Available online at www.sciencedirect.com
ScienceDirect
Journal of Molecular Catalysis B: Enzymatic 50 (2008) 69–73
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

# *N*-Acetylhexosamine triad in one molecule: Chemoenzymatic introduction of 2-acetamido-2-deoxy-β-D-galactopyranosyluronic acid residue into a complex oligosaccharide

Pavla Bojarová [a,c], Karel Křenek [a], Marek Kuzma [a], Lucie Petrásková [a], Karel Bezouška [a,c], Darius-Jean Namdjou [b,1], Lothar Elling [b], Vladimír Křen [a,*]

[a] *Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ 142 20 Prague 4, Czech Republic*
[b] *Laboratory for Biomaterials, Department of Biotechnology and Helmholtz-Institut for Biomedical Engineering, Aachen University, D 52074 Aachen, Germany*
[c] *Department of Biochemistry, Faculty of Sciences, Charles University Prague, Hlavova 8, CZ 128 40 Prague 2, Czech Republic*

Available online 11 September 2007

**Abstract**

A complex trisaccharide β-D-Gal*p*NAcA-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc (**3**) was prepared in a good yield (35%) in a transglycosylation reaction catalyzed by β-*N*-acetylhexosaminidase from *Talaromyces flavus* using *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-*galacto*-hexodialdo-1,5-pyranoside (**1**) as a donor followed by the *in situ* oxidation of the aldehyde functionality by $NaClO_2$. The disaccharide β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc (**2**) was used as galactosyl acceptor. A disaccharide β-D-Gal*p*NAcA-(1 → 4)-D-Glc*p*NAc (**4**; 39%) originated as a by-product in the reaction. Oligosaccharides comprising a carboxy moiety at C-6 are shown to be very efficient ligands to natural killer cell activation receptors, particularly to human receptor CD69. Thus, oxidized trisaccharide **3** is the best-known oligosaccharidic ligand to this receptor, with $IC_{50} = 2.5 \times 10^{-9}$ M. The presented method of introducing a β-D-Gal*p*NAcA moiety into carbohydrate structures is versatile and can be applied in the synthesis of other complex oligosaccharides.


*Keywords:* β-*N*-acetylhexosaminidase; *Talaromyces flavus*; Galactopyranosiduronic acid; Modified substrate; Natural killer cell; Transglycosylation

## 1. Introduction

In the past decades, the carbohydrate chemistry has seen its boom, evoked by the increasing need for new carbohydrate materials and by the dynamic development of glycomics. In nature, synthesis of carbohydrates is mostly accomplished by glycosyltransferases; however, typical hydrolytic enzymes – glycosidases – are now being used more often for the synthetic purposes, mainly due to a broader specificity (for both donors and acceptors), lower price and availability in a vast repertoire [1].

*N*-Acetylhexosamines (*N*-acetylglucosamine, GlcNAc; *N*-acetylgalactosamine, GalNAc; *N*-acetylmannosamine, ManNAc) are important building blocks in numerous *glyco*-structures, frequently with important biological activities, such as immunoactivity [2]. They often occur in various modified forms (sulfates, uronates, acylations, etc.) in nature.

Glycosidases are known to use modified glycosides as substrates in transglycosylation reactions [3]. Thus, β-*N*-acetylhexosaminidases have been applied in the synthesis of various *N*-acetylhexosamine-containing structures with modified glycosyl donors, for example those comprising 6-*O*-acetyl or *N*-acyl modifications [4]. Recently, we have demonstrated that some β-*N*-acetylhexosaminidases transfer 2-acetamido-2-deoxy-β-D-*galacto*-hexodialdo-1,5-pyranosyl moiety from *p*-nitrophenyl glycoside as a glycosyl donor, which opens the way (after aldehyde oxidation) to the synthesis of 2-acetamido-2-deoxy-β-D-galactopyranosyluronic acids (β-D-Gal*p*NAcA)

* Corresponding author.
*E-mail address:* kren@biomed.cas.cz (V. Křen).
[1] Present address: Institute for Biological Sciences (INB), National Research Council Canada (NRC), 100 Sussex Drive, Bldg. Sussex, K1A 0R6 Ottawa, Canada.

