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Mendeleev Commun., 2019, **29**, 254–255

Mendeleev Communications

Synthesis of GalNGcα1-3GalNAcα disaccharide and its interaction with human blood antibodies

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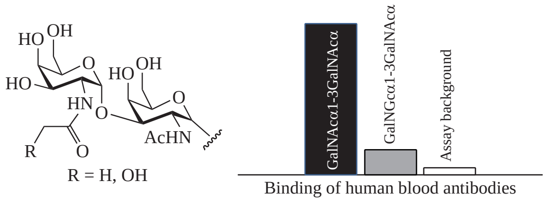
DOI: 10.1016/j.mencom.2019.05.004

We have synthesized GalNGcα1-3GalNAcα disaccharide, a glycoloyl analogue of GalNAcα1-3GalNAcα known as the core 5 motif of mucin-type glycoproteins, and investigated how this structural variation affects the interaction with human natural antibodies. In contrast to the case of the Neu5NGc/Neu5NAc pair, the additional glycoloyl OH group in GalNGcα1-3GalNAcα markedly reduced the antigenicity of the GalNGcα moiety.



The residues of 2-aminosugars, such as GlcN and GalN, as well as neuraminic acid Neu, which are contained in human glycoconjugates, typically have *N*-acetyl substituents because human biosynthetic machinery lacks enzymes synthesizing the corresponding *N*-glycoloyl (Gc) analogues. In contrast, most mammalian species are able to synthesize *N*-glycoloylneuraminic acid (Neu5Gc) in an amount at least equivalent to that of the *N*-acetylneuraminic acid (Neu5Ac) analogue.[1,2] In humans, Neu5NGc is supplied through consumption of red meat and can further accumulate in complex glycoconjugates. The amount of Neu5Gc in normal human tissues is typically below 1% of total sialic acids, and can be noticeably higher in cancer tissues.[3] Degradation of Neu5Gc in human organism is a metabolic source of UDP-GlcNGc and UDP-GalNGc, with the latter glycosyl donor participating in incorporation of glycoloyl moieties into human glycoconjugates.[4] The human immune system can distinguish between Neu5Ac- and Neu5Gc-containing antigens: antibodies do not recognize most versions of glycans with sialic acid residues, whereas the Neu5Gc-containing biomolecules are known as antigenic and immunogenic ones.[5] *N*-Glycoloylgalactosamine (GalNGc) has been already investigated,[6] though the oligosaccharides and glycoconjugates with its residues have not yet been described and their antigenicity in humans has not been examined. Here we present the chemical synthesis of the GalNGcα1-3GalNAcα disaccharide as an analogue of GalNAcα1-3GalNAcα which in turn is known as the core 5 motif of mucin-type glycoproteins, as well as describe the influence of Gc/Ac structural variation on the interaction with human natural antibodies[7] for this pair and for several analogous Gc/Ac saccharide pairs. All the oligosaccharides were synthesized as glycosides with the 3-aminopropyl spacer (sp), with a view to employing them for neoglycoconjugates preparation and glycoarray printing.

Glycosylation of acceptor **2**[8] (Scheme 1), bearing one hydroxyl group at C-3, with twofold excess of β-chloride **1** as a glycosyl donor[9] was performed in the presence of silver triflate, tetramethyl urea and molecular sieves 4 Å in dry dichloromethane at room temperature for 20 h.[10] After removal of the benzylidene group under acidic conditions, followed by acetylation and chromatographic purification on silica gel, disaccharides **3** and **3β** were obtained in 58 and 15% yields, respectively.

The conversion of the azido group at C-2 to RNH-group (R = = AcOCH$_2$CO or Ac) was carried out with AcCl or AcOCH$_2$COCl in the presence of PPh$_3$ in dry dichloromethane at room temperature,[†] similarly to the reported one-pot synthesis of glycosyl amides.[11]

After chromatographic purification, the resulting peracetylated oligosaccharides **4**, **5** and **5β** were analyzed by ^{1}H NMR spectroscopy. The complete assignment of their spectra using COSY-experiment confirmed the expected structure with $J_{1,2}$ ~ 3.5 Hz for H-1 in α-configuration, $J_{1,2}$ ~ 8 Hz for H-1 in β-configuration, and an upfield shift to 3.95–3.99 ppm for H-3a at glycosidic bond.

Deacetylation of the peracetylated oligosaccharides and removal of *N*-trifluoroacetyl groups, followed by purification on Dowex H$^+$ with elution by 1 M aq. NH$_3$, resulted in 3-aminopropyl glycosides **6**, **7** and **7β** in 20–100 mg amounts. Their structure was confirmed by mass spectrometry, ^{13}C and ^{1}H NMR spectroscopy (see Online Supplementary Materials).

N-Glycoloyl derivatives GalNGcβ-sp **8**, GalNGcα1-3-(Fucα1-2)Galβ-sp **9** and Galβ1-3GalNGcα-sp **10** were obtained in a similar way[†] from the previously synthesized corresponding 2-deoxy-2-azides[12,13] using routine method of deprotection. Their spectral data are given in Online Supplementary Materials.

