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Synthesis of two trisaccharides related to the triterpenoid saponins isolated from *Solanum lycocarpum*

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Dedicated to Professor Brindaban Chandra Ranu, Organic Chemistry Department, Indian Association for the Cultivation of Science, Jadavpur, Kolkata

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ABSTRACT

Chemical synthesis of two trisaccharides related to the triterpenoid saponins isolated from *Solanum lycocarpum* from commercially available D-Glc, D-Gal and L-Rha have been achieved by following concise and high-yielding route. The target trisaccharide **1** has been made by following a bis-glycosylation approach that has minimized the protecting group manipulations up to great extent. The trisaccharides have been synthesized in the form of their *p*-methoxyphenyl (OMP) glycosides to leave the scope for further glycoconjugates formation by the selective removal of the OMP glycoside and trichloroacetimidate chemistry. $La(OTf)_3$ has been used successfully as the promoter for the NIS mediated activation of thioglycosides.

1. Introduction

Steroidal saponins are secondary metabolites produced in plants in their regular growth and development programme. It is believed that this class of compounds helps to form a chemical barrier against fungal attack, and thus is very important for the defence mechanism in plants.[1] Although the biological importance of steroidal saponins has been recognised, the biosynthesis of saponins is still not understood completely. Saponins have also served as a source of medicines for a long time. One of the common features shared by this class of molecules is the presence of a branched oligosaccharide at the 3-OH position of the aglycon moiety.[2,3] It is evident that the glycosylation happens in a mature stage of the saponin biosynthesis and the oligosaccharides have a clear role to regulate the bioactivity of the saponin concerned. Therefore, chemical synthesis of saponins is required to elucidate the biosynthetic pathway and the activity.[4,5] Recently, Yoshikawa et al.[6] reported the structures of two new steroidal saponins, robenoside A and B from the Brazilian medicinal plant *Solanum lycocarpum* that showed reasonable antidiabetic properties. In continuation to our effort towards the synthesis of the oligosaccharides related to the bioactive saponins,[7] here we report the total synthesis of two trisaccharides related to the steroidal saponins isolated from *S. lycocarpum* in the form of their *p*-methoxyphenyl glycosides (Fig. 1).

2. Results and discussion

Synthesis of the target trisaccharide **1** was commenced with the known *p*-methoxyphenyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**3**)[8] (Scheme 1). *p*-Methoxyphenyl glycoside was preferred as the reducing end glycoside as it can be selectively converted to the corresponding trichloroacetimidate derivative after the formation of the target structure and allows further glycoconjugates formation. Compound **3** was selectively benzylated at the 3-position via stannylene chemistry by using Bu_2SnO in MeOH[9] followed by BnBr in the presence of CsF to afford *p*-methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**4**) in 73% yield. Further regioselective opening of the benzylidene acetal using triethylsilane in the presence of $BF_3{\cdot}Et_2O$[10] furnished the required diol, *p*-methoxyphenyl 3,6-di-*O*-benzyl-β-D-glucopyranoside (**5**) in 86% yield. At this point, a bis-glycosylation with two suitably protected rhamnose moieties would result the target trisaccharide **1**. Therefore, the diol **5** was reacted with excess (3 mol equiv) of known donor, *p*-tolyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**6**)[11] in the presence of *N*-iodosuccinimide (NIS) and $La(OTf)_3$.[12] The glycosylation reaction afforded the desired trisaccharide **7** in 62% isolated yield along with the (1→2)-disaccharide **8** in 20% yield. Surprisingly, no (1→4)-disaccharide

