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Synthesis of a tetrasaccharide related to the triterpenoid saponin Bellisoside isolated from *Bellis perennis* (compositae)

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Dedicated to Professor Sisir Chandra Rakshit, Burdwan University, West Bengal, India

ABSTRACT

A concise synthesis of a tetrasaccharide related to the triterpenoid saponins Bellisoside has been accomplished from commercially available monosaccharides through rational protecting group manipulations and stereoselective glycosylations. For the glycosylation reactions, H_2SO_4–silica has been successfully used as an alternative to conventional Lewis acids such as TfOH or TMSOTf. The target tetrasaccharide has been synthesized in the form of its *p*-methoxyphenyl glycoside which leaves scope for further glyco-conjugate formation through the selective deprotection of *p*-methoxyphenyl glycoside followed by trichloroacetimidate chemistry.

1. Introduction

Triterpenoid saponins are plant secondary metabolites synthesized routinely in the growth and development program in plants. Due to their intense anti-fungal properties, it is believed that this class of molecules act as a chemical barrier against fungal attacks in plants.[1] In addition to their role in the defence mechanism, triterpenoid saponins are used as a source of drugs against various diseases, mainly in the form of traditional medicines. Despite the importance associated with triterpenoid saponins, the actual mechanism of their biosynthesis and the actual roles in various biological events are yet to be fully understood. A common feature shared by all saponins is the presence of an oligosaccharide attached to the aglycone moiety.[2,3] Normally it is 3–4 sugars long and often branched and composed of D-Glc, D-Gal, D-Fuc, L-Rha, L-Ara, D-Xyl, etc.[4] It is believed that the glycosylation takes place in the later stage of the biosynthesis of the saponins and is essential for the bioactivity exerted by the saponins. Therefore, a clear understanding of the glycosylation and the elaboration of the oligosaccharide is needed in order to gain an insight into the saponins' biosynthesis and activity. Recently, Asada et al.[5] reported the isolation and characterization of six new triterpenoid saponins (Bellisosides) from *Bellis perennis* (compositae), the common Daisy. It is reported that the extracts are already being used in traditional medicines for the treatment of rheumatism and as expectorants.[6] Moreover, these saponins have shown cytotoxicity against HL-60 human promyelocytic leukemia cells.[5] In continuation of our efforts toward the synthesis of oligosaccharides related to the biodynamic saponins,[7] we herein report the total synthesis of the tetrasaccharide related to the saponins Bellisoside from commercially available monosaccharides through rational protecting group manipulation and stereoselective glycosylations. For the glycosylations, thioglycosides and glycosyl trichloroacetimidates are used as glycosyl donors and are activated with H_2SO_4 immobilized on silica in conjunction with *N*-iodosuccinimide (for thioglycosides) or alone (for trichloroacetimidates) (see Fig. 1).

2. Results and discussion

Careful consideration of the retrosynthetic analysis revealed that a (2+2) scheme is the best option to construct the target tetrasaccharide **1**. Therefore, a reducing end disaccharide acceptor, α-L-Rha-(1→2)-β-D-Fuc and a suitably activated non-reducing end disaccharide, α-L-Rha-(1→3)-β-D-Xyl were planned. The acceptor disaccharide was further disconnected to a suitably protected L-Rha donor and a D-Fuc acceptor. These two monosaccharide units can be prepared from commercially available L-Rha and D-Fuc through rational protecting group manipulations. Further disconnection of the donor disaccharide revealed that a suitable D-Xyl acceptor is required where the 3-OH position is free. It is difficult to design a protocol for the synthesis of such an acceptor from commercially available D-Xyl. Thus, it was planned that diacetone D-Glc could be utilized to discriminate the 3-OH by suitable protection and eventually converted to a D-Xyl moiety. The other L-Rha donor can be synthesized from commercially available L-Rha (Scheme 1).

