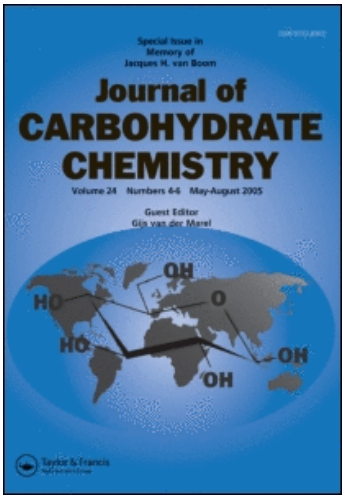

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Anup Kumar Misra[a]; Geetanjali Agnihotri[a]; Soni Kamlesh Madhusudan[a]; Pallavi Tiwari[a]
[a] Medicinal Chemistry Division, Central Drug Research Institute, Lucknow, India

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Practical Synthesis of Sulfated Analogs of Lactosamine and Sialylated Lactosamine Derivatives[#]

Anup Kumar Misra,[*] Geetanjali Agnihotri, Soni Kamlesh Madhusudan, and Pallavi Tiwari

Medicinal Chemistry Division, Central Drug Research Institute, Lucknow, India

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[*]Correspondence: Anup Kumar Misra, Medicinal Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226 001, India; E-mail: akmisra69@rediffmail.com.

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ABSTRACT

A series of β-D-Gal-(1 → 4)-β-D-GlcNAc-octyl, NeuAcα-(2 → 3)-β-D-Gal-(1 → 4)-β-D-GlcNAc-octyl, and their 6-*O*-sulfated and 6′-*O*-sulfated analogs (**1–6**) were synthesized in a concise manner starting from readily accessible monosaccharide intermediates. The syntheses involved formation of an orthogonally protected disaccharide and a trisaccharide from which all six compounds were derived.

Key Words: Sulfated lactosamine; Sialylated lactosamine; Sulfation.

INTRODUCTION

N-Acetyl lactosamine [Gal-β-(1 → 4)-GlcNAc] and its sialylated (α2,3 or α2,6) extention are quite common in cell surface glycans and glycolipids.[1] They are often modified to express differentiation antigens and functional oligosaccharides, such as Lewis X, sialyl Lewis X, which are found in human granulocytes and monocytes and acts as ligands for E-, P-, and L-selectins.[2] Sulfate groups in carbohydrates play important roles in conferring highly specific functions like cell–cell interactions, signal transduction, immunogenic recognition, and embryonic development in glycoproteins, glycolipids, and proteoglycans.[3] Keratan sulfate proteoglycan is a major component of the corneal stroma, which is composed of N-acetylated lactosamine repeating unit with sulfate residues at 6-*O* position of GlcNAc or Gal or in both, plays an important role in maintaining corneal transparency by organizing and providing proper hydration of the extracellular matrix.[4] The biosynthesis of keratan sulfate takes place by involvement of four recently cloned enzymes: β-*N*-acetylglucosaminyltransferase, β-galactosyltransferase, GlcNAc 6-*O*-sulfotransferase, and Gal 6-*O*-sulfotransferase.[5] All of them use *N*-acetyl lactosamine disaccharide as an acceptor to elongate the target glycosaminoglycans. Besides these, N-acetylated lactosamine acts as acceptors to a number of glycosyltransferases in the production of a number of glycoconjugates such as sialyl Lewis X, tumor related fucosylated poly-*N*-acetyl lactosamines, blood group antigens, etc., for example, α-fucosyltransferases (FucT IV and VII) require sialylated *N*-acetyl lactosamine and its sulfated analogs as acceptors to biosynthesize sialyl Lewis X and its sulfated analogs, ligands for selectins.[6]

