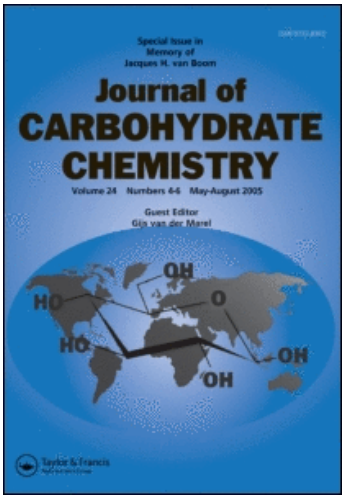

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Synthesis of Terminal Oligosaccharides from Type Specific Lipooligosaccharide of *Mycobacterium Tuberculosis*

Mukund K. Gurjar[a]; Sanjoy K. Das[a]; Prathama S. Mainkar[a]
[a] Indian Institute of Chemical Technology, Hyderabad, India

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SYNTHESIS OF TERMINAL OLIGOSACCHARIDES FROM TYPE SPECIFIC LIPOOLIGOSACCHARIDE OF *MYCOBACTERIUM TUBERCULOSIS*

Mukund K Gurjar,* Sanjoy K. Das and Prathama S. Mainkar

Indian Institute of Chemical Technology, Hyderabad 500 007, India

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ABSTRACT

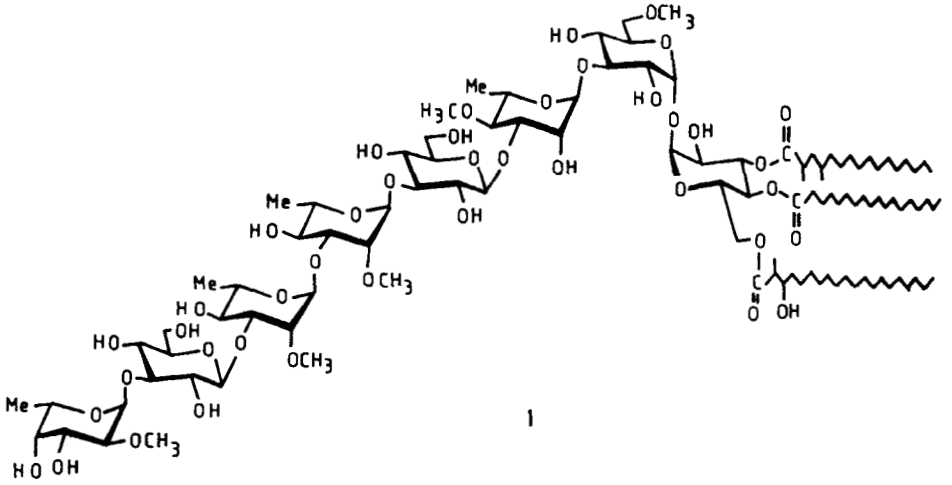
The coupling reaction between 1,3-di-*O*-acetyl-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranose (**9**) and methyl 4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (**4**) was carried out in the presence of boron trifluoride-etherate followed by deacetylation to give the disaccharide (**11**) containing a free 3' position. The second glycosylation reaction with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric salts followed by removal of benzyl and acetyl groups provided the trisaccharide **2**. The boron trifluoride catalysed condensation of 1,3,4-tri-*O*-acetyl-2-*O*-methyl-L-fucopyranose (**14**) and methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**15**) gave the disaccharide (**16**) from which the protecting groups were removed to form the disaccharide (**3**).

