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Chemical Modification of Lactose. III.[1] Syntheses of 4′-Acetamido-4′-deoxy-α-lactose and 6′-Acetamido-6′-deoxy-α-cellobiose

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Syntheses of 4′-acetamido-4′-deoxy-α-lactose (**12**) and 6′-acetamido-6′-deoxy-α-cellobiose (**19**) are described starting from 1,6-anhydro-β-cellobiose (**1**) with seven steps. The sequence of the reaction is as follows. Selective tritylation and successive acetylation of **1** afford 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-trityl-β-cellobiose (**2**), mp 191—192°, $[\alpha]_D^{23}$ −11°, in 45% yield. Detritylation of **2** with hydrogen bromide in acetic acid gives two products, crystalline 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-cellobiose (**3**) and sirupy 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-β-cellobiose (**4**), in a yield of approximately the equal amount. Sulfonylation of **3**, substitution of the sulfonate with sodium azide, reduction of the azido-group, acetolysis of the 1,6-anhydro ring, and deacetylation afford **12**. In the same manner, **19** is obtained from **4**.

In the preceding paper from this laboratory Tejima and Chiba[1] reported on the synthesis of 6-acetamido-6-deoxy-lactose which is a structural isomer of N-acetyl-lactosamine (4-β-D-galactopyranosyl-2-acetamido-2-deoxy-D-glucopyranose). It has been well known that N-acetyl-lactosamine is not only the repeating units in mucopolysaccharides, but also it has often been isolated from partial acid hydrolyzates of natural polysaccharides. Therefore, the authors considered that the analogous isomer of N-acetyl-lactosamine may be interesting from biological viewpoints. As an extension of the studies on the chemical modification of lactose, the authors now synthesized 4′-acetamido-4′-deoxy-α-lactose (**12**) in seven steps, starting from 1,6-anhydro-β-cellobiose (**1**). Synthesis of 6′-acetamido-6′-deoxy-α-cellobiose (**19**) was also described, because the compound was obtainable from the same intermediate.

The sequence of the reaction in this work is as follows. Selective tritylation and successive acetylation of **1** afford 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-trityl-β-cellobiose (**2**). Detritylation of **2** with hydrogen bromide in acetic acid gives two products, crystalline 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-β-cellobiose (**3**) and sirupy 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-β-cellobiose (**4**), in a yield of approximately the equal amount. Needless to say, the formation of **3** can be ascribed to the concomitant acetyl migration from C-4′ to C-6′ in **4**. Sulfonation of **3**, substitution of the sulfonate with sodium azide, reduction of the azido-group, acetolysis of the 1,6-anhydro ring, and deacetylation afford 4′-acetamido-4′-deoxy-α-lactose (**12**). The order of the two steps, reduction of azido-group and acetolysis, can be *vice versa*. The similar route is applied to **4** to afford 6′-acetamido-6′-deoxy-α-cellobiose (**19**). In the course of the preparation, valuable information on the properties of 1,6-anhydro-β-cellobiose has been acquired, and the preparation of several new compound is now reported in full detail.

Compound **2** is a useful intermediate for chemical modification of C-6′ in cellobiose[3] or synthesis of trisaccharides.[4] The product was first prepared by Lindberg and Selleby[3] and has been reported having mp 119—120° and $[\alpha]_D^{20}$ −50° ($CHCl_3$). The authors followed up the method several times, however, we could not separate the compound having the reported values. Therefore, in the first place preparation of **2** was investigated.

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1) Part II: S. Tejima and T. Chiba, *Chem. Pharm. Bull.* (Tokyo), 21, 546 (1973).
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya.*
3) B. Lindberg and L. Selleby, *Acta. Chem. Scand.*, 14, 1051 (1960).
4) N. Roy and T.E. Timell, *Carbohyd. Res.*, 6, 475 (1968).