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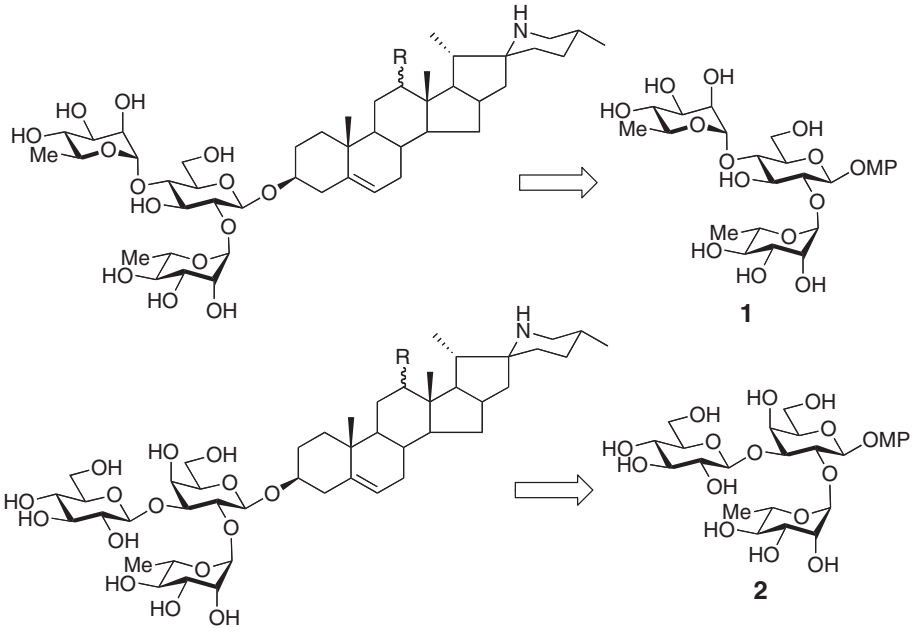
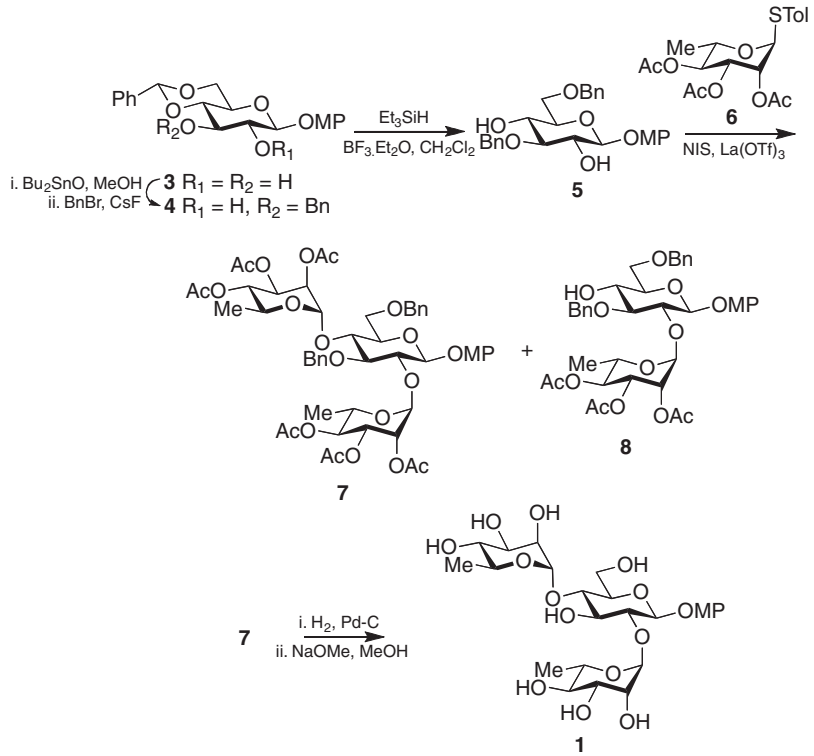


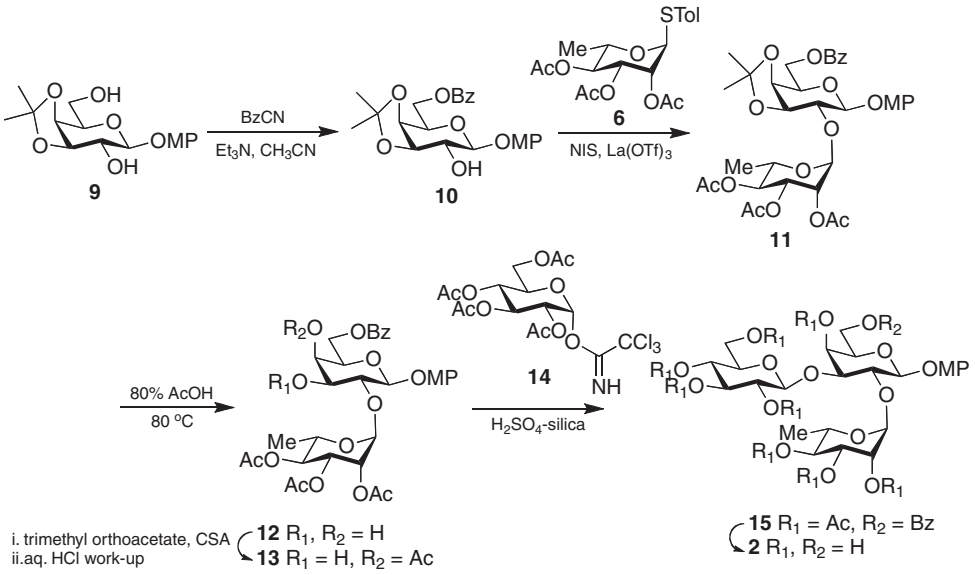
Figure 1. Structure of the triterpenoid saponins from *Solanum lycocarpum* and the synthetic targets.



Scheme 1. Synthesis of the trisaccharide **1**.

could be isolated that indicates the greater reactivity of 2-OH of the glucoside moiety in comparison to the 4-OH of the same. Finally, protected trisaccharide **7** upon catalytic hydrogenation using Pd-C under H_2 atmosphere[13] followed by transesterification using NaOMe in MeOH[14] afforded the target trisaccharide, *p*-methoxyphenyl α-L-rhamnopyranosyl-(1→2)-4-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**1**) in 72% overall yield (Scheme 1).