INTRODUCTION

It is estimated that 3 million persons die of tuberculosis every year out of 16 million active patients, while about 1 billion people have been infected with tuberculosis worldwide.[1] The highly immunoreactive glycolipids obtained from *Mycobacterium (M.) tuberculosis* (strain Canetti) belong to the trehalose containing lipooligosaccharides (LOS). Out of the two LOSs isolated, the simpler glycolipid (LOS-1) was assigned the structure : 2-*O*-Me-α-L-Fucp-(1→3)-β-D-Glcp-(1→3)-2-*O*-Me-α-L-Rhap-(1→3)-2-*O*-Me-α-L-Rhap-(1→3)-α-D-Glcp-(1→3)-4-*O*-Me-α-L-Rhap-(1→3)-6-*O*-Me-α-D-Glcp-(1→1)-tri-*O*

IICT Communication No. 3267

-acyl-α-D-Glcp (1), based on IR, NMR, FAB-Mass and chemical degradation studies.[2] The LOS-II contains a distal 4-amino-4,6-dideoxy-galactose residue for which the absolute configuration is yet not established. The antigenicity of glycolipids notably resides at the terminal oligosaccharide segment and therefore synthesis of these determinants formed the basis of our studies.[3] In this report, the synthesis of β-D-Glcp-(1 → 3)-2-*O*-Me-α-L-Rhap-(1 → 3)-2-*O*-Me-α-L-Rhap (2) and 2-*O*-Me-α-L-Fucp-(1 → 3)-α-D-Glcp (3), as methyl glycosides which constitute part structures of the larger oligosaccharide LOS-I, have been presented.



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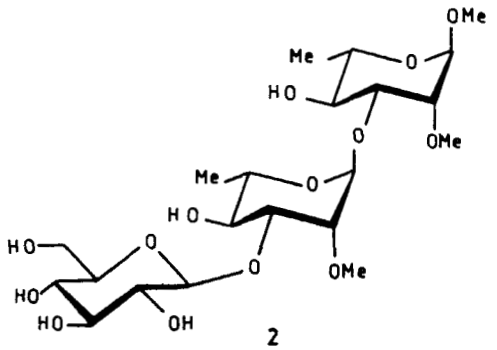

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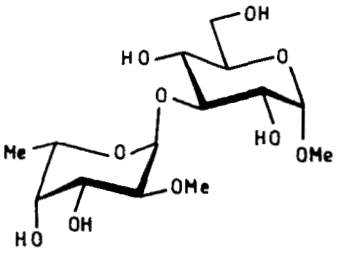
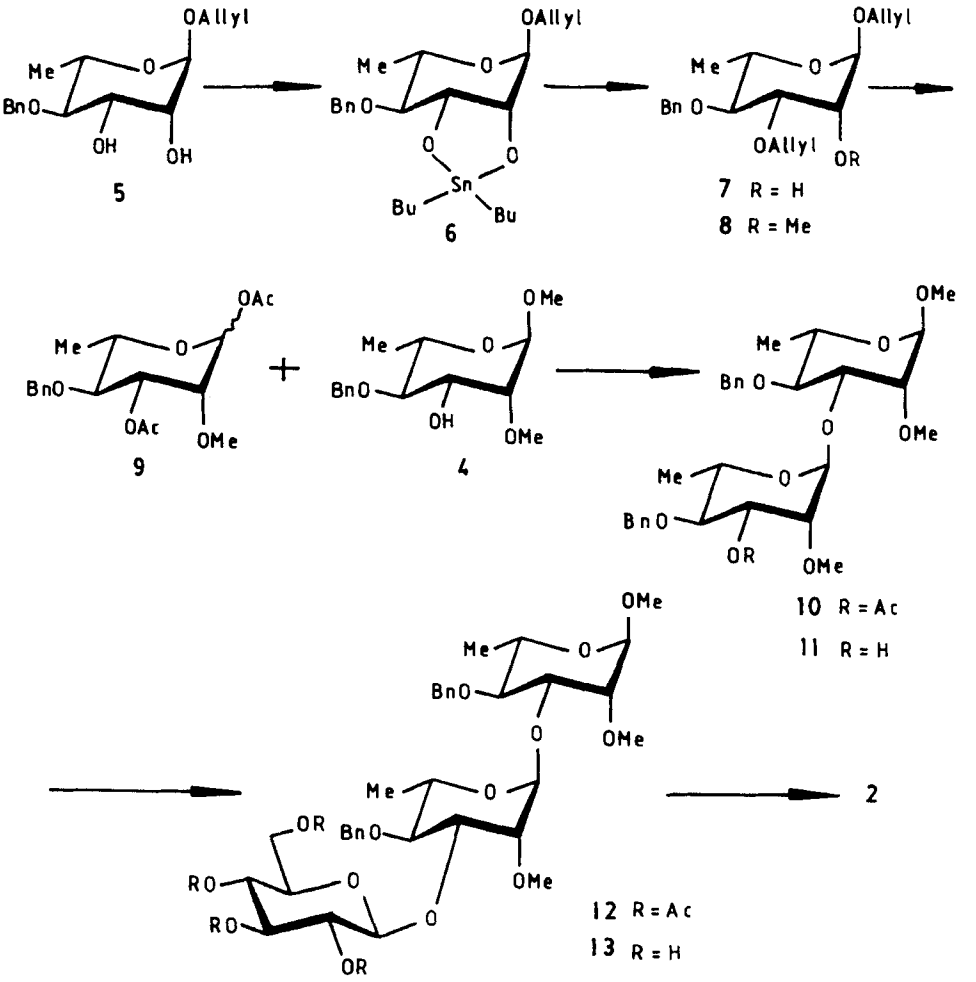


