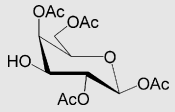
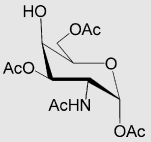
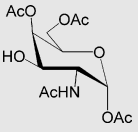
This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 40:60 *n*-hexane–ethyl acetate to provide the desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 17.20 min; yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.93 (d, J = 9 Hz; 1H-1), 5.38 (dd, J = 7.0, J = 3.5 Hz; 1H-3), 5.30 (dd, J = 10.8 Hz, J = 3.2 Hz, 1H-2), 4.20 (m, 1H-4; 2H-6a,b), 3.98 (m, 1H-5), 2.45 (bs, 1H-OH), 1.98–2.16 (4s, CH_3, 12H).

Table 1
Regioselective enzymatic mono-deprotection of per-*O*-acetylated glycopyranoses

Entry	Substrate[a]	Catalyst[b]	Activity[c]	Time[d] (h)	Yield[e](%)	Compound	Structure
a	**1**	CRL	4.53	5	96	**8**	
b	**2**	ANL	0.022	24	60	**9**	
c	**3**	CRL	0.20	48	95	**10**	
d	**4**	ANL	0.007	84	70	**11**	
e	**5**	CRL	2.71	8	96	**12**	
f	**6**	LECI	0.11	7	95	**13**	
g	**7**	CRL	0.09	120	80	**14**	

[a] 20 mM substrate.
[b] Lipases were immobilized on octyl-sepharose support; CRL: *Candida rugosa* lipase; ANL: *Aspergillus niger* lipase; LECI: lecitase phospholipase.
[c] The initial rate in μmol min^{-1} × g$^{-1}_{catalyst}$. It was calculated at 10–15% conversion.
[d] Reaction time to 100% conversion.
[e] Yield of the 6-hydroxy-tetraacetylated product at 100% conversion.

Table 2
Best conditions for the synthesis of 3- and 4-hydroxy-tetraacetylated monosaccharides by chemical migration at 4 °C from **8** to **14**

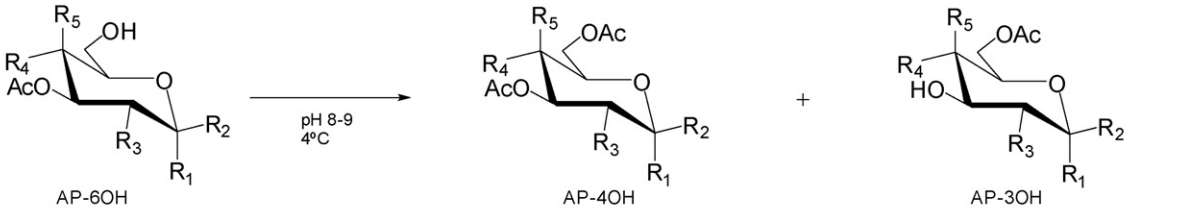
Entry	Substrate	pH	Time (h)	Compound:yield (%)	
a	**8**	8.5	5	 **15**: 80%	 **16**: 20%
b	**8**	9.5	7	 **15**: 70%	 **16**: 30%
c	**9**	8.5	8	 **17**: 70%	 **18**: 18%
d	**10**	8.5	3	 **19**: 79%	 **20**: 20%
e	**10**	9.5	3	 **19**: 66%	 **20**: 30%
f	**11**	8.5	10	 **21**: 78%	 **22**: 20%
g	**12**	8.5	3.5	 **23**: 42%	 **24**: 50%
h	**13**	8.5	1.5	 **25**: 45%	 **26**: 50%
i	**14**	9.5	2	 **27**: 42%	 **28**: 48%

2.3.19. 1,2,4,6-Tetra-O-acetyl-β-D-galactopyranose (26)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 40:60 *n*-hexane–ethyl acetate to provide the desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 23.37 min; yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.89 (d, J = 7 Hz; 1H-1), 5.40 (d, 1H-4), 5.15 (m, 1H-2), 4.30 (t, 1H-3), 3.80 (m, 2H-6a,b), 3.68 (m, 1H-5), 1.96–2.10 (4s, CH_3, 12H).