The synthesis of the required acceptor disaccharide started with the known *p*-tolyl 2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside[8] **2**. Acetylation of compound **2** using Ac_2O in pyridine[9] afforded the corresponding acetylated derivative **3** in 93% yield. Donor **3** was then glycosylated with the known acceptor,

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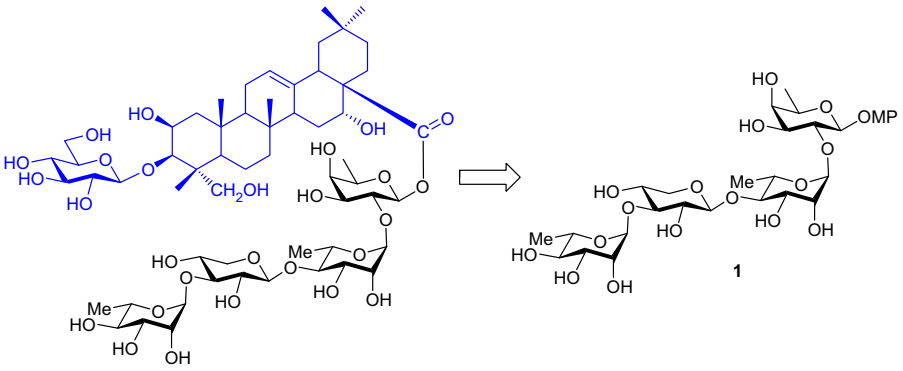
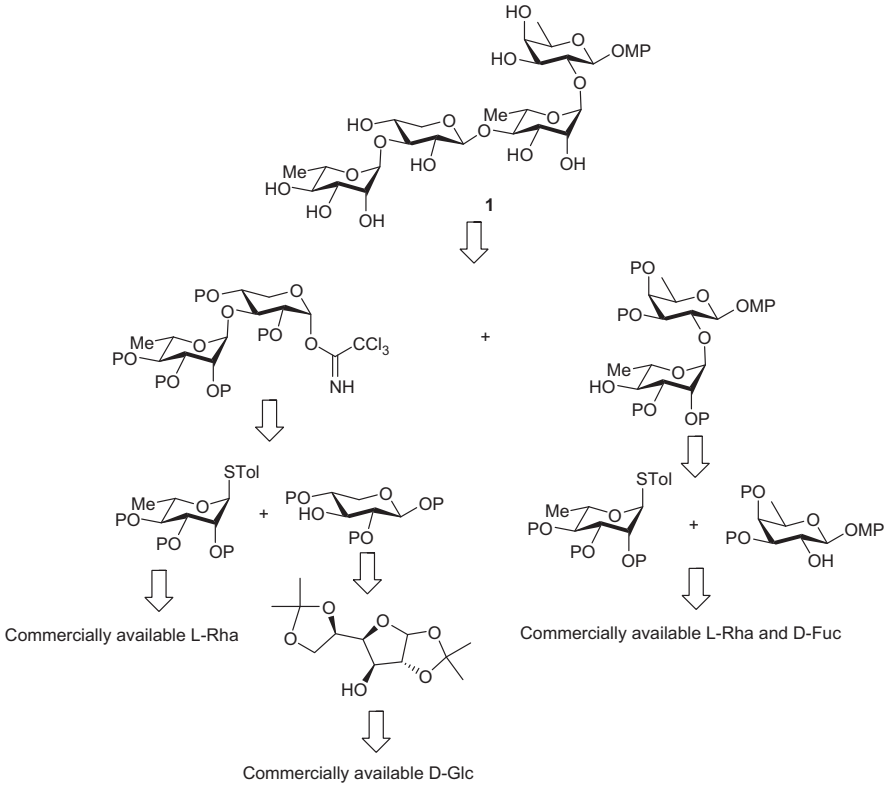


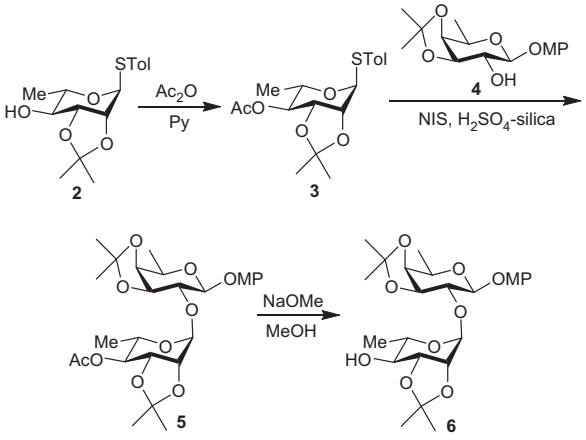
Figure 1. Structure of the triterpenoid saponin and synthetic target.



