[Chem. Pharm. Bull. 22(2) 398—403 (1974)]

UDC 517.458.2.057

Chemical Modification of Lactose. IV.[1] Syntheses of Disaccharide Derivatives Predominantly Modified at the C-6 Position in Lactose

Taku Chiba, Masanobu Haga, and Setsuzo Tejima

Faculty of Pharmaceutical Sciences, Nagoya City University[2]

(Received June 20, 1973)

The title compounds are required for the substrate specificity study on β-D-galactosidase (EC 3.2.1.23). Starting from 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-β-lactose, 4-O-β-D-galactopyranosyl-α-D-glucuronic acid, 6-O-methyl-α-lactose, methyl 3,6-anhydro-β-lactoside, and 6-deoxy-α-lactose were synthesized. 1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-xylo-hex-5-eno-pyranose (**13**) was also prepared by elimination of hydrogen iodide with silver fluoride in dry pyridine from 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-deoxy-6-iodo-β-lactose. In catalytic hydrogenation of **13**, regardless of the catalyst, a stereospecific hydrogenation proceeded in such a way that the 6-deoxy-L-ido isomer, 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-6-deoxy-α-L-idopyranose (**14**), was overwhelmingly predominant than the corresponding 6-deoxy-D-gluco isomer, 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-6-deoxy-β-D-glucopyranose. The result was the reverse with that reported in unsaturated monosaccharide series. Deacetylation of **14** afforded 4-O-β-D-galactopyranosyl-6-deoxy-L-idopyranose as a hygroscopic powder.

Recently, chemical modification of primary hydroxyl groups in non-reducing disaccharides such as sucrose and α,α-trehalose have been performed actively by Hough, *et al.*[3] In reducing disaccharides, similar works have been accomplished by many investigators on maltose,[4] phenyl α-maltoside,[5] methyl β-maltoside,[6] cellobiose,[7] and methyl β-cellobioside.[8] However, besides two papers reported from our laboratory[9] and recent publication by Vazquez, *et al.*,[10] little is known about lactose series. On the other hand, in the program of our enzymatic studies on the substrate specificity of β-D-galactosidase (EC 3.2.1.23) it became desirable to have a wide variety of modified lactoses as the substrates. From these two reasons we now designed the syntheses of the title compounds. In the course of the present studies, a novel reducing disaccharide, 4-O-β-D-galactopyranosyl-6-deoxy-L-idopyranose (**15**) was synthesized incidentally. In this paper, all compounds were synthesized from 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-β-lactose (**1**) which had been prepared from 1,6-anhydro-β-lactose,[9b] according to the procedure described in Part I.[9a] We report here the details leading to the syntheses of new disaccharide derivatives in the five subdivisions as mentioned below.

1) Part III: Y. Okamori, M. Haga, and S. Tejima, *Chem. Pharm. Bull.*, (Tokyo), **21**, 2538 (1973).
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya.*
3) See for example: L. Hough and K.S. Mufti, *Carbohyd. Res.*, **25**, 497 (1972); L. Hough, A.K. Palmer, and A.C. Richardson, *J. Chem. Soc. Perkin Trans.* **I**, 1972, 2513.
4) *a*) Y. Hirasaka, *Yakugaku Zasshi*, **83**, 960 (1962); *b*) G.G.S. Dutton and K.N. Slessor, *Can. J. Chem.*, **44**, 1069 (1966).
5) H. Arita, M. Isemura, T. Ikenaka, and Y. Matsushima, *Bull. Chem. Soc. Japan*, **43**, 818 (1970); H. Arita and Y. Matsushima, *J. Biochem.* (Tokyo), **69**, 409 (1971).
6) R.T. Sleeter and H.B. Sinclair, *J. Org. Chem.*, **35**, 3804 (1970).
7) *a*) B. Lindberg and L. Selleby, *Acta Chem. Scand.*, **14**, 1051 (1960); *b*) I. Johansson, B. Lindberg, and O. Theander, *ibid.*, **17**, 2019 (1963); *c*) S. Tejima and Y. Okamori, *Chem. Pharm. Bull.* (Tokyo), **20**, 2039 (1972).
8) M. Fujinaga and Y. Matsushima, *Bull. Chem. Soc. Japan*, **40**, 1706 (1967).
9) *a*) S. Tejima, *Carbohyd. Res.*, **20**, 123 (1971); *b*) S. Tejima and T. Chiba, *Chem. Pharm. Bull.* (Tokyo), **21**, 546 (1973).
10) I.M. Vazquez, I.M.E. Thiel, and J.O. Defferrari, *Carbohyd. Res.*, **26**, 351 (1973).

