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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, anomeric 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.

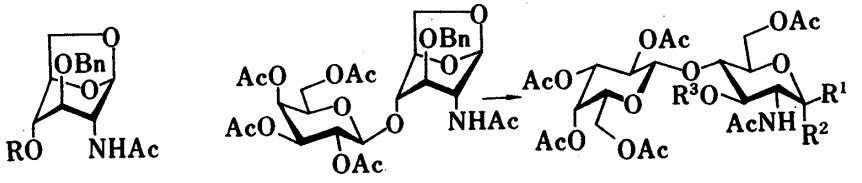
Condensation of 1,6-anhydro-3-*O*-benzyl-β-*N*-acetylglucosamine with acetobromogalactose by a conventional Koenigs–Knorr procedure, followed by selective acetolysis of the 1,6-anhydro-β-linkage, provided **5** and **6**. Debenzylation of **5** and **6** gave **8** and **9**, respectively.

Keywords——Koenigs–Knorr synthesis; 1,6-anhydro-3-*O*-benzyl-β-*N*-acetylglucosamine; 1,6-anhydro-β-*N*-acetyllactosamine derivative; anomeric octaacetyllactosamine; anomeric heptaacetyllactosamine; anomeric 3-*O*-benzyl-heptaacetyllactosamine

Numerous complex glycoconjugates of biological interest as well as oligosaccharides in human milk are composed of *N*-acetyllactosamine.[1] 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 anomeric 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**)[2] 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-*N*-acetyl-β-lactosamine (**3**) and a small amount of the *N*-acetylglucosamine derivative (**4**). The proton or carbon-13 nuclear magnetic resonance (^{1}H- or ^{13}C-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.[3] Such a side reaction is well documented in the Koenigs–Knorr condensation.[4]

As it has been found that benzyl ethers are readily cleaved by acetolyzing reagents,[5] the optimum conditions for selective cleavage of the 1,6-anhydro-β-linkage of **3** without affecting



1: R=H
4: R=Ac

3

5: R^1=H, R^2=OAc, R^3=Bn
6: R^1=OAc, R^2=H, R^3=Bn
7: R^1=H, R^2=OAc, R^3=Ac
8: R^1=H, R^2=OAc, R^3=H
9: R^1=OAc, R^2=H, R^3=H
10: R^1=OAc, R^2=H, R^3=Ac

Ac=acetyl
Bn=benzyl

Chart 1

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**).[1b] 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 ^{13}C-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,[6] and the results of ^{1}H-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.[2] Thin-layer chromatography (TLC) was performed on pre-coated silica gel plates of 0.25 or 0.5 mm thick (Kieselgel 60 F_{254}, Merck) using (A), $CHCl_3$–ether–MeOH (10:10:1, v/v); (B), $CHCl_3$–acetone (3:1). Detection was effected with anisaldehyde-H_2SO_4-EtOH spray reagent at 125°C,[7] 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 **1**[2] (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 $CHCl_3$, then filtered, and the filtrate was successively washed with ice-H_2O, aq. KI and $NaHCO_3$, and ice-H_2O. Desiccation ($MgSO_4$) 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%), $[\alpha]_D^{17}$ −84° (*c*=0.29, $CHCl_3$), as a foamy solid. ^{1}H-NMR ($CDCl_3$): 1.98, 2.02, 2.06, 2.11, 2.15 (15H, each s, OAc×4, NAc), 6.23 (1H, d, $J_{NH,2}$=10 Hz, NH, exchangeable with D_2O), 7.30 (5H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.26 ($^1J_{C\text{-}1\text{-}H\text{-}1}$=175.78 Hz, C-1), 99.07 ($^1J_{C\text{-}1'\text{-}H\text{-}1}$=156.25 Hz, C-1′). IR ν_{max}^{KBr} cm^{-1}: 3390 (NH), 1750 (OAc), 1674 (amide I), 1518 (amide II). TLC: *Rf* 0.45 (solvent A), 0.50 (B). *Anal.* Calcd for $C_{29}H_{37}NO_{14}$: 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, $[\alpha]_D^{19}$ −86.8° (*c*=0.24, $CHCl_3$), was isolated after removal of the solvent. ^{1}H-NMR ($CDCl_3$): 1.99, 2.10 (6H, each s, OAc, NAc), 5.38 (1H, s, H-1), 5.93 (1H, d, $J_{NH,2}$=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[3] by mixed mp, IR, and TLC. [lit. mp 115—116°C, $[\alpha]_D^{19}$ −93.8° (*c*=1, $CHCl_3$)].