A mixture of dried **1** (1 mole) in dry pyridine and freshly prepared trityl chloride (1.3 mole) was stirred for 48 hr at room temperature, then, in order to remove unreacted **1**, it was poured into ice-water, extracted with dichloromethane, and evaporated the solvent to afford a sirupy trityl ether. Without further purification, successive acetylation afforded **2**, mp 191—192°, $[\alpha]_D^{23}$ $-11°$ ($CHCl_3$), in 45% yield, starting from **1**. The product, chromatographically homogeneous, has a satisfactory elemental analysis. In the nuclear magnetic resonance (NMR) spectrum of **2**, fifteen-proton multiplet in the region of 7.20—7.50 ppm was observed, and assigned to the three phenyls. Three-proton singlet in the highest field (1.75 ppm) assigned to one acetyl which might be shifted to higher field owing to the effect of trityl; Horton, *et al.*[5] have reported that in 1,2,3,4-tetra-O-acetyl-6-O-trityl-α-D-glucopyranose the signal of one of the acetyl group is observed at exceptionally high field. Twelve proton singlet comes from the residual acetyls was observed at 2.00 ppm. Concering the apparent deviation of the values in physical constants between our product and that prepared by the original authors, we assumed that the latter might presumably be contaminated with starting material; lower melting point, more levorotatory value, and better yield of the latter would suggest the validity of the speculation.

In the course of detritylation of **2** with hydrogen bromide in acetic acid, as it has been pointed out by Roy and Timell,[6] a case of acetyl migration was encountered. Crystalline pentaacetate, mp 139—140° and $[\alpha]_D^{25}$ $-77°$ ($CHCl_3$) was afforded in 50% yield. The product, chromatographically homogeneous, had a satisfactory elemental analysis of 1,6-anhydropentaacetyl-cellobiose. The values of physical constants and the NMR data were in good agreement with those of 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-β-cellobiose (**3**), originally reported by Roy and Timell.[6] Compound **3** could not be converted into **2** by retritylation; this confirmed that the one hydroxyl was not that of primary alcohol. Therefore, the crystalline pentaacetate was tentatively assigned to **3**. Further proof of the structure will be mentioned later.

The mother liquid, from which **3** had been separated, was concentrated to afford a sirup. The yield was approximately the equal amount with **3**. The sirup was tentatively assigned to a crude 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-β-cellobiose (**4**). Further proof of the structure will be mentioned later.

On *p*-toluenesulfonylation or methanesulfonylation in the usual way, **3** yielded 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-O-*p*-tolylsulfonyl-β-cellobiose (**5**) or 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-O-methylsulfonyl-β-cellobiose (**6**), respectively. Treatment of **5** or **6** with sodium iodide in boiling acetonitrile did not cause replacement of the sulfonyloxy group by iodide; this confirmed that the sulfonyl group in **5** or **6** was not located on the primary alcohol group.

On the other hand, treatment of **6** with sodium benzoate in hexamethylphosphoric triamide (HMPA) at 115° for 13 hr caused replacement of the methylsulfonyloxy group by benzoyloxy, judging from the thin-layer chromatography (TLC). Deacetylation of the resulting product, followed by acetylation in the usual way, afforded hexa-O-acetyl-1,6-anhydro-β-lactose (lactosan hexaacetate) (**7**) in 51% yield; it was indistinguishable with an authentic sample[7] by mixed mp, TLC, and infrared (IR) spectrum. This result exactly supports the nucleophilic substitution proceeds involving inversion at C-4′, and the structure assigned to **3**, **5**, or **6** in which a hydroxyl in **3** and a sulfonyl-group in **5** or **6** are attached to C-4′.

It is of interest to describe that treatment of **5** with the same condition did not afford a homogeneous product, instead, a complex mixture was recognized by TLC. As it has been well known that, in nucleophilic substitution, tosyloxy-group is more reactive than mesyloxy.

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[5] D. Horton, J.B. Hughes, J.S. Jewell, K.D. Philips, and W.N. Turner, *J. Org. Chem.*, **32**, 1073 (1967).

[6] N. Roy and T.E. Timell, *Carbohyd. Res.*, **7**, 82 (1968).

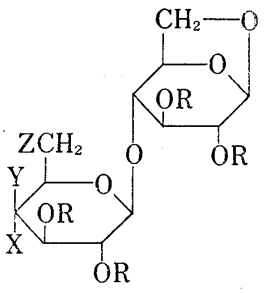
[7] S. Tejima, *Carbohyd. Res.*, **20**, 123 (1971).

Therefore, the tosyloxy in **5** is presumably eliminated as *p*-toluenesulfonic acid, and the resulting unsaturated compound may further be decomposed to a complex mixture.

