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Unusual Reactivity of Pyruvated Glycosyl Donors During Construction of Rhizobial Exopolysaccharide Fragments

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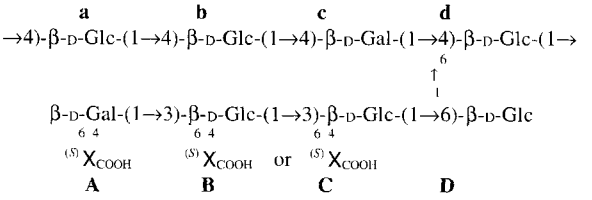
Abstract. Two pyruvated galactosyl donors, 2,3-di-*O*-benzoyl-4,6-*O*-[1-(*R*)-methoxycarbonyl(ethylidene)]-α-D-galactopyranosyl chloride (**6**) and trichloroacetimidate **13**, were coupled to position 3 of suitably protected mono and disaccharide benzyl glucoside acceptors. For both donors, an unusual high content of the α-(1→3)-linked products was obtained. The corresponding β-(1→3)-linked di- and trisaccharides are related to exopolysaccharides of *Rhizobium leguminosarum*.

Saprophytic bacteria of the genus *Rhizobium* are symbionts of several agriculturally important *Leguminosae* (pulse). This symbiosis enables the plant to assimilate atmospheric nitrogen (N_2-fixation). For invading the root hairs of the respective host plant, these bacteria produce large amounts of acidic exopolysaccharides (EPS) which are thought to be involved in the species specific infection mechanisms. However, much controvery is found in the literature if these EPS are in fact essential for the infection of leguminoses by rhizobial bacteria. Therefore, synthetic fragments related to the distinct EPS of various types of *Rhizobium* should be attractive tools for studying these symbiotic interactions.

Ongoing efforts in our laboratory toward the synthesis of biologically relevant saccharides prompted us to prepare a series of oligosaccharides related to EPS of various types of *Rhizobium* bacteria [1–3]. A significant feature of numerous rhizobial EPS is the presence of immunodominant pyruvic acid acetals [4–6]. For example, the repeating unit of the EPS of *Rhizobium leguminosarum* biovar *phaseoli* contains two such acidic acetals (Scheme 1) either at adjacent glycosyl residues **A** and **B** at the terminus of the side chain (**A**–**D**) or at residues **A** and **C**, respectively [7]. Similar adjacently double-pyruvated saccharide residues are found as well in EPS of *R. leguminosarum* biovars *trifolii* [8] and *viciae* [9] and in a glycolipid of *Mycobacterium smegmatis* [10,11] the synthesis of which has been previously reported from our laboratory [12, 13]. Here, we now describe syntheses of trisaccharide building blocks related to the side chain of EPS of *R. leguminosarum* biovar *phaseoli* having residues **A** and **C** pyruvylated.

Originally, it was planned to construct the crucial doubly pyruvated trisaccharide block related to the sequence **ABC** (Scheme 1) from a suitably protected disaccharide donor **AB** and a pyruvated monosaccharide acceptor **C**. For that purpose (cp. Scheme 2), 1,2,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside **1** [14] was first chloroacetylated [15] at position 3 followed by conversion of intermediate **2** into the corresponding glucosyl bromide **3**. Next, the latter bromide was transferred into benzyl β-D-glucopyranoside **4** which was subsequently dechloroacetylated, to afford acceptor **5**. Silver trifluoromethanesulfonate- (AgOTf) promoted condensation of the latter with pyruvated galactosyl chloride **6** [16,17], however, did not give the expected β-(1→3)-linked disaccharide. Instead, the α-(1→3)-linked disaccharide **7** was obtained in poor yield (33%) accompanied by hydrolysis products of the donor which were isolated as benzoate **8** after benzoylating workup of the crude reaction mixture. Similar α-selective glycosylations with pyruvated donor **6** have also been encountered previously [1] when less reactive glycosyl acceptors, like compound **5**, were used. The undesired



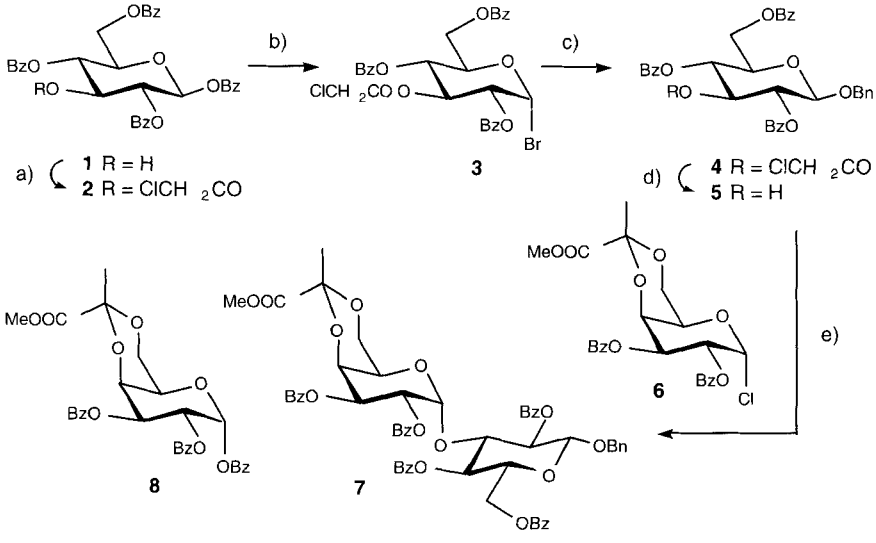
Scheme 1 Repeating unit of the exopolysaccharide of *Rhizobium leguminosarum* biovar *phaseoli* (CIAT 899) [7].

α-selectivity was previously thought to be either a result of a double diastereoselection [1, 18] during galactosylation with donor **6** or of a preferred attack of the acceptor from the convex side of the donor [1, 19] due to the presence of a conformationally restraining 4,6-pyruvate acetal and to be the more pronounced the more sterically demanding the acceptor appeared. However, no such steric demand could be attributed to the glycosyl acceptor **5** and the α-selectivity of its coupling with **6** must be regarded as an intrinsic property of the pyruvated galactosyl donor that can be circumvented only in special cases [1]. Thus, a different strategy was chosen for the synthesis of the desired trisaccharide block as follows.

