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Note

Synthesis of fluorescently labelled rhamnosides: probes for the evaluation of rhamnogalacturonan II biosynthetic enzymes

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Dedicated to Professor András Lipták on the occasion of his 75th birthday

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ABSTRACT

Three fluorescently labelled saccharides **10**–**12**, representing structures found in pectic glycan rhamnogalacturonan II (RG-II), were synthesised by chemical glycosylation of *O*-6 of diacetone-D-galactose followed by deprotection and reductive amination with amino-substituted fluorophore APTS. This convenient method installs a common aminogalactitol-based tether in order to preserve the integrity of the reducing end of specific carbohydrates of interest. APTS-labelled glycans prepared in this manner were purified by carbohydrate gel electrophoresis and subjected to capillary electrophoresis analysis, as a basis for the subsequent development of high sensitivity assays for RG-II-active enzymes.

Despite the ever-expanding repertoire of plant genome sequences and the essential role of carbohydrates in plant cell wall architecture,[1] detailed knowledge of the biosynthetic machinery required for cell wall polysaccharide biosynthesis is at best patchy and in places non-existent.[2] Much remains to be done in order to confirm the biological function of the ca. 2000 gene products[2] required for cell wall assembly. In terms of polysaccharide biosynthesis, in the model plant *Arabidopsis thaliana* an excess of 200 glycosyltransferases (GTs)[3] are probably required. However, across all plants GT substrate specificities have only been convincingly demonstrated for a dozen or so to date.[1d] A major bottle-neck lies in the lack of available oligosaccharide acceptor substrates and radiolabelled sugar nucleotide donor substrates. Addressing this issue, we have prepared a series for rhamnogalacturonan II (RG-II) fragments as methyl glycosides,[4] to enable assays with radiolabelled donor substrates. However, only a few relevant sugar nucleotides are readily accessible in radiolabelled form. We therefore, sought a complementary approach, based on acceptor substrates fluorescently labelled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS), which can be employed in conjunction with non-radiolabelled sugar nucleotides. Specifically, we opted for capillary electrophoresis (CE)-compatible materials, given the established high resolution and high sensitivity of CE coupled with laser-induced fluorescence detection (CE-LIF) for carbohydrate analysis.[5]

Typical protocols for fluorescent labelling of reducing sugars[6] make use of reductive amination reactions with amino-substituted chromophores. In the case of APTS labelling, the procedure has been shown to be highly reproducible and efficient (88% efficiency for maltose).[7] Inevitably the integrity of the reducing terminal sugar ring is lost in the process (Scheme 1, route A). We therefore, devised a means of installing an aldehyde-terminating tether to which the amino-fluorophore could be attached. For practical reasons, D-galactose was attractive since the readily available 1,2:3,4-di-*O*-isopropylidene-α-D-galactose (**1**) may serve initially as an acceptor for glycosylation. Subsequent removal of the acetonide protecting groups liberates a reducing terminus for conjugation to the fluorophore, generating an aminogalactitol spacer between the sugar of interest and the fluorophore without loss of the reducing terminal ring of the sugar in question (Scheme 1, route 2).[8]

L-Rhamnose, which occurs in several different places in the RG-II structure and is a putative glycosyl acceptor for a number of sugar nucleotide donors in the biosynthesis of plant cell walls, was chosen as a non-reducing end sugar for our initial targets. Since the minimum acceptor substrate structure required for GT activities may be represented by a larger oligosaccharide acceptor, we extended our choice of targets by selecting disaccharide β-Rha-(1→3′)-β-Api, which is a common fragment of two highly branched side chains of RG-II. Both α- and β-(1→6)-linked disaccharides, **10** and **11**, respectively, and trisaccharide **12** incorporating a Rha-(1→3′)-Api motif, were synthesised (Scheme 2) as compounds ready for derivatisation with the fluorophore. Glycosylation of