3

RESULTS AND DISCUSSION

To initiate the synthesis of the trisaccharide (2), the preparation of the disaccharide (11), containing a free OH group at 3'-position was first considered. The aglycone, methyl 4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (4), chosen for the present synthesis was earlier prepared[4] in

these laboratories from methyl α-L-rhamnopyranoside. The synthesis of the glycosyl donor 1,3-di-*O*-acetyl-4-*O*-benzyl-2-*O*-methyl-L-rhamnopyranose (**9**), was carried out as follows. The known[5] allyl 4-*O*-benzyl-α-L-rhamnopyranoside (*5*) and dibutyltin oxide were heated under reflux in benzene with azeotropic removal of water for 3 h and then the resulting dibutylstannylacetal derivative **6** was treated with allyl bromide in *N*,*N*-dimethylformamide at 90 °C to give the 3-*O*-allyl derivative **7**. The free OH group at C-2 was functionalised as its methyl ether (**8**) by using sodium hydride-

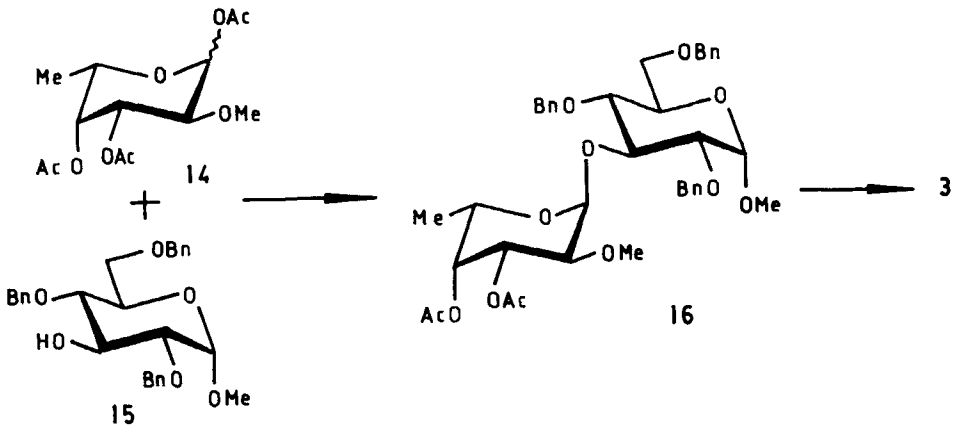


Scheme 1

methyl iodide in dry tetrahydrofuran. The next reaction was the removal of both the allyl groups for which **8** was first isomerised in the presence of tris(triphenylphosphine)rhodium (I) chloride followed by hydrolysis with mercury (II) chloride-mercury (II) oxide in aqueous acetone.[6] The resulting hemiacetal, after acetylation with acetic anhydride and pyridine, gave the diacetate (**9**).

