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ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 7240–7245

Concise synthesis of two trisaccharide analogs related to the glycone constituent of phanoside, a novel insulin releasing natural product☆

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Received 15 February 2007; revised 6 April 2007; accepted 26 April 2007
Available online 3 May 2007

Abstract—Two trisaccharides (**2** and **3**) related to the glycone part of phanoside, an insulin release stimulator, have been synthesized in excellent yield.

1. Introduction

Diabetes has been classified as insulin dependent (type 1) and noninsulin dependent (type 2). Noninsulin dependent (type 2) diabetes is the commonest form of diabetes constituting 90% of the diabetic population.[1,2] Its prevalence in more affluent societies is spectacular and of general concern.[3] It has broken the age barrier and also appears in younger people. Though its management through drugs is possible, drugs come together with serious side effects. Natural products can provide a better alternative as more effective anti-diabetics with less side effects. Recently, it has been reported that phanoside[4] (**1**, Fig. 1), a natural product isolated from the plant *Gynostemma pentaphyllum* is effective in stimulating insulin release, and is much more potent than well known anti-diabetic drugs, such as sulfonylureas.[4] As claimed in the original report, phanoside contains a branched trisaccharide glycone consisting of D-glucose, D-rhamnose, and D-lyxose.

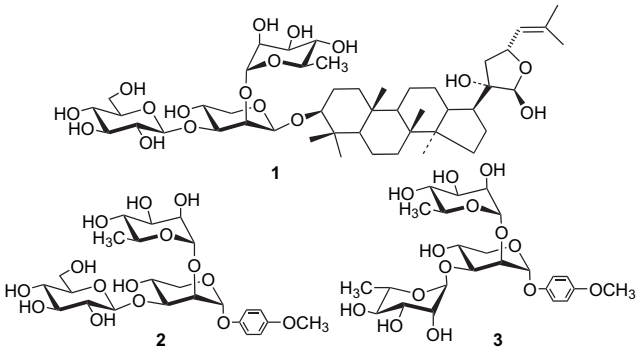

Figure 1. Structure of phanoside (**1**) and trisaccharide analogs of the glycone (**2** and **3**).

However, L-rhamnose is quite abundant in plant natural products and D-rhamnose is occasionally found in the microbial origin. Although the structural elucidation for the presence of D-rhamnose moiety instead of L-rhamnose was not clearly stated in the original paper, we were interested in synthesizing some analogous trisaccharide moieties related to the glycone part of phanoside containing a L-rhamnose moiety. Oligosaccharides containing D-lyxose are also scarce in nature. In order to understand the role of the glycone part of phanoside in enhancing the insulin release, it is essential to have higher quantities of several trisaccharide analogs related to the glycone part of phanoside. Hence it was thought to develop a synthetic route for the synthesis of two trisaccharide analogs (**2** and **3**) related to the glycone part of phanoside.

2. Results and discussion

The synthesis of target trisaccharide (**2**) as its 4-methoxyphenyl glycoside is demonstrated in Scheme 1. Treatment of D-lyxose tetraacetate (**4**) with 4-methoxy phenol in the presence of trifluoromethanesulfonic acid afforded 4-methoxyphenyl glycoside (**5**). Compound **5** was converted to compound **6** following a sequence of reactions consisting of deacetylation using sodium methoxide,[5] isopropylidenation[6] using 2,2-dimethoxypropane and *p*-toluenesulfonic acid, and benzylation using benzyl bromide under phase transfer conditions.[7] Removal of the isopropylidene group[8] from compound **6** using 80% aq acetic acid furnished compound **7**. Initially, a regioselective glycosylation strategy[9]

☆ C.D.R.I communication no. 7049.

Keywords: Carbohydrate; Glycosylations; Natural products; Insulin; Phanoside.

