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Synthesis of GalNAcβ1-4GlcNAcβ (LacdiNAc) *O*-sulfates

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GalNAcβ1-4GlcNAcβ and its mono- and di-O-sulfated derivatives have been synthesized as aminopropyl spacer glycosides allowing their covalent on-chip printing and conjugation with polymeric carriers.

O-Sulfation of glycans is known to be an important modification of glycoproteins and glycolipids; numerous sulfated motifs are known as ligands for siglecs and selectins, moreover, recent publications demonstrated that *O*-sulfate dramatically modulates galectin-binding properties of lactosamine (LacNAc) type chains and related glycans.[1] In this respect, GalNAcβ1-4GlcNAcβ (LacdiNAc) terminated glycans are much less studied (*e.g.*, refs. 2, 3) than the corresponding lactosamines, their chemical synthesis has not been described yet; it is worth noting that the substrate specificity of sulfotransferases allows generation of different sulfated LacdiNAcs to occur in mammalian cell. Only 4′-O-Su-GalNAcβ1-4GlcNAc (Su is sulfo derivative) was surely identified as a component of glycoproteins, it is known to be a termination of lutropin glycans that mediate the glycoprotein clearance by hepatic reticuloendothelial cell receptor,[4] and found as capping motif in several other glycoproteins.[5] Interestingly, the related motif, 4′-O-Su-Galβ1-4GlcNAcβ, was found to have one of the highest binding ranks in composition of printed glycan array[6] when antibodies of healthy donors were probed. High specificity of the antibodies is deduced from the minimal or no binding to isomeric sulfated lactosamines. Here we synthesized seven sulfated derivatives of GalNAcβ1-4GlcNAcβ, including 4′-O-Su-GalNAcβ1-4GlcNAcβ in order to clarify real specificity of the antibodies and their functional role. Next, 6-O-Su-derivative in the form of fluorescent probe demonstrated specific binding to adenocarcinoma cells;[7] isomeric sulfates of LacdiNAc have not been used for comparison, thus, these compounds are required to detail specificity of corresponding cell surface glycan-binding protein. Finally, LacdiNAc *O*-sulfates described here are currently used for probing of galectins.[8]

GalNAcβ1-4GlcNAcβ and its mono- or di-O-sulfated derivatives were synthesized (in 50–100 mg amounts) as aminopropyl spacer glycosides allowing their covalent on-chip printing and conjugation with polymeric carriers.

N-Trichloroethoxycarbonyl (Troc) derivative of galactosamine **1** has been used as a glycosyl donor because the *N*-Troc protection provides β-stereoselectivity of glycosylation, but at the same time transformation of the Troc-NH fragment into the target Ac-NH does not affect other protective groups.[9]

Selective O-sulfation, deprotection, and isolation of reaction products were performed according to techniques described in our previous papers.[10,11] As expected, the simplest and the most efficient way for obtaining O-sulfo derivatives proved to be the reaction of oligosaccharides having a single free OH group with $Py{\cdot}SO_3$ in dry pyridine at room temperature, namely, the synthesis of 6-O-Su-GalNAcβ1-4GlcNAcβ derivative **6** from **4** (86%) and 4-O-Su derivative **15** from **14** (79%). Only one product is formed in this case, which can be readily isolated by gel filtration on Sephadex LH-20 (eluent, H_2O) after deprotection. Preparation of disulfates from the corresponding diols proceeds analogously to **6** and **15**: 4′,6′-O-Su_2 derivative **12** from diol **11** (85%); 3,3′-O-Su_2 derivative **8** from diol **7** (68%), and 6,6′-O-Su_2 derivative **17** from diol **16** (75%).

According to our experience, selective sulfation at –10 to –20 °C leads to formation of a small amount of side products. Thus, sulfation of 4′,6′-diol **11** at lowered temperature resulted in formation of 6′-O-Su-GalNAcβ1-4GlcNAcβ derivative **13** (77%) and 4′,6′-disulfate **12** (9%), whereas 4′-O-Su derivative was not isolated. In the case of sulfation of diol **7**, 3-OH group in GlcNAc fragment proved to be so inactive that 3′-O-Su-GalNAcβ1-4GlcNAcβ **9** (62%) was practically the only reaction product, whereas the yield of 3,3′-O-Su_2 derivative **8** was less than 2%. Monosulfated derivatives can be readily separated from disulfated ones using anion-exchange chromatography on DEAE-Sephadex A-25 (elution with 0.01 M and 1 M aqueous AcOH, respectively).

Monosulfated derivatives **6**, **9**, **13** and **15** are internal salts; disulfated compounds **8**, **12** and **17** were converted to sodium salts using ion-exchange chromatography on Dowex Na^+ because in this form they are stable and can be stored for a long time (neutral pH value, non-volatile cation, which is not lost during freeze-drying).

The derivatives with one or two OH groups used in this study were obtained using standard procedures of carbohydrate chemistry: hydrogenolysis, deacetylation and acetylation, benzylidenation and debenzylidenation, reductive ring-opening of *O*-benzylidene acetals (see Scheme 1).

The purity of the target compounds was confirmed by analytical HPLC. When impurities were detected, the target compounds were purified using preparative reversed-phase HPLC.

