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Carbohydrate Research 338 (2003) 2083–2090
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Fast and efficient synthesis of a novel homologous series of L-fucosylated trisaccharides using the *Helix pomatia* α-(1→2)-L-galactosyltransferase

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Received 8 April 2003; received in revised form 1 July 2003; accepted 14 July 2003

Abstract

The α-(1→2)-L-galactosyltransferase from the albumen gland of the vineyard snail *Helix pomatia* exhibits high α-(1→2)-L-fucosyltransferase activity and can be used to transfer L-fucose from GDP-L-fucose to terminal, non-reducing D-galactose residues of an oligosaccharide, thus providing facile access to a range of H-antigen-containing oligosaccharides. The enzymatic glycosylation was applied here on a milligram scale to a series of disaccharide acceptor substrates. Apparently the site of interglycosidic linkage between the terminal and subterminal acceptor sugar units is of little or no consequence. The homologous series of trisaccharides thus produced were fully characterised by NMR analysis of their peracetates.

Keywords: α-(1→2)-L-Galactosyltransferase; *Helix pomatia*; α-(1→2)-L-Fucosylation; GDP-L-Fuc; α-(1→2)-L-Fucosylated oligosaccharide synthesis; H-Antigen

1. Introduction

Glycoconjugates—the bearers of biological information[1]—are macromolecules vital to eukaryotic life.[1] One of the most commonly occurring monosaccharide units in nature is L-fucose.[2] L-Fucosylated glycoconjugates are involved in many biological functions.[3–6] They are important components of human milk[5,7] and also constitute the major human ABO and Lewis blood group antigens.[8] The H blood group determinant, basis of the AB0 blood system, is defined by the disaccharide sequence L-Fuc-α-(1→2)-D-Gal.[9] In light of the biological relevance of these compounds, the development of methodologies allowing rapid and efficient access to H-antigen-containing carbohydrates are required.

Intelligent design of a synthetic pathway incorporating both chemical and enzymatic glycosylations can maximize the advantages of the chemical and enzymatic strategies and minimize eventual disadvantages. Glycosyltransferases are often characterised by high regio- and stereospecificity[1] and often achieve high glycosylation yields.[10–14] In the past, one of their main disadvantages was the price of the activated nucleotide donors, which are difficult and costly to synthesise, and this meant that syntheses with glycosyltransferases were either very expensive, or required the use of complicated in situ donor regeneration cycles.[12] However, recent large scale in vivo syntheses of sugar nucleotides have resulted in dramatic lowering of the prices of at least six

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doi:10.1016/S0008-6215(03)00344-6

of the eight native mammalian sugar nucleotides—so that GDP-L-Fuc (**1**) can now be purchased for a fraction of its previous cost.[†] The other main disadvantages of glycosyltransferases are that they are often rare, difficult to handle, labile, and/or membrane bound.[11–14] However, the highly-active α-(1→2)-L-galactosyltransferase which will be discussed within this work is readily available, extremely easy to handle and very stable. The enzyme is membrane-bound, but in this case that is not a disadvantage—in fact, the execution and work-up of reactions are particularly simple.

In Spring, the albumen gland of the vineyard snail *Helix pomatia* contains large amounts of a the highly-active α-(1→2)-L-galactosyltransferase (α-(1→2)-L-GalT), which recognises not only GDP-L-Gal but also GDP-L-Fuc.[15,16] Thus, the α-(1→2)-L-GalT can be used to transfer L-Fuc to terminal, non-reducing D-Gal residues of an oligosaccharide,[15,16] allowing the rapid synthesis of the subsequent L-Fuc-α-(1→2)-D-Gal-containing product. The purpose of this work was to apply the α-(1→2)-L-GalT to the homologous disaccharide series **2**–**5**, which differ only in the site of the interglycosidic linkage between the terminal galactopyranosyl and the subterminal glucopyranosyl moieties. This would demonstrate to what degree, if any, the *H. pomatia* α-(1→2)-L-GalT exhibits specificity for the site of interglycosidic linkage between the terminal and subterminal sugar moieties, and by doing so, would illustrate the significant synthetic potential of the α-(1→2)-L-GalT. These experiments were conducted on a milligram scale. In other instances, this method has been applied to oligosaccharide synthesis on a larger scale (5–10 mg)[17] and this will be reported in due course.

2. Results and discussion

The homologous series consisting of the four disaccharides D-Galβ-(1→2)-D-GlcβOMe (**2**), D-Galβ-(1→3)-D-GlcβOMe (**3**), D-Galβ-(1→4)-D-GlcβOMe (**4**) and D-Galβ-(1→6)-D-GlcβOMe (**5**) was obtained.[17,18] Each of the four disaccharides were acetylated under standard conditions (Scheme 1). The resulting peracetylated derivatives methyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**6**), methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-β-D-galactopyranoside (**7**), methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**8**) and methyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**9**) were subjected to structural analysis by 1D and 2D NMR.

