Tetrahedron: *Asymmetry* 21 (2010) 2413–2418

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Synthesis of a tetrasaccharide related to the triterpenoid saponin isolated from *Schima noronhae*

Priya Verma, Ritu Raj, Bimalendu Roy [†], Balaram Mukhopadhyay [*]

Indian Institute of Science Education and Research-Kolkata (IISER-K), Mohanpur Campus, PO BCKV Campus Main Office, Mohanpur, Nadia 741252, India

ARTICLE INFO

Article history:
Received 4 August 2010
Accepted 16 September 2010
Available online 18 October 2010

ABSTRACT

A concise synthesis of a tetrasaccharide related to the cell-growth inhibitory triterpenoid saponin isolated from *Schima noronhae* is reported. A late stage 2,2,6,6-tetramethylpiperidinyloxy (TEMPO)-mediated oxidation of a primary hydroxyl group to carboxylic acid has been achieved under phase-transfer conditions. Stereoselective glycosylations were carried out using thioglycoside or glycosyl trichloroacetimidate activation using sulfuric acid immobilized on silica (H_2SO_4–silica) in conjunction with *N*-iodosuccinimide and alone, respectively.

1. Introduction

Saponins are glycosylated secondary metabolites synthesized by plants during their routine programme of growth and development.[1] They posses intense antifungal properties and are thus believed to act as the natural chemical barriers in plants against fungal attack.[2] In addition to their antifungal properties, saponins have been exploited as food crops, for example, legumes and oats or sources of drugs, for example, ginseng and liquorice making them commercially attractive for diverse reasons.[3] However the details of the genetic machinery involved in the biosynthesis of these secondary metabolites have not yet been characterized. Despite the architectural diversity amongst the saponin structures isolated from different sources, one common feature that is shared by all saponins is the presence of a sugar chain attached to the 3-position of the aglycon moiety. These sugar chains are often branched and usually consist up to five sugar units. Normally the sugar units are selected from glucose, galactose, arabinose, xylose, rhamnose and glucuronic acid.[4] It is believed that the glycosylation of the aglycon moiety occurs in the final stage of the saponin biosynthesis and the biological function of the saponin depends largely on the glycosylation pattern. Therefore, it is essential to elucidate the exact biosynthesis of the oligosaccharide chain and identify the enzymes involved in the whole process to realize the source of saponin bioactivity.[5] For a better understanding of the glycosyltransferases involved and the order of events in the biosynthesis of saponins, the chemical synthesis of different sugar substrates will be useful.

Schima noronhae Reinw. ex Blume is a commonly grown plant in Southeast Asia and known as 'iju' in Japan.[6] The plant is used as a source of timber and the leaves as fodder crops. They are also used in traditional medicines in Indonesia and Malaysia, for example, the astringent corollas are used to treat uterine disorders and hysteria and also as an ointment to alleviate the symptoms of smallpox.[7] Recently, Ishibashi et al. reported two new triterpenoid saponins (Fig. 1) isolated from *S. noronhae* that showed potent cell-growth inhibitory activity against human cervical carcinoma cells (HeLa cells) and human colon carcinoma DLD1 cells.[8] Herein we report the chemical synthesis of the tetrasaccharide side chain **1** (Fig. 1) of the triterpenoid saponins isolated from *S. noronhae* in the form of its propargyl glycoside.

2. Results and discussion

Careful consideration of the retrosynthetic analysis suggested that a 2+2 strategy would be best suited for the construction of the target tetrasaccharide. Rational protecting group manipulations on the commercially available monosaccharides followed by stereoselective glycosylations were planned to prepare the required disaccharide synthons. Further coupling would result in the formation of the tetrasaccharide framework. A late stage oxidation would furnish the required carboxylic acid moiety (Fig. 2). The choice of propargyl glycoside at the reducing end opens up the possibility of further glycoconjugate formation through click chemistry or other metal catalyzed multi-component reactions.

The synthesis of the disaccharide donor **8** was started from the known *p*-methoxyphenyl β-D-galactopyranoside **2**.[9] The formation of the 4,6-benzylidene acetal using benzaldeylde dimethylacetal in the presence of catalytic CSA in acetonitrile[10] followed by

* Corresponding author. Tel.: +91 33 64513294; fax: +91 33 25873020.
E-mail address: sugarnet73@hotmail.com (B. Mukhopadhyay).
† Present address: Unité de Recherches en Biocatalyse (FRE-CNRS 2230), Faculté des Sciences et des Techniques, 2, rue de la Houssinière, B. P. 92208, 44322 Nantes cedex 3, France.