For a detailed mechanistic study of the above mentioned biosynthetic pathways to make a variety of glyconjugates involving recently cloned several glycosyltransferases and sulfotransferases enzymes, a large quantity of *N*-acetyl lactosamine and sialylated *N*-acetyl lactosamine and their sulfated analogs are required, although the synthesis of a reasonable quantities of complex oligosaccharides remains one of the most challenging areas of chemistry. Therefore, a concise, efficient synthetic methodology for the synthesis of *N*-acetyl lactosamine and sialylated *N*-acetyl lactosamine and their sulfated analogs would extend the scope to get a large access of these compounds. A few reports have been appeared in the literature regarding the synthesis of these classes of compounds and most of them required lengthy multi-step sequences.[7] Chemo-enzymatic synthesis shows great promise but requires access to a panel of glycosyltransferases and sulfotransferases.[8]

We report herein a practical, high yielding chemical synthesis of the octyl glycosides of *N*-acetyl lactosamine, sialylated *N*-acetyl lactosamine, and their 6-*O*-sulfated and 6′-*O*-sulfated analogs from the readily accessible protected monosaccharide precursors (**8–16**). The key feature in this synthetic protocol is the use of common intermediate (**10** and **17**) to get access to all target molecules (**1–6**) (Fig. 1).

RESULTS AND DISCUSSION

Glycosylation of octyl 2-acetamido-3-*O*-acetyl-2-deoxy-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (**8**), prepared from *N*-acetyl-D-glucosamine in six steps and 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate (**9**)[9] using trimethylsilyl trifluoromethanesulfonate (TMSOTf) in methylene chloride afforded the β-(1 → 4) linked disaccharide (**10**) in 78% yield which on treatment with sodium methoxide in methanol furnished β-D-octyl lactosamine disaccharide (**1**) in 93% yield. De-silylation of compound **10** using HF–pyridine[10] yielded disaccharide **11** in 78% that on

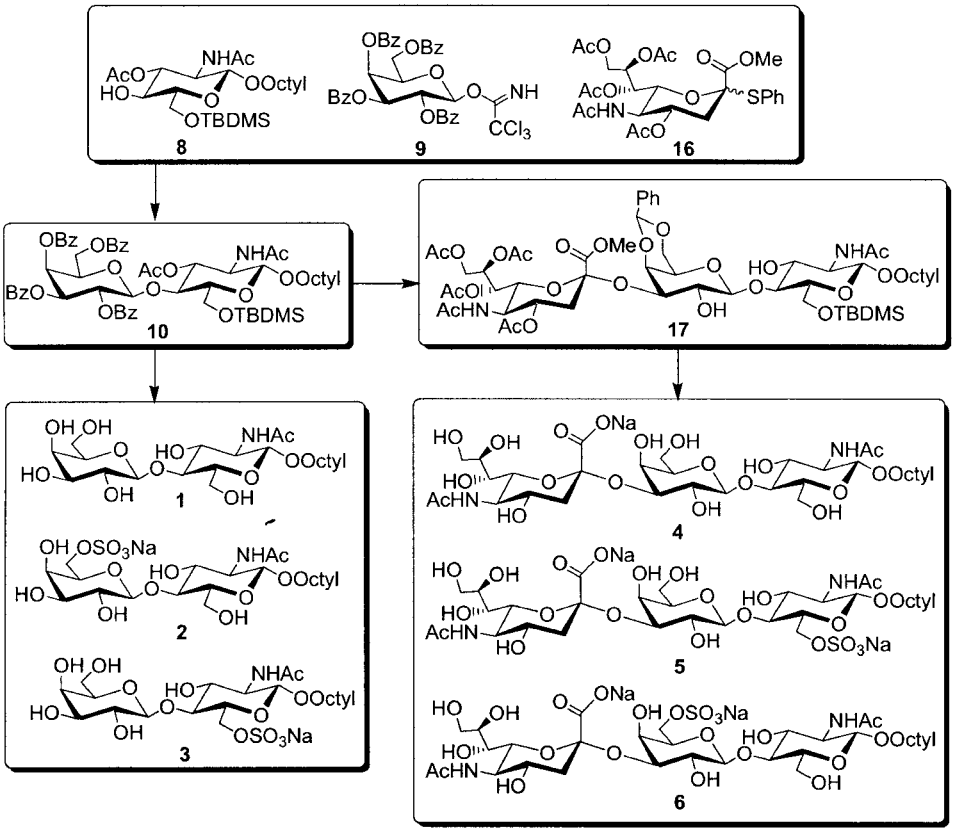
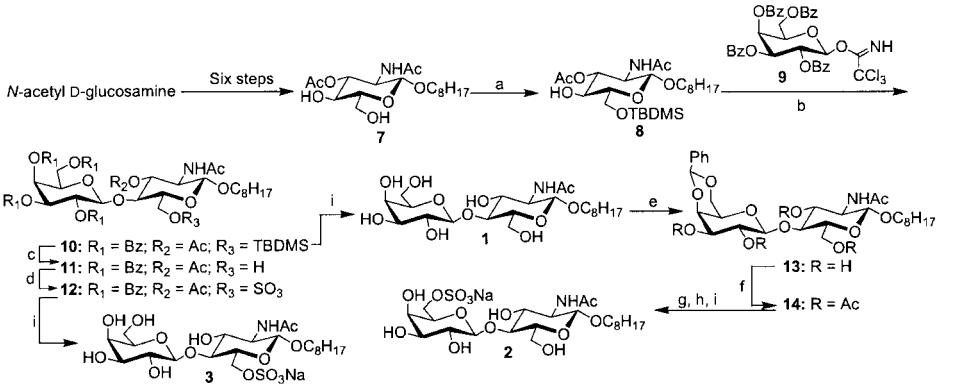


