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Note

Convergent synthesis of a trisaccharide as its 2-(trimethylsilyl)ethyl glycoside related to the flavonoid triglycoside from *Gymnema sylvestre*

Balaram Mukhopadhyay*,† and Robert A. Field*

Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK

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Abstract—The glycone part of the flavonoid triglycoside, kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside, has been synthesized in good yield and stereoselectivity using *N*-iodosuccinimide and $HClO_4$–silica promoted glycosylations of thioglycoside donors.

Keywords: Flavonoids; Antidiabetic; $HClO_4$–silica

Flavonoid glycosides constitute an important class of biomolecules and are isolated from fruits, vegetables and traditional medicinal plants.[1] They posses important biological activities in the growth and development of plants. Moreover, they are potent drug candidates having antimicrobial, anticancer or antioxidant properties.[2] The plant, *Gymnema sylvestre* (Retz.) Schult. (Asclepiadaceae), is widespread in India, China and Vietnam. It is well known for its leaves, called 'Gur-mar' in India, for the sweet taste suppressing activity[3] and is used as a folk medicine for the treatment of diabetes mellitus and in food additives against obesity.[4] Exploring the abundant resources of *G. sylvestre* in China, Yu et al.[5] recently reported the isolation and structural characterization of a new flavonol triglycoside, kaempferol 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (Fig. 1), which is an important biomolecule as a potential antidiabetic drug lead. Herein, we report a simple and high yielding stereoselective synthesis of the trisaccharide portion of this natural product as its 2-(trimethylsilyl)ethyl glycoside (**1**). The 2-(trimethylsilyl)ethyl glycoside of the target trisaccharide was particularly attractive because it can be converted to other glycosides via coupling to the aglycon moiety.

The synthesis was started from the known[6] 2-(trimethylsilyl)ethyl β-D-galactopyranoside (**2**). Selective protection of the primary hydroxyl group with *tert*-butyldiphenylsilyl group followed by acetylation of the other hydroxyl groups afforded the completely protected galactoside **3** in 85% yield over two steps (Scheme 1). Deprotection of the *tert*-butyldiphenylsilyl group using 1 M tetrabutylammonium fluoride[7] in THF at 60 °C furnished the required acceptor **4** in 78% yield. For the L-rhamnose moiety, the known *p*-tolyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (**5**)[8] was de-O-acetylated using sodium methoxide in methanol and converted to its 2,3-isopropylidene derivative **6** in 87% yield using 2,2-dimethoxypropane in the presence of $HClO_4$–silica.[9] Finally, the rhamnosyl donor **7** was generated by protecting the 4-hydroxyl group of **6** as a *p*-methoxybenzyl ether in 86% yield (Scheme 1).

N-Iodosuccinimide promoted glycosylation of acceptor **4** with donor **7** in the presence of $HClO_4$–silica[10] afforded the disaccharide **8** in 82% yield (Scheme 1). No degraded product due to the cleavage of the acid labile isopropylidene ketal or *p*-methoxybenzyl ether

* Corresponding authors. E-mail addresses: sugarnet73@hotmail.com; r.a.field@uea.ac.uk
† Present address: Medicinal and Process Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, PO Box 173, Lucknow 226 001, India.