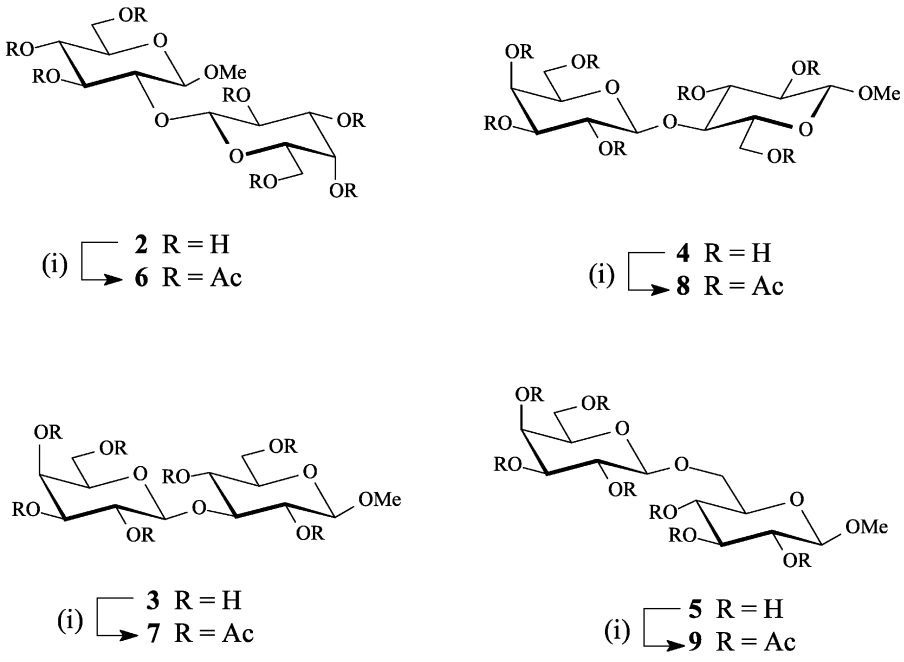
Having conducted successful initial specificity tests on the four disaccharides **2**–**5**,[17] the larger (milligram) scale α-(1→2)-L-fucosylations of this homologous series were begun with the incubation of D-Gal-β-(1→2)-D-GlcβOMe (**2**) with GDP-L-Fuc and the *H. pomatia* albumen gland sediment for 18 h, giving the α-(1→2)-L-fucosylated trisaccharide **10**. L-Fuc-α-(1→2)-D-Gal-β-(1→2)-D-GlcβOMe (**10**) was purified by Dionex HPAED-PAD and then peracetylated, giving the derivative **11** in 68% yield (based on the disaccharide acceptor). NMR analysis of **11** was able to confirm the site of L-fucosylation as being at the 2-hydroxyl group of the galactosyl ring, so that the newly formed trisaccharide **10** and its derivative **11** both contain an H-antigen moiety.

In an analogous manner, D-Gal-β-(1→3)-D-GlcβOMe (**3**) was reacted with the fucosyl donor **1** and α-(1→2)-L-GalT. The resulting L-Fuc-α-(1→2)-D-Gal-β-(1→3)-D-GlcβOMe (**12**) was purified by Dionex HPAEC-PAD and esterified, affording peracetate **13** as a yellow syrup in 71% yield, based on the disaccharide acceptor. After inspection of the 1D and 2D NMR spectra, the site of fucosylation was located as expected at the galactosyl 2-position, in α-configuration.

The next member of the homologous series, D-Gal-β-(1→4)-D-GlcβOMe (**19**), also underwent synthetic scale α-(1→2)-L-fucosylation with *H. pomatia* albumen gland sediment. The product **14** was purified by Dionex HPAEC-PAD and protected, giving the peracetylated trisaccharide **15** in 65% yield (based on the disaccharide acceptor). Structural analysis of compound **15** revealed the formation of an α-(1→2)-L-fucosyl linkage at the galactosyl 2-position.

Similarly, D-Gal-β-(1→6)-D-GlcβOMe (**5**) was incubated with GDP-L-Fuc and *H. pomatia* albumen gland sediment, forming the trisaccharide L-Fuc-α-(1→2)-D-Gal-β-(1→6)-D-GlcβOMe (**16**). The reaction mixture was purified, again by Dionex HPAEC-PAD methodology, and the relevant fractions were combined and lyophilised, then acetylated to afford the derivative **17** in 65% yield (based on the disaccharide acceptor). As in all previous cases, the new L-fucopyranosyl linkage was established as being α-(1→2) to the acceptor's terminal, non-reducing D-galactopyranosyl ring.

Comparison of the yields of the peracetylated trisaccharides **11**, **13**, **15** and **17** reveals that the *H. pomatia* albumen gland α-(1→2)-L-GalT exhibits little or no specificity for the site of the interglycosidic linkage between the terminal, non-reducing D-galactosyl moiety and the subterminal sugar unit. This confirms the observations made during small-scale Dionex experiments.[17] Not only did the enzyme recognise all four homologous disaccharides, but α-(1→2)-L-fucosylation occurred in all cases to a similar extent, as variation in the yields of the trisaccharides **11**–**17** lies within experimental error. Some of the NMR data for peracetylated disaccharides **6**, **7**, **8** and **9** and peracetylated trisaccharides **11**, **13**, **15** and **17** is compiled in Table 1, which illustrates the relevant chemical shifts of the trisaccharide protons and allows direct comparison with the