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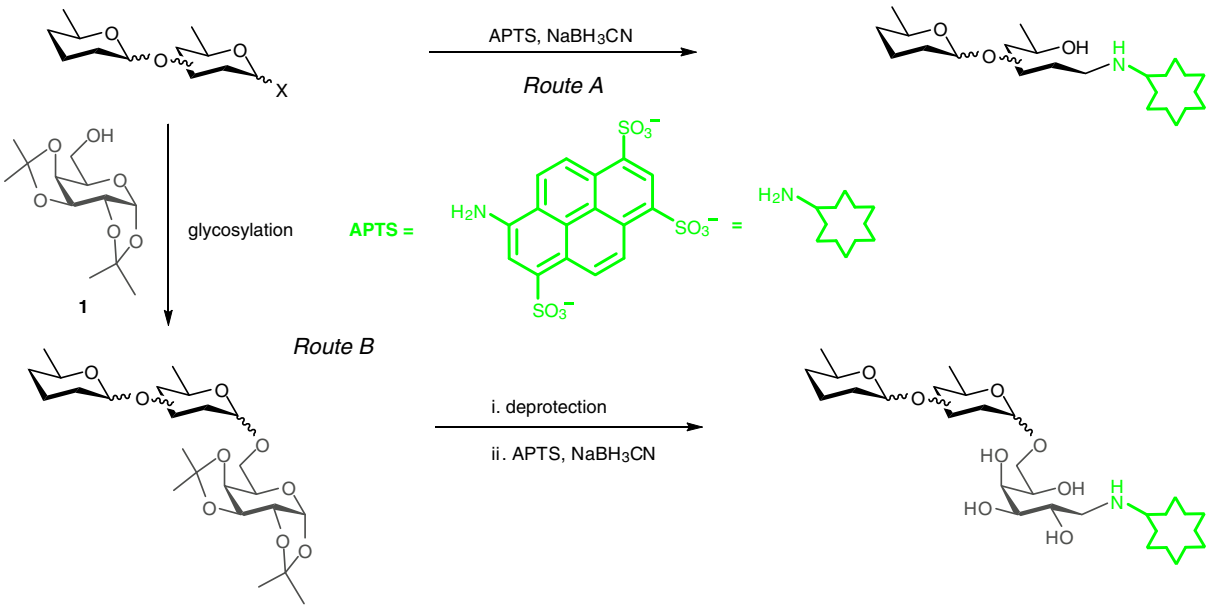
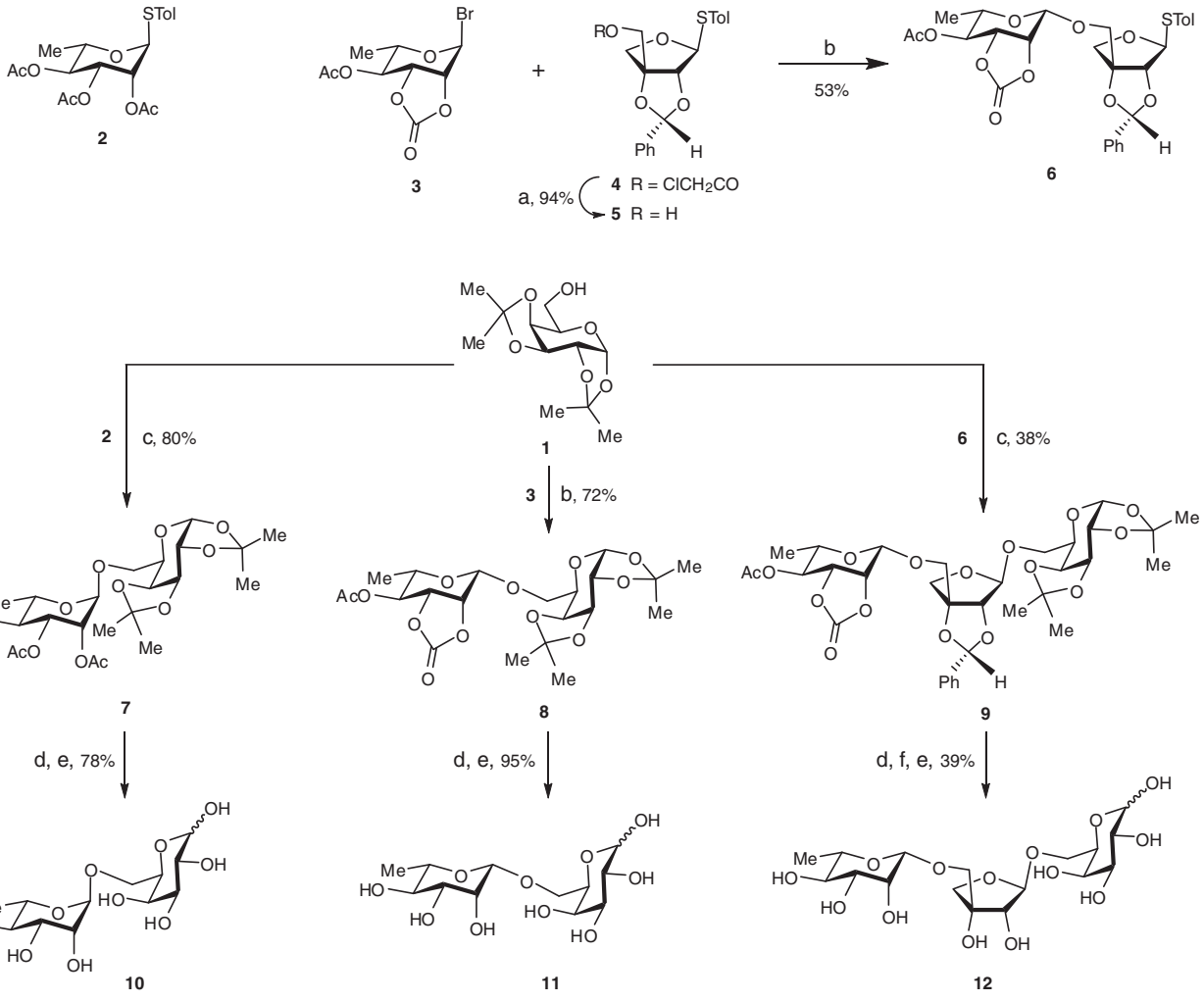