In order to abolish the problems encountered with the above outlined strategy and, furthermore, to test the unusual reactivity with other pyruvated galactosyl donors, the trisaccharide block **ABC** (Scheme 1) was now planned to be prepared from the pyruvated trichloroacetimidate **13** [1] and a suitably protected disaccharide acceptor related to the sequence **BC**, cp. Scheme 3. Donor **13** was chosen here instead of chloride **6** because it was expected from previous glycosylations [1] that **13** reacts more β-selectively.

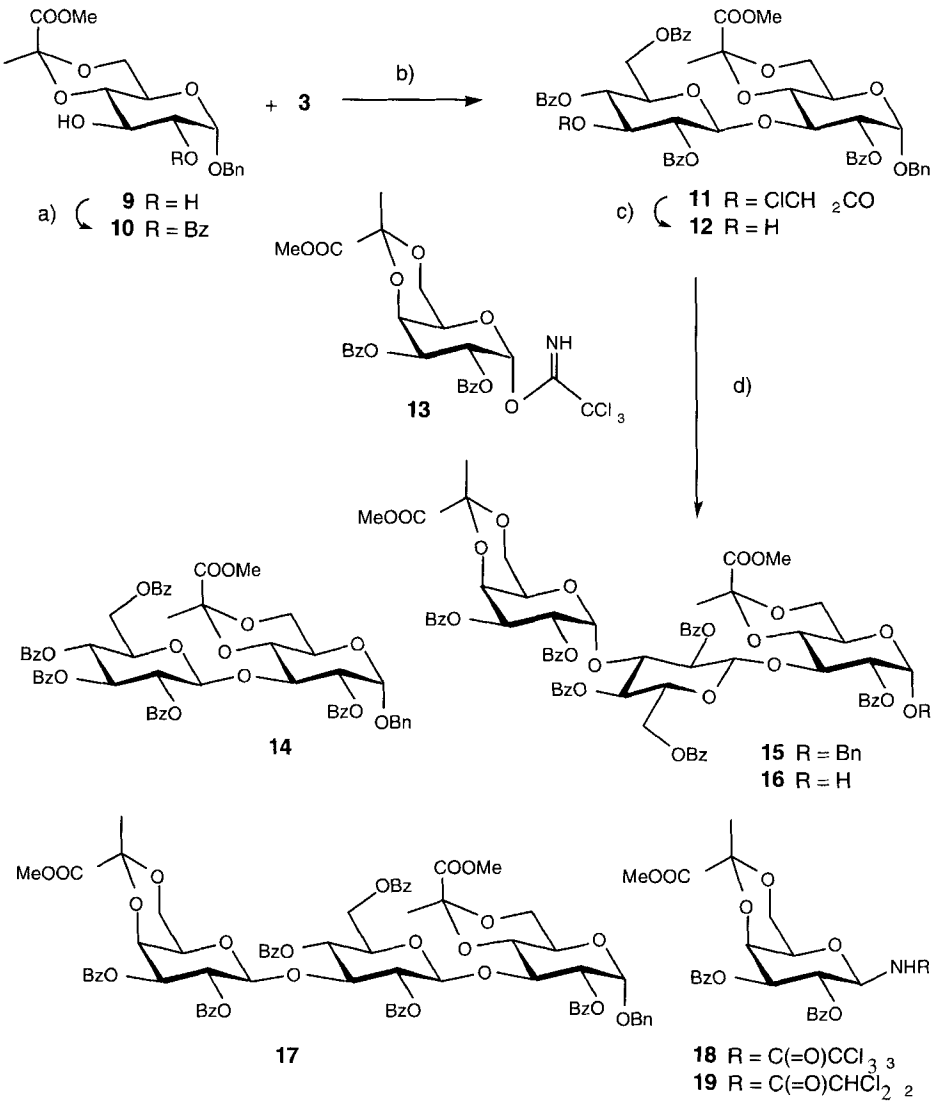
For the preparation of the needed disaccharide acceptor block, benzyl 4,6-*O*-[1-(*R*)-methoxycarbonyl-(ethylidene)]-α-D-galactopyranoside **9** [12] was first regioselectively benzoylated as previously described [13, 20] to afford compound **10**. Next, AgOTf-promoted condensation of the latter with bromide **3** proceeded in a clean reaction, to give β-(1→3)-linked disaccharide **11**, final dechloroacetylation of which at position 3' afforded acceptor **12**.

When donor **13** was coupled to thus prepared disaccharide acceptor **12**, a mixture of several compounds (TLC) was obtained in dichloromethane as the solvent. To this end, the crude mixture was benzoylated and chromatographed, to afford first compound **14** which was



Scheme 2 a) **1** [14] (1 eq.), $(ClCH_2CO)_2O$ (6.2 eq.), $NaHCO_3$ (1.2 eq.), DMF, 2 h, 25 °C, 87%; b) **2** (1 eq.), HBr (30% in AcOH), CH_2Cl_2, 1.5 h, 25 °C, 93%; c) benzyl alkohol (1 eq.), AgOTf (1.25 eq.), **3** (0.74 eq.), 2,4,6-trimethylpyridine (0.67 eq.), CH_2Cl_2, 10 min., 25 °C, 69%; d) **4** (1 eq.), thiourea (2 eq.), MeOH, 14 h, 25 °C, 6 h, 60 °C, 100%; e) 1) **5** (1 eq.), AgOTf (1.5 eq.), **6** [16] (0.88 eq.), 2,4,6-trimethylpyridine (0.7 eq.), CH_2Cl_2, 0.5 h, 25 °C; 2) pyridine/benzoyl chloride (2:1), 0.5 h, 25 °C, 69% **7**, 22% **8**.

formed from unreacted acceptor upon hydrolysis and rebenzoylation. Next, trisaccharides **15** and **17**, respectively, were obtained in a ratio of 1:1. This result was in contrast to the previous finding that under identical conditions, when condensed with benzyl 2-*O*-benzoyl-4,6-*O*-[1-(*S*)-methoxycarbonyl(ethylidene)]-α-D-gluco-