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doi:10.1016/j.tetasy.2010.09.002

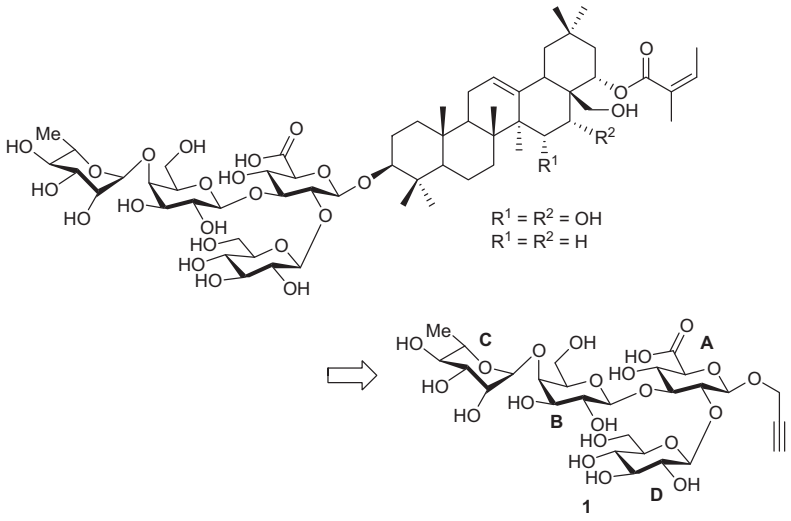


Figure 1. Structure of the triterpenoid and synthetic target.

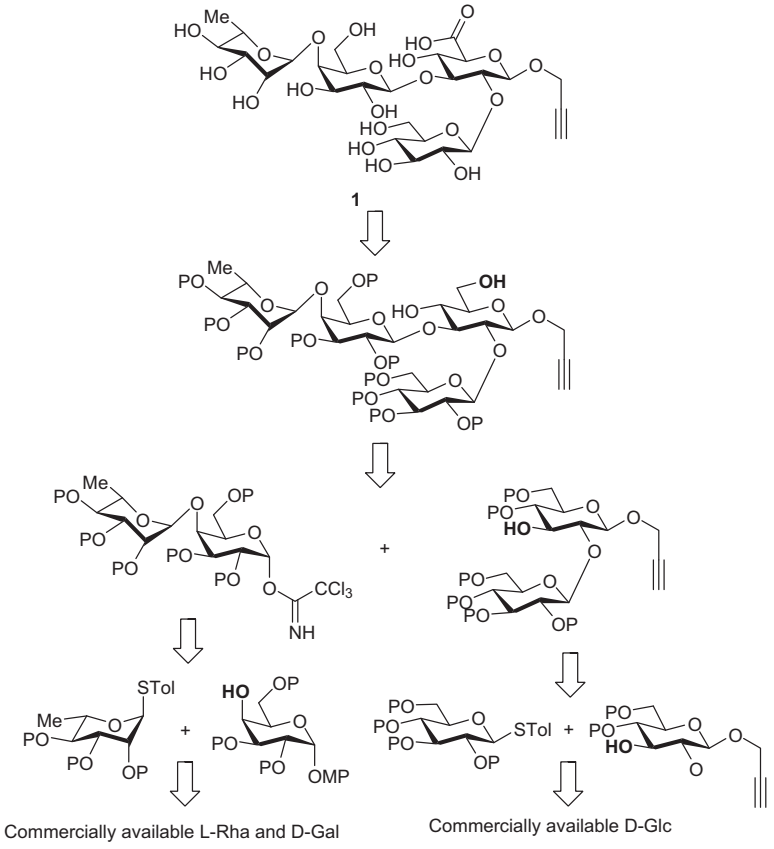
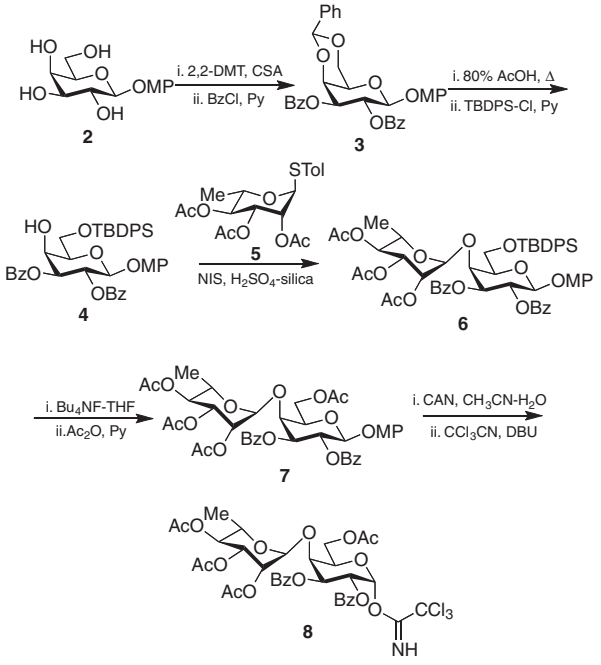


Figure 2. Retrosynthetic analysis of the target oligosaccharides.