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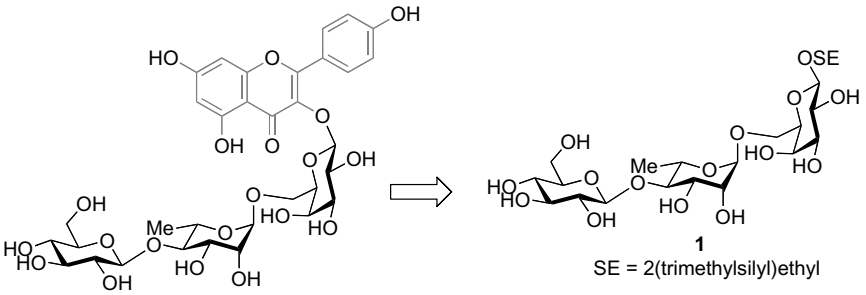
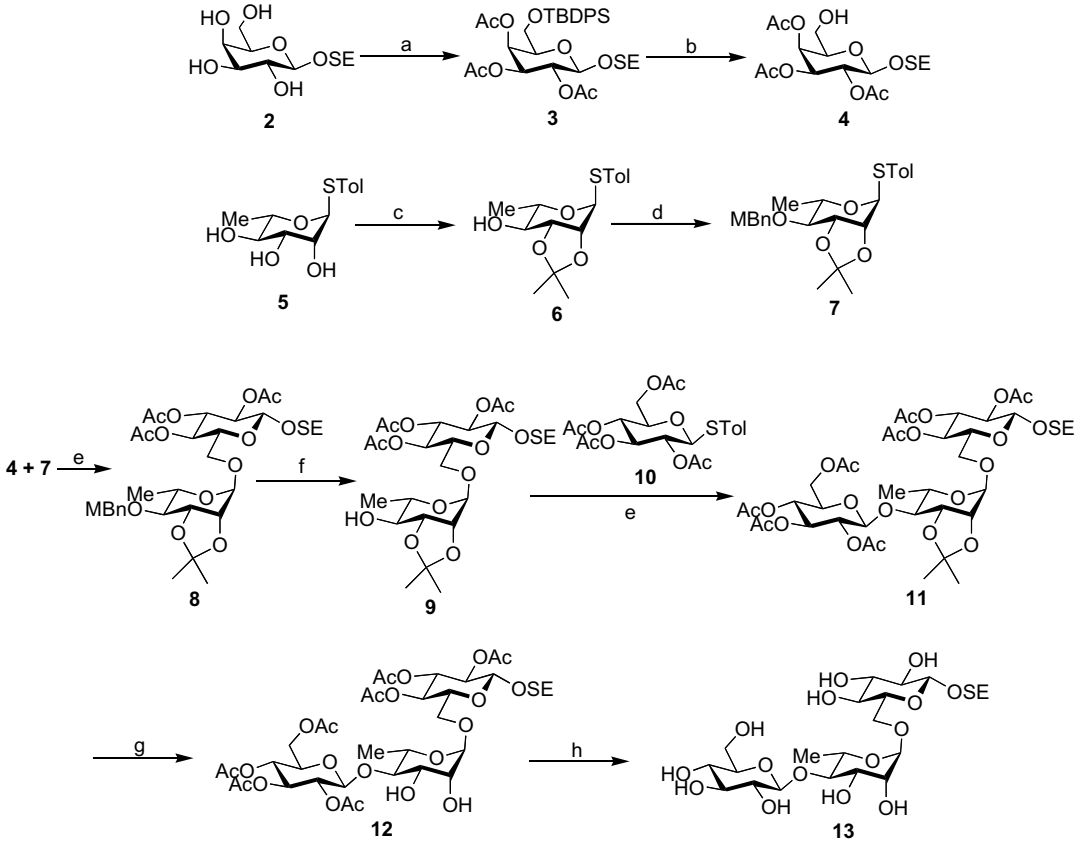


Figure 1. Structure of the flavonoid triglycoside from *Gymnema sylvestre*.



Scheme 1. Reagents and conditions: (a) (i) TBDPSCl, pyridine, rt, 6 h; (ii) Ac_2O, pyridine, rt, 3 h, 85% over two steps; (b) *n*-Bu_4NFTHF, 60 °C, 1 h, 78%; (c) 2,2-dimethoxypropane, acetone, $HClO_4$–silica, rt, 2 h, 87%; (d) *p*-methoxybenzyl chloride, NaH, DMF, rt, 2 h, 86%; (e) NIS, $HClO_4$–silica, CH_2Cl_2, rt, 45 min, 82%; (f) CAN, CH_3CN/H_2O (9:1), rt, 30 min, 87%; (g) $HClO_4$–silica, CH_3OH, 2 h, 91%; (h) $NaOCH_3$, CH_3OH, rt, 2 h, 95%.

was observed during glycosylation, which confirmed the applicability of the $HClO_4$–silica activation method to glycosylations with acid labile protecting groups.

Oxidative deprotection of the *p*-methoxybenzyl group using ceric ammonium nitrate[11] furnished the disaccharide acceptor **9** in 87% yield. This alcohol was then glycosylated with known donor, *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (**10**)[8] in the presence of *N*-iodosuccinimide and $HClO_4$–silica to give the blocked trisaccharide **11** in 87% yield. Deprotection of the isopropylidene ketal using $HClO_4$–silica in methanol[12] afforded the trisaccharide **12** in 91% yield. Finally Zémplen de-O-acetylation by catalytic sodium methoxide[13] in methanol furnished the target trisaccharide, 2-(trimethylsilyl)ethyl β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**13**) in 95% yield.