Scheme 3 a) [13]; b) **10** (1 eq.), AgOTf (1.5 eq.), **3** (1.2 eq.), 2,4,6-trimethylpyridine (0.8 eq.), CH_2Cl_2, 35 min., 25 °C, 85%; c) **11** (1 eq.), thiourea (2 eq.), CH_2Cl_2/MeOH, 19 h, 60 °C, 100%; d) 1) **12** (1 eq.), TMSOTf (0.1 eq.), **13** (1.1 eq.), CH_2Cl_2, 0.5 h, 0 °C; 2) pyridine/benzoyl chloride (2:1), 1 h, 25 °C, 44% **14**, 26% **15**, 26% crude **17**; 3) **12** (1 eq.), TMSOTf (0.1 eq.), **13** (1.1 eq.), MeCN, 0.5 h, −20 °C, **15/18** (38:62), 4) **15/18** (38:62), cat. Pd-C (10%), ethyl acetate, H_2, 48 h, 25 °C, 97% **19**, 100% **16**.

pyranoside **10** [1], donor **13** gave high yields of the corresponding β-(1→4)-linked product. Thus, the high α-selectivity cannot be attributed to acid catalyzed anomerisation under the reaction conditions. Similarly, when the condensation of **12** and **13** was performed in acetonitrile as the solvent (conditions which were previously found to give good β-selectivities [1]) no β-product **17** was obtained at all. Although homogeneous on TLC, the product of that reaction was shown to be a 38:62 mixture of the α-linked trisaccharide **15** and the rearranged imidate **18**. Once again, galactosyl amine **18** was also previously obtained from **13** in similar glycosylations [1, 17] when acetonitrile was used as the solvent. However, in order to separate the mixture here, the crude reaction product had to be hydrogenated prior to chromatography which converted **18** into the corresponding dichloro derivatives **19** and trisaccharide **15** into the 1-OH derivative **16**. This procedure appeared to be very useful in cases where rearranged imidates are formed during glycosylations. As was outlined above for the condensation of chloride **6** with glucoside **5**, the unselective condensation of the imidate **13** with disaccharide acceptor **12** was a consequence of the special properties of pyruvated galactosyl donors.

Finally, both trisaccharides **16** and **17** were subsequently transformed into trichloroacetimidates **20** and **21**, respectively which both can serve as donors for the synthesis of higher oligosaccharides.

In summary, it must be noted that the pyruvated galactosyl imidate **13** is a good donor for the introduction of a 4,6-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl residue in oligosaccharide syntheses only when special optimized acceptors and reaction conditions are chosen. In these cases, this donor behaves superior to other galactosyl donors [1]. However, as was outlined here, α-linked products may be obtained in large amounts as well.

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Experimental

NMR data were extracted from spectra measured in solutions of $CDCl_3$ (with TMS as an internal standard) at 25 °C with a Bruker AC 250F spectrometer. Proton signal assignments were made by first order analysis of the spectra. Of the two magnetically non-equivalent geminal protons at C-6, the one resonating at lower field was designated 6a-H and the one resonating at higher field was designated 6b-H. Carbon-signal assignments were made by mutual comparison of the spectra and by comparison with spectra of related compounds. Optical rotations were measured at 25 °C with a Perkin-Elmer automatic polarimeter, Model 241. Melting points were measured with a Büchi apparatus, Model SMP-20. Thin-layer chromatography (TLC) was performed on precoated plastic sheets, Polygram SIL UV_{254}, 40 × 80 mm (Macherey–Nagel) using appropriately adjusted mixtures of carbon tetrachloride-acetone for developing. Detection was effected with UV light, where applicable and by charring with 5% sulfuric acid in ethanol. Preparative chromatography was performed by elution from columns of Silica Gel 60 (Merck) using carbon tetrachloride-acetone mixtures as solvent. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 2 kPa, ≤0 °C.

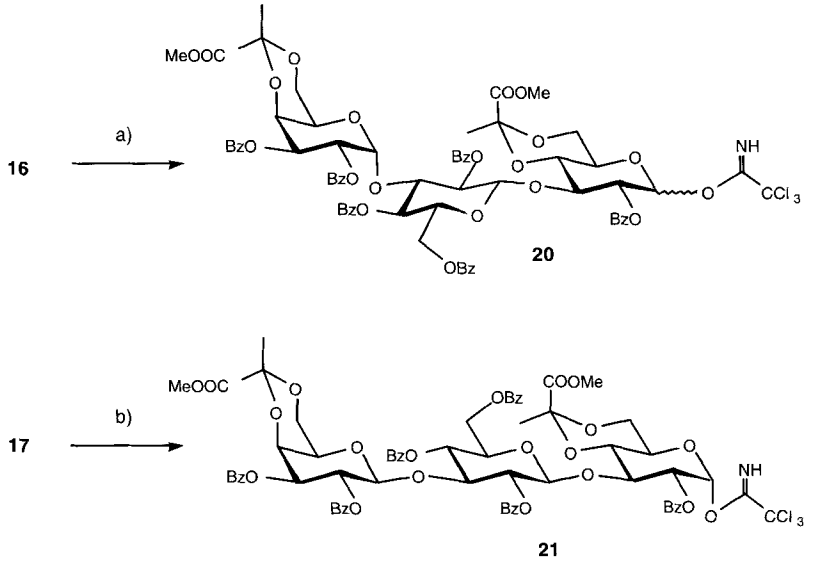
1,2,4,6-Tetra-O-benzoyl-3-O-chloroacetyl-β-D-glucopyranoside (**2**)

Chloroacetanhydride (3.55 g, 20.8 mmol) was added at room temperature to a mixture of **1** [14] (2.0 g, 3.35 mmol) and $NaHCO_3$ (0.33 g, 3.97 mmol) in DMF (5 ml), and the mixture was stirred for 2 h whereupon the product crystallized. The mixture was diluted with ice-H_2O, stirred for another 0.5 h, and the product was isolated by filtration. Recrystallisation from EtOH-CH_2Cl_2 afforded **2** (1.91 g, 87%). *M.p.* 213 °C; $[\alpha]_D$ = +5.3 (c = 1.2, $CHCl_3$). – ^{1}H NMR: δ/ppm = 6.24 (d, 1 H, $J_{1,2}$ = 7.8 Hz, 1-H), 5.85 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, 3-H), 5.75 (dd, 1 H, 2-H), 5.71 (t, 1 H, $J_{4,5}$ = 9.5 Hz, 4-H), 4.64 (dd, 1 H, $J_{5,6a}$ = 2.9 Hz, $J_{6a,6b}$ = –12.3 Hz, 6a-H), 4.48 (dd, 1 H, $J_{5,6b}$ = 4.6 Hz, 6b-H), 4.35 (ddd, 1 H, 5-H), 3.88 (s, 2 H, CH_2Cl). – ^{13}C NMR: δ/ppm = 92.5 (C-1), 74.2, 73.0, 70.5 (C-2,3,5), 68.9 (C-4), 62.5 (C-6), 40.2 (CH_2Cl).

