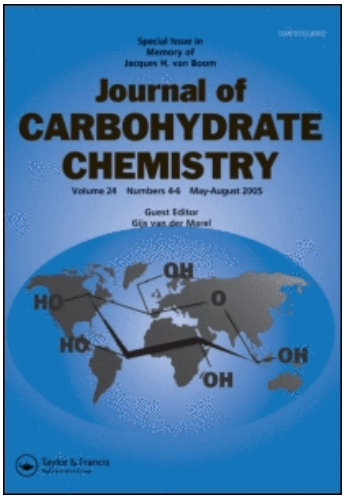

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An Improved Method for the Synthesis of 3.6-Di-O-Methyl-D-Glucose: Preparation of the Neo-Glycoprotein Containing 3,6-Di-O-Methyl-β-D-Glucopyranosyl-Groups

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AN IMPROVED METHOD FOR THE SYNTHESIS OF 3,6-DI-O-METHYL-D-GLUCOSE: PREPARATION OF THE NEO-GLYCOPROTEIN CONTAINING 3,6-DI-O-METHYL-β-D-GLUCOPYRANOSYL GROUPS

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ABSTRACT

3,6-Di-O-methyl-D-glucose, the non-reducing terminal sugar of the phenolic glycolipid-I, elaborated by Mycobacterium leprae, has been synthesized by a simple procedure and in high yield. 3-O-Methyl-D-glucose was converted to the corresponding benzyl glycoside and then tosylated to give benzyl 3-O-methyl-6-O-tosyl-β-D-glucopyranoside. Displacement of tosyl group with sodium methoxide followed by debenzylation afforded 3,6-di-O-methyl-D-glucose in high yield. Condensation of the acetobromo derivative of 3,6-di-O-methyl-D-glucose with 8-ethoxycarbonyloctanol gave 8-ethoxycarbonyloctyl 2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranoside. This was then deacetylated, converted to hydrazide, and finally coupled to bovine serum albumin via the acyl azide intermediate. The neo-glycoprotein containing the 3,6-di-O-methyl-β-D-glucopyranosyl group is useful for serodiagnosis of leprosy.

INTRODUCTION

In the recent past, the isolation and characterization of the specific glycolipid antigen of Mycobacterium leprae,[1-3] the so called phenolic glycolipid-I (PGL-I) has immensely contributed towards

the serodiagnosis of leprosy. PGL-I is a triglycosylphenolic diacylphthiocerol which contains the following trisaccharide; 3,6-di-O-methyl-β-D-glucopyranosyl-(1 ⟶ 4)-2,3-di-O-methyl-α-L-rhamnopyranosyl-(1⟶2)-3-O-methyl-α-L-rhamnopyranose.

The serological activity[4-6] of PGL-I and its dissected parts have been tested against hyperimmune anti-M. leprae rabbit antiserum and sera from leprosy patients by enzyme linked immunosorbent assay (ELISA). The principal specificity has been found to reside in the non-reducing terminal 3,6-di-O-methyl-β-D-glucopyranosyl group. Thus 3,6-di-O-methyl-β-D-glucopyranose coupled to bovine serum albumin,[7] is highly reactive with the leprosy sera. However, the neo-glycoproteins containing the di- or tri- saccharide,[8] similar to that of native PGL-I, showed higher sensitivity and selectivity. Thus 3,6-di-O-methyl-D-glucose is an essential sugar for the synthesis of any of the glycoproteins. We have developed a simple method for the synthesis of 3,6-di-O-methyl-D-glucose which has been synthesized earlier using different methods.[7,9-12] The 3,6-di-O-methyl-D-glucose, conjugated to bovine serum albumin via an 8-(ethoxycarbonyl) octyl linker arm,[13,14] can be used for diagnosis of leprosy at an early stage of infection.[4-6,15,16]