1. Experimental

1.1. General methods

All reagents and solvents were dried prior to use according to standard methods.[14] Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on Silica Gel 60-

F254 (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 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 60 (Fluka). Optical rotations were measured at the sodium D-line at ambient temperature, with a Perkin Elmer 141 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on either a Varian Unity plus spectrometer at 300 and 75 MHz or 400 and 100 MHz, respectively.

1.2. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranoside (3)

To a solution of **2** (2 g, 7.1 mmol) in dry pyridine (20 mL) was added *tert*-butyldiphenylsilyl chloride (2.2 mL, 8.5 mmol) and the mixture was stirred at rt for 6 h. When TLC (1:1, *n*-hexane/EtOAc) showed complete conversion of the starting material, Ac_2O (15 mL) was added and stirring was continued for 3 h. After complete conversion (TLC), the solvent was evaporated under reduced pressure and co-evaporated with toluene. The crude product thus obtained was purified by column chromatography (3:1, *n*-hexane/EtOAc) to afford compound **3** (3.8 g, 85%) as a glass. $[\alpha]_D^{25}$ +16.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.63–7.34 (m, 10H, ArH), 5.56 (d, 1H, $J_{3,4} = 3.0$ Hz, H-4), 5.17 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 10.5$ Hz, H-2), 5.03 (dd, 1H, $J_{2,3} = 10.5$ Hz, $J_{3,4} = 3.0$ Hz, H-3), 4.44 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 3.97 (m, 1H, $OCH_2CH_2Si(CH_3)_3$), 3.77 (m, 2H, H-6a, H-6b), 3.67 (m, 1H, H-5), 3.51 (m, 1H, $OCH_2CH_2Si(CH_3)_3$), 2.08, 2.04, 2.00 (3s, 9H, $3COCH_3$), 1.06 (s, 9H, *t*-$BuC(Ph)_2Si(CH_3)_3$), 1.02 (m, 2H, $OCH_2CH_2Si(CH_3)_3$), 0.01 (s, 9H, $OCH_2CH_2Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ: 170.4, 170.3, 169.6 ($3COCH_3$), 135.7, 135.6, 133.0, 132.9, 129.9, 129.8, 129.1, 128.3, 127.8 (ArC), 100.7 (C-1), 73.3, 71.4, 69.3, 67.4, 67.1, 61.2 (C-6), 26.6, 20.7, 20.5, 18.9, 17.8, −1.6 ($OCH_2CH_2Si(CH_3)_3$); HRMS calcd for $C_{34}H_{52}NO_9Si$ $[M+NH_4]^+$: *m*/*z* 646.3411. Found: *m*/*z* 646.3414.

1.3. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (4)

To a solution of compound **3** (3 g, 4.8 mmol) in dry THF (20 mL) was added 1 M *n*-Bu_4NF in THF (5 mL) and the solution was stirred at 60 °C for 1 h. When TLC (3:1, *n*-hexane/EtOAc) showed complete conversion of the starting material, the solvent was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 mL) and washed with H_2O (2 × 30 mL); the organic layer was collected, dried (Na_2SO_4) and evaporated to a syrup. Purification of the crude product by column chromatography (2:1, *n*-hexane/EtOAc) afforded compound **4** (1.5 g, 78%) as a colourless syrup. $[\alpha]_D^{25}$ +22.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.36 (d, 1H, $J_{3,4} = 3.0$ Hz, H-4), 5.21 (dd, 1H, $J_{1,2} = 8.1$ Hz, $J_{2,3} = 10.2$ Hz, H-2), 5.02 (dd, 1H, $J_{2,3} = 10.2$ Hz, $J_{3,4} = 3.0$ Hz, H-3), 4.43 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1), 3.97 (m, 1H, $OCH_2CH_2Si(CH_3)_3$), 3.77 (m, 2H, H-6a, H-6b), 3.67 (m, 1H, H-5), 3.51 (m, 1H, $OCH_2CH_2Si(CH_3)_3$), 2.08, 2.04, 1.99 (3s, 9H, $3COCH_3$), 0.93 (m, 2H, $OCH_2CH_2Si(CH_3)_3$), 0.01 (s, 9H, $OCH_2CH_2Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 171.4, 170.9, 169.9 ($3COCH_3$), 100.8 (C-1), 73.5, 71.6, 69.5, 67.3, 67.1, 60.2 (C-6), 20.7, 20.6, 20.5 ($3COCH_3$), 17.8 ($OCH_2CH_2Si(CH_3)_3$), −1.6 ($OCH_2CH_2Si(CH_3)_3$); HRMS calcd for $C_{17}H_{34}NO_9Si$ $[M+NH_4]^+$: *m*/*z* 424.2003. Found: *m*/*z* 424.2005.

