Syntheses of 2-Acetamido-2-deoxy-4-O-β-D-galactopyranosyl-
D-glucopyranose (N-Acetyllactosamine) Derivatives

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In order to provide useful key intermediates for syntheses of complex oligosaccharides, anomic 1,2,3,4,6,6′-hexa-O-acetyl-N-acetyllactosamines (8:α, and 9:β) and the corresponding 3-O-benzyl ethers (5:α, and 6:β) were synthesized.

Condensation of 1,6-anhydro-3-O-benzyl-β-N-acetylglucosamine with acetobromogalactose by a conventional Koenigs−Knorr procedure, followed by selective acetylation of the 1,6-anhydro-β-linkage, provided 5 and 6. Debenzylation of 5 and 6 gave 8 and 9, respectively.

Keywords—Koenigs−Knorr synthesis; 1,6-anhydro-3-O-benzyl-β-N-acetylglucosamine; 1,6-anhydro-β-N-acetyllactosamin derivative; anomic octaacetyllactosamine; anomic heptaacetyllactosamine; anomic 3-O-benzyl-heptaacetyllactosamine

Numerous complex glycoconjugates of biological interest as well as oligosaccharides in human milk are composed of N-acetyllactosamine. In complex oligosaccharides, sugar chains often branch at the C-3 position of N-acetyllactosamine. In order to provide useful key intermediates for syntheses of complex oligosaccharides, we developed syntheses of the anomic acetylated lactosamine derivatives having a benzyl or an unprotected hydroxyl group at the C-3 position. The results are reported here.

Condensation of 2-acetamido-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranose (1) with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (acetobromogalactose) (2) by a conventional Koenigs−Knorr procedure provided acetylated 1,6-anhydro-3-O-benzyl-β-N-acetyl-β-lactosamine (3) and a small amount of the N-acetyllactosamine derivative (4). The proton or carbon-13 nuclear magnetic resonance (D- or 13C-NMR) and infrared (IR) spectra were consistent with the assigned structures. Compound (4) resulted from trans-acetylation of 1 with 2, and the structure was confirmed by comparison with an authentic sample. Such a side reaction is well documented in the Koenigs−Knorr condensation.

As it has been found that benzyl ethers are readily cleaved by acetylozying reagents, the optimum conditions for selective cleavage of the 1,6-anhydro-β-linkage of 3 without affecting...
the benzyl group were investigated. After several trials, treatment of 3 with boron trifluoride etherate-acetic anhydride for 5 min at 0°C was found to be satisfactory; a longer reaction time at room temperature resulted in the formation of fully acetylated N-acetyl-α-lactosamine (7). Under these conditions, 3 provided the α-acetate (5) and β-acetate (6) in the yield ratio of ca. 2:1 together with unreacted 3, which was recycled. The configurations of 5 and 6 were determined from specific rotations and the chemical-shift values due to the anomeric carbons in 13C-NMR.

Debenzylation of 5 and 6 yielded the anomeric hexa-O-acetyl-N-acetyllactosamines (8: α-anomer, and 9: β-anomer), and acetylation of 8 and 9 gave the anomeric hepta-O-acetyl-N-acetyllactosamines (7: α-anomer, and 10: β-anomer), respectively. The melting point and specific rotation of 10 were in good agreement with the literature values, and the results of 1H-NMR spectroscopy also supported the assigned structure.

**Experimental**

Unless otherwise indicated, instruments and chromatographic conditions used in the experimental section were the same as before. Thin-layer chromatography (TLC) was performed on pre-coated silica gel plates of 0.25 or 0.5 mm thick (Kieselgel 60 F254, Merck) using (A), CHCl3-ether-MeOH (10:10:1, v/v); (B), CHCl3-acetone (3:1). Detection was effected with anisaldehydese-H2SO4-EtOH spray reagent at 125°C, or by ultraviolet (UV) irradiation at 254 nm.

2′,3′,4′,6′-Tetra-O-acetyl-1,6-anhydro-3-O-benzyl-N-acetyl-β-lactosamine (3) and 2-Acetamido-4-O-acetyl-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranose (4) — A solution of 2 (1.53 g, 3.72 mmol) in benzene (8 ml) was added to a suspension of 1H (240 mg, 0.79 mmol), Hg(CN)2 (1.4 g), and Drierite (0.5 g) in nitromethane (8 ml). After being stirred overnight at 55°C, the mixture was diluted with CHCl3, then filtered, and the filtrate was successively washed with ice-H2O,aq. KI and NaHCO3, and ice-H2O. Desiccation (MgSO4) and removal of the solvent provided a syrup, which was chromatographed on a column with hexane–ether (1:4). The fractions having Rf 0.45 (solvent A) were re-chromatographed with benzene–EtOAc (2:1) to give 3 (262.4 mg, 53%), [α]D20 -84° (c = 0.29, CHCl3), as a foamy solid. 1H-NMR (CDCl3): 1.98, 2.02, 2.06, 2.11, 2.15 (15H, each s, OAc×4, NAc), 6.23 (1H, d, JNH=10 Hz, NH, exchangeable with D2O), 7.30 (5H, s, aromatic protons). 13C-NMR (CDCl3): 101.26 (1JCH=175.78 Hz, C-1), 99.07 (1JCH=156.25 Hz, C-1'), IR νmax cm−1: 3390 (NH), 1750 (OAc), 1674 (amide I), 1518 (amide II). TLC: Rf 0.45 (solvent A), 0.50 (B).