For the synthesis of the target trisaccharide **2**, known *p*-methoxyphenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**9**)[15] was selectively benzoylated at the primary position by using an equimolar amount of BzCN in the presence of catalytic Et_3N in CH_3CN[16] to furnish the desired 2-ol derivative, *p*-methoxyphenyl 6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**10**) in 88% yield (Scheme 2). Glycosylation of rhamnosyl donor **6** with 2-ol acceptor **10** in the presence of NIS and $La(OTf)_3$ afforded the



Scheme 2. Synthesis of the trisaccharide **2**.

disaccharide **11** in 83% yield. Further, hydrolysis of the isopropylidene ketal using 80% aq. AcOH at 80 °C[17] followed by orthoesterification with trimethyl orthoacetate[18] in the presence of CSA and subsequent rearrangement of the orthoester by aq HCl work-up furnished the disaccharide acceptor, *p*-methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (**13**). Glycosylation of the disaccharide acceptor **13** with known 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**14**)[19] in the presence of H_2SO_4–silica[20] afforded the protected trisaccharide *p*-methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-6-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-galactopyranoside (**15**) in 81% yield. It is worth noting that when the final glycosylation was tried with *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**16**), only >15% isolated yield of the desired trisaccharide was obtained from a very messy product mixture. Finally, global deprotection of the trisaccharide **15** through transesterification using NaOMe in MeOH furnished the target trisaccharide, *p*-methoxyphenyl α-L-rhamnopyranosyl-(1→2)-3-*O*-(β-D-glucopyranosyl)-β-D-galactopyranoside (**2**) in 83% yield.

3. Conclusion

In conclusion, we have synthesized two trisaccharides related to the triterpenoid saponin isolated from *S. lycocarpum*. The trisaccharides have been synthesized as their *p*-methoxyphenyl glycosides to leave the scope open for further glycoconjugates formation. Synthesis of the trisaccharide **1** has been accomplished by following a bis-glycosylation strategy that minimized the protecting group manipulations.

4. Experimental

4.1. General

All reagents and solvents were dried prior to use according to standard methods.[21] Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on Silica Gel 60-F_{254} with detection by fluorescence and/or by charring following immersion in a 10% ethanolic solution of sulfuric acid. An orcinol dip, prepared by the careful addition of concentrated sulfuric acid (20 mL) to an ice-cold solution of 3,5-dihydroxytoluene (360 mg) in EtOH (150 mL) and H_2O (10 mL), was used to detect deprotected compounds by charring. Flash chromatography was performed with Silica Gel 230–400. Optical rotations were measured at the sodium D-line at ambient temperature. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 500 MHz and 125 MHz, respectively.

4.2. *p*-Methoxyphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (4)

A mixture of known *p*-methoxyphenyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**3**) (5.0 g, 13.3 mmol) and Bu_2SnO (3.6 g,14.7 mmol) in dry MeOH (30 mL) was refluxed for 3 h. The resulting solution was concentrated in vacuo, co-evaporated with toluene and the residue was dried under vacuum for 30 min. The residue was dissolved in dry DMF (20 mL) and BnBr (2.1 mL, 13.3 mmol) was added followed by CsF (2.0 g, 13.3 mmol) and the solution was stirred at 80 °C for 6 h. After evaporating the solvents in vacuo the residue was dissolved in CH_2Cl_2 (40 mL) and washed successively with H_2O (50 mL) and brine (50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated in vacuo. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (3:1) as eluent to afford pure compound **4** (4.3 g, 73%) as white foam. $[\alpha]_D^{25}$ +92 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.50–7.28 (m, 10H, ArH), 7.02 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.83 (d, 2H, C_6H_4OMe), 5.59 (s, 1H, C*H*Ph), 4.96 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.89 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.85 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.36 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.82 (t, 1H, $J_{3,4}$, $J_{4,5}$ 10.5 Hz, H-4), 3.77 (s, 3H, $C_6H_4OCH_3$), 3.75 (m, 3H, H-2, H-3, H-6b), 3.54 (m, 1H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.4, 151.0, 138.1, 137.0, 128.9, 128.2 (2 × C), 128.1 (2 × C), 127.9 (2 × C), 127.6, 125.9 (2 × C), 118.6 (2 × C), 114.4 (2 × C) (ArC), 102.7 (*C*HPh), 101.2 (C-1), 81.0, 80.5, 74.6, 73.7, 68.5, 66.2, 55.4 ($C_6H_4OCH_3$). HRMS calcd for $C_{27}H_{28}O_7Na$ $(M+Na)^+$: 487.1733, found: 487.1731.

