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Preparation of linear oligosaccharides by a simple monoprotective chemo-enzymatic approach

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ABSTRACT

A monoprotective approach, involving acetyl ester as unique protective group in oligosaccharides synthesis, has been developed. Starting from peracetylated monosaccharides and glycals, by using an efficient and selective chemo-enzymatic 'one-pot' strategy (a regioselective hydrolysis catalyzed by immobilized lipases followed by a chemical acyl migration), different carbohydrate acceptors, only protected with acetyl ester, can be achieved. If combined with the use of an acetylated glycosyl donor, the glycosylation reaction with these glycosyl acceptors leads to peracetylated oligosaccharides. These compounds can be directly used as intermediates for the synthesis of glycopeptides used as antitumoral vaccines and, at the end of the process, can be easily fully deprotected in only one step. Thus, these key building blocks have been successfully used in glycosylation reactions for an efficient construction of peracetylated disaccharides, such as the biological relevant lactosamine, in multigram scale. Subsequently, glycosylation with the 3OH-tetraacetyl-α-D-galactose, used as carbohydrate acceptor, allowed the synthesis of a peracetylated *N*-trisaccharidic precursor of the lacto-*N*-*neo*-tetraose antigen. Extending this strategy to a 3OH-di-acetyl galactal, one peracetylated precursor of the T tumor-associated carbohydrate antigen has been synthesized.
This efficient approach, characterized by the use of the acetyl ester as only protecting group during all the synthetical steps expected, represents an easy and efficient alternative to the classical synthetic methods in carbohydrate chemistry that involve several protecting group manipulation.

1. Introduction

Carbohydrates have been found to participate in many biological processes and it is now well established that protein- and lipid-bounded saccharides play essential roles in many molecular processes impacting eukaryotic biology and pathology.[1–4] Because their numerous linkage isomers and branching events, the carbohydrates are characterized by a complex structural diversity, far greater than that of other biopolymers such as nucleic acids or proteins.[5,6] Moreover, they are often present as heterogeneous mixtures in nature requiring complex and low-yielding procedures for their extraction from natural sources.[7] Therefore, the chemical synthesis represents an obligatory choice to obtain pure oligosaccharides. This approach, besides the great challenge of the stereoselective glycosydic bond formation,[8] is affected by another relevant difficulty: the preparation of the glycosyl acceptor.

In fact, the synthesis of a sugar building block bearing a strategically positioned free hydroxyl group (carbohydrate acceptor) requires multistep transformations that involve several protecting groups.[9] Recently, some elegant and efficient methodologies for the synthesis of *N*-glycans[10] and other interesting biological oligosaccharides,[11] based on the chemical manipulation of selected protecting groups, have been reported.

The development of selective processes expecting the use of only one protecting group for the construction of both carbohydrate donor and acceptor (monoprotective approach) would be an highly attractive strategy to carry out a sustainable synthesis with high efficiency. For example, peracetylated oligosaccharides are used as practice building blocks in the construction of glycopeptide-based multiantigenic cancer vaccines[12] or in the preparation of inhibitors of the glycosyltransferases involved in the over-expression of the tumoral endogenous cell surface glycoconjugates.[13] These compounds are normally prepared by using various protecting groups that are removed at the end of the multistep process before to proceed with the final per-O-acetylation.

In this context, *O*-acetylated pyranoses bearing only one free hydroxyl group (AP) could be considered useful building blocks in

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the synthesis of peracetylated oligosaccharides. In fact, by using acetyl moiety as the only protecting group for the construction of both glycosyl donor and acceptor, could be possible to directly obtain the peracetylated target compounds. This strategy results in a simplification of the entire synthetic pathway,[14] strongly reducing the protection/deprotection steps required by the use of different protecting groups and avoiding the final peracetylation reaction.

However, this approach has not really been investigated so far mainly because of the problems related to the achievement of the desired APs used as glycosyl acceptor. In fact, using a peracetylated sugar as starting material, only a chemical deprotection at the anomeric position can be easily performed.[15] By classical chemical procedures the selective ester removal from others positions results much more difficult.

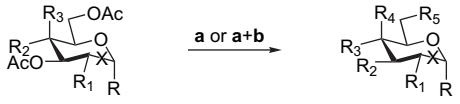
Considering that the glycosidic bond normally occurs at the C-3, C-4, or C-6, we focused our attention on the preparation of AP building blocks deprotected in these positions. Recently,[14,16–19] we have described, starting from peracetylated precursors, the '*one-pot*' chemo-enzymatic synthesis—combining a highly regioselective enzymatic hydrolysis at C-6 position with a temperature- and pH-controlled acyl migration—of a small library of different α/β acetylated carbohydrate acceptors regioselectively deprotected at C-6 or C-4 position. The kind of the enzyme immobilization procedure applied for the biocatalyst preparation strongly influences the regioselectivity, reaction rate, and the yields of these processes.[16,17]

In this work, we have studied and developed a simple chemo-enzymatic monoprotective approach for the synthesis of di- and trisaccharides involved in the structure of lacto-*N*-*neo*-tetraose[20] and one precursor of the tumor-associated carbohydrate antigen T[21]—being the transformation into its antigenic form well described,[22]—directly in their fully acetylated form (Scheme 1).

