Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2018, **28**, 421–422

Mendeleev Communications

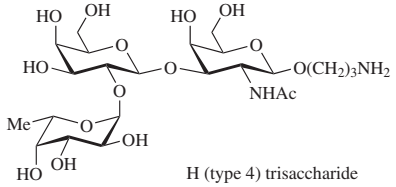
Synthesis of H (type 4) trisaccharide, key structural fragment of globo-H and fucosyl-GM1 cancer-associated antigens

Galina V. Pazynina, Svetlana V. Tsygankova, Ivan M. Ryzhov, Alexander S. Paramonov and Nicolai V. Bovin*

M. M. Shemyakin–Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russian Federation. Fax: +7 495 330 5592; e-mail: professorbovin@yandex.ru

DOI: 10.1016/j.mencom.2018.07.027

A convenient synthesis of Fucα1-2Galβ1-3GalNAcβ-OCH$_2$-CH$_2$CH$_2$NH$_2$ (trisaccharide H type 4) is described. This glycan is the terminal part of glycosphingolipids globo-H and fucosyl-GM1 known as cancer-associated carbohydrate antigens.



H (type 4) trisaccharide

According to Landsteiner's blood group rules, all humans regardless of blood group do not have antibodies to H-antigen. The biosynthetic precursors of blood group A and B glycans are trisaccharide terminations Fucα1-2Galβ1-4GlcNAcβ known as H type 2, and Fucα1-2Galβ1-3GlcNAcβ known as H type 1. They are mandatory (with the exception of so-called Bombey phenotype) components of endothelial, epithelial and red blood cells,[1] found in the composition of fairly long chains of glycoproteins and glycolipids. Type 3 form of H glycan, Fucα1-2Galβ1-3GalNAcα, is also a normal component of mucin-type glycoproteins, this trisaccharide is attached directly to Ser or Thr of polypeptide backbone. Type 4 glycan, Fucα1-2Galβ1-3GalNAcβ, is known only as the terminal part of glycosphingolipids; two of them, globo-H and fucosyl-GM1 (Figure 1), are cancer-associated carbohydrate antigens.[2]

Both globo-H and fucosyl-GM1 antigens have been the objects of attention in the development of antitumor vaccines and therapeutic monoclonal antibodies[2–6] over the past decade. The blood of healthy individuals contains a big variety of anti-glycan antibodies,[7] including natural (which are not the result of an immune response) antibodies against H type 4 trisaccharide and globo-H hexasaccharide.[8,9] The presence of natural antibodies against the motif of H trisaccharide makes it difficult to interpret the results when developing the therapeutic vaccines, as well as regarding theranostics strategies when the treatment is corrected in accordance with the permanent diagnostic monitoring. This means that the reagents will be in demand for the detection and affinity isolation of anti-H antibodies. Taking this into account, we propose a simple synthesis of H type 4 trisaccharide in the spacer-armed (with a primary NH_2 group convenient for bioconjugation) form.

Glycosylation[10] of the glycosyl acceptor **2** (Scheme 1)[11] bearing a hydroxyl group at C-3 with 6-*O*-benzyl galactosyl donor **1** in the presence of silver triflate,[12] tetramethylurea and molecular sieves 4 Å in dry dichloromethane at room temperature for 20 h with two-fold excess of the donor related to the acceptor led to disaccharide **3** (64%). The Zemplen de-O-acetylation and following acetonation gave 3′,4′-*O*-isopropylidene derivative **4** (42%) with one hydroxyl group at C-2 galactose unit.

Glycosylation of disaccharide **4** with benzylated L-fucose bromide (it was prepared from the corresponding thioglycoside[13] by treatment with bromine in anhydrous dichloromethane[10]) was carried out in a manner similar to the synthesis of disaccharide **3**, the yield of trisaccharide **5** was 72%. The isopropylidene and benzylidene groups were removed under acidic conditions (80% aq. AcOH, 70 °C, 2 h), subsequent hydrogenolysis followed by acetylation and chromatographic purification led to peracetate **6** (54%). In both glycosylations, the side anomers were not detected.