1) Synthesis of 4-O-β-D-Galactopyranosyl-α-D-glucuronic Acid

A glacial acetic acid solution of **1** was oxidized with potassium permanganate at room temperature, during which the progress of oxidation was judged by thin-layer chromatography (TLC) on silica gel. When the starting material disappeared, excess permanganate was removed by sodium oxalate. From the reaction mixture, 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucuronic acid (**2**) was isolated in 61% yield as a hygroscopic amorphous powder.

Treatment of **2** with diazomethane in ether afforded crystalline methyl 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucuronate (**3**) in 64% yield. In the nuclear magnetic resonance (NMR) spectrum of **3**, a three-proton singlet, corresponding to the methyl in carboxylic acid ester, appeared at τ 6.10. Twenty one-proton singlets at τ 7.83, 7.90, and 8.02 were assigned to the seven methyls in seven acetyls.

Deacetylation of **2** with sodium methoxide in methanol afforded 4-O-β-D-galactopyranosyl-α-D-glucuronic acid which crystallized as monohydrate (**4**). Compound (**4**) mutarotated in water from +62.7° to +45.5° for 24 hr. Therefore, the anomeric configuration was assigned to α. In reducing disaccharides uronic acid derivatives have been synthesized starting from maltose[4a] and cellobiose.[7,8]

2) Synthesis of 6-O-Methyl-α-lactose

Methylation of **1**, using diazomethane–boron trifluoride etherate reagent, gave crystalline 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-O-methyl-β-lactose (**5**) in 72% yield. The procedure was a slight modification which had been employed for the preparation of 6-O-methyl-D-glucose[11] or 2-O-methyl-D-mannose.[12] In the NMR spectrum of **5**, a three-proton singlet, corresponding to the methoxy group, appeared at τ 6.52. Deacetylation of **5** with sodium methoxide in methanol afforded 6-O-methyl-α-lactose (**6**) as hygroscopic crystals. Compound (**6**) mutarotated in water from +73.1° to +53.8°. Therefore, the anomeric configuration was assigned to α.

3) Synthesis of Methyl 3,6-Anhydro-β-lactoside (9)

According to a slight modification of the procedure employed for the preparation of methyl 3,6: 3′,6′-dianhydro-β-cellobioside,[13] methyl 2,3,2′,3′,4′,6′-hexa-O-acetyl-6-O-methanesulfonyl-β-lactoside (**7**),[9b] was treated with excess sodium methoxide in methanol at 0°. After neutralization and evaporation of the solvent, the residue was acetylated to afford methyl 2,2′,3′,-4′,6′-penta-O-acetyl-3,6-anhydro-β-lactoside (**8**) in 78% yield. The specific rotation changed remarkably from −14.9° (compound (**7**)) to −92.5° (compound (**8**)). The NMR spectrum and the elemental analysis were in good agreement with those of the assigned composition. Deacetylation of **8** afforded methyl 3,6-anhydro-β-lactoside (**9**) as a hygroscopic powder which consumed 1.88 molar quantity of sodium metaperiodate with a concomitant formation of 0.84 molar quantity of formic acid. After acid hydrolysis of **9**, D-galactose was identified as the sole reducing sugar by paper partition chromatography (PPC).

