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Efficient synthesis of methyl lycotetraoside, the tetrasaccharide constituent of the tomato defence glycoalkaloid α-tomatine

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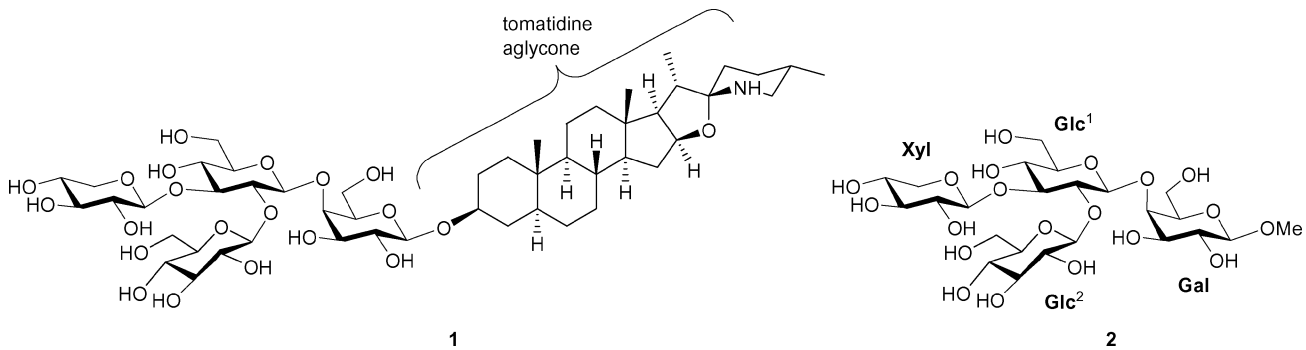
Branched oligosaccharide lycotetraose, β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranose, is a key constituent of many steroidal saponins, including glycoalkaloid α-tomatine, which is involved in protection of plants from invading pathogens. A new synthesis of the methyl β-lycotetraoside (**2**) is described. Key steps of the synthesis include two successive glycosylation reactions of disaccharide acceptor methyl (4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside with readily available benzoylated trichloroacetimidates of α-D-glucopyranose and α,β-D-xylopyranose. This scheme allows sequential glycosylation in one-pot on account of the convenient *in situ* removal of a *p*-methoxybenzyl protecting group under the acid conditions of the first glycosylation step. Following deprotection, tetrasaccharide **2** was obtained in 19% yield over eight steps.

Introduction

Saponins are a structurally diverse family of glycosylated triterpenes and steroids constitutively present in a variety of plant species[1] and marine organisms.[2] They possess a wide range of pharmacological activities; some such compounds are known to be the active principles in traditional herbal medicines.[3] The promising pharmaceutical properties of saponins have prompted the search for new compounds of this type from natural sources, followed by investigation of their biological and pharmacological activities.[1] Since isolation of saponins from plants is a formidable task, considerable efforts have also been devoted to their chemical synthesis.[4] In the plant kingdom, saponins often play a role as antimicrobials toxic to various pathogens, including fungi, and thus contribute to plant resistance to infection.[5] The proposed mechanism of saponin antimicrobial activity is believed to be based on complexation of sterols in the pathogen membrane, causing a loss of the membrane integrity and hence cell lysis.[6] The main saponin of tomato plants, the steroidal glycoalkaloid α-tomatine,[7] has a potent fungicidal activity *in vivo*.[8] However, this activity can be completely suppressed by some infectious fungi, which produce α-tomatine-detoxifying enzymes known as tomatinases.[9] These enzymes are glycosyl hydrolases which can remove one or more sugar residues from α-tomatine leading to a modified saponin or aglycone (tomatidine), which are substantially less toxic to fungi. Furthermore, it has recently been shown that degradation products resulting from tomatinase action suppress other mechanisms of plant resistance to fungal attack.[10] To gain insight into the mechanism of action of α-tomatine-detoxifying enzymes, the complete oligosaccharide structure of α-tomatine as well as fragments are needed. α-Tomatine **1** consists of a branched tetrasaccharide, β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-D-galactose, attached to O-3 of the steroidal aglycone, tomatidine (Fig. 1). This tetrasaccharide, known as lycotetraose,[11] is a recurrent glycone motif of glycoalkaloids found in a very wide variety of plant and marine species,[12] including members of the *Solanaceae* family[13] such as tomatoes, potatoes and other plants important in the human diet. Preparative enzymatic synthesis of lycotetraose from readily available α-tomatine was recently reported by our laboratory.[14] In this synthesis, the target tetrasaccharide was liberated from the glycoalkaloid by cleavage of the β-galactoside linkage between lycotetraose and tomatidine by a recombinant *endo*-glycosidase from the plant pathogen *Fusarium oxysporum* f. sp. *lycopersici*.[15] Continuing our efforts[16] on synthesis of oligosaccharide components of saponins important for plant defence mechanisms, we describe herein the chemical synthesis of branched methyl lycotetraoside **2**. The flexibility of the route described offers potential for construction of lycotetraose analogues.

Syntheses of lycotetraose[17] and lycotetraose-containing saponin desgalactotigonin[18] have been reported in the literature. Takeo and co-workers[17] synthesised lycotetraose as a reducing



1 **2**

Fig. 1 Structure of α-tomatine **1** and methyl lycotetraoside **2**.

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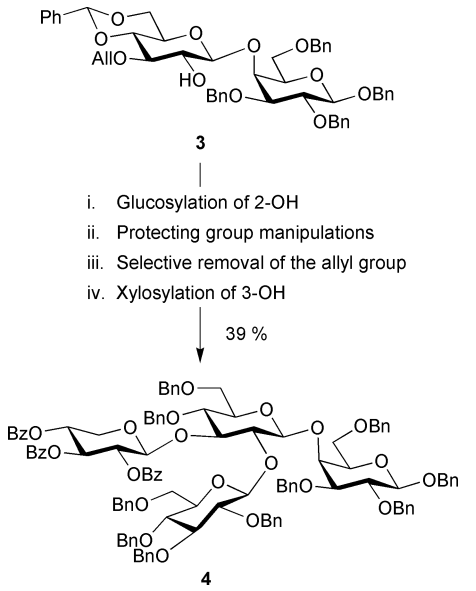
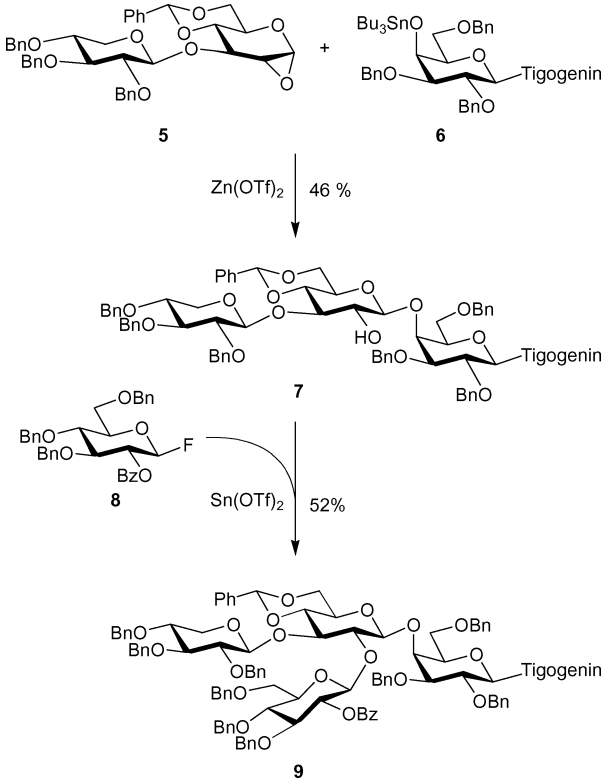
sugar in 12 steps in an overall 13% yield. The key building block in their synthesis was disaccharide derivative **3** (Scheme 1), which was 2-*O*-glycosylated, then 3-*O*-deprotected and 3-*O*-glycosylated to give protected lycotetraoside **4**. Danishefsky and Randolph[18] commenced the synthesis of desgalactotigonin **9** with the preparation of a steroid glycoside in the form of stannyl ether derivative **6**, which served as a glycosyl acceptor in the next step (Scheme 2). Coupling **6** with disaccharide epoxide **5** produced trisaccharide **7** having a conveniently deprotected 2-OH group in the second glucopyranosyl residue. However, glycosylation of this position in compound **7** proved to be difficult; the authors only succeeded with the use of glucopyranosyl fluoride **8**, which was "armed" by the presence of three benzyl protecting groups. Using this reaction scheme, the synthesis of tetrasaccharide saponin **9** was achieved in 13% overall yield, though this yield is calculated for the linear reaction sequence, which does not consider the lengthy syntheses of glycosyl donors **5** and **8**.