Scheme 1. Strategy for the preparation of fluorescent glycans suitable for carbohydrate-active enzyme analysis CE-LIF.



Scheme 2. Synthesis of oligosaccharides **10**–**12** using glycosylation of **1** with glycosyl donors **2**, **3** and **6**. Reagents and conditions: (a) Et_3N–MeOH (95:5), 20 °C, 4 h; (b) Ag_2O, MS 4 Å, CH_2Cl_2, 20 °C, 20 h; (c) NIS, Me_3SiOTf, CH_2Cl_2, −20 to 0 °C, 3 h; (d) 90% CF_3CO_2H, 20 °C; (e) 0.1 M NaOMe, MeOH; (f) H_2-Pd/C, EtOH–EtOAc 1:1, 20 °C.

alcohol **1**[9] with thioglycoside **2**[10] in the presence of NIS-Me_3SiOTf afforded α-rhamnoside **7**[11] in 80% yield (Scheme 2). β-Rhamnosylation of **1**, which was carried out with bromide **3**[12] under heterogeneous conditions[4a,12] involving insoluble Ag_2O as a promoter, led

to disaccharide **8**[12] in 72% yield (Scheme 2). NMR spectroscopic characteristics of **7** and **8** were consistent with literature data, thus confirming the α- and β-configuration, respectively, for rhamnosidic linkages in these compounds. Thioapiofuranoside derivative **5**,[13] prepared in 94% yield by selective deprotection (Et_3N–MeOH) of the 3′-position of chloroacetate **4**, was glycosylated with bromide **3** using the β-rhamnosylation approach (used to prepare disaccharide **8**) to yield disaccharide **6** (53%) (Scheme 2). This disaccharide was reacted with alcohol **1** in the presence of NIS and $HClO_4$ adsorbed on silica[14] to afford target trisaccharide **9** in 38% yield.[15] The β-configuration of the newly formed apiofuranosidic bond in **9** was confirmed by the appearance of the anomeric proton as a singlet in ^{1}H spectra (δ 5.26 ppm) and the position of the anomeric carbon in ^{13}C NMR spectra (δ 106.8 ppm), which are characteristic for 1,2-*trans*-furanosidic linkages.[16]

Deprotection of compounds **7** and **8** was achieved by hydrolysis of isopropylidene groups in 90% CF_3CO_2H and subsequent *trans*-esterification with NaOMe in MeOH to give unprotected glycans **10** and **11**, which were used without further purification in the fluorescent labelling step. Trisaccharide **12** was synthesised from protected derivative **9** in a similar way, but Pd-catalysed hydrogenation was included as an additional step to ensure complete removal of the benzylidene acetal group. Purification of **12** was performed by gel permeation chromatography in water. NMR spectra of deprotected oligosaccharides **10**–**12** indicated the presence of two species corresponding to two anomeric forms of the reducing end galactopyranose residue: signals of H-1 Gal and C-1 Gal appeared as distinct peaks (α/β ~1.5:1) both in ^{1}H and ^{13}C NMR spectra of these compounds.