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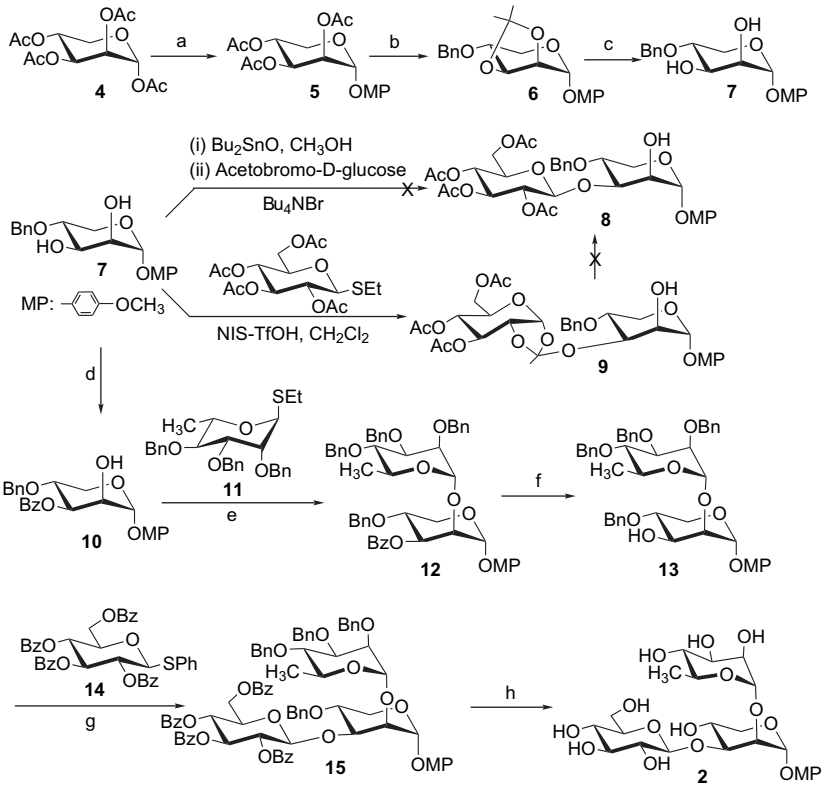
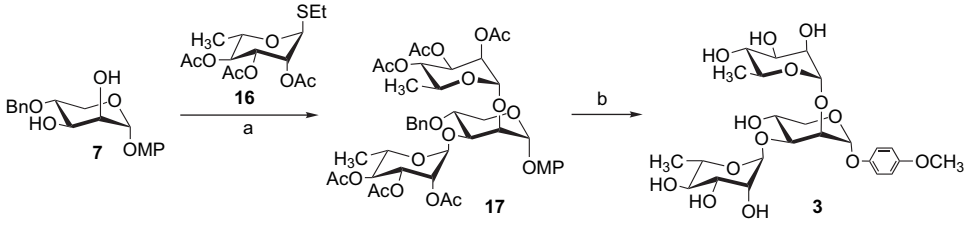
doi:10.1016/j.tet.2007.04.089



Scheme 1. Reagents: (a) 4-(OCH_3)–C_6H_5OH, TfOH, CH_2Cl_2, 2 h, 10 °C, 90%; (b) (i) CH_3ONa, CH_3OH, rt, 3 h; (ii) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, DMF, 6 h, 71% in two steps; (iii) benzyl bromide, tetrabutylammonium iodide, 50% aq NaOH, CH_2Cl_2, rt, 3 h, 90%; (c) 80% aq AcOH, 60 °C, 45 min, 93%; (d) (i) Bu_2SnO, CH_3OH, reflux, 6 h; (ii) benzoyl chloride, toluene, rt, 8 h, 80%; (e) *N*-iodosuccinimide, TfOH, CH_2Cl_2, −30 °C, 45 min, 78%; (f) 0.1 M CH_3ONa, CH_3OH, rt, 3 h, quantitative; (g) *N*-iodosuccinimide, TfOH, CH_2Cl_2, −30 °C, 45 min, 72%; (h) (i) CH_3ONa, CH_3OH, rt, 12 h; (ii) H_2, $Pd(OH)_2$–C, CH_3OH, rt, 24 h, 61% in two steps.

was attempted to prepare disaccharide **8**, through the formation of stannylidene acetal of compound **7**, followed by treatment with peracetylated glucosyl bromide. After several trials, no disaccharide **8** could be isolated and starting material was recovered. Another regioselective glycosylation was carried out using ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside under *N*-iodosuccinimide–triflic acid promoted glycosylation,[10] but only some orthoester **9** could be isolated instead of disaccharide **8**. Following literature reports,[11] the rearrangement of orthoester **9** to the required disaccharide **8** also failed and compound **7** was recovered. It was then decided to selectively protect the 3-hydroxy group of compound **7** with the 4-methoxybenzyl group via stannylidene acetal formation. Unfortunately, a mixture of products was isolated upon treatment of compound **7** with dibutyltin oxide followed by treatment with 4-methoxybenzyl chloride. Finally, stannylidene acetal formation of compound **7** followed by treatment with benzoyl chloride[12] yielded selectively benzoyl protected D-lyxose derivative **10** in excellent yield. Glycosylation of compound **10** with ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**11**), prepared from L-rhamnose in three steps, with *N*-iodosuccinimide and triflic acid[10] afforded the disaccharide derivative **12**. The presence of two anomeric signals at 97.4 (C-1) and 97.0 (C-1′) in the ^{13}C NMR spectrum confirmed the formation of **12**. Following an earlier literature report,[13] the configuration of the L-rhamnosyl linkage of compound **12** was confirmed by the partially decoupled ^{13}C NMR spectrum, in which the C-1/H-1 coupling constant ($J_{C\text{-}1/H\text{-}1}$) for L-rhamnosyl linkage was found to be 172.0 Hz indicating the formation of a α-linkage. Removal of the benzoyl group using sodium methoxide gave compound **13**, which on glycosylation with phenyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**14**)[14] using *N*-iodosuccinimide and trifluoromethanesulfonic acid furnished trisaccharide derivative **15**. The presence of three anomeric signals at 101.8 (C-1″), 99.7 (C-1), and 97.4 (C-1′) in the ^{13}C NMR spectrum, confirmed the formation of trisaccharide derivative **15**.



