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EFFICIENT SYNTHESIS OF 3′-GLYCOSYLATED LacNAc-BASED OLIGOSACCHARIDES

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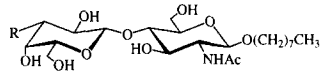
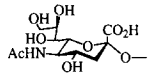
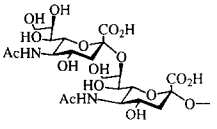
Abstract: LacNAc-based oligosaccharides, including sialyl-(2→3)-LacNAc, dimeric sialyl-(2→3)-LacNAc, trimeric sialyl-(2→3)-LacNAc, β-glucuronyl-(1→3)-LacNAc, and 3-sulfo-β-glucuronyl-(1→3)-LacNAc, were synthesized efficiently from a single protected LacNAc derivative having both OH-3′ and 4′ unprotected.

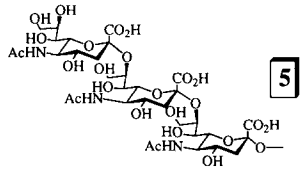
Glycoscience has experienced a surge of interest in recent years as the biological roles of various carbohydrates have been elucidated.[1] This has led to an increased demand for oligosaccharides for biological studies.[2] Systematic studies on the binding of oligosaccharides with proteins have shown the general trend that the ligand bound to a protein is usually no larger than a tri- or tetrasaccharide. Many ligands are LacNAc derivatives,[3] synthetic LacNAc-based oligosaccharides are therefore widely sought for biological studies. Here we report the efficient synthesis of five 3′-glycosylated LacNAc-based oligosaccharides: the parent LacNAc disaccharide **1**, sialyl-(2→3)-LacNAc trisaccharide **2**, dimeric sialyl-(2→3)-LacNAc tetrasaccharide **3**, trimeric sialyl-(2→3)-LacNAc pentasaccharide **4**, β-glucuronyl-(1→3)-LacNAc trisaccharide **5**, and 3-sulfo-β-glucuronyl-(1→3)-LacNAc trisaccharide **6**.

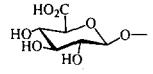


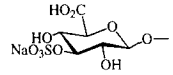
1 R= OH

2 R= 

3 R= 

4 R= 

5 R= 

6 R= 

The hydrophobic octyl group was selected as the aglycone to simplify the isolation of the deblocked products on C-18 resin.[4] Glycosylation of octyl 3,6-di-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranoside (**7**)[5] with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**8**) in the presence of AgOTf and 4 Å molecular sieves

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afforded the desired β-linked disaccharide. Subsequent *O*-deacetylation provided disaccharide **9** in 71% yield (two steps). After hydrogenation, the LacNAc disaccharide **1** was obtained in 75% yield. Treatment of compound **9** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid, followed by benzylation with BnBr/NaH and mildly acidic hydrolytic cleavage of the isopropylidene group afforded the suitably protected glycosyl acceptor **10** in 58% yield (three steps).

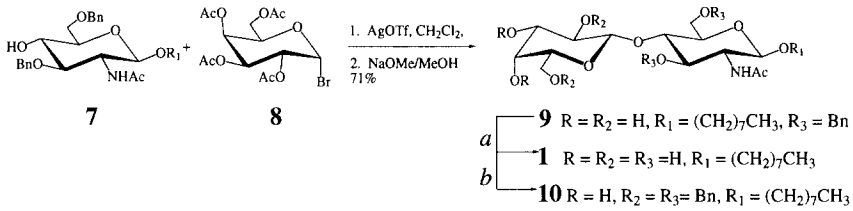

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Figure 1: (a) Pd/C, H_2, MeOH, 75%; (b) 1. $Me_2C(OMe)_2$, CSA; 2. NaH, BnBr, *n*-Bu_4NI; 3. AcOH-H_2O (8:2), 52% (three steps).

From diol **10**, we synthesized sialyl LacNAc, dimeric sialyl LacNAc and trimeric sialyl LacNAc derivatives for use as substrates for polysialyltransferase.[6] Glycosylation of **10** with the sialyl donor **11**[7] in acetonitrile at -35 °C for 10 h, in the presence of NIS/TfOH and 3 Å powdered molecular sieves, afforded the desired α-glycoside **12** in 51% yield. *O*-Deacetylation with sodium methoxide in methanol, followed by saponification of the methyl ester and catalytic hydrogenolysis of the benzyl groups (10% Pd/C), yielded the 3′-sialyl LacNAc trisaccharide **2** in 65% yield (three steps).