1.4. *p*-Tolyl 2,3-*O*-isopropylidene-4-*O*-(4-methoxybenzyl)-1-thio-α-L-rhamnopyranoside (7)

To a mixture of compound **5** (2 g, 7.4 mmol) in dry acetone (30 mL), 2,2-dimethoxypropane (1.4 mL, 11.1 mmol) was added followed by $HClO_4$–silica[15] (50 mg) and the mixture stirred at rt for 2 h. When TLC (1:1, *n*-hexane/EtOAc) showed complete conversion of the starting material, the solution was neutralized with Et_3N and the solvent was evaporated and the residue was re-dissolved in DMF. NaH (530 mg, 11.1 mmol, 50% in mineral oil) was added followed by *p*-methoxybenzyl chloride (1.3 mL, 9.6 mmol) and stirring continued at rt for 2 h until the reaction was complete (TLC; 2:1, *n*-hexane/EtOAc). The mixture was then diluted with CH_2Cl_2 (50 mL), washed with H_2O (3 × 50 mL). The organic phase was collected, dried (Na_2SO_4) and evaporated. The crude residue was purified by flash chromatography (2:1, *n*-hexane/EtOAc) to give compound **7** (2.7 g, 86%) as colourless syrup. $[\alpha]_D^{25}$ −133.1 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.38–6.86 (m, 8H, ArH), 5.68 (s, 1H, H-1), 4.84, 4.60 (2d, AB system, $J = 11.2$ Hz, $CH_2C_6H_4OCH_3$), 4.35 (m, 2H, H-2, H-3), 4.16 (m, 1H, H-5), 3.81 (s, 3H, $CH_2C_6H_4OCH_3$), 3.29 (dd, 1H, $J_{3,4} = 7.2$ Hz, $J_{4,5} = 9.9$ Hz, H-4), 2.33 (s, 3H, $SC_6H_4CH_3$), 1.53, 1.39 (2s, isopropylidene CH_3), 1.23 (d, 3H, $J_{5,6} = 6.2$ Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 159.3, 137.8, 132.5, 130.5, 129.8, 129.6, 113.7 (ArC), 109.4, 84.1 (C-1), 81.1 (C-4), 78.4 (CH_2–C_6H_4–OCH_3), 76.6 (C-2), 72.6 (C-3), 66.1 (C-5), 55.2 (CH_2–C_6H_4–OCH_3), 27.9, 26.3 (isopropylidene CH_3), 20.9 ($SC_6H_4CH_3$), 17.8 (CCH_3); HRMS calcd for $C_{24}H_{34}NO_5S$ $[M+NH_4]^+$: *m*/*z* 448.2158. Found: *m*/*z* 448.2156.

1.5. 2-(Trimethylsilyl)ethyl 2,3-di-*O*-isopropylidene-4-*O*-(4-methoxybenzyl)-α-L-rhamnopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (8)

A solution of compound **4** (1.2 g, 2.95 mmol), compound **7** (1.65 g, 3.8 mmol) and activated 4 Å molecular sieves

