Syntheses of Novel Sulfated Glycans for Cell-Adhesion Interaction Studies

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Abstract: Stereoselective syntheses of two 3-*O*-Gal sulfated trisaccharides GalNAcβ(1–4)[(3-SE)-Gal]β(1–3)GalNAcα-*O*-All and GalNAcβ(1–4)[(3-SE)-Gal]β(1–4)Glcβ-*O*-All were accomplished through the use of three novel glycosyl acceptors, namely, allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-galactopyranoside, methyl 2,6-di-*O*-benzoyl-3-*O*-naphthylmethyl-α-D-galactopyranoside and allyl 6-*O*-acetyl-2-*O*-benzoyl-3-*O*-naphthylmethyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside. These sulfated trisaccharides were expected to act as potential reference compounds for human β4GalNAc transferase and can be effectively used as antigens when linked to KLH.

Key words: glycosylations, oligosaccharides, stereoselective synthesis, sulfated glycans, sulfotransferases

Biosynthesis of oligosaccharides, polysaccharides, and glycoconjugates is mainly carried out by a large family of enzymes known as glycosyltransferases (Glycosyl-Ts).[1] Aberrant glycosylation and overexpression of carbohydrate structures leads to the growth of tumor cells with different adhesion properties.[2] It is a well-established fact that the changes in the structures of cancer-associated glycans are driven by the expression and activity alterations of Glycosyl-Ts involved in their biosynthesis.[3] Since, sulfotransferases (Sulf-Ts) and sialyltransferases (STs) can compete with GTs for the same sites during an assembly of glycans,[4] it became important to study the role of both the enzyme families in the development of malignant cells. Based on this principal, over the years, the prime objective of our laboratory has been to develop the chemistry for understanding the enzymic machinery of glycans, especially *O*-glycans.[4,5] It is evident from the literature reports[6] and our previous biochemical investigations[5] that Sulf-Ts are highly specific enzymes which incorporate a sulfate ester (SE) to a specific position of a specific oligosaccharide acceptor. For instance, two distinct types of Gal:3-*O*-sulfotransferases (Gal3Sulf-Ts) in tumor tissues and cancer cells demonstrated distinctive acceptor preferences. Enzymes from breast cancer cells prefer to sulfate 3-O position of Gal in the Galβ1→3GalNAcα moiety of the mucin core 2 structure. In contrast, enzymes from colon cancer cell lines and colon tumor tissues prefer to act on the Galβ1→4GlcNAcβ moiety.[5b,c] These studies have led us to identify whether human β4GalNAc-transferase has the capability to generate GalNAcβ(1–4)[(3-SE)-Gal]β sequence. Herein, we report the syntheses of GalNAcβ(1-4)-[(3-SE)-Gal]β(1-3)GalNAcα(1-*O*)-All (**1**) and GalNAcβ(1-4)-[(3-SE)-Gal]β(1-4)Glcβ(1-*O*)-All (**6**) as potential reference compounds that can be effectively used as antigens when linked to keyhole limpet hemocyanin (KLH) under conventional conditions.[7]

As envisaged from the retrosynthetic scheme (Figure 1), coupling between 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→4)-2,6-di-*O*-benzoyl-3-*O*-naphthylmethyl-D-galactopyranosyl trichloroacetimidate (**2**) and allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-galactopyranoside (**3**) would yield the desired sulfated trisaccharide **1**. The disaccharide donor **2** could in turn be obtained by the reaction of known 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-galactopyranosyl trichloroacetimidate (**4**)[8] with methyl 2,6-di-*O*-benzoyl-3-*O*-naphthylmethyl-α-D-galactopyranoside (**5**). Similarly, synthesis of second target molecule **6** necessitates the use of *N*-trichloroethoxycarbonyl (*N*-Troc) Gal imidate **4** and suitably protected lactose acceptor **7** having free 4′-OH accessible for glycosylation reaction. In order to introduce acetamido groups in the final trisaccharides **1** and **6** (examples of β-glycosides), *N*-Troc-protected glycosyl donor **4** was preferred as it is known to furnish β-glycosides in high yield with good β-stereoselectivity.[8,9] Also their transformation into acetamido group is straightforward.[10] To accomplish the introduction of sulfate group at the 3′-OH of Gal unit of trisaccharides **1** and **6**, protection with 2-naphthylmethyl (NAP) group proved to be highly suitable for our purpose owing to its stability under various reaction conditions, viz. acetal hydrolysis, glycosylation, and ester deprotection. Also, its chemoselective removal under DDQ oxidative conditions as required in the later part of our synthetic strategy is an added advantage.[11] We opted to make the allyl glycoside derivatives **1** and **6** because of their ease in attachment to gold nanoparticles and KLH following standard protocols.[7,12] We intend to utilize these compounds for developing monoclonal antibodies and for discovery of carbohydrate-binding aptamers.