1381-1177/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2007.09.002

[5]. Reactive aldehyde functionality can also be used to label *glyco*-structures by deuteration or tritiation (reduction with $NaBD_4$ or $NaBT_4$, respectively), for conjugation with amine- or hydrazine-carrying labels, etc. So far, no glycosyltransferase was found to synthesize such structures that occur rarely in nature, mostly as components of the capsular polysaccharides of some microorganisms [6].

The structures containing β-D-Gal*p*NAcA moiety were shown to be among the best-known ligands to the activation receptors of natural killer cells (NK cells), particularly of CD69 (human), resulting in immunostimulation. Here, the combination of the β-D-Gal*p*NAcA with other hexosamines in one molecule proved to be essential [7].

In this paper, we demonstrate the versatility of the enzymatic preparation of β-D-Gal*p*NAcA structures by the synthesis of a complex oligosaccharide containing GlcNAc and ManNAc residues that possesses a high immunostimulating activity.

## 2. Experimental

### *2.1. Synthesis of 2-acetamido-2-deoxy-β-D-galactopyranosyluronic acid-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-mannopyranose (**3**)*

Donor glycoside **1** (31 mg, 91 μmol) prepared under the catalysis of galactose oxidase from *Dactylium dendroides* [5] and acceptor disaccharide **2** (15 mg, 35 μmol), prepared by alkaline epimerization of chitobiose [8], were dissolved in citrate/phosphate buffer (0.05 M, pH 5.0, 1180 μL). The extracellular β-*N*-acetylhexosaminidase from *Talaromyces flavus* CCF 2686 (10.8 U) prepared as described previously [9] was added and the mixture was shaken at 35 °C. The reaction was monitored by TLC (AcOEt:MeOH:$H_2O$, 7:3:1 and propane-2-ol:$H_2O$:$NH_4OH$ aq., 7:2:1). After 5 h, the reaction was stopped by heating at 100 °C for 2 min. The reaction mixture was cooled to room temperature and $NaClO_2$ (26 mg, 287 μmol) was added in three portions. After the oxidation was complete (TLC; as above and $CH_2Cl_2$:MeOH:$H_2O$, 5:4:1) the reaction mixture was centrifuged (13,500 rpm, 10 min), concentrated *in vacuo* to ca. 80% volume (to remove free $Cl_2$) and loaded onto a Bio Gel P2 (BioRad, USA) column (2.5 cm × 80 cm; water 12.3 mL/h). Acceptor **2** was partially recovered (6 mg, 141 μmol). Title trisaccharide **3** was obtained as a white solid; yield: 4.8 mg (7.5 μmol, 35% referred to consumed acceptor **2**). According to NMR, compound **3** was a mixture of two anomers ($\alpha/\beta = 1.33$).

α-Anomer of **3**: $^1$H NMR (399.87 MHz, $D_2O$, 303 K) $\delta$ 1.799 (3H, s, Ac), 1.821 (3H, s, Ac), 1.827 (3H, s, Ac), 3.32* (1H, m, H-5′), 3.405 (1H, dd, $J = 2.6$, 11.9 Hz, H-6′d), 3.489 (1H, dd, $J = 4.6$, 11.9 Hz, H-6u), 3.522 (1H, dd, $J = 9.2$, 9.6 Hz, H-4), 3.522 (1H, dd, $J = 8.2$, 10.4 Hz, H-2′), 3.553 (1H, m, H-3′), 3.36* (1H, m, H-4′), 3.556 (1H, dd, $J = 3.3$, 10.9 Hz, H-3″), 3.561 (1H, dd, $J = 2.1$, 11.9 Hz, H-6d), 3.606 (1H, m, H-6′u), 3.635 (1H, ddd, $J = 2.1$, 4.6, 9.6 Hz, H-5), 3.701 (1H, dd, $J = 8.4$, 10.9 Hz, H-2″), 3.835 (1H, d, $J = 0.9$ Hz, H-5″), 3.894 (1H, dd, $J = 4.7$, 9.2 Hz, H-3), 4.004 (1H, dd, $J = 0.9$, 3.3 Hz, H-4″), 4.086 (1H, dd, $J = 1.8$, 4.7 Hz, H-2), 4.262 (1H, d, $J = 8.4$ Hz, H-1″), 4.342 (1H, d, $J = 8.2$ Hz, H-1′), 4.869 (1H, d, $J = 1.8$ Hz, H-1). *HMQC readout. $^{13}$C NMR (100.55 MHz, $D_2O$, 303 K, HMQC and HMBC readouts) $\delta$ 22.2 (Ac), 22.4 (Ac), 22.5 (Ac), 52.4 (C-2″), 53.0 (C-2), 55.2 (C-2′), 60.3 (C-6), 60.4 (C-6′), 67.8 (C-3), 69.3 (C-4″), 70.7 (C-5), 71.1 (C-3″), 72.5 (C-3′), 74.8 (C-5′), 75.5 (C-5″), 77.2 (C-4), 80.1 (C-4′), 93.0 (C-1), 101.6 (C-1′), 101.7 (C-1″), 174.5 (C-6″), 174.9 (2′-CO), 175.0 (2-CO), 175.1 (2″-CO).