(2.0 g) in dry CH_2Cl_2 (25 mL) was stirred at rt under a nitrogen atmosphere for 3 h. Then NIS (1.1 g, 4.9 mmol) was added followed by $HClO_4$–silica (50 mg) and the mixture was allowed to stir at rt for 45 min, until TLC (3:1, *n*-hexane/EtOAc) showed complete disappearance of acceptor. The mixture was diluted with CH_2Cl_2 (25 mL) and filtered through Celite. The filtrate was successively washed with $Na_2S_2O_3$ (3×50 mL), $NaHCO_3$ (2×50 mL) and brine (50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (3:1, *n*-hexane/EtOAc) to give **8** (1.7 g, 82%) as a white foam. $[\alpha]_D^{25}$ +16.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.25, 6.83 (2d, 4H, $CH_2C_6H_4OCH_3$), 5.37 (d, 1H, $J_{3,4}=3.3$ Hz, H-4), 5.15 (dd, 1H, $J_{1,2}=7.8$ Hz, $J_{2,3}=10.8$ Hz, H-2), 4.96 (dd, 1H, $J_{2,3}=10.8$ Hz, $J_{3,4}=3.3$ Hz, H-3), 4.91 (s, 1H, H-1′), 4.74, 4.51 (2d, AB system, $CH_2C_6H_4OCH_3$), 4.43 (d, 1H, $J_{1,2}=7.8$ Hz, H-1), 4.15 (dd, 1H, $J_{2',3'}=5.7$ Hz, $J_{3',4'}=6.9$ Hz, H-3′), 4.05 (d, 1H, $J_{2',3'}=5.7$ Hz, H-2′), 3.98 (m, 1H, O–$CH_2CH_2Si(CH_3)_3$), 3.79 (m, 1H, H-5), 3.74 (s, 3H, $CH_2C_6H_4OCH_3$), 3.62–3.43 (m, 4H, H-5′, H-6[a], H-6b, O–$CH_2CH_2Si(CH_3)_3$), 3.11 (dd, 1H, $J_{3',4'}=7.5$ Hz, $J_{4',5'}=9.9$ Hz, H-4′), 2.08, 2.00, 1.92 (3s, 9H, $3\times COCH_3$), 1.44, 1.30 (2s, 6H, isopropylidene CH_3), 1.17 (d, 3H, $J_{5,6}=6.2$ Hz, CCH_3), 0.89 (m, 2H, O–$CH_2CH_2Si(CH_3)_3$), −0.03 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 170.2 (2), 169.3 ($3\times COCH_3$), 159.1, 130.5, 129.5, 113.5 (ArC), 109.0, 100.6 (C-1), 97.4 (C-1′), 80.1, 78.3, 75.6, 72.2, 71.6, 71.1, 68.9, 67.3, 65.0 ($OCH_2CH_2Si(CH_3)_3$), 64.7 (C-6), 55.0 ($CH_2C_6H_4OCH_3$), 27.7, 26.0 (isopropylidene CH_3), 20.5, 20.4, 20.3 ($3\times COCH_3$), 17.7 (O–$CH_2CH_2Si(CH_3)_3$), 17.6 (C–CH_3), −1.7 ($Si(CH_3)_3$); HRMS calcd for $C_{34}H_{56}NO_{14}Si$ $[M+NH_4]^+$: *m*/*z* 730.3470. Found: *m*/*z* 730.3472.

1.6. 2-(Trimethylsilyl)ethyl 2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (9)

To a solution of compound **8** (1.5 g, 2.1 mmol) in CH_3CN and H_2O (9:1, 30 mL), was added CAN (2.3 g, 4.2 mmol) and the mixture was stirred at rt for 30 min. When TLC (2:1, *n*-hexane/EtOAc) showed complete conversion of the starting material to a slower running component, the mixture was diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ (2×50 mL) and brine (2×50 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated. The syrupy residue was purified by flash chromatography (2:1, *n*-hexane/EtOAc) to afford pure compound **9** (1.1 g, 87%) as a colourless glass. $[\alpha]_D^{25}$ +13.0 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.38 (d, 1H, $J_{3,4}=3.3$ Hz, H-4), 5.14 (dd, 1H, $J_{1,2}=7.8$ Hz, $J_{2,3}=10.8$ Hz, H-2), 4.98 (dd, 1H, $J_{2,3}=10.8$ Hz, $J_{3,4}=3.3$ Hz, H-3), 4.94 (s, 1H, H-1′), 4.45 (d, 1H, $J_{1,2}=7.8$ Hz, H-1), 4.04 (d, 1H, H-2′), 3.98 (m, 2H, H-3′, O–$CH_2CH_2Si(CH_3)_3$), 3.82 (m, 1H, H-5), 3.67–3.37 (m, 4H, H-5′, H-6a, H-6b, O–$CH_2CH_2Si(CH_3)_3$), 3.31 (m, 1H, H-4′), 2.10, 2.00, 1.92 (3s, 9H, $3\times COCH_3$), 1.45, 1.28 (2s, 6H, isopropylidene CH_3), 1.20 (d, 3H, $J_{5',6'}=6.2$ Hz, CCH_3), 0.89 (m, 2H, O–$CH_2CH_2Si(CH_3)_3$), −0.03 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 170.4, 170.3, 169.4 ($3\times COCH_3$), 109.4, 100.7 (C-1), 97.5 (C-1′), 78.2, 75.5, 74.2, 71.5, 71.2, 69.0, 67.4, 65.9 ($OCH_2CH_2Si(CH_3)_3$), 64.9 (C-6), 27.8, 26.0 (isopropylidene CH_3), 20.6, 20.5, 20.3 ($3\times COCH_3$), 17.7 (O–$CH_2CH_2Si(CH_3)_3$), 17.1 (C–CH_3), −1.7 ($Si(CH_3)_3$); HRMS calcd for $C_{26}H_{48}NO_{13}Si$ $[M+NH_4]^+$: *m*/*z* 610.2895. Found: *m*/*z* 610.2897.