Scheme 2. Reagents: (a) *N*-iodosuccinimide, TfOH, CH_2Cl_2, −30 °C, 45 min, 68%; (b) (i) CH_3ONa, CH_3OH, rt, 12 h; (ii) H_2, $Pd(OH)_2$–C, CH_3OH, rt, 10 h, 65% in two steps.

Conventional debenzoylation followed by hydrogenolysis[15] of trisaccharide **15** afforded target trisaccharide **2** in 62% yield.

In a separate experiment, another analogous trisaccharide **3** as its 4-methoxyphenyl glycoside was prepared following Scheme 2. Glycosylation of compound **7** with ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (**16**)[16] using *N*-iodosuccinimide and trifluoromethanesulfonic acid furnished analogous trisaccharide derivative **17**. Formation of compound **17** was confirmed by ^{1}H, ^{13}C, and partially decoupled ^{13}C NMR spectra. Saponification followed by hydrogenolysis of trisaccharide **17** furnished the trisaccharide **3**, which was confirmed by NMR and mass spectroscopies.

3. Conclusions

In summary, two trisaccharide analogs related to the glycone constituent of phanoside, a novel insulin releasing natural product have been synthesized as 4-methoxyphenyl glycosides in a very concise manner.

4. Experimental

4.1. General procedure

All the reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulfate (2% $Ce(SO_4)_2$ in 2 M H_2SO_4) sprayed plates on a hot plate. Silica gel of 230–400 mesh was used for column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker Advance DPX 300 MHz using TMS as an internal reference. Chemical shift values were expressed in δ parts per million. Elemental analysis was carried out on a Carlo ERBA-1108 analyzer. Optical rotations were measured at 25 °C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity are used in many reactions.

4.2. 4-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-D-lyxopyranoside (5)

To a solution of 1,2,3,4-tetra-*O*-acetyl-α-D-lyxopyranose (**4**, 6.0 g, 18.8 mmol) and 4-methoxy phenol (2.81 g, 22.6 mmol) in dry CH_2Cl_2 (25 mL) was added trifluoromethanesulfonic acid (150 μL) at 0 °C and the reaction mixture was allowed to stir at 10 °C for 2 h. The reaction mixture was diluted with CH_2Cl_2 and the organic layer was washed successively with water, aq $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude reaction product by column chromatography on silica gel using hexane–EtOAc (4:1) furnished pure compound **5** (6.5 g, 90%) as thick syrup; $[\alpha]_D^{25}$ +31.1 (*c* 1.2, $CHCl_3$); IR (neat): 1715, 1509, 1372, 1222, 1044, 831 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.00–6.96 (m, 2H, ArH), 6.83–6.73 (m, 2H, ArH), 5.50 (dd, *J*=9.9 and 3.6 Hz, 1H, 3-H), 5.39 (dd, *J*=3.3 and 2.4 Hz each, 1H, 2-H), 5.30 (d, *J*=2.4 Hz, 1H, 1-H), 5.27–5.19 (m, 1H, 4-H), 3.90 (dd, *J*=11.1 and 5.7 Hz, 1H, 5-H_a), 3.77 (s, 3H, OCH_3), 3.76–3.71 (m, 1H, 5-H_b), 2.18, 2.04 (2s, 9H, 3COCH_3); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 169.5, 169.4, 169.3 (3*C*OCH$_3$), 155.4, 149.9, 117.9 (2C), 114.6 (2C), 96.8 (C-1), 69.5, 68.5, 66.8, 60.2 (C-5), 55.4 (O*C*H$_3$), 20.7 (3C, CO*C*H$_3$); ESI-MS: *m*/*z*=405.1 [M+Na]$^+$. Anal. Calcd for $C_{18}H_{22}O_9$ (382.1): C, 56.54; H, 5.80. Found: C, 56.30; H, 6.10.