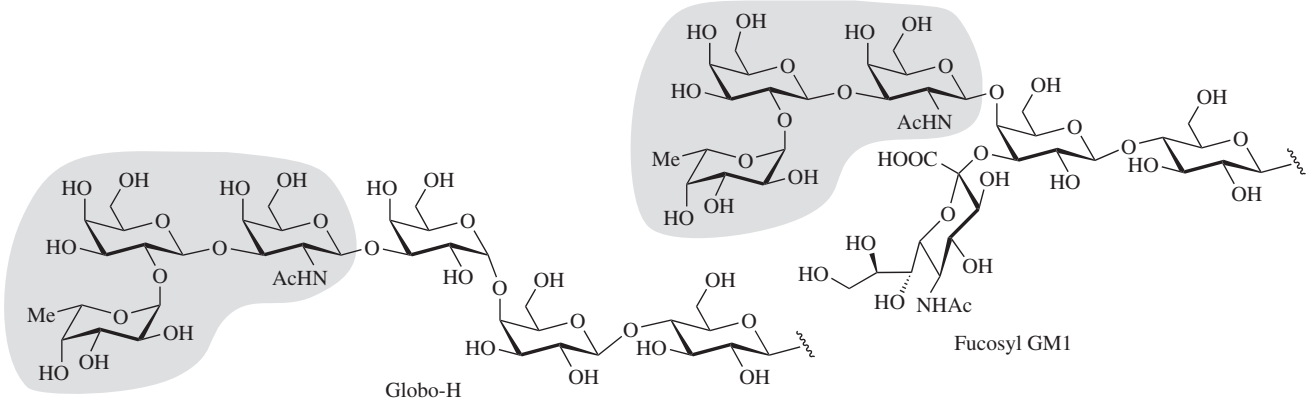
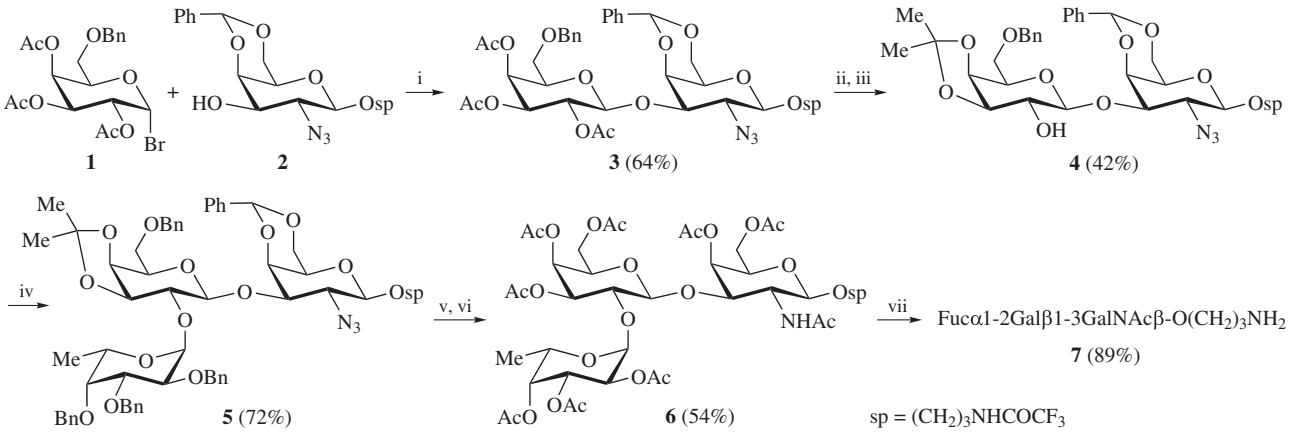


Figure 1 Trisaccharide motif Fucα1-2Galβ1-3GalNAcβ is shared by two cancer-associated glycosphingolipids, globo-H and fucosyl-GM1.



Scheme 1 *Reagents and conditions*: i, AgOTf, TMM, CH_2Cl_2, 16 h; ii, 0.05 M MeONa/MeOH, 1 h; iii, $Me_2C(OMe)_2$, TsOH; iv, 'Bn_3FucBr', AgOTf, TMM, CH_2Cl_2, 16 h; v, 80% aq. AcOH, 70 °C, 2 h; vi, H_2, Pd/C, MeOH, then Ac_2O/Py, 24 h; vii, 0.1 M MeONa/MeOH, 1 h, then 0.1 M aq. NaOH, 16 h.

Deacetylation of the peracetate **6** and removal of the *N*-trifluoroacetyl group followed by purification and isolation on Dowex H^+ resin (elution with 1 M aq. NH_3) gave fucosylated 3-aminopropyl glycoside **7** (89%). High-resolution ^{1}H NMR spectroscopy using COSY experiment proved the structure of peracetylated trisaccharide **6**, in particular, $J_{1,2}$ 8.3 Hz for H-1a, $J_{1,2}$ 7.7 Hz for H-1b in β-configuration and $J_{1,2}$ 3.5 Hz for H-1c in α-configuration; upfield shifts of proton H-3a (δ 4.91 ppm) and H-2b (δ 3.85 ppm) at glycosidic bonds. The structure of target compound **7** was confirmed by high-resolution ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.†

Trisaccharide **7** in composition of the 600-component glycoarray[14] allowed us to profile human antibodies[9] and characterize the specificity of galectins.[15]

This work was supported by the Russian Science Foundation (project no. 14-50-00131).