Recently, the azido-group has retained its place as the most used precursor of the amino-group in synthesis, introduction is easily performed by sulfonyloxy-group displacement with sodium azide, and the method is also available to the preparation of amino disaccharides.[1] Treatment of **5** with sodium azide in HMPA at 90—100° for 10 hr caused replacement of the sulfonyloxy-group by azide, and yielded crystalline 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-azido-4′-deoxy-*β*-lactose (**8**) in 74% yield. The difference shown between the substitution by sodium azide and that of sodium benzoate may be attributable to the strong nucleophilicity of the former reagent.

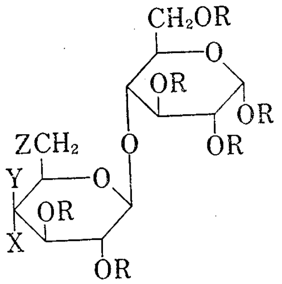
Catalytic reduction of **8** using Raney nickel, followed by acetylation, afforded crystalline 1,6-anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-acetamido-4′-deoxy-*β*-lactose (**9**).

It has been well-known that 1,6-anhydro ring in disaccharides is subjected to acetolysis without cleavage of the monosaccharide linkage to afford fully acetylated *α*-acetate;[8] 1,6-anhydro-6-thio ring is also permitted acetolysis which has been reported from our laboratory.[9] Acetolysis of **8** permitted opening of the 1,6-anhydro ring, during which the specific rotation changes rapidly from minus to plus while the azido-group remains unchanged, and yielded 1,2,3,6,2′,3′,6′-hepta-O-acetyl-4′-azido-4′-deoxy-*α*-lactose (**10**) in 88% yield. Catalytic reduction of **10** and successive acetylation, followed by silica gel column chromatography of the resulting sirup, yielded 1,2,3,6,2′,3′,6′-hepta-O-acetyl-4′-acetamido-4′-deoxy-*α*-lactose (**11**) in 60% yield. Compound **11** was also obtainable by acetolysis of **9**, while the yield was not improved. Deacetylation of **11** with sodium methoxide yielded 4′-acetamido-4′-deoxy-*α*-lactose (**12**) as a hygroscopic powder. Compound **12** mutarotated in water from +48° to +38° for 24 hr. After hydrochloric acid hydrolysis, two spots, *Rf* 0.16 and 0.40, positive with aniline hydrogen phthalate reagent, were identified by paper partition chromatography (PPC), and other spot could not be identified. The less mobile spot showed, in addition, positive ninhydrin test. In comparison with reference compounds, the spots were identified as 4-amino-4-deoxy-D-galactose hydrochloride and D-glucose, respectively.



1: R=Y=H, X=Z=OH
2: R=Ac, X=OAc, Y=H, Z=OTr
3: R=Ac, X=OH, Y=H, Z=OAc
4: R=Ac, X=OAc, Y=H, Z=OH
5: R=Ac, X=OTs, Y=H, Z=OAc
6: R=Ac, X=OMs, Y=H, Z=OAc
7: R=Ac, X=H, Y=Z=OAc
8: R=Ac, X=H, Y=N_3, Z=OAc
9: R=Ac, X=H, Y=NHAc, Z=OAc
13: R=Ac, X=OAc, Y=H, Z=OTs
14: R=Ac, X=OAc, Y=H, Z=OMs
15: R=Ac, X=OAc, Y=H, Z=I
16: R=Ac, X=OAc, Y=H, Z=N_3
Ac=acetyl, Tr=trityl,
Ts=tosyl, Ms=mesyl

Chart 1



10: R=Ac, X=H, Y=N_3, Z=OAc
11: R=Ac, X=H, Y=NHAc, Z=OAc
12: R=H, X=H, Y=NHAc, Z=OH
17: R=Ac, X=OAc, Y=H, Z=N_3
18: R=Ac, X=OAc, Y=H, Z=NHAc
19: R=H, X=OH, Y=H, Z=NHAc

Chart 2

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8) E.M. Montgomery, N.K. Richtmyer, and C.S. Hudson, *J. Am. Chem. Soc.*, **65**, 1848 (1943).
9) S. Tejima and Y. Okamori, *Chem. Pharm. Bull.* (Tokyo), **20**, 2036 (1972).