Scheme 1 Key glycosylation steps in the synthesis of the lycotetraose by Takeo *et al.*[17]



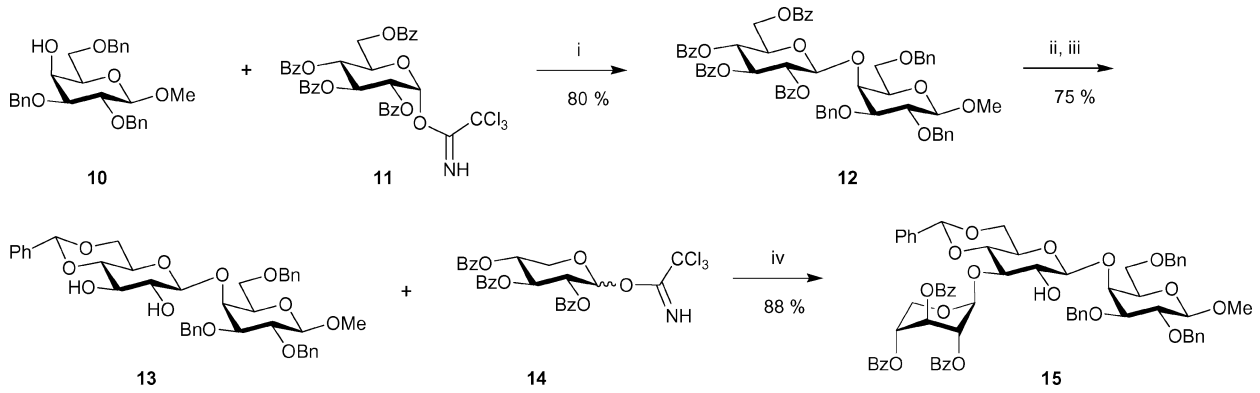
Scheme 2 Key glycosylation steps in the synthesis of desgalactotigonin by Danishefsky *et al.*[18] Tigogenin denotes a steroidal aglycone.

Results and discussion

In our hands, synthesis of methyl lycotetraoside **2** (Fig. 1) began with the construction of β-Glc^1-(1→4)-β-Gal disaccharide unit. Glucosylation of the known galactose derivative **10**[19] with trichloroacetimidate **11**[20] in the presence of TMSOTf in CH_2Cl_2 gave β-linked disaccharide **12** in 80% yield (Scheme 3). De-*O*-benzoylation of **12** with NaOMe in MeOH followed by 4,6-*O*-benzylidenation gave diol **13** in 75% yield over two steps.[21] The relative reactivity of the 2-OH and 3-OH groups in glycosylation of 4,6-*O*-benzylidene derivatives of glucopyranosides is known to be dependent on many factors,[22] amongst which are the nature and anomeric orientation of an aglycone, the method of glycosylation and the structure of the glycosyl donor. With the expectation of higher reactivity of the 3-OH in **13**, which was based on steric considerations, diol **13** was treated with 1.1 equiv. of xylopyranosyl donor **14**[23] in the presence of TMSOTf, producing a single regioisomer, **15**, in 81% yield. The regioselectivity was established by comparison of the ^{1}H NMR spectra of trisaccharide **15** and its 2′-*O*-acetylated derivative. The resonance of H-2′ in the spectra of **15** appeared ~1 ppm upfield of the corresponding signal in the spectra of the 2′-acetate of **15**, thus confirming the presence of a (1→3)-linkage in the trisaccharide. Coupling constants observed for the xylopyranosyl residue ($J_{1,2} = 3.3$ Hz, $J_{2,3} = J_{3,4} = 5.1$ Hz) indicated that the protected trisaccharide **15** has the desired β-configuration, but its pyranose ring adopts an unusual $^1C_4$ conformation instead of the more common $^4C_1$ form. Similar conformational behaviour for benzoylated xylopyranosyl residues has been observed before.[24]

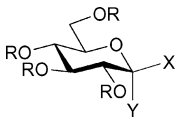
To complete the construction of the target tetrasaccharide, the remaining 2′-OH group in trisaccharide **15** needed to be β-glucosylated. In an attempt to complete this task, a number of readily available acylated glucopyranosyl donors were investigated. Reactions of **15** were carried out with glycosyl bromide **16**, trichloroacetimidates **14** and **17**, thioglycoside **18** and selenoglycoside **19** in the presence of typical promoters (Table 1). However, these attempts failed to produce the desired tetrasaccharide, giving only hydrolysed donor and the unchanged trisaccharide. Apparently, steric hindrance of the 2′-position makes trisaccharide **15** a poor glycosyl acceptor. The reactivity limitations which were observed previously in the glycosylation of trisaccharide acceptor **7** (similar to **15**) were overcome by Danishefsky *et al.* by application of more potent, partly benzylated glucosyl fluoride **8**.[18] However, in our hands, this partly "armed" donor, which required seven steps for its own preparation, did not react satisfactorily with alcohol **15** using conditions described in the literature. Further attempts to glycosylate the 2′-position in trisaccharide **15** were made using readily available fully benzylated thioglucoside **20**,[25] known to be a very reactive "armed" glycosyl donor.[26] This glycosylation was carried out in the presence of MeCN, which is known to provide β-stereoselctivity through the stereoselective kinetic formation of a reactive α-nitrilium ion intermediate.[27] Once again, the route was unsuccessful, in this case because of the complex mixture of products formed in this reaction which made separation impractical.