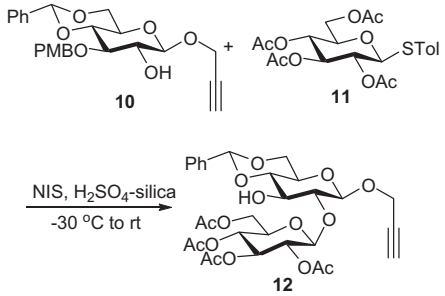
treatment with benzoyl chloride and pyridine in dichloromethane[11] afforded *p*-methoxyphenyl 4,6-*O*-benzylidene-2,3 di-*O*-benzoyl-β-D-galactopyranoside **3** in 81% yield. Hydrolysis of the benzylidene acetal using 80% AcOH at 80 °C[12] followed by selective protection of the primary hydroxyl group using TBDPS-Cl in pyridine[13] afforded the acceptor, *p*-methoxyphenyl 2,3 di-*O*-benzoyl 6-*O*-*tert*-butyldiphenylsilyl β-D-galactopyranoside **4** in 87% yield. Acceptor **4** was then glycosylated with the known donor, *p*-tolyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside **5**[14] using NIS in the presence of H_2SO_4–silica to furnish the disaccharide **6** in 85% yield.[15] Our previous experience with the oxidative cleavage of the *p*-methoxyphenyl group revealed that the OTBDPS moiety is not compatible with this transformation. Therefore, the TBDPS moiety was removed using Bu_4NF-THF[16] to afford compound **7** in 87% yield and subsequently acetylated using Ac_2O in pyridine to afford the disaccharide **8** in 93% yield. Next, CAN-mediated oxidative

cleavage[17] of the *p*-methoxyphenyl group followed by reaction with trichloroacetonitrile in the presence of DBU[18] furnished the required disaccharide donor, 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate **9** in 81% yield (Scheme 1).

In a separate experiment, a known propargyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **10**[19] was coupled with a known, *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **11**[14] using NIS in the presence of H_2SO_4–silica at −30 °C. Once the completion of the glycosylation was confirmed by TLC (*n*-hexane–EtOAc, 3:1), a slight excess of H_2SO_4–silica was added and the reaction was allowed to warm up to room temperature. The acid-labile *p*-methoxybenzyl group was hydrolysed[19] to furnish the required disaccharide acceptor, propargyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-β-D-glucopyranoside **12** in 83% overall yield (Scheme 2).

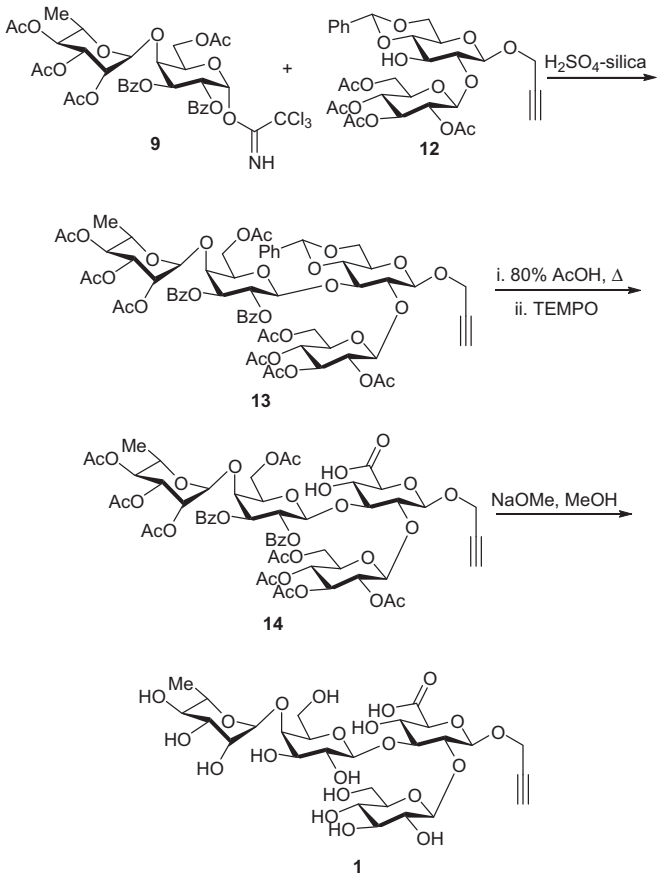


Scheme 1. Synthesis of the disaccharide donor.



Scheme 2. Synthesis of the disaccharide acceptor.

Final glycosylation between disaccharide donor **9** and the disaccharide acceptor **12** using H_2SO_4–silica at 0 °C afforded the tetrasaccharide **13** in 87% yield. Hydrolysis of the benzylidene acetal using 80% AcOH at 80 °C followed by TEMPO oxidation[20] installed the required carboxylic acid moiety **14** in 78% overall yield. Finally, Zemplén de-O-acylation[21] using NaOMe in MeOH furnished the target tetrasaccharide **1** in 89% yield (Scheme 3).