4.3. 4-Methoxyphenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-α-D-lyxopyranoside (6)

A solution of compound **5** (5.0 g, 13.1 mmol) in 0.05 M sodium methoxide in methanol (30.0 mL) was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+), filtered, and the filtrate was evaporated to dryness to give a glassy solid in quantitative yield (3.16 g). The dried mass thus obtained was dissolved in anhydrous DMF (15 mL) and 2,2-dimethoxypropane (2.4 mL, 18.5 mmol) was added to it followed by the addition of *p*-toluene sulfonic acid (~100 mg) to make the solution acidic (pH~2). After stirring at room temperature for 6 h, the reaction mixture was neutralized with triethylamine and filtered through the Celite bed. The filtrate was evaporated to dryness and the crude mass was purified over SiO_2 using hexane–EtOAc (3:1) to afford pure 4-methoxyphenyl 2,3-*O*-isopropylidene-α-D-lyxopyranoside (2.77 g, 71%), which was dissolved in CH_2Cl_2 (20 mL), and benzyl bromide (1.6 mL, 14.0 mmol) followed by 50% aq KOH solution (5 mL) and tetrabutylammonium iodide (~100 mg) were added to it. After stirring at room temperature for 3 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL). The organic layer was washed successively with water, aq NaCl, and water, dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography of the crude product over silica gel using hexane–EtOAc (5:1) furnished pure compound **6** (3.25 g, 90%) as syrup; $[\alpha]_D^{25}$ +60.0 (*c* 1.2, $CHCl_3$); IR (neat): 2359, 1597, 1375, 1225, 1073 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33–7.20 (m, 5H, ArH), 6.95–6.89 (m, 2H, ArH), 6.79–6.73 (m, 2H, ArH), 5.42 (d, *J*=1.5 Hz, 1H, 1-H), 4.72 (d, *J*=12.0 Hz, 1H, CH_2C$_6$H$_5$), 4.61 (d, *J*=12.0 Hz, 1H, CH_2C$_6$H$_5$), 4.34–4.30 (m, 1H, 2-H), 4.27 (dd, *J*=5.7 and 1.8 Hz, 1H, 3-H), 3.73 (s, 3H, OCH_3), 3.63–3.60 (m, 3H, 4-H and 5-$H_{a,b}$), 1.45, 1.37 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 155.1, 150.3, 138.2, 128.3 (2C), 127.8 (2C), 127.7, 117.8 (2C), 114.6 (2C), 109.3 (*C*(CH$_3$)$_2$), 97.0 (C-1), 77.5, 75.4 (Ph*C*H$_2$), 74.5, 71.9, 59.5 (C-5), 55.4 (O*C*H$_3$), 28.1 (C(*C*H$_3$)$_2$), 26.5 (C(*C*H$_3$)$_2$); ESI-MS: *m*/*z*=409.2 [M+Na]$^+$. Anal. Calcd for $C_{22}H_{26}O_6$ (386.2): C, 68.38; H, 6.78. Found: C, 68.20; H, 6.95.

4.4. 4-Methoxyphenyl 4-*O*-benzyl-α-D-lyxopyranoside (7)

Compound **6** (3.0 g, 7.77 mmol) was dissolved in 80% aq acetic acid (25 mL) and the reaction mixture was stirred at 60 °C for 45 min. The reaction mixture was evaporated and co-evaporated with toluene under reduced pressure. Purification of the crude reaction product using a short pad of SiO_2 using hexane–EtOAc (3:1) as the eluant furnished compound **7** (2.5 g, 93%) as yellow syrup; $[\alpha]_D^{25}$ +50.2 (*c* 1.2, $CHCl_3$); IR (neat): 2927, 2368, 2595, 1352, 1029, 673 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.25 (m, 5H, ArH), 6.93 (d, *J*=9.0 Hz, 2H, ArH), 6.77 (d,

J=9.0 Hz, 2H, ArH), 5.30 (br s, 1H, 1-H), 4.64–4.60 (m, 2H, CH_2Ph), 4.09–4.07 (m, 2H, 2-H and 3-H), 3.75 (s, 3H, OCH_3), 3.80–3.62 (m, 3H, 4-H and 5-$H_{a,b}$); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 155.1, 150.4, 137.9, 128.6 (2C), 127.8 (3C), 117.8 (2C), 114.6 (2C), 98.6 (C-1), 75.3, 72.5 (PhCH_2), 70.5, 70.3, 61.0 (C-5), 55.5 (OCH_3); ESI-MS: m/z=369.1 [M+Na]$^+$. Anal. Calcd for $C_{19}H_{22}O_6$ (346.1): C, 65.88; H, 6.40. Found: C, 65.70; H, 6.62.

