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Enzymatic glycosylation using 6-O-acylated sugar donors and acceptors: β-*N*-acetylhexosaminidase-catalysed synthesis of 6-*O*,*N*,*N*′-triacetylchitobiose and 6′-*O*,*N*,*N*′-triacetylchitobiose

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Abstract

p-Nitrophenyl 6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside (**5a**) was used as the glycosyl donor in a β-*N*-acetylhexosaminidase-catalysed (from *Penicillium brasilianum*) glycosylation of GlcNAc yielding 6′-*O*,*N*,*N*′-triacetylchitobiose (**6**), while 6-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranose (**3a**) served as a selectively protected acceptor in a transglycosylation reaction catalysed by the same enzyme to yield 6-*O*,*N*,*N*′-triacetylchitobiose (**4**).
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Keywords: β-*N*-Acetylhexosaminidase; Enzymatic acylation; Chitobiose; Acylated glycosyl donor

1. Introduction

In the last decade dynamic development of glycosciences has brought new fundamental findings from molecular glycobiology, molecular glycoimmunology and related frontier areas, while, in the meantime, demands for new glycostructures have stimulated the development of efficient syntheses of these compounds. Enzymatic methods are widely exploited in carbohydrate chemistry nowadays.[1] Besides glycosyltransferases and glycosidases, other enzymes such as lipases, proteases and amidases are also used in this area, and the synthetic diversity of these enzymes can be broadened by the use of non-natural substrates (xenosubstrates). For instance, glycosyltransferases are able to accept synthetically modified sugar nucleoside donors, e.g., various deoxysugars and thio analogues,[1] as well as completely different nucleosides, e.g., UDP-glucose with β-(1 → 4)-galactosyltransferase.[2] Glycosyltransferases keep their main synthetic advantage with these xenosubstrates that is to combine regioselectivity and absolute stereoselectivity. However, these enzymes have certain disadvantages, such as high price (both the enzymes and substrates), low number of different biocatalysts available, sensitivity to environmental stress (typical for intracellular enzymes, more-

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over membrane bound) and last, but not least, limitations in respect of the acceptors: they are rather stringent towards the distal one to two saccharide moieties of the acceptor, so that non-sugar acceptors can hardly be glycosylated by these enzymes (with the obvious exception of glycosyltransferases specific for a particular aglycon).

Contrary to glycosyltransferases, glycosidases, especially the extracellular ones, are quite robust enzymes, resistant to environmental stress (cosolvents, salts, pH, temperature, etc.,) and easier to handle. Glycosidases of microbial origin can be prepared in a relatively easy way from different sources, so that, libraries of these biocatalysts can be made available. Additionally, these enzymes can often be used for the glycosylation of non-sugar acceptors. Main drawbacks of glycosidases are lower yields and poorer regioselectivity. Specifically, using pyranosidic acceptors, quite often glycosylation, takes place at the primary OH group besides at one (or more) of the secondary OHs, thus lowering the yield of the desired product and producing complex and hardly separable reaction mixtures. To overcome this problem we have developed an approach that exploits selectively enzymatic 6-O-acylated glycosyl acceptors, and we have demonstrated its usability by accomplishing the enzymatic synthesis of the trisaccharide iso-globotriose from 6′-O-acylated lactose.[3]

Chitooligomers and some of their derivatives have been identified as strong activating ligands of the natural killer cell-activating receptor (NKR-P1),[2] making the preparation of these compounds and of their derivatives important. In this paper we report, for the first time, the use of 6-O-acylated-β-D-2-deoxy-2-acetamidoglucopyranosides, both as acceptors and as donors, in the synthesis of selectively acylated chitobioses catalysed by β-*N*-acetylhexosaminidases.

2. Materials and methods

General methods.—Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel F_{254} plates (Merck) using the solvent system 7:2:1 (v/v) isopropanol–water–concd NH_3. The spots were visualised by UV light and by charring with 10% H_2SO_4 in EtOH.