Figure 1. Synthesis of octyl glycosides of *N*-acetyl lactosamine, sialylated *N*-acetyl lactosamine, and their 6-*O*-sulfated and 6′-*O*-sulfated analogs from monosaccharide precursors (**8–16**).

sulfation[11] (sulfur trioxide–pyridine complex; $SO_3 \cdot Pyr$) followed by saponification gave the 6-*O*-sulfate **3** in 74% yield. Treatment of compound **1** with benzaldehyde dimethylacetal in presence of *p*-toluenesulfonic acid furnished octyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside (**13**) which gave compound **14** after conventional acetylation using acetic anhydride–pyridine in 78% yield in two steps. Removal of the benzylidene acetal from compound **14** through catalytic hydrogenolysis under neutral condition[11] followed by selective sulfation of the 6′-hydroxyl group ($SO_3 \cdot Pyr$) and saponification afforded 6′-*O*-sulfate derivative **2** in 72% overall yield in three steps (Sch. 1).

Sialylation of the disaccharide triol acceptor **15**, obtained from compound **13** by selective silylation of 6-hydroxyl group (TBDMSCl/imidazole),[12] using the *N*-acetyl-neuraminic acid donor[13] **16** and NIS/TfOH as promoter[14] afforded trisaccharide **17** in 56% yield. Characteristic proton and carbon signals in the ^1H and ^{13}C NMR spectra [δ 5.43 (s, PhC*H*), 5.05 (d, $J = 8.0$ Hz, H-1′), 4.60 (dd, $J = 7.8$ Hz, H − 1), 2.76 (dd, $J = 12.0$ Hz and 4.5 Hz, H-3″$_e$), and 1.75 (t, $J = 12.0$ Hz, H-3″$_a$)] confirmed the structure of **17**. In order to improve the yield of the glycosylation, several other sialyl donors and various promoters were examined but the yields were found to be similar. Trisaccharide derivative **17** has been used as a common scaffold for the preparation of two sulfated analogs **5** and **6**. De-benzylidenation under catalytic hydrogenolysis followed by removal of *tert*-butyldimethylsilyl group and acetyl groups together by using sodium methoxide afforded sialylated lactosamine trisaccharide **4** in 82% yield. Use of acidic condition to remove the benzylidene acetal resulted in some removal of acid sensitive sialic acid residue.