2.3.20. 2-Deoxy-2-acetamido-1,3,6-tri-O-acetyl-α-D-galactopyranose (27)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was



R_1= OAc, R_2= H, R_3=OAc,R_4=OAc,R_5=H **8**	R_1= OAc, R_2= H, R_3=OAc,R_4=OH,R_5=H **15**	R_1= OAc, R_2= H, R_3=OAc,R_4=OAc,R_5=H **16**
R_1= H, R_2= OAc, R_3=OAc,R_4=OAc,R_5=H **9**	R_1= H, R_2= OAc, R_3=OAc,R_4=OH,R_5=H **17**	R_1= H, R_2= OAc, R_3=OAc,R_4=OAc,R_5=H **18**
R_1= OAc, R_2= H, R_3=NHAc,R_4=OAc,R_5=H **10**	R_1= OAc, R_2= H, R_3=NHAc,R_4=OH,R_5=H **19**	R_1= OAc, R_2= H, R_3=NHAc,R_4=OAc,R_5=H **20**
R_1= H, R_2= OAc, R_3=NHAc,R_4=OAc,R_5=H **11**	R_1= H, R_2= OAc, R_3=NHAc,R_4=OH,R_5=H **21**	R_1= H, R_2= OAc, R_3=NHAc,R_4=OAc,R_5=H **22**
R_1= OAc, R_2= H, R_3=OAc,R_4=H,R_5= OAc **12**	R_1= OAc, R_2= H, R_3=OAc,R_4=H,R_5= OH **23**	R_1= OAc, R_2= H, R_3=OAc,R_4=H,R_5= OAc **24**
R_1= H, R_2= OAc, R_3=OAc,R_4=H,R_5=OAc **13**	R_1= H, R_2= OAc, R_3=OAc,R_4=H,R_5=OH **25**	R_1= H, R_2= OAc, R_3=OAc,R_4=H,R_5=OAc **26**
R_1= OAc, R_2= H, R_3=NHAc,R_4=H,R_5=O Ac **14**	R_1= OAc, R_2= H, R_3=NHAc,R_4=H,R_5=OH **27**	R_1= OAc, R_2= H, R_3=NHAc,R_4=H,R_5=O Ac **28**

Scheme 2. Chemical acyl migration of 6-hydroxy products **8**–**14**.

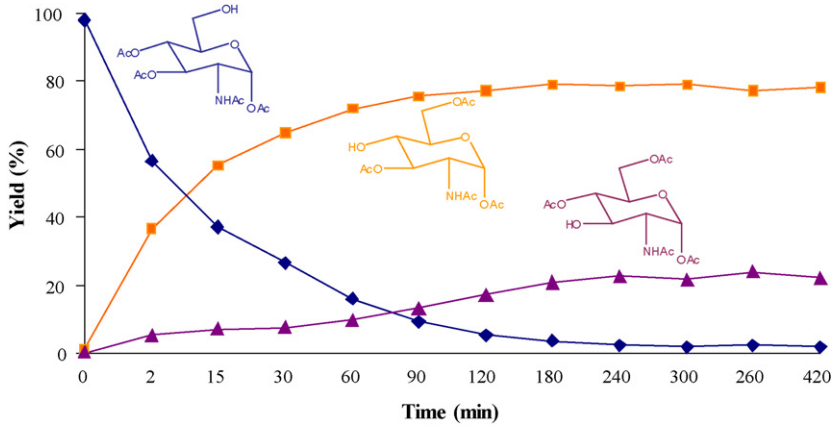


Fig. 2. Acyl chemical migration profile of **10** at pH 8.5 and 4 °C.

performed with 95:5 dichloromethane–methanol to provide the desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 13.27 min; yield: 42%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.20 (d, J = 3.7 Hz, 1H-1), 5.52 (d, J = 9 Hz, 1H-NH), 5.19 (dd, J = 9.2 Hz, J = 11 Hz; 1H-3), 4.83 (ddd, J = 11 Hz, J = 3.6 Hz; 1H-2), 4.27 and 4.36 (2dd, J = 6.3 Hz, J = 11.3 Hz; 2H-6), 4.1 (m, 1H-5), 4.07 (m, 1H-4), 1.96–2.18 (4s, CH_3, 12H).