^{1}H- and ^{13}C NMR spectra were recorded on a Varian INOVA-400 spectrometer (399.91 and 100.57 MHz, respectively) in D_2O at 30 °C. Carbon signal multiplicity was determined by an attached proton test (APT) experiment. Manufacturer's software was used for 2D NMR (COSY, HOM2DJ, ROESY, HMQC, HMBC).

Positive ion electrospray ionisation spectra (ESI-MS) were recorded on a double-focusing instrument Finnigan MAT 95 (Finnigan MAT, Bremen, D) with BE geometry. Samples dissolved in 2:1 (v/v) MeOH–water were continuously infused through a stainless capillary held at 3.3 kV into Finnigan ESI source via linear syringe pump at a flow rate of 40 μL/min.

For high-resolution experiments the instrument was tuned to a resolution of about 8000 (10% valley definition) and the measurements were carried out by the peak-matching method using a mixture of polypropylene glycols (average $M_r = 725$) as an internal standard.

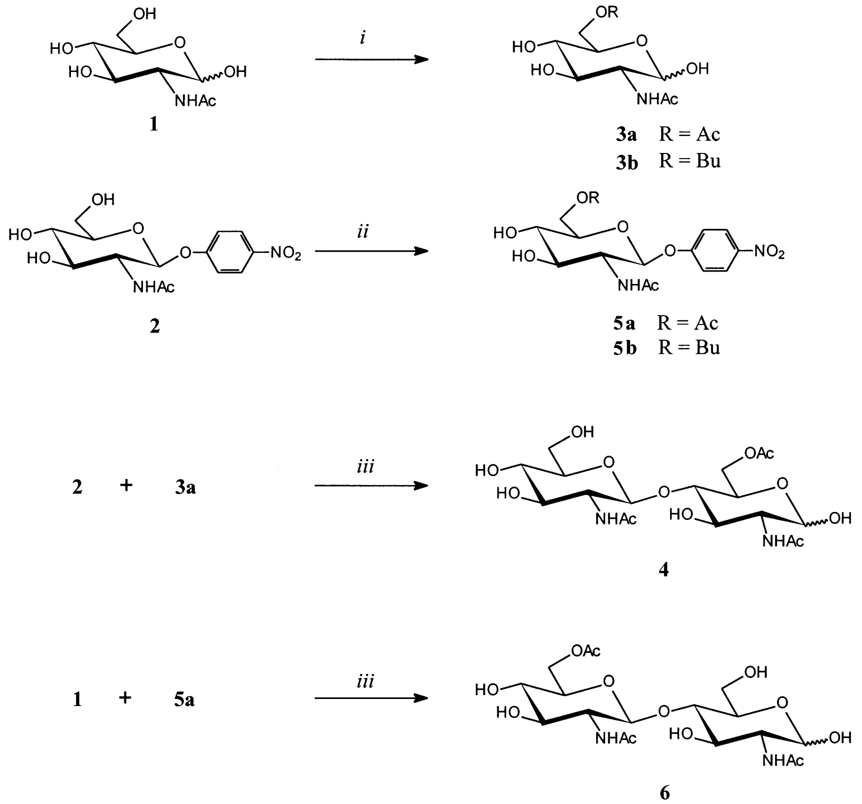
Enzyme preparation.—Filamentous fungi used for the preparation of the β-*N*-acetylhexosaminidases (EC 3.2.1.52) originated from the public collection CCF (Czech Collection of Fungi, Department of Botany, Charles University, Prague, Czech Republic). The enzymes were prepared by a standard procedure;[4] the medium after the cultivation was filtered off, cooled to 4 °C and satd with $(NH_4)_2SO_4$ to 80% saturation. The precipitate containing high specific activity of the respective β-*N*-acetylhexosaminidase (typically 50–100 U/mg protein) was used. Prior to the transglycosylation experiments it was necessary to test esterase contamination in the enzymes used. Compound **3a**, that was hydrolysed to **1** by some of the enzymatic preparations, was used as a testing substrate (monitored by TLC). β-*N*-Acetylhexosaminidases from *Aspergillus oryzae* and *P. brasilianum* were partially purified to remove the esterases. This task was accomplished by phenyl-sepharose chromatography: 100 U of the respective enzyme was dissolved in 10 mL of buffer A (0.6 M $(NH_4)_2SO_4$ in 50 mM phosphate buffer, pH 6.8). The sample was centrifuged and the clear solution was loaded on the phenyl sepharose (Pharmacia) column equilibrated with buffer A (12 × 3.5 cm). The column was eluted with a linear gradient of buffer A and buffer A without $(NH_4)_2SO_4$. The enzyme void of the esterase(s) activity was eluted at the beginning of chromatography. The enzyme was precipitated by $(NH_4)_2SO_4$ (80% saturation), typical recovery was 60%, specific activity was ca 50% higher than in the starting material.