In the next step, synthesis of 6′-acetamido-6′-deoxy-α-cellobiose will be described below. As described in the earlier part of this paper, crude 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-β-cellobiose (**4**) was obtainable during detritylation of **2**. On *p*-toluenesulfonylation or methanesulfonylation in the usual way, **4** yielded the corresponding monotosylate or monomesylate in 68 or 75% yield, respectively, which was tentatively assigned to 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-*p*-tolylsulfonyl-β-cellobiose (**13**) or 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-methylsulfonyl-β-cellobiose (**14**). Treatment of **13** or **14** with sodium iodide in boiling acetonitrile caused replacement of the sulfonyloxy group by iodide, and crystalline 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-deoxy-6′-iodo-β-cellobiose (**15**) was obtained in 80% yield; this confirmed that the sulfonyl-group in **13** or **14** was located on the primary alcohol group. Final proof of the structure of compound **4**, **13**, **14**, or **15** was obtained by an unequivocal structure of the final product (**19**).

Introduction of acetamido group in C-6′ was performed similarly with the method for the preparation of **12**; azide substitution of **13** to yield 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-azido-6′-deoxy-β-cellobiose (**16**), acetolysis of **16**, catalytic reduction of the resulting 1,2,-3,6,2′,3′,4′-hepta-O-acetyl-6′-azido-6′-deoxy-α-cellobiose (**17**) and finally, deacetylation of 1,2,3,6,2′,3′,4′-hepta-O-acetyl-6′-acetamido-6′-deoxy-α-cellobiose (**18**) yielded 6′-acetamido-6′-deoxy-α-cellobiose (**19**). Compound **19** crystallized as monohydrate and mutarotated in water from +29° to +25° for 24 hr. The structure was undoubtedly confirmed from the fact that, after hydrochloric acid hydrolysis of **19**, D-glucose and 6-amino-6-deoxy-D-glucose hydrochloride were identified by PPC.

Recent publications from our laboratory[1,7,9] have shown the usefulness of 1,6-anhydro-β-derivatives of lactose and cellobiose for chemical modification of the parent reducing disaccharides. The present paper adds further example of the usefulness of 1,6-anhydro-β-cellobiose for the purpose. Finally, it is of interest to notice that the first half of this paper introduced lactose derivatives starting from cellobiose which is unable to be metabolized by higher animals; on the contrary, lactose is one of the most available sugars for animals. Another method of conversion of cellobiose into lactose has been described by Kuzuhara and Emoto.[10]

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and uncorrected. Solutions were evaporated in a rotary evaporator below 40° under diminished pressure. Optical rotations were measured with a Yanagimoto Model OR-10 polarimeter. IR spectra were recorded with a Jasco Model IR-S spectrometer. NMR spectra were recorded at 60 MHz with a Jeol Model JNM-MH-60 spectrometer. Tetramethylsilane was used as the internal standard in $CDCl_3$. Chemical shifts are given on ppm scale. TLC on Silica Gel GF_{254} (E. Merck, Darmstadt, Germany) activated at 110° was performed with solvent systems (A) 6: 1 (v/v) $CHCl_3$-acetone, (B) 2: 1 ether–benzene, (C) 2: 1 $CHCl_3$-acetone, and (D) 2: 1 70% iso-PrOH–AcOEt. Detection was effected with H_2SO_4 or UV light (short wave length). Column chromatography was performed on a column of Wako-gel C-200 as the adsorbent, with 1 g of the mixture to be separated per 20 g of adsorbent. PPC was performed on Toyo Filter Paper No. 50 by the ascending method, with 6: 4: 3 (v/v) *n*-BuOH–pyridine–H_2O by the procedure of Ueda,[11] and detection was effected with (A) 0.2% ninhydrin solution dissolved in H_2O–saturated *n*-BuOH,[12] (B) aniline hydrogen phthalate.[13]

1,6-Anhydro-β-cellobiose (1)——Crystalline hexa-O-acetyl-1,6-anhydro-β-cellobiose (cellobiosan hexaacetate)[9] was deacetylated according to the method of Montogomery, *et al.*[8] to afford **1** as a hygroscopic powder which completely desiccated in a vacuum desiccator over P_2O_5.

1,6-Anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-trityl-β-cellobiose (2)——A mixture of **1** (12 g) and freshly prepared trityl chloride[14] (13.5 g) in dry pyridine (70 ml), protected from moisture, was stirred for 2 days

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[10] H. Kuzuhara and S. Emoto, *Agr. Biol. Chem.*, **30**, 122 (1966).

[11] M. Ueda, *Yakugaku Zasshi*, **90**, 1322 (1970).

[12] D. Aminoff and W.T.J. Morgan, *Nature*, **162**, 579 (1948); A.R. Patton and P. Chism, *Anal. Chem.*, **23**, 1683 (1951).