2. Results and discussion

2.1. Chemo-enzymatic synthesis of regioselectively deprotected acetylated carbohydrate acceptors

Product **3** is directly obtained by regioselective enzymatic hydrolysis of **1**, catalyzed by immobilized lipase from *Candida rugosa* (99% yield) as previously described.[16–19] Subsequently, after the removal of the solid biocatalyst, a controlled chemical acyl migration can be induced avoiding the isolation of **3** (Scheme 2), to obtain, in '*one-pot*', the acetylated AP **4** bearing a free hydroxy group at C-4 position (75% yield). Performing this process at pH 8.5 and 4 °C the presence of AP-3OH **5** has been limited to about 20% and the two regioisomers can be easily purified by flash chromatography. After isolation the AP **4** was obtained in gram scale (Scheme 2). The use of α anomer of peracetylated glucosamine, as starting material for the achievement of **3** and **4**, resulted essential to obtain good results. In fact, it has been reported that the hydrolysis of the β anomer[14] proceeds very slow, obtaining low yields in high reaction time. It is important also to remark that the yields of APs **3** and **4** are strictly depending from the efficiency of the enzymatic catalyst utilized during the hydrolysis. In fact, as previously reported,[23] when the enzyme is used in the free form (not immobilized), it results completely inactive mainly because its instability due to the presence of the organic cosolvent in the reaction medium (necessary for the complete substrate dissolution during the preparative process, i.e., up to 20 g/L[14,19]). Immobilizing the lipase on a hydrophobic supports (octyl-Sepharose),[24] it was possible to obtain a stable and active biocatalyst, in these conditions, permitting to perform a complete and regioselective hydrolysis of the *C*-6 acetyl ester of **1**, maintaining a good residual activity of the enzyme derivative even in the presence of the organic cosolvent.



1 R, R_2=OAc; R_1=NHAc; R_3=H; X=Single;

3 R, R_2, R_3=OAc; R_1=NHAc; R_4=H; R_5=OH; X=Single;
4 R, R_2, R_5=OAc; R_1=NHAc; R_3=OH; R_4=H; X=Single;
5 R, R_3, R_5=OAc; R_1=NHAc; R_2=OH; R_4=H; X=Single;

2 R, R_1,R_3=OAc; R_2=H; X=Single;

6 R, R_1, R_2, R_4=OAc; R_3=H; R_5=OH; X=Single;
7 R, R_1, R_2, R_5=OAc; R_3=H; R_4=OH; X=Single;
8 R, R_1, R_4, R_5=OAc; R_3=H; R_2=OH; X=Single;

9 R, R_1, R_3=H; R_2=OAc; X=Double

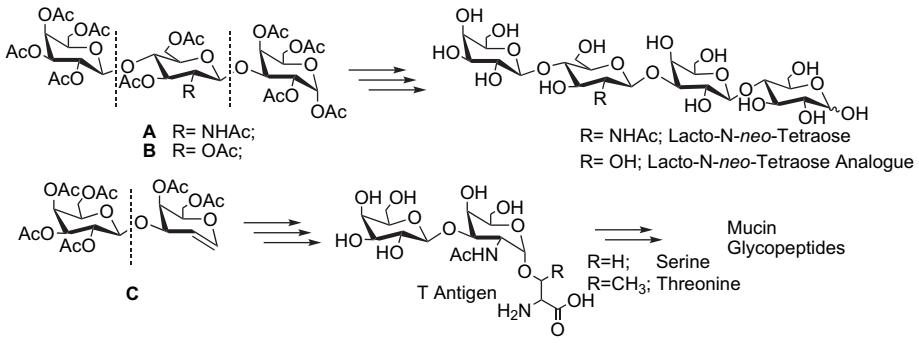
11 R, R_1, R_4=H; R_2, R_3=OAc; R_5=OH; X=Double
12 R, R_1, R_4=H; R_3,R_5=OAc; R_2=OH; X=Double

10 R, R_1, R_2=H; R_3=OAc; X=Double

13 R, R_1, R_3=H; R_2,R_4=OAc; R_5=OH; X=Double
14 R, R_1, R_3=H; R_4, R_5=OAc; R_2=OH; X=Double

Scheme 2. (a) Enzymatic hydrolysis: immobilized lipase, 20% CH_3CN in 50 mM KH_2PO_4 solution, pH 4, [substrate]: 20 mM, *T*: 25 °C; (b) Chemical acyl migration: 20% CH_3CN in 50 mM KH_2PO_4 solution, pH 8.5–9.5, [substrate]: 20 mM, *T*: 25 °C. Route **a** for products **3**, **6**, and **11**–**14**. Route **a**+**b** for products **4**, **5**, **7**, and **8**.

A similar approach was studied for the peracetylated α-D-galactopyranose **2**. In this case, the enzymatic regioselective hydrolysis was performed by screening several immobilized lipases in order to obtain a complete and regioselective deprotection at the *C*-6 position (Scheme 2). The *C. rugosa* lipase (CRL) resulted the best biocatalyst in terms of activity and regioselectivity (Table 1) leading



Scheme 1. Peracetylated precursors **A**, **B**, and **C** of biological interesting oligosaccharides and glycopeptides.

Table 1
Regioselective enzymatic hydrolysis of peracetylated galactopyranose **2**

Entry	Biocatalyst[a]	Time (h)	Vh[b] (UI/g)	Conversion (%)	Product (yield %)
1	CRL	8	2.71	>98	**6** (>98)
2	PFL	48	<0.1	6	**6** (<5)
3	PCL	48	<0.1	15	**6** (8)
4	CALB	48	<0.1	<5	N.D.
5	RML	48	<0.1	<5	N.D.
6	PPL	48	<0.1	<5	N.D.