4) Synthesis of 6-Deoxy-α-lactose (12)

Catalytic reduction of 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-deoxy-6-iodo-β-lactose (**10**)[9a] at room temperature under atmospheric pressure in the presence of triethylamine and a Raney nickel catalyst afforded crystalline 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-deoxy-β-lactose (**11**) in 59% yield. In the NMR spectrum, a three-proton doublet ($J_{5,6}$=5 Hz) appeared at τ 8.62 which corresponded to the methyl at C-5. Deacetylation of **11** afforded 6-deoxy-α-lactose

11) E.J. Bourne, I.R. McKinley, and H. Weigel, *Carbohyd. Res.*, **25**, 516 (1972).
12) J.O. Deferrari, E.G. Gros, and I.M.E. Thiel, "Methods in Carbohydrate Chemistry," Vol. VI, Academic Press., New York and London, 1972, p. 365.
13) F.H. Newth, S.D. Nicholas, F. Smith, and L.F. Wiggins, *J. Chem. Soc.*, **1949**, 2550.

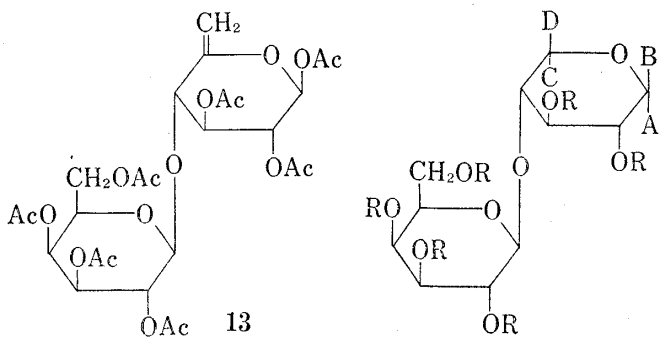
(**12**) as a hygroscopic crystal. Compound (**12**) mutarotated in water from +53.7° to +40.7°. Therefore, the anomeric configuration was assigned to α.

5) Synthesis of 4-O-β-D-Galactopyranosyl-6-deoxy-L-idopyranose (15)

Unsaturated sugars have been focused considerable attentions owing to the potential utility in the chemical modification of sugars. However, few papers have been published on unsaturated disaccharides, compared with those of monosaccharides. In lactose,[14] besides the classical papers on lactal and 2-hydroxylactal, no paper has been reported in the literature. According to the recent communication,[15] D-galactal strongly inhibited β-D-galactosidase. Therefore, unsaturated lactose derivatives may be interesting as a substrate of β-D-galactosidase. From these reasons, the authors projected synthesis of the compound having a double bond between C-5 and C-6 in lactose.

A mixture of **10** and silver fluoride in dry pyridine was stirred, with exclusion of light, for 22 hr at room temperature to induce a double bond by elimination of hydrogen iodide. The procedure was originally developed by Helferich and Himmen[16] for the preparation of 5,6-D-glucosene, and later, other examples of 5,6-D-glucosene formation have been reported in monosaccharides.[17]

After the termination of the stirring, the partially purified reaction mixture was submitted to preparative TLC to isolate 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-xylo-hex-5-eno-pyranose (**13**) in 43% yield. In the infrared (IR) spectrum of **13** in a Nujol mull indicated a peak at 1668 cm^{-1} corresponding to the carbon-carbon double bond. Catalytic hydrogenation of **13** was performed in order to confirm the structure by transformation of **13** to an authentic 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-deoxy-β-lactose (**11**). Treatment of an ethyl acetate solution of **13** with hydrogen in the presence of a palladium catalyst afforded two products, as judged by TLC. Therefore, after removal of the catalyst, the separation was performed using silica gel column chromatography. From the faster moving eluate, small amounts of crystals, mp 221—222° and $[\alpha]_D^{17}$ −10° were isolated which were indistinguishable with an authentic 1,2,3,2′,3′,4′,6′-hepta-O-acetyl-6-deoxy-β-lactose (**11**) by mixed melting point, TLC, and IR. From the second effluent of the same solvent, large amounts of another crystals, mp 134—135° and $[\alpha]_D^{13}$ −35°, were separated. In the NMR spectrum a three-proton doublet ($J_{5,6}$=7 Hz) appeared at τ 8.73 with 21-proton singlets (seven acetyls) at τ 7.77, 7.82, 7.87, and 7.95. The elemental analysis was in good agreement with that of heptaacetyl-monodeoxy-lactose. Therefore, the crystals were assigned to 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-6-deoxy-α-L-idopyranose (**14**).