[13] S.M. Partridge, *Nature*, **164**, 443 (1949).

[14] W.E. Bachmann, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, 1955, p. 841.

at room temperature, and poured into ice-water (700 ml). The mixture was extracted with CH_2Cl_2 (3 × 150 ml), and the extracts were combined, washed with H_2O, dried ($MgSO_4$), and evaporated to a sirup which acetylated with Ac_2O (50 ml) and dry pyridine (50 ml). After being kept overnight, the mixture was poured into ice-water (500 ml). The resulting precipitate was collected, dried in the air, and recrystallized from EtOH to give pure **2** (12.5 g, 45%), mp 191—192°, $[\alpha]_D^{23}$ −11° (c=1.1, $CHCl_3$) (lit.[3] mp 119—120°, $[\alpha]_D^{20}$ −50° (c =1.1, $CHCl_3$). NMR ($CDCl_3$) δ ppm: 1.75 (3H, s, CH_3CO), 2.00 (12H, s, $4CH_3CO$), 7.20—7.50 (15H, m, $3C_6H_5$). *Anal.* Calcd. for $C_{41}H_{44}O_{15}$: C, 63.40; H, 5.67. Found: C, 63.36; H, 5.73.

1,6-Anhydro-2,3,2′,3′,6′-penta-O-acetyl-*β*-cellobiose (3)——To a solution of **2** (7 g) in glacial AcOH (35 ml) was added a saturated solution of HBr in AcOH (35 ml). The mixture was shaken for 45 sec, poured into ice-water (300 ml), and the resulting trityl bromide removed by filtration. The filtrate was extracted with CH_2Cl_2 (3 × 50 ml), organic layer washed successively with saturated aq. $NaHCO_3$ and H_2O, dried ($CaCl_2$), and evaporated to dryness to afford a sirup which crystallized from EtOH–ether. The crystals (2.4 g, 50%) had mp 139—140° and $[\alpha]_D^{25}$ −77° (c=1, $CHCl_3$) (lit.[6] mp 142°, $[\alpha]_D^{25}$ −80°). NMR ($CDCl_3$) δ ppm: 2.10 (15H, s, $5CH_3CO$). *Anal.* Calcd. for $C_{22}H_{30}O_{15}$: C, 49.44; H, 5.65. Found: C, 49.49; H, 5.93.

The filtrate, from which **3** had been separated, was reserved for the preparation of **4** as mentioned later.

Tritylation of Compound 3——To a solution of **3** (100 mg) in dry pyridine (1 ml) was added freshly prepared trityl chloride (150 mg). The mixture was stirred for 48 hr, during which the reaction checked by TLC. No tritylation proceeded, and recovered **3** from the reaction mixture.

1,6-Anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-O-*p*-tolylsulfonyl-*β*-cellobiose (**5**)——Compound **3** (2.1 g) was added to a solution of TsCl (2 g) in pyridine (7 ml) at −10°. After it had been stirred for 1 hr, the mixture was kept overnight at 5°, and then poured into ice-water. The resulting precipitate was collected, dried in the air, and recrystallized from EtOH to give pure **5** (2.3 g, 85%), mp 155—156°, $[\alpha]_D^{19}$ −60.2° (c=1, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{36}O_{17}S$: C, 50.58; H, 5.27. Found: C, 50.28; H, 5.41.

1,6-Anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-O-methylsulfonyl-*β*-cellobiose (**6**)——Compound **3** (500 mg) was added to a solution of MsCl (0.5 ml) in pyridine (4 ml) at −10°. After it had been stirred for 1 hr, the mixture was kept overnight at 5°, poured into ice-water (100 ml), and extracted with CH_2Cl_2 (2 × 30 ml). The extracts were combined, washed successively with 1M H_2SO_4, saturated aq. $NaHCO_3$ and H_2O, dried ($CaCl_2$), and evaporated to dryness to afford a sirup which crystallized from EtOH. The crystals were collected and recrystallized from EtOH to give pure **6**, mp 174—175°, $[\alpha]_D^{14}$ −62.7° (c=1, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_{17}S$: C, 45.10; H, 5.27. Found: C, 44.93; H, 5.39.

Reaction of Sodium Iodide with Compound 5 or 6——A mixture of **5** or **6** (100 mg in each case) and NaI (100 mg) in acetonitrile (5 ml) was boiled under reflux, during which the reaction checked by TLC (solvent A) every 1 hr. The starting material (**5** or **6**) remained unchanged for 12 hr, which was recovered completely from the reaction mixture.