Reaction conditions: 20% CH_3CN in KH_2PO_4 50 mM buffered solution; pH 4; [substrate]: 20 mM; *T*: 25 °C.

[a] Lipases were immobilized on octyl-Sepharose; 1 g of enzyme derivative was added; lipases from: *C. rugosa* (CRL); *Rhizomucor miehei* (RML); *Pseudomonas fluorescens* (PFL); *Candida antarctica B* (CALB); *P. cepacia* (PCL); Pancreas porcine (PPL).

[b] Vh=initial hydrolysis rate in $\mu mol\ min^{-1} \times g^{-1}_{catalyst}$ calculated between 10 and15% of conversion. N.D.: not determined.

to the achievement of product **6** in quantitative yields (99%) within a short reaction time (entry 1). The other enzymes tested were almost inactive or poorly regioselective (Table 1).

Also after hydrolysis of substrate **2**, the isolation of AP-6OH **6** can be avoided and the solution directly used to obtain APs with free hydroxy group in other positions. In fact, after removing the solid biocatalyst by filtration, a very mild controlled acyl migration was induced; differently from what observed in the acyl migration on the glucosidic structures (probably due to the steric hindrance of the axial substituent at C-4), in this case, the AP-3OH **8** bearing a free hydroxyl group in position C-3 (Scheme 2) resulted as the main product. Selecting the reaction conditions (4 °C and pH 9.5), the amount of the 4-hydroxy derivative **7** was about 30% and product **8** was easily isolated (50% yield) by selective crystallization from the mixture of the regioisomers in gram scale and high purity (>95% by HPLC analysis). Both regioisomers **7** and **8**, after purification, have been characterized by ^1H NMR and 2D-COSY (see Section 4).

Also for the preparation of acetylated glycals bearing only one free hydroxy group by enzymatic hydrolysis of tri-*O*-acetyl glucal **9** and galactal **10** (Scheme 2), several immobilized lipases have been tested. The results obtained (Table 2) showed that CRL was the best biocatalyst in terms of regioselectivity for both substrates, permitting the obtainment of the C-6 hydroxy derivatives **11** and **13** in a very short reaction times (entries 1 and 7) and nearly quantitative yields.

In the case of tri-*O*-acetyl glucal **9**, the others immobilized enzymes tested (Table 2) showed complete regioselectivity toward the C-3 position achieving product **12** in nearly quantitative yields (95–99%, entries 2–5). Only pancreas porcine lipase (PPL, entry 6) resulted inactive. Even toward tri-*O*-acetyl-galactal **10**, the others immobilized lipases studied resulted selective for the C-3 position but the regioselectivity was lower if compared to that obtained in the hydrolysis of **9** (entries 8, 9, and 11). In fact, formation of product **13**, bearing a free hydroxyl group at C-6 position, was also observed. The *Pseudomonas cepacia* lipase (PCL) resulted the best catalyst in terms of activity and regioselectivity (Table 2) allowing to obtain the diacetylated galactal **14**, bearing a free hydroxyl group at the C-3 position in 90% yields (entry 9). CAL-B and PPL were inactive (entries 10 and 12).

Table 2
Regioselective enzymatic hydrolysis of peracetylated glycals **9** and **10**

Entry	Substrate	Biocatalyst[a]	Time (h)	Vh[b] (UI/g)	Conversion (%)	Products (%)
1	**9**	CRL	4	7.73	95	**11** (95)
2	**9**	PFL	4	7.46	95	**12** (95)
3	**9**	PCL	4	18.64	98	**12** (98)
4	**9**	CALB	4	8.88	>98	**12** (>98)
5	**9**	RML	24	4.74	98	**12** (98)
6	**9**	PPL	48	<0.1	<5	N.D.
7	**10**	CRL	5	3.73	95	**13** (90)
8	**10**	PFL	24	1.12	90	**14** (81)/**13** (9)
9	**10**	PCL	24	4.63	>98	**14** (91)/**13** (7)
10	**10**	CALB	48	<0.1	<5	N.D.
11	**10**	RML	24	10.8	97	**14** (75)/**13** (22)
12	**10**	PPL	48	<0.1	<5	N.D.

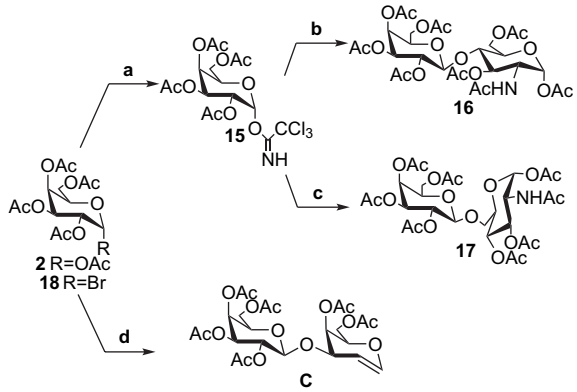
Reaction conditions: 20% CH_3CN in KH_2PO_4 50 mM buffered solution; pH 4; [substrate]: 20 mM; *T*: 25 °C.

