

## Synthesis of blocked trisaccharide analogue related to the repeating unit of the *O*-antigen form *E. coli* : 025

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Methyl 2-O-benzoyl-4, 6-O-benzylidene-O- $\alpha$ -D-glucopyranoside **1** was condensed with rhamnose-1-thio donor **2** in the presence of NIS/TMSOTf to yield the disaccharide methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O- $\alpha$ -D-glucopyranoside **3**. Acidic hydrolysis of compound **3** followed by tritylation, deacylation and benzylation yields the disaccharide methyl 2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-6-O-trityl-O- $\alpha$ -D-glucopyranoside **6**. Compound **6** on detritylation yields the acceptor methyl 2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O- $\alpha$ -D-glucopyranoside **7** which on condensation with known glucose-1-thio donor **8** in the presence of NIS/TMSOTf yields the linear protected trisaccharide methyl 2,3,4,6-tetra-O-acetyl-O- $\beta$ -D-glucopyranosyl-(1-6)-2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O- $\alpha$ -D-glucopyranoside **9**.

**Keywords:** Carbohydrates, synthesis, regioselective, stereoselective, glycosylation, trisaccharide

*Escherichia coli* is a group of gram-negative bacteria that colonizes at infant's gastro intestinal tract within hours of birth<sup>1</sup>. The subdivision of the immunologically active site of the bacterial surface structure was introduced by Kauffmann<sup>2</sup>. He has characterized *E. coli* based on the serotyping scheme. There are three types of antigens, which are as follows: (i) somatic *O*-antigen, (ii) capsular *K*-antigen and (iii) flagellar *H*-antigen. Initially he described 25 *O*, 55 *K* and 20 *H* antigens<sup>3</sup>. Currently the number of *O*-antigens has reached 173, *K*-antigens 103 and *H*-antigens 56 (Ref. 4).

The somatic *O*-antigens are composed of lipopolysaccharide complexes, which is part of the cell wall structure of the *E. coli*. The immunogenicity of the polysaccharide repeating units appears from the *O* antigens. Kauffmann and Vahlne introduced the term *K*-antigen as a symbol for envelop of capsular antigens<sup>5</sup>. *K*-antigens are acidic polysaccharides, serologically different from *O*-antigens. The antigenic diversity of *H*-antigen is based on the different types of flagellin present in the flagellar structure. The *O*, *K* and *H*-antigens can be found in nature in many of the possible combinations. The final number of *E. coli* serotype is very high viz. 50,000-100,000 or more<sup>6</sup>. However, the number of pathogenic serotypes is limited.

*E. coli* is generally confined to the intestinal lumen; however, in a debilitated or immunosuppressed host or when the bacteria are introduced to other tissues, even normal "non-pathogenic" strains of *E. coli* can cause infection<sup>7</sup>. *E. coli* infections may be limited to the mucosal surfaces or can disseminate throughout the body. The three general clinical syndromes caused by pathogenic *E. coli* strains are urinary tract infection, sepsis/meningitis, and enteric/diarrhoeal diseases<sup>2</sup>.

In view of the increasing drug resistance to the bacterial infections and potential importance of artificial antigens, selective chemical synthesis of immunodominant blocks of the *O*-antigens has gained considerable interest for their evaluation as potential synthetic antigens. This will help in designing synthetic antigens for precise diagnosis and protection and these substructures may ultimately provide a tool to study the structure-function relationship<sup>8</sup>.

### Results and Discussion

This paper illustrates the synthesis of the linear trisaccharide building block comprising of rhamnose, glucose and glucose present at the reducing end of the pentasaccharide repeating unit of the *O*-LPS (*O*-lipopolysaccharide) of *E. coli* 0:25 (Ref. 9) whose structural framework is as follows (**Figure 1**).