Hexa-O-acetyl-1,6-anhydro-*β*-lactose (Lactosan Hexaacetate) (7) from Compound 6——A mixture of of **6** (220 mg) and sodium benzoate (200 mg) in freshly distilled HMPA (13 ml) was heated in an oil bath at 115°, during which the reaction checked by TLC (solvent A); the starting material (**6**) had disappeared after 13 hr. The mixture was poured into ice-water (200 ml), extracted with ether (3 × 50 ml), the extracts were combined, washed with H_2O, dried ($CaCl_2$), and evaporated to a sirup. To a solution of the sirup in dry MeOH (15 ml) was added 1M sodium methoxide in MeOH (0.5 ml), and the mixture stirred for 1 hr to complete deacetylation as checked by TLC. The mixture was neutralized with AcOH and evaporated to dryness which acetylated overnight with Ac_2O (2 ml) and pyridine (4 ml). After evaporation of the mixture to dryness, the residue was dissolved in CH_2Cl_2 (15 ml), filtered, and the filtrate evaporated to give a sirup which crystallized from EtOH. The crystals were collected, air-dried, and recrystallized from AcOEt to give pure **7** (105 mg, 51%), mp 206—208°; it was indistinguishable with authentic **7** (mixed mp, IR, and TLC).

1,6-Anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-azido-4′-deoxy-*β*-lactose (**8**)——A mixture of **5** (2 g) and NaN_3 (1.6 g) in HMPA (10 ml) was heated under stirring in an oil bath at 90—100° for 10 hr. After cooling to room temperature, the mixture was diluted with AcOEt (150 ml), and washed with H_2O (3 × 50 ml). The organic layer was evaporated to afford a sirup which crystallized on adding EtOH and scratching the inside of the flask. The crystals were collected, and recrystallized from EtOH to give pure needles (1.2 g, 74%), mp 164—165°, $[\alpha]_D^{13}$ −80° (c=1.2, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 2100 (N_3). *Anal.* Calcd. for $C_{22}H_{29}O_{14}N_3$: C, 47.23; H, 5.22; N, 7.51. Found: C, 47.37; H, 5.24; N, 7.52.

1,6-Anhydro-2,3,2′,3′,6′-penta-O-acetyl-4′-acetamido-4′-deoxy-*β*-lactose (**9**)——A solution of **8** (250 mg) in 20 ml of 1: 3 (v/v) AcOEt–MeOH was treated with freshly prepared Raney nickel catalyst and the suspension shaken with hydrogen at room temperature and pressure; theoretical amount of hydrogen absorbed for 3 hr. After removal of the catalyst by filtration and washing of the residue with warm MeOH, the combined filtrate and washings were concentrated to give a sirup which acetylated with Ac_2O (1.5 ml) and pyridine (3 ml) overnight at room temperature. The sirup, obtained by repeated azeotropic distillation with toluene, crystallized from EtOH to give pure needles (150 mg, 58%), mp 229—230°, $[\alpha]_D^{13}$ −45.5° (c=1.5, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{33}O_{15}N$: C, 50.09; H, 5.78; N, 2.43. Found: C, 50.28; H, 5.90; N, 2.53.

1,2,3,6,2′,3′,6′-Hepta-O-acetyl-4′-azido-4′-deoxy-*α*-lactose (**10**)——Compound **8** (500 mg) was dissolved in the acetolysis mixture (10 ml; 1: 70: 30 (v/v) H_2SO_4–Ac_2O–AcOH). After stirring for 2 hr at room temperature, the solution was poured into ice-water (350 ml). The resulting precipitate was collected, and the air-dried powder recrystallized from EtOH to afford pure **10** (518 mg, 88%), mp 189—199°, $[\alpha]_D^{16}$ $+14.1°$ (c=1.3, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 2100 (N_3). *Anal.* Calcd. for $C_{26}H_{35}O_{17}N_3$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.04; H, 5.19; N, 6.45.