β-Anomer of **3**: $^1$H NMR (399.87 MHz, $D_2O$, 303 K) $\delta$ 1.817 (3H, s, Ac), 1.825 (3H, s, Ac), 1.839 (3H, s, Ac), 3.222 (1H, ddd, $J = 2.1$, 4.9, 9.7 Hz, H-5), 3.32* (1H, m, H-5′), 3.36* (1H, m, H-4′), 3.418 (1H, dd, $J = 2.7$, 11.9 Hz, H-6′u), 3.425 (1H, dd, $J = 9.5$, 9.7 Hz, H-4), 3.463 (1H, dd, $J = 4.9$, 11.9 Hz, H-6u), 3.511 (1H, dd, $J = 8.2$, 10.5 Hz, H-2′), 3.556 (1H, dd, $J = 3.3$, 10.9 Hz, H-3″), 3.563 (1H, m, H-3′), 3.606 (1H, m, H-6′d), 3.607 (1H, dd, $J = 2.1$, 11.9 Hz, H-6d), 3.691 (1H, dd, $J = 4.5$, 9.5 Hz, H-3), 3.701 (1H, dd, $J = 8.4$, 10.9 Hz, H-2″), 3.833 (1H, d, $J = 0.9$ Hz, H-5″), 4.004 (1H, dd, $J = 0.9$, 3.3 Hz, H-4″), 4.235 (1H, dd, $J = 1.6$, 4.5 Hz, H-2), 4.259 (1H, d, $J = 8.4$ Hz, H-1″), 4.318 (1H, d, $J = 8.2$ Hz, H-1′), 4.765 (1H, d, $J = 1.6$ Hz, H-1). *HMQC readout. $^{13}$C NMR (100.55 MHz, $D_2O$, 303 K, HMQC and HMBC readouts) $\delta$ 22.3 (Ac), 22.4 (Ac), 22.5 (Ac), 52.4 (C-2″), 53.7 (C-2), 55.2 (C-2′), 60.2 (C-6), 60.4 (C-6′), 69.3 (C-4″), 71.0 (C-3), 71.1 (C-3″), 72.4 (C-3′), 74.8 (C-5′), 75.3 (C-5), 75.5 (C-5″), 76.7 (C-4), 80.1 (C-4′), 93.2 (C-1), 101.6 (C-1′), 101.7 (C-1″), 174.5 (C-6″), 174.9 (2′-CO), 175.1 (2″-CO), 175.9 (2-CO).

MS (ESI): *m/z*: 642.1 $[M+H]^+$, 664.2 $[M+Na]^+$, 665.2 $[M+Na+H]^+$, 680.3 $[M+K]^+$, 686.2 $[M+2Na+H]^+$. $[\alpha]_D^{23} = +8.9$ ($c = 0.27$ in water).

Besides the desired product **3**, formation of by-product **4** was observed (3.6 mg, 8.2 μmol, 39%). The spectral characteristics of **4** are in accordance with the published data [5].

