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Synthesis of mono-, di- and tri-O-sulfated *N*-acetyllactosamines in a form suitable for glycochip printing

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A convenient and highly efficient technique for the selective sulfation of lactosamine derivatives has been suggested.

O-Sulfated glycans of glycoproteins and glycolipids frequently act as ligands for a diverse range of human and microbial glycan binding proteins including CD1 restricted T cell receptors,[1] chemokines,[2] laminins,[3] toxins[4] and many other important proteins as siglecs, galectins, selectins,[5] *etc*. In composition of glycolipid and glycoprotein glycans, the O-sulfo group (Su) can mask antigenic and lectin-binding sites, and at the same time to be an essential moiety for lectin binding or to enhance affinity of 'regular' sulfate-free glycans. Printed glycan microarray where carbohydrate ligands are covalently attached to a chip surface allows extending information on the specificity of carbohydrate-binding proteins and accelerating enormously obtaining of this information.[6,7] The most typical positions of Su in composition of glycoproteins and glycolipids are 3-O-Gal, 6-O-Gal, 4-O-GalNAc and 6-O-GlcNAc. Here, we describe the synthesis of ten derivatives of *N*-acetyllactosamine (Galβ1-4GlcNAc) with one or more O-Su groups (Table 1) and two sialylated (3′ and 6′) derivatives of Galβ1-4-GlcNAc with an O-sulfo group. All compounds are obtained in the form of $–OCH_2CH_2CH_2NH_2$ or $–OCH_2CH_2NH_2$ glycosides, suitable for construction of covalent ('printed') microarrays.

The protocol described before[8] was used with slight changes for obtaining sulfated lactosamine derivatives.

$Py{\cdot}SO_3$ (3 mmol) was suspended in a saccharide (0.5 mmol) solution in 5 ml of dry pyridine. Reaction mixture was stirred for 1–3 h upon reaction completion (TLC control) at room temperature (when it is necessary to carry out exhaustive sulfation) or at –10 to –20 °C (when selective sulfation is carried on). $NaHCO_3$ (6 mmol) was added followed by reaction mixture stirring for 15 min, addition of 50 ml of MeOH, filtration after 30 min, and washing of the residue on filter with MeOH (5×50 ml). Combined filtrate was concentrated *in vacuo* and co-evaporated several times with toluene. The main part of non-carbohydrate admixtures was removed from the reaction mixture by gel filtration on Sephadex LH-20 (elution with MeOH, 0.5% Py). After standard deprotection (H_2, Pd/C; MeONa/MeOH; $NaOH/H_2O$) ion-exchange chromatography on DEAE-Sephadex A-25 ($Ac^–$ form) was performed: monosulfates were eluted with 0.01 M Py·AcOH in water and disulfates, with 1 M Py·AcOH solution in water. The substances were dried by evaporation and purified by gel filtration on Sephadex LH-20 (elution with H_2O). The Na^+ salts of the synthesized sulfated derivatives were prepared by ion-exchange chromatography on DOWEX 50WX4 cationite (Na^+ form). Target products were obtained as white powders after evaporation and freeze drying.

In case of insufficient purity of the synthesized monosulfates (*e.g.*, compounds **14** and **17**), they were subjected to chromatography on silica gel (Pr^iOH:MeCN:H_2O:Py = 4:3:2:0.1). In case of disulfates, it is possible to additionally purify the substances by trituration with MeOH (compound **16**) or subsequent purification on DEAE-Sephadex A-25, Sephadex LH-20, DOWEX-Na^+ (compound **15**).

Low yield of compound **18** (52%) is related to the necessity of purification of the reaction mixture after introduction of the second sulfate. In all the other cases, the total yields were higher than 70%. The structures of all synthesized compounds were confirmed by mass spectrometry and ^{1}H NMR spectroscopy. Incorporation of the HSO_3 group leads to a weak field shift of neighbour protons (Table 1) thus unambiguously confirming their position. Synthesis of compounds **19**[8] and **21**[9] was described earlier with smaller yields.

In case when the starting oligosaccharide has only one OH group (compounds **3**,[†] **4a**, **5**,[†] **6**, **7**,[8] **8**,[‡] **9**[9]), sulfation was carried out at room temperature. Reaction was completed for 1 h in case of primary hydroxyls (at C-6 of galactose in compound **6** or glucosamine in compounds **4a**, **7**, **8**, **9**) or for 2 h in case of secondary hydroxyl (at C-4 of galactose in compound **3** or at C-3 of galactose in compound **5**). Disulfation of diols **2**[§] and **4**[‡] was also performed at room temperature; reaction was totally completed in 2 h. Sulfation proceeded selectively at –10 to –20 °C: only 6-O-Su-derivative was formed from 4,6-diol **2** in 2 h, whereas 3-O-Su-derivative was mainly formed from 3,4-diol **4** (additionally, about 10% unidentified admixture was separated by chromatography on silica gel). Sulfation of unprotected lactosamine **1**[1] at –10 to –20 °C led in 3 h to formation of a mixture of di- and tri-O-sulfates (~1:1) separated by ion-exchange chromatography on DEAE-Sephadex A-25 ($Ac^–$ form) after azide group hydrogenolysis. Di-O-sulfate **10** was eluted first by a 1 M solution of Py·AcOH in water followed by tri-O-sulfate **11**.