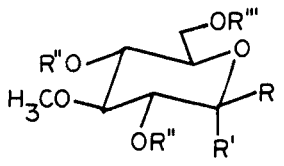
RESULTS AND DISCUSSION

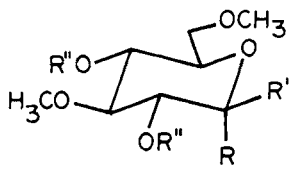
3-O-Methyl-D-glucose was synthesized from D-glucose, and was used as the starting compound.[17,18] Acetylation of 3-O-methyl-D-glucose with acetic anhydride-pyridine gave an anomeric mixture of 1,2,4,6-tetra-O-acetyl-3-O-methyl-D-glucopyranose **1** in ~100% yield. Bromination of **1** with anhydrous hydrogen bromide in acetic acid gave 2,4,6-tri-O-acetyl-3-O-methyl-α-D-glucopyranosyl bromide **2** in 96% yield. Condensation of **2** with a slight excess of benzyl alcohol in the presence of mercuric cyanide in dichloromethane gave benzyl 2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranoside **3** in 82% yield. Compound **3** was then deacetylated with sodium methoxide to give benzyl 3-O-methyl-β-D-glucopyranoside **4**.

Tosylation[19] of **4** using freshly crystallized tosyl chloride (1.05 equivalent) in the presence of 4-dimethylaminopyridine and triethyl-

amine in anhydrous dichloromethane gave benzyl 3-O-methyl-6-O-tosyl-β-D-glucopyranoside **5** in 88% yield after crystallization from diethyl ether-n-hexane. Treatment of **5** with 3M sodium methoxide in methanol gave benzyl 3,6-di-O-methyl-β-D-glucopyranoside **6** in 75% yield, after purification by column chromatography. Catalytic hydrogenolysis of **6** over 10% Pd-C gave 3,6-di-O-methyl-D-glucose **7** in 92% yield, which was crystallized from dichloromethane containing a few drops of methanol. 3,6-Di-O-methyl-D-glucose was converted to the corresponding alditol acetate[20] and was analyzed by GLC, using authentic 1,2,4,5-tetra-O-acetyl-3,6-di-O-methyl-D-glucitol as a standard.

3,6-Di-O-methyl-D-glucose was acetylated using acetic anhydride and pyridine to give 1,2,4-tri-O-acetyl-3,6-di-O-methyl-D-glucopyranose **8**. The anomeric mixture of **8** was brominated using anhydrous hydrogen bromide in acetic acid just prior to the coupling reaction (74%) due to high instability of 2,4-di-O-acetyl-3,6-di-O-methyl-α-D-glucopyranosyl bromide **9**. Condensation of **9** with 8-ethoxycarbonyloctanol under modified Helferich condition[21] using mercuric cyanide-mercuric bromide (5:2) in anhydrous dichloromethane gave 8-(ethoxycarbonyl) octyl 2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranoside **10** in 75%





	R	R'	R''	R'''
1.	H,OAc		Ac	Ac
2.	H	Br	Ac	Ac
3.	OBn	H	Ac	Ac
4.	OBn	H	H	H
5.	OBn	H	H	Ts
6.	OBn	H	H	CH_3
7.	H, OH		H	CH_3

	R	R'	R''
8.	H,OAc		Ac
9.	Br	H	Ac
10.	H	L-OEt	Ac
11.	H	L-OMe	H
12.	H	L-NH-NH_2	H
13.	H	L-N_3	H
14.	H	L-NH-BSA	H

L= O-$(CH_2)_8$-CO-

yield. Zemplén deacetylation[22] of **10** afforded 8-(methoxycarbonyl)octyl 3,6-di-O-methyl-β-D-glucopyranoside **11** in 94% yield. Conversion of compound **11** into its hydrazide by using hydrazine hydrate gave **12** in 80% yield after crystallization from ethyl acetate-n-hexane. The hydrazide **12** was converted into its acyl azide **13**, and this, in turn, was conjugated to the bovine serum albumin (BSA) to give O-(3,6-di-O-methyl-β-D-glucopyranosyl)-(1 ⟶ 9)-oxynonanoyl-BSA **14**. The amount of 3,6-di-O-methyl-D-glucose, incorporated to BSA (30 mmols of sugar per mmol of BSA), was estimated by the phenol-sulfuric acid method.[23]