### *2.2. Synthesis of 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-mannopyranose (**5**)*

Disaccharide **2** (45 mg, 0.11 mmol) and *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside (25.1 mg, 0.074 mmol) were dissolved in a mixture of citrate–phosphate buffer (0.05 M, pH 5.0, 1 mL) and acetonitrile (0.1 mL). β-*N*-Acetylhexosaminidase from *A. oryzae* CCF 1066 (10 U) was added and the mixture was incubated at 37 °C for 2 h. The reaction was stopped by heating (100 °C for 10 min) and, after removal of the *p*-nitrophenol by extraction with $Et_2O$ (2 × 1 mL), the mixture was fractionated by gel filtration (BioGel P2, 2.6 cm × 80 cm, flow rate 12 mL/h, eluted with $H_2O$). The isolated yield of the title compound **5** was 19.1 mg (41% referred to consumed donor). Inter-residue heteronuclear couplings observed in HMBC confirmed the linkages of sugar units in compound **5**. NMR and MS spectra were identical to those published previously [10].

### 2.3. Synthesis of other compounds

Other oligosaccharides used in this study were prepared as follows: 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose was prepared as described previously [11] and *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranosiduronic acid was prepared from aldehyde **1** by oxidation with $NaClO_2$ [5].

### 2.4. Spectral characterization

Positive-ion mass spectra were recorded on an LCQ Deca ion trap mass spectrometer (Thermo, San Jose, USA) equipped with nanoelectrospray ion source. Samples dissolved in 30% aqueous acetonitrile/1% formic acid were sprayed directly from borosilicate emitters (Proxeon, DK). The spray voltage was set at 1.2 kV and the heated capillary was kept at 150 °C. Full scan spectra were acquired over the *m*/*z* range 150–1000 Da. Optical rotation was measured on a Perkin-Elmer 241 polarimeter at 589 nm.

NMR spectra were measured on a Varian$^{UNITY}$*Inova* 400 MHz spectrometer (399.87 and 100.55 MHz, respectively) in $D_2O$ at 303 K. Residual signal of solvent was used as an internal standard ($\delta_H$ 4.508 ppm). Carbon chemical shifts in $D_2O$ were referred to acetone ($\delta_C$ 30.50 ppm). $^1$H NMR, COSY, HMQC, and HMBC spectra were measured using standard manufacturers' software (Varian Inc., Palo Alto, U.S.A.). Selective 1D-TOCSY was measured using sequence published by Uhrín and Barlow [12]. $^1$H NMR spectrum was zero filled to four-fold data points and multiplied by window function (two-parameter double-exponential Lorentz–Gauss function) before Fourier transformation to improve resolution. Chemical shifts are given in δ-scale [ppm], and coupling constants in Hz. Digital resolution allowed us to report proton chemical shifts to three and coupling constants to one decimal place. The carbon chemical shifts were read out from HMQC (protonated carbons) and HMBC (quaternary carbons) and are accurate to one decimal place. The proton spin systems of each sugar moiety were assigned by COSY, TOCSY and 1D-TOCSY. The assignment was transferred to carbons by HMQC. The anomeric configuration of mannose was deduced from direct coupling constants $J_{C\text{-}1,\,H\text{-}1}$ (173, 163 Hz, for α- and β-, respectively) observed in coupled HMQC. The position of substitution of sugar moiety was confirmed by long-range heteronuclear correlations in the HMBC. The correlation between H-1′ and C-4 or H-1″ and C-4′ in HMBC spectrum and downfield resonating C-4 and C-4′ confirmed the 1 → 4 linkages.

### 2.5. Test of affinity to NK cell activation receptors

All prepared compounds were tested for their affinity towards two NK cell activation receptors, NKR-P1 and CD69 proteins, in their monomeric soluble forms. NKR-391 protein, the major activation receptor of rat NK-cells, was expressed and purified as described previously [2,13]. CD69CWTY protein that contained the soluble ligand-binding domain of the earliest activation receptor of lymphocytes and NK-cells, CD69 antigen, was prepared as described earlier [14,15]. The identity and homogeneity of the proteins was verified using SDS-PAGE under both reducing and nonreducing conditions, N-terminal sequencing (10 cycles of automated Edman degradation), and by Fourier transform-ion cyclotron resonance mass spectrometry (APEX-Q, Bruker Daltonics, Bremen, D.). The proteins were radiolabelled with Na$^{125}$I using Iodogen kit (Pierce, Rockville, IL, U.S.A). Binding and inhibition assays were performed as described previously [5].