Using similar procedures, condensation of **10** with the dimeric sialyl donor **13**[8] gave the expected tetrasaccharide; after deacetylation and saponification, **14** was isolated in 25% yield (two steps). The ^1H NMR signals at 2.90 ppm (dd, 1H) and 2.70 ppm (dd, 1H) indicated α-glycoside formation. Catalytic hydrogenolysis (10% Pd/C) of the benzyl groups in methanol, followed by purification on Sephadex LH-20, yielded the 3′-dimeric sialyl LacNAc tetrasaccharide **3** in 50% yield.

Coupling the trimeric sialyl acid donor **15**[9] with acceptor **10** for 24 h under the conditions described above, afforded the pentasaccharide. After deacetylation and saponification, pentasaccharide **16** was isolated in 20% yield (three steps). The ^1H NMR signals at 2.91 ppm (dd, 1H), 2.71 ppm (dd, 1H) and 2.55 ppm (dd, 1H) supported the α-glycosidic linkage. After hydrogenation on Pd/C (MeOH/AcOH, 2 days), the final 3′-trimeric sialyl LacNAc **4** was obtained in 40% yield after purification on Sephadex LH-20.

Sulfated and unsulfated β-glucuronyl-(1→3)-LacNAc trisaccharides were required as substrates and standards in a study on the enzymatic sulfation of β-glucuronyl-(1→3)-LacNAc derivatives.[10] The glycosylation of **10** with glucuronyl imidate **17**[11] was performed at -20 °C in the presence of 0.15 equiv of BF_3/etherate and afforded the 1→3 linked trisaccharide **18** in 51% yield. After acetylation, the signal for H-4 of the galactosyl