1,2,3,6,2′,3′,6′-Hepta-O-acetyl-4′-acetamido-4′-deoxy-*α*-lactose (11)——i) From Compound **10**: Catalytic reduction of **10** (500 mg) in AcOEt–MeOH (40 ml) and successive acetylation of the resulting 4′-amino compound were performed, similarly with the method described for the preparation of **9**, to afford a sirup. The sirup was dissolved in $CHCl_3$, and the solution chromatographed on a column of silica gel using 6:1 (v/v) $CHCl_3$–acetone as the elution solvent. Evaporation of the solvent afforded a sirup which crystallized from ether and collected by filtration. The product (310 mg, 60%) had mp 110—121°, $[\alpha]_D^{16}$ $+37.7°$ (c=1, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 1670, 1520 (CONH). *Anal.* Calcd. for $C_{28}H_{39}O_{18}N$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.24; H, 5.62; N, 2.05.

ii) From Compound **9**: Compound **9** (90 mg) was dissolved in the acetolysis mixture (2 ml). After stirring for 3 hr at room temperature, the solution was poured into ice-water (50 ml), neutralized with $NaHCO_3$, and extracted with CH_2Cl_2 (2 × 20 ml). The organic layer was washed with H_2O, dried ($CaCl_2$), and concentrated to a sirup which dissolved in $CHCl_3$ and chromatographed on a column of silica gel as described in i). The resulting crystals (60 mg, 57%), mp 113—121°, were indistinguishable with the product, prepared by method i).

4′-Acetamido-4′-deoxy-*α*-lactose (12)——To a suspension of **11** (130 mg) in dry MeOH (10 ml) was added 1 M methanolic sodium methoxide (0.1 ml) at room temperature. The mixture was stirred, with exclusion of moisture, for 1 hr to complete deacetylation as checked by TLC. Dry Dowex 50 W × 8 (H^+) resin was added, and the suspension stirred for 30 min, filtered, and the filtrate evaporated to give a sirup which crystallized from EtOH–ether. The crystals were collected and purified by precipitation from the ethanolic solution, followed by addition of ether. Pure **12** (48 mg, 68%), had mp 165—167° (decomp.) and $[\alpha]_D^{14}$ $+48°$ (5 min)$\rightarrow$$+38°$ (24 hr, constant) (c=0.5, H_2O). *Anal.* Calcd. for $C_{14}H_{25}O_{11}N \cdot H_2O$: C, 41.90; H, 6.78; N, 3.48. Found: C, 41.81; H, 7.19; N, 3.50.

PPC of Acid Hydrolyzate of Compound 12——A mixture of **12** (40 mg) and 1 M HCl (2 ml) in a sealed tube was heated at 110—115° for 10 hr. After treatment with charcoal, the mixture was evaporated to dryness. The residue was dissolved in a small amount of H_2O, in which only two spots, *Rf* 0.16 and 0.40, were identified by PPC. The former was positive reagent (A) and (B) tests, while showed different *Rf* value with 2-amino-2-deoxy-D-glucose hydrochloride (*Rf* 0.31) or 6-amino-6-deoxy-D-glucose hydrochloride[15] (*Rf* 0.22). In comparison with reference compounds, the former was identified to 4-amino-4-deoxy-D-galactose hydrochloride and the latter to be D-glucose.

1,6-Anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-*p*-tolylsulfonyl-*β*-cellobiose (13)——Sirupy 1,6-anhydro-2,3,2′,3′,4′-penta-O-acetyl-*β*-cellobiose (**4**) (2.3 g) was prepared after evaporation of the solvent from the filtrate in the preparation of **3**.

To a chilled solution of the sirup in dry pyridine (8 ml) was added TsCl (2 g). The mixture was similarly treated as described for the preparation of **5** to give pure **13** (2 g, 68%), mp 155—156°, $[\alpha]_D^{23}$ $-31.8°$ (c=1, $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{36}O_{17}S$: C, 50.58; H, 5.27. Found: C, 50.37; H, 5.35.

1,6-Anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-O-methylsulfonyl-*β*-cellobiose (14)——Sirupy **4** (360 mg) was treated, under external cooling, with dry pyridine (2 ml) and MsCl (0.5 ml) for 15 hr, and the product was isolated as described for the preparation of **5**. Recrystallization from AcOEt gave pure **14** (310 mg, 75%), mp 198—199°, $[\alpha]_D^{14}$ $-44.8°$ (c=1.3, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{32}O_{17}S$: C, 45.10; H, 5.27. Found: C, 44.95; H, 5.39.