Scheme 3. Synthesis of the tetrasaccharide, oxidation and deprotection.

3. Conclusion

In conclusion, we have accomplished the total synthesis of the tetrasaccharide glycone part of the cell-growth inhibitory triterpenoid saponin isolated from *S. noronhae* as its propargyl glycoside. The propargyl glycoside at the reducing end will provide scope in making various types of glycoconjugates to assess the biological activity of the oligosaccharide in question.

4. Experimental

4.1. Preparation of H_2SO_4–silica

To a slurry of silica gel (10 g, 200–400 mesh) in dry diethyl ether (50 mL) was added commercially available concentrated H_2SO_4 (1 mL), and the slurry was shaken for 5 min. The solvent was evaporated under reduced pressure, resulting in free flowing H_2SO_4–silica, which was dried at 110 °C for 3 h and then used for the reactions.

4.2. *p*-Methoxyphenyl 6-*O*-(*tert*-butyldiphenylsilyl)-2,3-di-*O*-benzoyl-β-D-galactopyranoside 4

To the mixture of known *p*-methoxyphenyl-β-D-galactopyranoside **2** (3.0 g, 10.4 mmol) in dry acetonitrile (25 mL) was added benzaldehydedimethyl acetal (2.4 mL, 15.8 mmol) followed by catalytic amount of CSA (pH: 3–4) at room temperature and the reaction mixture was allowed to stir for 30 min. After completion of the reaction as shown by TLC (CH_2Cl_2–MeOH; 15:1), the solution was neutralized with Et_3N and the solvent was evaporated in vacuo to afford *p*-methoxyphenyl 4,6-*O*-benzylidene-β-D-galactopyranoside as a

crude product. To a slurry of this product in dry dichloromethane (30 mL) was added pyridine (3.4 mL, 41 mmol) and the mixture was placed in an ice bath. To the cold suspension was added benzoyl chloride (3.65 mL, 31.4 mmol); the exothermic reaction started immediately; the reaction mixture was allowed to stir at 0 °C for 30 min and then the temperature was raised to room temperature. The mixture was further allowed to stir for 3 h when TLC (*n*-hexane–EtOAc; 4:1) showed complete conversion of the starting material. The solvents were evaporated in vacuo and the residue was dissolved in dichloromethane (40 mL) and washed successively with H_2O (2 × 50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated to a syrup. The crude compound was purified by flash chromatography using *n*-hexane–EtOAc (4:1) to afford pure compound 3[22] (4.9 g, 81%) as a white solid compound. $[\alpha]_D^{25} = +107$ (*c* 1.2, $CHCl_3$). HRMS calcd for $C_{34}H_{30}O_9Na$ $(M+Na)^+$: 605.1788; found 605.1785.

A solution of compound 3 (3.0 g, 5.1 mmol) in AcOH–H_2O (9:1, 30 mL) was stirred at 85 °C for 2 h when TLC showed complete conversion of the starting material to a slower moving spot. The solvents were evaporated in vacuo and the residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to afford a pure diol (2.4 g, 93%) as a white solid. To a solution of the diol[23] (2.4 g, 4.8 mmol) in dry pyridine (25 mL) was added TBDPS-Cl (1.8 mL, 7.15 mmol) and the solution was stirred for 12 h at room temperature. Solvents were evaporated in vacuo and the residual syrup was purified by flash chromatography using *n*-hexane–EtOAc (5:1) as eluent to afford pure compound **4** (3.0 g, 87%) as a white foam. $[\alpha]_D^{25} = +107$ (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.03 (2d, 4H, *J* 8.6 Hz, COC_6H_5), 7.76–7.37 (m, 16H, ArH), 7.01, 6.76 (2d, 4H, *J* 8.7 Hz, $C_6H_4OCH_3$), 6.05 (dd, 1H, *J* 9.3, 7.9 Hz, H-2), 5.40 (dd, 1H, *J* 9.3, 3.1 Hz, H-3), 5.15 (d, 1H, *J* 7.9 Hz, H-1), 4.51 (br s, 1H, H-4), 4.06–3.88 (m, 3H, H-5, H-6^a, H-6^b), 3.76 (s, 3H, $C_6H_4OCH_3$), 2.98 (br s, 1H, OH), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 166.0, 165.4 (COC_6H_5), 155.5, 151.4, 135.7(2), 135.6(2), 133.4, 133.1, 132.8, 132.5, 132.4, 129.9(3), 129.7(2), 129.5, 129.1(2), 128.4(2), 128.3(2), 127.8(3), 118.9(2), 114.4(2) (ArC), 101.3 (C-1), 74.5, 74.4, 69.6, 68.2, 63.6 (C-6), 55.6 ($C_6H_4OCH_3$), 26.8(3) ($C(CH_3)_3$), 19.1 ($C(CH_3)_3$). HRMS calcd for $C_{43}H_{49}O_9SiNa$ $(M+Na)^+$: 755.2653; found 755.2650.