Reductive amination is a commonly used two-step, one-pot method to introduce a chromophore into a carbohydrate molecule. During the first step, a chromophore with a primary amino group reacts reversibly with the reducing carbohydrate to form a Schiffs base. A stable chromophore–carbohydrate derivative is subsequently obtained by reduction of the Schiff's base with sodium cyanoborohydride. To purify the resulting APTS-derivatised glycan samples, the PACE (polysaccharide analysis using carbohydrate gel electrophoresis) method was used.[17] After electrophoretic separation, bands on the gel corresponding to the APTS-derivatised glycan were identified under a UV-light lamp, cut from the gel, extracted with water and desalted with a PD-10 desalting column. Quantification of materials purified in this manner was carried out using the previously reported molar extinction coefficient for APTS-derivatised maltoheptaose,[5a] with compound purity being assessed by CE-LIF, calibrated against an APTS-glucose standard [retention times of reductively aminated APTS adducts: Glc 3.89 min; α-Rha-Gal **10**: 4.46 min; β-Rha-Gal **11**: 4.33 min; β-Rha-β-Api*f*-Gal **12**: 4.77 min].

In summary, herein we report a convenient strategy for the generation of fluorescently-labelled glycans related to rhamnogalacturonan II that are suitable for use in glycosyltransferase assay development. Details of the biological evaluation of these materials will be reported in due course.

1. Experimental

1.1. General methods

All reagents, including 8-aminopyrene-1,3,6-trisulfonic acid (APTS), were used as purchased without further purification. Reactions were carried out in dry solvents using septa and syringes for addition of reagents. The SiO_2-supported $HClO_4$-catalyst (~0.5 mmol of the acid per 1 g of powder) was prepared using Merck silica gel (15–40 μm) according to procedure reported in Ref. 14b. TLC was performed on pre-coated aluminium plates (Silica Gel 60 F_{254}, Merck). Column chromatography was performed on Biotage SP4 purification system using silica gel pre-packed cartridges. Optical rotations were measured using a Perkin–Elmer 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 22 °C with a Bruker Avance III spectrometer at 400 and 100 MHz, respectively. ESI-MS were obtained on the DecaXPplus ion trap mass spectrometer (Thermo). Purification of APTS-labelled carbohydrates was performed using the carbohydrate gel electrophoresis (PACE) method.[17] Desalting of aqueous solutions of APTS-labelled oligosaccharides was carried out on PD-10 desalting columns (GE Healthcare).

1.2. *p*-Tolyl 2,3-*O*-(*R*)-benzylidene-3-*C*-hydroxymethyl-1-thio-β-D-erythrofuranoside (5)

A solution of *p*-tolyl 2,3-*O*-(*R*)-benzylidene-3-*C*-(2-chloroacetoxy)methyl-1-thio-β-D-erythrofuranoside (**4**)[13] (400 mg, 0.95 mmol) in MeOH–Et_3N (95:5, 10 mL) was stirred for 4 h at 20 °C, the concentrated residue was purified by crystallisation (hexane–Et_2O) to give alcohol **7**, 308 mg (94%), mp 101–102 °C; $[\alpha]_D^{20}$ −246 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53–7.45 (m, 2H, aromatics), 7.43–7.31 (m, 5H, aromatics), 7.11 (d, 2H, *J* 7.9 Hz, aromatics), 5.91 (s, 1H, PhC*H*), 5.76 (s, 1H, H-1), 4.58 (s, 1H, H-2), 4.16 (m, 2H, H-4a, H-4b), 3.90 (m, 2H, H-3′a, H-3′b), 2.47 (t, 1H, *J* 5.9 Hz, OH), 2.30 (s, 3H, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 136.2, 132.9, 130.1, 130.0, 129.2, 128.5, 127.2 (aromatics), 106.6 (PH*C*H), 93.0 (C-3), 92.6 (C-1), 87.4 (C-2), 73.0 (C-4), 63.6 (C-3′), 21.2 (Me). ESI MS: *m/z* 344.9 $[M+H]^+$; calcd for $C_{19}H_{20}O_4S$ 345.12.