Table 1
Affinity of carbohydrate ligands to two NK cell activation receptor, NKR-P1A (rat), and CD69 (human), expressed in the logarithmic scale ($-\log IC_{50}$)

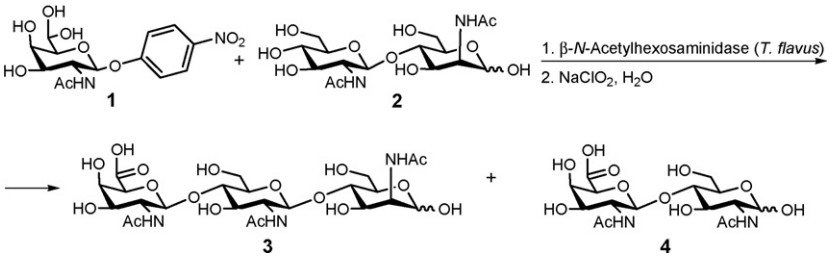
| Compound | NKR-P1 | CD69 |
|---|---|---|
| GlcNAc (standard) | 6.4 | 4.5 |
| *p*NP-β-D-Gal*p*NAcA | 7.5 | 4.6 |
| β-D-Gal*p*NAc-(1→4)-D-Glc*p*NAc | 6.8 | 3.9 |
| β-D-Gal*p*NAcA-(1→4)-D-Glc*p*NAc (**4**) | 7.2 | 7.4 |
| β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Man*p*NAc (**5**) | 9.8 | 7.8 |
| β-D-Gal*p*NAcA-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Man*p*NAc (**3**) | 9.8 | 8.6 |

Data are average values from three independent experiments.

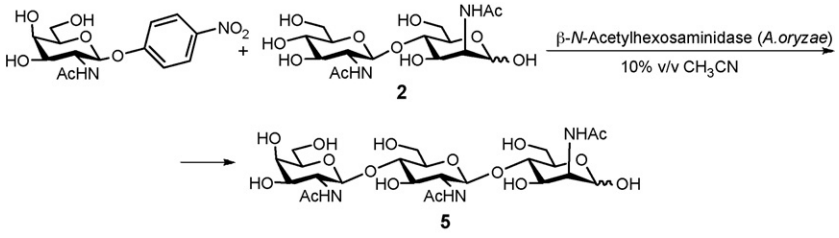
## 3. Results and discussion

We have recently presented a method of the enzymatic synthesis of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-*galacto*-hexodialdo-1,5-pyranoside (**1**) by oxidation of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside (*p*NP-GalNAc). This one-pot reaction was catalyzed by galactose oxidase (*D. dendroides*)–catalase system in a tube reactor and had a virtually quantitative yield [5]. We have also demostrated that compound **1** can act as a glycosyl donor in a transglycosylation reaction catalyzed by fungal β-*N*-acetylhexosaminidase from *T. flavus*. The galactosylation of GlcNAc acceptor and subsequent selective oxidation with $NaClO_2$ yielded β-D-Gal*p*NAcA-(1 → 4)-D-Glc*p*NAc (**4**). Product **4** was among the best-reported ligands to the activation receptors of NK cells, particularly of CD69 (human), which is obviously caused by the presence of the carboxylic moiety at C-6 (*cf.* the unoxidized analogue, β-D-Gal*p*NAc-(1 → 4)-D-Glc*p*NAc, Table 1). As this concept is very promising for the construction of highly efficient complex ligands to NK cell activation receptors, we decided to transfer the β-D-Gal*p*NAcA moiety to the disaccharide acceptor, β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc (**2**), which is one of the strongest NK cell activating disaccharides (with NKR-P1 protein, $IC_{50} = 7.9 \times 10^{-9}$ M, *i.e.*, 25-fold stronger than GlcNAc). [2] Thus, we aimed to prove the versatility of transglycosylation reactions with donor **1** and, moreover, synthesize a unique modified trisaccharide with superior immunoactivity.

Acceptor disaccharide **2** is quite a rare compound. We have recently developed an efficient preparatory method based on alkali-catalyzed C-2 epimerization of chitobiose followed by borate-saturated gel filtration [8]. Acceptor **2** was inert towards cleavage by many β-*N*-acetylhexosaminidases, contrary to its C-2 epimer chitobiose [11]. However, β-*N*-acetylhexosaminidase




Scheme 1. Synthesis of β-D-Gal*p*NAcA-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc (**3**).