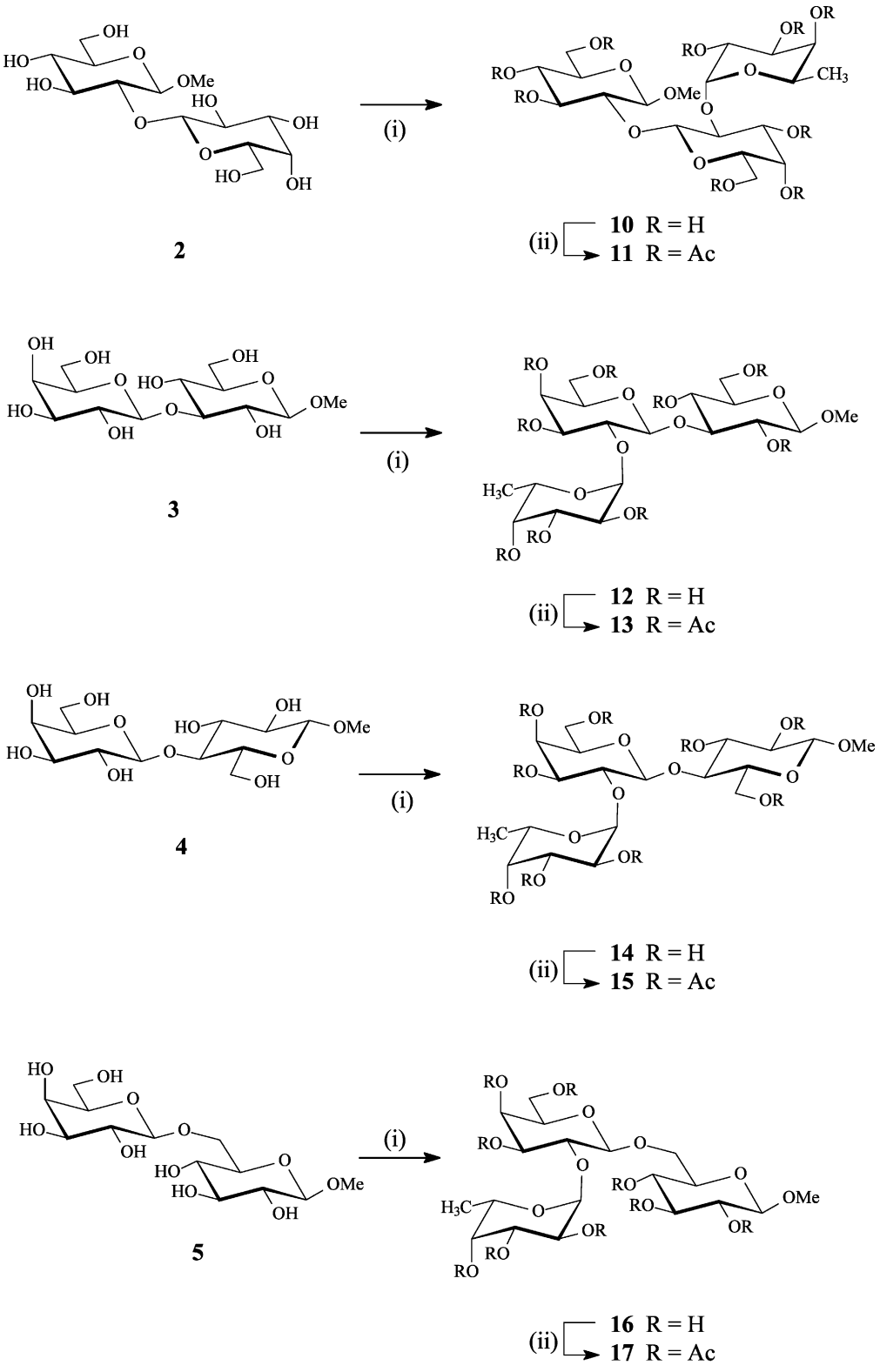
Scheme 1. (i) $(CH_3CO)_2O$, pyridine, 80 °C, 2 h.

corresponding protons in the disaccharide and monosaccharide precursors. The upfield shifts of the H-2′ protons in the trisaccharides, relative to the H-2′ shifts of the appropriate disaccharide acceptor, was a strong indication for the site of L-fucosylation (Scheme 2).

Having established that the site of interglycosidic linkage between the acceptor terminal and subterminal sugar moieties is of little consequence on a synthetic scale as well as on an analytical scale, it can be seen that the synthetic value of larger scale *H. pomatia* α-(1→2)-L-GalT L-fucosylation reactions is considerable. In light of the fact that *H. pomatia* α-(1→2)-L-GalT-mediated L-fucosylation reactions are also as efficient with oligosaccharides bearing different subterminal sugar units, such as D-Gal-β-(1→4)-D-Man[17,19] or L-Fuc-α-(1→2)-D-Gal-β-(1→3)-D-GalβOMe,[17,19] one may conclude that the *H. pomatia* albumen gland sediment is an extremely useful synthetic tool. This sediment offers a very reactive α-(1→2)-L-galactosyltransferase with a pleasingly flexible acceptor specificity, without compromising the enzyme's regio and stereospecificity. The snail *H. pomatia* is commonly found in Europe, and if the albumen gland is harvested in early spring, the snails represent a convenient and inexpensive enzyme source. With the recent decrease in price of GDP-L-Fuc and UDP-D-Gal, and the ready availability and convenient handling of the *H. pomatia* α-(1→2)-L-GalT, even researchers with no previous experience of glycosyltransferases should feel free to exploit this synthetic method.

Table 1
Comparison of ^{1}H NMR shifts for relevant protons of peracetylated trisaccharide derivatives **11**, **13**, **15** and **17**, the corresponding protons in peracetylated acceptor precursors **6**, **7**, **8** and **9**, and methyl β-D-glucopyranoside

Linkage type	Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside		Disaccharide peracetates			Trisaccharide peracetate		
	Proton	ppm		Proton	ppm		Proton	ppm
β-(1→2)	H-2	4.92	**6**	H-2	3.67	**11**	H-2	3.94
				H-2′	5.16		H-2′	3.92
β-(1→3)	H-3	5.16	**7**	H-3	3.83	**13**	H-3	3.92
				H-2′	5.00		H-2′	3.71
β-(1→4)	H-4	5.03	**8**	H-4	3.74	**15**	H-4	3.87–3.81
				H-2′	5.04		H-2′	3.87–3.81
	H-6a	4.20	**9**	H-6a	3.84–3.81	**17**	H-6a	3.84–3.79
β-(1→6)	H-6b	4.08		H-6b	3.55		H-6b	3.70–3.59
				H-2′	5.14		H-2′	3.90



Scheme 2. (i) GDP-L-Fuc **1**, *H. pomatia* albumen gland α-(1→2)-L-GalT, calf intestine alkaline phosphatase, $MnCl_2$, NaN_3, Tris/HCl buffer (pH 7.6), 28 °C, 18 h; (ii) $(CH_3CO)_2O$, pyridine, 80 °C, 2 h.