10 : A=C=H, B=OAc, D=CH_2I, R=Ac
11 : A=C=H, B=OAc, D=CH_3, R=Ac
12 : A=OH, B=C=R=H, D=CH_3
14 : A=D=H, B=OAc, C=CH_3, R=Ac

Chart 1

It was quite interesting to notice that the amount of yield of the 6-deoxy-L-ido isomer (**14**) (55 mg) was

14) J.R. Clamp, L. Hough, J.L. Hickson, and R.L. Whistler, "Advances in Carbohydrate Chemistry," Vol. 16, Academic Press, New York and London, 1961, p. 159.
15) Y.C. Lee, *Biochem. Biophys. Res. Commun.*, **35**, 161 (1969).
16) B. Helferich and E. Himmen, *Ber.*, **61**, 1825 (1928).
17) M.G. Blair, "Methods in Carbohydrate Chemistry," Vol. II, Academic Press, New York and London, 1963, p. 415.

far more predominant than that of the 6-deoxy-D-gluco isomer (**11**) (5 mg). Accordingly, the catalytic hydrogenation using other catalyst than palladium was further investigated. The use of a Raney nickel afforded the 6-deoxy-L-ido isomer (**14**) in 62% yield as the sole product, while a platinum oxide resulted **11** (7%) and **14** (73%). Therefore, regardless of the catalyst, stereospecific hydrogenation proceeded in such a way that the 6-deoxy-L-ido isomer was overwhelmingly predominant.

According to the literature, the opposite result has been reported on hydrogenation of the corresponding 5,6-D-glucosene; hydrogenation of 1,2,3,4-tetra-O-acetyl-6-deoxy-β-D-xylo-hex-5-enopyranose affords a mixture in which 6-deoxy-D-gluco isomer is always predominant than 6-deoxy-L-ido isomer.[18] Thus, the apparent discrepancy in reactivities between unsaturated monosaccharide and disaccharide needs to be investigated further.

Deacetylation of **14** afforded 4-O-β-D-galactopyranosyl-6-deoxy-L-idopyranose (**15**) as a hygroscopic powder. The product is a two anomeric mixture and the crystalline sugar has not been obtained.

Studies on the hydrolysis of the prepared compounds with β-D-galactosidase will be reported in the near future.

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 in a 0.5 dm tube. IR spectra were recorded with a Jasco Model IR-S spectrophotometer. 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 the τ scale. TLC on Silica gel GF_{254} (E. Merck, Darmstadt, Germany) activated at 110° was performed with solvent systems (A) 3: 1 (v/v) $CHCl_3$–acetone, (B) 2: 1 ether–benzene, (C) ether, 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,[19] and detection was effected with Tollens reagent.[20]

1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucuronic Acid (2)——1,2,3-2′,3′,4′,6′-Hepta-O-acetyl-β-lactose (1) was prepared according to the method as described in Part I.[9a]