Trisaccharide **17** was converted to 6-hydroxylated trisaccharide **19** in 77% overall yield after a sequence of transformation, which involves removal of benzylidene acetal

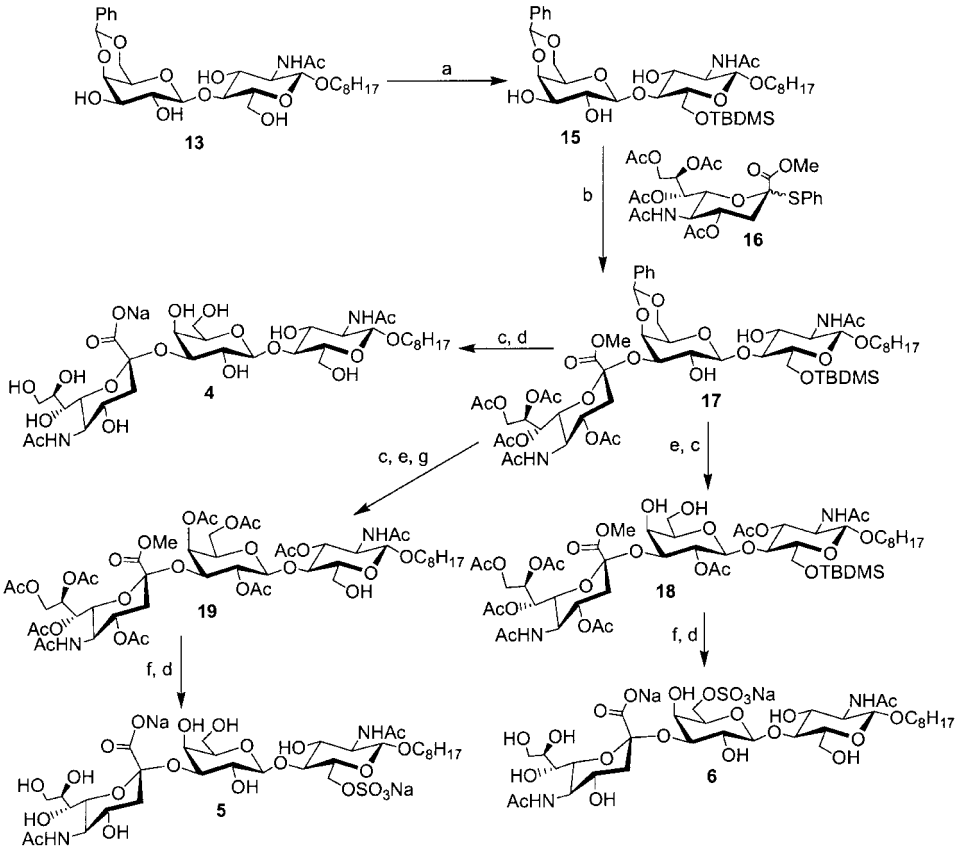


Scheme 1. Reagents: (a) TBDMS–Cl, imidazole, DMF, rt, 7 hr, 72%; (b) TMSOTf, CH_2Cl_2, MS-4Å, −10°C to rt, 3 hr, 78%; (c) HF–pyridine, THF, 0–5°C, 4 hr, 78%; (d) $SO_3 \cdot Pyr$ complex, pyridine, 6 hr, then Dowex 50W X8 (Na^+), 74%; (e) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, rt, 3 hr; (f) Ac_2O, pyridine, rt, 12 hr, 78% in two steps; (g) H_2, $Pd(OH)_2$–C (20%), MeOH, rt, 24 hr; (h) $SO_3 \cdot Pyr$ complex, pyridine, 6 hr, then Dowex 50W X8 (Na^+), 72% in two steps; (i) 0.1 M MeONa, MeOH, rt, 12 hr, 93%.

under hydrogenolytic condition, conventional acetylation followed by removal of silyl protection using HF–pyridine. Sulfation of 6-hydroxyl group of compound **19** followed by deacetylation furnished target 6-*O*-sulfated trisaccharide **5** in 78% yield. In another approach, trisaccharide **17** was converted to 4′,6′-dihydroxylated trisaccharide **18** by conventional acetylation followed by removal of benzylidene acetal using 10% Pd–C/H2 in 82% yield. Selective sulfation of 6′-hydroxyl group of **18** using $SO_3 \cdot Pyr$ followed by removal of silyl protection and acetyl group in one step under saponification condition furnished 6′-*O*-sulfated trisaccharide **6** in 74% yield (Sch. 2).

