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Synthesis of disaccharides for the study of human blood antibodies capable of recognizing the inner Glcβ1-3GalNAc disaccharide fragment of bacterial polysaccharides

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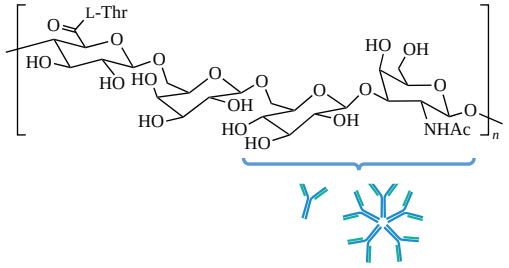
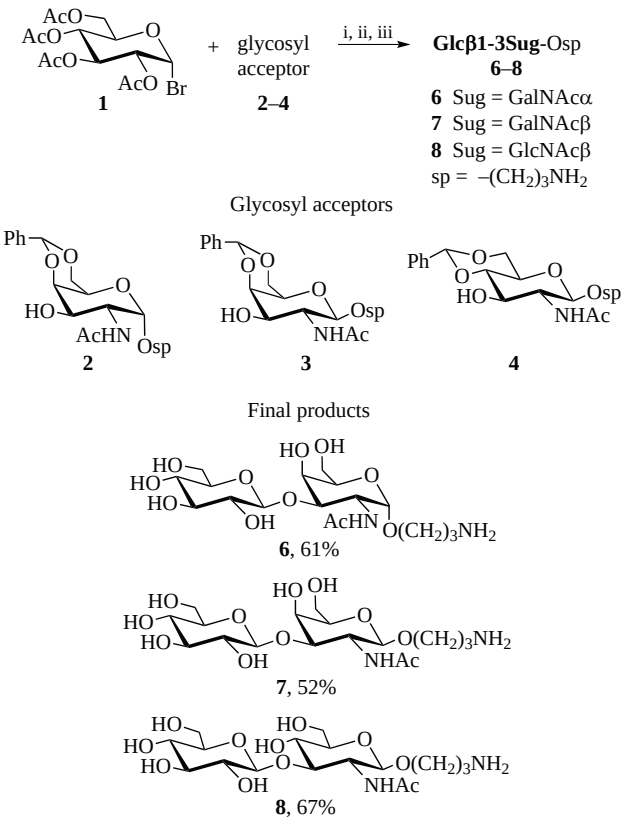
Disaccharides with the terminal Glcβ1-3 motif were synthesized as probes for studying human blood antibodies. An antibody isolated using Glcβ1-3GalNAcα–Sepharose was found to bind the inner part of the polysaccharide [–4GlcA6LThr3Acβ1-6Galβ1-6*Glcβ1-3GalNAc6Acβ*1–]$_n$ as evidenced by the use of a printed glycan array and inhibition assays.



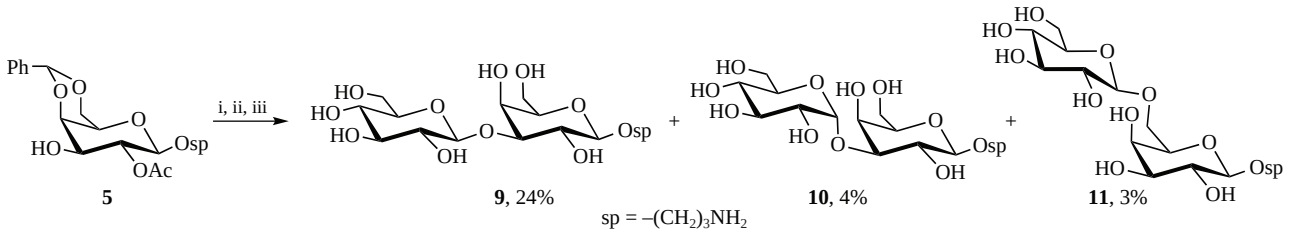
Keywords: glycan synthesis, natural antibodies, printed glycan array, bacterial polysaccharides, epitope.