1.3. *p*-Tolyl 3-*C*-(4-*O*-acetyl-2,3-*O*-carbonyl-β-L-rhamnopyranosyloxymethyl)-2,3-*O*-(*R*)-benzylidene-1-thio-β-D-erythrofuranoside (6)

A mixture of bromide **3** (240 mg, 0.81 mmol), alcohol **7** (85 mg, 0.25 mmol), MS 4 Å (500 mg) and Ag_2O (280 mg, 1.21 mmol) in CH_2Cl_2 (10 ml) was stirred for 20 h at 24 °C. The solids were removed by filtration, the filtrate was concentrated and the residue was purified by column chromatography (hexane–EtOAc, 4:1→2:3) to give disaccharide **6** (73 mg 53%): $[\alpha]_D^{20}$ −112 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.56 (m, 2H, aromatics), 7.38 (m, 5H, aromatics), 7.13 (m, 2H, aromatics), 5.99 (s, 1H, PhC*H*), 5.77 (s, 1H, H-1 Api), 5.45 (dd, 1H, *J* 8.7, 5.5 Hz, H-4 Rha), 5.12 (d, 1H, *J* 2.6 Hz, H-1 Rha), 4.82 (m, 2H, H-2 and H-3 Rha), 4.62 (s, 1H, H-2 Api), 4.29–4.18 (m, 3H, H-3′a, H-3′b and H-4a Api), 3.92 (d, 1H, *J* 10.9 Hz, H-4b Api), 3.81 (dq, 1H, *J* 8.6, 6.3 Hz, H-5 Rha), 2.33 (s, 3H, $C_6H_4CH_3$), 2.11 (s, 3H, Ac), 1.33 (d, 3H, *J* 6.3 Hz, H-6 Rha); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9 (CH_3CO), 153.4 (C=O), 138.1, 136.2, 133.0, 129. 9, 128.3, 127.3 (aromatics), 106.7 (PhCH), 95.3 (C-1 Rha), 92.65 (C-3 Api), 91.6 (C-1 Api), 87.3 (C-2 Api), 76.1, 72.9 (C-2 and C-3 Rha), 72.2 (C-4 Rha), 71.2 (C-4 Api), 70.5 (C-5 Rha), 69.1 (C-3′ Api), 21.1 (Me), 20.8 (Ac), 19.6 (C-6 Rha); ESI MS: *m/z* 581.4 $[M+Na]^+$; calcd for $C_{28}H_{30}O_{10}SNa$ 581.59.

1.4. 1,2:3,4-Di-*O*-isopropylidene-6-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-D-galactopyranose (7)

Me_3SiOTf (21 μL, 0.11 mmol) was added to a stirred mixture of alcohol **1** (266 mg, 1.02 mmol), thioglycoside **2** (450 mg, 1.14 mmol), NIS (276 mg, 1.23 mmol) and MS 4 Å (500 mg) in CH_2Cl_2 (10 mL) at −20 °C. The stirring continued for 3 h at −20 to 10 °C, Et_3N (0.5 mL) was added, the mixture was diluted with CH_2Cl_2 and solids were filtered off. The filtrate was extracted with 10% aqueous $Na_2S_2O_3$, the organic extract was washed with satd $NaHCO_3$ solution and H_2O, dried and concentrated in vacuo. The residue was purified by column chromatography (hexane–EtOAc, 4:1→3:2) to give the title compound **4** (440 mg, 80%): mp 112–114 °C (hexane), $[\alpha]_D^{20}$ −89.5 (c 1.0 $CHCl_3$) (lit.[11] mp 114–115, $[\alpha]_D^{20}$

−89); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.51 (d, 1H, *J* 5.0 Hz, H-1 Gal), 5.32–5.21 (m, 2H, H-2 and H-3 Rha), 5.07 (pt, 1H, *J* 7.9 Hz, H-4 Rha), 4.81 (d, 2H, *J* 0.9 Hz, H-1 Rha), 4.63 (dd, 2H, *J* 2.4, 7.9 Hz, H-3 Gal), 4.36–4.26 (m, 2H, H-2 and H-4 Gal), 4.06–3.95 (m, 2H, H-5 Rha, H-5 Gal), 3.86 (dd, 3H, *J* 7.4, 9.6 Hz, H-6a Gal), 3.59 (dd. 3H, *J* 6.2, 9.6 Hz, H-6b Gal), 2.16 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.56 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$), 1.34 (m, 6H, $C(CH_3)_2$), 1.21 (d, 3H, *J* 6.3 Hz, H-6 Rha); ^{13}C NMR (100 MHz, $CDCl_3$): δ 109.2, 108.7 ($C(CH_3)_2$), 97.3 (C-1 Rha), 96.2 (C-1 Gal), 71.2, 70.8, 70.62, 70.58, 69.8, 69.2 (C-2, C-3, C-4 Rha and C-2, C-3, C-4 Gal), 66.3, 66.2 (C-5 Rha, C-5 Gal), 65.3 (C-6 Gal), 26.1, 26.0, 25.0, 24.4 ($C(CH_3)_2$), 21.0, 20.9, 20.8, 20.75 (CH_3CO), 17.2 (C-6 Rha).