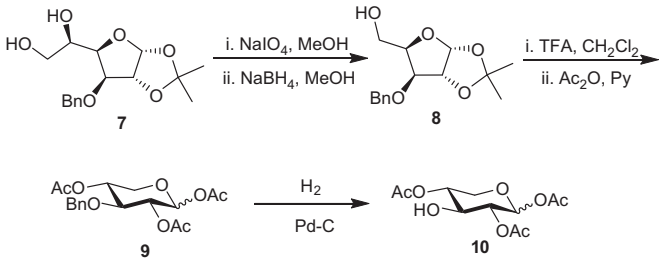
Scheme 1. Retrosynthetic analysis for the target oligosaccharides.

p-methoxyphenyl 3,4-*O*-isopropylidene-β-D-fucopyranoside[7a] **4** using *N*-iodosuccinimide in the presence of H_2SO_4–silica[10] furnishing the disaccharide, *p*-methoxyphenyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranoside **5** in 87% yield. When the same glycosylation reaction between donor **3** and acceptor **4** was carried out using NIS and TMSOTf, only 71% yield was obtained. Moreover, some degradation product was isolated which was proved to be due to the hydrolysis of one of the two isopropylidene groups. Next, Zemplén de-*O*-acetylation[11] using NaOMe in MeOH resulted in the required disaccharide acceptor *p*-methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranoside **6** in 91% yield (Scheme 2).

The synthesis of the required D-Xyl acceptor commenced with the known, 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[12] **7**. Oxidation using sodium periodate in methanol[13] followed by sodium borohydride reduction[14] afforded 3-*O*-benzyl-1,2-*O*-isopro-



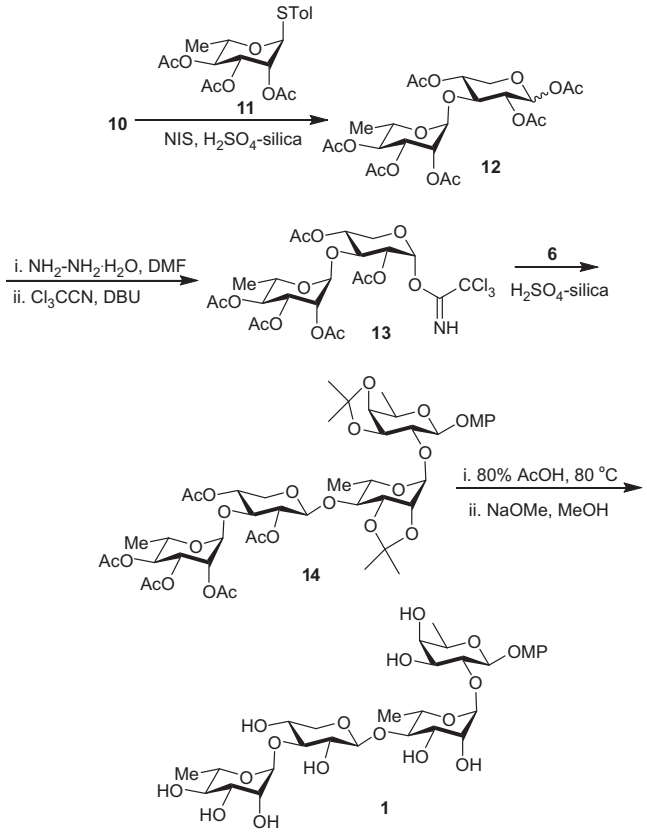
Scheme 2. Synthesis of the disaccharide acceptor **6**.



Scheme 3. Synthesis of the xylosyl acceptor **10**.

pylidene-α-D-xylofuranose **8** in 81% overall yield. The 1,2-isopropylidene acetal was cleaved using 90% aqueous trifluoroacetic acid in CH_2Cl_2 and the resulting compound was subsequently acetylated using Ac_2O in pyridine to furnish 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-D-xylopyranose **9** in 64% yield over two steps as an anomeric mixture. The benzyl group was selectively deprotected using H_2 in the presence of palladium on activated carbon to give the required acceptor, 1,2,4-tri-*O*-acetyl-D-xylopyranose **10** in 75% yield (Scheme 3). The use of TMSOTf instead of H_2SO_4–silica was unsuccessful in producing better results (68% yield).