Syntheses of starting materials required for the construction of sulfated trisaccharide **1** is depicted in Scheme 1. 4,6-*O*-Benzylidene protection of allyl α-D-GalNAc (**8**) followed by hydrolysis of its acetamido group provided amine **9** in 70% yield over two steps.[13] Troc protection of **9** using TrocCl and $NaHCO_3$ afforded the required acceptor **3** in reasonable yield. The poor yield of **3** may be attributed to the low solubility of free amine **9** in the

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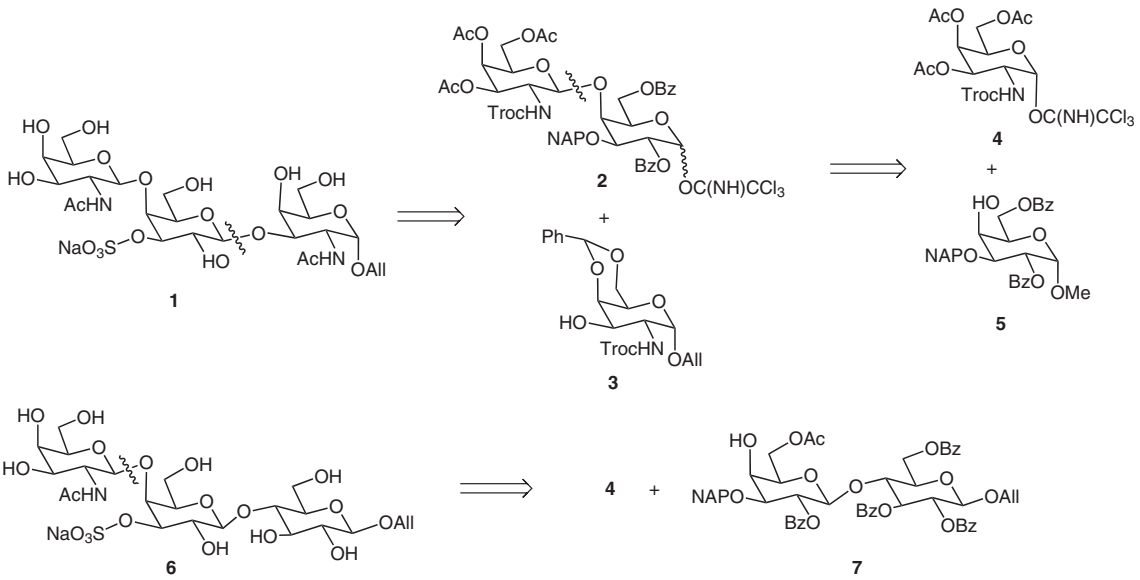
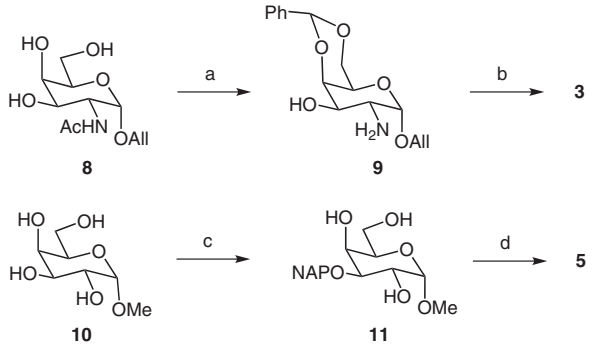


Figure 1 Retrosynthetic analysis of target sulfated trisaccharides **1** and **6**

reaction medium (water). Attempts to improve the yield of **3** in the above reaction are ongoing. Similarly, 3-*O*-NAP galactoside **5** was prepared from commercially available methyl α-D-galactoside (**10**). Reaction of **10** with NAPBr in presence of Bu_4NI and Bu_2SnO brought about the regioselective introduction of NAP to afford methyl 3-*O*-NAP-α-D-galactoside (**11**) in a very high yield (96%).[11] It is worth mentioning here that the 3-*O*-NAP group could be chemoselectively removed at the final stage of the synthetic strategy to endure sulfation. Selective benzoylation at 2- and 6-OH of **11** was successfully achieved using BzCl (2 equiv) in pyridine at –30 °C to provide the desired acceptor **5** in 80% yield (Scheme 1).

