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Synthesis of blood group Forssman pentasaccharide GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ–R

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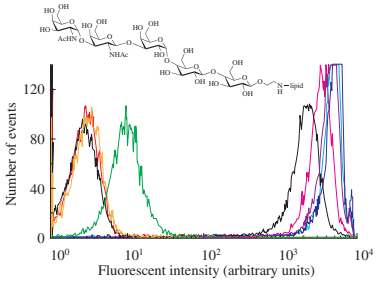
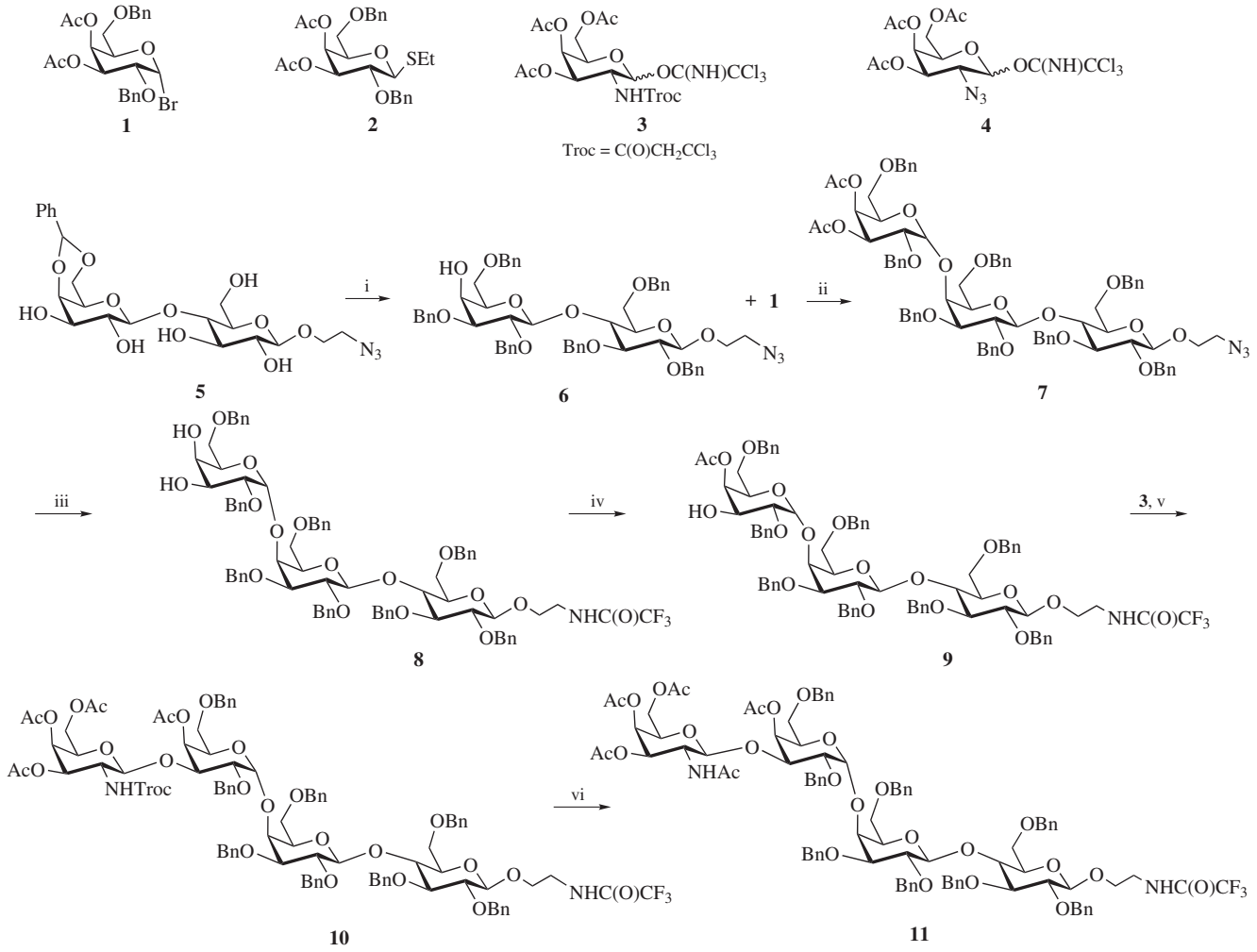
Synthesis of the glycan part of Forssman glycolipid GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc–Cer in the form of 2-aminoethyl glycoside has been carried out. The glycoside has been converted into a lipophilic derivative capable of controlled inserting into erythrocytes. The obtained surface-modified cells, termed kodecytes, revealed a high level of the blood group system FORS serological activity.