To a mixture of **1** (3 g, 4.7 mmole) in glacial AcOH (30 ml) was added $KMnO_4$ (1.8 g, 7.6 mmole) in portions under stirring at room temperature. After the termination of addition of $KMnO_4$, the stirring was allowed to continue. The reaction course was controlled by TLC on silica gel with solvent system 3: 10: 5 (v/v) EtOH–*n*-BuOH–H_2O. After 32 hr when the oxidation appeared to be optimal, as judged by TLC, excess $KMnO_4$ was removed by addition of sodium oxalate. The reaction mixture was poured into ice-H_2O (100 ml), filtered, and extracted with CH_2Cl_2 (3×20 ml). As the resulting organic layer was contaminated with some impurities as judged by TLC (solvent A), it was extracted with sat. $NaHCO_3$ solution (4×20 ml). The combined extracts were then poured into ice-H_2O (100 ml) containing glacial AcOH (25 ml), and the solution was extracted again with CH_2Cl_2 (4×20 ml); the resulting CH_2Cl_2-solution was chromatographically homogeneous. The solution was washed with H_2O, dried ($CaCl_2$), and concentrated to give a hygroscopic amorphous powder (1.9 g, 61%), $[\alpha]_D^{23}$ −15.2° (c=1.05, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH). TLC: *Rf* 0.66 (solvent D). *Anal.* Calcd. for $C_{26}H_{34}O_{19}$: C, 48.00; H, 5.27. Found: C, 48.20; H, 5.02.

Methyl 1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucuronate (3)——To a chilled solution of **2** (1.5 g) in dry ether (10 ml) at 0° was added CH_2N_2 in ether until a faint yellow color persisted. After the mixture was kept overnight at room temperature, the solvent was removed by evaporation to give a sirup which crystallized by addition of EtOH. The crystals were collected by filtration and recrystallized from EtOH to give pure **3** (980 mg, 64%), mp 130°, $[\alpha]_D^{23}$ −13.2° (c=1.82, $CHCl_3$). NMR ($CDCl_3$) τ: 6.10 (3H, s, $COOCH_3$), 7.83, 7.90, 8.02 (21H, s, 7×AcO). TLC: *Rf* 0.64 (solvent A), 0.31 (B), 0.39 (C). *Anal.* Calcd. for $C_{27}H_{36}O_{19}$: C, 48.80; H, 5.46. Found: C, 48.61; H, 5.40.

18) L. Hough, R. Khan, and B.A. Otter, "Deoxy Sugars," American Chemical Society, Washington, D.C., 1968, p. 123.
19) M. Ueda, *Yakugaku Zasshi*, **90**, 1322 (1970).
20) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).

4-O-β-D-Galactopyranosyl-α-D-glucuronic Acid Monohydrate (4)——To a chilled solution of **2** (2 g) in dry MeOH (20 ml) was added 0.5 M methanolic sodium methoxide (8 ml) at 0° and the mixture was stirred, with exclusion of moisture, for 1 hr. After about 15 min, a pale yellow precipitate was formed and the complete deacetylation was judged by TLC. Dry Amberlite IR-120 (H^+) resin was added and the suspension was stirred for 30 min, filtered, and the filtrate was concentrated to dryness to give a sirup which was dissolved in a small amount of aq. MeOH. Crystallization was induced after addition of about five fold volume of EtOH and kept for 24 hr in a refrigerator. The crystals were collected by filtration and dried in the air to afford **4** (0.6 g, 55%), mp 159—160°, $[\alpha]_D^{11}$ +62.7° (5 min)⟶+45.5° (24 hr) (c=1.11, H_2O). TLC: *Rf* 0.39 (solvent D). *Anal.* Calcd. for $C_{12}H_{20}O_{12}\cdot H_2O$: C, 38.51; H, 5.92. Found: C, 38.45; H, 5.87.