For the synthesis of the above mentioned trisaccharide building block, we started with the available methyl 2-O-benzoyl-4,6-O-benzylidene-O- $\alpha$ -D-glucopyranoside<sup>10</sup> **1** which served as acceptor, and was condensed with earlier synthesized ethyl 2,3,4 tri-O-acetyl-1-thio- $\alpha$ -L-rhamnopyranoside<sup>11</sup> donor **2** in the presence of NIS and TMSOTf as promoter<sup>12</sup> to yield methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O- $\alpha$ -D-glucopyranoside **3** as crystals in 80% yield. The <sup>1</sup>H NMR spectrum of the compound **3** showed a doublet ( $J_{1,2} = 3.9$  Hz) for one proton at  $\delta$  5.06 of the H-1 proton of glucose and a singlet at  $\delta$  5.04 for the H-1' proton of rhamnose along with a singlet for three methoxy proton at  $\delta$  3.38. The nature of compound **3** as tri-O-acetyl derivative is established from the appearance of three singlets of three protons each at 1.93, 1.90 and 1.88 respectively in addition to the doublet ( $J = 6.0$  Hz) at  $\delta$  0.76 for the secondary methyl group of rhamnose. The structure of the synthesized compound was also supplemented by <sup>13</sup>C NMR values showing the two anomeric signals at  $\delta$  98.0 and 97.7 for glucose and rhamnose moieties along with a signal at 55.4 methoxy carbon and at 16.6 for the secondary methyl of rhamnose. This synthesized disaccharide **3** is also confirmed by its FAB-MS with  $M^+$  at  $m/z$  at 658.

Removal<sup>13</sup> of the acetal ring with aq. acetic acid gave ethyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-O- $\alpha$ -D-glucopyranoside **4** as syrup in 80% yield. The primary hydroxyl group was silylated using TBDPSCl and pyridine<sup>14</sup> to give methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-6-O-*tert*-butyldiphenylsilyl -O- $\alpha$ -D-glucopyranoside **5a** which was expected to serve as an acceptor for the preparation of the branched trisaccharide comprising of glucose, rhamnose and GlcNAc by condensing it with the available ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-1-thio- $\beta$ -D-glucopyranoside<sup>15</sup> using NIS and TMSOTf as promoter. However, the attempt failed due to the undesired self condensation of the donor. Consequently, the strategy was changed to synthesize another trisaccharide present in the same pentasaccharide, comprising of glucose, rhamnose and glucose for which we proceeded as follows (**Scheme I**).

Tritylation<sup>16</sup> of **4** with trityl chloride gave methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-6-O-trityl-O- $\alpha$ -D-glucopyranoside **5b** as syrup in 65% yield. Deacylation of **5b** with NaOMe<sup>17</sup> in MeOH followed by benzylolation<sup>18</sup> with NaH and