The xylosyl acceptor **10** was then glycosylated with the known rhamnosyl donor, *p*-tolyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside[15] **11** using *N*-iodosuccinimide in the presence of H_2SO_4–silica to furnish the disaccharide, 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-1,2,4-tri-*O*-acetyl-D-xylopyranose **12** in 76% yield. Next, the anomeric *O*-acetate group was selectively cleaved using hydrazine hydrate and acetic acid[16] in dimethyl formamide and the hemiacetal thus obtained was reacted with trichloroacetonitrile in the presence of DBU[17] to afford the desired disaccharide donor **13** in 71% overall yield. Glycosylation of the disaccharide acceptor **6** with the trichloroacetimidate donor **13** in the presence of H_2SO_4–silica furnished the protected tetrasaccharide **14** in 74% yield. When TMSOTf was used for trichloroacetimidate activation, the yield was only 61% and again degraded products, due to hydrolysis of the isopropylidene acetal, were observed. Hydrolysis of the isopropylidene acetals using 80% acetic acid at 80 °C[18] followed by Zemplén de-*O*-acetylation finally gave the target tetrasaccharide **1** in 80% overall yield (Scheme 4).



Scheme 4. Synthesis of the target tetrasaccharide **1**.

3. Conclusion

In conclusion, the synthesis of the tetrasaccharide glycone part of the triterpenoid saponin Bellisoside has been accomplished in the form of its *p*-methoxyphenyl glycoside. The choice of the glycoside leaves scope for further glyco-conjugate formation by selective cleavage of the *p*-methoxyphenyl glycoside followed by trichloroacetimidate chemistry. Once more, H_2SO_4–silica proved to be a better alternative than the conventional Lewis acids such as TfOH or TMSOTf for the activation of the thioglycoside and trichloroacetimidates. Since the protecting group manipulation strategies and glycosylation steps were selective and high-yielding, the present synthetic strategy is capable of a reasonably large scale preparation.

4. Experimental

4.1. General

All the reagents and solvents were dried prior to use according to standard methods.[19] Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on silica gel 60-F_{254} (Merck or Whatman) 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 cm^3) to an ice-cold solution of 3,5-dihydroxytoluene (360 mg) in EtOH (150 cm^3) and water (10 cm^3), was used to detect the deprotected compounds by charring. Flash chromatography was performed with silica gel 230–400 mesh (Merck, India). Optical rotations were measured at the sodium D-line at ambient temperature, with a Perkin Elmer 141 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer at 500 and 125 MHz, respectively, using Me_4Si or CH_3OH as the internal standards, as appropriate.

4.1.1. *p*-Tolyl 4-*O*-acetyl-2,3-*O*-isopropylidene-1-thio-α-L-rhamnopyranoside 3

To a solution of compound **2** (2.5 g, 8.1 mmol) in dry pyridine (20 mL), Ac_2O (5 mL) was added and the solution was stirred at room temperature for 3 h when TLC (*n*-hexane–EtOAc, 4:1) showed complete conversion of the starting material to a faster moving spot. The solvents were evaporated in vacuo and co-evaporated with toluene to remove residual pyridine. The syrupy residue thus obtained was purified by flash chromatography using *n*-hexane–EtOAc (4:1) as the eluent to afford pure compound **3** (2.6 g, 93%) as a colorless gel. $[\alpha]_D^{25} = +93$ (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36, 7.13 (2d, 4H, S–C_6H_4–CH_3), 5.69 (s, 1H, H-1), 4.93 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{4,5}$ 8.0 Hz, H-4), 4.34 (d, 1H, $J_{2,3}$ 2.5 Hz, H-2), 4.22 (m, 2H, H-3, H-5), 2.34 (s, 3H, S–C_6H_4–CH_3), 2.12 (s, 3H, $COCH_3$), 1.57, 1.25 (2s, 6H, isopropylidene–CH_3), 1.11 (d, 3H, $J_{5,6}$ 6.5 Hz, C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1 ($COCH_3$), 138.1, 132.5(2), 129.9(2), 129.3 (Ar*C*), 110.0 (isopropylidene–C), 84.0 (C-1), 76.5, 75.6, 74.6, 65.5, 27.7, 26.5 (2 × isopropylidene–CH_3), 21.2 (S–C_6H_4–CH_3), 21.1 ($COCH_3$), 16.9 (C–CH_3). HRMS calcd for $C_{18}H_{24}O_5SNa$ $(M+Na)^+$: 375.1242, found: 375.1239.