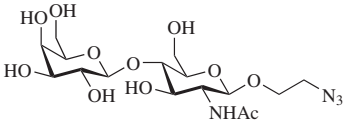
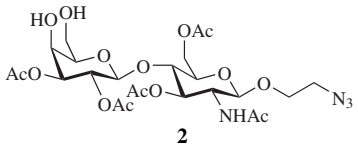
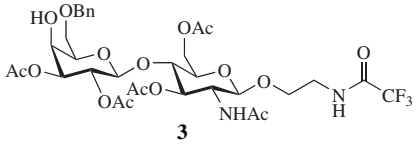
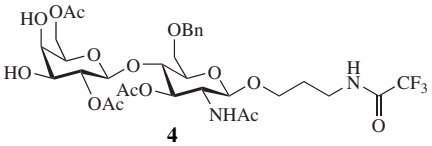
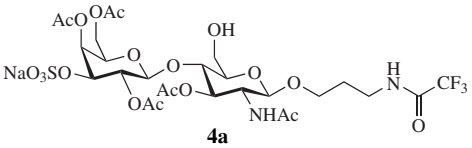
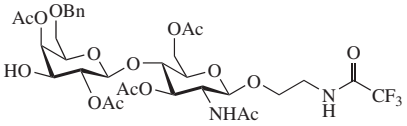
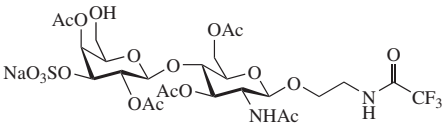
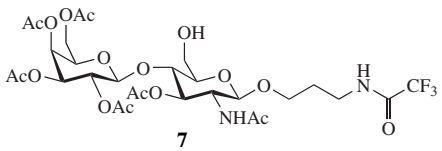
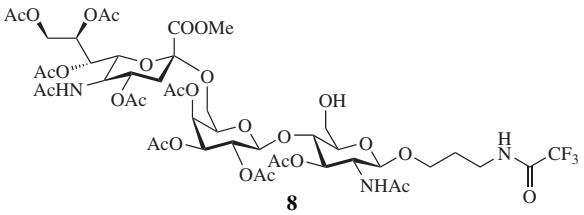
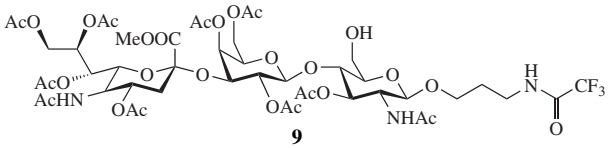
The first step in obtaining di-O-sulfate **16** was selective sulfation at 3-OH of the galactose fragment of compound **4** at

[†] Compounds **3** and **5** were obtained from Consortium for Functional Glycomics (www.functionalglycomics.org).

[‡] Compound **4** was obtained from 3-fluoroacetamidopropyl-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside[8] by sequential de-O-acetylation, benzylidenation, acetylation and debenzylidenation. Compound **4** is a side product. The main product, 3-fluoroacetamidopropyl-2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-4-*O*-(2,3-di-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside was subjected to sialylation[10] and hydrogenolysis yielding trisaccharide **8**.

[§] Compound **2** was synthesized from **1** by sequential benzylidenation, O-acetylation and debenzylidenation. For spectral characteristics of oligosaccharides, see Online Supplementary Materials.

Table 1 Synthesized sulfated saccharides, their properties and yields.