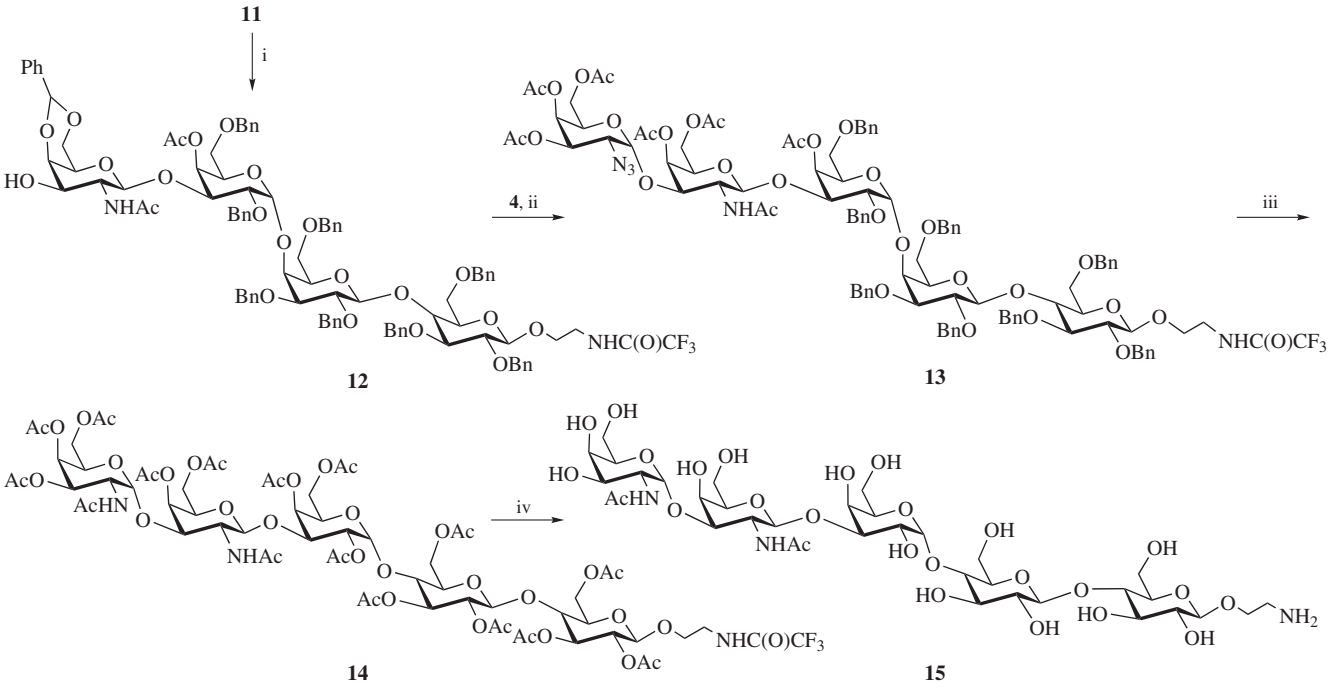


Forssman glycolipid GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glc–Cer defines the rare human blood group system designated as FORS or no. 31[1] and based on the presence or absence of Forssman antigen (Fs) on the surface of an erythrocyte membrane, resulting in FORS1+ and FORS1– phenotypes. Until recently, Fs has been considered as a heterophile- or xeno-antigen, because it is a known component of the sheep erythrocyte membrane, and humans have anti-Fs antibodies. In humans, Fs antigen is occasionally identified in malignant carcinomas.[2] By now, only three unrelated families with the FORS1+ phenotype have been recognized, and blood samples from only two unrelated FORS1+ individuals have been explored at the molecular level.[1] To investigate further natural antibodies to Fs-related glycans, here we report on the synthesis and serological activity of Forssman pentasaccharide (Fs-5) antigen, using our data on the binding of anti-Forssman antibodies.[3]