2-Acetamido-4-O-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (6-O,N,N′-triacetylchitobiose) (**4**).— Compound **2** (20 mg, 0.058 mmol), **3a** (130 mg, 0.5 mmol)[5] and $(NH_4)_2SO_4$ (16 mg, 0.12 mmol) were dissolved in a mixture of McIlvaine buffer (1 mL, 50 mM, pH 5.5) and acetonitrile (0.3 mL). β-*N*-Acetylhexosaminidase from *P. brasilianum* (CCF 2155) (10 U) was added and the mixture was incubated at 37 °C for 2 h. The reaction was stopped by heating in boiling water and, after removing the *p*-nitrophenol liberated (2 × 0.5 mL of Et_2O), the mixture was fractionated by gel filtration (BioGel P2, column 2.5 × 90 cm) to afford the single disaccharidic product **4** as a white amorphous powder (4.5 mg, 16.5%) (related to the donor); $[\alpha]_D^{25} = -64.7°$ (*c* 0.29; water); *m*/*z* 467.0 $[M+H]^+$ (Anal. Calcd for $C_{18}H_{31}N_2O_{12}$ 466.18); ^1H NMR (D_2O, 30 °C) (anomer rate: α:β = 80:20): δ [α anomer] 5.085 (d, $J_{1,2}$ 2.3 Hz, H-1), 4.519 (d, $J_{1',2'}$ 8.4 Hz, H-1′), 4.349 (dd, $J_{5,6a}$ 1.9, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.126 (dd, $J_{5,6b,}$ 5.3, $J_{6a,6b}$ 11.5 Hz, H-6b), 4.067 (ddd, $J_{5,6a}$ 1.9, $J_{5,6b}$ 5.3, $J_{4,5}$ 9.8 Hz, H-5), 3.939 (dd, $J_{5',6'a}$ 2.3, $J_{6'a,6'b}$ 11.8 Hz, H-6′a), 3.878 (m, H-2), 3.878 (m, H-3), 3.657 (dd, $J_{5',6'b}$ 6.5, $J_{6'a,6'b}$ 11.8 Hz, H-6′b), 3.686 (dd, $J_{1',2'}$ 8.4, $J_{2',3'}$ 10.3 Hz, H-2′), 3.520 (dd, $J_{3',4'}$ 8.4, $J_{2',3'}$ 10.3 Hz, H-3′), 3.486 (m, H-4), 3.381(ddd, $J_{5',6'a}$ 