[a] Lipases were immobilized on octyl-Sepharose support; 1 g of enzyme derivative was added; lipases from: *C. rugosa* (CRL); *R. miehei* (RML); *P. fluorescens* (PFL); *C. antarctica B* (CALB); *P. cepacia* (PCL); Pancreas porcine (PPL).

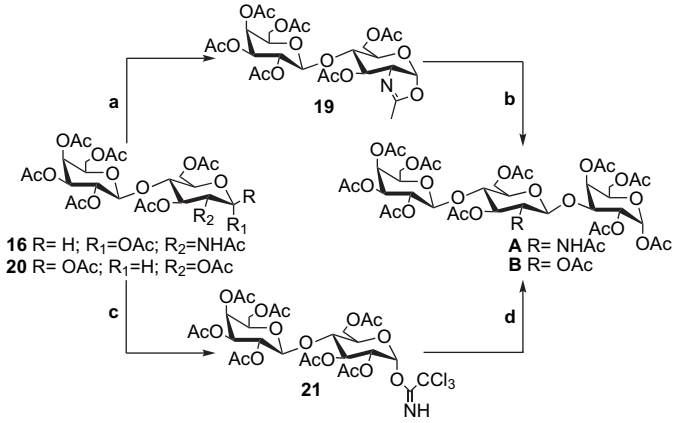
[b] Vh=initial hydrolysis rate in $\mu mol\ min^{-1} \times g^{-1}_{catalyst}$ calculated between 10 and 15% of conversion. N.D.: not determined.

2.2. Synthesis of peracetylated di-, trisaccharides, and T antigen precursor

The different monodeprotected acetylated building blocks **3**, **4**, and **8** were utilized in the construction of different peracetylated di- and trisaccharides (Schemes 3 and 4). 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-1,3,6,-tetra-*O*-acetyl-α-D-glucopyranose **16** was prepared (55% yield) by coupling the glycosyl acceptor **4** with the 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate **15** (prepared as previously



Scheme 3. (a) (1) $NH_2NH_2 \cdot AcOH$, DMF, 50 °C, 1 h; (2) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 1 h, [(1) and (2) 92% yield]; (b) compound **4**, $BF_3 \cdot OEt_2$, CH_2Cl_2, −20 °C, MS 4 Å, 5 h, 55% yield; (c) compound **3**, $BF_3 \cdot OEt_2$, CH_2Cl_2, −20 °C, MS 4 Å, 65%, 5 h. MS=molecular sieves, DMF=*N*,*N*-dimethylformamide, DBU=1,8-diazabicyclo[5.4.0]undec-7-ene; (d) compound **14**, AgOTf, 2,4,6-trimethylpyridine, CH_2Cl_2, −78 °C, 60% yield. AgOTf=silver trifluoromethanesulfonate.



Scheme 4. (a) Compound **16**, TMSOTf, 50 °C, $ClCH_2CH_2Cl$, 4 h; 75% yield; (b) compound **8**, CSA, $ClCH_2CH_2Cl$, 80 °C, MS 4 Å, 6 h, 50% yield; (c) (1) compound **20**, $NH_2NH_2 \cdot AcOH$, DMF, 50 °C, 1 h; (2) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 1 h, [(1) and (2) 75% yield]; (d) compound **8**, $BF_3 \cdot OEt_2$, CH_2Cl_2, −20 °C, MS 4 Å, 5 h, 50% yield. TMSOTf=trimethylsylil trifluoromethanesulfonate, CSA=camphor sulfonic acid, MS=molecular sieves, DMF=*N*,*N*-dimethylformamide, DBU=1,8-diazabicyclo[5.4.0]undec-7-ene.

reported[25]) in the presence of boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) as activating agent (Scheme 3, a and b). Following the same approach, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2-deoxy-2-acetamido-1,3,4,-tetra-*O*-acetyl-α-D-glucopyranose **17** was synthesized in 65% yield (Scheme 3, a and c). After flash chromatography isolation, both disaccharides were characterized by ^{1}H NMR spectroscopy and 2D-COSY.

After the study of the synthesis of the peracetylated disaccharides **16** and **17**, the chain elongation, using the AP-3OH **8** as carbohydrate acceptor, was investigated achieving the preparation of peracetylated trisaccharides **A** and **B** (Scheme 4). The synthesis of compound **A** was initially investigated by using trichloroacetimidate-activated lactosamine as carbohydrate donor. However, during the glycosylation with acceptor **8**, ($BF_3 \cdot OEt_2$ as promoting agent, at −20 °C) the activated lactosaminyl imidate was converted into the oxazoline derivative **19** without formation of the desired product **A** (data not shown). Thus, **16** was directly transformed into the oxazoline **19** and subsequently used like carbohydrate donor in the glycosylation reaction with **8** (Scheme 4, a and c). Starting from **16**, product **19** was prepared (75% yield as previously reported[14]) in the presence of trimethylsilyl triflate (TMSOTf) and, after purification by flash chromatography, used in the acid-catalyzed reaction with the AP-3OH **8** to provide the per-*O*-acetylated trisaccharide **A** in 50% yield.

After the preparation of the fully acetylated oligosaccharide **A**, the synthesis of its analogue **B** was studied, starting from commercial octaacetyl-β-D-lactopyranose **20**. In fact, the peracetylated product **B** was obtained by a glycosylation reaction between the heptaacetyl-α-D-lactopyranosyl trichloroacetimidate **21** (prepared according to the previously reported procedure[25]) with the C-3 AP **8** in the presence of boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$) in 55% yield (Scheme 4, c and d).