Because of the lack of reactivity of the 2′-OH group in trisaccharide **15** for glycosylation, an alternative route to the construction of branched-chain tetrasaccharide **2** was investigated. In this route the 2′-OH of the β-Glc^1-(1→4)-β-Gal disaccharide needed to be glycosylated first to produce β-Glc^2-(1→2)-β-Glc^1-(1→4)-β-Gal trisaccharide. Attachment of xylopyranose residue to this trisaccharide would then furnish target branched tetrasaccharide β-Glc^2-(1→2)-[β-Xyl-(1→3)]-β-Glc^1-(1→4)-β-Gal. This approach required a temporary protecting group to be installed in the 3′-position of the β-Glc^1-(1→4)-β-Gal disaccharide, which can be achieved using 3-*O*-*p*-methoxybenzylated



Scheme 3 *Reagents and conditions*: (i) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 1 h; (ii) NaOMe, MeOH, 20 °C, 30 h; (iii) $PhCH(OMe)_2$, TsOH, MeCN, 20 °C, 3 h; (iv) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 1 h.

Table 1 Glycosyl donors used in the attempted glycosylation of trisaccharide **15**



Donor	X	Y	R	Promotor
14	H	$OC(N{=}H)CCl_3$	Bz	TMSOTf
16	H	Br	Bz	AgOTf
17	H	$OC(N{=}H)CCl_3$	Ac	TMSOTf
18	SEt	H	Bz	NIS/TfOH
19	SePh	H	Bz	NIS/TfOH
20	SEt	H	Bn	NIS/TfOH

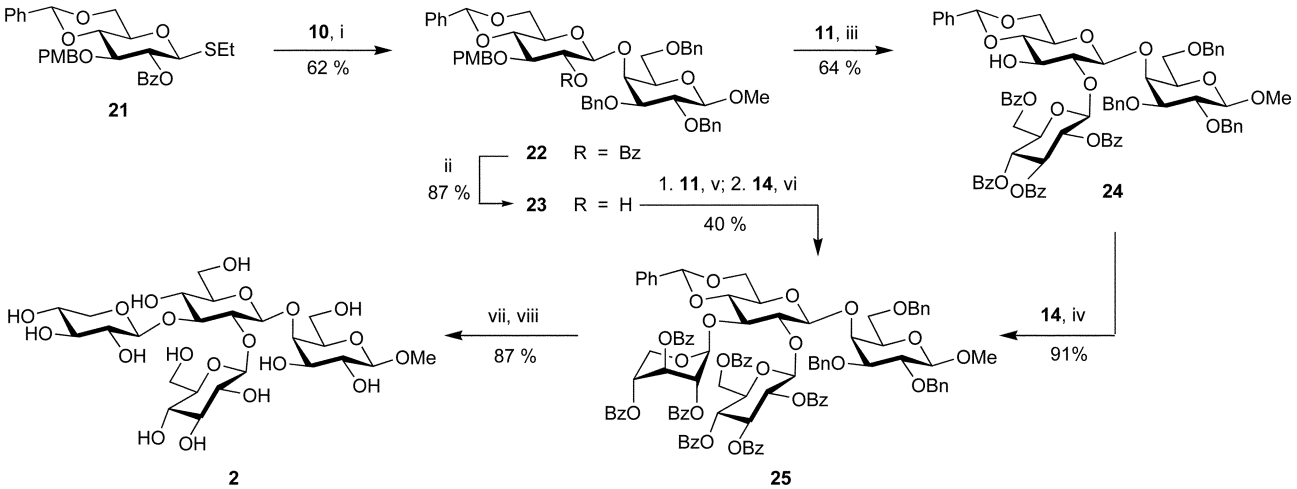
β-glucopyranosyl donor **21**[20b] as a precursor of the β-Glc[I] unit (Scheme 4). Furthermore, it was demonstrated by Nilsson *et al.*[28] that an *O-p*-methoxybenzyl (PMB) group is removable *in situ*, which is useful for "one-pot multistep" assemblies of complex oligosaccharides. Coupling of glycosyl donor **21** with acceptor **10** promoted by NIS/TfOH afforded disaccharide **22** in 62% yield. Disaccharide glycosyl acceptor **23**, prepared in 87% yield by debenzoylation of **22**, was subsequently glycosylated with glucopyranosyl trichloroacetimidate **11** in the presence of TMSOTf. Under these conditions, formation of the trisaccharide was accompanied by a significant loss of the PMB protecting group in the product, giving a partially deprotected trisaccharide **24**. Treatment of the reaction mixture with an excess of TMSOTf led to **24** as the sole trisaccharide product, which was isolated in 64% yield. Finally, glycosylation of **24** with xylopyranosyl donor **14** afforded protected tetrasaccharide **25** in 91% yield.

The possibility of one-pot synthesis of tetrasaccharide **25** starting from disaccharide **23** was also explored. In this approach, glycosylation of **23** with **10** (1.5 equiv.) was carried out in the presence of NIS/TMSOTf (0.2 equiv.) until complete consumption of the glycosyl acceptor, as judged by TLC. Addition of more TMSOTf (0.8 equiv.) allowed complete conversion of the initially formed trisaccharide into **24**. Xylopyranosyl trichloroacetimidate **11** (3.0 equiv.) was finally added to the reaction mixture, affording tetrasaccharide **25**, which was easy to isolate from the mixture, in an overall 40% yield. Deprotection of **25** by catalytic hydrogenation followed by debenzoylation gave the target, methyl lycotetraoside **2**, in an 87% yield.

The structure of tetrasaccharide **2** was confirmed by the presence of four anomeric signals both in ^1H and ^{13}C NMR spectra. On the basis of $J_{H\text{-}1,H\text{-}2}$ coupling constants (7–8 Hz) it can be concluded that in deprotected tetrasaccharide **2** the β-D-xylopyranosyl residue has the expected $^4C_1$ conformation. Overall, NMR data obtained for the synthetic methyl glycoside are consistent with those for authentic lycotetraose.[14,29]

Conclusion

In summary, we have developed a convenient route to the construction of lycotetraose, the saccharide component of plant defence glycoalkaloid α-tomatine, in the form of methyl glycoside. This oligosaccharide, as well its smaller trisaccharide fragments



Scheme 4 *Reagents and conditions*: (i) NIS, TfOH, CH_2Cl_2–Et_2O (1 : 1), 4 Å MS, 0 °C, 1 h; (ii) NaOMe, MeOH, 20 °C, 30 h; (iii) 1. TMSOTf (0.07 equiv.), 4 Å MS, CH_2Cl_2, 0 °C, 1 h, 2. TMSOTf (0.3 equiv.); (iv) TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C; (v) 1. TMSOTf (0.14 equiv.), 4 Å MS, CH_2Cl_2, 0 °C, 30 min, 2. TMSOTf (0.5 equiv.), (vi) TMSOTf, 4 Å MS, CH_2Cl_2, 20 °C, 20 min; (vii) Pd–C, H_2, EtOH, EtOAc, 24 h; (viii) NaOMe, MeOH, 20 °C, 17 h.

which were prepared *en route* to the complete structure, will be useful probes for studying the mechanism of α-tomatine action in protecting plants against pathogenic fungi. The reaction scheme applied for the synthesis of lycotetroside **2** was based on readily available monosaccharide building blocks and led to the target product in 19% yield over eight steps. It was demonstrated that the branched structure of lycotetraose β-Glc^2-(1→2)-[β-Xyl-(1→3)]-β-Glc^1-(1→4)-Gal can only be assembled efficiently by first attaching a β-Glc^2 unit to the 2′-position of β-Glc^1-(1→4)-Gal disaccharide, followed by attaching β-Xyl to the 3′-position of trisaccharide β-Glc^2-(1→2)-β-Glc^1-(1→4)-Gal. The reverse order of glycosylation, involving attachment of β-Glc residue to the 2′-position of readily available trisaccharide β-Xyl-(1→3)-β-Glc^1-(1→4)-Gal, failed because of the very low reactivity of the 2′-position in the glycosyl acceptor based on this trisaccharide. The reaction sequence used for the preparation of lycotetroside **2** is applicable to syntheses of related oligosaccharides, whilst the one-pot double glycosylation approach described offers scope for compound library synthesis.