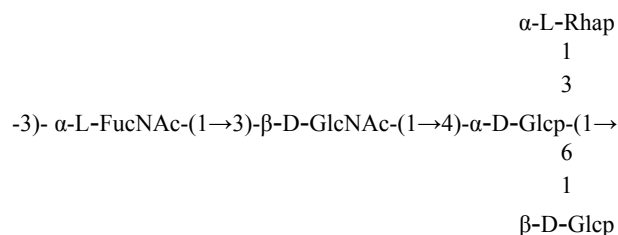
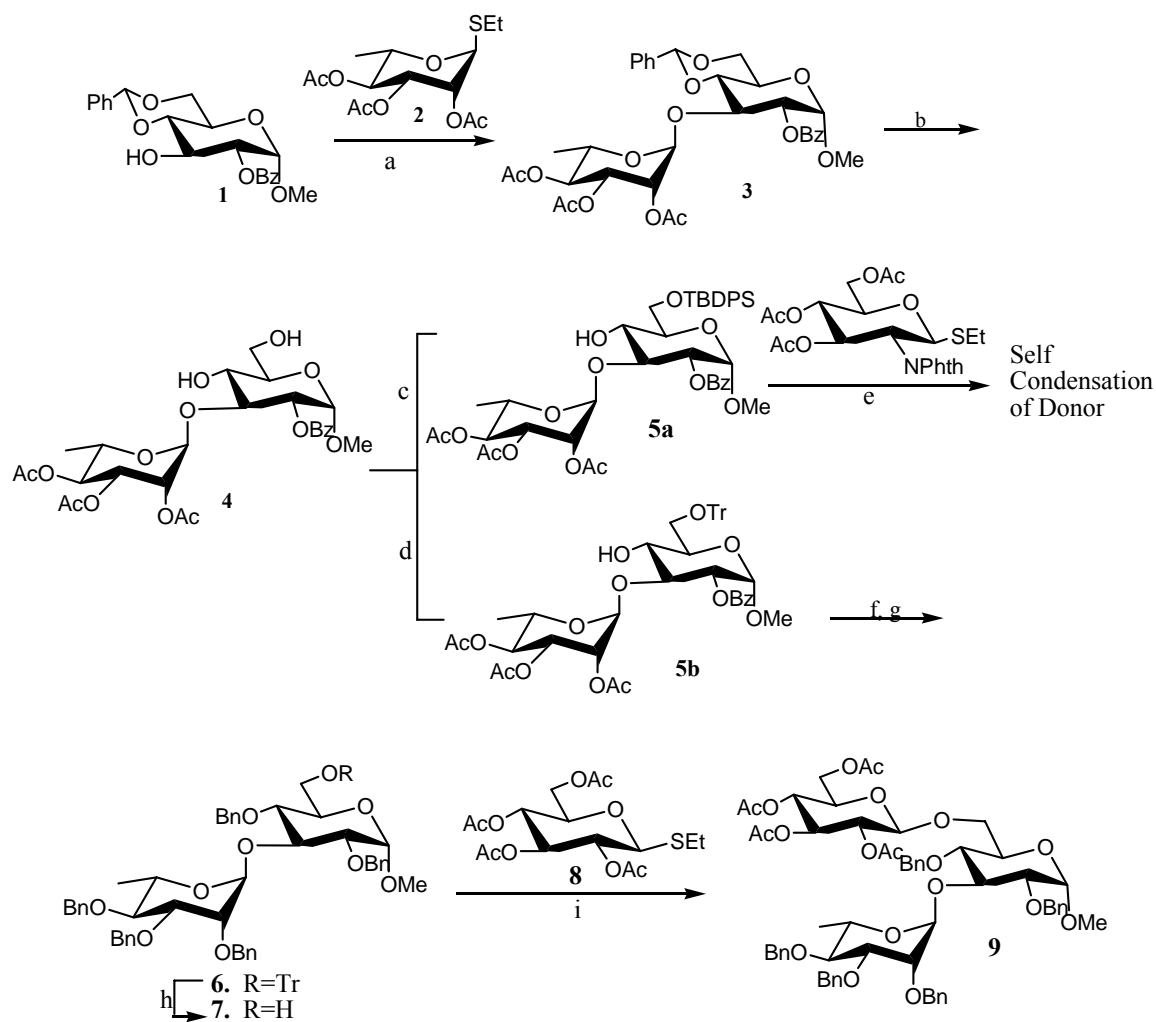