2.3, $J_{5',6'b}$ 6.5, $J_{4',5'}$ 9.8 Hz, H-5′), 3.319 (dd, $J_{3',4'}$ 8.4, $J_{4',5'}$ 9.8 Hz, H-4′), 2.092, 2.001, 1.992 (3 × s, 3 × Ac); δ [β anomer] 4.605 (d, $J_{1,2}$ 8.2 Hz, H-1), 4.512 (d, $J_{1',2'}$ 8.4 Hz, H-1′), 4.393 (dd, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.101 (dd, $J_{5,6b}$ 5.7, $J_{6a,6b}$ 11.7 Hz, H-6b), 3.939 (dd, $J_{5',6'a}$ 2.3, $J_{6'a,6'b}$ 11.9 Hz, H-6′a), 3.657 (dd, $J_{1,2}$ 8.2, $J_{2,3}$ 10.2 Hz, H-2), 3.686 (dd, $J_{1',2'}$ 8.4, $J_{2',3'}$ 10.3 Hz, H-2′), 3.647 (dd, $J_{5',6'b}$ 6.7, $J_{6'a,6'b}$ 11.9 Hz, H-6′b), 3.599 (ddd, $J_{5,6a}$ 2.1, $J_{5,6b}$ 5.7, $J_{4,5}$ 9.9 Hz, H-5), 3.520 (dd, $J_{3,4}$ 8.4, $J_{2,3}$ 10.2 Hz, H-3), 3.503 (dd, $J_{3',4'}$ 8.5, $J_{2',3'}$ 10.3 Hz, H-3′), 3.488 (dd, $J_{3,4}$ 8.4, $J_{4,5}$ 9.9 Hz, H-4), 3.381 (ddd, $J_{5',6'a}$ 2.3, $J_{5',6'b}$ 6.7, $J_{4',5'}$ 9.9 Hz, H-5′), 3.309 (dd, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.9 Hz, H-4′), 2.092, 2.001, 1.994 (3 × s, 3 × Ac). ^{13}C NMR (D_2O, 30 °C): δ [α anomer] 171.5, 171.5, 172.7 (3 × $CH_3\mathit{CO}O$), 103.5 (C-1′), 92.5 (C-1), 82.3 (C-4), 78.4 (C-5′), 75.9 (C-3′), 71.2 (C-3), 72.4 (C-4′), 69.1 (C-5), 64.7 (C-6), 62.8 (C-6′), 57.9 (C-2′), 55.6 (C-2), 21.0, 23.4, 23.0 (3 × $\mathit{C}H_3COO$); δ [β anomer] 171.5, 171.5, 172.7 (3 × $CH_3\mathit{CO}O$), 103.5 (C-1′), 97.4 (C-1), 82.3 (C-4), 78.4 (C-5′), 75.9 (C-3′), 75.7 (C-3), 74.1 (C-5), 72.4 (C-4′), 64.8 (C-6), 63.2 (C-6′), 57.9 (C-2′), 55.6 (C-2), 21.0, 23.4, 23.0 (3 × $\mathit{C}H_3COO$).