Experimental

General

Reactions were carried out in dry solvents using septa and syringes for addition of reagents. Dry CH_2Cl_2 and MeCN were prepared by distillation from CaH_2, MeOH was distilled from $Mg(OMe)_2$ and stored over 4 Å molecular sieves. Cation-exchange resins were washed with water and dry MeOH before use. TLC was performed on precoated aluminium plates (Silica Gel 60 F_{254}, Merck). Spots were visualized by exposure to UV light or by immersion in 5% ethanolic H_2SO_4 followed by heating to 150 °C. Solutions of reaction products were dried over $MgSO_4$ and solvents were evaporated under reduced pressure at 25–40 °C. Column chromatography was performed on silica gel (40–70 μm, BDH-Merck). Optical rotations were measured at 25 °C using a Perkin–Elmer 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 24 °C with a Varian Unity Plus spectrometer at 400 and 100 MHz, respectively, using TMS (for solution in $CDCl_3$) or Me_2CO (δ 49.9, for solutions in D_2O) as internal standards. Resonance assignments were made with the aid of gCOSY and gHSQC experiments. In cases where spectral dispersion was poor, only selected diagnostic NMR data are given; other spectral features were in accord with the proposed structures. Accurate electrospray ionisation mass spectra (HR ESI-MS) were obtained using positive ionization mode on a Finnigan MAT 900 XLT mass spectrometer. For compounds with molecular masses over 1000, low resolution ESI-MS were obtained on the same instrument and experimental data were matched to theoretical isotope patterns.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-galactopyranoside (12). Galactoside acceptor **10**[19] (0.47 g, 1.01 mmol), glucosyl trichloroacetimidate **11**[20] (1.13 g, 1.52 mmol), and 4 Å MS (1.5 g) were stirred in CH_2Cl_2 (10 ml) at room temperature for 1 h. The reaction mixture was cooled to 0 °C, TMSOTf (18 μl, 0.10 mmol) was added and the reaction was left stirring for 1 h. Et_3N (0.5 ml) was added, the mixture was filtered through Celite, diluted with CH_2Cl_2 (15 ml), washed with water (3 × 10 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (toluene–EtOAc 12 : 1 → 9 : 1) gave disaccharide **12** as a colourless oil (0.85 g, 80%); $[\alpha]_D$ +35 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.26 (1 H, dd, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.7 Hz, H-2 Gal), 3.43 (1 H, dd, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.8 Hz, H-3 Gal), 3.49 (3 H, s, OMe), 3.50 (1 H, dd, $J_{5,6b}$ = $J_{5,6b}$ = 5.6 Hz, H-5 Gal), 3.66–3.71 (2 H, m, H-6a Gal, OCH_2Ph), 3.80 (1 H, dd, $J_{5,6b}$ = 5.6 Hz, $J_{6a/6b}$ = 10.1 Hz, H-6b Gal), 4.01–4.06 (2 H, m, H-4 Gal, H-5 Glc), 4.17 (1 H, d, $J_{1,2}$ = 7.7 Hz, H-1 Gal), 4.32 (1 H, d, J_{gem} = 10.8 Hz, OCH_2Ph), 4.41–4.47 (2 H, m, H-6a Glc), 4.50 (1 H, d, J_{gem} = 11.7 Hz, OCH_2Ph), 4.58 (1 H, dd, $J_{5,6b}$ = 3.3 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6b Glc), 4.62 (1 H, d, J_{gem} = 11.9 Hz, OCH_2Ph), 4.67 (1 H, d, J_{gem} = 11.9 Hz, OCH_2Ph), 5.24 (1 H, d, $J_{1,2}$ = 8.0 Hz, H-1 Glc), 5.57 (1 H, dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.8 Hz, H-2 Glc), 5.72 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4 Glc), 5.92 (1 H, t, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3 Glc), 7.04–7.58 (27 H, m, Ph), 7.84–8.07 (8 H, m, Ph); δ_C (100 MHz, $CDCl_3$): 57.3 (OMe), 62.9 (C-6 Glc), 69.8 (C-4 Glc), 69.8 (C-6 Gal), 72.2 (C-5 Glc), 72.4 (C-2 Glc), 73.1 (C-3 Glc), 73.6 (C-5 Gal), 73.7, 73.8 (O*C*H_2Ph × 2), 74.9, 75.0 (C-4 Gal, O*C*H_2Ph), 79.9 (C-2 Gal), 81.3 (C-3 Gal), 101.9 (C-1 Glc), 104.8 (C-1 Gal), 125.6–130.4, 133.3–133.7, 138.1–138.9 (4 × quat. Ph), 165.4–166.3 (4 × CO); ESI MS: found *m*/*z* 1060.4 $[M + NH_4]^+$ calcd. for $C_{62}H_{62}O_{15}N$ 1060.6.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-β-D-glucopyranosyl)-β-D-galactopyranoside (13). To a solution of protected disaccharide **12** (0.85 g, 0.815 mmol) in MeOH (10 ml) was added sodium (10 mg), and the mixture was stirred at room temperature for 30 h. The solution was neutralised with Amberlite IR-120 (H^+), filtered and the filtrate concentrated *in vacuo*. The residue was dissolved in MeCN (10 ml), treated with benzaldehyde dimethyl acetal (0.18 ml, 1.22 mmol) and *p*-toluenesulfonic acid (~25 mg) and stirred at room temperature for 3 h. The mixture was then neutralised with Et_3N, dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (hexane–EtOAc 5 : 1 → 2 : 1) gave diol **12** as a colourless oil (0.46 g, 75%); $[\alpha]_D$ −4 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.33 (1 H, m, H-5 Glc), 3.47–3.63 (8 H, m, H-3 Gal, H-5 Gal, H-6a Gal, H-2 Glc, H-4 Glc, OMe), 3.69–3.81 (H-2 Gal, H-6b Gal, H-3 Glc, H-6a Glc), 3.95 (1 H, d, $J_{4,5}$ = 3.1 Hz, H-4 Gal), 4.10 (1 H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b Glc), 4.25 (1 H, d, $J_{1,2}$ = 7.7 Hz, H-1 Gal), 4.46–4.53 (3 H, m, H-1 Glc, OCH_2Ph), 4.69 (1 H, d, J_{gem} = 11.0 Hz, OCH_2Ph), 4.71 (1 H, d, J_{gem} = 12.0 Hz, OCH_2Ph), 4.80 (1 H, d, J_{gem} = 12.0 Hz, OCH_2Ph), 4.90 (1 H, d, J_{gem} = 11.0 Hz, OCH_2Ph), 5.50 (1 H, s, C*H*Ph), 7.23–7.49 (20 H, m, Ph); δ_C (100 MHz, $CDCl_3$): 57.3 (OMe), 66.8 (C-5 Glc), 68.5 (C-6 Gal), 68.8 (C-6 Glc), 72.7, 73.6 (O*C*H_2Ph), 73.7, 74.3 (O*C*H_2Ph), 75.4 (O*C*H_2Ph), 75.7, 78.3, (C-4 Gal), 79.9, 80.5, 102.1 (C-1 Glc), 105.0 (C-1 Gal), 106.1 (*C*HPh), 126.2–129.5 (Ph), 137.2–138.6 (4 × quat. Ph); HR ESI MS: found *m*/*z* 732.3371 $[M + NH_4]^+$ calcd. for $C_{41}H_{50}O_{11}N$ 732.3378.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranoside (15). Disaccharide **12** (0.15 g, 0.21 mmol), xylosyl imidate **14**[23] (0.16 g, 0.270 mmol) and 4 Å MS (0.30 g) were stirred in CH_2Cl_2 (30 ml) for 1 h at room temperature. The reaction mixture was cooled to 0 °C, TMSOTf (2 μl, 0.01 mmol) was added and the reaction was left stirring for 1 h. Et_3N (0.2 ml) was added and the flask was allowed to warm to room temperature. The mixture was filtered through Celite, diluted with CH_2Cl_2 (15 ml), washed with water (3 × 20 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (hexane–EtOAc 6 : 1 → 3 : 1) gave trisaccharide **15** as a colourless oil (0.21 g, 88%); $[\alpha]_D$ −33 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.36 (1 H, td, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 5.0 Hz, H-5 Glc), 3.44–3.80 (12 H, m, OMe, H-2 Gal, H-3 Gal, H-5 Gal, H-6a Gal, H-6b Gal, H-2 Glc, H-3 Glc, H-6a Glc, H-5a Xyl), 3.91 (1 H, d, *J* = 3.1 Hz, H-4 Gal), 4.10 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ = 9.0 Hz, H-4 Glc), 4.17 (1 H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b Glc), 4.24 (1 H, d, $J_{1,2}$ = 7.5 Hz, H-1 Gal), 4.42 (1 H, d, $J_{1,2}$ = 7.7 Hz, H-1 Glc), 4.45–4.53 (2 H, m, OCH_2Ph), 4.61–4.73 (3 H, m, H-5b Xyl, OCH_2Ph), 4.77 (1 H, d, J_{gem} = 11.9 Hz, OCH_2Ph), 4.89 (1 H, d, J_{gem} = 11.9 Hz, OCH_2Ph), 5.17–5.20 (1 H, m, H-4 Xyl), 5.40 (1 H, dd, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 5.1 Hz, H-2 Xyl), 5.50 (1 H, d, $J_{1,2}$ = 3.3 Hz, H-1 Xyl), 5.54 (1 H, s, PhC*H*), 5.64 (1 H, t, $J_{2,3}$ = 5.1 Hz, H-3 Xyl), 7.14–7.55 (29 H, m, Ph), 8.00–8.08 (6 H, m, Ph); δ_C (100 MHz, $CDCl_3$): 57.2 (OMe), 59.7 (C-5 Xyl), 67.0 (C-5 Glc), 68.4, 68.6, 68.6, 68.9, 69.2 (C-6 Gal, C-6 Glc, C-1 Xyl, C-2 Xyl, C-3 Xyl),