Scheme 2. Synthesis of β-D-Gal*p*NAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc (**5**).

from *T. flavus*, which is the most efficient enzyme in transferring the modified galactosyl moiety of aldehyde **1**, shows a slight degree of cleavage of **2** [11]. Additionally, a high activity of the enzyme is required in the reaction mixture (substrate **1** is cleaved more slowly than unmodified *p*NP-GalNAc) and a high concentration of **2** is needed to compete with a mere hydrolysis of the donor. As a result, acceptor **2** was partially cleaved during the reaction and disaccharide **4** was formed as a by-product. This is not a serious problem because subsequent purification by gel filtration leads to a complete separation of the mixture including recuperation of the unreacted substrates. Both trisaccharide **3** and disaccharide **4** were isolated in a high yield (35 and 39%, respectively), which indicates an exceptionally efficient glycosylation of acceptor disaccharide (ca. 74% of the acceptor present were glycosylated). The reaction is outlined in Scheme 1.

The transglycosylation reaction was optimized as for the donor/acceptor ratio and the required enzyme activity. The enzymatic reaction was followed by *in situ* oxidation of the aldehydic group to a carboxyl by $NaClO_2$. Interestingly, this method is rather selective and can be used with reducing sugars, which are not oxidized at the anomeric position.

The affinity of novel trisaccharide **3** to two NK cell activation receptors, rat NKR-P1 and human CD69 (their monomeric soluble forms) was tested, and the results were compared to other C6-oxidized compounds as well as to the unoxidized trisaccharide analogue **5**, which was prepared as shown in Scheme 2.

The binding assay was performed as described previously [5]. D-Mannose served as a negative (noninhibitory) control carbohydrate, and GlcNAc as a positive control, providing $IC_{50}$ values of $4 \times 10^{-7}$ M (NKR-P1A) and $3.2 \times 10^{-5}$ M (CD69). All inhibition assays were performed in triplicates. The results for NKR-P1 and CD69 receptors are summarised in Table 1 as $-\log IC_{50}$.

These results clearly show that the binding potential of carbohydrate ligands increases with the presence of a carboxy moiety at C6 (compare compounds β-D-Gal*p*NAc-(1 → 4)-D-Glc*p*NAc *vs.* **4** and **5** *vs.* **3**), which is in accordance with our presumption and previous results [5]. Another increase in binding capacity is brought about by prolonging the carbohydrate chain by one hexosamine unit (compare β-D-Gal*p*NAc-(1 → 4)-D-Glc*p*NAc *vs.* **5** and **4** *vs.* **3**). These improvements are more pronounced with CD69 human receptor. Thus, the novel oxidized trisaccharide **3** proves to be the best-reported oligosaccharidic ligand to CD69 human NK cell activation receptor with $IC_{50} = 2.5 \times 10^{-9}$ M, which corresponds to 12,800-fold stronger binding than that of GlcNAc standard and 16-fold stronger than of the previously reported oxidized disaccharide **4**.

## 4. Conclusions

A versatile method of introducing a β-D-Gal*p*NAcA moiety into carbohydrate structures is presented, using aldehyde **1** as a donor in a transglycosylation reaction catalyzed by β-*N*-acetylhexosaminidase from *T. flavus*. Thus, a modified trisaccharide of β-D-Gal*p*NAcA-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-D-Man*p*NAc (**3**) was prepared in a high yield (35%), together with a by-product, β-D-Gal*p*NAcA-(1 → 4)-D-Glc*p*NAc (**4**; 39%). The prepared compounds were tested as ligands to natural killer activation receptors, NKR-P1 (rat) and CD69 (human) and their binding potential was compared to several analogous and standard compounds. The results confirmed that the presence of a carboxy moiety at C6, as well as the prolongation of a carbohydrate chain (disaccharide → trisaccharide), significantly enhance the binding capacity to these receptors. Their potential immunostimulating effect can be further extended by their presentation on multivalent scaffolds. As

a result, oxidized trisaccharide **3** proved to be the best-known ligand to CD69 human activation receptor, with $IC_{50} = 2.5 \times 10^{-9}$ M, which is 12,800-fold more efficient than GlcNAc.

