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Synthesis of blood group pentasaccharides ALe^y, BLe^y and related tri- and tetrasaccharides

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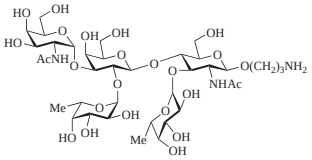
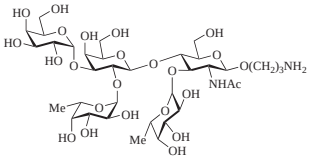
Pentasaccharides GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)-GlcNAc (ALe^y) and Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)-GlcNAc (BLe^y), as well as forms GalNAcα1-3Galβ1-4(Fucα1-3)GlcNAc and GalNAcα1-3Galβ1-4GlcNAc were synthesized as molecular tools for studying natural antibodies directed against blood group A and B antigens.



Blood group related glycan ALe^y



Blood group related glycan BLe^y

The antigenic determinants of blood group ABH system[1] proved to be terminal tetrasaccharide motifs GalNAcα1-3(Fucα1-2)-Galβ1-4GlcNAc- (A) and Galα1-3(Fucα1-2)Galβ1-4GlcNAc- (B), which determine agglutination of erythrocytes by human alloantibodies.[2] In addition to these motifs, red blood cells possess a number of related structures, particularly, the corresponding pentasaccharides with an 'additional' fucose moiety at O^3 of glucosamine fragment. The role of these pentasaccharides for transfusion and transplantation remains unclear.

Incomplete blood group B related structures Galα1-3Galβ1-4GlcNAc- ('Galili') and Galα1-3Galβ1-4(Fucα1-3)GlcNAc- are well known as xenoantigens playing important roles in the innate antiviral immunity. Corresponding blood group A related tri- and tetrasaccharides, GalNAcα1-3Galβ1-4GlcNAc- and GalNAcα1-3Galβ1-4(Fucα1-3)GlcNAc-, were not identified in normal human glycoproteins and glycollipids until now. This work was aimed at obtaining molecular tools for investigation of natural antibodies to the mentioned above pentasaccharides A and B, as well as GalNAcα1-3Galβ1-4(Fucα1-3)GlcNAc and GalNAcα1-3Galβ1-4GlcNAc glycans, in functioning of the human innate humoral immunity. Thus, compounds **1**–**6** were herein synthesized. Donors **7**–**9** were used in the syntheses.

GalNAcα1-3Galβ1-4GlcNAcβ-OR
1

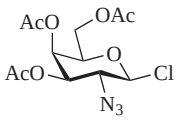
GalNAcα1-4Galβ1-4GlcNAcβ-OR
2

GalNAcα1-3Galβ1-4(Fucα1-3)GlcNAcβ-OR
αGalNAcLe^x
3

Galα1-3Galβ1-4(Fucα1-3)GlcNAcβ-OR
αGalLe^x
4

GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-OR
ALe^y
5

Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-OR
BLe^y
R = $(CH_2)_3NH_2$ **6**



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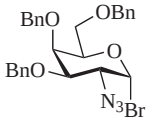

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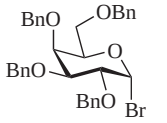
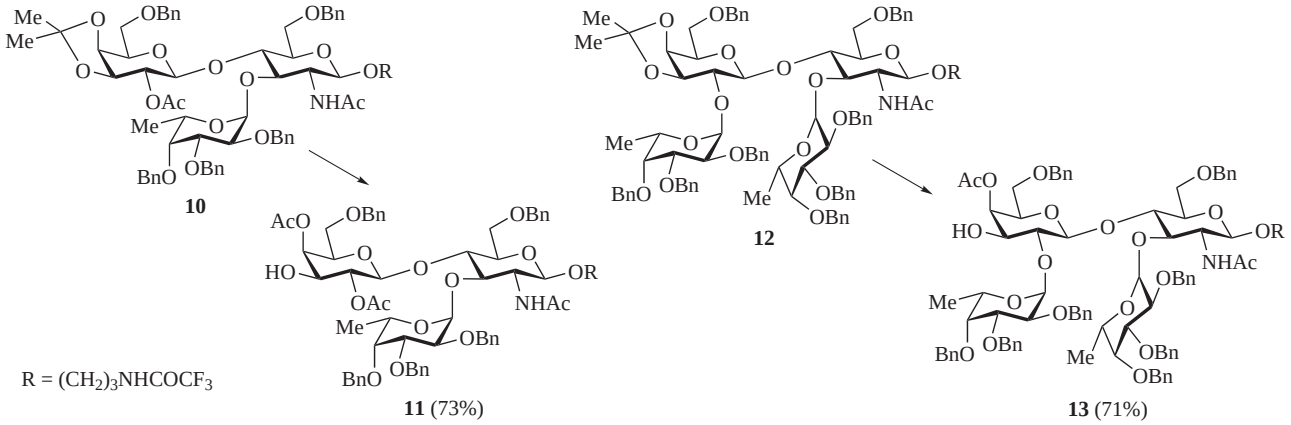