4.3. *p*-Methoxyphenyl 3,6-di-*O*-benzyl-β-D-glucopyranoside (5)

To a solution of compound **4** (1.5 g, 3.2 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C, triethylsilane (6.0 mL, 38 mmol) was added followed by addition of $BF_3.Et_2O$ (790 μL, 6.4 mmol). The reaction was stirred at 0 °C till the TLC showed the complete conversion of the reactant (2 h). The reaction mixture was diluted with CH_2Cl_2 (15 mL) and successively washed with H_2O (30 mL) and $NaHCO_3$ (30 mL). The organic layer was collected, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated and the crude material was purified by flash chromatography using *n*-hexane–EtOAc (2:1) to afford pure compound **5** (1.3 g, 86%). $[\alpha]_D^{25}$ +101 (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.40–7.32 (m, 10H, ArH), 7.02 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.80 (d, 2H, C_6H_4OMe), 4.99 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.85 (d, 1H, *J* 11.5 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.60 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.55 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.36 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.78 (s, 3H, $C_6H_4OCH_3$), 3.76 (m, 2H, H-2, H-6b), 3.73 (t, 1H, $J_{2,3}$, $J_{3,4}$ 10.0 Hz, H-3), 3.58 (m, 1H, H-5), 3.48 (t, 1H, $J_{3,4}$, $J_{4,5}$ 10.0 Hz, H-4), 2.73 (br s, 1H, O*H*), 2.61 (br s, 1H, O*H*). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.4, 151.1, 138.5, 137.8, 128.6 (2 × C), 128.4 (2 × C), 128.0 (2 × C), 127.9, 127.6, 127.4 (2 × C), 118.6 (2 × C), 114.5 (2 × C) (ArC), 102.0 (C-1), 83.6, 74.8, 74.4, 74.0, 73.6, 71.3, 70.1, 55.6 ($C_6H_4OCH_3$). HRMS calcd for $C_{27}H_{30}O_7Na$ $(M+Na)^+$: 489.1889, found: 489.1887.

4.4. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3,6-di-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (7)

A mixture of compound **5** (1.0 g, 2.1 mmol), compound **6** (2.5 g, 6.3 mmol) and MS 4 Å (2 g) in dry CH_2Cl_2 (30 mL) was stirred under nitrogen for 1 h. NIS (1.8 g, 8.2 mmol) was added and the mixture was cooled to 0 °C followed by addition of $La(OTf)_3$ (100 mg). The mixture was allowed to stir at 0 °C for 30 min when TLC showed complete consumption of the acceptor **5**. The mixture was immediately filtered through a pad of Celite. The filtrate was diluted with CH_2Cl_2 (20 mL) and washed successively with $Na_2S_2O_3$ (2 × 50 mL), $NaHCO_3$ (2 × 50 mL) and brine (50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by flash chromatography using *n*-hexane–EtOAc (2:1 to 1:1) to afford pure compound **7** (1.3 g, 62%) as colourless foam and **8** (315 mg, 20%) as light yellow oil.

Compound **7**: $[\alpha]_D^{25}$ +73 (*c* 0.9, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 7.31–7.27 (m, 10H, ArH), 6.98 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.82 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.24 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 5.0 Hz, H-2′), 5.22 (dd, 1H, $J_{2',3'}$ 5.0 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.20 (m, 2H, H-2″, H-3″), 5.19 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 5.02 (d, 1H, $J_{1'',2''}$ 1.5 Hz, H-1″), 5.01 (d, 1H, 12.0 Hz, CH_2Ph), 4.97 (t, 2H, *J* 10.0 Hz, H-4′, H-4″), 4.91 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.85 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.56 (2d, 2H, AB system, *J* 12.0 Hz, CH_2Ph), 4.34 (m, 1H, H-5′), 4.05 (t, 1H, $J_{3,4}$, $J_{4,5}$ 8.5 Hz, H-4), 4.01 (m, 2H, H-2, H-5″), 3.79 (s, 3H, $C_6H_4OCH_3$), 3.78–3.73 (m, 3H, H-3, H-6a, H-6b), 3.56 (m, 1H, H-5), 2.09, 2.01, 1.99, 1.92, 1.87, 1.85 (6s, 18H, 6 × $COCH_3$), 1.21 (d, 3H, *J* 6.0 Hz, C–CH_3), 0.86 (d, 3H, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.2, 170.1, 170.0, 169.9, 169.8, 169.4 (6 × $COCH_3$), 155.3, 151.0, 137.9, 137.5, 128.2 (2 × C), 128.0 (2 × C), 127.5 (2 × C), 127.4, 127.1, 126.1 (2 × C), 118.2 (2 × C), 114.6 (2 × C) (ArC), 100.2 (C-1), 98.2 (C-1′), 97.2 (C-1″), 83.8, 75.1, 74.4, 74.3, 73.1, 70.8, 70.6, 69.9, 69.3, 69.2, 68.9, 68.1, 66.8, 66.7, 55.6 ($C_6H_6OCH_3$), 20.9, 20.8, 20.7, 20.6, 20.5, 20.4 (6 × $COCH_3$), 17.2, 17.1 (2 × C–CH_3). HRMS calcd for $C_{51}H_{62}O_{21}Na$ $(M+Na)^+$: 1033.3681, found: 1033.3678.