The glycosylation reactions were then studied in detail with the aim to improve the yields. In particular, the synthesis of the biological relevant peracetylated lactosamine **16** was investigated in different reaction conditions, studying the influence of parameters such as molar ratio between donor/acceptor, temperature, or the promoter used. The yields reported in Table 3 have been evaluated after purification of product **16** (purity >95% by ^{1}H NMR). The results of this glycosylation study (Table 3) have shown that the yield of product **16** was strongly improved, up to 70%, by using a combination of two different promoting agents such as boron trifluoride diethyl etherate and bismuth (III) triflate,[26] at room temperature (entry 4). This tandem catalysis system ($BF_3 \cdot OEt_2$/$Bi(OTf)_3$) has been tested also for the synthesis of the trisaccharide **B** (Table 3, entry 8) improving the yield up to 65%.

Both the finals products **A** and **B** have successfully been prepared with a simple, efficient, and practical synthetical route that involves the use of the acetoxy moiety as only protecting group. In fact, the trisaccharide **A** was achieved after four steps and 20% overall yield (starting from the peracetylated pyranoside **1**); equally, the construction of product **B** totally request two synthetical steps with about 33% overall yield (starting from the peracetylated pyranoside **2**).

Similarly to the procedure optimized with different APs, C-3 hydroxy diacetylated galactal **14** was studied in the construction of the fully acetylated precursor **C** of the T tumor-associated carbohydrate antigen.[22]

In this case, intermediate **14**—obtained by enzymatic hydrolysis catalyzed by immobilized PCL as above described—was used, after purification, as carbohydrate acceptor in the glycosylation reaction, promoted by silver triflate (AgOTf), with the commercial tetra-acetyl-α-D-galactosyl bromide **18** as carbohydrate donor (Scheme 3, d). Compound **C** was obtained in 54% overall yield from the commercial tri-*O*-acetyl galactal **10**.

3. Conclusion

The present manuscript describes an efficient monoprotective procedure for the preparation of peracetylated oligosaccharides involving acetyl ester as unique protecting group. A chemo-enzymatic approach in the preparation of regioselective monodeprotected peracetylated monosaccharides and glycals combined with glycosylation methods have permitted to synthesize biologically relevant di- and trisaccharides in high overall yields strongly reducing the synthetic protection/deprotection steps required with other chemical procedures.

The use of this monoprotective strategy may represent an elegant and efficient alternative, in carbohydrate chemistry, to obtain even more complex oligosaccharides, than those reported in this work,[27] with a high relevance for biochemical, biophysical, or biological investigations.

4. Experimental section

4.1. General

All the immobilized lipases were purchased from Innovate Biotechnology s.r.l. (Rivalta Scrivia, Tortona (Al), Italy). The pH of the solutions during the enzymatic reactions was kept constant by using an automatic titrator 718 Stat Tritino from Metrohm (Herisau, Switzerland). Reagents and chemicals were purchased from Fluka, Aldrich, Pharmacia Biotech (Uppsala, Sweden), Carbosynth Limited (Newbury, United Kingdom) and used without further purification. Solvents were dried by standard methods. Molecular sieves were activated prior to use by heating under high vacuum. The chemical reactions were monitored by thin layer chromatography on coated aluminum plates (silica gel 60 GF_{254}, Merck, Darmstadt). Detection was performed by charring with a 5% H_2SO_4 in ethanol. Flash chromatography was performed on silica gel 60 (230–400 mesh, Merck, Darmstadt). HPLC analyses were performed using 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. HPLC column was a Gemini-C18 (250 mm×4.6 mm and 5 μm Ø) from Phenomenex–Chemtek (Chemtek Analitica, Anzola Emilia, Bologna, Italy). NMR data were recorded on a Bruker AMX 400 instrument. Coupling constants are reported in hertz.

Table 3
Glycosylation studies for the synthesis of peracetylated lactosamine **16** and trisaccharide **B** with different reaction conditions

Entry	Acceptor (equiv)	Donor (equiv)	Promoter (equiv)	Time (h)	*T* (°C)	Product (%)
1	**4** (1)	**15** (1.5)	$BF_3 \cdot OEt_2$ (1.5)	7	RT	**16** (36)
2	**4** (1)	**15** (1.5)	$BF_3 \cdot OEt_2$ (1.5)	5	−20	**16** (41)
3	**4** (1)	**15** (3.5)	$BF_3 \cdot OEt_2$ (3.5)	6.5	−20	**16** (33)
4	**4** (1)	**15** (2.5)	$BF_3 \cdot OEt_2$ (2), $Bi(OTf)_3$ (0.5)	5	RT	**16** (70)
5	**4** (1)	**15** (1.5)	$CF_3SO_3Si(CH_3)_3$	6	−50	**16** (<5)
6	**4** (1)	**15** (2)	CSA	6	0	**16** (<5)
7	**8** (1)	**21** (1.5)	$BF_3 \cdot OEt_2$ (1.5)	5	−20	**B** (55)
8	**8** (1)	**21** (2.5)	$BF_3 \cdot OEt_2$ (2), $Bi(OTf)_3$ (0.5)	5	−20	**B** (65)

CSA=camphor sulfonic acid.

Electrospray mass spectroscopy (ESI-MS) was performed in methanol on a LCQ-DECA Thermo Finnigan.