$C_{36}H_{29}ClO_{11}$	Calcd.:	C 64.24	H 4.34	Cl 5.27
(673.1)	Found:	C 64.03	H 4.36	Cl 5.30.

2,4,6-Tri-O-benzoyl-3-O-chloroacetyl-α-D-glucopyranosyl bromide (**3**)

HBr 30% in acetic acid (1.8 ml) was added at room temperature to a stirred solution of **2** (1.67 g, 2.48 mmol) in CH_2Cl_2 (10 ml) and stirring was continued for 1.5 h. The solution was successively washed with ice-cold H_2O and aqueous $NaHCO_3$ solution, dried and concentrated. Chromatography (10:1 CCl_4/acetone) afforded **3** (1.46 g, 93%). $[\alpha]_D$ = +138.5 (c = 0.8, $CHCl_3$). – ^{1}H NMR: δ/ppm = 6.82 (d, 1 H, $J_{1,2}$ = 4.1 Hz, 1-H), 6.05, 5.69 (2 t, 2 × 1 H, $J_{2,3}$ = $J_{3,4}$ = $J_{4,5}$ = 9.9 Hz, 3,4-H), 5.23 (dd, 1 H, 2-H), 4.62–4.68 (m, 2 H, 5,6a-H), 4.48 (dd, 1 H,



Scheme 4 a) **16** (1 eq.), K_2CO_3 (1.7 eq.), Cl_3CCN (2.9 eq.), CH_2Cl_2, 10 h, 25 °C, 75%; b) 1) crude **17** (1 eq.), cat. Pd–C (10%), ethyl acetate, H_2, 12 h, 25 °C; 2) cat. K_2CO_3, Cl_3CCN (16.6 eq.), CH_2Cl_2, 10 h, 25 °C, 58%.

$J_{5,6b}$ = 4.7 Hz, $J_{6a,6b}$ = −12.7 Hz, 6b-H), 3.87 (s, 2 H, CH_2Cl). – ^{13}C NMR: δ/ppm = 86.5 (C-1), 72.5, 72.0, 70.0 (C-2,3,5), 67.8 (C-4), 61.8 (C-6), 40.2 (CH_2Cl).

$C_{29}H_{24}BrClO_9$	Calcd.:	C 55.13	H 3.83	Hal 11.82
(631.9)	Found:	C 54.93	H 3.84	Hal 11.26.

Benzyl 2,4,6-tri-O-benzoyl-3-O-chloroacetyl-β-D-glucopyranoside (**4**)

A mixture of benzyl alcohol (0.45 ml, 4.3 mmol), AgOTf (1.38 g, 5.4 mmol) and 3Å molecular sieves (0.5 g) in CH_2Cl_2 (5 ml) was stirred under Ar at room temperature for 0.5 h. To this mixture, a solution of **3** (2.0 g, 3.17 mmol) and 2,4,6-trimethylpyridine (348 mg, 2.87 mmol) in CH_2Cl_2 (5 ml) was added, stirring was continued for 10 min, and neutralized by addition of 2,4,6-trimethylpyridine. The mixture was filtered through a layer of Celite, the filtrate was successively washed with aqueous $Na_2S_2O_3$ and $NaHCO_3$ solutions, dried and concentrated. Chromatography (10:1 CCl_4/acetone) of the residue afforded **4** (1.45 g, 69%). $[\alpha]_D$ = −20.1 (c = 0.9, $CHCl_3$). – ^1H NMR: δ/ppm = 5.68–5.44 (m, 3 H, 2,3,4-H), 4.88 (d, 1 H, J = −12.5 Hz, CH_2Ph), 4.76 (d, 1 H, $J_{1,2}$ = 7.8 Hz, 1-H), 4.67 (d, 1 H, CH_2Ph), 4.64 (dd, 1 H, $J_{5,6a}$ = 3.3 Hz, $J_{6a,6b}$ = −12.1 Hz, 6a-H), 4.49 (dd, 1 H, $J_{5,6b}$ = 5.1 Hz, 6b-H), 4.05 (m, 1 H, 5-H), 3.84 (s, 2 H, CH_2Cl). – ^{13}C NMR: δ/ppm = 98.9 (C-1), 74.4, 72.1, 71.6 (C-2,3,5), 70.0 (CH_2Ph), 69.6 (C-4), 63.0 (C-6), 40.2 (CH_2Cl).

$C_{36}H_{31}ClO_{10}$	Calcd.:	C 65.61	H 4.74	Cl 5.38
(659.1)	Found:	C 64.44	H 4.84	Cl 5.46.

Benzyl 2,4,6-tri-O-benzoyl-β-D-glucopyranoside (**5**)

A solution of thiourea (198 mg, 2.6 mmol) in MeOH (5 ml) was added at room temperature to a solution of **4** (0.86 g, 1.3 mmol) in a mixture of CH_2Cl_2 (2 ml) and MeOH (10 ml) and the solution was stirred for 14 h at room temperature and for 6 h at 60 °C. The mixture was concentrated, the residue redissolved in CH_2Cl_2, washed with aqueous $NaHCO_3$ solution, dried and concentrated. Chromatography (5:1 CCl_4/acetone) of the residue afforded **5** (0.77 g, 100%). $[\alpha]_D$ = −38.2 (c = 1.1, $CHCl_3$); ^1H NMR: δ/ppm = 5.41 (d, 1 H, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.5 Hz, 4-H), 5.29 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.3 Hz, 2-H), 4.88 (d, 1 H, J = −12.5 Hz, CH_2Ph), 4.71 (d, 1 H, 1-H), 4.67 (d, 1 H, CH_2Ph), 4.65 (dd, 1 H, $J_{5,6a}$ = 3.1 Hz, $J_{6a,6b}$ = -12.3 Hz, 6a-H), 4.46 (dd, 1 H, $J_{5,6b}$ = 12.1 Hz, 6b-H), 3.96 (ddd, 1 H, 5-H), 3.06 (d, 1 H, J = 5.9 Hz, OH); ^{13}C NMR: δ = 98.8 (C-1), 74.9, 74.0, 72.4, 72.0 (C-2,3,4,5), 70.3 (CH_2Ph), 63.4 (C-6).