Anal. Calcd for C24H30O11N: C, 55.86; H, 5.98; N, 2.25. Found: C, 55.58; H, 6.01; N, 2.17.

From the fractions having Rf 0.46 (solvent A), 4 (42 mg, 15.7%), mp 115–116°C, [α]D20 -86.8° (c = 0.24, CHCl3), was isolated after removal of the solvent. 1H-NMR (CDCl3): 1.99, 2.10 (6H, each s, OAc, NAc), 5.38 (1H, s, H-1), 5.93 (1H, d, JNH=8 Hz, NH), 7.32 (5H, s, aromatic protons). TLC: Rf 0.46 (solvent A), 0.53 (B). The product was indistinguishable from authentic 2-acetamido-4-O-acetyl-1,6-anhydro-3-O-benzyl-2-deoxy-β-D-glucopyranose by mixed mp, IR, and TLC. [lit. mp 115–116°C, [α]D20 -93.8° (c = 1, CHCl3), 1,2,3,4,6,6′-Hexa-O-acetyl-3-benzyl-N-acetyl-α- and β-lactosamines (5 and 6) — A solution of 3 (98 mg) in ice-cold acetylation reagent [boron trifluoride etherate–Ac2O (1:25, v/v)] was stirred for 5 min at 0°C. A piece of ice was then added, and the mixture was stirred for 2 h to decompose excess Ac2O. After neutralization with NaHCO3, the whole was extracted with CHCl3. The extracts were washed with H2O, dried (MgSO4), and concentrated to a syrup. On preparative TLC with solvent A, 3 (41 mg, 41.8%) was recovered from the zone having Rf 0.45, and recycled. Compound 6 (19 mg, 16.5%), [α]D20 -29° (c = 0.48, CHCl3), was isolated as a foamy solid from the zone having Rf 0.41. 1H-NMR (CDCl3): 2.00, 2.02, 2.04, 2.05, 2.09, 2.12, 2.16 (21H, each s, OAc×6, NAc), 4.73 (1H, d, J1=2=6 Hz, H-1', β-Gal), 5.80 (1H, d, J2=4 Hz, H-1, β-Glc), 6.27 (1H, d, JNH=10 Hz, NH), 7.36 (5H, s, aromatic protons). 13C-NMR (CDCl3): 99.65 (1JC-1=158.69 Hz, C-1'), 92.00 (1JC-1=173.33 Hz, C-1). IR νmax cm−1: 3380 (NH), 1750 (OAc), 1667 (amide I), 1540 (amide II). TLC: Rf 0.41 (solvent A), 0.46 (B). Anal. Calcd for C33H34N2O17·1/2H2O: C, 53.95; H, 6.04; N, 1.91. Found: C, 53.68; H, 5.92; N, 1.96.

From the zone having Rf 0.33 (solvent A), 5 (40.7 mg, 34.8%), [α]D20 +54° (c=0.2, CHCl3), was isolated as a glassy mass. 1H-NMR (CDCl3): 1.96, 1.99, 2.08, 2.10 (21H, each s, OAc×6, NAc), 6.12 (1H, d, J1=4 Hz, H-1, α-Glc), 7.38 (5H, s, aromatic protons). 13C-NMR (CDCl3): 101.11 (1JC-1=158.69 Hz, C-1'), 90.25 (1JC-1=178.22 Hz, C-1). IR νmax cm−1: 3380 (NH), 1750 (OAc), 1665 (amide I), 1535 (amide II). TLC: Rf 0.33 (solvent A), 0.41 (B). Anal. Calcd for C33H34N2O17·1/2H2O: C, 53.30; H, 6.10; N, 1.88. Found: C, 53.32; H, 5.86; N, 1.88.