72.6, 74.3, 75.5 (3 × O*C*H$_2$Ph), 76.6, 77.5, 78.7, 79.2, 80.0, 80.3, 98.7 (C-1 Xyl), 101.7 (*C*HPh), 104.9 (C-1 Gal), 106.6 (C-1 Glc), 126.1–130.3, 133.4–133.6 (3 × quat. Ph), 137.2–138.6 (4 × quat. Ph), 165.2–165.9 (3 × CO); ESI MS: found *m*/*z* 1181.6 [M + Na]$^+$ calcd. for $C_{67}H_{66}O_{18}Na$ 1181.4.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-*O*-benzoyl-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-β-D-galactopyranoside (22). Thioglycoside **21**[20b] (0.25 g, 0.466 mmol), alcohol **10**[19] (0.23 g, 0.489 mmol) and 4 Å MS (0.50 g) were stirred in CH_2Cl_2 (10 ml) at room temperature for 1 h and the mixture was cooled to 0 °C. A stock-solution of NIS/cat. TfOH was prepared by the addition of TfOH (20 µL, 226 µmol) to NIS (0.46 g, 2.04 mmol) in CH_2Cl_2–Et_2O (1 : 1, 20 ml). A portion of this solution (4.7 ml), was added to the flask and the mixture was stirred for 1 h at 0 °C. Et_3N (0.2 ml) was added, the reaction mixture was allowed to warm to room temperature, filtered through Celite, diluted with EtOAc (20 ml), washed with 10% aq. $Na_2S_2O_3$ (20 ml), 10% aq. $NaHCO_3$ (20 ml) and water (20 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (toluene–EtOAc 15 : 1) gave disaccharide **22** as an oil (0.27 g, 62%); $[\alpha]_D$ +47 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.23 (1 H, dd, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2 Gal), 3.36 (1 H, dd, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.8 Hz, H-3 Gal), 3.40–3.49 (5 H, m, H-5 Gal, H-5 Glc, OMe), 3.55 (1 H, d, J_{gem} = 11.0 Hz, OC*H*$_2$Ph), 3.64 (1 H, dd, $J_{5,6a}$ = 5.3 Hz, $J_{6a,6b}$ = 9.3 Hz, H-6a Gal), 3.69 (3 H, s, OMe), 3.71–3.81 (4 H, m, H-6b Gal, H-6a Glc, H-3 Glc, H-4 Glc), 4.03 (1 H, d, $J_{3,4}$ = 2.8 Hz, H-4 Gal), 4.14 (1 H, d, $J_{1,2}$ = 7.6 Hz, H-1 Gal), 4.21–4,27 (2 H, m, H-6b Glc, OC*H*$_2$Ph), 4.54 (2 H, s, OC*H*$_2$Ph), 4.55 (2 H, s, OC*H*$_2$Ph), 4.66 (1 H, d, J_{gem} = 11.9, OC*H*$_2$Ph), 4.73 (1 H, d, J_{gem} = 11.9, OC*H*$_2$Ph), 4.99 (1 H, d, $J_{1,2}$ = 8.1 Hz, H-1 Glc), 5.27 (1 H, dd, $J_{1,2}$ = $J_{2,3}$ = 8.1, H-2 Glc), 5.60 (1 H, s, C*H*Ph), 6.51 (2 H, d, $J_{A,B}$ = 8.8 Hz, $CH_2C_6H_4OMe$), 7.01–7.54 (25 H, m, Ph), 7.89 (2 H, d, $J_{A,B}$ = 8.8 Hz, $CH_2C_6H_4OMe$); δ_C (100 MHz, $CDCl_3$): 55.0 (OMe), 58.5 (OMe), 66.2 (C-5 Glc), 68.7 (C-6 Glc), 69.1 (C-6 Gal), 73.1, 73.4, 73.5 (× 2) (3 × O*C*H$_2$Ph), 73.7 (C-2 Glc), 74.1 (C-4 Gal), 74.6, 77.3, 79.4 (C-2 Gal), 81.3 (C-3 Gal), 81.7, 101.3 (*C*HPh), 102.3 (C-1 Glc), 104.5 (C-1 Gal), 113.6 ($CH_2C_6H_4OMe$), 126.1–130.3 (Ph), 132.8, 137.4–138.9 (quat. Ph), 159.2 ($CH_2C_6H_4OMe$), 165.0 (CO); HR ESI MS: found *m*/*z* 956.4216 [M + NH$_4$]$^+$ calcd. for $C_{56}H_{62}O_{13}N$ 956.4218.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-β-D-glucopyranosyl)-β-D-galactopyranoside (23). To a solution of protected disaccharide **22** (0.35 g, 0.373 mmol) in MeOH (6 ml) was added sodium (~10 mg), and the solution was stirred at room temperature for 30 h. The solution was neutralized with Amberlite IR-120 (H$^+$), filtered and the filtrate was concentrated *in vacuo* to afford the alcohol **23** as a colourless oil (0.27 g, 87%); $[\alpha]_D$ +25 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.20–3.26 (1 H, m, H-5 Glc), 3.38–3.58 (8 H, m, H-3 Gal, H-5 Gal, H-6a Gal, H-3 Glc, H-4 Glc, OMe), 3.61–3.66 (3 H, m, H-2 Gal, H-2 Glc, H-6a Glc), 3.67–3.74 (4 