The synthesis of Fs-5 as well as its terminal shorter fragments was reported,[4] however this strategy was not appropriate here due to the additional need in core (inner) fragments of Fs-5, namely tri- and tetrasaccharides of the globo series (Gb3 and Gb4), as well as the necessity for oligosaccharides to bear ω-amino spacers for the planned further biological application. For this reason we chose a synthetic strategy based on a one-by-one elongation (Scheme 1) of the carbohydrate chain, starting from spacer-armed lactose derivative **6**, which had been prepared from 2-azidoethyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside **5**[5] by benzylation and subsequent reductive ring opening of the resulting benzylidene acetal.[6] Introduction of an α-D-galactopyranosyl moiety was achieved using α-galactosyl bromide **1**, derived from compound **2** similarly to the method reported,[7] as a glycosyl donor. Glycosylation of lactoside **6** with bromide **1** in dichloromethane in the presence of AgOTf stereoselectively gave the desired trisaccharide **7** in 93% yield. The configuration of the formed glycosidic bond was confirmed using ^{1}H NMR spectroscopy. The anomeric proton of the α-galactopyranoside moiety appeared at 5.15 ppm as a doublet with a coupling constant of 3.4 Hz. Further protecting group manipulations, namely the Zemplen deacetylation, reduction of azide moiety, N-trifluoroacetylation and ortho esterification with subsequent selective orthoester rearrangement,[8] allowed us to obtain glycosyl acceptor **9** (see Scheme 1) in an appropriate yield. Next, the reaction of trisaccharide **9** with trichloroacetimidate **3**, bearing the *N*-(2,2,2-trichloroethoxy)carbonyl (Troc) moiety,[9] using TMSOTf as a promoter under standard glycosylation conditions[7] afforded tetrasaccharide **10** in 71% yield. In its ^{1}H NMR spectrum, the anomeric proton of the 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranoside moiety appeared at 4.06 ppm as a doublet with a coupling constant of 8.3 Hz. Tetrasaccharide **10** was treated with tetra-*n*-butylammonium fluoride (TBAF) to cleave the *N*-Troc group,[10] and the resulting mixture was N-acetylated to give tetrasaccharide **11**. The subsequent Zemplen deacetylation of compound **11** (Scheme 2) and introduction of benzylidene group resulted in glycosyl acceptor **12**. The presence of the intact AcO group in Gal^{IV} after the Zemplen deacetylation was confirmed by the downfield shift of H-4^{IV} in the ^{1}H NMR spectrum of compound **12**. Its subsequent glycosylation with trichloroacetimidate **4** in diethyl ether using TMSOTf gave a multicomponent mixture of products. The desired pentasaccharide **13** was isolated after debenzylidenation and acetylation in 21% yield for the three steps. The anomeric proton of the 2-azido-2-deoxy-α-D-galactopyranoside moiety appeared in its ^{1}H NMR spectrum at 5.26 ppm as a doublet with a coupling constant of 3.5 Hz. The reduction of azido group in compound **13** using DTT in aqueous DMF followed by N-acetylation, the Zemplen deacetylation, hydrogenolysis over 10% Pd/C and subsequent acetylation resulted in peracetylated pentasaccharide **14** in 45% yield for the four steps. The structure of compound **14** was confirmed by ^{1}H–^{1}H COSY NMR spectroscopy. Complete deacetylation and removal of trifluoroacetyl group in compound