Compound **8**: ^1H NMR (500 MHz, $CDCl_3$) δ: 7.34–7.26 (m, 10H, ArH), 6.97 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.82 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.33 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.0 Hz, H-2′), 5.26 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 5.24 (dd, 1H, $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.06 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 4.89 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.83 (2d, 2H, AB system, *J* 11.5 Hz, CH_2Ph), 4.55 (2d, 2H, AB system, *J* 12.0 Hz, CH_2Ph), 4.34 (m, 1H, H-5′), 3.86 (dd, 1H, $J_{1,2}$ 7.5 Hz, $J_{2,3}$ 9.0 Hz, H-2), 3.78 (s, 3H, $C_6H_4OCH_3$), 3.72 (m, 3H, H-4, H-6a, H-6b), 3.62 (t, 1H, $J_{2,3}$, $J_{3,4}$ 8.5 Hz, H-3), 3.57 (m, 1H, H-5), 2.62 (br s, 1H, O*H*), 2.07, 2.01, 1.97 (3s, 9H, 3 × $COCH_3$), 1.19 (d, 3H, *J* 6.0 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1, 170.0, 169.9 (3 × $COCH_3$), 155.3, 151.0, 138.1, 137.6, 128.5 (2 × C), 128.4 (2 × C), 127.9 (2 × C), 127.9, 127.8, 127.7 (2 × C), 118.1 (2 × C), 114.6 (2 × C) (ArC), 100.1 (C-1), 98.0 (C-1′), 85.5, 76.6, 75.5, 73.9, 73.7, 72.5, 71.0, 70.3, 69.6, 69.2, 66.5, 55.6 ($C_6H_4OCH_3$), 20.8, 20.7, 20.6 (3 × $COCH_3$), 17.2 (C–CH_3). HRMS calcd for $C_{39}H_{46}O_{14}Na$ $(M+Na)^+$: 761.2785, found: 761.2787.

4.5. *p*-Methoxyphenyl α-L-rhamnopyranosyl-(1→2)-4-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (1)

The protected trisaccharide **7** (1 g, 1.0 mmol) was dissolved in CH_3OH and 20% Pd-C (150 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite bed and evaporated to dryness. The residue was dissolved in dry MeOH (10 mL), NaOMe (0.5 M in MeOH, 1.0 mL) was added and the solution was stirred at room temperature for 4 h. The solution was neutralised with DOWEX 50 W H^+ resin and filtered through a cotton plug. The filtrate was evaporated and washed with CH_2Cl_2 (5 ml) removing the methyl benzoate impurity to afford the target trisaccharide **1** (412 mg, 72%). $[\alpha]_D^{25}$ +102 (*c* 0.8, H_2O). ^1H NMR (500 MHz, D_2O) δ: 6.96 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.88 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.06 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 5.03 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.75 (d, 1H, $J_{1'',2''}$ 1.5 Hz, H-1″), 3.95 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.0 Hz, H-2′), 3.91 (m, 1H, H-5′), 3.87 (dd, 1H, $J_{1'',2''}$ 1.5 Hz, $J_{2'',3''}$ 3.5 Hz, H-2″), 3.81 (m, 1H, H-5″), 3.73 (dd, 1H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$12.5 Hz, H-6a), 3.69 (s 3H, $C_6H_4OCH_3$), 3.68 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.0 Hz, H-3), 3.63 (m, 2H, H-3′, H-3″), 3.58 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.0 Hz, H-4), 3.54 (dd, 1H, $J_{5,6b}$ 2.5 Hz, $J_{6a,6b}$12.5 Hz, H-6b), 3.52 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2), 3.47 (m, 1H, H-5), 3.35 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 3.32 (t, 1H, $J_{3'',4''}$, $J_{4'',5''}$ 10.0 Hz, H-4″), 1.15 (d, 3H, *J* 6.5 Hz, C–CH_3), 1.09 (d, 3H, *J* 6.5 Hz, C–CH_3). ^{13}C NMR (125 MHz, D_2O) δ: 154.8, 150.5, 117.9 (2 × C), 115.3 (2 × C) (ArC), 101.6 (C-1), 101.0 (C-1′), 99.2 (C-1″), 79.9, 76.9, 75.2, 75.0, 72.0, 70.5, 70.3, 70.2, 69.1, 69.0, 60.1, 55.9 ($C_6H_6OCH_3$), 49.0, 16.7 (C–CH_3), 16.5 (C–CH_3). HRMS calcd for $C_{25}H_{38}O_{15}Na$ $(M+Na)^+$: 601.2108, found: 601.2105.