1,2′,3′,4′,6,6′-Hexa-*O*-acetyl-3-*O*-benzyl-*N*-acetyl-α- and β-lactosamines (5 and 6)——A solution of **3** (98 mg) in ice-coid acetolyzing reagent [boron trifluoride etherate–Ac_2O (1:25, v/v) 2.5 ml] 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 Ac_2O. After neutralization with $NaHCO_3$, the whole was extracted with $CHCl_3$. The extracts were washed with H_2O, dried ($MgSO_4$), 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%), $[\alpha]_D^{18}$ −29.2° (*c*=0.48, $CHCl_3$), was isolated as a foamy solid from the zone having *Rf* 0.41. ^{1}H-NMR ($CDCl_3$): 2.00, 2.02, 2.04, 2.05, 2.09, 2.12, 2.16 (21H, each s, OAc×6, NAc), 4.73 (1H, d, $J_{1',2}$=6 Hz, H-1′, β-Gal), 5.80 (1H, d, $J_{1,2}$= 4 Hz, H-1, β-Glc), 6.27 (1H, d, $J_{NH,2}$=10 Hz, NH), 7.36 (5H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 99.65 ($^1J_{C\text{-}1'\text{-}H\text{-}1}$=158.69 Hz, C-1′), 92.00 ($^1J_{C\text{-}1\text{-}H\text{-}1}$=173.33 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1750 (OAc), 1667 (amide I), 1540 (amide II). TLC: *Rf* 0.41 (solvent A), 0.46 (B). *Anal.* Calcd for $C_{33}H_{43}NO_{17}\cdot 1/2H_2O$: 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%), $[\alpha]_D^{21}$ +54° (*c*=0.2, $CHCl_3$), was isolated as a glassy mass. ^{1}H-NMR ($CDCl_3$): 1.96, 1.99, 2.08, 2.10 (21H, each s, OAc×6, NAc), 6.12 (1H, d, $J_{1,2}$=4 Hz, H-1, α-Glc), 7.38 (5H, s, aromatic protons). ^{13}C-NMR ($CDCl_3$): 101.11 ($^1J_{C\text{-}1'\text{-}H\text{-}1}$=158.69 Hz, C-1′), 90.25 ($^1J_{C\text{-}1\text{-}H\text{-}1}$=178.22 Hz, C-1). IR ν_{max}^{KBr} cm^{-1}: 3380 (NH), 1750 (OAc), 1665 (amide I), 1535 (amide II). TLC: *Rf* 0.33 (solvent A), 0.41 (B). *Anal.* Calcd for $C_{33}H_{43}NO_{17}\cdot H_2O$: 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 acetolyzing 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, $[\alpha]_D^{20}$ +62.1° (*c*=0.23, $CHCl_3$), 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[1b] by IR, mixed mp, and TLC. [lit. mp 230—231°C, $[\alpha]_D^{22}$ +50.1° (*c*=0.96, $CHCl_3$)].

1,2',3',4',6,6'-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%), $[\alpha]_D^{16}$ +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 ν_{max}^{KBr} 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 $C_{26}H_{37}NO_{17}\cdot H_2O$: 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_2O (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',6,6'-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%), $[\alpha]_D^{17}$ +18.8° (*c*=0.36, $CHCl_3$), as a foamy solid. ^{1}H-NMR ($CDCl_3$): 1.99, 2.08, 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 ν_{max}^{KBr} 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 $C_{26}H_{37}NO_{17}\cdot 2H_2O$: C, 46.50; H, 6.15; N, 2.09. Found: C, 46.41; H, 5.91; N, 1.91.

1,2',3,3',4',6,6'-Hepta-*O*-acetyl-*N*-acetyl-β-lactosamine (10)——Acetylation of **9** (16.6 mg) with Ac_2O (0.5 ml) and pyridine (1 ml) overnight at room temperature was carried out. The mixture was concentrated to povide crude **10** (16.7 mg, 99.6%), which was crystallized from benzene–hexane as fine needles, mp 109—112°C, $[\alpha]_D^{20}$ −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* 0.23 (solvent A), 0.25 (B). [lit.[6] mp 108—110°C, $[\alpha]_D^{20}$ −7.05° (*c*=0.95, $CHCl_3$)].

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References and Notes

1) *a*) R. Kornfeld and S. Kornfeld, *Annu. Rev. Biochem.*, **45**, 217 (1976); *b*) T. Takamura, T. Chiba, and S. Tejima, *Chem. Pharm. Bull.*, **27**, 721 (1979), and the references cited therein.
2) Y. Itoh and S. Tejima, *Chem. Pharm. Bull.*, **30**, 3383 (1982).
3) S. Oguri, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, **28**, 3196 (1980).
4) J.-C. Jacquinet and P. Sinaÿ, *J. Chem. Soc., Perkin Trans. 1*, **1979**, 314.
5) C.M. McCloskey, *Advan. Carbohyd. Chem.*, **12**, 137 (1957).
6) B.A. Dmitriev, Yu.A. Knirel, and N.K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1973**, 2365.
7) E. Stahl and U. Kaltenbach, *J. Chromatogr.*, **5**, 351 (1961).