H, m, H-6b Gal, OMe), 3.87 (1 H, d, $J_{3,4}$ = 2.9 Hz, H-4 Gal), 4.01–4.09 (1 H, m, H-6b Glc), 4.18 (1 H, d, $J_{1,2}$ = 7.7 Hz, H-1 Gal), 4.36 (1 H, d, $J_{1,2}$ = 7.5 Hz, H-1 Glc), 4.40–4.46 (2 H, m, OC*H*$_2$Ph), 4.60–4.67 (2 H, m, OC*H*$_2$Ph), 4.75 (2 H, s, OC*H*$_2$Ph), 4.75–4.84 (2 H, m, OC*H*$_2$Ph), 5.45 (1 H, s, C*H*Ph), 6.76 (2 H, d, $J_{A,B}$ = 8.8 Hz, $CH_2C_6H_4OMe$), 7.16–7.41 (22 H, m, Ph); δ_C (100 MHz, $CDCl_3$): 55.5 (OMe), 57.3 (OMe), 66.9 (C-5 Glc), 68.5 (C-6 Gal), 68.9 (C-6 Glc), 72.7, 73.6 (O*C*H$_2$Ph), 74.2 (O*C*H$_2$Ph), 74.3 (O*C*H$_2$Ph), 75.6 (O*C*H$_2$Ph), 75.9, 78.3 (C-4 Gal), 80.1, 80.2, 80.6, 80.9, 101.5 (*C*HPh), 105.0 (C-1 Gal), 106.6 (C-1 Glc), 113.9 ($CH_2C_6H_4OMe$), 126.3–131.9 (Ph), 137.5–138.7 (quat. Ph), 159.4 ($CH_2C_6H_4OMe$); HR ESI MS: found *m*/*z* 852.3954 [M + NH$_4$]$^+$ calcd. for $C_{49}H_{54}O_{12}N$ 852.3954.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranoside (24). Trichloroacetimidate **11**[20] (0.11 g, 0.149 mmol), disaccharide **23** (83 mg, 0.10 mmol) and 4 Å MS (0.20 g) were stirred in CH_2Cl_2 (5 ml) at room temperature for 1 h. The reaction mixture was cooled to 0 °C, TMSOTf (2 µl, 0.01 mmol) was added and the reaction was stirred until TLC (EtOAc–hexane 1 : 1) showed the complete disappearance of **23** (~1 h). TMSOTf (8 µl, 0.04 mmol) was added and stirring continued until the major product (R_f = 0.66) disappeared and a new product (R_f = 0.32) was detected by TLC. Et_3N (0.2 ml) was added, the mixture was filtered through Celite, diluted with CH_2Cl_2 (15 ml), washed with water (3 × 10 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (hexane–EtOAc 5 : 1 → 2 : 1) gave trisaccharide **24** as a colourless oil (83 mg, 64%); $[\alpha]_D$ −11 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 3.19 (1 H, m, H-5 Glc^1), 3.42 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ = 8.9 Hz, H-4 Glc^1), 3.44–3.60 (7 H, m, H-6a Glc^1, H-3 Gal, H-5 Gal, H-6a Gal, OMe), 3.64–3.75 (3 H, m, H-6b Gal, H-2 Glc^1, H-3 Glc^1), 4.04–4.27 (3 H, m, H-2 Gal, H-6b Glc^1, H-5 Glc^2), 4.20 (1 H, d, $J_{3,4}$ = 2.6 Hz, H-4 Gal), 4.30 (1 H, d, $J_{1,2}$ = 7.7 Hz, H-1 Gal), 4.44 (2 H, s, OC*H*$_2$Ph), 4.61 (1 H, dd, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a Glc^2), 4.65 (1 H, dd, $J_{5,6b}$ = 3.7 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6b Glc^2), 4.66 (1 H, d, J_{gem} = 12.3 Hz, OC*H*$_2$Ph), 4.91 (1 H, d, J_{gem} = 12.3 Hz, OC*H*$_2$Ph), 5.03 (1 H, d, J_{gem} = 11.0 Hz, OC*H*$_2$Ph), 5.09 (1 H, d, J_{gem} = 11.0 Hz, OC*H*$_2$Ph), 5.12 (1 H, d, $J_{1,2}$ = 6.8 Hz, H-1 Glc^1), 5.32 (1 H, d, $J_{1,2}$ = 7.9 Hz, H-1 Glc^2), 5.42 (1 H, s, C*H*Ph), 5.56 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4 Glc^2), 5.58 (1 H, dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.5 Hz, H-2 Glc^2), 5.89 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3 Glc^2), 7.19–7.58 (32 H, m, Ph), 7.75–7.77 (2 H, m, Ph), 7.86–7.89 (Ph), 7.97–8.01 (4 H, m, Ph); δ_C (100 MHz, $CDCl_3$): 57.3, 64.3 (C-6 Glc^2), 65.7 (C-5 Glc^1), 68.8 (C-6 Glc^1), 69.5 (C-6 Gal), 70.6, 71.5, 72.4, 73.0, 73.4, 73.6, 73.9, 75.4, 79.6, 81.1, 81.7, 82.5, 100.6 (C-1 Glc^1), 101.3 (C-1 Glc^2), 102.1 (*C*HPh), 105.4 (C-1 Gal), 126.5–133.4 (Ph), 133.08–133.4 (Ph), 137.1, 138.5–139.3 (4 × quat. Ph), 165.4 (2 × CO), 166.0 (CO), 166.3 (CO); ESI MS: found *m*/*z* 1315.5 [M + Na]$^+$ calcd. for $C_{75}H_{72}O_{20}Na$ 1315.5.