4.6. *p*-Methoxyphenyl 6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (10)

To a suspension of compound **9** (4.1 g, 12.5 mmol) in dry CH_3CN (30 mL), BzCN (1.5 mL, 12.5 mmol) was added followed by Et_3N (20 μL) and allowed to stir at 0 °C. The solution became clear within 15 min and the TLC (*n*-hexane–EtOAc; 2:1) showed complete consumption of the starting material. MeOH (2 mL) was added to quench the reaction. Solvents were evaporated in vacuo and the residue was purified by flash chromatography using 2:1 *n*-hexane–EtOAc to afford pure *p*-methoxyphenyl-6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside **10** (4.8 g, 88%). $[\alpha]_D^{25}$ +79 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.08–6.67 (m, 9H, Ar*H*), 4.72 (dd, 1H, $J_{6a,6b}$ 11.5 Hz, $J_{5,6a}$ 3.0 Hz, H-6a), 4.67 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.62 (m, 1H, H-6b), 4.24 (m, 2H, H-3, H-4), 3.89 (t, 1H, $J_{1,2}$, $J_{2,3}$ 8.5 Hz, H-2), 3.73 (s, 3H, C_6H_4–OCH_3), 3.72 (m, 1H, H-5), 1.59, 1.43 (2s, 6H, 2 × isopropylidene-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.2 (*C*OPh), 155.4, 151.0, 133.1, 129.8,

129.7 (2 × C), 128.4 (2 × C), 118.6 (2 × C), 114.4 (2 × C) (ArC), 110.8 [$C(CH_3)_2$], 101.6 (C-1), 78.9, 73.3, 73.2, 71.4, 63.6, 55.5 (C_6H_4–OCH_3), 26.3, 22.6 (2 × isopropylidene CH_3). HRMS calcd for $C_{23}H_{26}O_8Na$ $(M+Na)^+$: 453.1525, found: 453.1521.

4.7. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-6-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (11)

A mixture of compound **10** (2 g, 4.65 mmol), compound **6** (2.2 g, 5.5 mmol) and MS 4 Å (2 g) in dry CH_2Cl_2 (25 mL) was stirred under nitrogen for 1 h. NIS (1.6 g, 7.4 mmol) was added and the mixture was cooled to 0 °C followed by addition of $La(OTf)_3$ (100 mg). The mixture was allowed to stir at 0 °C for 30 min. when TLC showed complete consumption of the acceptor **10**. The mixture was filtered through a pad of Celite. The filtrate was diluted with CH_2Cl_2 (20 mL) and washed successively with $Na_2S_2O_3$ (2 × 50 mL), $NaHCO_3$ (2 × 50 mL) and brine (50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by flash chromatography using *n*-hexane–EtOAc (2:1) to afford pure compound **11** (2.6 g, 83%) as white foam. $[\alpha]_D^{25}$ +113 (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.06–7.44 (m, 5H, ArH), 6.97 (d, 2H, *J* 9.5 Hz, C_6H_4OMe), 6.66 (d, 2H, *J* 9.5 Hz, C_6H_4OMe), 5.33 (dd, 1H, $J_{1',2'}$ 2.0 Hz, $J_{2',3'}$ 3.5 Hz, H-2′), 5.26 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1′), 5.24 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.10 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 4.74 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.69 (dd, 1H, $J_{5,6a}$ 4.0 Hz, $J_{6a,6b}$12.5 Hz, H-6a), 4.62 (dd, 1H, $J_{5,6b}$ 11.5 Hz, $J_{6a,6b}$12.5 Hz, H-6b), 4.35 (m, 1H, H-5′), 4.31 (dd, 1H, $J_{2,3}$ 7.0 Hz, $J_{3,4}$ 2.0 Hz, H-3), 4.25 (br d, 1H, $J_{3,4}$ 2.0 Hz, H-4), 4.21 (m, 1H, H-5), 4.00 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 7.0 Hz, H-2), 3.71 (s, 3H, $C_6H_4OCH_3$), 2.16, 2.03, 1.96 (3s, 9H, 3 × $COCH_3$), 1.57, 1.35 (2s, 6H, 2 × isopropylidene-CH_3), 1.24 (d, 3H, *J* 6.0 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1, 170.0, 169.9 (3 × $COCH_3$), 166.1 (COPh), 155.5, 151.2, 133.2, 129.8, 129.7 (2 × C), 128.4 (2 × C), 118.6 (2 × C), 114.4 (2 × C) (ArC), 111.0 [$C(CH_3)_2$], 100.2 (C-1), 95.9 (C-1′), 79.8, 75.0, 73.5, 71.2, 71.0, 69.5, 69.2, 66.4, 63.6, 55.5 ($C_6H_4OCH_3$), 27.8, 26.3 (2 × isopropylidene CH_3), 20.9, 20.8, 20.7 (3 × $COCH_3$), 17.3 (C–CH_3). HRMS calcd for $C_{35}H_{42}O_{15}Na$ $[M+Na]^+$: 725.2421, found: 725.2423.