4.5. 4-Methoxyphenyl 3-*O*-benzoyl-4-*O*-benzyl-α-D-lyxopyranoside (10)

A solution of compound **7** (2.5 g, 7.22 mmol) and dibutyltin oxide (2.16 g, 8.67 mmol) in anhydrous methanol (20 mL) was refluxed for 3 h. The reaction mixture was concentrated and co-evaporated with toluene several times. The dried mass was redissolved in dry toluene and benzoyl chloride (930 μL, 7.92 mmol) was added to it, and the reaction mixture was allowed to stir at room temperature for 8 h. The reaction was quenched by the addition of methanol and the reaction mixture was concentrated. Column chromatography of the crude product over silica gel using hexane–EtOAc (4:1) furnished pure compound **10** (2.6 g, 80%) as glassy solid; $[\alpha]_D^{25}$ +40.8 (c 1.2, $CHCl_3$); IR (KBr): 2938, 2365, 1695, 1594, 1286, 1093, 1006, 705 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.08–8.05 (m, 2H, ArH), 7.61–7.56 (m, 1H, ArH), 7.48–7.43 (m, 2H, ArH), 7.28–7.14 (m, 5H, ArH), 7.02 (d, J=8.7 Hz, 2H, ArH), 6.82 (d, J=8.4 Hz, 2H, ArH), 5.64 (dd, J=8.1 and 3.0 Hz, 1H, 3-H), 5.34 (d, J=3.0 Hz, 1H, 1-H), 4.65 (s, 2H, CH_2Ph), 4.34 (t, J=2.7 and 2.7 Hz, 1H, 2-H), 4.09–4.02 (ddd, J=8.1, 5.4, and 2.7 Hz, 1H, 4-H), 3.90–3.82 (m, 2H, 5-H_{ab}), 3.78 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 165.4 (*C*OPh), 155.1, 150.2, 137.9, 133.2 (2C), 129.9, 129.8 (2C), 128.4 (2C), 128.3 (2C), 127.8, 127.7, 117.9 (2C), 114.5 (2C), 98.9 (C-1), 73.3, 72.6, 72.3, 69.0, 61.8 (C-5), 55.4 (OCH_3); ESI-MS: m/z=473.2 [M+Na]$^+$. Anal. Calcd for $C_{26}H_{26}O_7$ (450.1): C, 69.32; H, 5.82. Found: C, 69.14; H, 6.04.

4.6. 4-Methoxyphenyl 2,3,4-tri-*O*-benzyl-α-L-rhamno-pyranosyl-(1→2)-3-*O*-benzoyl-4-*O*-benzyl-α-D-lyxo-pyranoside (12)

To a solution of compound **10** (1.0 g, 2.22 mmol) and compound **11** (1.3 g, 2.66 mmol) in anhydrous CH_2Cl_2 (15 mL) was added powdered MS (4 Å, 2.0 g) and the mixture was stirred under argon for 1 h. *N*-Iodosuccinimide (658 mg, 2.92 mmol) was added to the reaction mixture and cooled to −30 °C. Trifluoromethanesulfonic acid (25 μL, 0.29 mmol) was added to the reaction mixture at −30 °C and the reaction mixture was allowed to stir for 30 min at −30 °C. The reaction mixture was filtered through a Celite bed and the organic layer was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$, and water in succession, dried (Na_2SO_4), and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane–EtOAc (7:1) afforded pure disaccharide derivative **12** (1.5 g, 78%) as syrup; $[\alpha]_D^{25}$ −27.1 (c 1.2, $CHCl_3$); IR (neat): 2367, 1595, 1351, 1057 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.06–8.03 (m, 2H, ArH), 7.54–7.50 (m, 1H, ArH), 7.35–7.25 (m, 22H, ArH), 7.02–6.96 (m, 2H, ArH), 6.78 (d, J=9.0 Hz, 2H, ArH), 5.62 (dd, J=8.4 and 3.0 Hz, 1H, 3-H), 4.96 (br s, 1H, 1-H), 4.90 (d, J=11.1 Hz, 1H, CH_2Ph), 4.79 (d, J=12.3 Hz, 1H, CH_2Ph), 4.69–4.60 (m, 5H, 2CH_2Ph, 1′-H), 4.58 (d, J=12.6 Hz, 1H, CH_2Ph), 4.53 (d, J=11.0 Hz, 1H, CH_2Ph), 4.33–4.28 (m, 1H, 2-H), 4.0–3.92 (m, 1H, 5′-H), 3.88 (s, 3H, OCH_3), 3.86–3.84 (m, 2H, 5-$H_{a,b}$), 3.83 (t, J=4.5 and 4.5 Hz, 1H, 2′-H), 3.81–3.78 (m, 1H, 4-H), 3.76 (dd, J=9.0 and 3.1 Hz, 1H, 3′-H), 3.51 (t, J=9.3 and 9.3 Hz, 1H, 4′-H), 0.98 (d, J=6.3 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 165.5 (*C*OPh), 154.4, 151.1, 139.3–114.9 (Ar C), 97.4 (C-1), 97.0 (C-1′), 80.6, 80.0, 75.7, 75.3 (*C*H_2Ph), 73.7 (*C*H_2Ph), 73.2 (*C*H_2Ph), 72.8 (*C*H_2Ph), 72.7, 72.3 (2C), 68.9, 62.3 (C-5), 57.0 (OCH_3), 17.8 (*C*H_3); ESI-MS: m/z 889.5 [M+Na]$^+$. Anal. Calcd for $C_{53}H_{54}O_{11}$ (866.3): C, 73.42; H, 6.28. Found: C, 73.25; H, 6.50.