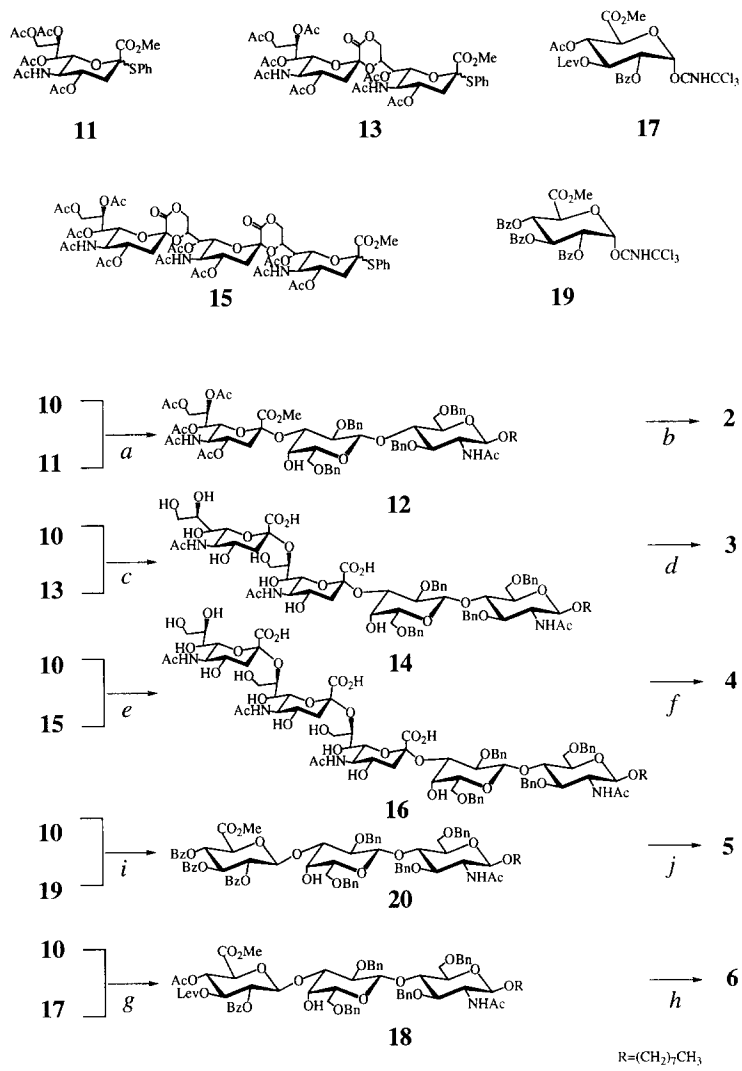

Figure 2: (a) NIS (2 equiv), TfOH (0.2 equiv), CH_3CN, 3 Å MS, -35 °C, 2 h, (51%); (b) 1. Pd/C, MeOH/AcOH (1:1), H_2; 2. MeONa/MeOH, 12 h; 3. NaOH/H_2O/MeOH , (1.-3.: 65%); (c) 1. NIS (3 equiv), TfOH (0.2 equiv), CH_3CN, 3 Å MS, -35 °C, 24 h; 2. MeONa/MeOH/H_2O, 24 h, (1.-2.: 25%); (d) Pd/C, MeOH/AcOH, H_2, (50%); (e) 1. NIS (3 equiv), TfOH (0.2 equiv), CH_3CN, 3 Å MS, -35 °C, 24 h; 2. MeONa/MeOH/H_2O, 24 h , (1.-2.: 20%); (f) Pd/C, MeOH/AcOH, H_2, (40%); (g) BF_3/ether (0.15 equiv), CH_2Cl_2, 4 Å MS, -20 °C, 4 h , (51%); (h) 1. NH_2NH_2.AcOH, EtOH, rt, 1 h; 2. SO_3.Et_3N, DMF, 40 °C, 12 h; 3. MeONa/MeOH/H_2O; 4. Pd/C, MeOH, H_2, (1.-4. 55%); (i) BF_3/ether (0.15 equiv), CH_2Cl_2, 4 Å MS, -20 °C, 2 h, (70%); (j) 1. MeONa/MeOH/H_2O; 2. Pd/C, MeOH, H_2, (1.-2. 45%).

moiety was shifted from 4.01 ppm (d, J = 2.7 Hz, 1H) to 4.79 ppm (d, J = 2.7 Hz, 1H), confirming the 1→3 linkage. Selective removal of the levulinoyl group was achieved with hydrazine-monoacetate. Treatment of the product with excess sulfur trioxide trimethylamine complex in DMF at 40 °C followed by deacetylation with methanolic sodium methoxide, saponification of the methyl ester with sodium hydroxide and catalytic hydrogenolysis (Pd/C, 10%) afforded 3-*O*-sulfo-β-glucuronyl-(1→3)-LacNAc **6** in 55% yield after purification on Sephadex LH-20 (four steps). Deprotection of **18** by hydrogenation, deacetylation and saponification gave β-glucuronyl-(1→3)-LacNAc **5** in 62% yield (three steps).

The perbenzoyl glucuronyl donor **19** was prepared from methyl [2-(trimethylsilyl)ethyl β-D-glucopyranoside]uronate[11] in 70% yield by benzoylation, removal of the 2-(trimethylsilyl) ether group, and formation of the trichloroacetimidate. Glycosylation of **10** with **19** in the presence of BF_3/etherate at -20 °C afforded the 1→3 linked β-glycoside **20** in 70% yield. After catalytic hydrogenolysis (10% Pd/C) of the benzyl groups in methanol, *O*-debenzoylation with sodium methoxide in methanol, and subsequent saponification of methyl ester group with sodium hydroxide, the trisaccharide **5** was obtained in 45% yield (three steps).

In conclusion, the partially protected LacNAc diol **10** was found to be an efficient acceptor for the synthesis of biologically important 3′-glycosylated LacNAc-based oligosaccharides.

Acknowledgment

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References and notes

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Spectral data for selected new compounds:

1, ^{1}H NMR (300 MHz, CD_3OD): δ 4.38 (1H, J = 7.6 Hz, H-1), 4.40 (1H, J = 7.6 Hz, H-1′), 1.95 (s, 3H, NHCOCH_3), 0.88 (t, 3H, J = 6.6 Hz, CH_3).

2, ^{1}H NMR (300 MHz, CD_3OD): δ 4.44 (d, 1H, J = 7.8 Hz, H-1), 4.36 (d, 1H, J = 8.1 Hz, H-1′), 4.04 (dd, 1H, $J_{3',2'}$ = 9.6 Hz, $J_{3',4'}$ = 3 Hz, H-3′), 2.75 (dd, 1H, H-3″eq), 2.00 (s, 3H, NHCOCH_3), 1.95 (s, 3H, NHCOCH_3), 0.89 (t, 3H, J = 6.6Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 102.9, 101.8, 100.9 (C-1, C-1′ and C-1″).

3, ^{1}H NMR (300 MHz, CD_3OD): δ 4.44 (d, 1H, J = 7.2 Hz, H-1), 4.38 (d, 1H, J = 8.0 Hz, H-1′), 2.48 (m, 2H, H-3″eq and H-3‴eq), 2.02 (s, 6H, 2×NHCOCH_3), 1.96 (s, 3H, NHCOCH_3), 0.90 (t, 3H, J = 6.6 Hz, CH_3); ESMS: *m/z* 1077 $[M]^+$.

4, ^{1}H NMR (500 MHz, CD_3OD): δ 4.40 (d, 1H, J = 7.7 Hz, H-1), 4.38 (d, 1H, J = 8.0 Hz, H-1′) 2.89 (dd, 1H), 2.49 (dd, 1H), 2.45 (dd, 1H), 2.04 (s, 3H, NHCOCH_3), 2.03 (s, 3H, NHCOCH_3), 2.01 (s, 6H, 2×NHCOCH_3), 0.89 (t, 3H, J = 6.7 Hz, CH_3); ESMS: *m/z* 1367 $[M]^+$.