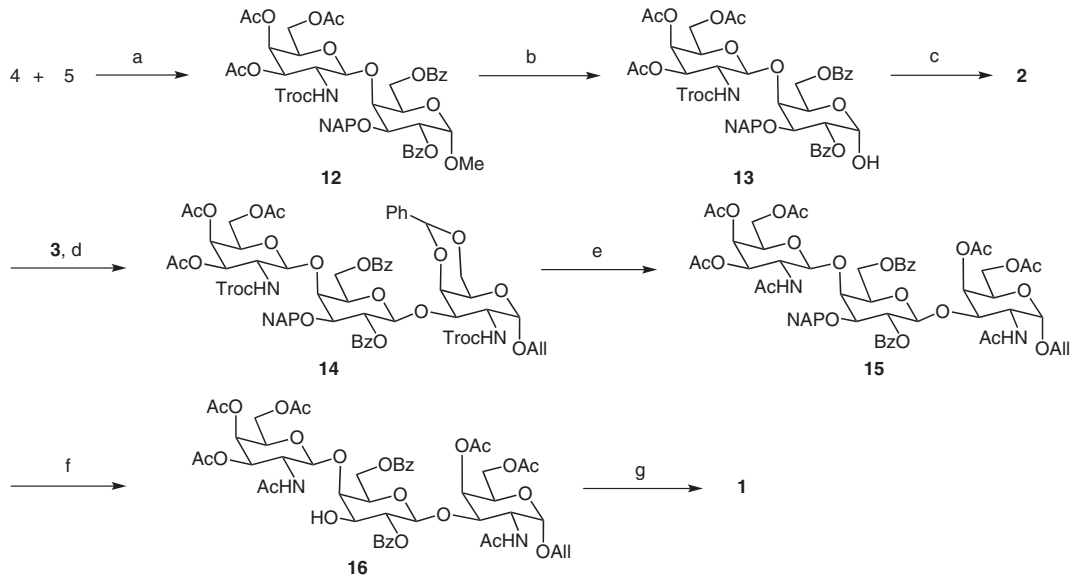
With the required starting materials in hand, we turned our attention on the synthesis of one of the target molecules **1**. Condensation of alcohol **5** with imidate **4** was performed under standard glycosylation conditions using TMSOTf



Scheme 1 Preparation of the key building blocks **3** and **5**. *Reagents and conditions*: a) i) $PhCH(OMe)_2$, PTSA, DMF, r.t., overnight; ii) 30% KOH solution, 1,4-dioxane–EtOMe (5:3 v/v), refluxed at 120 °C, 18 h (70%, 2 steps); b) TrocCl, $NaHCO_3$, Et_2O–H_2O (1:1 v/v), r.t., 1.5 h (40%); c) Bu_2SnO–dry benzene, refluxing, 4 h, then NAPBr, *n*-Bu_4NI, 80–85 °C, 18 h (96%). d) BzCl, pyridine, –30 °C, 6 h (80%).

as the catalyst[14] to provide the desired β-(1→4) linked disaccharide **12** in 86% yield (Scheme 2). In order to synthesize disaccharide donor **2**, compound **12** was first subjected to acetolysis[15] with AcOH–Ac_2O–H_2SO_4 followed by removal of the resulting anomeric acetate by hydrazinium acetate[16] to afford the 1-hydroxy compound **13** in a good yield of 75% over two steps. Compound **13** upon further treatment with trichloroacetonitrile in the presence of DBU[8] gave the desired trichloroacetimidate **2** in a 78% yield. Under the similar conditions as mentioned for the synthesis of **12**, coupling between **2** and **3** was carried out successfully to obtain the β-(1→3) linked trisaccharide **14** in moderate yield. Subsequent removal of 4,6-*O*-benzylidene ring of **14** followed by conversion of NHTroc into NHAc[10] afforded **15** in 69% over three steps (Scheme 2). Chemoselective removal of NAP from **15** was effected using DDQ to afford **16** in 75% yield.[11] Finally, sulfation using SO_3–pyridine complex in DMF[17] followed by complete deprotection using NaOMe/MeOH, neutralization with IR-120 (H^+) resin, and passage through IR-120 (Na^+) resin afforded the title compound **1**[18] as a white solid in 95% yield (over two steps, Scheme 2).