4.2. Enzymatic regioselective hydrolysis of different *O*-peracetylated substrates

Substrates **1**, **2**, **9**, and **10** (2 mmol) were added to 100 mL solution of phosphate buffer 50 mM with 20% acetonitrile at pH 5, and the reaction was initialized by adding 5 g of the selected biocatalyst. The reaction was performed at this pH in order to avoid the chemical acyl migration in the per-*O*-acetylated carbohydrates hydrolysis.[18] The reaction mixture was kept under mechanical stirring at 25 °C. During the reaction, the pH was kept constant by automatic titration. Hydrolysis reactions were followed by HPLC. 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 crude product was purified by flash chromatography.

4.3. Chemical pH-acyl migration process

After the enzymatic hydrolytic reaction, the immobilized biocatalyst was removed by simple filtration. The solutions containing the 6-OH derivatives **3** or **6** were incubated at different pHs (8.5 and 9.5, respectively) and 4 °C. 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 crude product was purified by flash chromatography.

4.4. 2-Deoxy-2-acetamido-1,3,4-tri-*O*-acetyl-α-D-glucopyranose (3)

This compound was synthesized following the general procedure of enzymatic hydrolysis above described and purified by silica gel flash chromatography; elution of the was performed with dichloromethane/methanol 95:5. Yield: 98%. R_f=0.32 (dichloromethane/methanol 95:5). HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) at pH 4, flow rate 1.0 mL/min; t_R=5.70 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.19 (d, $J_{1,2}$=3.32 Hz, 1H, H-1), 5.61 (d, 1H, NH), 5.30 (t, 1H, H-3), 5.16 (t, 1H, H-4), 4.46 (m, 1H, H-2), 3.81 (m, 1H, H-5), 3.59 and 3.71 (2dd, 2H, 2H-6a,6b), 2.20 (s, 3H, Ac), 2.05–2.11 (2s, 6H, Ac,), 1.96 (s, 3H, Ac). The hydroxyl proton was not observed due to broadening of the corresponding signal.

4.5. 2-Deoxy-2-acetamido-1,3,6-tri-*O*-acetyl-α-D-glucopyranose (4)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel flash chromatography; elution was performed with dichloromethane/methanol 95:5 to provide the desired product. Yield: 75%. R_f=0.39 (dichloromethane/methanol 95:5). HPLC analysis: 15% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R=7.5 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.15 (d, $J_{1,2}$=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 (br s, 1H-OH), 2.19 (s, CH_3, 3H), 2.13–2.15 (2s, CH_3, 6H), 1.95 (s, CH_3, 3H).

4.6. 2-Deoxy-2-acetamido-1,4,6-tri-*O*-acetyl-α-D-glucopyranose (5)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel flash chromatography; elution was performed with dichloromethane/methanol 95:5 to provide the desired product. Yield: 20%. R_f=0.25 (dichloromethane/methanol 95:5). HPLC analysis: 15% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R=9.3 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.19 (d, $J_{1,2}$=3.28 Hz, 1H, H-1), 5.96 (d, 1H, NH), 5.0 (t, 1H, H-4), 4.35 (ddd, 1H, H-2), 4.09–4.30 (2dd, 2H, 2H-6a/6b), 3.99 (m, 1H, H-5), 3.8 (t, 1H, H-3), 2.00–2.30 (4s, 12H, Ac).

4.7. 1,2,3,6-Tetra-*O*-acetyl-α-D-galactopyranose (7)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described and purified by silica gel flash chromatography; elution was performed with *n*-hexane/ethyl acetate 1:1 to provide the desired product. Yield: 30%. R_f=0.34 (*n*-hexane/ethyl acetate 4:6). HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R=16.7 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.37 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.45 (dd, 1H, H-3), 5.31 (dd, 1H, H-2), 4.39 (m, 1H, H-5), 4.09–4.23 (m, 3H, H-4 and 2H-6a/6b), 2.45 (br s, 1H-OH), 2.02–2.18 (4s, 12H, Ac).

4.8. 1,2,4,6-Tetra-*O*-acetyl-α-D-galactopyranose (8)

This compound was synthesized following the general procedure of the chemo-enzymatic synthesis above described. Purification has been performed by selective crystallization at room temperature with an *n*-hexane/ethyl acetate 4:6 mixture. Yield: 50%. R_f=0.21 (*n*-hexane/ethyl acetate 4:6). HPLC analysis: 20% acetonitrile in phosphate buffer (10 mM) pH 4, flow rate 1.0 mL/min; t_R=18.8 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.33 (d, $J_{1,2}$=3.68 Hz, 1H, H-1), 5.45 (d, 1H, H-4), 5.18 (dd, 1H, H-2), 4.30 (t, 1H, H-3), 4.05 and 4.27 (2dd, 2H-6a,b), 4.15 (m, 1H, H-5), 2.00–2.20 (4s, 12H, Ac).