1,2,3,3′,4′,6,6′-Hepta-O-acetyl-N-acetyl-α-lactosamine (7) — A solution of 3 (20 mg) in acetylating reagent (0.5 ml) was stirred for 3 d at room temperature. The mixture was treated as described for the preparation of 5 and 6. On preparative TLC with solvent A, crude 7 was separated from the zone having Rf 0.23. Pure 7 (11.9 mg, 54.8%), mp 228–230°C, [α]D20 +62.1° (c=0.23, CHCl3), was crystallized from 2-PrOH.
as prisms. $^1$H-NMR (CDCl$_3$): 1.93, 1.96, 2.06, 2.09, 2.11, 2.15, 2.18 (24H, each s, OAc×7, NAc), 4.55 (1H, d, J$_{1,2}$=7 Hz, H-1', β-Gal), 5.74 (1H, d, J$_{NH,2}$=9 Hz, NH), 6.10 (1H, d, J$_{1,2}$=4 Hz, H-1, α-Glc). TLC: RF 0.23 (solvent A), 0.29 (B). The product was indistinguishable from authentic hepta-O-acetyl-N-acetyl-α-lactosamine(8) by IR, mixed mp, and TLC. [lit. mp 230—231°C, [α]$_D$ +50.1° (c=0.96, CHCl$_3$)],

$1,2,3,4,6$-Hexa-O-acetyl-N-acetyl-α-lactosamine (8) — Hydrogenolytic debenzylation of 5 (24.8 mg) in MeOH (2.5 ml) with 10% Pd on charcoal (25 mg) was carried out at room temperature under atmospheric pressure. After removal of the catalyst and solvent, 8 (19.3 mg, 91.3%), [α]$_D$ +79.8° (c=0.23, CHCl$_3$), was obtained as a foamy solid. $^1$H-NMR (CDCl$_3$): 1.98, 2.00, 2.06, 2.08, 2.09, 2.12, 2.16 (21H, each s, OAc×6, NAc), 4.59 (1H, d, J$_{1,2}$=7 Hz, H-1', β-Gal), 5.60 (1H, d, J$_{NH,2}$=10 Hz, NH), 6.15 (1H, d, J$_{1,2}$=3 Hz, H-1, α-Glc). IR $\nu_{max}$ cm$^{-1}$: 3470 (OH), 3380 (NH), 1753 (OAc), 1672 (amide I), 1540 (amide II). TLC: RF 0.16 (solvent A), 0.18 (B). Anal. Calcd for C$_{28}$H$_{37}$NO$_{17}$·H$_2$O: C, 47.78; H, 6.01; N, 2.14. Found: C, 47.64; H, 5.53; N, 2.04.

Acetylation of 8 (27 mg) with Ac$_2$O (0.5 ml) and pyridine (1 ml) provided the octaacetate (28.4 mg, 98.6%), which was indistinguishable from 7 by IR, mixed mp, and TLC.

$1,2,3,4,6$-Hexa-O-acetyl-N-acetyl-β-lactosamine (9) — Debenzylation of 6 (20.5 mg) with 10% Pd on charcoal (20 mg) in MeOH (2 ml) was carried out as described for 8 to provide 9 (16.6 mg, 88.5%), [α]$_D$ +18.8° (c=0.36, CHCl$_3$), as a foamy solid. $^1$H-NMR (CDCl$_3$): 1.99, 2.02, 2.12, 2.22 (21H, each s, OAc×6, NAc), 4.57 (1H, d, J$_{1,2}$=8 Hz, H-1', β-Gal), 5.62 (1H, d, J$_{NH,2}$=8 Hz, NH), 5.69 (1H, d, J$_{1,2}$=8 Hz, H-1, β-Glc). IR $\nu_{max}$ cm$^{-1}$: 3480 (OH), 3390 (NH), 1750 (OAc), 1668 (amide I), 1525 (amide II). TLC: RF 0.21 (solvent A), 0.16 (B). Anal. Calcd for C$_{28}$H$_{37}$NO$_{17}$·2H$_2$O: C, 46.50; H, 6.15; N, 2.09. Found: C, 46.41; H, 5.91; N, 1.91.

$1,2,3,4,6,6$-Hepta-O-acetyl-N-acetyl-β-lactosamine (10) — Acetylation of 9 (16.6 mg) with Ac$_2$O (0.5 ml) and pyridine (1 ml) overnight at room temperature was carried out. The mixture was concentrated to provide crude 10 (16.7 mg, 99.6%), which was crystallized from benzene–hexane as fine needles, mp 109—112°C, [α]$_D$ +11.3° (c=0.35, CHCl$_3$). $^1$H-NMR (CDCl$_3$): 1.96, 1.97, 2.05, 2.09, 2.11, 2.15 (24H, each s, OAc×7, NAc), 4.51 (1H, d, J$_{1,2}$=8 Hz, H-1', β-Gal), 5.64 (1H, d, J$_{1,2}$=8 Hz, H-1, β-Glc), 5.88 (1H, d, J$_{NH,2}$=9 Hz, NH). TLC: RF 0.25 (solvent A), 0.25 (B). [lit. $^3$H mp 108—110°C, [α]$_D$ +7.05° (c=0.95, CHCl$_3$)].

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References and Notes