The coupling reaction[7] of **4** with **9** was promoted with boron trifluoride etherate to give the disaccharide (**10**) whose structure was proven by the NMR studies. For example, in the ^{13}C NMR studies, the anomeric carbons were located at 97.77 ($J_{C\text{-}1,H\text{-}1}$ = 170 Hz), and 99.35 ($J_{C\text{-}1',H\text{-}1'}$ = 167 Hz) ppm which were consistent with the assigned structure. The removal of the acetate group from **10** under Zemplen condition gave the requisite disaccharide (**11**), suitable for further glycosylation reaction at 3'-position.

The next coupling reaction of **11** with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide[8] was carried out in the presence of mercury (II) cyanide and mercury (II) bromide to afford the trisaccharide derivative **12** which was isolated after deacetylation as the tetraol (**13**). In the ^{13}C NMR spectrum of **13**, the requisite anomeric signals were located at 97.79 ($J_{C\text{-}1,H\text{-}1}$ = 167 Hz), 99.22 ($J_{C\text{-}1',H\text{-}1'}$ = 174 Hz) and 103.79 ($J_{C\text{-}1'',H\text{-}1''}$ = 157 Hz) ppm. Finally, the benzyl groups from **13** were cleaved by hydrogenolysis over palladium-carbon to afford the trisaccharide (**2**). In the ^{1}H NMR spectrum of **2**, the anomeric protons resonated at 4.55 (doublet, $J_{1'',2''}$ = 8.1 Hz), 4.70 (singlet) and 5.05 (singlet) ppm.



Scheme 2

The synthesis of methyl 3-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-α-D-glucopyranoside (**3**), which constitutes the terminal disaccharide segment of LOS-I was then investigated. The coupling reaction of 1,3,4-tri-*O*-acetyl-2-*O*-methyl-L-fucopyranose (**14**)[9] and methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (**15**)[10] was carried out in the presence of boron trifluoride etherate to afford the disaccharide (**16**). In the ^{13}C NMR spectrum of **16**, the following chemical shifts and the coupling constants: 96.25 ($J_{C-1,H-1}$=175 Hz) and 97.6 ($J_{C-1',H-1'}$ = 171 Hz) ppm were observed for anomeric carbons. Removal of the acetyl groups by Zemplen reaction and the benzyl groups by hydrogenolysis gave the requisite disaccharide **3** whose ^1H NMR spectrum revealed two doublets at 4.66 ($J_{1,2}$ = 1.5 Hz) and 5.22 ($J_{1',2'}$ = 3.0 Hz) ppm for the anomeric protons.

EXPERIMENTAL

General Procedures. Optical rotations were determined at 25 °C with JASCO DIP 370 polarimeter. NMR were recorded on Varian Gemini 200 MHz and Varian Unity 400 MHz spectrophotometers with tetramethylsilane as an internal standard. Mass spectra were recorded on a CFC-21-110B. TLC was performed on Silica Gel G (Merck) and detection being either by charring with α-naphthol or UV detector. Column chromatography was performed on silica gel (Mesh-60-120) purchased from Acme Chemical Company, Bombay.