EXPERIMENTAL

General procedures. Melting points were determined in a sulfuric acid bath and are uncorrected. Optical rotations were measured with a Jasco, DIP-360 polarimeter and NMR spectra were recorded with a Jeol FX-100 spectrometer. GLC was performed using packed columns (6m x 4mm) of 3% ECNSS-M on Gas-Chrom Q at 170 °C and 3% OV-225 on Gas-Chrom Q at 170 °C in an Hewlett-Packard 5730A gas chromatograph fitted with a flame ionization detector. TLC was performed on silica gel-G (BDH,India). Spots were made visible by spraying the plates with 10% sulfuric acid, followed by heating. Column chromatography was performed on silica gel 60-120 mesh (SISCO, India). The following solvent systems (v/v) were used: A, 1:1 n-hexane-diethyl ether; B, 1% methanolic dichloromethane; C, 2:1 ethyl acetate-toluene; D, 9:1 dichloromethane-methanol; E, 3:1 toluene-ethyl acetate; F, 8:1 ethyl acetate-methanol. Solutions were evaporated, at a temperature <50 °C, under diminished pressure.

Benzyl 3-O-methyl-β-D-glucopyranoside (4). 3-O-Methyl-D-glucose (32.8 g), synthesized from D-glucose as described elsewhere,[17,18] was acetylated with acetic anhydride (25 mL) and pyridine (25 mL) to give 1,2,4,6-tetra-O-acetyl-3-O-methyl-D-glucose **1** as a syrup (61.2 g, ~100%); TLC R_f 0.43 (A). To a chilled (at 0 °C) solution of **1** in dry dichloromethane (10 mL) and freshly distilled acetic acid (60 mL), was added a saturated (at 0 °C) solution of hydrogen bromide in acetic acid (120 mL). The mixture was kept for 3 h at 0 °C and then diluted with dichloromethane (200 mL). The solution was washed

successively with iced water twice, aqueous sodium bicarbonate, and water. The organic layer was dried ($MgSO_4$), filtered, and concentrated to a syrup to give 2,4,6-tri-O-acetyl-3-O-methyl-α-D-glucopyranosyl bromide **2** (62 g, 96%); TLC R_f 0.62 (A).

To a stirred mixture of dry benzyl alcohol (35 mL, 338 mmol), mercuric cyanide (41 g, 162 mmol), and powdered molecular sieves 4 A° (20 g) in dry dichloromethane (150 mL) was added dropwise over a period of 1 h, a solution of **2** (62 g, 162 mmol) in dry dichloromethane (150 mL). The mixture was stirred for 16 h at room temperature, filtered through a layer of celite, and the residue was washed with dichloromethane. The combined filtrate and washings were washed successively with water, aqueous potassium bromide, aqueous sodium bicarbonate, and water, and dried ($MgSO_4$). The solution was concentrated, and remaining benzyl alcohol was removed *in vacuo* at 80 °C by codistillation with water. The resulting white mass was recrystallized from ethanol to give benzyl 2,4,6-tri-O-acetyl-3-O-methyl-β-D-glucopyranoside **3** as needles (54.5 g, 82%): TLC R_f 0.7 (B); mp 106-108 °C (lit.[7] mp 88-91 °C); $[\alpha]_D^{25}$ - 62.7° (c 2.5, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.40-7.32 (m, 5H, Ph), 5.18-4.98 (m, 2H, ring CH), 4.72 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, $PhCH_2$), 4.44 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.24-4.16 (m, 2H, ring CH), 3.66-3.44 (m, 2H, ring CH), 3.38 (s, 3H, OCH_3), 2.09, 2.07, 2.06 (s, each 3H, OAc); ^{13}C NMR ($CDCl_3$) δ 170.60-169.13 (CH_3-CO-O-), 136.96 (Ph,α-C), 128.36, 127.83, 127.65 (Ph), 99.57 (C-1), 81.32 (C-3), 72.08, 72.00 (C-5, C-2), 70.50 (CH_2-Ph), 69.21 (C-4), 62.36 (C-6), 58.85 (OCH_3), 20.77 (CH_3-CO-).