1.5. 6-*O*-(4-*O*-Acetyl-2,3-*O*-carbonyl-β-L-rhamnopyranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (8)

Reaction of glycosyl acceptor **1** (300 mg, 1.15 mmol) with glycosyl bromide **3** (500 mg, 1.69 mmol) was carried out in the presence of Ag_2O (500 mg, 2.16 mmol) and MS 4 Å (3.0 g) in essentially the same way as for the synthesis of **6**. The product was purified by column chromatography (toluene–acetone, 4:1) to give disaccharide **8** (390 mg, 72%); $[\alpha]_D^{20}$ −16 (*c* 1.0, $CHCl_3$) (lit.[12] $[\alpha]_D$ −10.2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.51 (d, 1H, *J* 5.0 Hz, H-1 Gal), 5.42 (m, 1H, H-4 Rha), 4.97 (br s, 1H, H-1 Rha), 4.78 (m, 2H, H-2 and H3 Rha), 4.62 (dd, 1H, *J* 7.9, 2.4 Hz, H-3 Gal), 4.31 (dd, 1H, *J* 5.0, 2.4 Hz, H-2 Gal), 4.29 (dd, 2H, *J* 7.9, 2.0 Hz, H-4 Gal), 4.02 (m, 1H, H-5 Gal), 3.94 (dd, 1H, *J* 9.8, 6.1 Hz, H-6a Gal), 3.79–3.72 (m, 2H, H-5 Rha, H-6b Gal), 2.11 (s, 3H, Ac), 1.54 (s, 3H, $C(CH_3)_2$), 1.43 (s, 3H, $C(CH_3)_2$), 1.35 (s, 3H, $C(CH_3)_2$), 1.33 (s, 3H, $C(CH_3)_2$), 1.29 (d, 3H, *J* 6.3 Hz, H-6 Rha); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0 (CH_3CO), 153.6 (C=O), 109.2, 108.8 ($C(CH_3)_2$), 96.2 (C-1 Gal), 95.5 (C-1 Rha), 76.3, 72.5 (C-2 and C-3 Rha), 71.4 (C-4 Rha), 70.7, 70.55, 70.5 (C-2, C-3 and C-4 Gal), 70.2 (C-5 Rha), 67.4 (C-6 Gal), 66.1 (C-5 Gal), 26.1, 26.0, 25.0, 24.4 ($C(CH_3)_2$), 20.8 (CH_3CO), 19.4 (C-6 Rha); ESI MS: *m/z* 497.4 $[M+Na]^+$; calcd for $C_{21}H_{30}O_{12}Na$ 497.44.

1.6. 6-*O*-(3-*C*-(4-*O*-Acetyl-2,3-*O*-carbonyl-β-L-rhamnopyranosyl-oxymethyl)-2,3-*O*-(*R*)-benzylidene-β-D-erythrofuranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (9)