4.7. 4-Methoxyphenyl 2,3,4-tri-*O*-benzyl-α-L-rhamno-pyranosyl-(1→2)-4-*O*-benzyl-α-D-lyxopyranoside (13)

A solution of compound **12** (1.5 g, 1.73 mmol) in 0.1 M sodium methoxide in methanol (10.0 mL) was stirred at room temperature for 3 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+), filtered, and the filtrate was evaporated to dryness to give disaccharide derivative **13** as syrup (1.31 g); $[\alpha]_D^{25}$ −16.0 (c 1.2, $CHCl_3$); IR (neat): 2926, 2369, 1592, 1351 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.18 (m, 20H, ArH), 6.89 (d, J=9.0 Hz, 2H, ArH), 6.76 (d, J=9.0 Hz, 2H, ArH), 5.05 (d, J=3.8 Hz, 1H, 3-H), 4.93 (br s, 1H, 1-H), 4.90 (d, J=11.1 Hz, 1H, CH_2Ph), 4.74–4.59 (m, 8H, 4CH_2Ph, 1′-H), 4.08–4.01 (m, 1H, 5′-H), 4.0–3.96 (m, 1H, 2-H), 3.86–3.80 (m, 2H, 2′-H and 4-H), 3.76 (s, 3H, OCH_3), 3.75 (dd, J=9.0 and 2.7 Hz, 1H, 3′-H), 3.68–3.56 (m, 3H, 5-$H_{a,b}$ and 4′-H), 1.32 (d, J=6.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 155.2–127.7 (Ar C), 117.9 (2C), 114.5 (2C), 98.5 (C-1), 97.8 (C-1′), 80.3, 79.4, 76.1, 75.4 (2C, *C*H_2Ph), 75.2 (*C*H_2Ph), 72.9 (*C*H_2Ph), 72.4, 72.3, 70.2, 69.1, 61.8 (C-5), 55.4 (OCH_3), 18.1 (*C*H_3); ESI-MS: m/z 785.3 [M+Na]$^+$. Anal. Calcd for $C_{46}H_{50}O_{10}$ (762.3): C, 72.42; H, 6.61. Found: C, 72.25; H, 6.80.

4.8. 4-Methoxyphenyl [2,3,4-tri-*O*-benzyl-α-L-rhamno-pyranosyl-(1→2)]-[2,3,4,6-tetra-*O*-benzoyl-β-D-gluco-pyranosyl-(1→3)]-4-*O*-benzyl-α-D-lyxopyranoside (15)

To a solution of compound **13** (1.0 g, 1.31 mmol) and phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**14**, 1.1 g, 1.6 mmol) in anhydrous CH_2Cl_2 (10 mL) was added powdered MS (4 Å, 1.5 g) and the mixture was stirred under argon for 1 h. *N*-Iodosuccinimide (390 mg, 1.73 mmol) was added to the reaction mixture and cooled to −30 °C. Trifluoromethanesulfonic acid (15 μL, 0.17 mmol) was added to the reaction mixture at −30 °C and the reaction mixture was allowed to stir for 30 min at −30 °C. After completion of the reaction, the reaction mixture was filtered through a Celite bed and the organic layer was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated to a syrupy mass. Column chromatography of the crude product over SiO_2 using hexane–EtOAc (5:1) afforded pure trisaccharide derivative **15** (1.26 g, 72%); $[\alpha]_D^{25}$ +10.6 (c 1.2, $CHCl_3$); IR (neat): 2924, 2368, 1595, 1351, 1097, 708 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.10–8.04 (m, 2H, ArH), 7.85–7.80 (m, 4H, ArH), 7.74–7.71 (m, 2H, ArH), 7.52–7.46 (m, 4H, ArH), 7.42–7.22