3. Experimental

3.1. General methods

TLC was performed on silica gel 60-coated aluminium sheets (E. Merck) using the given eluent mixtures. Spots were visualised by spraying with 10% H_2SO_4 in EtOH and subsequent heating. Free oligosaccharide purification was performed using Dionex HPAEC-PAD (Carbopac 100, 9 mm, 100 mM aqueous NaOH), Dionex GmbH, Idstein, Germany. Optical rotations were measured on a Perkin–Elmer Polarimeter 243, with $[\alpha]_D$ values given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed by the microanalytical laboratory of the University of Hamburg. NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer. Chemical shifts are referred to the sovents used. Maldi-TOF spectra were measured on a Bruker Biflex-II spectrometer with DHB as matrix. All yields of α-(1→2)-L-fucosylation reactions are given based on the amount of unprotected disaccharide acceptor offered for α-(1→2)-L-fucosylation.

3.2. Procedure A: preparation of *H. pomatia* albumen gland sediment

The *H. pomatia* albumen glands (2.0 g) were homogenised in Tris/HCl buffer (50 mM, pH 7.6, 5 mL) in a Potter-Elvehjem homogeniser, then centrifuged at 4000 rpm for 45 min at 4 °C. The supernatant was removed from the pellet and discarded. This process was repeated five times with 30 min centrifugation at 4000 rpm, whereupon the final albumen gland sediment (approx 2000 μL) was rehomogenised and either used immediately or stored at −70 °C. 2.81 μmol of acceptor is reacted with 300 μL of albumen gland sediment. One snail can provide one albumen gland, which has an average weight of approx 2.0 g. This means that roughly 18.7 μmol of acceptor can be α-(1→2)-L-fucosylated per snail (6.7 mg of disaccharide acceptor per snail). No further activity values can be given for this α-(1→2)-L-galactosyltransferase, because it has not yet been completely purified. Frequently repeated experiments have shown that this value is, however, highly reproducible. Albumen gland sediment which has been frozen and stored must be rehomogenised before use.

3.3. Procedure B: peracetylation of oligosaccharides

The saccharide (1–5 mg) was dissolved in acetic anhydride (1 mL) and pyridine (200 μL) and heated to 80 °C for 2 h. The reaction mixture was then evaporated to dryness in vacuo, diluted with dichloromethane (2 mL), washed with bidistilled water (2 × 2 mL), dried ($MgSO_4$), filtered, and evaporated to dryness in vacuo.

3.4. Methyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (6)

The disaccharide D-Galβ-(1→2)-D-GlcβOMe (**2**, 5.0 mg, 0.015 mmol)[17,18] was acetylated according to Procedure B, giving the title compound **6** (6.2 mg, 65%) as a yellow glass after purification by preparative TLC ($CHCl_3$–acetone 9:1, developed thrice). $[\alpha]_D^{20}$ +4.5° (*c* 0.31 $CHCl_3$), $C_{27}H_{38}O_{18}$ (650.21 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 673.50$ $[M+Na]^+$, 689.50 $[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.37 (dd, 1 H, H-4′, $J_{3',4'} = 3.6$, $J_{4',5'} = 0.8$), 5.19 (t, 1 H, H-3, $J_{2,3} = 9.4$, $J_{3,4} = 9.4$), 5.16 (dd, 1 H, H-2′, $J_{1',2'} = 8.1$, $J_{2',3'} = 10.2$), 4.98 (vt, 1 H, H-4, $J_{3,4} = 9.4$, $J_{4,5} = 9.6$), 4.95 (dd, 1 H, H-3′, $J_{2',3'} = 10.2$, $J_{3',4'} = 3.6$), 4.74 (d, 1 H, H-1′, $J_{1',2'} = 8.1$), 4.42 (d, 1 H, H-1, $J_{1,2} = 7.6$), 4.29 (dd, 1 H, H-6a, $J_{5,6a} = 4.8$, $J_{6a,6b} = 12.5$), 4.20 (dd, 1 H, H-6a′, $J_{5',6a'} = 6.4$, $J_{6a',6b'} = 11.2$), 4.15–4.10 (m, 2 H, H-6b, H-6b′, $J_{5',6b'} = 6.6$, $J_{6a',6b'} = 11.2$), 3.89 (m, 1 H, H-5′), 3.69 (m, 1 H, H-5), 3.67 (dd, 1 H, H-2, $J_{1,2} = 7.6$, $J_{2,3} = 9.4$), 3.56 (s, 3 H, OCH_3), 2.19, 2.15, 2.11, 2.07, 2.04, 2.00, 1.97 (7 × s, each 3 H, C*H*$_3$COO) ppm, ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 102.57 (C-1), 100.70 (C-1′), 77.93, 71.49 (C-2, C-5), 73.33 (C-3), 71.18 (C-3′), 70.89 (C-5′), 69.03 (C-2′), 68.50 (C-4), 66.35 (C-4′), 61.79, 61.52 (C-6, C-6′), 57.05 (OCH_3), 21.50–19.80 (*C*H$_3$COO) ppm.