The Glcβ1-3Sug[1] oligosaccharide fragment is not found in human glycoproteins or glycolipids but occurs in bacterial polysaccharides, particularly, of *Escherichia coli*.[2] A structural motif with a galactose replacing a glucose residue, Galβ1-3GalNAcα (known as TF-antigen[3]), is often found in glycoproteins, and Galβ1-3GalNAcβ (known as $T_{ββ}$ antigen[4]) in human gangliosides. Natural (preexisting) antibodies against both TF and $T_{ββ}$ antigens are considered as tumor-associated,[5] and are formed as a result of B1 lymphocyte priming[6] with bacterial polysaccharides of the gut microbiota. We supposed that natural antibodies that bind to Galβ1-3GalNAc epitope of glycoproteins or gangliosides were generated by the immune system against an isomeric Glcβ1-3GalNAc of bacterial polysaccharides. To test this hypothesis, synthesis of disaccharides Glcβ1-3Sug-O$(CH_2)_3NH_2$ (Sug = GalNAcβ, GalNAcα, GlcNAcβ, Galβ) was performed and the interaction of these disaccharides and bacterial polysaccharides with antibodies of human peripheral blood has been studied.

All compounds were obtained as 3-aminopropyl glycosides (Schemes 1 and 2) suitable for printing onto the printed glycan array (PGA). Glycosylation of glycosyl acceptors **2**,[7] **3**,[8] **4**,[9] **5**[10] containing a hydroxy group at C-3 with bromide **1**, obtained from the corresponding peracetate,[11] was performed in the presence of silver triflate and *N*,*N*,*N*′,*N*′-tetramethylurea (TMU) in dry dichloromethane at room temperature with a two-fold excess of the donor related to the acceptor, according to the general procedure.[12] The reaction products were isolated by silica gel chromatography following debenzylidenation and acetylation. Glycosylation of acceptor **5** gave an ortho ester, whose decomposition on heating in acetic acid afforded a mixture of products separated as peracetates by chromatography



Scheme 1 *Reagents and conditions*: i, AgOTf, TMU, CH_2Cl_2, MS-4 Å, room temperature, 20 h; ii, 80% aq. AcOH, 70 °C, 2 h, then Ac_2O/Py; iii, 0.1 M MeONa/MeOH, 30 min, then 0.1 M aq. NaOH, 16 h.



Scheme 2 *Reagents and conditions*: i, bromide **1**, AgOTf, TMU, CH_2Cl_2, MS-4 Å, room temperature, 20 h; ii, 80% aq. AcOH, 70 °C, 2 h, then Ac_2O/Py; iii, 0.1 M MeONa/MeOH, 30 min, then 0.1 M aq. NaOH, 16 h.

on silica gel. Deacetylation and removal of the *N*-trifluoroacetyl group followed by purification on Dowex H^+ (elution with 1 M NH_4OH) resulted in 3-aminopropyl glycosides **6**–**8** in 40–100 mg (52–67%) overall yields (see Scheme 1). Nothing of α-anomers was formed in these experiments. In case of acceptor **5**, 47 mg (24%) of the target disaccharide **9** was obtained along with 8 mg (4%) of an α-anomer **10** and 6 mg (3%) of an 1→6 isomer **11** (see Scheme 2). The yields of the target disaccharides were calculated over all reaction steps and purification on the glycosyl acceptor (recovery of acceptor **2**–**4** 10–20% and **5** ~60% in the form of peracetates was not taken into account). The structures of the target disaccharides were confirmed by electrospray ionization mass spectrometry as well as ^{13}C and ^1H NMR spectroscopy, in particular by $J_{1,2}$ 3.7–4.0 Hz for H-1 in the α-configuration and $J_{1,2}$ 7.9–8.5 Hz for H-1 in the β-configuration. The spectral data are given in Online Supplementary Materials.

The synthesized disaccharides were printed onto activated slides together with other ~400 oligosaccharides and ~200 bacterial polysaccharides. A therapeutic immunoglobulin preparation containing IgG, IgM, and IgA from 1000+ healthy donors (commercial name CIP, complex immunoglobulin preparation[13]) was applied to the glycoarray (in the form of a microchip); the results are presented in Figure 1. The disaccharides demonstrated a modest-to-high level of binding with antibodies, the highest level (comparable with that of the Fs disaccharide known to be one of the highest binders[14]) being observed for **6** and **7**. Similar high binding values of disaccharides **6** and **7** (IgM) were observed when serum of individual healthy donors was probed (data not shown).