Six target compounds (**1–6**) were prepared in 4 g scale following the above mentioned reaction sequences. Selected ^1H and ^{13}C NMR and MS data for key compounds are presented below.[a] Compounds **1–6** have been evaluated as acceptors for several enzymes including various sulfotransferase and fucosyl transferase acceptors.[2,3]

[a]Partial ^1H NMR (300 MHz, D_2O): the following common signals for the octyl aglycon were observed in D_2O solution: δ 1.60–1.40 (m, 2H, OCH_2CH_2), 1.30–1.10 [m, 10 H, $OCH_2CH_2(CH_2)_5$-CH_3], 0.85 (t, 3H, octyl CH_3). H-1 indicates the anomeric proton of the GlcNAc residue, H-1′ the anomeric proton of the Gal residue and onwards. **1**: δ 4.40 (d, $J_{1,2} = 8.1$ Hz, 1H, H-1), 4.38 (d, $J_{1',2'} = 7.0$ Hz, 1H, H-1′), 4.16 (m, 2H, H-2 and H-2′), 1.96 (s, 3H, NHAc); ^{13}C NMR: δ 173.6, 105.2, 102.8, 81.0, 77.2, 76.6, 74.9, 74.4, 72.7, 70.8, 70.5, 62.6, 62.0, 56.8, 55.3, 39.1, 30.8, 30.6, 27.2, 23.8, 23.1, 14.5; TOFMS: calcd. for $C_{22}H_{41}O_{11}N$ ($M + Na^+$) 518.5; found 518.5. **2**: δ 4.48 (d, $J_{1,2} = 8.4$ Hz, 1H, H-1), 4.40 (d, $J_{1',2'} = 7.5$ Hz, 1H, H-1′), 4.18 (dd, 2H, H-$6'_{a,b}$), 2.07 (s, 3H, NHAc); ^{13}C NMR: δ 175.0, 103.3, 101.6, 79.7, 75.2, 73.3, 72.9, 72.8, 72.6, 71.3, 71.1, 68.8, 67.7, 60.8, 55.7, 31.7, 29.1, 28.9, 25.6, 22.8, 22.6, 14.0; TOFMS: calcd. for $C_{22}H_{40}O_{14}NSNa$ ($M + Na^+$) 620.2; found 620.2. **3**: 4.50 (d, $J_{1,2} = 7.5$ Hz, 1H, H-1), 4.44 (d, $J_{1',2'} = 8.1$ Hz, 1H, H-1′), 4.34 (bs, 2H, H-$6_{a,b}$), 1.99 (s, 3H, NHAc); ^{13}C NMR: δ 175.5, 103.1, 101.7, 77.9, 75.8, 73.1, 73.0, 72.9, 72.8, 71.5, 71.2, 69.2, 66.9, 61.6, 55.7, 31.7, 29.1, 28.9, 25.6, 22.8, 22.6, 14.0; TOFMS: calcd. for $C_{22}H_{40}O_{14}NSNa$ ($M + Na^+$) 620.2; found 620.2. **4**: δ 4.55 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1), 4.46 (d, $J_{1',2'} = 7.2$ Hz, 1H, H-1), 4.06 (dd, $J = 3.0$ Hz and 9.9 Hz, 1H, H-3), 2.70 (dd, $J = 4.5$ Hz and 12.3 Hz, 1H, H-$3''_e$), 1.98 (s, 6H, 2NHAc), 1.75 (t, $J = 12.0$ Hz, 1H, H-$3''_a$); ^{13}C NMR: δ 175.6, 174.9, 174.5, 103.1, 101.6, 100.4, 78.8, 76.0, 75.7, 75.3, 73.4, 73.0, 72.8, 71.1, 69.9, 68.9, 68.6. 