Compound **3** (54.4 g) in absolute methanol (350 mL) was treated with methanolic 0.02 M sodium methoxide (150 mL). The solution was kept for 2 h at room temperature when TLC R_f 0.3 (B) showed complete conversion of starting compound. It was then made neutral with Dowex 50-WX8 (H^+) ion-exchange resin, filtered, and concentrated to a syrup, which crystallized from dichloromethane-n-hexane to give **4** (36.2 g, 96%): mp 108-109 °C; $[\alpha]_D^{25}$ - 65.1° (c 1.5, CH_2Cl_2) [lit.[7] mp 106 °C; $[\alpha]_D$ - 55.26° (c 2.87, $CHCl_3$)]; ^1H NMR (CD_3OD) δ 7.60-7.21 (m, 5H, Ph), 4.78 (ABq, 2H, $J_{A,B}$ = 12 Hz, $PhCH_2$), 4.34 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.62 (s, 3H, OCH_3); ^{13}C NMR (CD_3OD) δ 138.86 (Ph, α-C), 129.15, 129.03, 128.56 (Ph), 103.12

(C-1), 87.67 (C-3), 77.67 (C-5), 74.80 (C-2), 71.64 (CH_2-Ph), 71.06 (C-4), 62.63 (C-6), 60.93 (OCH_3).

Benzyl 3-O-methyl-6-O-tosyl-β-D-glucopyranoside (5). A mixture of **4** (6.8 g, 24.0 mmol), freshly crystallized tosyl chloride (4.8 g, 25.2 mmol), 4-dimethylaminopyridine (144 mg), freshly distilled triethylamine (6 mL), dimethylformamide (6 mL), and anhydrous dichloromethane (120 mL) was stirred at room temperature under nitrogen for 4 h. The mixture was then stirred for 1 h in the presence of ice, diluted with dichloromethane (100 mL). The organic layer was then washed with ice-cold 2M hydrochloric acid, saturated aqueous sodium bicarbonate, water, dried ($MgSO_4$), and concentrated to dryness (9.8 g, 93%). The product **5** was recrystallized from n-hexane-diethyl ether (9.3 g, 88%): TLC R_f 0.65 (C); mp 100-101 °C; $[\alpha]_D^{25}$ -53.4° (c 1.53, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.95-7.32 (m, 9H, Ph), 4.70 (ABq, 2H, $J_{A,B}$ = 12 Hz, $PhCH_2$), 4.32 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.62 (s, 3H, OCH_3), 2.62 (d, 1H, OH), 2.42 (s, 3H, CH_3).

Anal. Calcd for $C_{21}H_{26}O_8S$: C, 57.52; H, 5.98. Found : C, 57.52; H, 6.2.