4.9. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-1,3,4,-tetra-*O*-acetyl-α-D-glucopyranose (16)

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyltrichloroacetimidate **15**[25] (3 g, 6.08 mmol; 5.032 g, 10.2 mmol when it is used the tandem catalysis promoting system $BF_3{\cdot}OEt_2$/$Bi(OTf)_3$) and 2-deoxy-2-acetamido-1,3,6-tri-*O*-acetyl-α-D-glucopyranose **4** (1.41 g, 4.06 mmol) were dissolved in dry CH_2Cl_2 (30 mL), and the promoting agent (with only $BF_3{\cdot}OEt_2$: 747 μL, 6.08 mmol; using the tandem catalysis promoting system $BF_3{\cdot}OEt_2$/$Bi(OTf)_3$: 1 mL, 8.12 mmol of $BF_3{\cdot}OEt_2$/1.332 g, 2.03 mmol of $Bi(OTf)_3$) was slowly added over 10 min in the presence of activated molecular sieves 4 Å (1 g) at −20 °C under nitrogen. After stirring at −20 °C for 5 h, the mixture was allowed to warm up to room temperature, diluted with $CHCl_3$ (30 mL), filtered through Celite, washed with satd aq $NaHCO_3$ (3×50 mL), and brine (1×50 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue purified by silica gel flash chromatography (dichloromethane/ethanol 40:1) to provide the product as a glassy white solid. Yield: 55% (70% using the tandem catalysis promoting system $BF_3{\cdot}OEt_2$/$Bi(OTf)_3$). R_f=0.23 (toluene/methanol 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.07 (d, $J_{1,2}$=3.52 Hz, 1H, H-1), 5.61 (br d, 1H, NH), 5.35 (dd, 1H, H-4′), 5.22 (br dd, 1H, H-3), 5.10 (dd, 1H, H-2′), 4.95 (dd, 1H, H-3′), 4.51 (d, J=7.89 Hz, 1H, H-1′), 4.30–4.43 (m, 2H, H-2, H-6b), 4.03–4.16 (m, 3H, H-6a′, H-6b′ and H-6a), 3.79–3.92 (m, 3H, H-4, H-5 and H-5′), 1.90–2.10 (8s, 24H, Ac). MS (ESI)$^+$ m/z: calcd for $C_{28}H_{39}NO_{18}$: 677.21, found: 700.20 [M+Na^+].

4.10. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2-deoxy-2-acetamido-1,3,4,-tetra-*O*-acetyl-α-D-glucopyranose (17)

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyltrichloroacetimidate **15** (213 mg, 0.43 mmol) and 2-deoxy-2-acetamido-1,3,4-tri-*O*-acetyl-α-D-glucopyranose **3** (100 mg, 0.28 mmol) were dissolved in dry CH_2Cl_2 (2 mL), and $BF_3\cdot OEt_2$ (0.053 mL, 0.43 mmol) was slowly added within 10 min 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 diluted with $CHCl_3$ (10 mL), filtered through Celite, washed with satd aq $NaHCO_3$ (3×10 mL) and brine (1×10 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue purified by silica gel flash chromatography (dichloromethane/ethanol 40:1) to provide the product as a glassy white solid. Yield: 65%. R_f=0.25 (dichloromethane/ethanol 40:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.17 (d, $J_{1,2}$=3.7 Hz, 1H, H-1), 5.58 (br d, 1H, NH), 5.40 (br dd, 1H, H-4′), 5.19 (m, 2H, H-2′ and H-3), 5.02 (m, 2H, H-4 and H-3′), 4.48 (d, $J_{1,2}$=7.93 Hz, 1H, H-1′), 4.44 (m, 1H, H-2), 4.23–4.07 (2dd, 2H, H-6a′ and H-6b′), 4–3.87 (m, 3H, H-5, H-5′ and H-6a), 3.5 (dd, 1H, H-6b), 2.22–2.05 (8s, 24H, Ac). MS (ESI)$^+$ *m*/*z*: calcd for $C_{28}H_{39}NO_{18}$: 677.21, found: 700.32 [M+Na$^+$].

4.11. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-3,6-di-*O*-acetyl-β-D-glucopyranosyl-(1→3)-1,2,4,6-tetra-*O*-acetyl-α-D-galactopyranose (A)

A solution of the oxazoline **19** (75 mg, 0.121 mmol) in freshly distilled dichloroethane (2.5 mL) at 80 °C, in the presence of activated molecular sieves 4 Å under nitrogen, was treated sequentially with camphor sulfonic acid (CSA) (11.3 mg, 0.048 mmol) followed by AP-3OH carbohydrate acceptor **8** (338.4 mg, 0.971 mmol). The resulting solution was maintained at 80 °C and after 6 h was left to reach the ambient temperature. The reaction mixture was then diluted with $CHCl_3$ (10 mL), washed with a saturated solution of $NaHCO_3$ (3×10 mL), followed by brine (2×10 mL), then dried over Na_2SO_4. The organic phase was filtered, concentrated in vacuo, and the residue was purified by flash chromatography (*n*-hexane/ethyl acetate 1:9) to afford the target compound **A**. Yield: 55%. R_f=0.24 (*n*-hexane/ethyl acetate 1:9). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.30 (d, $J_{1,2}$=3.58 Hz, 1H, H-1), 5.48 (br d, 1H, NH), 5.45 (m, 1H, H-4), 5.37 (dd, 1H, H-4″), 5.26 (dd,1H, H-2), 5.19 (br t, 1H, H-3′), 5.13 (dd, 1H, H-2″), 5 (dd, 1H, H-3″), 4.77 (d, *J*=7.22 Hz, 1H, H-1′), 4.55 (d, *J*=7.88 Hz, 1H, H-1″), 4.27 (t,1H, H-5), 3.97–4.2 (m, 7H, 2H-6a/b, 2H-6′a–b, 2H-6″a–b and H-3), 3.9 (t, 1H, H-4′), 3.84 (t, 1H, H-5″), 3.67 (dd, 1H, H-2′), 3.58 (br d, 1H, H-5′), 1.94–2.23 (11s, 33H, CH_3-CO). ^{13}C NMR (400 MHz, $CDCl_3$): δ=170.47, 170.36, 170.14, 169. 93, 169.75, 169.66, 169.53, 169.08, 168.83, 100.95, 100.42, 89.74, 77.25, 76.93, 76.61, 75.41, 72.40, 72.10, 71.77, 70.68, 70.57, 70.01, 69.39, 69.01, 69, 68.97, 67.38, 66.58, 61.85, 60.68, 60.25, 54.43, 53.82, 32.10, 30.05, 29.82, 21.32, 21.07. MS (ESI)$^+$ *m*/*z*: calcd for $C_{40}H_{55}NO_{26}$: 965.86, found: 988.30 [M+Na]$^+$