3.5. Methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (7)

The disaccharide D-Galβ-(1→3)-D-GlcβOMe (**3**, 4 mg, 0.011 mmol)[17,18] was acetylated according to Procedure B, giving the title compound **7** as a yellow oil (5.9 mg, 82%). $[\alpha]_D^{20}$ −21.0° (*c* 0.29 $CHCl_3$), $C_{27}H_{38}O_{18}$ (650.21 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z =$ 673.07 $[M+Na]^+$, 689.05 $[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.29 (dd, 1 H, H-4′, $J_{3',4'} = 3.8$, $J_{4',5'} \cong$ 1.0), 5.00 (dd, 1 H, H-2′, $J_{1',2'} = 7.9$, $J_{2',3'} = 10.4$), 4.91 (dd, 1 H, H-2, $J_{1,2} = 8.1$, $J_{2,3} = 9.7$), 4.89 (vt, 1 H, H-4, $J_{3,4} = 9.4$, $J_{4,5} = 9.4$), 4.87 (dd, 1 H, H-3′, $J_{2',3'} = 10.4$, $J_{3',4'} = 3.8$), 4.48 (d, 1 H, H-1′, $J_{1',2'} = 7.9$), 4.23 (d, 1 H, H-1, $J_{1,2} = 8.1$), 4.14 (dd, 1 H, H-6a, $J_{5,6a} = 4.6$, $J_{6a,6b} =$ 12.5), 4.13–4.09 (m, 2 H, H-6a′, H-6b, $J_{6a',6b'} = 10.9$, $J_{6a,6b} = 12.5$), 3.98 (dd, 1 H, H-6b′, $J_{5',6b'} = 7.4$, $J_{6a',6b'} = 10.9$), 3.83 (vt, 1 H, H-3, $J_{2,3} = 9.7$, $J_{3,4} = 9.4$), 3.83–3.79 (m, 1 H, H-5′), 3.63–3.58 (m, 1 H, H-5), 3.40 (s, 3 H, OCH_3), 2.08, 2.05, 2.02, 2.00, 1.97, 1.95, 1.89 (7 × s, each 3 H, C*H*$_3$COO) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 101.95 (C-1), 101.61 (C-1′), 78.85 (C-3), 73.02 (C-2), 72.29 (C-5), 71.49 (C-4/C-3′), 70.88 (C-5′), 69.03, 68.98 (C-2′ and C-3′/C-4), 67.22 (C-4), 62.64 (C-6), 61.31 (C-6′), 57.05 (OCH_3), 21.37, 21.19, 21.15, 21.07, 21.03, 20.95, 20.89 (each *C*H$_3$COO) ppm.

3.6. Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (8)

The commercially available disaccharide methyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**4**, 5 mg, 0.014 mmol) was acetylated according to Procedure B and purified by preparative TLC ($CHCl_3$–acetone 9:1, developed four times) giving the title trisaccharide **8** as a pale yellow glass (6.5 mg, 72%). $[\alpha]_D^{20}$ −4.4° (*c* 0.32 $CHCl_3$), $C_{27}H_{38}O_{18}$ (650.21 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 673.39$ $[M+Na]^+$, 689.40 $[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.28 (dd, 1 H, H-4′, $J_{3',4'} = 3.6$, $J_{4',5'} \cong 1.0$), 5.13 (vt, 1 H, H-3, $J_{2,3} = 9.4$, $J_{3,4} = 9.2$), 5.04 (dd, 1 H, H-2′, $J_{1',2'} = 7.6$, $J_{2',3'} = 10.4$), 4.88 (dd, 1 H, H-3′, $J_{2',3'} = 10.4$, $J_{3',4'} = 3.6$), 4.81 (dd, 1 H, H-2, $J_{1,2} = 7.9$, $J_{2,3} = 9.4$), 4.43 (d, 1 H, H-6a, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$), 4.42 (d, 1 H, H-1′, $J_{1',2'} = 7.6$), 4.32 (d, 1 H, H-1, $J_{1,2} = 7.9$), 4.03–3.99 (m, 3 H, H-6a′, H-6b, H-6b′, $J_{5,6b} = 4.8$, $J_{5',6a'} = 6.4$, $J_{5',6b'} = 7.4$, $J_{6a',6b'} = 11.2$), 3.80 (vt, 1 H, H-5′), 3.74 (vt, 1 H, H-4, $J_{3,4} = 9.2$, $J_{4,5} = 9.7$), 3.56–3.52 (m, 1 H, H-5), 3.41 (s, 3 H, OCH_3), 2.08, 2.05, 1.99, 1.90 (4 × s, each 3 H, C*H*$_3$COO), 1.98 (s, 9 H, 3 × C*H*$_3$COO) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 101.79 (C-1), 101.51 (C-1′), 76.70 (C-4), 73.28 (C-3), 73.05 (C-5), 72.08 (C-2), 71.42 (C-3′), 71.12 (C-5′), 69.56 (C-2′), 67.04 (C-4′), 62.41 (C-6), 61.22 (C-6′), 57.40 (OCH_3), 21.26, 21.21, 21.15, 21.04, 20.91 (*C*H$_3$COO) ppm.

3.7. Methyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (9)

The disaccharide methyl 6-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**5**, 35 mg, 0.089 mmol)[17,18] was peracetylated according to Procedure B, giving the title compound **9** as a clear glass (51 mg, 79%) after purification by preparative TLC ($CHCl_3$–acetone 9:1, developed thrice). $[\alpha]_D^{20}$ −11° (*c* 1 $CHCl_3$), $C_{27}H_{38}O_{18}$ (650.21 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 672.88$ $[M+Na]^+$, 688.86 $[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.32 (dd, 1 H, H-4′, $J_{3',4'} = 3.2$, $J_{4',5'} = 0.7$), 5.14 (dd, 1 H, H-2′, $J_{1',2'} = 7.9$, $J_{2',3'} = 10.4$), 5.12 (vt, 1 H, H-3, $J_{2,3} = 9.8$, $J_{3,4} = 9.5$), 4.92 (dd, 1 H, H-3′, $J_{2',3'} = 10.4$, $J_{3',4'} = 3.2$), 4.87 (dd, 1 H, H-2, $J_{1,2} = 8.2$, $J_{2,3} = 9.8$), 4.82 (vt, 1 H, H-4, $J_{3,4} = 9.5$, $J_{4,5} = 10.1$), 4.49 (d, 1 H, H-1′, $J_{1',2'} = 7.9$), 4.33 (d, 1 H, H-1, $J_{1,2} = 8.2$) 4.10 (dd, 1 H, H-6a′, $J_{5',6a'} = 6.6$, $J_{6a',6b'} = 11.4$), 4.05 (dd, 1 H, H-6b′, $J_{5',6b'} = 6.9$, $J_{6a',6b'} = 11.4$), 3.84–3.81 (m, 2 H, H-5′, H-6a, $J_{5,6a} = 2.2$, $J_{6a,6b} = 11.0$), 3.63 (m, 1 H, H-5), 3.55 (dd, 1 H, H-6b, $J_{5,6b} = 7.6$), 3.44 (s, 3 H, OCH_3), 2.08, 1.99, 1.99, 1.98, 1.96, 1.92, 1.91 (7 × s, each 3 H, C*H*$_3$COO) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 103.29 (C-1), 103.09 (C-1′), 73.78 (C-5), 73.24 (C-3), 71.63 (C-2), 71.36, 71.09 (C-5′, C-3′), 70.02 (C-4), 69.22 (C-2′), 69.00 (C-6), 67.34 (C-4′), 62.13 (C-6′), 57.40 (OCH_3), 21.73, 20.69, 20.45 (*C*H$_3$COO) ppm.