Figure 1

BnBr gave methyl 2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-6-O-trityl-O- $\alpha$ -D-glucopyranoside **6** as syrup in 73% yield. The <sup>1</sup>H NMR spectrum of the compound showed a doublet ( $J_{1,2} = 3.6$  Hz) for one proton at  $\delta$  4.71 for the H-1 proton of glucose, a singlet for one proton at  $\delta$  4.66 for the H-1' proton of rhamnose, a singlet for three methoxy proton at  $\delta$  3.39. It also showed a multiplet of forty aromatic protons in the region  $\delta$  7.51-6.78 and a doublet ( $J = 6.0$  Hz) at  $\delta$  1.03 for the secondary methyl group of rhamnose. The above synthesized disaccharide is also confirmed by its FABMS with  $M^+$  at  $m/z$  1032. Detritylation<sup>19</sup> of **6** with aq. acetic acid gave methyl 2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O- $\alpha$ -D-glucopyranoside **7** as syrup in 60% yield. The <sup>1</sup>H NMR spectrum of the compound showed a singlet for one proton at  $\delta$  4.65 for the H-1' proton of rhamnose, a doublet ( $J_{1,2} = 3.3$  Hz) at  $\delta$  4.54 for the H-1 proton of glucose and a singlet for three methoxy proton at  $\delta$  3.29. It also showed a multiplet for twenty five proton in the region  $\delta$  7.48-7.12 for the aromatic protons along with a doublet ( $J = 6.0$  Hz) at  $\delta$  1.13 for the C-6 methyl proton of rhamnose, which was also supplemented by the <sup>13</sup>C NMR showing the peaks at  $\delta$  98.5 and 97.5 for the two anomeric carbons of glucose and rhamnose. The above synthesized compound **7** is also confirmed by its FABMS with  $(M+H)^+$  at  $m/z$  at 791.

The above deprotected disaccharide **7** served as acceptor which is condensed with the earlier synthesized ethyl 2,3,4,6 tetra-O-acetyl-1-thio- $\beta$ -D-glucopyranoside donor<sup>20</sup> in the presence of NIS and TMSOTf (Ref. 12) to yield methyl 2,3,4,6-tetra-O-acetyl-O- $\beta$ -D-glucopyranosyl-(1-6)-2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O- $\alpha$ -D-glucopyranoside **8** as syrup in 45% yield (**Scheme I**). The <sup>1</sup>H NMR spectrum of the compound showed a singlet for one proton at  $\delta$  4.65 for the H-1' proton of rhamnose, a doublet ( $J_{1,2} = 7.8$  Hz) for one proton at  $\delta$  4.59 for the H-1'' proton of glucose, a doublet ( $J_{1,2} = 3.0$  Hz) for one proton at  $\delta$  4.57 for the H-1 proton of glucose, and a singlet for the three



**Scheme I** — (a) NIS/TMSOTf/CH<sub>2</sub>Cl<sub>2</sub>/MS-4Å/0°C/20 min; (b) 80% aq. AcOH/80°C/6 hr; (c) TBDPSCI/Pyridine 0°C, RT; (d) trityl chloride/ Pyr/60°C/8 hr; (e) NIS/TMSOTf/CH<sub>2</sub>Cl<sub>2</sub>/MS-4Å/0°C/20 min; (f) NaOMe/MeOH (1M)/RT/4 hr; (g) NaH/BnBr/DMF/RT/6 hr; (h) 80% aq. acetic acid/80°C/6 hr; (i) NIS/TMSOTf/CH<sub>2</sub>Cl<sub>2</sub>/MS-4Å/0°C/20 min.

methoxy proton at  $\delta$  3.29. The presence of four singlets of three protons each at  $\delta$  2.04, 2.02, 2.00, 1.94 could be attributed to four acetoxy groups. It also showed a doublet ( $J = 6.0$  Hz) at  $\delta$  1.08 for the secondary methyl group of rhamnose. The above synthesized compound is also supported by its <sup>13</sup>C NMR spectrum showing the three anomeric carbon peaks at 101.5, 98.5 and 97.5 for C-1'', C-1 and C-1'. This synthesized compound was also confirmed by its FABMS which showed (M+H)<sup>+</sup> at  $m/z$  1121.

## Experimental Section

All reactions were monitored by TLC on silica gel G (E. Merck). Column chromatography was performed over silica gel (SRL, 60-120 mesh). All solvents were distilled before use and evaporation of solvents were conducted at 40°C unless otherwise

stated. Optical rotation were measured at 25°C on AA-5 series polarimeter. <sup>1</sup>H NMR spectra were recorded on a Bruker DPX 300 spectrometer using CDCl<sub>3</sub> as solvent (TMS as internal standard). Melting points were determined on a Buchi 540 m.p. apparatus. The final synthesized compound was confirmed by 2D HSQC experiment. Mass spectra were recorded on Mass spectrometer (Jeol SX 102) for FABMS.

### Methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-4,6-O-benzylidene-O- $\alpha$ -D-glucopyranoside, 3

A mixture of 1 (600 mg, 1.6 mmole), 2 (572 mg, 1.7 mmole) and 4Å molecular sieves (1.2 g) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was cooled under nitrogen to 0°C and stirred for 10 min. NIS (500 mg, 2.2 mmole) and

TMSOTf (155  $\mu$ l, 0.85 mmole) were successively added, the mixture was stirred for 20 min, neutralized by the addition of triethylamine and filtered through a layer of celite. The filtrate was washed with aq.  $\text{Na}_2\text{S}_2\text{O}_3$  solution, dried and concentrated. Chromatography (hexane:ethylacetate, 3:1) of the residue afforded **3** as crystal (857 mg, 80%), m.p. 162-64°C,  $[\alpha]_{\text{D}}^{25} + 44^\circ$  (*c*, 1.0,  $\text{CHCl}_3$ ).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  8.01-7.32 (m, 10H, aromatic protons), 5.59 (s, 1H,  $-\text{CHC}_6\text{H}_5$ ), 5.23 (dd, 1H,  $J_{1,2} = 3.6$  Hz,  $J_{2,3} = 9.6$  Hz, H-2), 5.06 (d, 1H,  $J_{1,2} = 3.9$  Hz, H-1), 5.09-5.05 (m, 2H, H-2', H-3'), 5.04 (bs, 1H, H-1'), 4.89 (t, 1H,  $J_{3,4,5} = 9.9$  Hz, H-4'), 4.44-4.31 (m, 2H, H-6a, 6b), 4.21-4.08 (m, 1H, H-5'), 3.98-3.90 (m, 1H, H-5), 3.81 (t, 1H,  $J_{2,3,4} = 10.2$  Hz, H-3), 3.71 (t, 1H,  $J_{3,4,5} = 9.3$  Hz, H-4), 3.38 (s, 3H,  $-\text{OCH}_3$ ), 1.93, 1.90, 1.88 (s, 3H each,  $3 \times -\text{OCOCH}_3$ ), 0.76 (d, 3H,  $J = 6.0$  Hz,  $6'-\text{CH}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  169.8 ( $2 \times -\text{COCH}_3$ ), 169.1 ( $-\text{COCH}_3$ ), 165.5 ( $-\text{COPh}$ ), 137.1-126.3 (aromatic carbons), 101.9 ( $-\text{CHPh}$ ), 98.0 (C-1), 97.7 (C-1'), 79.5, 74.7 (2C), 73.2, 71.0, 69.4, 68.9 (2C), 66.2, 55.4 ( $-\text{OCH}_3$ ), 20.7, 20.6, 20.5 ( $3 \times -\text{COCH}_3$ ), 16.6 (C-6' Rha); FABMS  $[\text{M}]^+$  at  $m/z$  658. Anal. Calcd for  $\text{C}_{33}\text{H}_{38}\text{O}_{14}$ : C, 60.18; H, 5.82. Found C, 60.12; H, 5.86%.

#### Methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-O- $\alpha$ -D-glucopyranoside, **4**

A solution of **3** (600 mg, 0.91 mmole) in aq. acetic acid (80%, 6 mL) was warmed at 80°C for 1 hr followed by evaporation of solvents. Chromatography (hexane:ethylacetate, 3:2) of the residue afforded **4** as syrup (416 mg, 80%),  $[\alpha]_{\text{D}}^{25} + 33.6^\circ$  (*c*, 0.4,  $\text{CHCl}_3$ ).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  8.07-7.42 (m, 5H, aromatic protons), 5.24 (dd, 1H,  $J_{1,2} = 3.6$  Hz,  $J_{2,3} = 9.6$  Hz, H-2), 5.09-5.04 (m, 3H, H-2', H-3', H-4'), 5.01 (s, 1H, H-1'), 4.97 (d, 1H,  $J_{1,2} = 3.6$  Hz, H-1), 4.20-4.08 (m, 2H, H-6a, 6b), 3.95-3.85 (m, 2H, H-5', H-5), 3.72-3.51 (m, 2H, H-3, H-4), 3.38 (s, 3H,  $-\text{OCH}_3$ ), 2.03, 2.02, 1.89 (s, 3H each,  $3 \times -\text{OCOCH}_3$ ), 1.25 (d, 3H,  $J = 6.3$  Hz,  $6'-\text{CH}_3$ ). Anal. Calcd for  $\text{C}_{26}\text{H}_{34}\text{O}_{14}$ : C, 54.73; H, 6.01. Found C, 54.65; H, 6.07%.

#### Methyl 2,3,4-tri-O-acetyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2-O-benzoyl-6-O-trityl-O- $\alpha$ -D-glucopyranoside, **5b**

To a stirred solution of **4** (400 mg, 0.61 mmole) in pyridine (3 mL) was added triphenyl methyl chloride (254 mg, 0.091 mmole) at 0°C and the reaction-mixture was stirred for 8 hr at 60°C. The solution was

dissolved in chloroform and filtered to remove excess of trityl chloride, washed with water, dried and concentrated to give yellow syrup. Chromatography (hexane:ethylacetate, 8:2) of the residue afforded **5b** as syrup (372 mg, 65%),  $[\alpha]_{\text{D}}^{25} + 29.1^\circ$  (*c*, 0.6,  $\text{CHCl}_3$ ).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  8.06-7.23 (m, 20H, aromatic protons), 5.24 (dd, 1H,  $J_{1,2} = 3.6$  Hz,  $J_{2,3} = 9.6$  Hz, H-2), 5.11 (d, 1H,  $J_{1,2} = 3.6$  Hz, H-1), 5.09-4.87 (m, 3H, H-2', H-3', H-4'), 5.00 (bs, 1H, H-1'), 4.21-4.07 (m, 2H, H-6a, 6b), 3.80-3.64 (m, 2H, H-5', H-5), 3.52-3.42 (m, 2H, H-3, H-4), 3.39 (s, 3H,  $-\text{OCH}_3$ ), 2.02, 1.97, 1.88 (s, 3H each,  $3 \times -\text{OCOCH}_3$ ), 1.19 (d, 3H,  $J = 6.3$  Hz,  $6'-\text{CH}_3$ ); Anal. Calcd for  $\text{C}_{45}\text{H}_{48}\text{O}_{14}$ : C, 66.49; H, 5.95. Found C, 66.43; H, 5.98%.

#### Methyl 2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-6-O-trityl-O- $\alpha$ -D-glucopyranoside, **6**

Compound **5b** (300 mg, 0.37 mmole) was treated with NaOMe in MeOH (1M, 3 mL) and the solution was allowed to stir at RT for 4 hr. The solution was made neutral by the addition of Amberlite IR-120 ( $\text{H}^+$ ) resin, filtered, and the solvent was evaporated to provide deacylated product (172 mg, 80%). To the solution of this crude deacylated product (172 mg, 0.3 mmole) in DMF (2 mL) were added NaH (60% oil coated, 148 mg, 3.7 mmole) and BnBr (0.26 mL, 2.2 mmole), and the mixture was stirred at RT for 6 hr. MeOH (2 mL) was then added to destroy the excess reagents, the reaction-mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (10 mL) and washed with water, dried with anhyd.  $\text{Na}_2\text{SO}_4$  and concentrated to give the crude syrup. Chromatography (hexane:ethylacetate, 17:3) of the residue afforded **6** as syrup (222 mg, 73%),  $[\alpha]_{\text{D}}^{25} + 7.5^\circ$  (*c*, 0.7,  $\text{CHCl}_3$ ).