Allyl 3-*O*-Allyl-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranoside (8). A solution of **5** (5.0 g, 17.0 mmol) and dibutyltin oxide (5.0 g, 20.4 mmol) in benzene (75 mL) was heated under reflux with azeotropic removal of water for 3 h. Solvent was removed, and the residue was diluted with dry *N*,*N*-dimethylformamide (50 mL) followed by the addition of allyl bromide (3.08 g, 25.5 mmol). The reaction mixture was heated at 90 °C for 3 h and then concentrated. The residue was dissolved in ethyl acetate, washed with water, dried and concentrated. The residue was purified by column chromatography on silica gel by eluting with ethyl acetate-light petroleum (1:4) to give **7** (4.54 g, 81%): $[\alpha]_D$ -7.2° (*c* 1.0, chloroform); ^1H NMR ($CDCl_3$) δ 1.28 (d, 3H, J = 6.3 Hz, 5-CH_3), 3.37 (t, 1H, J = 9.2 Hz, H-4), 4.61, 4.86 (ABq, 2H, Ph$\underline{CH_2}$), 4.82 (s, 1H, H-1), 5.25 (m, 4H, 2xCH_2=), 5.9 (m, 2H, 2xCH=), 7.3 (s, 5H, Ph).

To **7** (4.0 g, 12.0 mmol) in dry tetrahydrofuran (40 mL) sodium hydride (0.54 g, 80% dispersion in oil) was added. After 2 h, methyl iodide (1.12

mL) was introduced, the reaction mixture stirred for 5 h, decomposed with methanol and concentrated. The residue in chloroform was washed with water, dried and concentrated to afford a residue which was purified on silica gel by eluting with ethyl acetate-light petroleum (1:9) to give **8** (3.6 g, 86%): $[\alpha]_D$ -55° (*c* 0.9, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.26 (d, 3H, $J_{5,6}$ = 6.5 Hz, 5-CH_3), 3.40 (t, 1H, $J_{3,4}$ = 8.8 Hz, H-4), 3.48 (s, 3H, OMe), 4.54, 4.86 (ABq, 2H, PhC$\underline{H}_2$), 4.75 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1), 5.2 (m, 4H, 2xC$\underline{H}_2$=),5.9 (m, 2H, 2xCH=), 7.3 (m, 5H, Ph).

Anal. Calcd for $C_{20}H_{28}O_5$: C, 69.0; H, 8.0. Found: C, 68.7; H, 7.9.

1,3-Di-*O*-Acetyl-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranose (9). A mixture of **8** (3.0 g, 8.62 mmol) and tris(triphenylphosphine) rhodium (I) chloride (150 mg) in ethanol-benzene-water (7:3:1, 75 mL) was heated under reflux for 18 h, and then filtered and concentrated. The residue was diluted with ethyl acetate and washed with 1N hydrochloric acid, sodium bicarbonate solution, water, dried and concentrated. The residue was dissolved in 1:1 aqueous acetone (40 mL), and mercury (II) chloride (0.75 g) and mercury (II) oxide (0.3 g) were added. The reaction mixture was stirred for 2 h, filtered and concentrated. The residue was chromatographed on silica gel by eluting with ethyl acetate-light petroleum (1:2) to give the diol which was treated with acetic anhydride (2.4 mL), pyridine (2.5 mL) and *N*,*N*-4-dimethylaminopyridine (25 mg) in chloroform (15 mL). After 12 h and usual work-up, the crude product was purified by column chromatography on silica gel by using ethyl acetate-light petroleum (1:4) to give **9** (1.73 g, 57%): $[\alpha]_D$ -52.5°(*c* 0.3, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 3H, $J_{5,6}$ = 6.4 Hz, 5-CH_3), 2.08, 2.14 (2s, 6H, 2xOAc), 3.48 (s, 3H, OMe), 4.66 (ABq, 2H, PhC$\underline{H}_2$), 5.16 (dd, 1H, $J_{2,3}$ = 2.8, $J_{3,4}$ = 8.8 Hz, H-3), 6.09 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1), 7.3 (m, 5H, Ph).