Starting compound	Product	*T*/°C	*t*/h	Yield (%)[a]	Selected ^{1}H NMR data for the product
 **1**	6-O-Su-Galβ1-4(6-O-Su)-GlcNAcβO(CH$_2$)$_2$NH$_2$ **10**	–10 to –20	3	37	4.472 (dd, H-6a), 4.343 (dd, H-6b), 4.221 (m, H-6′a,6′b)
	3,6-di-O-Su-Galβ1-4-(6-O-Su)GlcNAcβO(CH$_2$)$_2$NH$_2$ **11**			31	4.494 (dd, H-6a), 4.339 (dd, H-6b), 4.391 (dd, H-3′), 4.366 (dd, H-4′), 4.240 (m, H-6′a,6′b)
 **2**	6-O-Su-Galβ1-4-GlcNAcβO(CH$_2$)$_2$NH$_2$ **12**	–10 to –20	2	73	4.190 (m, H-6′a,6′b)
	4,6-di-O-Su-Galβ1-4-GlcNAcβO(CH$_2$)$_2$NH$_2$ **13**	22		71	4.726 (dd, H-4′), 4.385 (dd, H-6′a), 4.227 (dd, H-6′b), 4.139 (ddd, H-5′)
 **3**	4-O-Su-Galβ1-4-GlcNAcβO(CH$_2$)$_2$NH$_2$ **14**	22	2	59	4.693 (dd, H-4′)
 **4**	3,4-di-O-Su-Galβ1-4-GlcNAcβO(CH$_2$)$_3$NH$_2$ **15**	22	2	54	4.976 (dd, H-4′), 4.440 (dd, H-3′)
 **4a**	3-O-Su-Galβ1-4-(6-O-Su)-GlcNAcβO(CH$_2$)$_3$NH$_2$ **16**	22	1	55	4.466 (dd, H-6a), 4.343 (dd, H-6b), 4.367 (dd, H-3′), 4.316 (dd, H-4′)
 **5**	3-O-Su-Galβ1-4-GlcNAcβO(CH$_2$)$_2$NH$_2$ **17**	22	2	64	4.357 (dd, H-3′), 4.312 (dd, H-4′)
 **6**	3,6-di-O-Su-Galβ1-4-GlcNAcβO(CH$_2$)$_2$NH$_2$ **18**	22	1	52	4.381 (dd, H-3′), 4.362 (dd, H-4′), 4.238 (m, H-6′a,6′b)
 **7**	Galβ1-4(6-O-Su)-GlcNAcβO(CH$_2$)$_3$NH$_2$ **19**	22	1	79	4.439 (dd, H-6a), 4.343 (dd, H-6b)
 **8**	Neu5Acα2-6Galβ1-4-(6-O-Su)GlcNAc-βO(CH$_2$)$_3$NH$_2$ **20**	22	1	98	4.471 (dd, H-6a), 4.314 (dd, H-6b)
 **9**	Neu5Acα2-3Galβ1-4-(6-O-Su)GlcNAc-βO(CH$_2$)$_3$NH$_2$ **21**	22	1	92	4.508 (dd, H-6a), 4.406 (dd, H-6b)

[a]Total yield of target product per sulfation and deprotection stages.

–10 to –20 °C. Then, the obtained monosulfate was acetylated with acetic anhydride/pyridine. After chromatography on silica gel (eluent, $CHCl_3$:MeOH:Py = 3:1:0.01) followed by hydrogenolysis over Pd/C in methanol, lactosamine derivative **4a** with one OH group at the 6-position of the glucosamine moiety was obtained. This derivative was quantitatively sulfated for 1 h at room temperature. After deprotection and standard purification procedures, di-O-sulfate **16** was obtained in 76% yield with ~90% purity. Trituration with methanol gave rise to 96% purity compound; yield, 55%.

Disulfate **18** was also obtained by sequential sulfation. Mono-OH derivative of lactosamine **6** was obtained by sulfation of compound **5** followed by hydrogenolysis of 6-OBn group of galactose moiety. After chromatography on silica gel (eluent, $CHCl_3$:MeOH:Py = 4:1:0.01), compound **6** was sulfated at room temperature. Disulfate was isolated from the reaction mixture by chromatography (eluent, $CHCl_3$:MeOH:Py = 2:1:0.01). After deprotection and purification, target product **18** was obtained in a total yield of 52%.

The structures of synthesized oligosaccharides were confirmed by ^{1}H NMR data using homonuclear correlation spectroscopy and conventional analysis of coupling constants (see Online Supplementary Materials). The position of the sulfate group was deduced from the characteristic down-field shifts of corresponding protons.

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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.2008.09.002.

References

1 G. De Libero and L. Mori, *Nat. Rev. Immunol*., 2005, **5**, 485.
2 R. Sandhoff, H. Grieshaber, R. Djafarzadeh, T. P. Sijmonsma, A. E. Proudfoot, T. M. Handel, H. Wiegandt, P. J. Nelson and H. J. Grone, *Biochim. Biophys. Acta*, 2005, **1687**, 52.
3 J. F. Talts, Z. Andac, W. Gohring, A. Brancaccio and R. Timpl, *EMBO J.*, 1999, **18**, 863.
4 E. Rousset, J. Harel and J. D. Dubreuil, *Infect. Immun*., 1998, **66**, 5650.
5 A. Aruffo, W. Kolanus, G. Walz, P. Fredman and B. Seed, *Cell*, 1991, **67**, 35.
6 O. Blixt, S. Head, T. Mondala, C. Scanlan, M. E. Huflejt, R. Alvarez, M. C. Bryan, F. Fazio, D. Calarese, J. Stevens, N. Razi, D. J. Stevens, J. J. Skehel, I. van Die, D. R. Burton, I. A. Wilson, R. Cummings, N. Bovin, C.-H. Wong and J. C. Paulson, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 17033.
7 V. I. Dyukova, N. V. Shilova, O. E. Galanina, A. Yu. Rubina and N. V. Bovin, *Biochim. Biophys. Acta*, 2006, **1760**, 603.
8 T. V. Ovchinnikova, E. V. Shipova, M. A. Sablina, G. A. Pazynina, I. S. Popova, A. B. Tuzikov and N. V. Bovin, *Mendeleev Commun*., 2002, 213.
9 G. V. Pazynina, M. A. Sablina, A. B. Tuzikov, A. A. Chinarev and N. V. Bovin, *Mendeleev Commun*., 2003, 245.
10 G. Pazynina, A. Tuzikov, A. Chinarev, P. Obukhova and N. Bovin, *Tetrahedron Lett*., 2002, **43**, 8011.

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