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta$  7.51-6.78 (m, 40H, aromatic protons), 4.95 (d, 1H,  $J = 11.1$  Hz,  $-\text{CHHC}_6\text{H}_5$ ), 4.71 (d, 1H,  $J_{1,2} = 3.6$  Hz, H-1), 4.66 (bs, 1H, H-1'), 4.59 (d, 1H,  $J = 10.8$  Hz,  $-\text{CHHC}_6\text{H}_5$ ), 4.55 (d, 1H,  $J = 2.1$  Hz, H-2'), 4.52 (d, 1H,  $J = 12.0$  Hz,  $-\text{CHHC}_6\text{H}_5$ ), 4.43 (d, 1H,  $J = 11.1$  Hz,  $-\text{CHHC}_6\text{H}_5$ ), 4.27-4.09 (m, 6H,  $3 \times -\text{CH}_2\text{C}_6\text{H}_5$ ), 3.95-3.42 (m, 9H, H-6a, 6b, H-5', H-5, H-2, H-3, H-3' H-4, H-4'), 3.39 (s, 3H,  $-\text{OCH}_3$ ), 1.03 (d, 3H,  $J = 6.0$  Hz,  $6'-\text{CH}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  138.4-127.6 (aromatic carbons), 98.2 (C-1), 97.3 (C-1'), 81.0 (2C), 80.9, 79.7, 75.1 (2C), 72.4, 72.1, 72.0, 70.9, 70.6 (2C), 68.1, 61.4 (2C), 55.2 ( $-\text{OCH}_3$ ), 17.6 (C-6' Rha); FABMS  $[\text{M}]^+$  at

$m/z$  1032. Anal. Calcd for  $C_{67}H_{68}O_{10}$ : C, 77.88; H, 6.63. Found C, 77.79; H, 6.71%.

**Methyl 2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O- $\alpha$ -D-glucopyranoside, 7**

A solution of **6** (200 mg, 0.19 mmole) in acetic acid:water (4:1, 2 mL) was warmed at 80°C for 1 hr. The reaction mixture was then concentrated by co-evaporation of toluene under reduced pressure. Chromatography (*n*-hexane:ethylacetate, 8:2) of the residue afforded **7** (92 mg, 60%) as syrup,  $[\alpha]_D^{25} + 23^\circ$  (*c*, 1.0,  $CHCl_3$ ).  $^1H$  NMR ( $CDCl_3$ , 300 MHz):  $\delta$  7.48-7.12 (m, 25H, aromatic protons), 4.94 (d, 1H,  $J = 10.8$  Hz,  $-CHHC_6H_5$ ), 4.76 (d, 1H,  $J = 10.5$  Hz,  $-CHHC_6H_5$ ), 4.65 (bs, 1H, H-1'), 4.62-4.56 (m, 1H,  $-CHHC_6H_5$ ), 4.54 (d, 1H,  $J_{1,2} = 3.3$  Hz, H-1), 4.50 (d, 1H,  $J = 2.1$  Hz, H-2') 4.47-4.41 (m, 2H,  $-CH_2C_6H_5$ ), 4.29-4.13 (m, 5H,  $5 \times -CHHC_6H_5$ ), 3.95-3.42 (m, 9H, H-6a, 6b, H-5', H-5, H-2, H-3, H-3', H-4, H-4'), 3.29 (s, 3H,  $-OCH_3$ ), 1.13 (d, 3H,  $J = 6.0$  Hz,  $6'-CH_3$ );  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  138.6-127.4 (aromatic carbons), 98.5 (C-1), 97.5 (C-1'), 80.9, 80.7, 79.8, 75.1 (2C), 72.6, 72.1, 71.9, 70.8, 70.7 (2C), 68.2, 61.5 (2C), 55.1 ( $-OCH_3$ ), 17.8 (C-6' Rha); FABMS  $[M + H]^+$  at  $m/z$  at 791. Anal. Calcd for  $C_{48}H_{54}O_{10}$ : C, 72.89; H, 6.88. Found C, 72.81; H, 6.94%.