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

A mixture of the acceptor **4** (1.3 g, 1.8 mmol), donor **5** (925 mg, 2.3 mmol) and MS 4 Å (2.0 g) in dry CH_2Cl_2 (25 mL) was stirred under an N_2 atmosphere for 30 min. Next, NIS (675 mg, 3.0 mmol) was added followed by H_2SO_4–silica (50 mg) and the mixture was allowed to stir at rt for 45 min when TLC (*n*-hexane–EtOAc, 3:1) showed complete conversion of the acceptor. The mixture was filtered through a pad of Celite® and the filtrate was washed successively with aq $Na_2S_2O_3$ (2 × 30 mL), saturated aq $NaHCO_3$ (2 × 30 mL) and H_2O (30 mL). The organic layer was collected, dried (Na_2SO_4) and filtered. The solvents were evaporated in vacuo and the residue was purified by flash chromatography using *n*-hexane–EtOAc (4:1) as eluent to afford pure disaccharide **6** (1.5 g, 85%) as a colourless gel. $[\alpha]_D^{25} = +97$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.94 (d, 4H, *J* 8.0 Hz, ArH), 7.70 (m, 4H, ArH), 7.51–7.32 (m, 12H, ArH), 6.96, 6.70 (2d, 4H, ArH), 5.94 (dd, 1H, $J_{3',4'}$ 9.5 Hz, $J_{4',5'}$ 10.0 Hz, H-4′), 5.46 (dd, 1H, $J_{2',3'}$ 1.5 Hz, $J_{3',4'}$ 9.5 Hz, H-3′), 5.44 (m, 1H, H-2′), 5.35 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.12 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 4.89 (t, 1H, $J_{1,2}$, $J_{2,3}$ 9.5 Hz, H-2), 4.85 (br s, 1H, H-1′), 4.35 (br s, 1H, H-4), 4.08 (dd, 1H, $J_{5,6a}$ 8.0 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.89–3.46 (m, 2H, H-5′, H-6b), 3.74 (m, 1H, H-5), 3.71 (s, 3H, CH_2–C_6H_5–OCH_3), 2.02, 1.98, 1.94 (3s, 9H, 3 × $COCH_3$), 0.86 (d, 3H, $J_{5',6'}$ 6.0 Hz, C–CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 169.9, 165.7, 164.9 (3 × $COCH_3$), 165.7, 164.9 (2 × COPh), 155.3, 151.3, 135.6(2), 135.5(2), 133.4, 133.0, 132.9, 132.8, 130.0(2), 129.9, 129.8, 129.7(2), 129.4, 128.5(2), 128.4, 128.2(2), 127.8(3), 127.7, 118.3(2), 114.4(2), 100.8 (C-1), 99.2 (C-1′), 75.6, 74.2, 73.8, 70.9, 70.3, 70.2, 69.6, 68.8, 67.3, 63.2 (C-6), 55.5 (CH_2–C_6H_5–OCH_3), 26.7 [$C(CH_3)_3$], 20.9, 20.8, 20.7 (3 × $COCH_3$), 17.4 (C–CH_3). HRMS calcd for $C_{55}H_{60}O_{16}SiNa$ $(M+Na)^+$: 1027.3548; found 1027.3542.

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

To a solution of compound **6** (1.4 g, 1.4 mmol) in dry THF (10 mL), Bu_4NF in THF (2.8 mL, 2.8 mmol) was added and the solution was allowed to stir at rt for 6 h. Then the solvents were evaporated to dryness. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (2:1) to afford pure compound **7** (930 mg, 87%) as a light yellow syrup. $[\alpha]_D^{25} = +85$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.93–7.31 (m, 10H, ArH), 6.93, 6.75 (2d, 4H, ArH), 5.94 (dd, 1H, $J_{3',4'}$ 8.0 Hz, $J_{4',5'}$ 10.5 Hz, H-4′), 5.48 (dd, 1H, $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 8.0 Hz, H-3′), 5.47 (m, 1H, H-2′), 5.38 (dd, 1H, $J_{2,3}$ 9.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 5.19 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.97 (t, 1H, $J_{1,2}$, $J_{2,3}$ 8.5 Hz, H-2), 4.43 (d, 1H, $J_{1',2'}$ 3.0 Hz, H-1′), 4.02–3.97 (m, 3H, H-5′, H-6a, H-6b), 3.81 (m, 1H, H-5), 2.05, 1.95, 1.94 (3 × $COCH_3$), 1.20 (d, 3H, $J_{5',6'}$ 6.5 Hz, C–CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.0, 169.6, 169.3 (3 × $COCH_3$), 165.7, 165.0 (2 × COPh), 155.5, 151.0, 133.5, 133.1, 129.9(2), 129.7(2), 129.3, 128.5(2), 128.4, 128.3(2), 118.5(2), 114.5(2) (ArC), 100.9 (C-1), 99.4 (C-1′), 74.8, 74.1, 73.6, 70.9, 69.5, 69.4, 68.9, 68.0, 61.2 (C-6), 55.5 ($OC_6H_5OCH_3$), 20.7, 20.6, 20.5 (3 × $COCH_3$), 17.3 (C–CH_3).