A mixture of glycosyl acceptor **1** (33.9 mg, 0.13 mmol), disaccharide donor **2** (72.8 mg, 0.13 mmol), NIS 35.2 mg (0.16 mmol) and MS 4 Å (100 mg) in CH_2Cl_2 (4 mL) was stirred for 30 min at −20 °C, then $HClO_4/SiO_2$ (7 mg, prepared according to[14b]) was added and the stirring continued for 30 min at −20 to 0 °C. The reaction was quenched with addition of Et_3N (0.1 ml), the mixture was diluted with CH_2Cl_2 and filtered. The filtrates were washed with 10% aqueous $Na_2S_2O_3$, satd $NaHCO_3$ and water, organic layer was dried and concentrated. TLC analysis of the residue indicated the presence of two products, the first having R_f = 0.51 and the second having R_f = 0.32 (hexane–EtOAc 3:7), which were separated by column chromatography (hexane–EtOAc 7:3→3:7). The first compound was identified as trisaccharide **12** (34.3 mg, 38%), $[\alpha]_D^{20}$ −45 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (m, 2H, Ph), 7.37 (m, 3H, Ph), 5.95 (s, 1H, C*H*Ph), 5.55 (d, 1H, *J* 5.1 Hz, H-1 Gal), 5.41 (m, 1H, H-4 Rha), 5.28 (s, 1H, H-1 Api), 5.12 (br s, 1H, H-1 Rha), 4.77 (m, 2H, H-2 and H-3 Rha), 4.61 (dd, 1H, *J* 7.9, 2.4 Hz, H-3 Gal), 4.49 (s, 1H, H-2 Api), 4.32 (dd, 1H, *J* 5.1, 2.4 Hz, H-2 Gal), 4.21 (dd, 1H, *J* 7.9, 1.9 Hz, H-4 Gal), 4.18 (d, 1H, *J* 11.1 Hz, H-4a Api), 4.14–4.09 (m, 3H, H-3′a Api, H-6a and H-6b Gal), 4.03 (m, 1H, H-5 Gal), 4.00 (d, 1H, *J* 10.3 Hz, H-3′b Api), 3.89 (d, 1H, *J* 11.1 Hz, H-4b Api), 3.76 (m, 1H, H-5 Rha), 2.04 (s, 1H, Ac), 1.55 (s, 3H, $C(CH_3)_2$), 1.45 (s, 3H, $C(CH_3)_2$), 1.33 (s, 6H, $C(CH_3)_2$), 1.30 (d, 2H, *J* 6.3 Hz, H-6 Rha); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9 (CH_3CO), 153.4 (C=O), 136.4, 129.8, 128.3, 127.4 (Ph), 106.8 (C-1 Api), 106.4 (PhCH), 96.3 (C-1 Gal), 95.1 (C-1 Rha), 91.1 (C-3 Api), 86.5 (C-2 Api), 76.2, 72.2 (C-2 and C-3 Rha), 73.5 (C-3′ Api), 71.3 (C-4 Rha), 71.1 (C-4 Gal), 70.7 (C-3 Gal), 70.4 (C-2 Gal), 69.4 (C-4 Api), 66.9 (C-5 Gal), 66.3 (C-5 Rha), 60.4 (C-6 Gal), 26.0, 24.9, 24.5 ($C(CH_3)_2$), 21.1 (CH_3CO), 20.8 (Ac), 19.4 (C-6 Rha); ESI MS: *m/z* 712.2 $[M+NH_4]^+$; calcd for $C_{33}H_{42}O_{16}{\cdot}NH_4$ 712.72.

1.7. 6-*O*-(α-L-Rhamnopyranosyl)-D-galactopyranose (10)

Compound **7** (120 mg, 0.23 mmol) was dissolved in 90% aqueous CF_3CO_2H (1.5 mL), the solution was stirred for 15 min at 22 °C, toluene (5 mL) was added and solvents were evaporated in vacuum. The residue was dissolved in toluene and concentrated to dryness; this operation was repeated twice. The product (95 mg), R_f = 0.44 (CH_2Cl_2–MeOH 9:1) was dissolved in dry MeOH (1.0 mL), then 0.02 M NaOMe (1.0 mL) was added and after 1 h at 22 °C the reaction mixture was neutralised by addition of Amberlite IR120 (H^+). The resin was removed by filtration, the filtrates were concentrated in vacuum and the residue was freeze-dried from water to give disaccharide **10** as white powder (59 mg, 78%); ^{1}H NMR (400 MHz, D_2O): δ 5.17 (d, *J* 3.6 Hz, H-1 α-Gal), 4.74 (br s, H-1 Rha), 4.48 (d, *J* 7.9 Hz, H-1 β-Gal), 1.21 (d, *J* 6.2 Hz, H-6 Rha); ^{13}C NMR (100 MHz, D_2O) δ 100.6, 100.4 (J_{C-H} 175.8 Hz, C-1 Rha), 96.4 (C-1 β-Gal), 92.3 (C-1 α-Gal), 73.4, 72.7, 72.0, 71.8, 70.2, 70.0, 69.9, 69.4, 69.1, 69.0, 68.8, 68.6, 68.2; HRESI MS: *m/z* 349.1104 $[M+Na]^+$; calcd for $C_{12}H_{22}O_{10}Na$ 349.1105.