4.1.2. *p*-Methoxyphenyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranoside 5

A mixture of donor **3** (2.0 g, 5.7 mmol), acceptor **4** (1.4 g, 4.4 mmol) and MS 4 Å (1.5 g) in dry CH_2Cl_2 (20 mL) was stirred under nitrogen for 30 min. NIS (1.7 g, 7.4 mmol) was added followed by H_2SO_4–silica (50 mg) and the mixture was allowed to stir for 45 min at 5–10 °C when TLC (*n*-hexane–EtOAc, 4:1) showed complete conversion of the acceptor. The mixture was filtered through a pad of Celite® and the filtrate was washed successively with aq $Na_2S_2O_7$ (2 × 30 mL), aq $NaHCO_3$ (2 × 30 mL), and brine (30 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was purified by flash chromatography using *n*-hexane–EtOAc (4:1) as the eluent to give pure disaccharide **5** (2.1 g, 87%) as a white foam. $[\alpha]_D^{25} = +83$ (*c* 1.2, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.92, 6.83 (2d, 4H, $OC_6H_4OCH_3$), 5.58 (s, 1H, H-1′), 4.88 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{4,5}$ 8.0 Hz, H-4′), 4.71 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.23 (dd, 1H, $J_{2,3}$ 7.0 Hz, $J_{3,4}$ 4.4 Hz, H-3), 4.16 (d, 1H, $J_{2,3}$ 4.4 Hz, H-2′), 4.10 (m, 2H, H-3′, H-5′), 4.03 (dd, 1H, $J_{3,4}$ 4.4 Hz, $J_{4,5}$ 2.5 Hz, H-4), 4.02 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 7.0 Hz, H-2), 3.95 (m, 1H, H-5), 3.77 (s, 3H, $OC_6H_4OCH_3$), 2.08 (s, 3H, $COCH_3$), 1.57, 1.56, 1.35, 1.34 (4s, 12H, 4 × isopropylidene–CH_3), 1.43 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6), 1.16 (d, 3H, $J_{5',6'}$ 6.0 Hz, H-6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.1 ($COCH_3$), 155.2, 151.4, 118.2(2), 114.5(2) (Ar*C*), 110.2, 109.7 (2 × isopropylidene–*C*), 99.9 (C-1), 96.0 (C-1′), 80.0, 76.3, 76.0, 75.8, 74.6, 74.5, 69.0, 64.0, 55.6 ($OC_6H_4CH_3$), 28.0, 27.7, 26.4(2) (4 × isopropylidene–CH_3), 21.0 ($COCH_3$), 16.8, 16.6 (2 × C–CH_3). HRMS calcd for $C_{27}H_{38}O_{11}Na$ $(M+Na)^+$: 561.2312, found: 561.2308.

4.1.3. *p*-Methoxyphenyl 2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranoside 6

To a solution of disaccharide **5** (1.5 g, 2.8 mmol) in dry MeOH (20 mL) was added NaOMe in MeOH (2 mL, 0.5 M) and the solution was allowed to stir at room temperature for 2 h when TLC (*n*-hexane–EtOAc, 4:1) showed complete conversion of the starting material to a slightly slower moving spot. The solution was neutralized by DOWEX 50 W H^+ resin, filtered through cotton wool, and the filtrate was evaporated in vacuo. The residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) to give pure compound **6** (1.3 g, 91%) as a white foam. $[\alpha]_D^{25} = +102$ (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.91, 6.78 (2d, 4H, Ar*H*), 5.52 (s, 1H, H-1′), 4.69 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.21 (dd, 1H, $J_{2,3}$ 7.0 Hz, $J_{3,4}$ 5.5 Hz, H-3), 4.14 (d, 1H, $J_{2',3'}$ 5.5 Hz, H-2′), 4.02 (m, 4H, H-2, H-3′, H-4, H-5′), 3.92 (m, 1H, H-5), 3.73 (s, 3H, $OC_6H_4OCH_3$), 3.40 (t, 1H, $J_{3',4'}$, $J_{4',5'}$ 8.5 Hz, H-4′), 2.82 (br s, 1H, O*H*), 1.56, 1.51, 1.33, 1.32 (4s, 12H, 4 × isopropylidene–CH_3), 1.41 (d, 3H, $J_{5,6}$ 6.5 Hz, H-6), 1.29 (d, 3H, $J_{5',6'}$ 6.5 Hz, H-6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.1, 151.3, 118.0(2), 114.5(2) (Ar*C*), 110.1, 109.3 (2 × isopropylidene–*C*), 99.9 (C-1), 95.8 (C-1′), 79.9, 78.4, 76.3, 75.8, 74.5, 74.3, 68.9, 65.9, 60.3, 55.5 ($OC_6H_4OCH_3$), 27.9(2), 26.4, 26.1 (4 × isopropylidene–CH_3), 17.2 (C-6), 16.6 (C-6′). HRMS calcd for $C_{25}H_{36}O_{10}Na$ $(M+Na)^+$: 519.2206, found: 519.2202.