$C_{34}H_{30}O_9$	Calcd.:	C 70.09	H 5.19
(582.6)	Found:	C 69.79	H 5.20.

Benzyl O-{2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl-(ethylidene)]-α-D-galactopyranosyl}-(1→3)-2,4,6-tri-O-benzoyl-β-D-glucopyranoside (**7**) *and 1,2,3-tri-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-α-D-galactopyranose* (**8**)

A mixture of **5** (0.58 g, 1.0 mmol), AgOTf (385 mg, 1.5 mmol) and 3Å molecular sieves (0.5 g) in CH_2Cl_2 (5 ml) was stirred under Ar at room temperature for 0.5 h. To this mixture, a solution of **6** [16] (0.43 g, 0.88 mmol) and 2,4,6-trimethylpyridine (85 mg, 0.7 mmol) in CH_2Cl_2 (5 ml) was added and stirring was continued for 0.5 h. Pyridine (1 ml) and benzoyl chloride (0.5 ml) was added and stirring was continued for 0.5 h. The mixture was diluted with CH_2Cl_2 and successively washed with aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution, dried and concentrated. Chromatography (10:1 CCl_4/acetone) of the residue afforded first **7** (0.31 g, 34%); *m.p.* 184–187 °C (acetone/*n*-hexane); $[\alpha]_D$ = −49.4 (c = 1.1, $CHCl_3$). – ^1H NMR (significant signals): δ/ppm = 5.67 (dd, 1 H, $J_{2,3}$ = 10.9 Hz, 2'-H), 5.52 (d, 1 H, $J_{1,2}$ = 3.8 Hz, 1'-H), 5.49 (t, 1 H, $J_{2,3}$ = 9.1 Hz, 2-H), 4.85, 4.62 (2 d, 2 H, J = −12.5, CH_2Ph), 4.28 (dd, 1 H, $J_{4,5}$ = 3.2 Hz, 4'-H), 3.90 (dd, 1 H, $J_{5,6a}$ = 5.6 Hz, 5-H), 3.54 (s, 3 H, $COOCH_3$), 3.43 (dd, 1 H, $J_{6a,6b}$ = -12.5 Hz, 6a'-H), 3.12b (d, 1 H, 6b'-H), 1.43 (s, 3 H, CH_3). – ^{13}C NMR (significant signals): δ/ppm = 169.8 (CO_2), 98.8, 98.8 (C-1,1'), 98.5 (C_{acetal}), 77.9 (C-3), 72.8, 72.5, 72.2, (C-2,4',5), 70.1 (CH_2Ph), 69.3 (C-3'), 69.2 (C-2'), 67.0 (C-4), 64.6 (C-6'), 63.6 (C-6), 52.3 ($COOCH_3$), 25.6 (CH_3).

$C_{58}H_{52}O_{18}$	Calcd.:	C 67.18	H 5.05
(1037.0)	Found:	C 66.75	H 5.05.

Eluted next was **8** (0.11 g, 22%), identical to the previously described compound [16].

Benzyl O-(2,4,6-tri-O-benzoyl-3-O-chloroacetyl-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl(ethylidene)]-α-D-glucopyranoside (**11**)

A suspension of **10** [13] (472 mg, 1.03 mmol), AgOTf (395 mg, 1.54 mmol) and 3Å molecular sieves (0.5 g) in CH_2Cl_2 (5 ml) was treated for 35 min with a solution of **3** (0.78 g, 1.23 mmol) and 2,4,6-trimethylpyridine (100 mg, 0.83 mmol) in CH_2Cl_2 (7 ml) as described for the preparation of compound **7**. Chromatography (10:1 CCl_4/acetone) afforded **11** (0.89 g, 85%). $[\alpha]_D$ = +71.0 (c = 1.5, $CHCl_3$). – ^1H NMR (significant signals): δ/ppm = 3.55 (t, 1 H, $J_{3,4}$ = 9.9 Hz, 3-H), 3.83 (s, 3 H, $COOCH_3$), 3.79 (s, 2 H, CH_2Cl), 1.42 (s, 3 H, CH_3). – ^{13}C NMR: δ/ppm = 170.0 (CO_2), 99.3 (C_{acetal}), 98.8 (C-1'), 95.9 (C-1), 74.8, 74.7, 74.1, 73.7 (C-3,3',4,5'), 72.2, 71.7 (C-2, 2'), 69.9 (CH_2Ph), 69.4 (C-4'), 65.2 (C-6), 63.2 (C-6'), 62.5 (C-5), 52.8 ($COOCH_3$), 40.2 CH_2Cl), 25.0 (CH3).

$C_{53}H_{49}ClO_{18}$	Calcd.:	C 63.06	H 4.89	Cl 3.51
(1009.4)	Found:	C 63.21	H 4.95	Cl 3.50.

Benzyl O-(2,4,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl(ethylidene)]-α-D-glucopyranoside (**12**)

A solution of **11** (0.74 g, 0.73 mmol) in a 1:4 mixture of CH_2Cl_2/MeOH (10 ml) was treated for 19 h at 60 °C with thiourea (111 mg, 1.46 mmol) in MeOH (2 ml) as described for the preparation of compound **5**. Chromatography (5:1 CCl_4/acetone) afforded **12** (0.68 g, 100%). $[\alpha]_D$ = +68.7 (c = 1.2, $CHCl_3$). – ^1H NMR (significant signals): δ/ppm = 5.50 (t, 1 H, $J_{2,3}$ = 9.9 Hz, 2'-H), 5.34 (d, 1 H, $J_{1,2}$ = 6.5 Hz, 1-H), 5.20–5.08 (m, 2 H, $J_{1,2}$ = 8.7 Hz, 1'-H, $J_{2,3}$ = 8.0 Hz, 4'-H), 4.66 (dd, 1 H, $J_{6a,6b}$ = -12.1 Hz, 6a'-H), 4.64, 4.43 (2 d, 2 H, J = −12.2, CH_2Ph), 4.54 (t, 1 H, $J_{4,5}$ = 9.5 Hz, 4-H), 4.44 (dd, 1 H, $J_{5,6b}$ = 7.3 Hz, 6b'-H), 4.16 (dd, 1 H, $J_{5,6a}$ = 3.3 Hz, 5-H), 4.07–3.90 (m, 2 H, $J_{5,6a}$ = 3.3 Hz, 5'-H, $J_{6a,6b}$ = −9.9 Hz, 6a-H), 3.82 (dd, 1 H, $J_{5,6b}$ = 5.3 Hz, 6b-H), 3.82 (s, 3 H, $COOCH_3$), 3.69 (t, 1 H, $J_{3,4}$ = 9.5 Hz, 3-H), 3.58 (t, 1 H, $J_{3,4}$ = 10.1 Hz, 3'-