Methyl 4-*O*-Benzyl-2-*O*-methyl-3-*O*-(3-*O*-acetyl-4-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (10). To a stirred solution of **4** (0.45 g, 1.59 mmol), **9** (0.61 g, 1.73 mmol) and 4Å molecular sieves (1.0 g) in dry dichloromethane (25 mL) at 0 °C was added freshly distilled boron trifluoride etherate (30 μL). After 2 h, potassium carbonate (0.3 g) was added, filtered and the filtrate washed with water, dried and concentrated. The residue was chromatographed on silica gel by eluting with ethyl acetate-light petroleum (1:3) to give **10** (0.55 g, 60%): $[\alpha]_D$ -36.5° (*c* 1.9, chloroform); ^{1}H NMR ($CDCl_3$) δ 1.29, 1.32 (2d, 6H, $J_{5,6}$ = $J_{5',6'}$ = 6.0 Hz, 5-CH_3, 5'-CH_3), 2.04 (s, 3H, OAc), 3.15, 3.35, 3.50 (3s, 9H, 3xOMe), 4.62, 4.85 (ABq, 2H,

$PhC\underline{H}_2$), 4.65 (s, 1H, H-1), 4.69 (ABq, 2H, $PhC\underline{H}_2$), 4.98 (d, 1H, $J_{1',2'}$ = 1.0 Hz, H-1'), 5.22 (dd, 1H, $J_{2',3'}$ = 2.5, $J_{3',4'}$ = 9.4 Hz, H-3'), 7.3 (m, 10H, 2xPh); ^{13}C NMR ($CDCl_3$) δ 97.7 ($J_{C-1,H-1}$ = 170 Hz) and 99.35 ($J_{C-1',H-1'}$ = 167 Hz).

Anal. Calcd for $C_{31}H_{42}O_{10}$: C, 64.8; H, 7.3. Found: C, 64.5; H, 7.1.

Methyl 4-O-Benzyl-2-O-methyl-3-O-[4-O-benzyl-2-O-methyl-3-O-(β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (13). Compound **10** (0.43 g, 0.75 mmol), methanol (15 mL) and sodium (25 mg) were stirred for 12 h and then worked up in a usual fashion to give **11** (0.4 g, 100 %). To a stirred solution of **11** (0.40 g, 0.75 mmol), 4Å molecular sieves (0.8 g), mercury (II) cyanide (0.17 g), mercury (II) bromide (0.06 g) in dry dichloromethane (20 mL) was added 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (0.61 g, 1.5 mmol). After 5 h, the reaction mixture was worked up in a usual fashion, and then the residue was chromatographed on silica gel by using ethyl acetate-light petroleum (1:3) to give **12** (0.36 g) which was dissolved in methanol (12 mL) and sodium (25 mg) was added. After 12 h and usual work-up, the residue was chromatographed on silica gel by eluting with ethyl acetate-light petroleum (7:3) to give **13** (0.36 g, 56%): $[\alpha]_D$ -28° (*c* 2.26, methanol), ^1H NMR ($CDCl_3$) δ 1.27, 1.29 (2d, 6H, $J_{5,6}$ = $J_{5',6'}$ = 6.0 Hz, 5-CH_3, 5'-CH_3), 3.23, 3.36, 3.46 (3s, 9H, 3xOMe), 5.08 (s, 1H, H-1'), 7.3 (m, 10H, 2xPh); ^{13}C NMR ($CDCl_3$) δ 97.79 ($J_{C-1,H-1}$ = 167 Hz), 99.22 ($J_{C-1',H-1'}$ = 174 Hz) and 103.79 ($J_{C-1'',H-1''}$ = 157 Hz).

Anal. Calcd for $C_{35}H_{50}O_{14}$: C, 60.5; H, 7.2. Found: C, 60.0; H, 7.0.