4.8. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-6-*O*-benzoyl-β-D-galactopyranoside (12)

A solution of compound **11** (2.8 g, 3.9 mmol) in 80% AcOH (30 mL) was stirred at 80 °C for 2 h when TLC (*n*-hexane–EtOAc; 1:1) showed complete conversion of the starting material to a slower moving spot. Solvents were evaporated in vacuo and the residue thus obtained was purified by flash chromatography using gradient mixture of *n*-hexane and EtOAc (2:1 to 1:1) to afford pure compound **12** (2.4 g, 92%) as white foam. $[\alpha]_D^{25}$ +76 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.04–7.44 (m, 5H, ArH), 6.97 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.68 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.38 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.0 Hz, H-2′), 5.34 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 5.23 (dd, 1H, $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.09 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 4.82 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.63 (dd, 1H, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$12.5 Hz, H-6a), 4.55 (dd, 1H, $J_{5,6b}$ 7.5 Hz, $J_{6a,6b}$12.5 Hz, H-6b), 4.31 (m, 1H, H-5′), 4.01 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.5 Hz, H-2), 3.96 (br s, 1H, H-4), 3.89 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 1.0 Hz, H-3), 3.87 (m, 1H, H-5), 3.72 (s, 3H, $C_6H_4OCH_3$), 3.51 (br s, 1H, O*H*), 3.21 (br s, 1H, O*H*), 2.13, 2.02, 1.98 (3s, 9H, 3 × $COCH_3$), 1.21 (d, 3H, *J* 6.0 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.5 (2 × C), 170.0 (3 × $COCH_3$), 166.5 (COPh), 155.3, 151.1, 133.3, 129.8 (2 × C), 129.6, 128.5 (2 × C), 118.3 (2 × C), 114.4 (2 × C) (ArC), 100.6 (C-1), 97.9 (C-1′), 75.9, 74.2, 72.5, 70.9, 69.7, 69.4, 69.1, 66.7, 63.1, 55.6 ($C_6H_4OCH_3$), 20.9, 20.8, 20.7 (3 × $COCH_3$), 17.3 (C–CH_3). HRMS calcd for $C_{32}H_{38}O_{15}Na$ $[M+Na]^+$: 685.2108, found: 685.2106.

4.9. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (13)

To a solution of **12** (2.4 g, 3.6 mmol) in dry CH_3CN (2.5 mL), trimethyl orthoacetate (803 μL, 4.68 mmol) was added followed by CSA (50 mg) and the mixture was allowed to stir at room temperature until complete conversion of the starting material was evident by TLC (2:1 *n*-hexane–EtOAc). After 45 min, the solution was neutralised by Et_3N and evaporated in vacuo. The resulting syrupy mass was dissolved in CH_2Cl_2 (30 mL) and washed successively with 1 N HCl (3 × 50 mL) to rearrange the orthoester to corresponding 4-*O*-acetate, followed by wash with aq. $NaHCO_3$ (2 × 50 mL) and brine (50 mL). Organic layer was separated, dried (Na_2SO_4) and evaporated to syrup. The crude product was purified by flash chromatography (4:1 *n*-hexane–EtOAc) to give pure disaccharide acceptor **13** (2.2 g, 87%) as colourless foam. $[\alpha]_D^{25}$ +93 (*c* 1.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.04–7.44 (m, 5H, ArH), 6.97 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.70 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.38 (br d, 1H, $J_{3,4}$ 1.5 Hz, H-4), 5.35 (dd, 1H, $J_{1',2'}$ 2.0 Hz, $J_{2',3'}$ 3.5 Hz, H-2′), 5.30 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1′), 5.23 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.09 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 4.88 (d, 1H, $J_{1,2}$ 7.0 Hz, H-1), 4.45 (dd, 1H, $J_{5,6a}$ 11.5 Hz, $J_{6a,6b}$12.5 Hz, H-6a), 4.39 (dd, 1H, $J_{5,6b}$ 5.5 Hz, $J_{6a,6b}$12.5 Hz, H-6b), 4.35 (m, 1H, H-5′), 4.08–4.03 (m, 3H, H-2, H-3, H-5), 3.73 (s, 3H, $C_6H_4OCH_3$), 3.03 (br s, 1H, O*H*), 2.19, 2.12, 2.02, 1.97 (4s, 12H, 4 × $COCH_3$), 1.25 (d, 3H, *J* 6.0 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.4, 170.3 (2 × C), 170.0 (4 × $COCH_3$), 166.0 (COPh), 155.4, 151.0, 133.3, 129.7 (2 × C), 129.5, 128.5 (2 × C), 118.2 (2 × C), 114.5 (2 × C) (ArC), 100.5 (C-1), 98.1 (C-1′), 75.3, 73.3, 71.1, 70.9, 70.2, 69.6, 69.3, 66.6, 62.4, 55.6 ($C_6H_4OCH_3$), 20.9, 20.8 (2 × C), 20.7 (4 × $COCH_3$), 17.3 (C–CH_3). HRMS calcd for $C_{34}H_{40}O_{16}Na$ $(M+Na)^+$: 727.2214, found: 727.2211.