1,2,3,2′,3′,4′,6′-Hepta-O-acetyl-6-O-methyl-β-lactose (5)——To a chilled mixture of **1** (1 g) in dry CH_2Cl_2 (5 ml) and boron trifluoride etherate (0.1 ml) was added CH_2N_2 in CH_2Cl_2 at 0° until a faint yellow color persisted. After the solution was allowed to stand for 1.5 hr at 0°, the resulting polymethylene was removed by filtration. The filtrate was successively washed with aq. $NaHCO_3$ solution and H_2O, dried ($CaCl_2$), and the solvent was removed by evaporation to afford a sirup which crystallized by addition of EtOH. The crystals were collected by filtration and recrystallized from EtOH to give pure **5** (737 mg, 72%), mp 180—181°, $[\alpha]_D^{11}$ −3.1° (c=1.27, $CHCl_3$). NMR ($CDCl_3$) τ: 6.52 (3H, s, OMe), 7.79, 7.88, 7.90, 7.98 (21H, s, 7×AcO). TLC: *Rf* 0.66 (solvent A), 0.54 (B), 0.48 (C). *Anal.* Calcd. for $C_{27}H_{38}O_{18}$: C, 49.85; H, 5.89. Found: C, 49.79; H, 5.93.

6-O-Methyl-α-lactose (6)——To a solution of **5** (500 mg) in dry MeOH (10 ml) was added 0.5 M methanolic sodium methoxide (0.2 ml) at room temperature. The mixture was similarly treated as described for the preparation of **4** to afford a sirup which crystallized from a small amount of MeOH. The crystals were collected by filtration and recrystallized from MeOH to give hygroscopic crystals (197 mg, 72%), mp 184—185°, $[\alpha]_D^{16}$ +73.1° (5 min)⟶+53.8° (22 hr) (c=0.52, H_2O). TLC: *Rf* 0.40 (solvent D). *Anal.* Calcd. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 43.75; H, 6.93.

Methyl 2,2′,3′,4′,6′-Penta-O-acetyl-3,6-anhydro-β-lactoside (8)——Methyl 2,3,2′,3′,4′,6′-hexa-O-acetyl-6-O-methanesulfonyl-β-lactoside (**7**) was prepared according to the method as described in Part II.[9b]

To an ice-cold solution of **7** (1 g, 1.5 mmole) in dry $CHCl_3$ (5 ml) was added dry MeOH (5 ml) containing Na (0.4 g, 17.4 mmole) at 0°. After the mixture was allowed to stand overnight at 0°, the excess alkali was neutralized with glacial AcOH. The suspension was concentrated to dryness and the residue was acetylated with Ac_2O (5 ml) and pyridine (5 ml) for 24 hr at room temperature. The mixture was poured into ice-H_2O (100 ml), extracted with CH_2Cl_2 (3×20 ml), and then processed in the usual way to give a sirup which crystallized from EtOH. The crystals were collected by filtration and recrystallized from EtOH to give pure **8** (625 mg, 78%), mp 193—194°, $[\alpha]_{23}^{D}$ −92.5° (c=1.06, $CHCl_3$). NMR ($CDCl_3$) τ: 6.51 (3H, s, OMe), 7.75, 7.79, 7.83, 7.93 (15H, s, 5×AcO). TLC: *Rf* 0.30 (solvent A), 0.23 (B), 0.26 (C). *Anal.* Calcd. for $C_{23}H_{32}O_{15}$: C, 50.37; H, 5.88. Found: C, 50.40; H, 5.90.

Methyl 3,6-Anhydro-β-lactoside (9)——To a suspension of **8** (550 mg) in dry MeOH (10 ml) was added 0.5 M methanolic sodium methoxide (0.2 ml) at room temperature. The mixture was similarly treated as described for the preparation of **4** to afford a hydroscopic powder (320 mg, 95%), $[\alpha]_D^{16}$ +5.6° (c=1.08, H_2O), TLC: *Rf* 0.55 (solvent D). *Anal.* Calcd. for $C_{13}H_{22}O_{10}\cdot H_2O$: C, 43.82; H, 6.79. Found: C, 43.54; H, 6.83.