Methyl 2-O-Methyl-3-O-[2-O-methyl-3-O-(β-D-glucopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (2). A solution of **13** (0.10 g, 0.14 mmol), 10% palladium-carbon (20 mg) in methanol (10 mL) was stirred under a hydrogen atmosphere for 24 h, filtered and concentrated. The residue was purified by column chromatography on silica gel by using chloroform-methanol (9:1) (0.07 g, 94%): $[\alpha]_D$ -35° (*c* 1.25, methanol); ^1H NMR ($CDCl_3$+CD_3OD) δ 1.31 (t, 6H, $J_{5,6}$ = $J_{5',6'}$ = 6.5 Hz, 5-CH_3, 5'-CH_3), 3.37, 3.45, 3.47 (3s, 9H, 3x OMe), 4.55 (d, 1H, $J_{1'',2''}$ = 8.1 Hz, H-1''), 4.70 (s, 1H, H-1), 5.05 (s, 1H, H-1'); MS : $\underline{m/z}$ 351 (M^+-Glcp), 339 (M^+-$(OMe)_2$-Rhap).

Anal. Calcd for $C_{21}H_{38}O_{14}$: C, 49.0; H, 7.4. Found: C, 48.3; H, 7.3.

Methyl 2,4,6-Tri-O-benzyl-3-O-(3,4-di-O-acetyl-2-O-methyl-α-L-fucopyranosyl)-α-D-glucopyranoside (16). To a stirred solution of 1,3,4-tri-O-acetyl-2-O-methyl-L-fucopyranose[9] (**14**) (0.30 g, 1.0 mmol), methyl 2,4,6-tri-O-benzyl-α-D-glucopyranoside[10] (**15**) (0.55 g, 1.18 mmol) and 4Å molecular sieves

(0.6 g) in dichloromethane (30 mL) at 0 °C was added boron trifluoride etherate (100 µ L). After stirring at room temperature for 3 h, the reaction mixture was worked up as described above for compound **10.** The residue was chromatographed on silica gel with ethyl acetate-light petroleum (3:7) to give **16** (0.38 g, 54%): $[\alpha]_D$ -40° (*c* 2.4, chloroform); ^{1}H NMR ($CDCl_3$) δ 0.56 (d, 3H, $J_{5',6'}$ = 6.3 Hz, 5'-CH_3), 2.04, 2.10 (2s, 6H, 2xOAc), 3.33, 3.40 (2s, 6H, 2xOMe), 4.91 (bs, 1H, H-4'), 5.31 (dd, 1H, $J_{2',3'}$ = 10.5, $J_{3',4'}$ = 2.1 Hz, H-3'), 5.69 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 7.3 (m, 15H, 3xPh); ^{13}C NMR ($CDCl_3$) δ96.25 ($J_{C\text{-}1,H\text{-}1}$ = 175 Hz), 97.6 ($J_{C\text{-}1',H\text{-}1'}$ = 171 Hz).

Methyl 3-O(2-O-Methyl-α-L-fucopyranosyl)-α-D-glucopyranoside (3). Compound **16** (0.35 g, 0.49 mmol), methanol (20 mL) and sodium (50 mg) were stirred for 3 h, deionised by Amberlite IR 120 (H) resin and filtered. The filtrate was stirred over 10% Pd-C (50 mg) in a hydrogen atmosphere for 24 h. The catalyst was filtered off and the filtrate concentrated to afford a residue which was purified by column chromatography on silica gel by eluting with chloroform-methanol (10:1) to give **3** (0.098 g, 56%): $[\alpha]_D$ -17° (*c* 0.65, methanol); ^{1}H NMR ($CDCl_3$) δ 1.19 (d, 1H, J = 6.5 Hz, 5'-CH_3), 3.31, 3.44 (2s, 6H, 2xOMe), 4.66 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1'), 5.22 (d, 1H, $J_{1',2'}$ = 3.0 Hz, H-1); CI-MS : m/z 355 (M^++1).

Anal. Calcd for $C_{14}H_{26}O_{10}$: C, 47.5; H, 7.3. Found: C, 47.4; H, 7.3.

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