4.10. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzoyl-β-D-galactopyranoside (15)

A mixture of compound **13** (2 g, 2.8 mmol), compound **14** (2.2 g, 4.5 mmol) and MS 4 Å (2 g) in dry CH_2Cl_2 (25 mL) was stirred under nitrogen for 1 h. The reaction mixture was cooled to −10 °C followed by addition of H_2SO_4-silica (40 mg). The mixture was allowed to stir at 0 °C for 30 min. when TLC showed complete consumption of the acceptor **13**. The mixture was filtered through a pad of Celite and neutralised with Et_3N. The filtrate was diluted with CH_2Cl_2 (20 mL) and washed with H_2O. The organic layer was collected, dried (Na_2SO_4) and evaporated in vacuo. The residue was purified by flash chromatography using *n*-hexane–EtOAc (2:1) to afford pure compound **15** (2.3 g, 81%). $[\alpha]_D^{25}$ +81 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.06–7.45 (m, 5H, ArH), 6.96 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.66 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.43 (br d, 1H, $J_{3,4}$ 1.5 Hz, H-4), 5.32 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.5 Hz, H-2′), 5.26 (t, 1H, $J_{2'',3''}$, $J_{3'',4''}$ 9.5 Hz, H-3″), 5.23 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.16 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 5.13 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 10.0 Hz, H-4′), 5.10 (t, 1H, $J_{3'',4''}$, $J_{4'',5''}$ 9.5 Hz, H-4″), 4.89 (t, 1H, $J_{1'',2''}$ 7.5 Hz, $J_{2'',3''}$ 9.5 Hz, H-2″), 4.84 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.78 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.41 (m, 2H, H-6a, H-6b), 4.37 (dd, 1H, $J_{5'',6a''}$ 2.5 Hz, $J_{6a'',6b''}$12.0 Hz, H-6a″), 4.34 (m, 1H, H-5′), 4.12–4.06 (m, 4H, H-2, H-4, H-5, H-6b″), 3.72 (m, 1H, H-5″), 3.71 (s, 3H, $C_6H_4OCH_3$), 2.19, 2.15, 2.09, 2.04, 2.02, 2.01, 2.00, 1.97 (8s, 24H, 8 × $COCH_3$), 1.18 (d, 3H, *J* 6.5 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.8, 170.3 (2 × C), 170.1, 170.0 (2 × C), 169.4, 168.9 (8 × $COCH_3$), 155.5, 151.0, 133.2, 129.8 (2 × C), 129.7, 128.5 (2 × C), 118.2 (2 × C), 114.5 (2 × C) (ArC), 100.6 (C-1), 99.4 (C-

1″), 97.5 (C-1′), 77.8, 74.1, 72.3, 72.1, 71.8, 71.5, 70.7, 69.6, 69.3, 68.9, 68.3, 67.0, 62.7, 61.0, 55.6 ($C_6H_6OCH_3$), 20.8 (2 × C), 20.7 (2 × C), 20.6, 20.5 (2 × C), 20.4 (8 × $COCH_3$), 17.2 (C–CH_3). HRMS calcd for $C_{48}H_{58}O_{25}Na$ $(M+Na)^+$: 1057.3165, found: 1057.3163.

4.11. *p*-Methoxyphenyl α-L-rhamnopyranosyl-(1→2)-3-*O*-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (2)

To a solution of protected trisaccharide **15** (1 g, 1.0 mmol) in dry MeOH (15 mL), NaOMe (0.5 M in MeOH, 1.5 mL) was added and the solution was stirred at room temperature for 4 h. The solution was neutralised with DOWEX 50 W H^+ resin and filtered through a cotton plug. The filtrate was evaporated and washed with CH_2Cl_2 (5 ml) removing any impurity present to afford compound **2** (475 mg, 83%). $[\alpha]_D^{25}$ +91 (*c* 0.9, H_2O). ^1H NMR (500 MHz, D_2O) δ: 6.95 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 6.84 (d, 2H, *J* 9.0 Hz, C_6H_4OMe), 5.05 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 5.04 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.52 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.11 (br d, 1H, $J_{3,4}$ 3.0 Hz, H-4), 3.96 (m, 1H, H-5′), 3.93 (dd, 1H, $J_{1',2'}$ 1.5 Hz, $J_{2',3'}$ 3.0 Hz, H-2′), 3.88 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.0 Hz, H-3), 3.86–3.74 (m, 3H, H-2, H-3′, H-4′), 3.65 (s 3H, $C_6H_4OCH_3$), 3.64–3.55 (m, 4H, H-5, H-6a, H-6b, H-6a″), 3.39–3.21 (m, 5H, H-2″, H-3″, H-4″, H-5″, H-6b′), 1.05 (d, 3H, *J* 6.0 Hz, C–CH_3). ^{13}C NMR (125 MHz, D_2O) δ: 154.6, 150.4, 117.6 (2 × C), 115.1 (2 × C) (ArC), 104.1 (C-1″), 101.2 (C-1), 99.3 (C-1′), 82.9, 75.9, 75.8, 75.7, 74.8, 73.1, 71.9, 70.2 (2 × C), 69.4, 68.9, 68.6, 60.6, 60.5, 55.8 ($C_6H_6OCH_3$), 16.6 (C–CH_3). HRMS calcd for $C_{25}H_{38}O_{16}Na$ $(M+Na)^+$: 617.2058, found: 617.2061.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.08.004.

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