5, ^{1}H NMR (300 MHz, CD_3OD): δ 4.68 (d, 1H, J = 7.8 Hz, H-1), 4.52 (d, 1H, J = 7.6 Hz, H-1′), 4.50 (d, 1H, J = 7.0 Hz, H-1″), 4.11 (d, 1H, J < 1 Hz, H-4′), 1.95 (s, 3H, NHCOCH_3), 0.89 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 105.4, 105.4, 102.8 (C-1, C-1′ and C-1″); ESMS: *m/z* 670 $[M]^+$.

6, ^{1}H NMR (300 MHz, CD_3OD): δ 4.65 (d, 1H, H-1), 4.49 (m, 2H, H-1′ and H-1″), 4.30 (t, 1H, H-3″), 4.11 (d, 1H, J < 1 Hz, H-4″), 1.95 (s, 3H, NHCOCH_3), 0.89 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 105.4, 105.3, 102.9 (C-1, C-1′ and C-1″); ESMS: *m/z* 772 $[M]^+$.

9, ^{1}H NMR (300 MHz, CD_3OD): δ 4.45 (d, 1H, J = 7.8 Hz, H-1′), 4.39 (d, 1H, J = 7.8 Hz, H-1), 1.91 (s, 3H, NHCOCH_3), 0.88 (t, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 104.4, 102.8 (C-1 and C-1′).

10, ^{1}H NMR (300 MHz, CD_3OD): δ 4.38 (d, 1H, J = 7.2 Hz, H-1), 4.38 (d, 1H, J = 7.3 Hz, H-1′), 1.90 (s, 3H, NHCOCH_3), 0.90 (s, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 104.3, 102.8 (C-1, C-1′).

14, ^{1}H NMR (300 MHz, CD_3OD): δ 7.1-7.4 (m, 20H, 4×CH_2Ph), 2.64 (dd, 1H, H-3′eq), 2.59 (dd, 1H, H-3‴eq), 2.0 (s, 6H, 2×NHCOCH_3), 1.81 (s, 3H, NHCOCH_3), 0.90 (s, 3H, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 104.9, 103.8, 103.0, 102.5 (C-1, C-1′, C-2″ and C-2‴).

16, ^{1}H NMR (300 MHz, CD_3OD): δ 7.1-7.4 (m, 20H, 4×CH_2Ph), 2.90 (dd, 1H, H-3″eq), 2.70 (dd, 1H, H-3‴eq), 2.58 (dd, 1H, H-3⁗eq), 2.00 (s, 6H, 2×NHCOCH_3), 1.98 (s, 3H, NHCOCH_3), 1.80 (s, 3H, NHCOCH_3); 0.89 (t, 3H, J = 6.6 Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 104.0, 103.5, 103.0, 102.8, 102.2 (C-1, C-1′, C-2″. C-2‴and C-2⁗).

18, ^{1}H NMR (300 MHz, CD_3OD): δ 5.59 (t, 1H, J = 9.0 Hz, H-2″), 5.31 (d, 1H, J = 8.1 Hz, H-1″), 5.21 (t, 1H, J = 9.9 Hz, H-3″), 5.12 (d, 1H, J = 10.8 Hz, H-4″), 4.59 (d, 1H, J = 8.1 Hz, H-1′), 4.43 (d, 1H, J = 8.1 Hz, H-1), 4.01 (d, 1H, J = 2.7 Hz, H-4′), 3.59 (s, 3H, OCH_3), 2.06 (s, 3H, COCH_3), 1.99 (s, 3H, OCOCH_3), 1.96 (s, 3H, NHCOCH_3), 0.89 (t, 3H, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 102.51, 101.49, 99.34 (C-1, C-1′ and C-1″).

20, ^{1}H NMR (300 MHz, CD_3OD): δ 6.03 (t, 1H, J = 9.9 Hz), 5.58 (t, 1H, J = 9.0 Hz), 5.57 (d, 1H, J = 7.5 Hz, H-1″), 5.13 (d, 1H, J = 10.8 Hz, H-5″), 4.60 (d, 1H, J = 7.5 Hz, H-1), 4.30 (d, 1H, J = 7.5 Hz, H-1′), 3.50 (s, 3H, OCH_3), 1.96 (s, 3H, NHCOCH_3), 0.88 (t, 3H, J = 6.0 Hz, CH_3); ^{13}C NMR (300 MHz, CD_3OD): δ 103.86, 102.99, 102.76 (C-1, C-1′ and C-1″).