4.5. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranoside 8

Compound **7** (900 mg, 1.2 mmol) was dissolved in pyridine (8 mL) followed by the addition of Ac_2O (4 mL) and the solution was allowed to stir at rt for 1 h. The solvents were evaporated and the residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to give pure disaccharide **8** (880 mg, 93%) as a white foam. $[\alpha]_D^{25} = +108$ (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ: 7.97–7.34 (m, 10H, Ar*H*), 6.96, 6.78 (2d, *J* 9.0 Hz, OC_6H_4OMe), 5.95 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.5 Hz, H-2), 5.49 (dd, 1H, $J_{1',2'}$ 2.5 Hz, $J_{2',3'}$ 3.0 Hz, H-2′), 5.45 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.39 (dd, 1H, $J_{2',3'}$ 3.5 Hz, $J_{3',4'}$ 8.5 Hz, H-3′), 5.14 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.99 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 8.5 Hz, H-4′), 4.88 (d, 1H, $J_{1',2'}$ 2.5 Hz, H-1′), 4.47 (dd, 1H, $J_{5,6a}$ 6.5 Hz, $J_{6a,6b}$ 11.0 Hz, H-6a), 4.38 (br d, 1H, H-4), 4.26 (dd, 1H, $J_{5,6b}$ 6.0 Hz, $J_{6a,6b}$ 11.5 Hz, H-6b), 4.07 (bt, 1H, H-5), 4.02 (m, 1H, H-5′), 3.75 (s, 3H, $C_4H_4OCH_3$), 2.11, 2.06, 1.95, 1.90 (4s, 12H, 4 × $COCH_3$), 1.20 (d, 3H, $J_{5',6'}$ 6.0 Hz, C–CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.4, 170.0, 169.5, 169.3 (4 × $COCH_3$), 165.7, 164.9 (2 × COC_6H_5), 155.6, 151.1, 133.6, 133.1, 130(2), 129.7(2), 129.3, 128.6(2), 128.3(2), 118.8(2), 114.4(2) (ArC), 101.1 (C-1), 99.6 (C-1′), 74.3, 73.5, 71.9, 70.9, 69.6, 69.3, 68.9, 67.7, 62.5, 55.6 ($C_6H_4OCH_3$), 20.8, 20.7, 20.6(2) (4 × $COCH_3$), 17.3 (C–CH_3). HRMS calcd for $C_{41}H_{44}O_{17}Na$ $(M+Na)^+$: 831.2476; found 831.2474.

4.6. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate 9

To a solution of compound **8** (850 mg, 1.1 mmol) in CH_3CN–H_2O (9:1, 20 mL), CAN (1.2 g, 2.2 mmol) was added and the solution was stirred at room temperature for 30 min after which TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the starting

material to a slower moving spot. Solvents were evaporated in vacuo, and the residue was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (2 × 20 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated to a syrup. The crude hemiacetal thus obtained was dissolved in dry CH_2Cl_2 (20 mL), after which CCl_3CN (550 μL, mmol) was added followed by DBU (mL, mmol) and the solution was stirred at room temperature for 2 h when TLC (*n*-hexane–EtOAc, 3:1) showed complete conversion of the starting material to a faster moving spot. The solvents were evaporated in vacuo and the residue was purified by flash chromatography using 4:1 (*n*-hexane–EtOAc) as eluent to afford pure trichloroacetimidate derivative **9** (755 mg, 81%) as a colourless gel. The material was used directly for further glycosylation.

4.7. Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-4,6-*O*-benzylidene-β-D-glucopyranoside 12

