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Synthesis of glycoprotein N-chain core fragment GlcNAcβ1-4(Fucα1-6)GlcNAc

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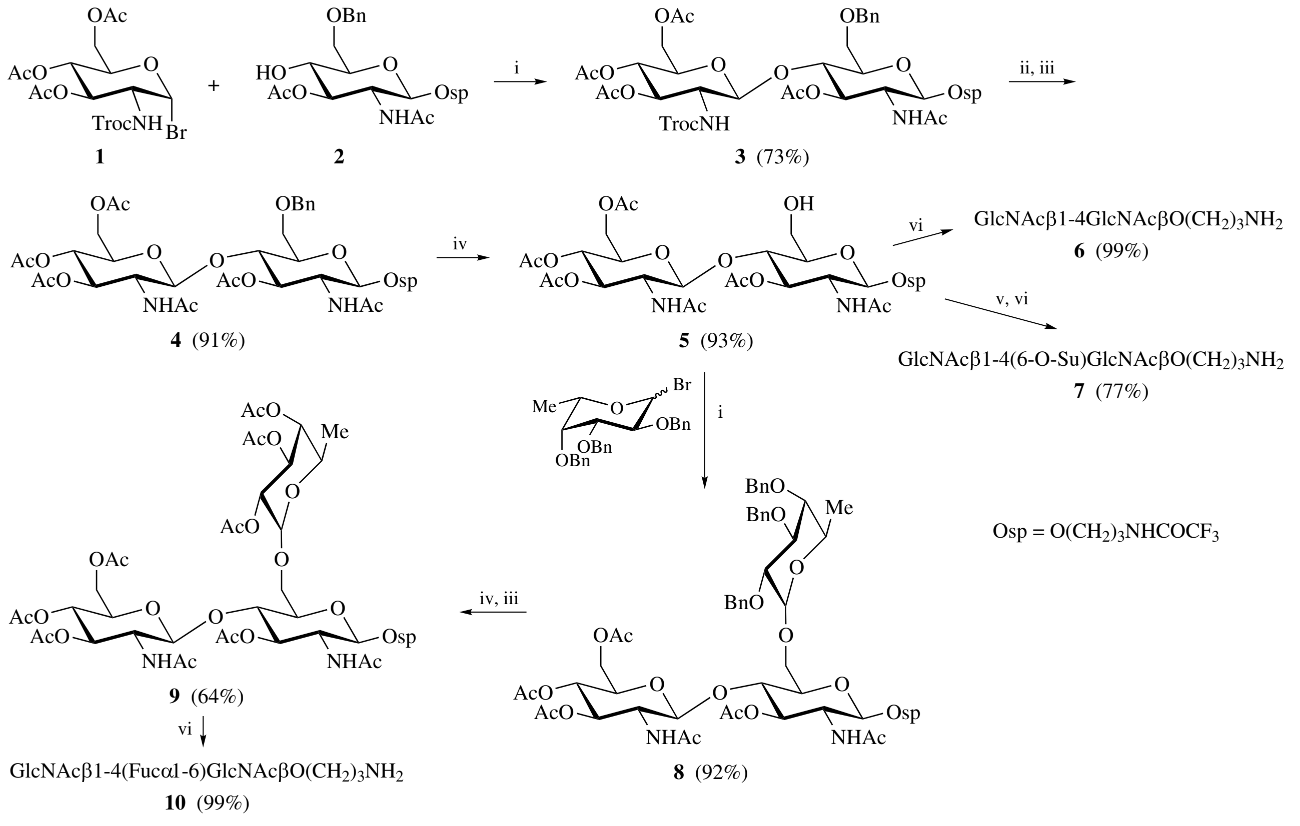
Synthesis of $Ac_3GlcNAc\beta(1\text{-}4)\text{-}3\text{-}Ac\text{-}6\text{-}BnGlcNTroc\beta\text{-}O(CH_2)_3NHCOCF_3$, a convenient precursor for the preparation of 6-substituted derivatives of disaccharide GlcNAcβ1-4GlcNAc, is described. α-Fucosylation of this precursor after the removal of protection groups leads to the title trisaccharide, whereas O-sulfation results in 6-O-sulfated derivative of chitobiose.