Thus, blood antibodies interact with the Glcβ1-3GalNAc fragment as such, without a polysaccharide 'context', while the aim of the study was to check the possibility of its recognition in the composition of a polysaccharide. Since CIP as well as blood serum contains a big variety of antibodies that can potentially distort the result of the interaction with the polysaccharide, a narrow fraction of antibodies was isolated from the CIP using Glcβ1-3GalNAcα–Sepharose 6FF. According to glycoarray data, these antibodies bound both disaccharides **6** and **7**, and those polysaccharides which include a Glcβ1-3GalNAcβ

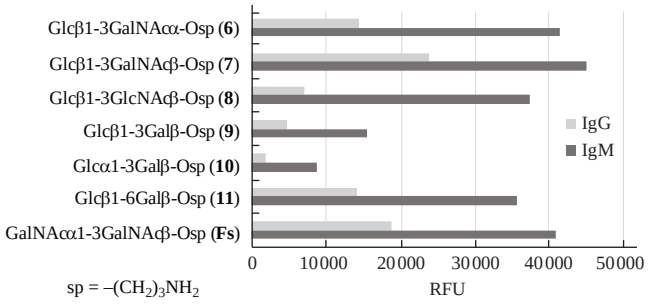


Figure 1 Binding of human antibodies (in composition of CIP) to the Glcβ1-3Sug disaccharides. Data of PGA analysis, the measured RFU (relative fluorescence units) values are proportional to binding intensity (scale maximum of this assay is ~65 000).

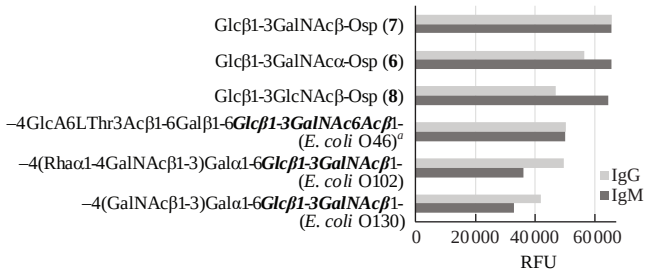


Figure 2 PGA analysis of the antibodies (IgG : IgM : IgA = 3 : 6 : 1) isolated using a Glcβ1-3GalNAcα–Sepharose 6FF adsorbent. Selected data are shown, for the array's highly binding ligands only. [a]The repeating unit of *E. coli* O46 has an *O*-acetyl substitution (~70% on Thr and ~15% on GalNAc).[2]

fragment (*E. coli* O46, O102, and O130) (Figure 2), but did not bind polysaccharides containing a Glcβ1-3GalNAcα fragment (*E. coli* O157, and O57) or a Glcβ1-3GlcNAc fragment (*P. penneri* 113, *P. mirabilis* O33, and *S. enterica* O60).

To confirm the binding to the inner region of the polysaccharide chain, an inhibitory ELISA was performed (see Online Supplementary Materials), according to which the $I_{50\%}$ value for free disaccharides **6** and **7** was determined as 200 and 50 μM, respectively. Assuming the molecular mass of the polysaccharide from *E. coli* O46 as 30 kDa (it is not known exactly), the found weight value of $I_{50\%}$ turns out to be equivalent to ~40 μM, which is similar. Immune surveillance of bacterial infection largely (and often decisively) targets lipopolysaccharides, predominantly at their spatially accessible terminal sites.[15] However, the data presented here indicate the possibility that human blood antibodies are capable of binding with good affinity to the internal, formally hidden sites of the polysaccharide chain. The antibodies studied here (a significant part of which are IgMs) are most likely natural (pre-existing) antibodies, that is, they perform a supervisory function over the emergence of infectious bacteria, so in the future it is necessary to find out, whether they are able to bind and block whole bacteria, where the internal part of polysaccharide chains is less accessible to interactions.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.06.011.

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