The structures of final compounds were confirmed by ^{1}H NMR spectroscopy data. Sulfation of the hydroxyl group resulted in the downfield shift of the signals of neighboring protons compared to GalNAcβ1-4GlcNAcβ (δ 3.51–3.93 ppm): δ 4.16 and 4.31 ppm for H-6a and H-6b; δ 4.46 ppm for H-3′; δ 4.57 ppm for H-3; δ 4.69 ppm for H-4′; δ 4.21 and 4.23 ppm for H-6′a and H-6′b. In the case of disulfates, signals of the corresponding protons can be slightly shifted by ±0.02 ppm compared to monosulfates (for details, see Online Supplementary Materials, spectral characteristics of oligosaccharides).

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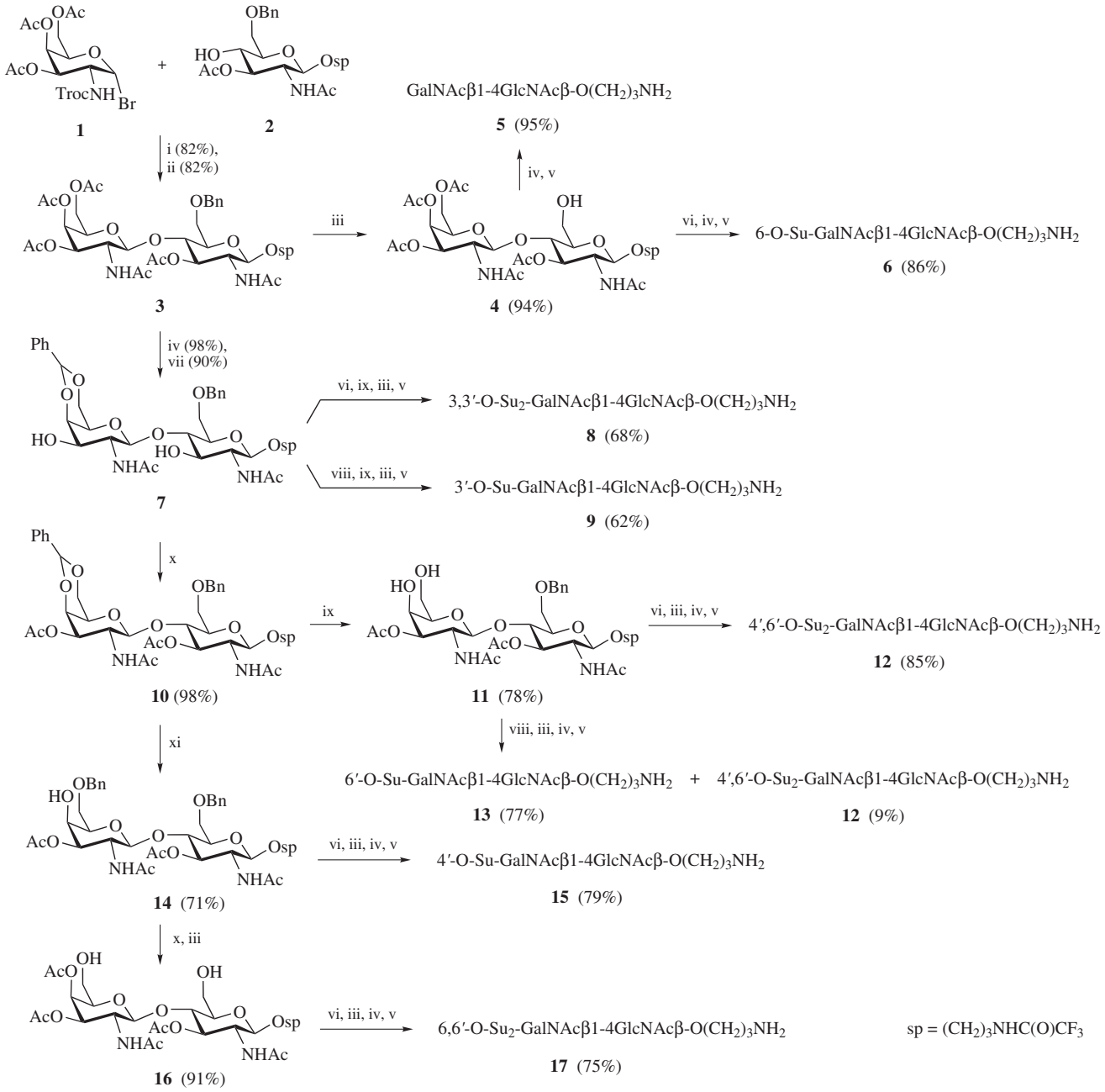

Scheme 1 *Reagents and conditions*: i, AgOTf/TMM, CH_2Cl_2, room temperature; ii, Zn/AcOH; iii, Pd/C, H_2, MeOH, 2 h; iv, 0.1 M NaOMe/MeOH, 2 h; v, 0.1 M NaOH/H_2O, 3 h; vi, Py·SO_3/Py, room temperature, 1 h; vii, $PhCH(OMe)_2$, TsOH, MeCN, 5 h; viii, Py·SO_3/Py, –15 to –20 °C, 5 h; ix, 80% aq. AcOH, 70 °C, 2 h; x, Ac_2O, Py, room temperature, 12 h; xi, $NaCNBH_3$/THF, MsOH, room temperature, 2 h.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2010.11.004.

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