A conservative linkage between polypeptide chain and N-glycan of glycoproteins is known to be GlcNAcβ1-4GlcNAcβ1-Asn fragment, where GlcNAc residue is linked to nitrogen of asparagine side chain. In plants, parasites and some other organisms a considerable number of these fragments is modified by L-fucose residue at O^3 of reducing glucosamine, whereas in humans and other mammals modification at O^6, *i.e.* GlcNAcβ1-4(Fucα1-6)-GlcNAcβ, is observed.[1] During the study of natural human antibodies using a glycoarray[2] we observed high titer levels of IgG and IgM antibodies to disaccharides GlcNAcβ1-4GlcNAcβ and Fucα1-3GlcNAcβ for all the donors.[3,4] Taking into consideration that we observed other antibodies capable of paradoxical binding with the core, *i.e.* the most hidden part of glycans,[5] it became necessary to test whether humans have natural antibodies to the full glycosylated core GlcNAcβ1-4(Fucα1-6)GlcNAcβ.

Here we report the synthesis of GlcNAcβ1-4GlcNAcβ and its sulfated and fucosylated at O^6 derivatives as aminopropyl spacer glycosides (in amounts 50–100 mg) (Scheme 1). This makes possible their use for printing on glycoarray, thus extending the repertoire of available synthetic glycans.[6–15]

Glycosylation of the glycosyl acceptor **2** bearing a hydroxyl group at C^4 (ref. 16) with a glycosyl bromide of *N*-trichloroethoxycarbonyl (Troc) derivative of glucosamine **1**[15] in the presence of silver triflate and tetramethyl urea with two-fold excess of donor related to acceptor afforded disaccharide **3** (73%). We have applied a recently described method[17] using tetrabutylammonium fluoride in THF to the replacement of TrocNH with AcNH. This approach proved to be more efficient and convenient than the traditional action of Zn in acetic acid; the yield was increased by 10% and the reaction proceeded



Scheme 1 *Reagents and conditions*: i, AgOTf, TMM, MS-4Å, CH_2Cl_2, room temperature, 20 h; ii, Bu_4NF, THF, 3 h; iii, Ac_2O/Py; iv, 10% Pd/C, H_2, 2 h; v, Py·SO_3, Py, room temperature, 3 h; vi, 0.05 M MeONa/MeOH, 1 h, then 0.05 M aq. NaOH, 16 h.

rapidly (3 h). The O→N migration of Ac group observed in this particular case did not complicate the further isolation because heterogeneity was easily improved by acetylation.[†]

Catalytic debenzylation in disaccharide **4** led to derivative **5** bearing hydroxy group at C^6 of the reducing residue of glucosamine (93%). Deacetylation and N-de(trifluoroacetylation) of compound **5** gave the corresponding 3-aminopropyl derivative of chitobiose **6** (99%).

Disaccharide **5** with a hydroxyl group at C^6 was sulfated with $Py{\cdot}SO_3$ in pyridine at room temperature.[9] Removal of protective groups furnished 6-O-sulfated derivative of chitobiose (77%).

Glycosylation of disaccharide **5** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide in the presence of silver triflate and tetramethyl urea in dichloromethane resulted in trisaccharide **8** (92%). Hydrogenolysis followed by acetylation provided peracetate **9** (64% after HPLC purification). Removal of protective groups led to the target product – fucosylated derivative of chitobiose **10** (99%).

The structures of all target compounds and key intermediates were confirmed by high-resolution NMR spectroscopy and mass spectrometry.

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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.2015.07.004.

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[†] A 1 M THF solution of tetrabutylammonium fluoride (0.5 ml) was added to solution of the Troc-derivative of oligosaccharide (0.1 mmol) in 1 ml of THF. The mixture was kept at room temperature for 3 h and evaporated *in vacuo*. After acetylation with the mixture of acetic anhydride (2 ml) with pyridine (3 ml) for 16–20 h, the reaction mixture was co-evaporated with toluene. The dry residue was dissolved in ethyl acetate (30 ml), washed with saturated aqueous solution of sodium chloride and evaporated *in vacuo*. The product was isolated by chromatography on silica gel.

For the spectral characteristics of compounds **5–7**, **9** and **10**, see Online Supplementary Materials.