## Acknowledgements

Support from the grants of COST Chemistry D25 (MŠMT OC 170), Czech Science Foundation no. 203/05/0172, MŠMT grant LC06010, research concepts AV0Z50200510 and MSM 21620808 and mobility grant AV ČR-DAAD PPP-D7-CZ26/04 05D/03/44448 (V.K. & L.E.) is gratefully acknowledged.

## References

[1] V. Křen, J. Thiem, Chem. Soc. Rev. 26 (1997) 463–474.
[2] P. Krist, E. Herkommerová-Rajnochová, J. Rauvolfová, T. Semeňuk, P. Vavrušková, J. Pavlíček, K. Bezouška, L. Petruš, V. Křen, Biochem. Biophys. Res. Commun. 287 (2001) 11–20.
[3] (a) T. Kimura, S. Takayama, H. Huang, C.-H. Wong, Angew. Chem., Int. Engl. Ed. 35 (1996) 2348–2350;
(b) D.A. MacManus, U. Grabowska, K. Biggadike, M.I. Bird, S. Davies, E.N. Vulfson, T. Gallagher, J. Chem. Soc. Perkin Trans. 1 (1999) 295–305.
[4] (a) L. Hušáková, S. Riva, M. Casali, S. Nicotra, M. Kuzma, Z. Huňková, V. Křen, Carbohydr. Res. 331 (2001) 143–148;
(b) P. Fialová, L. Weignerová, J. Rauvolfová, V. Přikrylová, A. Pišvejcová, R. Ettrich, M. Kuzma, P. Sedmera, V. Křen, Tetrahedron 60 (2004) 693–701.
[5] P. Fialová, D.-J. Namdjou, R. Ettrich, V. Přikrylová, J. Rauvolfová, K. Křenek, M. Kuzma, L. Elling, K. Bezouška, V. Křen, Adv. Synth. Catal. 347 (2005) 997–1006.
[6] (a) S.V.K.N. Murthy, M.A. Melly, T.M. Harris, C.G. Hellerquist, J.H. Hash, Carbohydr. Res. 117 (1983) 113–123;
(b) K. Amano, J.C. Williams, S.R. Missler, V.N. Reinhold, J. Biol. Chem. 262 (1987) 4740–4747.
[7] V. Křen, Enzymatic synthesis of carbohydrate ligands for natural killer cells. The way from ligand optimization towards anticancer glycodrugs, in: M.P. Schneider (Ed.), Chemical Probes in Biology, Kluwer, Amsterdam, 2003, pp. 379–389.
[8] L. Petrásková, A. Charvátová, V. Přikrylová, V. Kristová, J. Rauvolfová, L. Martínková, J. Jiménez-Barbero, N. Aboitiz, L. Petruš, V. Křen, J. Chromatogr. A 1127 (2006) 126–136.
[9] Z. Huňková, V. Křen, M. Ščigelová, L. Weignerová, O. Scheel, J. Thiem, Biotechnol. Lett. 18 (1996) 725–730.
[10] N. Aboitiz, F.J. Cañada, L. Hušáková, M. Kuzma, V. Křen, J. Jiménez-Barbero, Org. Biomol. Chem. 2 (2004) 1987–1994.
[11] L. Hušáková, E. Herkommerová-Rajnochová, T. Semeňuk, M. Kuzma, J. Rauvolfová, V. Přikrylová, R. Ettrich, O. Plíhal, K. Bezouška, V. Křen, Adv. Synth. Catal. 345 (2003) 735–742.
[12] D. Uhrín, P.N. Barlow, J. Magn. Reson. 126 (1997) 248–255.
[13] K. Bezouška, G. Vlahas, O. Horváth, G. Jinochová, A. Fišerová, R. Giorda, W.H. Chambers, T. Feizi, M. Pospíšil, J. Biol. Chem. 269 (1994) 16945–16952.
[14] J. Pavlíček, B. Sopko, R. Ettrich, V. Kopecký Jr., V. Baumruk, P. Man, V. Havlíček, M. Vrbacký, L. Martínková, V. Křen, M. Pospíšil, Biochemistry 42 (2003) 9295–9306.
[15] J. Pavlíček, D. Kavan, P. Pompach, P. Novák, O. Lukšan, K. Bezouška, Biochem. Soc. Trans. 32 (2004) 1124–1126.