4.1.4. 3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose 8

To a solution of known di-ol **7** (2.0 g, 6.4 mmol) in MeOH (25 mL) was added $NaIO_4$ (2.7 g, 12.8 mmol) at 0 °C and the mixture was allowed to stir for 3 h after which the temperature was slowly raised to 25 °C. The mixture was filtered and the solvents were evaporated in vacuo and the resulting syrup was dissolved in MeOH (20 mL) followed by the addition of $NaBH_4$ (970 mg, 25.6 mmol) at 0 °C. The mixture was stirred at 0–10 °C for 5 h. The excess $NaBH_4$ was neutralized by adding acetone and the solvents were evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (30 mL) and brine (30 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and evaporated. The residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) as the eluent to afford pure compound **8** (1.5 g, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.35–7.27 (m, 5H, Ar*H*), 5.94 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.66, 4.47 (2d, AB system, *J* 12.0 Hz, CH_2Ph), 4.61 (d, 1H, $J_{1,2}$, $J_{2,3}$ 3.5 Hz, H-2), 4.27 (m, 1H, H-4), 3.98 (d, 1H, $J_{2,3}$, $J_{3,4}$ 3.5 Hz, H-3), 3.90 (dd, 1H, $J_{4,5a}$ 6.0 Hz, $J_{5a,5b}$ 12.0 Hz, H-5a), 3.81 (dd, 1H, $J_{4,5b}$ 5.0 Hz, $J_{5a,5b}$ 12.0 Hz, H-5b), 2.86 (br s, 1H, O*H*), 1.47, 1.30 (2s, 6H, 2 × isopropylidene–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 137.3, 128.9(2), 128.1, 127.5(2) (Ar*C*), 111.7 (isopropylidene–*C*), 105.0 (C-1), 82.4, 82.3, 80.5, 71.9, 60.7 (C-5), 26.8, 26.3 (2 × isopropylidene–CH_3). HRMS calcd for $C_{15}H_{20}O_5Na$ $(M+Na)^+$: 303.1208, found: 303.1205.

4.1.5. 1,2,4-Tri-*O*-acetyl-3-*O*-benzyl-D-xylopyranose 9

To a solution of compound **8** (1.3 g, 4.6 mmol) in CH_2Cl_2 (20 mL) was added 90% aq TFA (4 mL) and the solution was stirred at room temperature for 30 min when TLC (*n*-hexane–EtOAc, 2:1) showed complete conversion of the starting material to a slower running spot. The solution was neutralized carefully by adding solid $NaHCO_3$ till the effervescence ceases and was then filtered. The solvents were evaporated in vacuo and the residue was dissolved in pyridine (15 mL). Ac_2O (3 mL) was added and the solution was stirred for 6 h at 10 °C. After removing the solvents by co-evaporating with toluene, the residue was dissolved in CH_2Cl_2 (20 mL) and washed with H_2O (30 mL) and brine (30 mL). The organic layer was separated, dried (Na_2SO_4), filtered, and evaporated. The residue thus obtained was purified by flash chromatography to afford compound **9** (1.1 g, 64%) as an anomeric mixture. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.22 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1$^\alpha$), 5.76 (d, 1H, $J_{1,2}$ 6.5 Hz, H-1$^\beta$), 4.71 (2d, AB system, 2H, *J* 11.0 Hz, CH_2Ph), 2.14, 2.02, 2.01 (3s, 9H, 3 × $COCH_3\alpha$), 2.10, 2.08, 2.07 (3s, 9H, 3 × $COCH_3\beta$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.3, 168.8, 168.7 (3 × $COCH_3$), 136.6, 127.7(2), 127.5(2), 127.4 (Ar*C*), 91.0 (C-1$^\alpha$), 88.7 (C-1$^\beta$), 20.1, 19.9, 19.8 (3 × $COCH_3$). HRMS calcd for $C_{18}H_{22}O_8Na$ $(M+Na)^+$: 389.1212, found: 389.1209.