All the synthesized glycoloyl derivatives were printed onto activated slides,[‡] and the assay with fluorescent detection was carried out as described.[14,15] Complex immunoglobulin preparation, a therapeutic immunoglobulin mixture containing IgG, IgM and IgA from 1000+ healthy donors, was used as a source of natural antibodies. As a result, the NGc derivative **6** bound human

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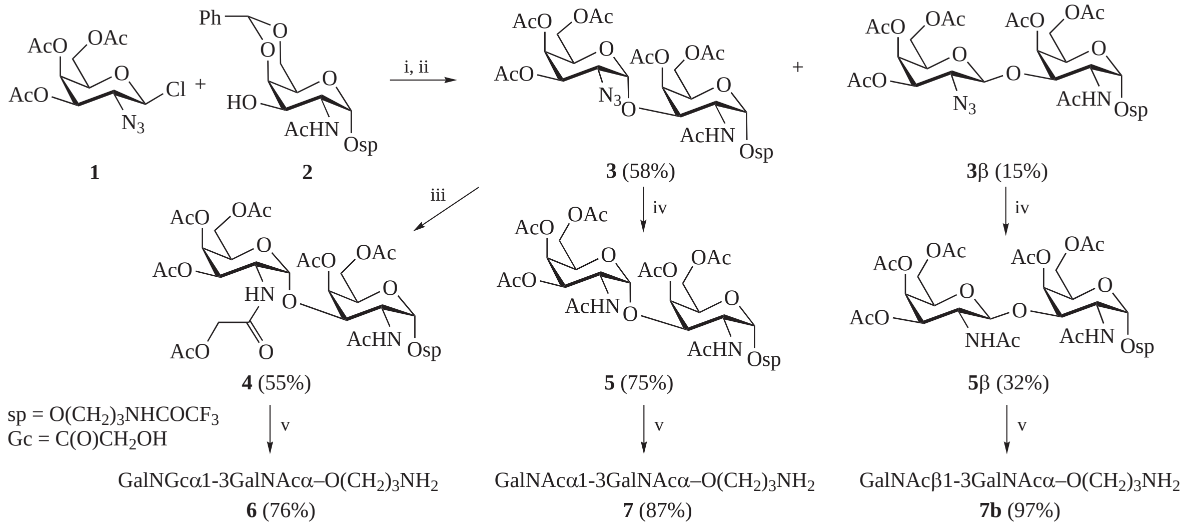
[†] To a solution of oligosaccharide azide (0.1 mmol) in dry methylene dichloride (5 ml), PPh$_3$ (0.4 mmol) and AcOCH$_2$COCl or AcCl (0.4 mmol) were added. The mixture was kept at room temperature for 5–10 h under TLC control and then co-evaporated with toluene *in vacuo* three times. The product was isolated by chromatography on silica gel. Yields 50–75%.

[‡] Semiotik LLC, Moscow, Russia. Total ~600 antigens at 6 replicates each.



Scheme 1 *Reagents and conditions*: i, AgOTf, tetramethylurea, MS 4 Å, CH_2Cl_2, room temperature, 20 h; ii, 80% aq. AcOH, 70 °C, 2 h, then Ac_2O/Py; iii, PPh_3, $AcOCH_2COCl$, CH_2Cl_2, room temperature, 10 h; iv, PPh_3, AcCl, CH_2Cl_2, room temperature, 10 h; v, 0.1 м MeONa/MeOH, 1 h, then 0.1 м aq. NaOH, 16 h.

Table 1 Binding of human natural antibodies to GalNGc-containing oligosaccharides and their GalNAc counterparts. Data of the printed glycans array.

Compound	Median fluorescence (arbitrary units)	Median absolute deviation
8 GalNGcβ-sp	811	78
11 GalNAcβ-sp	2563	537
6 GalNGcα1-3GalNAcα-sp	3010	285
7 GalNAcα1-3GalNAcα-sp	19348	2027
9 GalNGcα1-3(Fucα1-2)Galβ-sp	1134	266
12 GalNAcα1-3(Fucα1-2)Galβ-sp	4284	524
10 Galβ1-3GalNGcα-sp	538	126
13 Galβ1-3GalNAcα-sp	933	59

antibodies one order of magnitude weaker than its NAc counterpart **7** (Table 1). Therefore, it can be concluded that OH group of the NGc moiety abolishes the interaction of specific human anti-core 5 natural antibodies with the GalNGcα1-3GalNAcα antigen. Similarly, the lower binding of the NGc version compared with the NAc counterpart was observed for other pairs (see Table 1), namely compound **8** *vs.* β-galactosamine derivative **11**[12], compound **9** *vs.* A-trisaccharide **12**[13] as well as compound **10** *vs.* Thomsen–Friedenreich antigen **13**.[8] In the last pair, the NGc moiety is located in the inner GalNAcα monosaccharide residue. For these three pairs, the difference in binding is not as pronounced as for the pair of compounds **6** and **7**. Note that for the pair of Neu5Acα2-3Galβ1-4GlcNAc/Neu5Gcα2-3Galβ1-4GlcNAc, the opposite effect is described.[15,16]

In summary, the *N*-glycoloyl moiety of glycoconjugates is not perceived by the innate immunity system as a constitutional ‘alien’ motif, and therefore the antigenicity of Neu5Gc-glycoconjugates, which is discussed in terms of xenotransplantation[17] and tumor immunity,[18] could be reevaluated.

This work was supported by the Russian Science Foundation (grant no. 14-50-00131). The work of N.V.S. was supported by the Russian Foundation for Basic Research (grant no. 18-04-00749). Experiments were partially carried out using the equipment provided by the Institute of Bioorganic Chemistry core facility (CKP IBCh, supported by the Ministry of Education and Science of the Russian Federation, grant no. RFMEFI62117 X0018).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.05.004.

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Received: 24th December 2018; Com. 18/5789