3.8. Methyl 3,4,6-tri-*O*-acetyl-2-*O*-(2-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl]-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (11)

Methyl 2-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (**2**, 1.00 mg, 25 μg/μL, 2.81 μmol)[17,18] was added to GDP-L-Fuc (2.25 mg, 100 μg/μL, 3.74 μmol), 60 μL of $MnCl_2$ (100 mM), 12 μL of NaN_3 (10 mg/mL), 10 μL of calf intestine alkaline phosphatase (185 mU/μL), and 300 μL of albumen gland sediment, and incubated at 28 °C for 18 h with stirring. The reaction was terminated by dilution with bidistilled water to a total volume of 2 mL and centrifugation at 14,000 rpm for 10 min. The supernatant was removed, and the pellet washed with 500 μL bidistilled water and centrifuged at 14,000 rpm for 10 min, twice. The combined aqueous fractions were lyophilised, diluted to 400 μL and purified by semi-preparative HPAEC-PAD. The relevant fractions were combined, lyophilised and acetylated according to GP1. The reaction mixture was evaporated to dryness by flushing with nitrogen gas, and the resulting residue was dissolved in dichloromethane (2 mL) and washed with bidistilled water (2 × 3 mL). The organic phase was evaporated to dryness, giving the title compound **11** as a yellow syrup in 68% yield (1.7 mg). $[\alpha]_D^{20}$ −12.2° (*c* 0.09 $CHCl_3$), $C_{37}H_{52}O_{24}$ (880.28 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 903.49$ $[M+Na]^+$, 919.46 $[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.35 (dd, 1 H, H-3″/H-2″, $J_{2'',3''} = 10.7$), 5.30 (d, 1 H, H-1″, $J_{1'',2''} = 3.8$), 5.31–5.28 (m, 2 H, H-4′, H-4″, $J_{3',4'} = 4.1$, $J_{4',5'} = 1.0$, $J_{3'',4''} = 3$–4), 5.16 (dd, 1 H, H-3, $J_{2,3} = 8.5$, $J_{3,4} = 9.7$), 5.09 (vt, 1 H, H-4, $J_{3,4} = 9.7$, $J_{4,5} = 9.7$), 5.00–4.98 (m, 1 H, H-2″/H-3″, $J_{2'',3''} = 10.7$), 4.98 (dd, 1 H, H-3′, $J_{2',3'} = 9.8$, $J_{3',4'} = 4.1$), 4.66 (d, 1 H, H-1′, $J_{1',2'} = 7.6$), 4.45–4.41 (m, 1 H, H-5″), 4.43 (d, 1 H, H-1, $J_{1,2} = 8.2$), 4.29 (dd, 1 H, H-6a′, $J_{5',6a'} = 6.3$, $J_{6a',6b'} = 11.4$), 4.17 (dd, 1 H, H-6a, $J_{5,6a} = 4.7$, $J_{6a,6b} = 12.3$), 4.15–4.07 (m, 2 H, H-6b, H-6b′, $J_{5,\ 6b} = 7.3$, $J_{6a,6b} = 12.3$, $J_{5',6b'} = 6.6$, $J_{6a',6b'} = 11.4$), 3.94 (dd, 1 H, H-2, $J_{1,2} = 8.2$, $J_{2,3} = 8.5$), 3.92 (dd, 1 H, H-2′, $J_{1',2'} = 7.6$, $J_{2',3'} = 9.8$), 3.85 (m, 1 H, H-5′), 3.75 (ddd, 1 H, H-5, $J_{5,6a} = 4.7$, $J_{5,6b} = 7.3$, $J_{6a,6b} = 12.3$), 3.52 (s, 3 H, OCH_3), 2.18, 2.16, 2.13, 2.10, 2.08, 2.05, 2.03, 1.99, 1.97 (9 × s, each 3 H, C*H*$_3$COO), 1.16 (d, 3 H, H-6″, $J_{5'',6''} = 6.6$) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 102.20 (C-1), 100.86 (C-1′), 96.58 (C-1″), 75.43 (C-2), 74.36 (C-3), 74.10, 69.28, 67.67 (C-3′, C-2″, C-3″), 72.76 (C-2′), 72.20 (C-5), 71.42 (C-4″), 70.88 (C-5′), 68.74 (C-4), 67.40 (C-4′), 65.00 (C-5″), 62.01 (C-6), 61.73 (C-6′), 56.70 (OCH_3), 22.57–20.19 (*C*H$_3$COO), 15.55 (C-6″) ppm.