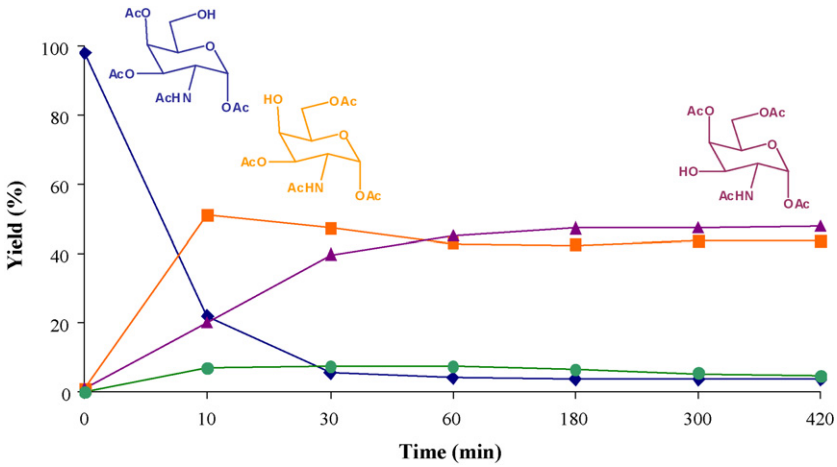


Fig. 3. Acyl chemical migration profile of **12** at pH 8.5 and 4 °C. Green circles: others. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

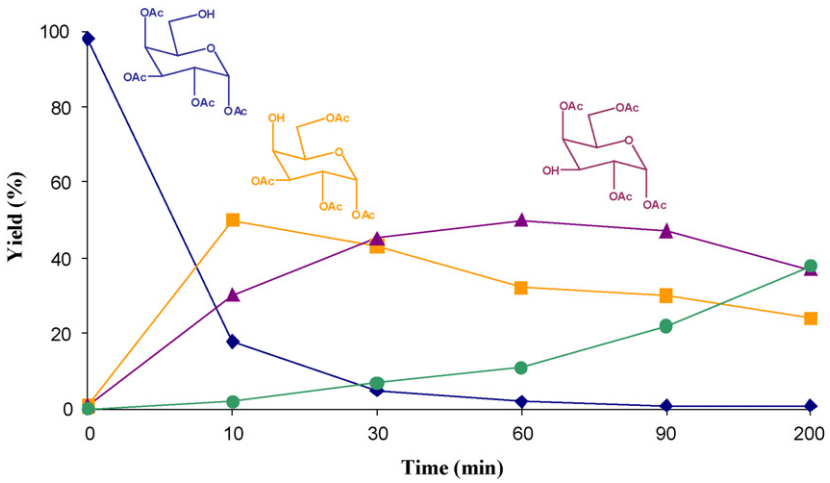


Fig. 4. Acyl chemical migration profile of **14** at pH 9.0 and 4 °C. Green circles: others. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2.3.21. 2-Deoxy-2-acetamido-1,3,6-tri-O-acetyl-α-D-galactopyranose (28)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel column chromatography. Elution was performed with 95:5 dichloromethane–methanol to provide the desired product. HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R = 17.06 min; yield: 48%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.22 (d, J = 3.46 Hz, 1H-1), 5.67 (d, J = 8.1 Hz, 1H-NH), 5.41 (bd, J = 2.5 Hz; 1H-4), 4.52 (ddd, J = 11.36 Hz, J = 8.1 Hz, J = 3.6 Hz; 1H-2), 4.07–4.15 (2dd, J = 6.7 Hz, J = 11.5 Hz; 2H-6a,b), 4.22 (bt, J = 6.7 Hz 1H-5), 4.01 (dd, J = 2.9, J = 7.9 Hz, 1H-3), 2.05–2.21 (4s, CH_3, 12H).

3. Results and discussion

First, the enzymatic regioselective hydrolysis of different α and β peracetylated-glycopyranoses (**1**–**7**) using several immobilized lipases (Scheme 1) was studied in order to obtain different products deprotected at the C-6 position. The CRL was the biocatalyst with the highest activity and regioselectivity in the hydrolysis of all α-anomers (Table 1) assayed as substrates; other enzymes tested were not active (results not shown). This enzyme displayed more than 30 times lower activity against the peracetylated glucosamine **3** and galactosamine **7** than that one expressed against **1** and **5**, respectively (Table 1). CRL was almost inactive towards all the β-anomer substrates (data not shown). For that reason several lipases have been tested and the most active ones are reported in Table 1.