(m, 22H, ArH), 7.20–7.07 (m, 4H, ArH), 6.91–6.86 (m, 2H, ArH) 6.57 (d, J=9.0 Hz, 2H, ArH), 6.46 (d, J=8.7 Hz, 2H, ArH), 5.88 (t, J=9.6, 9.6 Hz, 1H, 3″-H), 5.62 (t, J=9.6 and 9.6 Hz, 1H, 2″-H), 5.42 (t, J=9.6 and 9.6 Hz, 1H, 4″-H), 5.34 (d, J=8.1 Hz, 1H, 1″-H), 5.25 (br s, 1H, 1-H), 5.05 (d, J=11.1 Hz, 1H, CH_2Ph), 4.82–4.70 (m, 4H, 2CH_2Ph, 3-H), 4.66 (br s, 1H, 1′-H), 4.61 (d, J=11.6 Hz, 1H, CH_2Ph), 4.56–4.51 (m, 3H, CH_2Ph), 4.39 (t, J=3.6 and 3.6 Hz, 1H, 2-H), 4.30 (dd, J=12.0 and 5.1 Hz, 1H, 6″-H_a), 4.17–4.10 (m, 2H, 6″-H_b and 5′-H), 3.92 (dd, J=9.0 and 2.7 Hz, 1H, 3′-H), 3.84–3.82 (m, 2H, 5″-H and 2′-H), 3.81–3.77 (m, 1H, 4-H), 3.76 (s, 3H, OCH_3), 3.75–3.73 (m, 2H, 5-$H_{a,b}$), 3.72–3.68 (m, 1H, 4′-H), 1.42 (d, J=6.0 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 165.7, 165.6, 164.9, 164.8 (4COPh), 138.8–114.3 (Ar C), 101.0 (C-1″), 99.5 (C-1), 98.7 (C-1′), 80.9, 80.5, 75.7, 75.4, 74.7, 73.4, 72.7 (2C), 72.6 (2C, CH$_2$Ph), 72.5, 71.2 (2C, CH$_2$Ph), 69.4 (2C), 63.2 (C-6″), 62.6 (C-5), 55.4 (OCH$_3$), 18.5 (CH$_3$); ESI-MS: m/z 1363.5 [M+Na]$^+$. Anal. Calcd for $C_{80}H_{76}O_{19}$ (1340.5): C, 71.63; H, 5.71. Found: C, 71.45; H, 5.98.

4.9. 4-Methoxyphenyl [α-L-rhamnopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-α-D-lyxopyranoside (2)

A solution of compound **15** (1.0 g, 0.75 mmol) in 0.1 M sodium methoxide in methanol (25.0 mL) was stirred at room temperature for 12 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+), filtered, and the filtrate was evaporated to dryness to give a syrup, which was purified through Sephadex LH-20 using toluene as eluant to give 4-methoxyphenyl [2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl-(1→2)]-[β-D-glucopyranosyl-(1→3)]-4-*O*-benzyl-α-D-lyxopyranoside, which was dissolved in methanol (5 mL), and 20% $Pd(OH)_2$–C (300 mg) was added to it. The reaction mixture was stirred under hydrogen at room temperature for 18 h. The mixture was filtered through a Celite bed and concentrated to give target trisaccharide **2** (260 mg, 61%) as syrup; $[\alpha]_D^{25}$ +22 (c 1.2, CH_3OH); ^{1}H NMR (CD_3OD–D_2O, 300 MHz): δ 6.92–6.89 (m, 2H, ArH), 6.77–6.73 (m, 2H, ArH), 5.28 (d, J=4.8 Hz, 1H, 1′-H), 4.96 (br s, 1H, 1-H), 4.47 (d, J=7.8 Hz, 1H, 1″-H), 4.10–4.03 (m, 2H, 2′-H and 3′-H), 3.88–3.86 (m, 1H, 4′-H), 3.84–3.83 (m, 1H, 2-H), 3.82–3.77 (m, 3H, 6″-$H_{a,b}$ and 4″-H), 3.64 (s, 3H, OCH_3), 3.59–3.53 (m, 2H, 3-H and 5′-H), 3.35–3.27 (m, 2H, 3″-H and 4-H), 3.25–3.20 (m, 3H, 5″-H and 5-$H_{a,b}$), 3.17–3.14 (m, 1H, 2″-H), 1.22 (d, J=6.3 Hz, 3H, CH_3); ^{13}C NMR (CD_3OD–D_2O, 75 Hz): δ 154.0, 149.6, 116.4 (2C), 112.9 (2C), 101.8 (C-1″), 99.7 (C-1), 97.4 (C-1′), 77.1 (2C), 75.4 (2C), 75.0, 72.4 (2C), 71.2, 69.7, 69.4, 67.9, 60.0 (2C, C-5 and C-6″), 53.4 (OCH$_3$), 15.3 (CH$_3$); ESI-MS: m/z 587.2 [M+Na]$^+$. Anal. Calcd for $C_{24}H_{36}O_{15}$ (564.2): C, 51.06; H, 6.43. Found: C, 50.85; H, 6.65.

4.10. 4-Methoxyphenyl [2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)]-4-*O*-benzyl-α-D-lyxopyranoside (17)