3.9. Methyl 2,4,6-tri-*O*-acetyl-3-*O*-[3,4,6-tri-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside (13)

Methyl 3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**3**, 1.20 mg, 25 μg/μL, 3.37 μmol)[17,18] was added to GDP-L-Fuc (2.70 mg, 100 μg/μL, 4.49 μmol), 72 μL of $MnCl_2$ (100 mM), 14.4 μL of NaN_3 (10 mg/mL), 12 μL of calf intestine alkaline phosphatase (185 mU/μL), and 360 μL of albumen gland sediment, and incubated at 28 °C for 18 h with stirring. The reaction was diluted with bidistilled water (2000 μL) and centrifuged (14,000 rpm, 10 min). The supernatant was removed, and the pellet washed and centrifuged twice (each 500 μL bidistilled water, 14,000 rpm, 10 min). The combined aqueous fractions were lyophilised, diluted to 500 μL and purified by semi-preparative HPAEC-PAD. The relevant fractions were combined, lyophilised and acetylated according to GP1. The reaction mixture was evaporated to dryness by flushing with nitrogen gas, and the resulting residue was dissolved in dichloromethane (2 mL) and washed with bidistilled water (2 × 3 mL). The organic phase was evaporated to dryness, giving the title compound **13** as a yellow syrup in 71% yield (2.1 mg). $[\alpha]_D^{20}$ −29.5° (*c* 0.10 $CHCl_3$), $C_{37}H_{52}O_{24}$ (880.28 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 903.13$ $[M+Na]^+$, 919.09$[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.33 (dd, 1 H, H-3″, $J_{2'',3''} = 10.7$, $J_{3'',4''} = 3.4$), 5.24 (dd, 1 H, H-4″, $J_{3'',4''} = 3.4$, $J_{4'',5''} = 1.0$), 5.24–5.20 (m, 1 H, H-4′, $J_{3',4'} \approx 4.0$, $J_{4',5'} \approx 1.0$), 5.21 (d, 1 H, H-1″, $J_{1'',2''} = 3.8$), 4.91–4.85 (m, 4 H, H-2, H-4, H-3′, H-2″, $J_{1,2} = 7.6$, $J_{2,3} = 9.7$, $J_{3,4} = 9.7$, $J_{4,5} = 9.7$, $J_{2',3'} = 9.8$, $J_{3',4'} = 4.0$, $J_{1'',2''} = 3.8$, $J_{2'',3''} = 10.7$), 4.45 (d, 1 H, H-1′, $J_{1',2'} = 7.6$), 4.35 (m, 1 H, H-5″, $J_{4'',5''} \approx 1.0$, $J_{5'',6''} \approx 6.5$ Hz), 4.28 (d, 1 H, H-1, $J_{1,2} = 7.6$), 4.18–4.16 (m, 2 H, H-6a, H-6a′, $J_{5,6a} = 5.4$, $J_{6a,6b} = 12.3$, $J_{5',6a'} = 5.4$, $J_{6a',6b'} = 10.7$), 4.10 (m, 1 H, H-6b), 3.95 (dd, 1 H, H-6b′, $J_{5',6b'} = 8.2$, $J_{6a',6b'} = 10.7$), 3.92 (vt, 1 H, H-3, $J_{2,3} = 9.7$, $J_{3,4} = 9.7$), 3.79 (m, 1 H, H-5′), 3.71 (dd, 1 H, H-2′, $J_{1',2'} = 7.6$, $J_{2',3'} = 9.8$), 3.62 (m, 1 H, H-5), 3.41 (s, 3 H, OCH_3), 2.18, 2.07, 2.03, 2.00, 2.00, 2.00, 1.90, 1.89, 1.88 (9 × s, each 3 H, C*H*$_3$COO), 1.13 (d, 3 H, H-6″, $J_{5'',6''} = 6.5$) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 101.70 (C-1), 100.94 (C-1′), 96.33 (C-1″), 76.59 (C-3), 73.90, 68.95, 68.90, 68.81 (C-3, C-4, C-3′, C-2″), 72.39 (C-2′), 72.32 (C-5), 70.74, 70.60 (C-5′, C-3″), 67.43, 67.40 (C-4′, C-4″), 65.31 (C-5″), 62.69 (C-6), 61.24 (C-6′), 57.19 (OCH_3), 22.70–20.10 (*C*H$_3$COO), 15.82 (C-6″) ppm.

3.10. Methyl 2,3,6-tri-*O*-acetyl-4-*O*-(2-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl]-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (15)

Methyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**4**, 2.00 mg, 25 μg/μL, 5.61 μmol) was added to GDP-L-Fuc (4.50 mg, 100 μg/μL, 7.48 μmol), 120 μL of $MnCl_2$ (100mM), 24 μL of NaN_3 (10 mg/mL), 20 μL of calf intestine alkaline phosphatase (185 mU/μL), and 600 μL of albumen gland sediment, and incubated at 28 °C for 18 h with vigorous stirring. The reaction was terminated by dilution with bidistilled water (2866 μL), and divided into two similar portions. These were centrifuged at 14,000 rpm for 10 min. The supernatants were removed and combined, and the pellets were washed, each with 500 μL bidistilled water and centrifuged at 14,000 rpm for 10 min, twice. The combined aqueous fractions were lyophilised, diluted to 400 μL and purified by semi-preparative HPAEC-PAD. The relevant fractions were combined, lyophilised and acetylated according to GP1. The reaction mixture was evaporated to dryness by flushing with nitrogen gas, and the resulting residue was dissolved in dichloromethane (3 mL) and washed with bidistilled water (2 × 3 mL). The organic phase was evaporated to dryness, giving the title compound **15** as a yellow glass in 65% yield (3.2 mg). $[\alpha]_D^{20}$ −10.0° (*c* 0.16 $CHCl_3$), $C_{37}H_{52}O_{24}$ (880.28 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 903.27$ $[M+Na]^+$, 919.24 $[M+K]^+$, ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.38 (d, 1 H, H-1″, $J_{1'',2''} = 3.8$), 5.32 (m, 1 H, H-4′, $J_{3',4'} = 3.3$, $J_{4',5'} < 1.0$), 5.28 (m, 1 H, H-4″, $J_{4'',5''} < 1.0$), 5.15 (dd, 1 H, H-3′, $J_{2',3'} = 11.2$, $J_{3',4'} = 3.3$), 5.12 (t, 1 H, H-3, $J_{2,3} = 9.7$, $J_{3,4} = 9.7$), 5.00–4.95 (m, 2 H, H-2″, H-3″, $J_{1'',2''} = 3.9$, $J_{2'',3''} = 6.6$), 4.95 (dd, 1 H, H-2, $J_{1,2} = 7.9$, $J_{2,3} = 9.7$), 4.52 (dd, 1 H, H-6a, $J_{5,6a} = 2.0$, $J_{6a,6b} = 12.0$), 4.41 (d, 1 H, H-1′, $J_{1',2'} = 7.6$), 4.39 (d, 1 H, H-1, $J_{1,2} = 7.9$), 4.27 (dd, 1 H, H-6b, $J_{5,6b} = 5.9$, $J_{6a,6b} = 12.0$), 4.14 (dd, 1 H, H-6a′, $J_{5',6a'} = 6.9$, $J_{6a',6b'} = 11.2$), 4.07 (dd, 1 H, H-6b′, $J_{5',6b'} = 6.9$, $J_{6a',6b'} = 11.2$), 3.87–3.81 (m, 3 H, H-5′, H-4, H-2′), 3.65–3.60 (m, 2 H, H-5, H-5″, $J_{5'',6''} = 6.6$), 3.51 (s, 3 H, OCH_3), 2.23, 2.16, 2.15, 2.12, 2.10, 2.07, 2.06, 2.05, 2.00 (9 × s, each 3 H, C*H*$_3$COO), 1.22 (d, 3 H, H-6″, $J_{5'',6''} = 6.6$) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 101.72 (C-1), 100.21 (C-1′), 95.54 (C-1″), 74.35 (C-4), 73.45, 68.03 (C-2″, C-3″), 72.99 (C-5, C-5″), 71.54 (C-3), 71.20, 71.15, 71.11, 70.83 (C-2, C-2′, C-4′, C-5′), 62.01 (C-6), 60.92 (C-6′), 57.16 (OCH_3), 20.85, 20.65, 20.18 (*C*H$_3$COO), 15.53 (C-6″) ppm.