Scheme 3 outlines the synthesis of 3′-*O*-SO_3Na trisaccharide **6**. 3′-*O*-NAP protection of **17**[19] as described before provided **18** in a reasonable yield. 4,6-*O*-Benzylidene protection of **18** followed by conventional benzoylation furnished **19** in 70% yield over two steps. Finally, treatment of **19** with 80% AcOH cleaved the benzylidene ring to afford diol which was selectively acetylated at the 6′-OH (primary hydroxyl group) using 1 equivalent of Ac_2O in pyridine at low temperature to get the desired lactose acceptor **7** in 81% yield. TMSOTf-mediated coupling of **7** with imidate **4** provided the desired β-(1→4) linked trisaccharide **20** in a good yield (Scheme 3). Following the same strategy as described for **1**; conversion of NHTroc into NHAc, removal of NAP, sulfation, and



Scheme 2 Synthesis of trisaccharide **1**. *Reagents and conditions*: a) TMSOTf, 4 Å MS, dry CH_2Cl_2, N_2, –50 °C, 40 min (86%); b) i) Ac_2O–AcOH (11:9 v/v), H_2SO_4, 0 °C to –4 °C, overnight; ii) N_2H_2·HOAc, DMF, 50 °C, 2 h (75%, 2 steps); c) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 30 min (78%); d) TMSOTf, 4 Å MS, dry CH_2Cl_2, N_2, –50 °C to 0 °C, 30 min (55%); e) i) 80% aq AcOH, 55 °C, 30 min; ii) Cd powder, DMF–AcOH (2:1 v/v), r.t., overnight; iii) Ac_2O–pyridine (1:2 v/v), CH_2Cl_2, r.t., 70 h (69%, 3 steps); f) DDQ, CH_2Cl_2–MeOH (4:1 v/v), overnight (75%). g) i) SO_3–pyridine, dry DMF, 50 °C, 2 h; ii) NaOMe/MeOH, r.t., 24 h (95%, 2 steps).

finally complete deprotection and treatment with IR-120 (Na^+) resin, afforded the final sulfated trisaccharide **6**[20] as a white solid in an overall yield of 58% over five steps.

In summary, we have developed a concise and practical synthesis of two 3-*O*-Gal-sulfated trisaccharides **1** and **6** based on the rationally designed synthetic strategy. We argue that the sulfated compounds prepared here will be useful for cell-adhesion interaction studies which recognize sulfated glycans. Furthermore, attempts to explore the synthetic utility of intermediates **2**, **3**, and **7** for obtaining higher oligosaccharides with similar structures are currently being undertaken. These compounds will be further employed for examining the specificity of glycosyltransferases and sulfotransferases.

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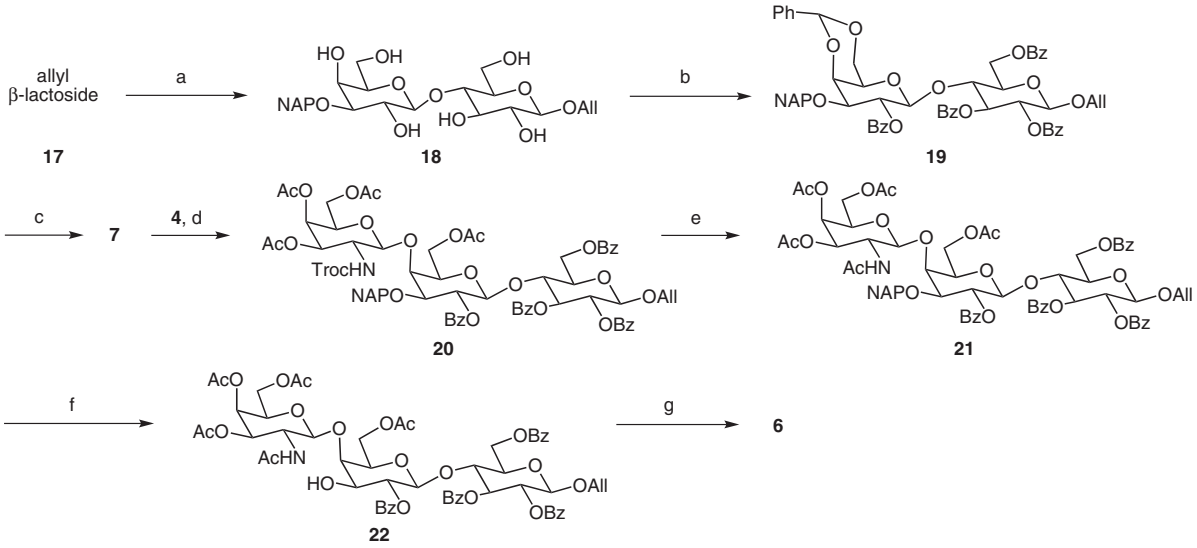