1.8. 6-*O*-(β-L-Rhamnopyranosyl)-D-galactopyranose (11)

Treatment of compound **8** (103 mg, 0.22 mmol) as described for synthesis of **10**, afforded disaccharide **11** as white powder (68 mg, 95%); ^{1}H NMR (D_2O, 400 MHz): δ 5.14 (br s, H-1 α-Gal), 4.56 (br s, H-1 Rha), 4.47 (d, 2H, *J* 7.9 Hz, H-1 β-Gal), 1.18 (m, H-6 Rha); ^{13}C NMR (100 MHz, D_2O) δ 99.9, 99.7 (C-1 Rha), 96.5 (C-1 β-Gal), 92.4 (C-1 α-Gal), HRESI MS: *m/z* 349.1105 $[M+Na]^+$; calcd for $C_{12}H_{22}O_{10}Na$ 349.1105.

1.9. 6-*O*-(3-*C*-(β-L-Rhamnopyranosyloxymethyl)-β-D-erythrofuranosyl)-D-galactopyranose (12)

A soln of disaccharide **9** (35 mg, 50 μmol) in 90% aqueous CF_3CO_2H (1.8 mL) was stirred for 15 min at 22 °C and then concentrated in vacuo several times with toluene (5 mL). The residue was dissolved in EtOH–EtOAc (1:1), cat. 10% Pd/C was added and the mixture was stirred under H_2 for 17 h at 22 °C. The catalyst was filtered off, solvents were evaporated under vacuum and the residue was treated with 0.1 M NaOMe in MeOH for 2 h at 22 °C. The mixture was neutralised with Amberlite IR-120 (H^+), the resin was filtered off and filtrates were concentrated. The residue was purified using gel permeation chromatography (Toyopearl HW-40S, 1.5 × 80 cm) in H_2O and the product was freeze-dried to give the title compound **12** as a white powder (9 mg, 39%): ^{1}H NMR (400 MHz, $CDCl_3$): δ (D_2O, 600 MHz): δ 5.10 (H-1 α-Gal), 4.95, 4.88 (H-1 Api), 4.51 (H-1 Rha), 4.49 (H-1 β-Gal), 1.15 (H-6 Rha); ^{13}C NMR (150 MHz, D_2O) δ 108.7 (C-1 Api), 100.3 (C-1 Rha), 96.4 (C-1 α-Gal), 92.2 (C-1 β-Gal), 16.6 (C-6 Rha); HRESI MS: *m/z* 481.1526 $[M+Na]^+$; calcd for $C_{17}H_{30}O_{14}Na$ 481.1528.

1.10. Typical procedure for derivatisation of oligosaccharide samples with APTS

An oligosaccharide (~0.2 μmol) was solubilised by vigorous stirring in a solution prepared by mixing of 0.2 M APTS in 15% AcOH (5 μl) and 1 M $NaBH_3CN$ in THF (5 μl). The resulting solution was incubated for 20 h at 37 °C and APTS-derivatised product was separated from unreacted oligosaccharide using gel electrophoresis.[17] A band corresponding to the APTS-derivatised oligosaccharide

was identified under UV-light, cut out from the gel and labelled saccharide was extracted with water. The aqueous extracts were desalted using a PD-10 column and concentrated to dryness. The labelled product was quantified spectrophotometrically using the previously reported molar extinction coefficient for APTS-derivatised maltoheptaose (17160 M^{-1} cm^{-1} at 455 nm).[5a]

1.11. Typical procedure for the CE-LIF analysis of oligosaccharide samples labelled with APTS

Analysis of APTS-labelled glycan samples was performed on a PA800 ProteomeLab instrument (Beckman Coulter), equipped with an N–CHO coated capillary (50 μm I.D, length to detector 40 cm, total length 50.2 cm) and laser-induced fluorescence detection system (excitation at 488 nm, emission at 520 nm). Samples were introduced into the capillary by pressure-injection (0.5 psi, 20 s) and separated in running buffer (25 mM LiOAc, 0.4% polyethylene oxide, pH 4.75)[5b] at 30 kV. Data were analysed with the PA-800 *plus* software (Beckman Coulter).

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