2-Acetamido-6-O-acetyl-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (6′-O,N,N′-triacetylchitobiose) (**6**).— Compound **5a** (20 mg, 0.05 mmol),[5] **1** (130 mg, 0.6 mmol) and $(NH_4)_2SO_4$ (16 mg, 0.12 mmol) were dissolved in a mixture of McIlvaine buffer (1 mL, 50 mM, pH 5.5) and MeCN (0.3 mL). β-*N*-Acetylhexosaminidase from *P. brasilianum* (CCF 2155) (10 U) was added and the mixture was incubated at 37 °C for 4.5 h. After heating at 100 °C, the reaction mixture was worked up as above yielding **6** as a white amorphous powder (5 mg, 21%) (related to the acylated donor); $[\alpha]_D^{25} = -95.7°$ (*c* 0.23; water); *m*/*z* 467.1 $[M+H]^+$ (Anal. calcd for $C_{19}H_{31}N_2O_{12}$ 466.18); ^1H NMR (D_2O, 30 °C) (anomer rate: α:β = 62:38): δ [α anomer] 5.102 (d, $J_{1,2}$ 2.8 Hz, H-1), 4.529 (d, $J_{1',2'}$ 8.5 Hz, H-1′), 4.492 (dd, $J_{5',6'a}$ 2.5, $J_{6',6'b}$ 11.9 Hz, H-6′a), 4.178 (dd, $J_{5',6'b}$ 7.3, $J_{6'a,6'b}$ 11.9 Hz, H-6′b), 3.900 (m, H-2), 3.896–3.818 (ddd, $J_{5,6a}$ 2.1, $J_{5,6b}$ 3.9, $J_{4,5}$ 10.8 Hz, H-5), 3.870 (m, H-3), 3.784 (dd, $J_{1',2'}$ 8.5, $J_{2',3'}$ 10.2 Hz, H-2′), 3.770 (dd, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 11.8 Hz, H-6a), 3.691 (dd, $J_{5,6b}$ 3.9, $J_{6a,6b}$ 11.8 Hz, H-6b), 3.648 (ddd, $J_{5',6'a}$ 2.5, $J_{5',6'b}$ 7.3, $J_{4',5'}$ 9.7 Hz, H-5′), 3.542 (dd, $J_{3,4}$ 8.0, $J_{4,5}$ 10.8 Hz, H-4), 3.514 (dd, $J_{3',4'}$ 8.8, $J_{2',3'}$ 10.2 Hz, H-3′), 3.372 (dd, $J_{3',4'}$ 8.8, $J_{4',5'}$ 9.7 Hz, H-4′), 2.089, 2.035, 1.989 (3 × s, 3 × Ac); δ [β anomer] 4.584 (d, $J_{1,2}$ 8.3 Hz, H-1), 4.526 (d, $J_{1',2'}$ 8.5 Hz, H-1′), 4.481 (dd, $J_{5',6'a}$ 2.5, $J_{6'a,6'b}$ 11.9 Hz, H-6′a), 4.178 (dd, $J_{5',6'b}$ 7.3, $J_{6'a,6'b}$ 11.9 Hz, H-6′b), 3.93 (m, H-6a), 3.784 (dd, $J_{1,2}$ 8.5, $J_{2,3}$ 10.2 Hz, H-2), 3.686 (dd, $J_{1',2'}$ 8.4, $J_{2',3'}$ 10.3 Hz, H-2′), 3.68 (m, H-6b), 3.648 (ddd, $J_{5',6'a}$ 2.5, $J_{5',6'b}$ 7.3, $J_{4',5'}$ 9.7 Hz, H-5′), 3.514 (dd, $J_{3,4}$ 8.8, $J_{2,3}$ 10.2 Hz, H-3), 3.503 (dd, $J_{3',4'}$ 8.5, $J_{2',3'}$ 10.3 Hz, H-3′), 3.394 (ddd, $J_{5,6a}$ 2.0, $J_{5,6b}$ 4.5, $J_{4,5}$ 10.3 Hz, H-5), 3.360 (dd, $J_{3,4}$ 8.8, $J_{4,5}$ 9.7 Hz, H-4), 3.309 (dd, $J_{3',4'}$ 8.5, $J_{4',5'}$ 9.9 Hz, H-4′), 1.993, 2.035, 2.082 (3 × s, 3 × Ac). ^{13}C NMR (D_2O, 30 °C): δ [α anomer] 174.1, 173.8, 173.1 ($3 \times CH_3\mathit{CO}O$), 103.86 (C-1′), 95.56 (C-1), 83.53 (C-4), 75.96 (C-3′), 74.49 (C-5′), 72.54 (C-4′), 71.47 (C-5 interchangeable), 71.19 (C-3 interchangeable), 65.12 (C-6′), 62.12 (C-6), 57.56 (C-2′), 55.73 (C-2), 23.33, 22.87, 20.93 ($3 \times \mathit{C}H_3COO$); δ [β anomer] 174.1, 173.8, 173.1 ($3 \times CH_3\mathit{CO}O$), 103.86 (C-1′), 97.3 (C-1), 83.11 (C-4), 75.96 (C-3′), 75.65(C-5′), 72.54 (C-4′), 76.42 (C-3), 65.12 (C-6′), 62.91 (C-6), 57.76 (C-2′), 58.4 (C-2), 23.33, 22.87, 20.93 ($3 \times \mathit{C}H_3COO$).