Benzyl 3,6-di-O-methyl-β-D-glucopyranoside (6). To a solution of **5** (1.43 g, 3.26 mmol) in dry methanol (10.9 mL), 3 M sodium methoxide in methanol (5.4 mL, 16.3 mmol) was added and the reaction mixture heated under reflux for 5 h. It was then cooled, diluted with cold water (5 mL), and concentrated to near dryness. The residue was diluted with dichloromethane (25 mL), washed successively with cold 2M hydrochloric acid, aqueous sodium bicarbonate, and water, dried ($MgSO_4$) and concentrated. TLC R_f 0.45 (C) showed formation of a new compound. The product was purified by silica gel column chromatography (C) to give **6** (0.73 g, 75%). Analytical sample was prepared by recrystallization from ethyl acetate-n-hexane: mp 95-96 °C, $[\alpha]_D^{25}$ -71.3° (c 2.2, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.33 (s, 5H, Ph), 4.78 (ABq, 2H, $J_{A,B}$ = 12.0 Hz, CH_2-Ph), 4.36 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.62, 3.38 (s, each 3H, OMe), 2.82 (d, 1H, OH), 2.42 (d, 1H, OH).

Anal. Calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found : C, 60.32; H, 7.56.

3,6-Di-O-methyl-D-glucose (7). Compound **6** (3.2 g) was dissolved in methanol and hydrogenated in the presence of 10% Pd-C (0.16 g) for 6 h at room temperature and normal pressure. The catalyst was separated by filtration of the mixture through a celite pad, and washed with methanol. The combined filtrate and washings were evaporated to give **7** (2.05 g, 92%). 3.6-Di-O-methyl-D-glucose was crystallized from dichloromethane containing few drops of methanol (1.6 g, 78%). A further 0.35 g of material was obtained upon crystallization of the mother liquors (88%, total yield) : TLC R_f 0.2 (D); mp 115-117 °C; $[\alpha]_D^{25}$ + 62° (c 1.5, H_2O) [lit.[9] mp 113-116 °C; $[\alpha]_D^{18}$ + 61.6° (c 1, H_2O)]; ^1H NMR (CD_3OD) δ 5.12-5.04 (m, 1H, H-1), 3.62, 3.34 (s, each 3H, OMe).

3,6-Di-O-methyl-D-glucose **7** (2 mg) was then converted to its alditol acetate,[20] and analyzed by GLC by comparing with an authentic sample of 1,2,4,5-tetra-O-acetyl-3,6-di-O-methyl-D-glucitol. The chromatogram showed a major peak for 1,2,4,5-tetra-O-acetyl-3,6-di-O-methyl-D-glucitol (97%).

8-(Methoxycarbonyl)octyl 3,6-di-O-methyl-β-D-glucopyranoside (11). 3,6-Di-O-methyl-D-glucose **7** (0.5 g) was acetylated with acetic anhydride (1 mL) and pyridine (1 mL) to give 1,2,4-tri-O-acetyl-3,6-di-O-methyl-D-glucopyranose anomers **8**. To a chilled (to 0 °C) solution of **8** (0.8 g) in dichloromethane (0.2 mL) and freshly distilled acetic acid (0.8 mL) was added a saturated solution of hydrogen bromide (at 0 °C) in acetic acid (1.6 mL). The mixture was stirred for 0.5 h at 0 °C when TLC R_f 0.65 (A) showed almost complete conversion of starting compound. The solution was washed successively with cold water, aqueous sodium bicarbonate, and water, dried ($MgSO_4$) and concentrated to give 2,4-di-O-acetyl-3,6-di-O-methyl-α-D-glucopyranosyl bromide **9** (0.63 g, 74%) as a syrup.

To a stirred solution of 8-ethoxycarbonyloctanol (0.36 g, 1.78 mmol) in dichloromethane (4 mL) containing mercuric cyanide (0.36 g, 1.43 mmol), mercuric bromide (0.2 g, 0.55 mmol) and dry powdered molecular sieve 4 A° (0.4 g) was added a solution of **9** (0.63 g, 1.77 mmol) in dry dichloromethane (2 mL). The mixture was stirred for 16 h at room temperature under nitrogen. The solids were filtered off and washed with dichloromethane. The combined filtrate and

washings was washed successively with cold water, M potassium bromide, and water, dried ($MgSO_4$) and concentrated to dryness. The residue was then purified by silica gel column chromatography (E) to give 8-(ethoxycarbonyl)octyl 2,4-di-O-acetyl-3,6-di-O-methyl-β-D-glucopyranoside **10** (0.63 g, 75%); TLC R_f 0.62 (E).