H), 1.52 (s, 3 H, CH_3). – ^{13}C NMR: δ/ppm = 170.2 (CO_2), 99.4 (C_{acetal}), 98.3 (C-1'), 95.9 (C-1), 75.5 (C-4), 74.6 (C-5'), 74.2, 73.9, 73.6 (C-2, 2', 3), 72.6 (C-3'), 71.5 (C-4'), 69.9 (CH_2Ph), 65.3 (C-6), 63.7 (C-6'), 62.5 (C-5), 52.9 ($COOCH_3$), 25.2 (CH_3).

$C_{51}H_{48}O_{17}$ Calcd.: C 65.66 H 5.19
(932.9) Found: C 65.47 H 5.34.

Benzyl O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl-(ethylidene)]-α-D-glucopyranoside (**14**) and *benzyl O-{2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-α-D-galactopyranosyl}-(1→3)-2,4,6-tri-O-benzoyl-β-D-glucopyranosyl-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl-(ethylidene)]-α-D-glucopyranoside* (**15**) and *benzyl O-{2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-β-D-galactopyranosyl}-(1→3)-2,4,6-tri-O-benzoyl-β-D-glucopyranosyl-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl-(ethylidene)]-α-D-glucopyranoside* (**17**) and *trichloroacetamido 2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl-(ethylidene)]-β-D-galactopyranoside* (**18**)

(a) A solution of **13** [1] (230 mg, 0.37 mmol) in CH_2Cl_2 (2 ml) was added at 0 °C during 0.5 h to a solution of **12** (0.32 g, 0.34 mmol) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 6 µl, 35 µmol) in CH_2Cl_2 (5 ml). The mixture was neutralized by addition of pyridine (1 ml), benzoyl chloride (0.5 ml) was added and the solution was stirred for 1 h at room temperature. The mixture was washed with aqueous $NaHCO_3$ solution, dried and concentrated. Chromatography (10:1 CCl_4/acetone) of the residue afforded first **14** (0.16 g, 44%). $[\alpha]_D$ = +87.1 (c = 1.2, $CHCl_3$); ^1H NMR (significant signals): δ = 5.83 (t, 1 H, $J_{3,4}$ = 9.3 Hz, 3-H), 5.68 (t, 1 H, $J_{4,5}$ = 9.6 Hz, 4'-H), 5.51 (dd, 1 H, $J_{2,3}$ = 9.1 Hz, 2'-H), 5.36 (d, 1 H, $J_{1,2}$ = 7.4 Hz, 1'-H), 5.13–5.08 (m, 1 H, $J_{2,3}$ = 9.8 Hz, 2-H), 5.09 (d, 1 H, $J_{1,2}$ = 3.8 Hz, 1-H), 4.65 (dd, 1 H, $J_{6a,6b}$ = –12.1 Hz, 6a'-H), 4.64, 4.43 (2 d, 2 H, J = –12.3 Hz, CH_2Ph), 4.53–4.40 (m, 1 H, $J_{5,6b}$ = 3.5 Hz, 6b'-H), 4.23 (m, 1 H, $J_{5,6a}$ = 4.9 Hz, 5'-H), 3.85 (s, 3 H, $COOCH_3$), 1.43 (s, 3 H, 2 CH_3). – ^{13}C NMR (significant signals): δ/ppm = 170.1 (CO_2), 99.3 (C_{acetal}), 99.0 (C-1'), 96.0 (C-1), 74.8, 74.2, 73.4 (C-3, 4, 5'), 73.3 (C-3'), 72.4, 71.9 (C-2, 2'), 69.7 (C-4), 65.3 (C-6), 63.4 (C-6'), 62.6 (C-5), 52.8 ($COOCH_3$), 25.1 (CH_3).

$C_{58}H_{52}O_{18}$ Calcd.: C 67.18 H 5.05
(1037.0) Found: C 66.96 H 5.15.

Eluted next was **15** (0.13 g, 26%). $[\alpha]_D$ = +102.1 (c = 1.6, $CHCl_3$). – ^1H NMR (significant signals): δ/ppm = 5.64 (dd, 1 H, $J_{2,3}$ = 11.0 Hz, 2-H), 5.54 (t, 1 H, $J_{2,3}$ = 9.3 Hz, 2''-H), 5.51 (d, 1 H, $J_{1,2}$ = 3.8 Hz, 1-H), 4.63, 4.41 (2 d, 2 H, J = –12.3, CH_2Ph), 4.37 (t, 1 H, $J_{3,4}$ = 8.3 Hz, 3'-H), 4.24 (d, 1 H, $J_{3,4}$ = 3.2, $J_{4,5}$ < 1 Hz, 4''-H), 3.94 (dd, 1 H, $J_{5,6a}$ = 4.7, $J_{6a,6b}$ = –10.2 Hz, 6a-H), 3.81 (dd, 1 H, $J_{5,6b}$ = 4.6 Hz, 6b-H), 3.83, 3.54 (2 s, 6 H, 2 $COOCH_3$), 3.33 (dd, 1 H, $J_{6a,6b}$ = –12.5 Hz, 6a''-H), 3.81 (dd, 1 H, 6b-H), 1.50, 1.43 (s, 6 H, 2 CH_3). – ^{13}C NMR (significant signals): δ/ppm = 170.2 (2 CO_2), 99.4, 98.4 (C_{acetal}), 98.9, 98.6 (C-1', 1''), 95.9 (C-1), 78.1 (C-3'), 74.3, 74.0, 73.3 (C-2, 2', 2''), 73.0, 72.2, 72.0 (C-3, 4, 4''), 69.8 (CH_2Ph), 69.1 (C-3''), 67.0 (C-4'), 65.3 (C-6), 64.5 (C-6''), 64.2 (C-6'), 62.5 (C-5''), 61.7 (C-5), 52.8, 52.3 (2 $COOCH_3$), 25.6 25.1 (2 CH_3).