Experiments were partially carried out using the equipment provided by the Institute of Bioorganic Chemistry core facility (CKP IBCH, supported by the Russian Ministry of Education and Science, grant no. RFMEFI62117X0018).

References

1 S. Marionneau, A. Cailleau-Thomas, J. Rocher, B. Le Moullac-Vaidye, N. Ruvoën, M. Clément and J. Le Pendu, *Biochimie*, 2001, **83**, 565.
2 S. Hakomori, *Adv. Exp. Med. Biol*., 2001, **491**, 369.
3 S. J. Danishefsky, Y.-K. Shue, M. N. Chang and C.-H. Wong, *Acc. Chem. Res*., 2015, **48**, 643.
4 Z. Zhou, G. Liao, S. S. Mandal, S. Suryawanshi and Z. Guo, *Chem. Sci*., 2015, **6**, 7112.
5 L. M. Krug, G. Ragupathi, C. Hood, M. G. Kris, V. A. Miller, J. R. Allen, S. J. Keding, S. J. Danishefsky, J. Gomez, L. Tyson, B. Pizzo, V. Baez and P. O. Livingston, *Clin. Cancer Res*., 2004, **10**, 6094.
6 P. O. Livingston, C. Hood, L. M. Krug, N. Warren, M. G. Kris, T. Brezicka and G. Ragupathi, *Cancer Immunol. Immunother*., 2005, **54**, 1018.
7 M. E. Huflejt, M. Vuskovic, D. Vasiliu, H. Xu, P. Obukhova, N. Shilova, A. Tuzikov, O. Galanina, B. Arun, K. Lu and N. Bovin, *Mol. Immunol*., 2009, **46**, 3037.
8 T. Pochechueva, S. Alam, A. Schötzau, A. Chinarev, N. V. Bovin, N. F. Hacker, F. Jacob and V. Heinzelmann-Schwarz, *J. Ovarian Res*., 2017, **10**, 8.
9 N. Bovin, P. Obukhova, N. Shilova, E. Rapoport, I. Popova, M. Navakouski, C. Unverzagt, M. Vuskovic and M. Huflejt, *Biochim. Biophys. Acta*, 2012, **1820**, 1373.
10 G. V. Pazynina, V. V. Severov and N. V. Bovin, *Russ. J. Bioorg. Chem*., 2008, **34**, 625 (*Bioorg. Khim*., 2008, **34**, 696).
11 T. V. Ovchinnikova, A. G. Ter-Grigoryan, G. V. Pazynina and N. V. Bovin. *Russ. J. Bioorg. Chem*., 1997, **23**, 55 (*Bioorg. Khim*., 1997, **23**, 61).
12 S. Hanessian and J. Banoub, in *Methods of Carbohydrate Chemistry*, eds. R. L. Whistler and J. N. BeMiller, Academic Press, New York, 1980, vol. 8, pp. 247–250.
13 H. Lönn, *Carbohydr. Res*., 1985, **139**, 105.
14 G. Pazynina, M. Sablina, T. Ovchinnikova, T. Tyrtysh, S. Tsygankova, A. Tuzikov, K. Dobrochaeva, N. Shilova, N. Khasbiullina and N. Bovin, *Carbohydr. Res*., 2017, **445**, 23.
15 Y. A. Knirel, H.-J. Gabius, O. Blixt, E. M. Rapoport, N. R. Khasbiullina, N. V. Shilova and N. V. Bovin, *Glycoconjugate J*., 2014, **31**, 7.

Received: 20th December 2017; Com. 17/5439

† ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE spectrometer (Bruker BioSpin MRI GmbH) at 303 K. Chemical shifts for characteristic protons are given with the use of HOD (δ 4.750), $CHCl_3$ (δ 7.270) as reference. The signals in ^{1}H NMR spectra were assigned using a technique of spin–spin decoupling (double resonance) and 2D-^{1}H,^{1}H-COSY experiments. The values of optical rotation were measured on a Perkin Elmer 341 digital polarimeter at 25 °C. Mass spectra were recorded on a MALDI-TOF Vision-2000 spectrometer using dihydroxybenzoic acid as a matrix.