1,6-Anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-deoxy-6′-iodo-*β*-cellobiose (15)——A mixture of **13** (100 mg) and NaI (100 mg) in acetonitrile (5 ml) was boiled for 1.5 hr under reflux. After cooling to room temperature, the mixture was poured into ice-water (50 ml), and extracted with CH_2Cl_2 (2 × 15 ml). The organic layer was washed with H_2O, dried ($CaCl_2$), and evaporated to a sirup which crystallized on addition of a small amount of EtOH. The crystals were collected, and recrystallized from EtOH to give pure **15** (75 mg, 80%), mp 179—181°, $[\alpha]_D^{16}$ $-23.4°$ (c=0.8, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{29}O_{14}I$: C, 40.99; H, 4.50. Found: C, 40.96; H, 4.48.

Compound **15** was also obtainable starting from **14**, except for the boiling period 3 hr.

1,6-Anhydro-2,3,2′,3′,4′-penta-O-acetyl-6′-azido-6′-deoxy-*β*-cellobiose (16)——A mixture of **13** (700 mg) and NaN_3 (500 mg) in HMPA (4 ml) was heated under stirring in an oil bath at 90—100° for 4 hr. The mixture was similarly treated as described for the preparation of **8** to afford a sirup which dissolved in benzene and chromatographed on a column of silica gel using 2:1 (v/v) ether-benzene as elution solvent. Removal of the solvent gave crystals which recrystallized from EtOH to give pure **16** (420 mg, 74%), mp 113—114°, $[\alpha]_D^{21}$ $-45.4°$ (c=1, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 2100 (N_3). *Anal.* Calcd. for $C_{22}H_{29}O_{14}N_3$: C, 47.23; H, 5.22; N, 7.51. Found: C, 46.97; H, 5.14; N, 7.50.

1,2,3,6,2′,3′,4′-Hepta-O-acetyl-6′-azido-6′-deoxy-*α*-cellobiose (17)——A mixture of **16** (300 mg) in acetolysis mixture (6 ml) was similarly treated as described for the preparation of **10**, to afford pure **17** (322 mg,

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[15] E. Hardegger, G. Zanetti, and K. Steiner, *Helv. Chim. Acta*, **46**, 282 (1963).

91%), mp 185—201°, $[\alpha]_D^{16}$ +36° (c=1.1, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 2100 (N_3). *Anal.* Calcd. for $C_{26}H_{35}O_{17}N_3$: C, 47.20; H, 5.33; N, 6.35. Found: C, 47.02; H, 5.34; N, 6.35.

1,2,3,6,2′,3′,4′-Hepta-O-acetyl-6′-acetamido-6′-deoxy-α-cellobiose (**18**)——Catalytic reduction of **17** (125 mg) and successive acetylation of the resulting 6′-amino compound were similarly performed as described for the preparation of **9** to afford a sirup which crystallized from EtOH–ether. The crystals (97 mg, 74%) had mp 196—198° and $[\alpha]_D^{17}$ +41.2° (c=1, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3280 (NH), 1650, 1535 (CONH). *Anal.* Calcd. for $C_{28}H_{39}O_{18}N$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.67; H, 6.05; N, 2.12.

6′-Acetamido-6′-deoxy-α-cellobiose Monohydrate (**19**)——Deacetylation of **18** (300 mg) was performed by the similar method described in the preparation of **12** to afford a sirup which solidified on addition of ether and scratching the inside of the flask. The hygroscopic powder, which was collected by rapid filtration, turned to a crystalline mass after left in the air; yield 120 mg (68%). The product had mp 211—214° (decomp.) and $[\alpha]_D^{15}$ +29° (5 min)→+25° (24 hr, constant) (c=1, H_2O). IR ν_{max}^{Nujol} cm^{-1}: 3280—3480 (OH, NH), 1655, 1565 (CONH). *Anal.* Calcd. for $C_{14}H_{25}O_{11}N \cdot H_2O$: C, 41.90; H, 6.78; N, 3.48. Found: C, 42.08; H, 6.64; N, 3.56.

PPC of Acid Hydrolyzate of Compound 19——Authentic 6-amino-6-deoxy-D-glucose hydrochloride (*Rf* 0.22) was prepared according to the method of Hardegger, *et al.*[15] D-Glucose (*Rf* 0.40), D-galactose (0.38), and 2-amino-2-deoxy-D-glucose hydrochloride (0.31) were used as the reference compounds. Acid hydrolysis of **19** (40 mg) and the working of the hydrolyzate were performed similarly with those of compound **12**, in which 6-amino-6-deoxy-D-glucose hydrochloride and D-glucose were identified.

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