4.1.6. 1,2,4-Tri-*O*-acetyl-D-xylopyranose 10

A solution of compound **9** (1.0 g, 2.7 mmol) in MeOH (100 mL) was injected with a flow rate of 1 mL/min through the Flow Hydrogenation Assembly (H-cube®, ThalesNano, Hungary) having 10% Pd-C cartridge. The temperature was set to 70 °C. The resulting solution thus obtained was evaporated to dryness and the residue was purified by flash chromatography using *n*-hexane–EtOAc (2:1) as the eluent to give compound **10** (565 mg, 75%) as an anomeric mixture. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.17 (s, 1H, H-1$^\alpha$), 5.60 (d, 1H, $J_{1,2}$ 7.0 Hz, H-1$^\beta$), 2.27 (br s, 1H, O*H*), 2.17, 2.04, 2.02 (3s, 9H, 3 × $COCH_3\alpha$), 2.12, 2.09, 2.07 (3s, 9H, 3 × $COCH_3\beta$). HRMS calcd for $C_{11}H_{16}O_8Na$ $(M+Na)^+$: 299.0743, found: 299.0739.

4.1.7. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl trichloroacetimidate 13

A mixture of acceptor **10** (500 mg, 1.8 mmol), donor **11** (950 mg, 2.4 mmol), and MS 4 Å (1.0 g) in dry CH_2Cl_2 (15 mL) was stirred under nitrogen for 30 min. Then H_2SO_4–silica (30 mg) was added and the mixture was stirred at room temperature for 45 min when TLC (*n*-hexane–EtOAc, 3:1) showed complete conversion of the acceptor. The mixture was neutralized with Et_3N and filtered through Celite®. The filtrate was evaporated and purified by flash chromatography to afford the disaccharide **12** (755 mg, 76%) as an anomeric mixture. Subsequently, disaccharide **12** (750 mg, 1.4 mmol) was dissolved in dry DMF (10 mL), hydrazine hydrate (60 μL, 1.7 mmol) was added followed by AcOH (97 μL, 1.7 mmol) and the solution was stirred at room temperature for 3 h. The solvents were evaporated, the residue was dissolved in CH_2Cl_2 (20 mL), and washed with H_2O (30 mL) and brine (30 mL). The organic layer was collected, dried (Na_2SO_4), filtered, and evaporate

in vacuo. The residue was further dissolved in dry CH_2Cl_2 (15 mL), Cl_3CCN (700 μL, 7.0 mmol) was added followed by DBU (210 μL, 1.4 mmol), and the solution was stirred at room temperature for 2 h. After evaporation of the solvents, the residue was purified by flash chromatography using *n*-hexane–EtOAc (3:1) as the eluent to afford pure trichloroacetimidate derivative **13** (630 mg, 71%). This trichloroacetimidate derivative was used for the final glycosylation without any further characterization.

4.1.8. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-fucopyranoside 14

A mixture of the disaccharide acceptor **6** (300 mg, 0.6 mmol), trichloroacetimidate donor **13** (600 mg, 0.9 mmol), and MS 4 Å (500 mg) in dry CH_2Cl_2 (10 mL) was stirred under nitrogen for 30 min. Next, H_2SO_4–silica (15 mg) was added and the mixture was allowed to stir at room temperature for 2 h when TLC (*n*-hexane–EtOAc, 2:1) showed complete consumption of the acceptor. The mixture was neutralized with Et_3N and filtered through Celite®. The filtrate was evaporated in vacuo and the residue was purified by flash chromatography using *n*-hexane–EtOAc (4:1 to 2:1 gradient) as the eluent to afford pure tetrasaccharide **14** (440 mg, 74%) as a white foam. $[\alpha]_D^{25} = +78$ (*c* 0.9, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.92, 6.87 (2d, 4H, Ar*H*), 5.52 (s, 1H, H-1‴), 5.16 (dd, 1H, $J_{2''',3'''}$ 2.5 Hz, $J_{3''',4'''}$ 9.5 Hz, H-3‴), 5.07 (m, 2H, H-2′, H-2‴), 5.02 (s, 1H, H-1′), 4.97 (dd, 1H, $J_{3''',4'''}$ 9.5 Hz, $J_{4''',5'''}$ 10.0 Hz, H-4‴), 4.91 (d, 1H, $J_{1'',2''}$ 7.0 Hz, H-1″), 4.91 (m, 1H, H-4″), 4.68 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.24 (dd, 1H, $J_{3',4'}$ 9.5 Hz, $J_{4',5'}$ 10.0 Hz, H-4′), 4.11 (dd, 1H, $J_{4'',5a''}$ 4.5 Hz, $J_{5a'',5b''}$ 11.5 Hz, H-5a″), 4.05 (m, 3H, H-2, H-3, H-2′), 3.94 (m, 3H, H-3′, H-4, H-5b″), 3.88 (t, 1H, $J_{2'',3''}$, $J_{3'',4''}$ 8.5 Hz, H-3″), 3.78 (s, 3H, $OC_6H_4OCH_3$), 3.74 (m, 1H, H-5), 3.52 (m, 1H, H-5′), 3.27 (m, 1H, H-5‴), 2.14, 2.12, 2.09, 2.07, 2.03 (5s, 15H, 5 × $COCH_3$), 1.56, 1.50, 1.45, 1.32 (4s, 12H, 4 × isopropylidene–CH_3), 1.29, 1.23, 1.17 (3d, 9H, *J* 6.5 Hz, 3 × C–CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.1, 169.0, 168.8, 168.6, 168.4 (5 × $COCH_3$), 154.1, 150.5, 116.8(2), 113.7(2) (Ar*C*), 109.2, 108.2 (2 × isopropylidene–*C*), 98.9 (C-1), 98.6 (C-1″), 97.2 (C-1′), 94.6 (C-1‴), 80.0, 78.8, 78.2, 78.0, 76.3, 76.1, 74.5, 71.6, 70.9, 70.0, 68.9, 68.7, 67.0, 64.2, 62.1, 55.7 ($OC_6H_4OCH_3$), 28.6, 28.5, 27.8, 27.6 (4 × isopropylidene–CH_3), 21.1, 20.9, 20.8, 20.7(2) (5 × $COCH_3$), 18.1, 17.9, 17.8 (3 × C–CH_3). HRMS calcd for $C_{46}H_{64}O_{23}Na$ $(M+Na)^+$: 1007.3736, found: 1007.3732.