1.7. 2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-isopropylidene-α-L-rhamnopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (11)

To a solution of disaccharide acceptor **9** (1.0 g, 1.7 mmol) and ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside **10** (1.0 g, 2.2 mmol) in dry CH_2Cl_2 (20 mL) was added activated powdered 4 Å molecular sieves (2.0 g) and the mixture was stirred under nitrogen for 2 h. NIS (640 mg, 2.9 mmol) was added, followed by $HClO_4$–silica (50 mg) and stirring was continued for 30 min until TLC (1:1, *n*-hexane/EtOAc) showed complete disappearance of the acceptor. The mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a Celite pad. The filtrate was washed successively with aq $Na_2S_2O_3$ (2×50 mL), satd aq $NaHCO_3$ (2×50 mL) and brine (50 mL). The organic layer was separated, dried (Na_2SO_4) and evaporated to a syrup. The crude product was purified by column chromatography (1:1, *n*-hexane/EtOAc) to afford pure **11** (1.4 g, 89%) as a white foam. $[\alpha]_D^{25}$ +21.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.36 (d, 1H, $J_{3,4}=3.6$ Hz, H-4), 5.15 (t, 1H, $J_{2'',3''}=J_{3'',4''}=9.3$ Hz, H-3″), 5.12 (dd, 1H, $J_{1,2}=8.1$ Hz, $J_{2,3}=10.5$ Hz, H-2), 4.96 (dd, 1H, $J_{1'',2''}=7.8$ Hz, $J_{2'',3''}=9.3$ Hz, H-2″), 4.93 (d, 1H, $J_{1'',2''}=7.8$ Hz, H-1″), 4.92 (dd, 1H, $J_{2,3}=10.5$ Hz, $J_{3,4}=3.6$ Hz, H-3), 4.91 (s, 1H, H-1′), 4.85 (dd, 1H, $J_{3'',4''}=J_{4'',5''}=9.3$ Hz, H-4″), 4.42 (d, 1H, $J_{1,2}=8.1$ Hz, H-1), 4.12 (dd, 1H, $J_{5'',6a''}=5.4$ Hz, $J_{6a'',6b''}=12.3$ Hz, H-6a″), 4.05 (dd, 1H, $J_{5'',6b''}=2.4$ Hz, $J_{6a'',6b''}=12.3$ Hz, H-6b″), 3.98–3.87 (m, 3H, H-2′, H-3′, O–$CH_2CH_2Si(CH_3)_3$), 3.80 (m, 1H, H-5), 3.63–3.43 (m, 5H, H-5′, H-5″, H-6a, H-6b, O–$CH_2CH_2Si(CH_3)_3$), 3.31 (m, 1H, H-4′), 2.04, 2.03, 2.02, 2.01, 2.00, 1.98, 1.96 (7s, 21H, $7\times COCH_3$), 1.43, 1.25 (2s, 6H, isopropylidene CH_3), 1.14 (d, 3H, $J_{5',6'}=6.2$ Hz, CCH_3), 0.86 (m, 2H, O–$CH_2CH_2Si(CH_3)_3$), −0.06 (s, 9H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): 170.5, 170.2 (2), 169.6, 169.4 (2), 169.3 ($7COCH_3$), 109.3, 100.6 (C-1), 98.9 (C-1″), 96.9 (C-1′), 78.6, 77.6, 75.5, 72.8, 71.6, 71.1, 68.9, 68.7, 67.3, 66.9, 64.2, 63.9 (C-6), 62.1 (C-6″), 27.7, 26.1 (isopropylidene