FAB-MS (pos.) Calcd. for $C_{75}H_{70}O_{26}$: 1387.36. Found: 1409 ($M + Na^+$).

Eluted next was crude **17** (0.13 g, 26%), contaminated by a very small amount of trichloroacetamide. – ^1H NMR (significant signals): δ/ppm = 5.58 (t, 1 H, $J_{4,5}$ = 9.1 Hz, 4'-H), 5.56 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, 2''-H), 5.29–5.18 (m, 2 H, 2'-H), 5.06 (d, 1 H, $J_{1,2}$ = 3.8 Hz, 1-H), 4.87–4.79 (m, 2 H, 2, 3'-H), 4.61 (dd, 1 H, $J_{6a,6b}$ = –11.9 Hz, 6a'-H), 4.61, 4.39 (2 d, 2 H, J = –12.2 Hz, CH_2Ph), 4.50 (dd, 1 H, $J_{5,6b}$ = 5.8 Hz, 6b'-H), 4.26 (d, 1 H, $J_{3,4}$ = 3.6, $J_{4,5}$ < 1 Hz, 4''-H), 4.14 (m, 1 H, $J_{5,6a}$ = 4.4 Hz, 5'-H), 3.92 (m, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$, = 4.9 Hz, $J_{5,6b}$ = 5.0 Hz, 5-H), 3.83, 3.55 (2 s, 6 H, 2 $COOCH_3$), 1.47, 1.21 (2 s, 6 H, 2 CH_3). – ^{13}C NMR (significant signals): δ/ppm = 170.4, 170.3 (2 CO_2), 100.1 (C-1''), 99.5, 98.4 (2 C_{acetal}), 98.7 (C-1'), 95.7 (C-1), 78.0 (C-3), 74.3, 74.1, 73.9, 73.8 (C-3', 5', 4, 4''), 72.9, 71.8 (C-2'', 2'), 69.8 (CH_2Ph), 69.0, 68.5 (C-2, 3''), 65.5 (C-5''), 65.3 (C-6), 64.4 (C-6', 6''), 62.5 (C-5), 52.9 52.2 (2 $COOCH_3$), 25.3, 25.0 (2 CH_3).

CI-MS Calcd. for $C_{75}H_{70}O_{26}$: 1387.36. Found: 1329 (M–COOMe + $2H^+$).

(b) A solution of **13** (500 mg, 0.81 mmol) in acetonitrile (2 ml) was added at once at –20 °C to a solution of **12** (0.69 g, 0.74 mmol) and TMSOTf (15 µl, 80 µmol) in acetonitrile (7 ml) and the solution was stirred for 0.5 h. The mixture was neutralized by addition of pyridine (0.5 ml), diluted with CH_2Cl_2, washed with aqueous $NaHCO_3$ solution, dried and concentrated. Chromatography (10:1 CCl_4/acetone) of the residue afforded a 38:62 mixture (determined by ^1H NMR) of compounds **15** and **18** (0.66 g, 36% **15** with respect to **12**, 56% **18** with respect to **13**).

O-{2,3-Di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-α-D-galactopyranosyl}-(1→3)-2,4,6-tri-O-benzoyl-β-D-glucopyranosyl-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl(ethylidene)]-D-glucopyranose (**16**) and *dichloroacetamido 2,3-di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-β-D-galactopyranoside* (**19**)

A suspension of a mixture of **15** and **18** (0.47 g), as described above, and Pd 10% on charcoal (0.5 g) in ethyl acetate (20 ml) was treated at room temperature for 48 h with H_2. The mixture was filtered and the filtrate was concentrated. Chromatography (10:1 CCl_4/acetone) of the residue afforded first **19** (0.18 g, 97%). *M.p.* 195 °C (acetone/*n*-hexane); $[\alpha]_D$ = +105.4 (c = 1.1, $CHCl_3$). – ^1H NMR: δ/ppm = 8.03–7.93 (m, 1 H, $J_{1,NH}$ = 9.1 Hz, NH), 5.93 (dd, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.5 Hz, 3-H), 5.85 (s, 1 H, $CHCl_2$), 5.80 (t, 1 H, $J_{1,2}$ = 9.1 Hz, 2-H), 5.36 (t, 1 H, 1-H), 4.61 (d, 1 H, $J_{4,5}$ < 1.0 Hz, 4-H), 4.16 (dd, 1 H, $J_{5,6a}$ = 1.5 Hz, $J_{6a,6b}$ = -13.1 Hz, 6a-H), 4.05 (dd, 1 H, $J_{5,6b}$ = 1.7 Hz, 6b-H), 3.73 (bs, 1 H, 5-H), 3.69 (s, 3 H, $COOCH_3$), 1.63 (s, 3 H, CH_3). – ^{13}C NMR: δ/ppm = 169.9 ($COOCH_3$), 164.6 (CONH), 98.8 (C_{acetal}), 79.3 (C-1), 72.1 (C-4), 69.1 (C-3), 68.3, 67.5 (C-2,5), 65.7 ($CHCl_2$), 65.0 (C-6), 52.6 ($COOCH_3$), 25.6 (CH_3).

$C_{26}H_{25}Cl_2NO_{10}$ Calcd.: C 53.62 H 4.33 Cl 12.18 N 2.41
(582.4) Found: C 53.50 H 4.40 Cl 12.31 N 2.33.

Eluted next was **16** (0.24 g, 100%). – ^1H NMR (significant signals): δ/ppm = 5.63 (dd, 1 H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 10.9 Hz, α-2''-H), 5.29 (dd, 1 H, $J_{3,4}$ = 3.4, Hz α-3''-H), 5.21 (bt, 1 H, $J_{1,2}$ = 8.1, Hz $J_{2,3}$ = 9.3, β-2-H), 5.11 (dd, 1 H, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 9.9 Hz, α-2-H), 4.23 (d, 1 H, $J_{4,5}$ < 1 Hz, α-4''-H), 3.83,

3.54 (2 s, 6 H, 2α-$COOCH_3$), 3.32 (dd, 1 H, $J_{5,6a}$ = 3.4, Hz $J_{6a,6b}$ = −11.9, Hz α-6a''-H), 3.10 (bd, 1 H, α-6b''-H), 1.49, 1.42 (2 s, 6 H, 2α-CH_3). – ^{13}C NMR (significant signals): δ/ppm = 169.9 (2 CO_2), 99.4, 98.4 (C_{acetal}), 98.7 (C-1', 1''), 91.0 (C-1), 78.1 (C-3, 3'), 74.3 (C-5'), 74.0 (C-4'), 73.5 (C-4), 73.2 (C-2'), 72.3 (C-1'), 69.3 (C-3''), 65.3 (C-6), 64.5 (C-6''), 64.1 (C-6'), 62.3 (C-5''), 61.7 (C-5), 52.9, 52.3 (2 $COOCH_3$), 25.6, 25.1 (2 CH_3).