4.1.9. *p*-Methoxyphenyl α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside 1

A solution of compound **14** (400 mg, 0.4 mmol) in 80% aq AcOH (10 mL) was stirred at 80 °C for 3 h. Then the solvents were evaporated and co-evaporated with toluene to remove residual AcOH. The residue was dissolved in MeOH (10 mL), after which NaOMe in MeOH (0.5 M, 1 mL) was added and the solution was allowed to stir at room temperature for 6 h. The solution was neutralized by DOWEX 50 W H^+ resin, filtered and evapoarated to afford pure target tetrasaccharide **1** (225 mg, 80%) as white amorphous powder. $[\alpha]_D^{25} = +63$ (*c* 0.8, $CHCl_3$). ^{1}H NMR (500 MHz, D_2O) δ: 5.00 (s, 1H, H-1‴), 4.96 (d, 1H, $J_{1'',2''}$8.0 Hz, H-1″), 4.73 (s, 1H, H-1′), 4.62 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 3.91-3.18 (m, 17H, H-2, H-2′, H-2″, H-2‴, H-3, H-3′, H-3″, H-3‴, H-4, H-4′, H-4″, H-4‴, H-5, H-5′, H-5a″, H-5b″, H-5‴), 3.75 (s, 3H, $OC_6H_4OCH_3$), 1.05, 1.00, 0.86 (3d, 9H, 3 × C–CH_3). ^{13}C NMR (125 MHz, D_2O) δ: 154.3, 150.2, 116.6(2), 113.9(2) (Ar*C*), 101.5 (C-1), 100.8 (C-1″), 99.7 (C-1′), 98.4 (C-1‴), 76.5, 76.3, 76.1, 75.8, 75.5, 75.1, 74.4, 71.8, 71,6, 70.3, 70.1, 68.7, 68.3, 67.6, 64.5, 60.1, 55.4 ($OC_6H_4OCH_3$), 17.9, 17.7, 17.1 (3 × C–CH_3). HRMS calcd for $C_{30}H_{46}O_{18}Na$ $(M+Na)^+$: 717.2582, found: 717.2577.

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10. 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 conc. 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 used for the reactions. For the use of H_2SO_4–silica in various carbohydrate reactions see: (a) Mandal, S.; Sharma, N.; Mukhopadhyay, B. *Synlett* **2009**, 3111–3114; (b) Roy, B.; Verma, P.; Mukhopadhyay, B. *Carbohydr. Res.* **2009**, *344*, 145–148; (c) Rajput, V. K.; Mukhopadhyay, B. *Tetrahedron Lett.* **2006**, *47*, 5939–5941; (d) Mukhopadhyay, B. *Tetrahedron Lett.* **2006**, *47*, 4337–4341; (e) Roy, B.; Mukhopadhyay, B. *Tetrahedron Lett.* **2007**, *48*, 3783–3787.
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