A mixture of acceptor **10** (1.3 g, 3.0 mmol), donor **11** (1.8 g, 3.9 mmol) and MS 4 Å (g) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen for 30 min at −30 °C. Next, NIS (1.1 g, 5.1 mmol) was added followed by H_2SO_4–silica (50 mg) and the mixture was stirred for another 45 min when TLC (*n*-hexane–EtOAc, 3:1) showed complete consumption of the acceptor. Then the temperature was slowly raised to rt (1 h) to cleave the *p*-methoxybenzyl group. The mixture was filtered through a pad of Celite® and the filtrate was washed successively with aq $Na_2S_2O_3$ (2 × 25 mL), saturated aq $NaHCO_3$ (2 × 25 mL) and H_2O (25 mL). The organic layer was collected, dried (Na_2SO_4) and filtered. The solvents were evaporated in vacuo and the residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) as an eluent to afford pure disaccharide **12** (1.6 g, 83%) as a white foam. $[\alpha]_D^{25} = +98$ (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.46–7.33 (m, 5H, Ar*H*), 5.52 (s, 1H, C*H*Ph), 5.21 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.5 Hz, H-3′), 5.11 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4′), 5.03 (dd, 1H, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 9.5 Hz, H-2′), 4.86 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.68 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.46, 4.34 (2dd, *J* 2.5 Hz, 15.0 Hz, O–CH_2–C≡CH), 4.32 (m, 1H, H-6^a), 4.27 (dd, 1H, $J_{5',6a'}$ 3.0 Hz, $J_{6a',6b'}$ 11.5 Hz, H-6a′), 4.21 (dd, 1H, $J_{5',6b'}$ 4.5 Hz, $J_{6a',6b'}$ 11.5 Hz, H-6b′), 3.91 (m, 1H, H-3), 3.78 (m, 1H, H-5′), 3.75 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 3.53–3.44 (m, 3H, H-2, H-5, H-6b), 3.14 (br d, 1H, $J_{2,OH}$ 2.5 Hz, O*H*), 2.51 (t, 1H, *J* 2.5 Hz, O–CH_2–C≡C*H*), 2.08, 2.04, 2.02, 1.98 (4s, 12H, 4 × COCH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.7, 170.5, 170.2, 169.4 (4 × *C*OCH$_3$), 136.8, 129.2, 128.2(2), 126.2(2) (Ar*C*), 101.7 (*C*HPh), 101.3 (C-1), 100.9 (C-1′), 83.5, 79.7, 78.4, 75.5, 72.6, 72.0(2), 71.9, 68.5, 68.1, 66.0, 62.0, 53.4, 20.8, 20.7, 20.6, 20.5 (4 × COCH_3). HRMS calcd for $C_{30}H_{36}O_{15}Na$ (M+Na)$^+$: 659.1952; found 659.1957.

4.8. Propargyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside 13

A mixture of donor **9** (750 mg, 0.9 mmol), acceptor **12** (480 mg, 0.75 mmol) and MS 4 Å (1.0 g) in dry CH_2Cl_2 (15 mL) was stirred under N_2 for 30 min. Next, H_2SO_4–silica (30 mg) was added and the solution was stirred for 45 min at 0 °C when TLC (*n*-hexane–EtOAc) showed complete conversion of the acceptor. The mixture was neutralized with Et_3N and filtered through a pad of Celite®. The filtrate was evaporated and the crude product was purified by flash chromatography using *n*-hexane–EtOAc (4:1) to afford pure tetrasaccharide **13** (865 mg, 87%) as a white foam. $[\alpha]_D^{25} = +79$ (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 8.05–7.32 (m, 15H, Ar*H*), 5.68 (dd, 1H, $J_{3'',4''}$ 9.5 Hz, $J_{4'',5''}$ 10.0 Hz, H-4″), 5.59 (s, 1H, C*H*Ph), 5.49 (m, 1H, H-2″), 5.32 (m, 3H, H-2″, H-3′, H-3″), 5.20 (t, 1H, $J_{3''',4'''}$, $J_{4''',5'''}$ 9.5 Hz, H-4‴), 5.08 (m, 2H, H-2‴, H-3‴), 4.94 (t, 1H, $J_{1',2'}$, $J_{2',3'}$ 8.5 Hz, H-2′), 4.87 (br s, 1H, H-1″), 4.86 (d, 1H, $J_{1'',2''}$ 9.0 Hz, H-1″), 4.85 (d, 1H, $J_{1''',2'''}$ 9.0 Hz, H-1‴), 4.54 (d, 1H, $J_{1,2}$ 7.0 Hz, H-1), 4.33 (m, 2H, OCH_2C≡CH), 4.31 (br d, 1H, H-4′), 4.30–3.90 (m, 8H, H-3, H-5, H-6a, H-6b, H-6a′, H-6b′, H-6a‴, H-6b‴), 3.76 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.0 Hz, H-4), 3.72 (m, 1H, H-5‴), 3.54 (m, 1H, H-5″), 3.42 (m, 1H, H-5′), 2.43 (t, 1H, *J* 1.5 Hz, OCH$_2$C≡C*H*), 2.10, 2.07, 2.06(2), 2.00, 1.98, 1.94, 1.93 (8 × COCH_3), 1.15 (d, 3H, *J* 6.5 Hz, C–CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.8, 170.2, 170.1, 170.0, 169.6, 169.5, 169.4, 169.3 (8 × *C*OCH$_3$), 165.8, 164.9 (2 × *C*OPh), 137.1, 133.6, 133.2, 130.1(2), 129.9(2), 129.4, 129.2, 128.5(2), 128.4(2), 128.3(2), 128.2, 126.0(2) (Ar*C*), 101.3 (*C*HPh), 100.11 (C-1‴), 99.8 (C-1′), 99.2 (C-1), 98.9 (C-1″), 80.8, 79.5, 78.6,77.5, 75.1, 73.9, 73.5, 73.0, 71.9, 71.8, 71.4, 71.0, 70.3, 69.5, 69.1, 68.8, 68.4, 67.7, 65.5, 62.6, 61.8, 60.4, 56.3, 29.7, 21.0, 20.8, 20.7(2), 20.6(2), 20.5, 20.3 (8 × COCH_3), 17.4 (C–CH_3). HRMS calcd for $C_{64}H_{72}O_{30}Na$ (M+Na)$^+$: 1343.4006; found 1343.4002.