3. Results and discussion

Transglycosylation of *N*-acetylglucosamine (GlcNAc, **1**) as the acceptor using *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (*p*-NP-GlcNAc, **2**) as the glycosyl donor in a reaction catalysed by, e.g., a β-*N*-acetylhexosaminidase from *A. oryzae*, gives mostly di-*N*,*N*′-acetylchitobiose and about 10% of its (1 → 6) isomer.[6] Separation of these compounds, either by selective cleavage of the (1 → 6) isomer by a β-*N*-acetylhexosaminidase from jack beans on by tedious carbon-Celite chromatography, is rather complicated. Therefore, **1** was selectively 6-O-acetylated by protease N (from *Bacillus subtilis*, Amano) (Scheme 1), suspended in DMF in the presence of the acyl donor trifluoro ethyl acetate, to give **3a** in 62% isolated yield (the corresponding 6-*O*-butanoate (**3b**) was obtained in 82% yield).[5] Compound **3a** was used as the acceptor in transglycosylation reactions (gly-



Scheme 1. Conditions: (i) protease N (Amano), trifluoroethyl acetate (or trifluoroethyl butanoate), DMF, 40 °C, 6 d. (ii) Novozym 435 (Novo), vinyl acetate (or vinyl butanoate), Py/Me_2CO, 30 °C, 4 d. (iii) β-*N*-Acetylhexosaminidase from *Penicillium brasilianum*, pH 5.5, 37 °C, 2–5 h.

cosyl donor-*p*-NP-GlcNAc) catalysed by a series of fungal β-*N*-acetylhexosaminidases (12 enzymes originating from the enzyme library[7] of the Laboratory of Biotransformation, Inst. of Microbiology, Prague), the best results being obtained with the enzyme isolated from *P. brasilianum*. The glycosylation occurred selectively at the C-4 OH position of the acceptor yielding 6-*O*,*N*,*N*′-triacetylchitobiose (**4**) as the exclusive disaccharidic product. The structure of **4** was confirmed by NMR, substitution at C-4 was deduced from the downfield shift of C-4 (compared to C-4′), and acetylation at C-6 from the downfield shift of C-6 (no acetyl migration during the reaction). The proton spectrum corroborated further the structure suggested for **4**.

We decided to extend our investigation using the 6-O-acyl derivatives of **2** as the sugar acceptors. Accordingly, **2** was selectively 6-O-acetylated with the use of the immobilised lipase B from *Candida antarctica* (Novozym 435, 3 mg/mL).[6] Vinyl acetate (10% v/v) served as the acetyl donor, the reaction proceeded in 7:11 Py–acetone at 30 °C for 4 days giving the quantitative conversion of a 30 mM solution of **2** into the product **5a** (61%, after crystallisation from AcOEt–MeOH). The 6-*O*-butanoyl derivative **5b** was prepared in an analogous manner (49% after crystallisation) Scheme 1.[5]

Preliminarily, fungal β-*N*-acetylhexosaminidases (Table 1) were tested for their hydrolytic activity towards the 6-O-acylated *p*-NP-glycoside **5a**. To our surprise, despite the presence of the acetyl moiety at C-6 OH, hydrolytic activity towards **5a** related to the unmodified substrate **2** ranged from 2 to 18% (Table 1). The enzymes with the highest reaction rates (11–18%) were screened for transglycosylation activity using GlcNAc as an acceptor (Table 1). The best enzyme for the transfer of the 6-*O*-acetyl-β-D-2-deoxy-2-acetamidoglucopyranosyl moiety originated again from *P. brasilianum*. This enzyme was also tested for the cleavage of the 6-O-butanoylated substrate **5b**. The hydrolytic activity was about 4% of that observed with **2**, thus the cleavage was negligible and no transglycosylation was observed. It is obvious that by choosing the appropriate size of the 6-*O*-acyl moiety it is possible to regulate the hydrolytic activity of the respective β-*N*-acetylhexosaminidase. Surprisingly, the use of modified donor substrates with glycosidases has not been exploited too much until now. Kimura et al.[8] used 6-oxo *p*-nitrophenyl β-galactopyranoside (prepared by the action of a galactose oxidase) as a donor in the β-galactosidase-catalysed synthesis of *N*-acetyllactosamine derivatives, while, more recently, the Gallagher group reported the results obtained with a series of C-6 modified *p*-NP β-galactopyranosides (e.g., 6-deoxy, 6-F, 6-allyl, 6-