*C*H$_3$), 20.4 (2), 20.3 (2), 20.2, 20.1, 20.0 (7CO*C*H$_3$), 17.7 (O–CH$_2$*C*H$_2$Si(CH$_3$)$_3$), 17.1 (C–*C*H$_3$), −1.7 (Si(*C*H$_3$)$_3$); HRMS calcd for $C_{40}H_{66}NO_{22}Si$ $[M+NH_4]^+$: *m*/*z* 940.3846. Found: *m*/*z* 940.3845.

1.8. 2-(Trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranoside (12)

Compound **11** (1.1 g, 1.2 mmol) was dissolved in CH_3OH (20 mL), $HClO_4$–silica (200 mg) was added and the mixture was stirred at rt for 2 h until TLC (1:1, *n*-hexane/EtOAc) showed complete conversion of the starting material to a slower running component. The mixture was filtered through Celite and the solvent was evaporated to give compound **12** (950 mg, 91%) as a white foam. $[\alpha]_D^{25}$ +27.0 (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.46 (d, 1H, $J_{3,4} = 2.4$ Hz, H-4), 5.32 (t, 1H, $J_{2'',3''} = J_{3'',4''} = 9.2$ Hz, H-3″), 5.19 (dd, 1H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.4$ Hz, H-2), 5.07 (dd, 1H, $J_{1'',2''} = J_{2'',3''} = 9.2$ Hz, H-2″), 4.97 (dd, 1H, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 2.4$ Hz, H-3), 4.95 (d, 1H, $J_{1'',2''} = 9.2$ Hz, H-1″), 4.90 (dd, 1H, $J_{3'',4''} = J_{4'',5''} = 9.2$ Hz, H-4″), 4.76 (s, 1H, H-1′), 4.48 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.25 (dd, 1H, $J_{5'',6a''} = 2.4$ Hz, $J_{6a'',6b''} = 12.3$ Hz, H-6a″), 4.15 (dd, 1H, $J_{5'',6b''} = 5.4$ Hz, $J_{6a'',6b''} = 12.3$ Hz, H-6b″), 4.01–3.79 (m, 5H, H-2′, H-3′, H-6a, H-6b, O–C*H*$_2$CH$_2$-Si(CH$_3$)$_3$), 3.70 (m, 1H, H-5), 3.61–3.51 (m, 3H, H-5′, H-5″, O–C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 3.43 (m, 1H, H-4′), 3.17 (br d, 1H, O*H*), 2.76 (br s, 1H, O*H*), 2.16, 2.08, 2.06, 2.05, 2.02, 1.98, 1.96 (7s, 21H, 7 × COC*H*$_3$), 1.23 (d, 3H, $J_{5',6'} = 6.2$ Hz, CC*H*$_3$), 0.93 (m, 2H, O–CH$_2$C*H*$_2$-Si(CH$_3$)$_3$), −0.02 (s, 9H, Si(C*H*$_3$)$_3$); ^{13}C NMR ($CDCl_3$): δ 170.9, 170.8 (2), 170.5, 170.3, 169.5, 169.4 (7*C*OCH$_3$), 100.9 (C-1), 99.4 (C-1″), 98.4 (C-1′), 80.4, 72.8, 71.6, 71.5, 71.4, 70.9, 69.4, 68.9, 68.5, 67.5, 67.0, 65.6, 64.2 (C-6), 61.8 (C-6″), 20.4 (2), 20.3 (2), 20.2, 20.1, 20.0 (7CO*C*H$_3$), 17.8 (O–CH$_2$*C*H$_2$Si(CH$_3$)$_3$), 17.1 (C–*C*H$_3$), −1.6 (Si(*C*H$_3$)$_3$); HRMS calcd for $C_{37}H_{62}NO_{22}Si$ $[M+NH_4]^+$: *m*/*z* 900.3533. Found: *m*/*z* 900.3535.