Scheme 1 *Reagents and conditions*: i, NaH, BnBr, DMF, room temperature, 12 h, then $NaBH_3CN$, $MeSO_3H$, MS 4 Å, THF, room temperature, 1 h; ii, AgOTf, TMU, MS 4 Å, CH_2Cl_2, room temperature, overnight; iii, MeONa, MeOH, room temperature, 2 h, then DTT, Et_3N, DMF–H_2O (4 : 1), room temperature, 2 h, then CF_3COOMe, Et_3N, MeOH, room temperature, 3 h; iv, $MeC(OEt)_3$, TsOH, CH_2Cl_2, room temperature, 2 h, then 80% aq. AcOH, room temperature, 0.5 h; v, TMSOTf, MS 4 Å, CH_2Cl_2, room temperature; vi, $(n\text{-}Bu)_4NF \cdot H_2O$, THF, room temperature, 3 h, then Ac_2O, Et_3N, room temperature, 2 h.



Scheme 2 *Reagents and conditions*: i, MeONa, MeOH, room temperature, 2 h, then $PhCH(OMe)_2$, TsOH, room temperature, 4 h; ii, TMSOTf, MS 4 Å, Et_2O, 4 °C, then 80% aq. AcOH, 80 °C, 2 h, then Ac_2O, pyridine, room temperature, 15 h; iii, DTT, Et_3N, DMF–H_2O (4 : 1), room temperature, 1 h, then Ac_2O, Et_3N, MeOH, room temperature, then MeONa, MeOH, room temperature, 2 h, then 10% Pd/C, H_2, MeOH, room temperature, 3 h, then Ac_2O, pyridine, room temperature, 12 h; iv, MeONa, MeOH, room temperature, 2 h, then aq. NaOH, room temperature, 2 h, then Dowex H^+, 5% aq. NH_3.

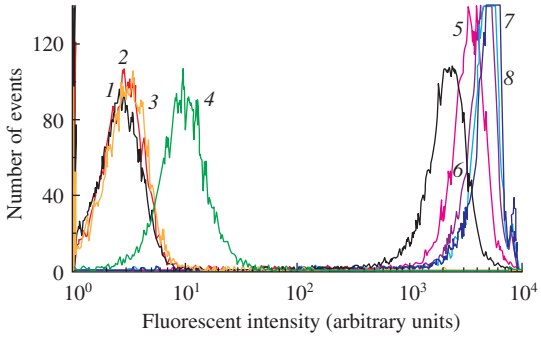


Figure 1 Flow cytometry results for the interaction of monoclonal anti-Forssman M1/22.25.8HL antibodies with: (*1*), (*2*) and (*3*) native erythrocytes of regular blood groups A, B and O as FORS1– negative controls; (*4*) native erythrocytes of FORS1+ donor with low content of Fs-5 glycolipid; (*5*), (*6*), (*7*) and (*8*) kodecytes with increasing from 3 to 25 μg load of the synthesized glycolipid into erythrocytes of regular blood group O.

14 by aqueous alkali followed by cation-exchange chromatography afforded the target Fs-5 as its 2-aminoethyl glycoside **15** in 90% yield.

Pentasaccharide Fs-5 was then converted to a lipophilic derivative, namely a synthetic glycolipid construct of function–spacer–lipid type using dioleoylphosphatidylethanolamine as a lipid part,[11] and then inserted into FORS1– erythrocytes to form glycan-modified surface. Reactivity of the obtained FORS1+ cells, termed kodecytes,[11] towards monoclonal anti-Forssman M1/22.25.8HL antibodies was compared with FORS1– cells and with erythrocytes of rare FORS1+ donor using flow cytometry (Figure 1). The high intensity of binding to all versions of Fs kodecytes, weak positive interaction with FORS1+ donor's erythrocytes and the lack of binding with negative controls, namely donor cells of regular blood groups A, B and O, clearly revealed the insertion of the synthesized construct into cell membrane as well as the dose-dependent specific mode of the interaction.

In summary, we have synthesized Forssman pentasaccharide in a form suitable for its bioconjugation, bound it with dioleoylphosphatidylethanolamine, inserted the conjugate into erythrocytes and demonstrated the specific interaction of the obtained kodecytes with monoclonal anti-Forssman antibodies.

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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.2019.09.034.

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