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*Mendeleev Commun.*, 2023, **33**, 107–108

Mendeleev Communications

# Synthesis of Sug1-4GalNAcα disaccharides and their interaction with human blood antibodies

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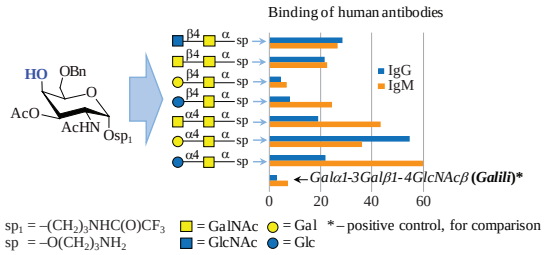
DOI: 10.1016/j.mencom.2023.01.033

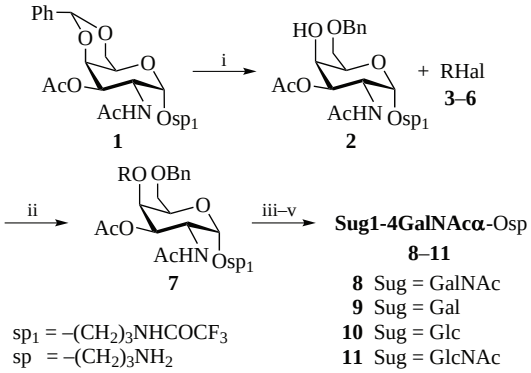
**Disaccharides Sug1-4GalNAcα-Osp (Sug = Gal, Glc, GalNAc, GlcNAc in α- or β-configuration) were synthesized as spacer (sp) glycosides. Their strong interaction with serum antibodies of healthy donors was demonstrated using a printed glycan array (PGA).**




*Keywords*: glycan synthesis, glycosylation, antigen, anti-glycan antibodies, printed glycan array (PGA).

The Sug(α or β)1-4GalNAcα fragment is not characteristic of human glycoproteins but is found in bacterial polysaccharides from *Shigella*,[1] *Escherichia*,[2,3] *Salmonella*[4] and *Proteus*,[5] as well as pathogenic fungi *Aspergillus*.[6–8] Our previous studies have shown unusually high titers of human peripheral blood antibodies to one of the glycans containing such a fragment, which are comparable to the most representative level of any anti-glycan natural antibodies[9] and leave no doubt of their importance for the functioning of innate immunity. In this work, disaccharides Sug1-4GalNAcα-O$(CH_2)_3NH_2$ (Sug = Gal, Glc, GalNAc, GlcNAc in α- or β-configuration) were synthesized and their interaction with antibodies of healthy donors was studied.

All compounds were obtained as 3-aminopropyl glycosides suitable for construction of the Printed Glycan Array (PGA).[10]




**Scheme 1** *Reagents and conditions*: i, $MeSO_3H$, $NaBH_3CN$, THF, MS 4 Å; ii, AgOTf, TMU, $CH_2Cl_2$, MS 4 Å, room temperature, 20 h; iii, $Bu_4NF$, THF, 3 h, then $Ac_2O$/Py (NHTroc → NHAc, in case of donor **6**); iv, $H_2$, 10% Pd/C, then $Ac_2O$/Py; v, 0.1 M MeONa/MeOH, 30 min; then 0.1 M aq. NaOH, 16 h. RHal = glycosyl donor, see Table 1.

The glycosyl acceptor **2** in all syntheses was α-galactosamine containing a hydroxyl group at C-4; it was obtained by reductive opening of the benzylidene ring[11] of monosaccharide **1**[12] in 82% yield (Scheme 1). Glycosylation of **2** with chloride **3**[13] or bromides **4**–**6**[14–16] (Table 1) was performed in the presence of silver triflate and *N*,*N*,*N*′,*N*′-tetramethylurea (TMU) in dry dichloromethane at room temperature with two-fold excess of the donor related to the acceptor according to the general procedure.[16] As a result, disaccharides **7** were isolated by silica gel chromatography in 60–90% yield. The peracetylated oligosaccharides were obtained after routine methods of deprotection (hydrogenolysis, replacement of TrocNH by AcNH-group[17] in case of donor **6**), acetylation, and purification using preparative reversed-phase HPLC (C18, MeCN–$H_2O$). Deacetylation, removal of *N*-trifluoroacetyl group followed by purification on Dowex $H^+$ (elution with 1 M aq. $NH_3$) resulted in 3-aminopropyl glycosides (**8α**, **9α**, **10β** and **11**) in 40–100 mg amounts and minor isomers (**8β**, **9β** and **10α**) in 1–10 mg amounts. Yields (cumulatively for all deblocking steps plus purification) ranged from 50 to 66%. Their structures were confirmed by mass spectrometry, $^{13}C$ and $^1H$ NMR spectroscopy, in particular $J_{1,2}$ 3.7–4.0 Hz for H-1 in α-configuration and $J_{1,2}$ 7.9–8.5 Hz for H-1 in β-configuration (see Online Supplementary Materials).