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-(4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-3-*O*-(2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl)-β-D-glucopyranosyl)-β-D-galactopyranoside (25).

Method A. Trisaccharide **24** (67 mg, 0.052 mmol), imidate **14** (62 mg, 0.104 mmol) and 4 Å MS (0.12 g) were stirred in CH_2Cl_2 (4 ml) at room temperature for 1 h. The reaction mixture was cooled to 0 °C, TMSOTf (2 µl, 0.01 mmol) was added and the reaction was stirred for 1 h at this temperature. Et_3N (0.2 ml) was added, the mixture was diluted with CH_2Cl_2 (15 ml), filtered through Celite, washed with water (3 × 10 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Column chromatography (toluene–EtOAc 17 : 1) gave tetrasaccharide **25** as a colourless oil (82 mg, 91%); $[\alpha]_D$ −3 (*c* 1.00, $CHCl_3$); δ_H (400 MHz, $CDCl_3$): 2.67 (1 H, dd, $J_{4,5a}$ = 7.5 Hz, $J_{5a,5b}$ = 12.1 Hz, H-5a Xyl), 2.89–3.01 (1 H, m, H-5 Glc^2), 3.21–3.28 (1 H, m, H-5 Glc^1), 3.44–3.69 (9 H, m, H-3 Gal, H-5 Gal, H-6a Gal, H-6b Gal, H-4 Glc^1, H-6a Glc^1, OMe), 3.87 (1 H, dd, $J_{1,2}$ = 7.3 Hz, $J_{2,3}$ = 9.2 Hz, H-2 Glc^1), 3.99 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3 Glc^1), 4.09 (1 H, s, H-4 Gal), 4.12–4.23 (3 H, m, H-2 Gal, H-6b Glc^1, H-5b Xyl), 4.23 (2 H, s, OC*H*$_2$Ph), 4.33 (1 H, d, $J_{1,2}$ = 7.1 Hz, H-1 Gal), 4.41 (1 H, dd, $J_{5,6b}$ = 3.7 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a Glc^2), 4.58–4.66 (2 H, m, H-6b Glc^2, OC*H*$_2$Ph), 4.92–4.98 (2 H, m, H-1 Glc^2, H-1 Xyl), 4.99 (1 H, d, J_{gem} = 12.1 Hz, OC*H*$_2$Ph), 5.13 (1 H, d, $J_{1,2}$ = 7.3 Hz, H-1 Glc^1), 5.16–5.24 (3 H, m, H-4 Xyl, OC*H*$_2$Ph), 5.48–5.60 (2 H, m, H-2 Xyl, C*H*Ph), 5.58 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4 Glc^2), 5.72–5.81 (3 H, m, H-2 Glc^2, H-3 Glc^2, H-3 Xyl), 7.01–8.21 (55 H, m, Ph); δ_C (100 MHz, $CDCl_3$): 57.4 (OMe), 61.2 (C-5 Xyl), 64.5 (C-6 Glc^2), 66.1 (C-5 Glc^1), 69.1 (C-6 Glc^1), 69.6 (C-6 Gal), 69.8 (C-4 Xyl), 71.0, 71.2, 71.5, 71.6, 71.8, 73.2 (× 2), 73.3, 73.5 (×2 O*C*H$_2$Ph), 76.1 (O*C*H$_2$Ph), 77.5, 79.1, 79.3, 80.4, 82.3, 99.5 and 99.7 (C-1 Xyl and C-1 Glc^2), 100.6 (C-1 Glc), 101.3 (*C*HPh), 105.2 (C-1 Gal), 126.2–130.3, 133.2–133.9, 137.4,

138.6, 139.1, 139.3 (Ph), 163.8–166.0 (7 × CO); ESI MS: found m/z 1760.6 $[M + Na]^+$ calcd. for $C_{101}H_{92}O_{27}Na$ 1760.6.

Method B. Disaccharide **23** (40 mg, 0.048 mmol), trichloroacetimidate **11** (53 mg, 0.072 mmol) and 4 Å MS (0.10 g) were stirred in CH_2Cl_2 (4 ml) at room temperature for 1 h. TMSOTf (2 µl, 0.01 mmol) was added at 0 °C, the reaction was stirred for 30 min, a second portion of TMSOTf (8 µl, 0.04 mmol) added, and the reaction stirred until TLC (EtOAc–hexane 1 : 1) indicated complete removal of the *p*-methoxybenzyl group, as described in the synthesis of **24**. A solution of xylopyranosyl donor **14** (58 mg, 0.096 mmol) in CH_2Cl_2 (1 ml) was added, the reaction was stirred at room temperature for 20 min, treated with Et_3N (0.5 ml), diluted with CH_2Cl_2 (15 ml) and filtered through Celite. The filtrate was washed with water (3 × 10 ml), dried ($MgSO_4$) and concentrated *in vacuo*. Purification of the residue by chromatography (toluene–EtOAc 17 : 1) gave the tetrasaccharide **25** as a colourless oil (33 mg, 40%).

Methyl 4-*O*-[2-*O*-(β-D-Glucopyranosyl)-3-*O*-(β-D-xylopyranosyl)-β-D-glucopyranosyl]-β-D-galactopyranoside (2). A solution of protected tetrasaccharide **25** (0.18 g, 0.104 mmol) in EtOH–EtOAc (1 : 1, 10 ml) was hydrogenated over 10% Pd/C (20 mg) for 17 h at room temperature, the mixture filtered through Celite and concentrated *in vacuo*. The residue was dissolved in MeOH (10 ml) and NaOMe (20 mg) added. The mixture was stirred for 24 h at room temperature, neutralized with Amberlite IR-120 (H^+), filtered and concentrated *in vacuo*. The residue was purified by filtration of the aqueous solution to afford methyl lycotetraoside **2** (45 mg, 87%); $[\alpha]_D$ −11 (*c* 1.00, MeOH); δ_H (400 MHz, D_2O): 3.55 (3 H, s, OMe), 4.31 (1 H, d, $J_{1,2}$ = 7.3 Hz, H-1), 4.69 (1 H, d, $J_{1,2}$ = 7.5 Hz, H-1), 4.72 (1 H, d, $J_{1,2}$ = 7.5 Hz, H-1), 5.00 (1 H, d, $J_{1,2}$ = 7.9 Hz, H-1); δ_C (100 MHz, D_2O): 57.9 (OMe), 61.3 (C-6), 61.5 (C-6), 61.8 (C-6), 65.9, 68.7, 70.0, 70.7, 72.3, 74.1, 74.3, 74.5, 75.0, 76.1, 76.4, 76.6, 77.3, 79.3, 80.2, 85.1, 102.7 (C-1), 103.5 (2 × C-1), 104.5 (C-1); HR ESI MS: found m/z 673.2163 $[M + Na]^+$ calcd. for $C_{24}H_{42}O_{20}Na$ 673.2162.