Table 1
Hydrolytic and transglycosylation activity of fungal β-*N*-acetylhexosaminidases towards *p*-nitrophenyl 6-*O*-acetyl-β-D-2-deoxy-2-acetamidoglucopyranoside (**5a**)

Source of β-*N*-acetylhexosaminidase	Relative hydrolytic activity [a] (%)	Transglycosylation activity [b]
Aspergillus flavipes (CCF 2026)	7.0	ND [c]
A. fumigatus (CCF1OS9)	6.5	ND
A. awamori (CCF 763)	1.4	ND
A. oryzae (CCF 3062)	4.5	ND
A. flavus (CCF1129)	3.2	ND
A. flavofurcatis (CCF3O61)	2.0	ND
A. parasiticus (CCF 1298)	11.0	+
A. oryzae (CCF 1066)	18.0	++
Penicillium purpurogenes var. *rubrisclerotium* (CCF 2985)	14.4	+
P. brasilianum (CCF 2155)	12.8	+++
P. spinulosum (CCF 2159)	13.5	+
P. oxalicum (CCF 1959)	12.1	++

[a] Hydrolytic activity towards the 6-O-acetylated substrate (**5a**) related to that of the natural substrate (**2**). Substrate concentration: 5 mM, pH 5.5, 37 °C.

[b] Acceptor GlcNAc. The product (**6**) formation was tested by TLC (DC-Alufolien Kieselgel 60 F_{254}, Merck, 7:2:1 *n*-propanol–water–ammonia solution, charring with 5% H_2SO_4 in EtOH).

[c] Not determined in the enzymes with the low hydrolysis rate.

propargyl, 6,7-epoxy).[9] From these few reports and from our present findings, it seems that modifications at sugar C-6 are quite tolerated by certain glycosidases, whereas modifications in other positions, especially at C-2, result in impairing the action of these enzymes.

In preparative transglycosylation reactions **5a** was used as a donor and GlcNAc (**1**) served as acceptor. Acetonitrile (30% v/v) was added as a co-solvent to improve the solubility of the substrates, and ammonium sulphate to improve the transglycosylation yields.[10] The products were separated by gel filtration to afford 6′-*O*,*N*,*N*′-triacetylchitobiose (**6**) as the only disaccharidic product. In both transglycosylation reactions, the glycosyl donors (**5a** or **2**) were used in ca 10 times lower amount than the acceptors to avoid formation of byproducts from the monosaccharides liberated from the donors (e.g., di-*N*,*N*′-acetylchitobiose). The structure of the product **6** was confirmed by NMR, C-6′ was shifted downfield compared to C-6 (acetylation) and C-4 was shifted downfield compared to C-4′ thus confirming the (1→4) linkage; proton spectrum confirmed further the structure.

We have also tested a combination of the 6-O-acetylated donor (**5a**) and the acceptor (**3a**) in the same reaction, but no traces of the expected 6,6′,*N*,*N*′-tetracetylchitobiose were formed by the action of a β-*N*-acetylhexosaminidase from *P. brasilianum*.

Apart from the first report on the transglycosylation reactions catalysed by β-*N*-acetylhexosaminidases using modified donors and acceptors, the present results described in this paper enable the synthesis of selectively protected chitooligosaccharides that could be used for further synthetic elaboration, e.g., by perbenzylation and deacetylation. Specifically, the use of 6-O-butanoylated *p*-NP-GlcNAc as an acceptor will enable enzymatic synthesis of *p*-NP-chitooligosaccharides, which are required for immunological and for enzymatic studies.[2]

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