4.12. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-1,2,4,6-tetra-*O*-acetyl-α-D-galactopyranose (B)

Hepta-*O*-acetyl-α-D-lactopyranosyltrichloroacetimidate (**21**) (336 mg, 0.431 mmol) and 1,2,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**8**) (100 mg, 0.287 mmol) were dissolved in dry CH_2Cl_2 (2 mL), and $BF_3\cdot OEt_2$ (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 diluted with $CHCl_3$ (10 mL), filtered through Celite, washed with satd aq $NaHCO_3$ (3×10 mL), and brine (1×10 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue purified by silica gel column chromatography (dichloromethane/ethanol 40:1) to provide the product **B** as a white solid. Yield: 55%. R_f=0.30 (dichloromethane/ethanol 40:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.30 (d, *J*=3.9 Hz, 1H, H-1), 5.49 (d, 1H, H-4), 5.36 (br d, 1H, H-4″), 5.09–5.17 (m, 2H, H-3′ and H-2), 5.28 (dd, 1H, H-2″), 4.98 (dd, 1H, H-3″), 4.78 (dd, 1H, H-2′), 4.66 (d, *J*=7.5 Hz, 1H, H-1′), 4.54 (d, *J*=7.92 Hz, 1H, H-1″), 3.99–4.2 (m, 7H, 2H-6a/6b, 2H-6a′/6b′, 2H-6a″/6b″ and H-3), 3.87–3.94 (m, 3H, H-5, H-5″ and H-4′), 3.6 (dt, 1H, H-5′), 1.94–2.23 (11s, 33H-OAc). ^{13}C NMR (400 MHz, $CDCl_3$): δ=170.44, 170.34, 170.12, 169.91, 169.72, 169.63, 169.50, 169.05, 168.80, 101.64, 100.36, 90.25, 77.7, 77.4, 77.1, 76.08, 73.10, 72.89, 72.40, 72.22, 71.34, 71.07, 69.65, 68.67, 66.99, 62.32, 61.12, 54.18, 53.84, 32.15, 31.35, 30.11, 29.68, 21.32, 21.07. MS (ESI)$^+$ *m*/*z*: calcd for $C_{40}H_{54}O_{27}$: 966.84, found: 989 [M+Na]$^+$.

4.13. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-*O*-acetyl-D-galactal (C)

To a solution of the 4,6-di-*O*-acetyl galactal **14** (676 mg, 3 mmol) in freshly distilled dichloromethane (25 mL) at room temperature, in the presence of activated molecular sieves 4 Å under nitrogen, were added the commercial bromide **18** (2.056 g, 5 mmol) and 2,4,6-trimethylpyridine (660 μL, 5 mmol). The solution was cooled to −78 °C, under mechanical stirring, and after 1 h silver triflate (1.541 g, 6 mmol dissolved in 10 mL of anhydrous toluene) was slowly added over 10 min. The suspension was kept for 1 h more at −78 °C then was subsequently allowed to warm up to room temperature within 2 h and left under mechanical stirring over night. The solution was diluted with CH_2Cl_2 (25 mL), filtered through Celite, washed with satd aq $NaHCO_3$ (2×40 mL), and brine (1×40 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel flash chromatography (dichloromethane/acetone 93:7) to provide the product **C** as a white glassy solid. Yield: 60%. R_f=0.36 (dichloromethane/acetone 93:7). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.41 (dd, $J_{1,2}$=6.1, 1.2 Hz, 1H, H-1), 5.38 (dd, 1H, H-4), 5.36 (m, 1H, H-4′), 5.2 (dd, 1H, H-2′), 5.02 (dd, 1H, H-3′), 4.82 (m, 1H, H-2), 4.59 (d, $J_{1,2}$=7.917 Hz, 1H, H-1′), 4.56 (m, 1H, H-3), 4.28–4.32 (m, 3H, H-5 and 2H-6a/6b), 4.08–4.16 (m, 2H, 2H-6a′/6b′), 3.9 (dt, 1H, H-5′), 2.2–1.98 (6s, 18H, OAc). MS (ESI)$^+$ *m*/*z*: calcd for $C_{24}H_{33}O_{15}$: 560.5, found: 583.1 [M+Na]$^+$.

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27. The reaction conditions here investigated should be applicable to other carbohydrates as well as it is indicated by our preliminary studies on D-galactosamine, D-mannose, D-mannosamine, and some disaccharides.