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References and notes

1 K. Hostettmann and A. Martson, *Saponins*, Cambridge University Press, Cambridge, UK, 1995.
2 M. Iorizzi, S. D. Marino and F. Zollo, *Curr. Org. Chem.*, 2001, **5**, 951–973.
3 *Saponins used in traditional and modern medicine*, ed. G. R. Waller and K. Yamasaki, Plenum Press, New York, 1996.
4 H. Pellissier, *Tetrahedron*, 2004, **60**, 5123–5162.
5 G. R. Fenwick, K. R. Price, C. Tsukamoto and K. Okubo, 'Saponins', in: *Toxic substances in crop plants*, ed. J. P. F. D'Mello, C. M. Duffus and J. H. Duffus, Royal Society of Chemistry, Cambridge, UK, 1991, pp. 285–327.
6 (*a*) E. A. J. Keukens, T. de Vrije, C. van den Boom, P. de Waard, H. H. Plasman, F. Thiel, V. Chupin, W. M. F. Jongen and B. de Kruijff, *Biochim. Biophys. Acta*, 1995, **1240**, 216–228; (*b*) E. A. J. Keukens, T. de Vrije, L. A. M. Jansen, H. de Boer, M. Janssen, A. I. P. M. de Kroon, W. M. F. Jongen and B. de Kruijff, *Biochim. Biophys. Acta*, 1996, **1279**, 243–250.
7 T. D. Fontaine, G. W. Irving, R. Ma, J. B. Poole and S. P. Doolittle, *Arch. Biochem.*, 1948, **18**, 467–475.
8 J. Roddick, *Phytochemistry*, 1974, **13**, 9–25.
9 (*a*) J. P. Morrissey and A. E. Osbourn, *Microbiol. Mol. Biol. Rev.*, 1999, **63**, 708–724; (*b*) A. Osbourn, *Trends Plant Sci.*, 1996, **1**, 4–9; (*c*) M. Ruiz-Rubio, A. Pérez-Espinosa, K. Lairini, T. Roldán-Arjona, A. Dipietro and N. Anaya, in: *Studies in natural products chemistry*, ed. Atta-ur-Rahman, Elsevier, Oxford, UK, 2001, vol. 25, pp. 293–326.
10 (*a*) K. Bouarab, R. Melton, J. Peart, D. Baulcombe and A. Osbourn, *Nature*, 2002, **418**, 889–892; (*b*) S. Ito, T. Eto, S. Tanaka, N. Yamauchi, H. Takahara and T. Ikeda, *FEBS Lett.*, 2004, **571**, 31–34.
11 R. Kuhn, I. Low and H. Trischmann, *Chem. Ber.*, 1957, **90**, 203–218.
12 110 saponins incorporating lycotetraose attached to O-3 of a steroidal aglycon are registered in the *Chem. Abstr.* database (May 2005).
13 (*a*) M. Friedman and G. M. McDonald, *Crit. Rev. Plant Sci.*, 1997, **16**, 55–132; (*b*) M. Friedman, *J. Agric. Food Chem.*, 2002, **50**, 5751–5780.
14 K. Woods, C. J. Hamilton and R. A. Field, *Carbohydr. Res.*, 2004, **339**, 2325–2328.
15 T. Roldán-Arjona, A. Pérez-Espinosa and M. Ruiz-Rubio, *Mol. Plant-Microbe Interact.*, 1999, **12**, 852–861.
16 B. Mukhopadhyay and R. A. Field, *Carbohydr. Res.*, 2004, **339**, 1285–1291.
17 K. Takeo, T. Nakaji and K. Shinmitsu, *Carbohydr. Res.*, 1984, **133**, 275–287.
18 (*a*) J. T. Randolph and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1993, **115**, 8473–8474; (*b*) J. T. Randolph and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1995, **117**, 5693–5700.
19 M. Sakagami and H. Hamana, *Tetrahedron Lett.*, 2000, **41**, 5547–5551.
20 (*a*) R. R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1984, 1343–1357; (*b*) R. Verduyn, M. Douwes, P. A. M. van der Klein, E. M. Mosinger, G. A. van der Marel and J. H. van Boom, *Tetrahedron*, 1993, **49**, 7301–7316.
21 Removal of *O*-benzoyl groups in tetrabenzoate **12** by Zemplen deacylation was unusually slow, requiring 30 h for completion. This slow de-*O*-benzoylation may be a consequence of the increased stability of a 2-*O*-benzoyl group on a galactopyranosyl residue. Similar behaviour of 2-*O*-acyl protecting groups was noticed previously,[17] in some cases removal of 2-benzoates being impossible, see: S. Deng, B. Yu, Y. Hui, H. Yu and X. Han, *Carbohydr. Res.*, 1999, **317**, 53–62.
22 (*a*) H. B. Mereyala, S. Bhavani and A. P. Rudradas, *Synth. Commun.*, 2004, **34**, 1057–1063; (*b*) B. Fraser-Reid, J. C. Lopez, K. V. Radhakrishnan, M. Mach, U. Schlueter, A. M. Gomez and C. Uriel, *J. Am. Chem. Soc.*, 2002, **124**, 3198–3199; (*c*) Y. Zeng and F. Z. Kong, *Carbohydr. Res.*, 2003, **338**, 843–849; (*d*) P. G. Evans, H. M. I. Osborn and W. G. Suthers, *Tetrahedron Lett.*, 2002, **43**, 7855–7857; (*e*) Y. Zeng, J. Ning and F. Kong, *Tetrahedron Lett.*, 2002, **43**, 3729–3733.
23 Xylopyranose trichloroacetimidate **14** was prepared as an anomeric mixture, the α-anomer adopting the $^1C_4$ conformation and the β-anomer the $^4C_1$ conformation: L. Q. Chen and F. Z. Kong, *Carbohydr. Res.*, 2002, **337**, 2335–2341.
24 (*a*) G. Gu, Y. Du and R. J. Linhardt, *J. Org. Chem.*, 2004, **69**, 5497–5500; (*b*) K. Ple, M. Chwalek and L. Voutquenne-Nazabadioko, *Tetrahedron*, 2005, **61**, 4347–4362.
25 G. H. Veeneman and J. H. van Boom, *Tetrahedron Lett.*, 1990, **31**, 275–278.
26 B. Fraser-Reid, Z. F. Wu, U. E. Udodong and H. Ottosson, *J. Org. Chem.*, 1990, **55**, 6068–6070.
27 (*a*) I. Braccini, C. Derouet, J. Esnault, C. Hervé du Penhoat, J.-M. Mallet, V. Michon and P. Sinaÿ, *Carbohydr. Res.*, 1993, **246**, 23–41; (*b*) K. Fukase, A. Hasuoka, I. Kinoshita, Y. Aoki and S. Kusumoto, *Tetrahedron*, 1995, **51**, 4923–4932.
28 S. Bhattacharyya, B. G. Magnusson, U. Wellmar and U. J. Nilsson, *J. Chem. Soc., Perkin Trans. 1*, 2001, 886–890.
29 W. Willker and D. Leibfritz, *Magn. Reson. Chem.*, 1992, **30**, 645–650.