1.9. 2-(Trimethylsilyl)ethyl β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (1)

To a methanolic solution of **12** (900 mg, 1.0 mmol), methanolic $NaOCH_3$ (0.5M, 0.5 mL) was added and the solution was stirred at rt for 2 h. The solution was neutralized with DOWEX 50W H^+ resin and filtered through cotton. The solvent was evaporated under reduced pressure to afford the target trisaccharide **1** (570 mg, 95%) as a white amorphous powder. $[\alpha]_D^{25}$ +12.0 (*c* 1.0, CH_3OH); ^{1}H NMR (CD_3OD): δ 4.73 (s, 1H, H-1′), 4.56 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 4.22 (d, 1H, $J_{1'',2''} = 6.3$ Hz, H-1″), 3.96 (m, 1H, O–C*H*$_2$CH$_2$-Si(CH$_3$)$_3$), 3.86–3.17 (m, 17H, H-2, H-2′, H-2″, H-3, H-3′, H-3″, H-4, H-4′, H-4″, H-5, H-5′, H-5″, H-6a, H-6b, H-6a′, H-6b″, O–C*H*$_2$CH$_2$Si(CH$_3$)$_3$), 1.33 (d, 3H, $J_{5',6'} = 6.2$ Hz, CC*H*$_3$), 0.96 (m, 2H, O–CH$_2$C*H*$_2$-Si(CH$_3$)$_3$), 0.02 (s, 9H, Si(C*H*$_3$)$_3$); ^{13}C NMR (CD_3OD): δ 105.8 (C-1), 104.4 (C-1″), 102.1 (C-1′), 83.7, 78.2, 78.0, 76.1, 75.0, 74.9, 72.5, 72.4, 72.1, 71.6, 70.4, 68.5, 68.0, 67.9, 62.8, 19.1 (O–CH$_2$*C*H$_2$Si(CH$_3$)$_3$), 18.2 (C–*C*H$_3$), −1.4 (Si(*C*H$_3$)$_3$); HRMS calcd for $C_{23}H_{48}NO_{15}Si$ $[M+NH_4]^+$: *m*/*z* 606.2793. Found: *m*/*z* 606.2794.

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Supplementary data

^{1}H and ^{13}C NMR spectra of compounds **1**, **3**, **4**, **6**, **7**, **8**, **9**, **11** and **12**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.03.019.

References

1. Bohm, B. A. *Introduction to Flavonoids*; Harwood Academic Press: Amsterdam, 1998.
2. Harborne, J. B.; Baxter, H. In *The Handbook of Natural Flavonoids*; John Wiley & Sons: Chichester, 1999; Vol. 1.
3. Kapoor, L. D. *CRC Handbook of Ayurvedic Medicinal Plants*; CRC: Boca Raton, FL, 1990; pp 200–201.
4. Porchezhian, E.; Dobriyal, R. M. *Pharmazie* **2003**, *58*, 5–9.
5. Liu, X.; Ye, W.; Yu, B.; Zhao, S.; Wu, H.; Che, C. *Carbohydr. Res.* **2004**, *339*, 891–895.
6. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629–5647.
7. Limberg, G.; Thiem, J. *Carbohydr. Res.* **1995**, *275*, 107–115.
8. Mukhopadhyay, B.; Kartha, K. P. R.; Russell, D. A.; Field, R. A. *J. Org. Chem.* **2004**, *69*, 7758–7760.
9. Mukhopadhyay, B.; Russell, D. A.; Field, R. A. *Carbohydr. Res.* **2005**, *340*, 1075–1080.
10. Mukhopadhyay, B.; Collet, B.; Field, R. A. *Tetrahedron Lett.* **2005**, *46*, 5923–5925.
11. (a) Johansson, R.; Samuelson, B. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2371; (b) Classon, B.; Garegg, P. J.; Samuelsson, B. *Acta. Chem. Scand., Ser. B* **1984**, *38*, 419.
12. Agarwal, A.; Vankar, Y. D. *Carbohydr. Res.* **2005**, *340*, 1661–1667.
13. Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 1254–1259.
14. Perrin, D. D.; Amarego, W. L.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: London, 1996.
15. Preparation of $HClO_4$–silica: Immobilized perchloric acid on silica was prepared by adding commercially available $HClO_4$ (0.3 mmol, as a 70% aqueous solution) to a slurry of silica gel (5 g, 200 mesh) in Et_2O (15 mL) and the solvents were removed under reduced pressure. The resulting free flowing powder was kept at 110 °C for 2 h and used directly in reactions.