The immobilized ANL was the most active and regioselective for the deprotection in the C-6 position in the hydrolysis of β-peracetylated-glycopyranoses **2** and **4** whereas the immobilized phospholipase lecitase (LECI) was the catalyst with the highest activity in the preparation of 6-hydroxy derivative **13** (Table 1). The activity of all biocatalysts towards the β-anomers was in general much lower than the activity of CRL towards the α-anomers (Table 1). The different regioselective α- or β-6-hydroxy-tetraacetylated glycopyranoses were isolated in multigram scale with high overall yields 60–96% (Table 1) and identified by ^{1}H NMR.

A very mild controlled acyl migration was subsequently studied to obtain the tetraacetylated glycopyranoses bearing a free hydroxyl group in C-3 or C-4 position, using the reaction conditions studied and reported in a previous publication [17]. Thus the 6-hydroxy derivatives **8**–**14**, in aqueous solution at pH 8.5–9.5 and 4 °C, were converted into products **15–28** (Scheme 2).

In fact, starting from glucopyranoses, such as the different anomers of tetracetylated glucose and glucosamine **8**–**11**, a high amount of the 4-hydroxy derivative was preferentially obtained (about 70–80%) at pH 8.5, with a low quantity (10–20%) of C-3 hydroxy product (entries a, c, d and f, Table 2). In the case of **8** and **10**, the yield of 3-hydroxy products **16** and **20** increased up to 30% (entries b and e, Table 2) by incubation at pH 9.5.

With the galactopyranosidic structures, using the different anomers of 6-hydroxy-tetracetylated galactose and galactosamine **12**–**14** (Table 2), a slightly higher amount of 3-hydroxy product was obtained respect to that one of the C-4 deprotected derivative (about 50% *vs.* 40–45%) (entries g–i, Table 2).

After a first analysis of the results, the yield of products obtained depended on the glycopyranose structure used as well as the reaction conditions applied. A comparison in the migration profile of compounds **10**, **12** and **14** was performed. In particular, for the compounds bearing an acetamido group at the C-2 position (Figs. 2 and 4), the concentration of acetylated pyranoses with a free hydroxy group in C-3 and in C-4 was stable even after reaching the maximum concentration value; on the other hand, when an acetoxy group is present at the C-2 position (Fig. 3) the concentration of compounds with a free hydroxy group in C-3 and C-4, after reaching a maximum value, decrease and other products (probably pyranoses bearing a free hydroxy group at the C-2 position) slowly appear.

The migration can be quenched by acidification (pH 4). After extraction in organic solvent the different 3- and 4-hydroxy derivatives were purified by flash chromatography, isolated and characterized by ^{1}H NMR spectroscopy.

4. Conclusion

In this work, immobilized lipases were highly regioselective towards the hydrolysis in C-6 position allowing, in many cases, yields up to 95%. The hydrolysis of the α-anomers peracetylated monosaccharides tested was particularly efficient using *C. rugosa* lipase. The hydrolysis of the β-anomers resulted much slower.

Thus, the regioselective hydrolysis of peracetylated α-monosaccharides, catalyzed by immobilized CRL, permitted to obtain the

AP-OH with an hydroxyl group in C-6 in yields up to 95%, with a good purity and without purification. Only in the case of peracetylated galactosamine **7** the reaction was slow and the final product (80% yield) needed purification.

The combination of this enzymatic hydrolysis with a subsequent mild acyl migration was applied to obtain the "one-pot" preparation of the AP-OH with an hydroxyl group in C-4 or C-3 position. In fact, according to the procedure previously reported [8], when the enzymatic hydrolysis is complete or almost complete, after filtration of the immobilized lipase, the aqueous solution of the AP-6OH, can be directly used for the preparation of an AP-OH bearing the free hydroxyl group at C-3 or C-4, by the controlled acyl migration induced by changing pH and temperature.

Therefore, this efficient and straightforward chemo-enzymatic approach permitted to prepare a small library of different regioisomers of monohydroxy acetylated α-glycopyranoses in good overall yields with a "one-pot" procedure. These products can been useful to synthesize fully peracetylated disaccharides by reaction with an activated sugar donor [15,17].

Similarly, activation of the obtained disaccharide and glycosylation with another AP-OH, could be considered for the preparation of longer oligosaccharides, with a high relevance for biochemical and biological investigations, only using acetyl moiety as protecting group. The results obtained in the study and optimization of this new synthetic strategy will be the object of forthcoming publications.

Acknowledgements

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