9

In our previous publication[3] we obtained A and B (type 2) pentasaccharides and 'Galili' trisaccharides and demonstrated that glycosylation of 3′,4′-diols of lactosamine and its fucosylated



R = $(CH_2)_3NHCOCF_3$

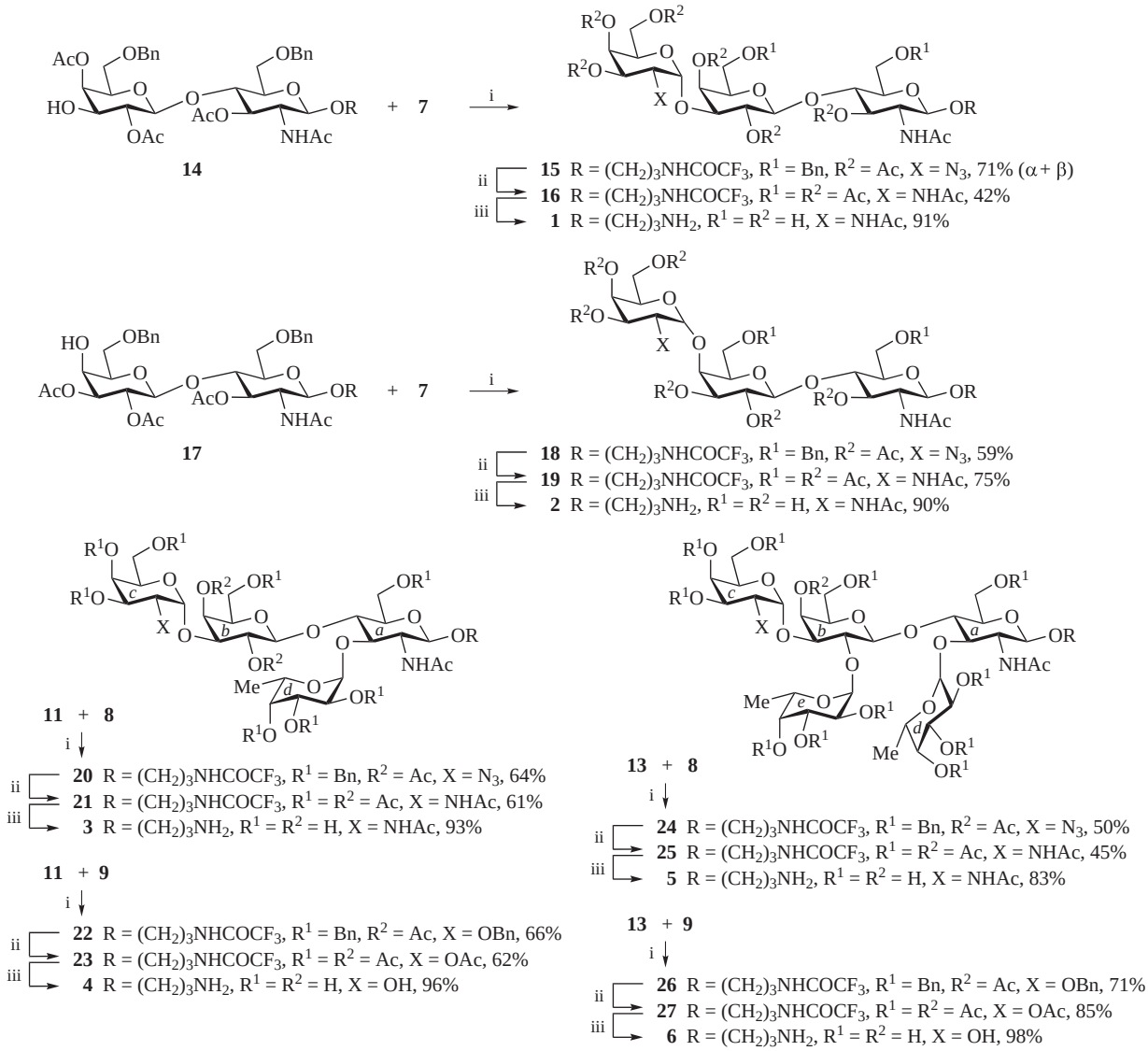
10

11 (73%)

12

13 (71%)

Scheme 1 *Reagents and conditions*: 3% TFA in $CHCl_3$, 3 h; $MeC(OEt)_3$, TsOH, MeCN, 1 h; 80% aq. AcOH, room temperature, 1 h.