68.0, 63.1, 61.6, 60.6, 55.7, 52.2, 40.2, 31.7, 29.1 (2C), 28.9, 25.6, 22.8, 22.6 (2C), 14.0; TOFMS: calcd. for $C_{33}H_{57}O_{19}N_2Na$ ($M + Na^+$) 831.8; found 831.8. **5**: δ 4.61 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1), 4.53 (d, $J_{1',2'} = 7.8$ Hz, 1H, H-1′), 4.36 (q, 2H, H-$6_{a,b}$), 4.11 (dd, $J = 3.3$ Hz and 9.1 Hz, 1H, H-2), 2.74 (dd, $J = 4.8$ and 12.0 Hz, 1H, H-$3''_e$), 2.01, 2.00 (2s, 6H, 2NHAc), 1.78 (t, $J = 12.0$ Hz, 1H, H-$3''_a$); ^{13}C NMR: δ 175.5, 175.0, 174.6, 102.6, 101.7, 100.3, 77.7, 75.9, 75.6, 73.4, 73.1, 72.9, 72.0, 71.2, 70.0, 69.0, 68.6, 68.0, 66.9, 63.0, 61.6, 55.8, 52.3, 40.1, 31.7, 29.1 (2C), 28.9, 25.6, 22.8, 22.6 (2C), 14.0; TOFMS: calcd. for $C_{33}H_{56}O_{22}N_2SNa_2$ ($M + Na^+$) 933.8; found 933.8. **6**: δ 4.56 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1), 4.48 (d, $J_{1',2'} = 7.8$ Hz, 1H, H-1′), 2.71 (dd, $J = 4.5$ Hz and 12.0 Hz, 1H, H-$3''_e$), 1.88 (s, 6H, 2NHAc), 1.76 (t, $J = 12$ Hz, 1H, H-$3''_a$); ^{13}C NMR: δ 175.5, 175.0, 174.4, 102.9, 101.6, 100.5, 79.7, 75.8, 75.2, 73.4, 73.2, 72.7, 72.3, 71.1, 69.8, 68.9, 68.7, 68.1, 68.0, 63.1, 60.8, 55.7, 52.2, 40.0, 31.7, 29.1 (2C), 28.9, 25.6, 22.8, 22.6 (2C), 14.0; TOFMS: calcd. for $C_{33}H_{56}O_{22}N_2SNa_2$ ($M + Na^+$) 933.8; found 933.8.



Scheme 2. Reagents: (a) TBDMS–Cl, imidazole, DMF, rt, 5 hr, 72%; (b) NIS, TfOH, CH_3CN–CH_2Cl2 (5 : 1), MS-3Å, −20°C, 18 hr, 56%; (c) H, $Pd(OH)_2$–C (20%), MeOH, rt, 32 hr; (d) 0.1 M MeONa, MeOH, rt, 12 hr, then a few drops of water, rt, 12 hr, 82% in two steps; (e) Ac_2O, pyridine, rt, 12 hr, quantitative; (f) $SO_3 \cdot$Pyr complex, pyridine, 0°C–rt, 8 hr, then Dowex 50W X8 (Na^+), 74%; (g) HF–pyridine, THF, 0–5°C, 4 hr, 78%.

CONCLUSION

In conclusion, the 6- and 6′-*O*-sulfated analogs of lactosamine and sialyllactosamine were synthesized following a practical high yielding procedure utilizing a common disaccharide scaffold. Most of the methodologies used in this synthetic scheme are very convenient and high yielding thus providing this report a potential alternative to the existing methods. This high yielding practical synthetic protocol for the synthesis of these classes of molecules will certainly add value to the glycobiology.

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