The synthesized disaccharides were printed onto activated slides together with other ~400 oligosaccharides and ~200 bacterial polysaccharides.[10] A therapeutic immunoglobulin preparation containing purified IgG, IgM, and IgA from 1000+ healthy donors (commercial name CIP, Complex Immunoglobulin Preparation) was applied to the array (in the form of a microchip[10]); the results are given in Table 2. For comparison, the values of binding of antibodies to two glycans, the level of

**Table 1** Glycosylation of acceptor **2**.

| Glycosyl donor (RHal) | Yield[a] of **7** (α+β) (%) | α/β[b] | Final product | Yield[c] of **8**–**11** (%) |
|---|---|---|---|---|
| $Ac_3GalN_3\beta Cl$ **3** | 60 | 10 : 1 | GalNAcα1-4GalNAcα-O$(CH_2)_3NH_2$ **8α** | 66 |
| | | | GalNAcβ1-4GalNAcα-O$(CH_2)_3NH_2$ **8β** | 7 |
| $Bn_4Gal\alpha Br$ **4** | 90 | 7 : 1 | Galα1-4GalNAcα-O$(CH_2)_3NH_2$ **9α** | 61 |
| | | | Galβ1-4GalNAcα-O$(CH_2)_3NH_2$ **9β** | 9 |
| $Ac_4Glc\alpha Br$ **5** | 70 | 47 : 1 | Glcβ1-4GalNAcα-O$(CH_2)_3NH_2$ **10β** | 52 |
| | | | Glcα1-4GalNAcα-O$(CH_2)_3NH_2$ **10α** | 1 |
| $Ac_3GlcNHTroc\alpha Br$ **6** | 65[d] | – | GlcNAcβ1-4GalNAcα-O$(CH_2)_3NH_2$ **11** | 56 |

[a]Yields of the target disaccharides are calculated on the quantity of glycosyl acceptor (return of acceptor 10–20% was not accounted). [b] α/β ratios in the isolated product by preparative reversed-phase HPLC. [c]Total deblocking and purification yield. [d]α-Anomer was not identified.

**Table 2** Binding of human antibodies (in composition of CIP) to Sug1-4GalNAcα-Osp disaccharides. Data of PGA analysis, the measured RFU (relative fluorescence units) values are proportional to binding intensity (scale maximum of this assay ~65,000).

| Compound | Oligosaccharide | IgG | IgM |
|---|---|---|---|
| **11** | GlcNAcβ1-4GalNAcα-Osp | 28,590 | 26,462 |
| **8β** | GalNAcβ1-4GalNAcα-Osp | 21,518 | 22,533 |
| **9β** | Galβ1-4GalNAcα-Osp | 4,334 | 6,721 |
| **10β** | Glcβ1-4GalNAcα-Osp | 8,067 | 24,417 |
| **8α** | GalNAcα1-4GalNAcα-Osp | 18,875 | 43,413 |
| **9α** | Galα1-4GalNAcα-Osp | **54,593** | 36,147 |
| **10α** | Glcα1-4GalNAcα-Osp | 21,803 | **60,444** |
| | GalNAcα1-3GalNAcβ-Osp (Fs) | 32,734 | 45,126 |
| | Galα1-3Galβ1-4GlcNAcβ-Osp (Galili) | 2,526 | 6,970 |

which in donors (in the CIP as well) is known to be high,[9] are given, namely GalNAcα1-3GalNAcβ (disaccharide termination of Fs glycolipid) and Galα1-3Galα1-4GlcNAcβ (Galili trisaccharide).

Synthesized disaccharides (except for **9β** and **10β**) exhibited high levels of IgG and IgM immunoglobulin binding comparable to Fs, which is known to be one of the highest in humans; moreover, in the case of **9α** (IgG) and **10α** (IgM) even higher. At the same time, level of binding with disaccharides (except for **9β**) is much higher than that of the trisaccharide Galili.

High values of binding of human antibodies to the disaccharides were observed in individual healthy donors as well (data not shown). Based only on the obtained data on the high titer antibodies, it is rather difficult to figure out the structure of the glycan that they are intended to recognize in reality. Therefore, we also attracted information from the literature references of the database[18] (Carbohydrate Structure Database including data on the structure of more than 30,000 polysaccharides from about 15,000 organisms), and as a result of their consideration with our data, we put forward an assumption about those pathogens (and their particular glycoconjugates) against which the identified antibodies are directed:

GalNAcβ1-4GalNAcα (**8β**) and GlcNAcβ1-4GalNAcα (**11**) are fragments of a capsular polysaccharide found in *Haemaphilus influenzae f*;

disaccharides GalNAcα1-4GalNAcα (**8α**) and Galα1-4GalNAcα (**9α**) are fragments of the exopolysaccharide galactosamingalactan of the fungal pathogen *Aspergillus fumigatus*;

disaccharide Glcα1-4GalNAcα (**10α**) is a fragment of a polysaccharide found in *Pseudomonas aeruginosa*.

This work was supported by Russian Science Foundation (grant no. 22-24-00398). NMR spectra were recorded using equipment provided by Moscow Core facility of the M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science.

*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.01.033.

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*Received: 22nd August 2022; Com. 22/6986*