$C_{68}H_{64}O_{26}$	Calcd.:	C 62.96	H 4.97	
(1927.2)	Found:	C 62.77	H 5.04.	

O-{2,3-Di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-α-D-galactopyranosyl}-(1→3)-2,4,6-tri-O-benzoyl-β-D-glucopyranosyl-(1®3)-2-O-benzoyl-4,6-O-[(S)-1-meth--oxycarbonyl(ethylidene)]-D-glucopyranosyl trichloroacetimidate (**20**)

A mixture of **16** (0.16 g, 0.12 mmol), K_2CO_3 (28 mg, 0.2 mmol) and trichloroacetonitrile (35 µl, 0.35 mmol) was stirred at room temperature for 10 h. The mixture was centrifuged and the supernatent solution was concentrated. Chromatography (10:1 CCl_4/acetone) of the residue afforded **20** (0.13 g, 75%) as a 67:33 α/β-mixture of anomers. – ^{1}H NMR (significant signals): δ/ppm = 6.50 (d, 1 H, $J_{1,2}$ = 3.8 Hz, α-1-H), 5.97 (d, 1 H, $J_{1,2}$ = 6.4, Hz β-1-H), 3.84, 3.54 (2 s, 6 H, 2α-$COOCH_3$), 1.52, 1.43 (2 s, 6 H, 2α-CH_3). – ^{13}C NMR (significant signals): δ/ppm = 169.9 (2 CO_2), 160.8 (CNH), 99.5, 98.4 (C_{acetal}), 98.6 (C-1', 1''), 93.7 (C-1), 90.6 (CCl_3), 78.0 (C-3), 74.1 (C-3'), 73.2 (C-4), 72.5, 72.2 (C-2, 2', 4'', 5'), 69.2 (C-2'', 3''), 67.0 (C-4'), 65.0 (C-6), 64.6 (C-6''), 64.0 (C-6'), 61.7 (C-5, 5''), 53.0, 52.3 (2 $COOCH_3$), 25.6, 25.0 (2 CH_3).

$C_{70}H_{64}Cl_3NO_{26}$	Calcd.:	C 58.32	H 4.47	Cl 7.38	N 0.97
(1441.6)	Found:	C 58.22	H 4.53	Cl 7.34	N 0.79.

O-{2,3-Di-O-benzoyl-4,6-O-[(R)-1-methoxycarbonyl(ethylidene)]-β-D-galactopyranosyl}-(1→3)-2,4,6-tri-O-benzoyl-β-D-glucopyranosyl-(1→3)-2-O-benzoyl-4,6-O-[(S)-1-methoxycarbonyl(ethylidene)]-D-glucopyranosyl trichloroacetimidate (**21**)

A suspension of crude compound **17** (90 mg, 60 µmol) and Pd 10% on charcoal (0.1 g) in ethyl acetate (10 ml) was treated at room temperature for 12 h with H_2. The mixture was filtered and the filtrate was concentrated. The residue was dissolved in CH_2Cl_2 (5 ml) and treated with a catalytic amount of K_2CO_3 and trichloroacetonitrile (1 ml, 1.0 mmol) as described for the preparation of compound **20**. Chromatography (10:1 CCl_4/acetone) afforded **21** (50 mg, 58%) as a 80:20 α/β-mixture of anomers. – ^{1}H NMR (significant signals of the α-anomer): δ/ppm = 6.44 (d, 1 H, $J_{1,2}$ = 3.8 Hz, 1-H), 5.59 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, 2''-H), 5.55 (t, 1 H, $J_{3,4}$ = 9.0 Hz, $J_{4,5}$ = 9.7 Hz, 4'-H), 5.28 (d, 1 H, $J_{1,2}$ = 7.9 Hz, 1'-H), 5.29–5.21 (m, 1 H, 2'-H), 5.14 (dd, 1 H, $J_{2,3}$ = 9.9 Hz, 2-H), 4.86 (d, 1 H, $J_{1,2}$ = 8.0 Hz, 1''-H), 4.83 (dd, 1 H, $J_{3,4}$ = 3.6 Hz, 3''-H), 4.65 (dd, 1 H, $J_{6a,6b}$ = −11.9 Hz, 6a'-H), 4.52 (dd, 1 H, $J_{5,6b}$ = 6.1 Hz, 6b'-H), 4.27 (d, 1 H, $J_{4,5}$ < 1 Hz, 4''-H), 4.19 (m, 1 H, $J_{5,6a}$ = 4.3 Hz, 5'-H), 4.03 (dd, 1 H, $J_{5,6a}$ = 4.9, Hz $J_{6a,6b}$ = −10.6 Hz, 6a-H), 3.86, 3.55 (2 s, 6 H, 2 $COOCH_3$), 1.48, 1.25 (2 s, 6 H, 2 CH_3). – ^{13}C NMR (significant signals of the α-anomer): δ/ppm = 170.3, 170.1 (2 CO_2), 160.6 (CNH), 100.2 (C-1''), 99.6 (C-1'), 98.4, 98.2 (C_{acetal}), 93.7 (C-1), 90.6 (CCl_3), 77.7 (C-3), 73.8, 73.8, 73.4, 73.0 (C-3', 5', 4, 4''), 72.6, 71.9 (C-2'', 2'), 70.5 (C-4'), 69.0, 69.0, 68.5 (C-2, 3'', 5), 65.0 (C-6, 6''), 64.4 (C-6'), 53.0, 52.2 (2 $COOCH_3$), 25.3, 24.9 (2 CH_3).

$C_{70}H_{64}Cl_3NO_{26}$	Calcd.:	C 58.32	H 4.47	N 0.97	
(1441.6)	Found:	C 57.85	H 4.58	N 0.90.	

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