To a solution of compound **7** (500 mg, 1.44 mmol) and ethyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**16**, 1.2 g, 3.60 mmol) in anhydrous CH_2Cl_2 (10 mL) was added powdered MS (4 Å, 1.0 g) and the mixture was stirred under argon for 1 h. *N*-Iodosuccinimide (890 mg, 3.97 mmol) was added to the reaction mixture and it was cooled to −30 °C. Trifluoromethanesulfonic acid (35 μL, 0.39 mmol) was added and the reaction mixture was allowed to stir for 30 min at −30 °C. After completion of the reaction, the reaction mixture was filtered through a Celite bed and the filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane–EtOAc (3:1) afforded pure trisaccharide derivative **17** (872 mg, 68%); $[\alpha]_D^{25}$ −26.1 (c 1.2, $CHCl_3$); IR (neat): 2935, 2366, 1752, 1594, 1375, 1225, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.28–7.14 (m, 5H, ArH), 6.97 (d, J=8.7 Hz, 2H, ArH), 6.78 (d, J=9.0 Hz, 2H, ArH), 5.35–5.24 (m, 5H, 1-H, 1′-H, 2′-H, 3′-H, and 4′-H), 5.13–4.97 (m, 6H, 1″-H, 2″-H, 3″-H, 4″-H, CH_2Ph), 4.20–4.09 (m, 4H, 5′-H, 5″-H, 2-H, 3-H), 4.04–3.87 (m, 2H, 5-$H_{a,b}$), 3.77 (s, 3H, OCH_3), 3.67–3.60 (m, 1H, 4-H), 2.18, 2.17, 2.16, 2.07, 2.06, 2.05 (6s, 18H, 6COCH_3), 1.22–1.24 (m, 6H, 2CH_3); ^{13}C NMR ($CDCl_3$, 75 Hz): δ 169.9, 169.7, 169.5, 169.4, 169.3, 169.2 (6COCH$_3$), 155.1–114.4 (Ar C), 98.5 (C-1), 97.3 (C-1′ and C-1″), 71.2, 70.6, 70.5 (2C), 70.2 (CH_2Ph), 69.8, 69.5, 69.1, 68.8, 68.7, 68.5, 67.1, 66.6 (C-5), 55.3 (OCH$_3$), 20.6 (2C, 2COCH$_3$), 20.5 (2C, 2COCH$_3$), 20.3 (2C, 2COCH$_3$), 17.3, 17.0 (2CH$_3$); ESI-MS: m/z 913.3 [M+Na]$^+$. Anal. Calcd for $C_{43}H_{54}O_{20}$ (890.3): C, 57.97; H, 6.11. Found: C, 57.75; H, 6.30.

4.11. 4-Methoxyphenyl [α-L-rhamnopyranosyl-(1→2)]-[α-L-rhamnopyranosyl-(1→3)]-α-D-lyxopyranoside (3)

To a solution of compound **17** (800 mg, 0.9 mmol) in methanol was added 20% $Pd(OH)_2$–C (100 mg) and the reaction mixture was stirred under hydrogen at room temperature for 10 h. The mixture was filtered through Celite bed and concentrated to give yellow syrup, which was dissolved in 0.1 M sodium methoxide in methanol (15.0 mL) and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was neutralized with Dowex 50W-X8 (H^+), filtered, and the filtrate was evaporated to dryness to give a syrup, which was purified through Sephadex LH-20 using 80% aq EtOH as eluant to give pure trisaccharide **3** (320 mg, 65%) as a viscous liquid; $[\alpha]_D^{25}$ −10 (c 1.2, CH_3OH); ^{1}H NMR (CD_3OD–D_2O, 300 MHz): δ 6.92 (d, J=8.7 Hz, 2H, ArH), 6.76 (d, J=9.0 Hz, 2H, ArH), 5.15 (br s, 1H, 1-H), 5.04 (d, J=2.6 Hz, 2H, 1′-H and 1″-H), 4.09–4.08 (m, 1H, 2′-H), 3.99–3.98 (m, 1H, 2″-H), 3.86–3.74 (m, 3H, 3′-H, 3″-H, and 2-H), 3.73–3.55 (m, 4H, 4′-H, 4″-H, 3-H, and 4-H), 3.65 (s, 3H, OCH_3), 3.39–3.29 (m, 4H, 5′-H, 5″-H, and 5-$H_{a,b}$), 1.23–1.13 (m, 6H, 2CH_3); ^{13}C NMR (CD_3OD–D_2O, 75 MHz): 156.7, 149.1, 119.1 (2C), 115.6 (2C), 100.1 (2C, C-1′ and C-1″), 94.5 (C-1), 73.8 (2C), 73.7 (2C), 72.2, 72.1 (2C), 72.0 (2C), 70.5, 70.4, 68.8 (C-5), 56.1 (OCH$_3$), 18.1, 17.9 (2CH$_3$); ESI-MS: m/z 571.2 [M+Na]$^+$. Anal. Calcd for $C_{24}H_{36}O_{14}$ (548.2): C, 52.55; H, 6.62. Found: C, 52.38; H, 6.85.

Acknowledgements

Instrumentation facilities from SAIF, CDRI are gratefully acknowledged. G.A. and P.K.M. thank CSIR, New Delhi for providing a Senior and Junior Research Fellowships,

respectively. This project was partly funded by Department of Science and Technology (DST), New Delhi (Project no. SR/FTP/CSA-10/2002), India.

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