Periodate oxidation of **9** was performed according to the procedure of Okui.[21] Compound (**9**) (70 mg) consumed 1.88 molar quantity of $NaIO_4$ with a concomitant formation of 0.84 molar quantity of H·COOH. The $NaIO_4$-consumptions (mole) at room temperature were as follows: 1,39 (15 min), 1.57 (30 min), 1.69 (1 hr), 1.88 (2 hr, constant), and 1.88 (18 hr).

PPC of Acid Hydrolysate of Compound (9)——A mixture of **9** (100 mg) and 1 M HCl (4 ml) in a sealed tube was heated at 110° for 5 hr. After treatment with charcoal, the mixture was evaporated to dryness and a trace of HCl was removed completely by repeated azeotropic distillation with EtOH and storage of the residue overnight in a desiccator over NaOH. The product was dissolved in a small amount of H_2O, in which D-galactose was identified as the sole reducing sugar (*Rf* 0.38) by PPC.

1,2,3,2′,3′,4′,6′-Hepta-O-acetyl-6-deoxy-β-lactose (11)——1,2,3,2′,3′,4′,6′-Hepta-O-acetyl-6-deoxy-6-iodo-β-lactose (**10**) was prepared according to the method as described in Part I.[9a]

Compound (**10**) (1 g, 1.3 mmole) was dissolved in AcOEt (10 ml) containing triethylamine (0.2 g, 2.0 mmole) and the mixture was shaken with hydrogen in the presence of freshly prepared Raney nickel catalyst at room temperature under atmospheric pressure. The theoretical amount of hydrogen was absorbed in 5 hr. After removal of the catalyst, the filtrate was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (30 ml) and successively washed with 1 M HCl, aq. $NaHCO_3$ solution, 10% aq. $Na_2S_2O_3$ solution, and H_2O, dried ($CaCl_2$), and concentrated to a sirup which was contaminated with some impurities by TLC (solvent A). The CH_2Cl_2-solution of the sirup was chromatographied on a column of silica gel using 10:1 (v/v) $CHCl_3$–acetone as elusion solvent. Evaporation of the solvent from the faster moving eluate afforded a sirup which crystallized from EtOH. The crystals were collected by filtration and recrystallized from EtOH

21) S. Okui, *Yakugaku Zasshi*, **75**, 1262 (1955).

to give pure **11** (492 mg, 59%), mp 221—222°, $[\alpha]_D^{17}$ −10.7° (c=1.03, $CHCl_3$). NMR ($CDCl_3$) τ: 7.79, 7.88, 7.91, 7.98 (21H, s, 7×AcO), 8.62 (3H, d, $J_{5,6}$=5 Hz, C-CH_3). TLC: *Rf* 0.70 (solvent A), 0.33 (B). 0.49 (C). *Anal.* Calcd. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.35; H, 5.85.

6-Deoxy-α-lactose (12)——To a suspension of **11** (300 mg) in dry MeOH (5 ml) was added 0.5 M methanolic sodium methoxide (0.1 ml) at room temperature. The mixture was stirred, with the exclusion of moisture, for 20 min to effect dissolution, and the solution was allowed to stand for 5 hr. The resulting precipitates were dissolved in a small amount of H_2O. To the solution was added dry Amberlite IR-120 (H^+) resin and the mixture stirred for 30 min. After removal of the resin by filtration, the filtrate was concentrated to afford a sirup which crystallized from a small amount of MeOH. The crystals were collected by filtration and dried in a vacuum desiccator to afford hygroscopic crystals (122 mg, 77%), mp 210—211°, $[\alpha]_D^{21}$ +53.7° ⟶ +40.7° (24 hr) (c=1.08, H_2O). TLC: *Rf* 0.49 (solvent D). *Anal.* Calcd. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.08; H, 6.69.