3.11. Methyl 2,3,4-tri-*O*-acetyl-6-*O*-(2-*O*-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl]-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (17)

To a mixture of GDP-L-Fuc (4.50 mg, 100 μg/μL, 7.48 μmol), 120 μL of $MnCl_2$ (100 mM), 24 μL of NaN_3 (10 mg/mL), 20 μL of calf intestine alkaline phosphatase (185 mU/μL), and 600 μL of albumen gland sediment was added methyl 6-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (**5**, 2.00 mg, 25 μg/μL, 5.61 μmol)[17,18] and the reaction mixture was incubated at 28 °C for 18 h

with vigorous stirring. The reaction was terminated by dilution with bidistilled water (2866 μL), and divided into two similar portions. These were centrifuged at 14,000 rpm for 10 min. The supernatants were removed and combined, and the pellets were each washed with bidistilled water (500 μL) and centrifuged at 14,000 rpm for 10 min, twice. The combined aqueous fractions were lyophilised, diluted to 400 μL and purified by semi-preparative HPAEC-PAD. The relevant fractions were combined, lyophilised and acetylated according to GP1. The reaction mixture was evaporated to dryness by flushing with nitrogen gas, and the resulting residue was dissolved in dichloromethane (3 mL) and washed with bidistilled water (2×3 mL). The organic phase was evaporated to dryness, giving the title compound **17** as a yellow glass in 65% yield (3.2 mg). $[\alpha]_D^{20}$ -10.6° (*c* 0.16 $CHCl_3$), $C_{37}H_{52}O_{24}$ (880.28 g mol^{-1}), MALDI-TOF (DHB, positive mode): $m/z = 903.46$ $[M+Na]^+$, 919.47 $[M+K]^+$, ^1H NMR (500 MHz; $CDCl_3$): δ 5.35 (d, 1 H, H-1″, $J_{1'',2''} = 4.1$), 5.28–5.26 (m, 3 H, H-4′, H-3″, H-4″), 5.15 (vt, 1 H, H-3, $J_{2,3} = 9.2$, $J_{3,4} = 9.2$), 4.96–4.91 (m, 3 H, H-2, H-3′, H-2″), 4.84 (vt, 1 H, H-4, $J_{3,4} = 9.2$, $J_{4,5} = 9.4$), 4.57–4.54 (m, 2 H, H-5″, H-1′, $J_{1',2'} = 7.6$), 4.45 (d, 1 H, H-1, $J_{1,2} = 7.9$), 4.12 (dd, 1 H, H-6a′, $J_{5',6a'} = 6.6$, $J_{6a',6b'} = 11.2$), 4.01 (dd, 1 H, H-6b′, $J_{5',6b'} = 6.4$, $J_{6a',6b'} = 11.2$), 3.90 (dd, 1 H, H-2′, $J_{1',2'} = 7.6$, $J_{2',3'} = 10.2$), 3.84–3.79 (m, 2 H, H-6a, H-5′), 3.70–3.59 (m, 2 H, H-5, H-6b), 3.44 (s, 3 H, OCH_3), 2.09, 2.06, 2.02, 1.99, 1.99, 1.98, 1.94, 1.91, 1.90 ($9\times$ s, each 3 H, CH_3COO), 1.10 (d, 3 H, H-6″, $J_{5'',6''} = 6.5$) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 102.07 (C-1′), 101.19 (C-1), 95.78 (C-1″), 74.27, 71.13, 68.67 (C-2, C-3′, C-3″), 74.14 (C-5), 73.20 (C-3), 71.63 (C-2′), 71.57, 67.92, 67.73 (C-4′, C-4″, C-3″), 71.13 (C-5′), 69.68 (C-4), 68.86 (C-6), 65.03 (C-5″), 61.82 (C-6′), 56.33 (OCH_3), 21.80–21.10 (CH_3COO), 16.32 (C-6″) ppm.

Acknowledgements

Support of this work by the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft (SFB 470) ist gratefully acknowledged.

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