Scheme 2 *Reagents and conditions*: i, AgOTf, TMM, CH_2Cl_2, 20 h; ii, 10% Pd/C, H_2, MeOH, then Ac_2O–Py 1:2, 16 h; iii, 0.05 M MeONa/MeOH, 1 h, then 0.05 M aq. NaOH, 16 h.

derivative proceeds non-selectively, this making more efficient the use of acceptors with one hydroxyl group at C^3 of galactose fragment. Scheme 1 depicts transformation of isopropylidene derivatives **10** and **12** into acceptors **11** and **13**. Disaccharide acceptors **14** and **17** (see Scheme 2) were prepared as described.[4]

Glycosylation (Scheme 2) was performed in dry dichloromethane in the presence of silver triflate, tetramethylurea and molecular sieves 4 Å at room temperature for 20 h and two-fold excess of donor (**7**[5] or **9**[6]) related to acceptor.[4] Donor **8**[7] was taken in three-fold excess related to the acceptors **11** and **13**, where the hydroxyl group at C^3 of the galactose fragment is spatially shielded with tribenzylfucose moiety. Even with this excess of donor about 40% of the acceptor remained non-glycosylated, whereas in other cases this value was less than 20%.

Lactosamine derivative **14** with one hydroxyl group at C^3 of galactose moiety was glycosylated with azido chloride of galactose **7**; product **15** was isolated chromatographically on silica gel as mixture of anomers (α/β = 3.0 determined by ^{1}H NMR spectroscopy). After the conversion of the azido group to NHAc, anomers **16** and **16β** displayed different chromatographic mobility allowing their separation and characterization to be performed.

Another lactosamine derivative with one hydroxyl group at C^4 of galactose moiety (**17**) was glycosylated with azido chloride of galactose **7** giving rise to trisaccharide **18** with an α1-4 linkage. A possible side product (β1-4) either was not observed.

Acceptors **11** and **13** were glycosylated with benzylated galactosyl bromide **9** to get the products of α-galactosylation (**22** and **26**) and with donor **8** for insertion of α-galactosamine fragment (synthesis of compounds **20** and **24**). Formation of the β-anomer was significant only in case of pentasacccharide **24** (**24β** was isolated from reaction mixture and characterized, α/β = 3.3), in other cases side β-anomers were not identified.

After hydrogenolysis, acetylation and chromatographic purification oligosaccharides protected as peracetates **16**, **16β**, **19**, **21**, **23**, **25**, **25β**, and **27** were characterized by high resolution ^{1}H NMR spectra. The complete assignment using COSY experiment proved their structure, in particular $J_{1,2}$ ~ 3.5 Hz for H-1c in α-configuration and $J_{1,2}$ ~ 8 Hz for H-1c in β-configuration in compounds **16β** and **25β**, upfield shift (δ 3.7–3.9 ppm) of proton H-3b at glycosidic bond compared to peracetate **19** acetylated at this position and glycosylated at C-4b (δ 4.923 ppm for H-3b and δ 4.095 ppm for H-4b).

Deacetylation of the peracetates and removal of the *N*-trifluoroacetyl groups followed by purification and isolation on Dowex H^+ (elution with 1 M aqueous NH_3) gave 3-aminopropylglycosides **1**–**6** (in amounts 20–100 mg); their structure was confirmed by mass spectrometry and ^{1}H NMR spectroscopy.

Pentasaccharides **5** and **6** were converted into lipophilic form (E. Yu. Korchagina, unpublished data) optimal for engineering of the so called KODEcytes, *i.e.* erythrocytes possessing addi-

tional alloantigens, particularly ALe^y and BLe^y antigens. This allowed us to characterize a number of monoclonal antibodies and polyclonal sera for their fine epitope specificity.[8] Surprisingly, trisaccharides **1** and **2** in composition of printed glycan array[9] revealed much higher titers of natural antibodies than the corresponding B-related glycans Galα1-3Galβ1-4GlcNAc- ('Galili') and Galα1-4Galβ1-4GlcNAc- (P_1) (N. R. Khasbiullina, unpublished data).

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Online Supplementary Materials

Supplementary data associated with this article (^{1}H NMR and MALDI-TOF mass spectra of the key synthesized compounds) can be found in the online version at doi:10.1016/j.mencom.2016.03.005.

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