1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-xylo-hex-5-eno-pyranose (13)—Dry silver fluoride (1 g, 7.9 mmole) was added to a solution of **10** (1 g, 1.3 mmole) in dry pyridine (8 ml) and the suspension was shaken, with the exclusion of light, for 22 hr at room temperature. The resulting black mixture was diluted with CH_2Cl_2 (50 ml), and it was poured into H_2O (100 ml) under stirring. After removal of the precipitates by filtration, the organic layer was separated, successively washed with 5% H_2SO_4, aq. $NaHCO_3$ solution, and H_2O, dried ($CaCl_2$), and concentrated to afford black residue which was dissolved in CH_2Cl_2. The CH_2Cl_2-solution was submitted to preparative TLC using ether as developing solvent. Detection of **13** on the plate was effected with irradiation of UV lamp (short wave length). The UV-positive sections were scraped up and the combined silica gel was extracted with AcOEt. Evaporation of the solvent from the extract afforded a sirup which crystallized from EtOH. The crystals were collected by filtration and recrystallized from EtOH to give pure **13** (358 mg, 43%), mp 168—169°, $[\alpha]_D^{18}$ −57.7° (c=1.04, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1668 (C=C). TLC: *Rf* 0.69 (solvent A), 0.40 (B), 0.53 (C). *Anal.* Calcd. for $C_{26}H_{34}O_{17}$: C, 50.49; H, 5.54. Found: C, 50.79; H, 5.67.

1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-6-deoxy-α-L-idopyranose (14)——1) Catalytic Reduction with Palladium Catalyst: A solution of **13** (100 mg) in AcOEt (5 ml) was added to Pd catalyst which had been freshly made by reduction of $PdCl_2$ (100 mg) in the same solvent.[22] On vigorous stirring at room temperature, the mixture absorbed the theoretical amount of hydrogen in 2.5 hr. The catalyst was removed by filtration and the solution concentrated to a sirup which showed two spots, *Rf* 0.27 (main) and 0.49 (minor) by TLC (solvent C). The sirup was dissolved in CH_2Cl_2 and the solution was applied on a column of silica gel using ether as elusion solvent. Evaporation of the solvent from the faster moving eluate afforded a sirup which crystallized from EtOH. The separated crystals (5 mg, 5%) were indistinguishable with **11** (mixed mp, IR, and TLC).

Another crystals (**14**) were separated from the second effluent of the same solvent. Recrystallization from EtOH gave pure **14** (55 mg, 55%), mp 134—135°, $[\alpha]_D^{13}$ −35° (c=0.40, $CHCl_3$). NMR ($CDCl_3$) τ: 7.77, 7.82, 7.87, 7.95 (21H, s, 7×AcO), 8.73 (3H, d, $J_{5,6}$=7 Hz, C-CH_3). TLC: *Rf* 0.58 (solvent A), 0.21 (B), 0.27 (C). *Anal.* Calcd. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.18; H, 5.66.

2) Catalytic Reduction with Platinum Oxide Catalyst: Instead of Pd catalyst, platinum oxide catalyst was adopted and hydrogenation of **13** (100 mg) was similarly performed according to the method as described in 1). After column chromatography of the resulting hydrogenation product, compound (**11**) (7 mg, 7%) and (**14**) (73 mg, 73%) were separated.

3) Catalytic Reduction with Raney Nickel Catalyst: In this case, Compound (**14**) (62 mg, 62%) was obtained as the sole hydrogenation product.

4-O-β-D-Galactopyranosyl-6-deoxy-L-idopyranose (15)——To a solution of **14** (100 mg) in dry MeOH (5 ml) was added 0.5 M methanolic sodium methoxide (0.1 ml) at room temperature. The mixture was similarly treated as described for the preparation of **4**, to afford hygroscopic amorphous powder (50 mg, 95%), $[\alpha]_D^{16}$ −7.1° (c=0.28, H_2O). TLC: *Rf* 0.51 (solvent D). *Anal.* Calcd. for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.30; H, 7.10.

Acknowledgement The authors are grateful to Miss. M. Ishiguro and K. Kamijo for elemental analyses and NMR measurement.

22) O.T. Schmidt and W. Starb, *Chem. Ber.*, **87**, 393 (1954).