Compound **6**: ^{1}H NMR (700 MHz, $CDCl_3$) δ: 1.159 (d, 3H, H-6c, $J_{5,6}$ 6.5 Hz), 1.889–1.937 (m, 2H, CH_2 sp), 1.993 (2), 2.001, 2.040, 2.051, 2.068, 2.104, 2.156, 2.173 (9s, 9×3H, COMe), 3.217 (ddd, 1H, H-2a, $J_{1,2}$ 8.3 Hz, $J_{2,3}$ 11.1 Hz, $J_{2,NH}$ 6.7 Hz), 3.485–3.573 (m, 2H, NCH_2 sp), 3.751–3.795 (m, 1H, OCH sp), 3.848 (dd, 1H, H-2b, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 10.0 Hz), 3.875 (br.t, 1H, H-5b, J 7.1 Hz), 3.893–3.913 (br.t, 1H, H-5a, J 6.2 Hz), 3.916–3.956 (m, 1H, OCH sp), 4.037 (dd, 1H, H-6′a, $J_{6',6''}$ 11.7 Hz, $J_{5,6'}$ 7.2 Hz), 4.124 (dd, 1H, H-6′b, $J_{6',6''}$ 11.2 Hz, $J_{5,6'}$ 7.1 Hz), 4.156 (dd, 1H, H-6″b, $J_{6',6''}$ 11.2 Hz, $J_{5,6''}$ 6.2 Hz), 4.175 (dd, 1H, H-6″a, $J_{6',6''}$ 11.7 Hz, $J_{5,6''}$ 5.1 Hz), 4.505–4.537 (m, 2H, H-5c, H-1b, $J_{1,2}$ 7.7 Hz), 4.911 (dd, 1H, H-3a, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 11.1 Hz), 4.952 (d, 1H, H-1a, $J_{1,2}$ 8.3 Hz), 4.980 (dd, 1H, H-2c, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.9 Hz), 4.986 (dd, 1H, H-3b, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.0 Hz), 5.254 (dd, 1H, H-4c, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1.0 Hz), 5.269 (dd, 1H, H-4b, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1.0 Hz), 5.320 (dd, 1H, H-3c, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 10.9 Hz), 5.410 (br.d, 1H, H-4a, J 3.4 Hz), 5.474 (d, 1H, H-1c, $J_{1,2}$ 3.5 Hz), 6.637 (d, 1H, N*H*Ac a, $J_{2,NH}$ 6.7 Hz), 7.203–7.265 (m, 1H, $NHCOCF_3$ sp). R_f 0.36 (C_6H_{12}–$CHCl_3$–Pr^{i}OH, 4:2:1). MS, *m/z*: 1019 (calc. for $[C_{41}H_{57}N_2F_3O_{24}]H^+$, *m/z*: 1019.32).

Fucα1-2Galβ1-3GalNAcβ-O(CH$_2$)$_3$NH$_2$ **7**. ^{1}H NMR (700 MHz, D_2O) δ: 1.225 (d, 3H, H-6c, $J_{5,6}$ 6.6 Hz), 1.897–2.009 (m, 2H, CH_2 sp), 2.071 (s, 3H, NCOMe), 3.103 (m≈t, 2H, NCH_2 sp, J 6.9 Hz), 3.631–3.832 (m, 11H), 3.848 (dd, 1H, H-3b, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 9.7 Hz), 3.910 (br.d, 1H, H-4b, J 3.4 Hz), 3.956 (dd, 1H, H-3a, $J_{3,4}$ 3.0 Hz, $J_{2,3}$ 11.0 Hz), 3.998 (dd, 1H, H-2a, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 11.0 Hz), 4.019–4.067 (m, 1H, OCH sp), 4.135 (br.d, 1H, H-4a, J 2.9 Hz), 4.233 (br.q, 1H, H-5c, $J_{5,6}$ 6.6 Hz), 4.352 (d, 1H, H-1a, $J_{1,2}$ 8.2 Hz), 4.633 (d, 1H, H-1b, $J_{1,2}$ 7.7 Hz), 5.261 (d, 1H, H-1c, $J_{1,2}$ 4.1 Hz). ^{13}C NMR (176 MHz, D_2O) δ: 102.66, 102.13, 99.21 (C-1a, C-1b, C-1c), 76.60, 76.02, 75.15, 74.91, 73.64, 71.89, 69.62, 69.18, 68.50, 68.10, 68.06, 66.81 (C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b, C-2c, C-3c, C-4c, C-5c, CH_2O sp), 61.04 (C-6a, C-6b), 51.47 (C-2a), 37.74 (NCH_2 sp), 26.74 (CH_2 sp), 22.29 (NCO*Me*), 15.32 (C-6c). R_f 0.48 (MeOH–1 M aq. Py·AcOH, 5:1). MS, *m/z*: 587 (calc. for $[C_{23}H_{42}N_2O_{15}]H^+$, *m/z*: 587.26). $[\alpha]_{546}$ –58 (*c* 0.6, MeCN–H_2O, 1:1).