Scheme 3 Synthesis of trisaccharide **6**. *Reagents and conditions*: a) i) Bu_2SnO–dry toluene, refluxing, 4 h, then NAPBr, *n*-Bu_4NI, 110–115 °C, 48 h (60%); b) i) $PhCH(OMe)_2$, PTSA, MeCN, r.t., 1 h; ii) BzCl, pyridine, r.t., overnight (70%, 2 steps); c) i) 80% AcOH, 75–80 °C, 6 h; ii) Ac_2O, DMAP, pyridine, –30 °C, 7 h (81%, 2 steps); d) TMSOTf, 4 Å MS, dry CH_2Cl_2, N_2, –50 °C, 3 h (76%); e) i) Cd powder, DMF–AcOH (2:1 v/v), r.t., overnight; ii) Ac_2O–py (1:2 v/v), CH_2Cl_2, r.t., 20 h (80%, 2 steps); f) DDQ, CH_2Cl_2–MeOH (4:1 v/v), overnight (76%); g) i) SO_3–pyridine, dry DMF, 50 °C, 4 h; ii) NaOMe/MeOH, r.t., 48 h (95%, 2 steps).

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(18) **Analytical Data for Compound 1**
^{1}H NMR (400 MHz, CD_3OD): δ = 1.97 (s, 3 H, NHCOCH_3), 2.03 (s, 3 H, NHCOCH_3), 3.93–3.41 (m, 16 H), 4.03–3.98 (m, 2 H), 4.11–4.09 (m, 1 H), 4.23–4.17 (m, 1 H), 4.39 (d, *J* = 7.6 Hz, 1 H, H-1′), 4.46–4.41 (m, 1 H), 4.63 (d, *J* = 8.4 Hz, 1 H, H-1″), 5.17 (dd, ^3J = 10.4 Hz, ^2J = 1.6 Hz, 1 H, $OCH_2CH{=}CH_{cis}H_{trans}$), 5.32 (dd, ^3J = 17.2 Hz, ^2J = 1.6 Hz, 1 H, $OCH_2CH{=}CH_{cis}H_{trans}$), 5.99–5.89 (m, 1 H, $OCH_2CH{=}CH_2$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 22.9 ($COCH_3$), 23.2 ($COCH_3$), 55.5, 61.8, 62.8, 62.9, 69.4, 70.3, 72.4, 72.8, 72.9, 74.7, 74.8, 75.8, 77.0, 77.9, 78.5 (C-3), 78.9 (C-3′), 98.2, 104.6, 106.5 (C-1, C-1′, C-1″), 117.8 ($CH{=}CH_2$), 135.7 ($CH{=}CH_2$), 174.2 ($COCH_3$), 175.4 ($COCH_3$) ppm. ESI-MS: *m/z* calcd for $C_{25}H_{41}N_2NaO_{19}S$: 728.2; found: 751.2 $[M + Na]^+$.

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(20) **Analytical Data for Compound 6**
^{1}H NMR (400 MHz, CD_3OD): δ = 2.06 (s, 3 H, NHCOCH_3), 3.26 (m, 1 H), 3.91–3.46 (m, 14 H), 4.16–4.01 (m, 2 H), 4.36–4.28 (m, 4 H, H-1, *J* = 8.0 Hz, incorporated with other protons), 4.44 (d, *J* = 7.6 Hz, 1 H, H-1′), 4.61 (d, *J* = 8.4 Hz, 1 H, H-1″), 5.16 (dd, ^3J = 10.8 Hz, ^2J = 1.2 Hz, 1 H, $OCH_2CH{=}CH_{cis}H_{trans}$), 5.32 (dd, ^3J = 17.6 Hz, ^2J = 1.6 Hz, 1 H, $OCH_2CH{=}CH_{cis}H_{trans}$), 5.99–5.93 (m, 1 H, $OCH_2CH{=}CH_2$) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 23.5 ($COCH_3$), 54.2, 61.4, 61.9, 62.7, 69.8, 70.9, 71.3, 73.8, 74.9, 75.7, 76.4, 76.6, 76.7, 76.8, 81.0 (C-3), 81.1 (C-3′), 103.4, 104.9, 105.0 (C-1, C-1′, C-1″), 117.6 ($CH{=}CH_2$), 135.8 ($CH{=}CH_2$), 174.9 ($COCH_3$) ppm. ESI-MS: *m/z* calcd for $C_{23}H_{38}NNaO_{19}S$: 687.2; found: 710.2 $[M + Na]^+$.