**Methyl 2,3,4,6-tetra-O-acetyl-O- $\beta$ -D-glucopyranosyl-(1-6)-2,3,4-tri-O-benzyl-O- $\alpha$ -L-rhamnopyranosyl-(1-3)-2,4-di-O-benzyl-O- $\alpha$ -D-glucopyranoside, 9**

Treatment of a mixture of **8** (28 mg, 0.07 mmole), and **7** (50 mg, 0.063 mmole) and 4 Å molecular sieves (80 mg) in  $CH_2Cl_2$  (2 mL) was cooled under  $N_2$  to 0°C and stirred for 10 min. NIS (21 mg, 0.9 mmole) and TMSOTf (6.0  $\mu$ l, 0.035 mmole) were successively added, the mixture was stirred for 20 min, neutralized by the addition of triethylamine and filtered through a layer of celite. The filtrate was washed with aq.  $Na_2S_2O_3$  solution, dried and concentrated. Chromatography (hexane:ethylacetate, 9:1) of the residue afforded **9** as syrup (33 mg, 45%),  $[\alpha]_D^{25} + 10^\circ$  (*c*, 1.0,  $CHCl_3$ ).

$^1H$  NMR ( $CDCl_3$ , 300 MHz):  $\delta$  7.37-7.17 (m, 25H, aromatic protons), 5.19-5.06 (m, 3H, H-2', H-3', H-4'), 4.98 (d, 1H,  $J = 10.8$  Hz,  $-CHHC_6H_5$ ), 4.70 (d, 1H,  $J = 10.2$  Hz,  $-CHHC_6H_5$ ), 4.65 (bs, 1H, H-1'), 4.59 (d, 1H,  $J_{1,2} = 7.8$  Hz, H-1'), 4.57 (d, 1H,  $J_{1,2} = 3.0$  Hz, H-1), 4.54-4.51 (m, 1H, H-2'), 4.49-4.38 (m, 5H,  $4 \times -CHHC_6H_5$ ,  $1 \times -CHHC_6H_5$ ), 4.27-4.05 (m, 5H,  $3 \times -CHHC_6H_5$ , H-6a'', 6b''), 3.92-3.60 (m, 8H,

H-5, H-2, H-6a, 6b, H-5'', H-3', H-4', H-5'), 3.45-3.34 (m, 2H, H-3, H-4), 3.29 (s, 3H,  $-OCH_3$ ), 2.04, 2.02, 2.00, 1.94 (s, 3H each,  $4 \times -OCOCH_3$ ), 1.08 (d, 3H,  $J = 6.0$  Hz,  $6'-CH_3$ );  $^{13}C$  NMR ( $CDCl_3$ ):  $\delta$  170.6, 170.5, 169.4, 169.1 ( $4 \times -COCH_3$ ), 138.9-127.4 (aromatic carbons), 101.5 (C-1''), 98.5 (C-1), 97.5 (C-1'), 80.7, 80.6, 79.6, 76.0, 75.3, 74.9, 74.8, 72.9, 72.7, 72.1, 71.8, 71.7, 71.9, 69.4, 68.3, 68.2, 67.9, 61.9, 61.8, 55.1 ( $-OCH_3$ ), 29.7 ( $2 \times -COCH_3$ ), 20.7, 20.6 ( $2 \times -COCH_3$ ), 17.8 (C-6 Rha); FABMS  $[M + H]^+$  at  $m/z$  at 1121. Anal. Calcd for  $C_{62}H_{72}O_{19}$ : C, 66.42; H, 6.47. Found C, 66.35; H, 6.52%.

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