4.9. Propargyl α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosyl-(1→3)-2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranosiduronic acid 1

A solution of compound **13** (850 mg, 0.65 mmol) in 80% aq AcOH (15 mL) was stirred at 80 °C for 2 h until TLC (*n*-hexane–EtOAc, 3:1) showed complete conversion of the starting material to a slower moving spot. The solvents were evaporated in vacuo and the syrupy residue was dissolved in 15 mL CH_2Cl_2. To this solution, 3 mL of H_2O was added followed by aq NaBr (1 M 0.3 mL), aq tetrabutylammonium bromide (1 M, 0.6 mL), TEMPO (27 mg) and saturated aq $NaHCO_3$ (1.6 mL) at 0 °C. To the resulting mixture, aq NaOCl (2.0 mL) was added and the mixture was allowed to stir for 1.5 h when the temperature was raised to room temperature. At this point TLC showed complete conversion of the starting material to a faster moving spot, presumably the corresponding aldehyde derivative. The mixture was neutralized with 1 M HCl (as required) to keep the pH of the mixture at 6–7. Then *tert*-butanol (9 mL), $NaOCl_2$ (600 mg in 2.3 mL H_2O) and NaH_2PO_4 (750 mg in 6 mL H_2O) were added and the mixture was allowed to stir at room temperature for another 4 h when TLC showed complete conversion. The mixture was diluted with saturated NaH_2PO_4 and the product was extracted with EtOAc. The organic layer was dried (Na_2SO_4) and evaporated. The crude product thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (1:1) to *n*-hexane–EtOAc (1:4) to afford compound **14** (625 mg, 78%). To a solution of compound **14** (300 mg, 0.24 mmol) in dry MeOH (10 mL), NaOMe in MeOH (0.5 M, 1 mL) was added and the solution was stirred at room temperature for 6 h. Excess NaOMe was neutralized with DOWEX 50 W H^+ resin, filtered through a cotton plug and the filtrate was evaporated to give pure target tetrasaccharide **1** (150 mg, 89%) as an amorphous white solid. $[\alpha]_D^{25} = +63$ (*c* 0.7, H_2O). ^{1}H NMR (D_2O, 500 MHz) δ: 5.09 (d, 1H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.74 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.70 (m, 2H, H-1, H-1‴), 4.39 (m, 2H, OCH_2C≡CH), 4.02 (dd, 1H, $J_{1'',2''}$ 1.5 Hz, $J_{2'',3''}$ 3.0 Hz, H-2″), 3.95 (br d, 1H, H-4′), 3.91–3.36 (m, 16H, H-2, H-2′, H-2‴, H-3, H-3′, H-3″, H-3‴, H-4, H-4″, H-5, H-5″, H-5‴, H-6a′, H-6b′, H-6a‴, H-6b‴), 3.34 (t, 1H, $J_{3''',4'''}$, $J_{4''',5'''}$ 8.5 Hz, H-4‴), 3.26 (m, 1H, H-5′), 2.85 (t, 1H, *J* 2.0 Hz, OCH$_2$C≡C*H*), 1.19 (d, 3H, *J* 6.5 Hz, C–CH_3). ^{13}C NMR (D_2O, 125 MHz) δ: 173.0 (COOH), 102.9 (C-1′), 102.4 (C-1‴), 101.8 (C-1), 99.7 (C-1″), 83.0, 79.6, 78.5, 76.7, 76.0, 75.7, 75.6, 75.4, 73.6, 73.3, 71.7, 71.0, 70.2, 70.0, 69.5, 69.3, 61.5, 60.6, 56.9, 48.9, 23.1, 16.5 (C–CH_3). HRMS calcd for $C_{27}H_{42}O_{21}Na$ (M+Na)$^+$: 725.2116; found 725.2113.

Acknowledgements

P.V. is thankful to the UGC, New Delhi, India, R.R. is thankful to the IISER-Kolkata and B.R. is thankful to the CSIR, New Delhi, India

for fellowship. The work is funded by the DST, New Delhi, India through grant SR/S1/OC-67/2009.

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