To a cooled solution of **10** (0.6 g) in dry methanol (6 mL) was added methanolic 0.02 M sodium methoxide (2 mL) and the resulting solution left overnight at room temperature. It was then made neutral with Dowex 50-WX8 (H^+) ion-exchange resin, filtered, and concentrated to a syrup **11** (0.45 g, 94%): TLC R_f 0.2 (E); 0.8 (F); $[\alpha]_D^{25}$ -25.1° (c 1.6, CH_3OH) [lit.[7] $[\alpha]_D^{25}$ -30° (c 1.0, CH_3OH)]; ^1H NMR (CD_3OD) δ 4.28 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.67, 3.66 (s, 3H each, CO_2Me, OMe), 3.38 (s, 3H, OMe).

8(Hydrazinocarbonyl)octyl 3,6-di-O-methyl-β-D-glucopyranoside (12). Compound **11** (0.42 g) in dry ethanol (7 mL) was treated with hydrazine hydrate (1 mL, 85%) for 36 h at room temperature. After evaporation of solvents, and codistillation of traces of hydrazine with ethanol, the residue was dried under vacuum to give **12** (0.36 g, 87%); TLC R_f 0.45 (F). Compound **12** was purified by crystallization from ethyl acetate-n-hexane (0.33 g, 80%): mp 104-106 °C; $[\alpha]_D^{25}$ -21.3° (c 1.4, CH_3OH) [lit.[7] mp 179 °C, $[\alpha]_D$ -70° (c 0.5, H_2O)]; ^1H NMR ($CDCl_3$) δ 6.96 (bs, 1H, NH), 4.25 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 3.62, 3.38 (s, each 3H, OMe), 2.12 (t, 2H, $-CH_2CO$), 2.65-1.25 (m, 12H, $-(CH_2)_6-$); ^{13}C NMR ($CDCl_3$) δ 178.22 (C=O), 102.96 (C-1), 85.88 (C-3), 74.65 (C-2), 73.71 (C-4), 72.49 (O-CH_2), 70.61 (C-5), 70.03 (C-6), 60.43 (OMe), 59.56 (OMe), 29.54, 29.01, 25.74, 25.39 [$(CH_2)_7$].

O-(3,6-Di-O-methyl-β-D-glucopyranosyl)-(1 ⟶ 9)-oxynonanoyl-BSA (14). A stirred solution of **12** (36 mg, 95 μmol) in dry dimethylformamide (1.2 mL) was cooled to -30 °C, and 3.6 M hydrochloric acid in dry 1,4-dioxane (215 μL) was added. A solution of t-butylnitrite in dry dimethylformamide (1:10, 345 μL) was added and the solution was stirred for 30 min at -30 °C. TLC (F) showed disappearance of **12** and formation of a new faster-moving component **13**. The excess of nitrous acid was neutralized with 0.5 M solution of sulfamic acid in dimethylformamide (345 μL). After 15 min the cold (-50 °C)

solution of the acyl azide **13** was added dropwise to a solution of BSA (69 mg) in an aqueous solution 0.08 M in $Na_2B_4O_7$ and 0.3 M in $KHCO_3$ (6.9 mL, pH 9.2) at 0 °C. The solution was stirred overnight at 0 °C and then dialyzed against five changes of distilled water in an Amicon ultrafiltration cell equipped with UM-10 membrane and freezed-dried to provide **14** as a white fluffy material (95 mg). The amount of hexose bound to protein was then estimated by the phenol-sulfuric acid method[23] using 3,6-di-O-methyl-D-glucose as a standard and the number of moles of bound hapten was calculated on the basis of a molecular weight for BSA of 65,000.

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