Synthesis of 4-(Methoxycarbonyl)phenyl β-Lactoside Derivatives Modified at C(6) or C(6′), and Evaluation of Their Inhibitory Activity on *Erythrina cristagalli* Lectin-Mediated Hemagglutination

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We report herein the synthesis of 4-(methoxycarbonyl)phenyl β-lactoside and eight derivatives modified at C(6) or C(6′). These compounds were evaluated in hemagglutination inhibition assay using the lectin from *Erythrina cristagalli*. None of the compounds showed enhanced activity as compared to lactose.

Introduction. – Lectins are carbohydrate-binding proteins other than immunoglobulins that display no enzymatic activity towards the recognized sugars. This protein class is widely spread in Nature and is generally involved in biological events mediated by their interactions with carbohydrates, like intracellular protein traffic, cell-cell adhesion, leucocytes recruitment to inflammatory sites, clearance of proteins from the circulatory system, and tumor metathesis [1]. Understanding the molecular basis of carbohydrate-lectin interactions is of major importance in order to develop inhibitors of these interactions in the search of new therapies for human diseases [2].

The lectins have agglutinating property as a result of their oligomeric structures bearing multiple carbohydrate binding sites that are responsible for their multivalent interactions with carbohydrates [1a]. This property is classically exploited in the hemagglutination inhibition assay (HIA), which is employed to assess the specificity of these proteins for mono- and oligosaccharides [3].

As plant lectins like the *Erythrina cristagalli* lectin (ECL) are easily available in pure form and in substantial amounts, they are often used in lectin-carbohydrate interaction studies [4]. ECL is a homodimeric lectin that shows specificity for the monosaccharides D-galactose and *N*-acetyl-D-galactosamine [5].

In this work, the synthesis of lactose derivatives and the evaluation of their interactions with ECL, compared to that of D-galactose (**1**) and lactose (**2**), using HIA is reported.

The aim of the work was to identify synthetic monovalent ligands that could bind to the lectin with higher affinity than lactose. These could be used in the synthesis of optimized multivalent ligands. Thus, the synthesis of derivatives of 4-(methoxycarbo-

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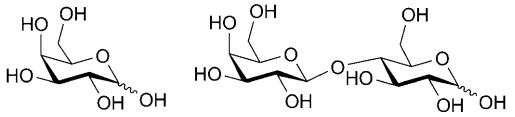
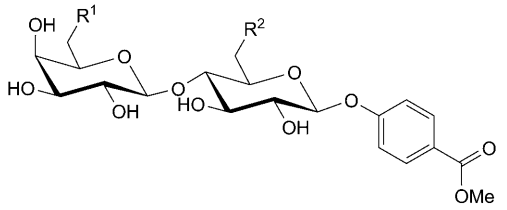


1 **2**

nyl)phenyl β-lactoside **3** modified at position 6 (**4a**–**4d**) and position 6′ (**5a**–**5d**) was undertaken. Lactoside **3** was chosen as starting material taking into account the ease of its preparation and the presence of a protected carboxy group that allows for the preparation of multivalent ligands with dendrimeric amines [6].



3 R^1 = OH, R^2 = OH
4a R^1 = OH, R^2 = H
4b R^1 = OH, R^2 = N_3
4c R^1 = OH, R^2 = NH_2
4d R^1 = OH, R^2 = NHAc
5a R^1 = H, R^2 = OH
5b R^1 = N_3, R^2 = OH
5c R^1 = NH_2, R^2 = OH
5d R^1 = NHAc, R^2 = OH

Results and Discussion. – Initially lactoside **3** was prepared from lactose (**2**) in four steps. Thus, peracetylation of lactose (Ac_2O/AcONa) followed by treatment with a solution of HBr in AcOH gave acetobromolactose. This was reacted with methyl 4-hydroxybenzoate in alkaline (Na_2CO_3) aqueous media [7] to give per-*O*-acetylated 4-(methoxycarbonyl)phenyl-β-lactoside, which was deacetylated with MeONa [8] to give lactoside **3** in 55% yield from lactose (*Scheme 1*).

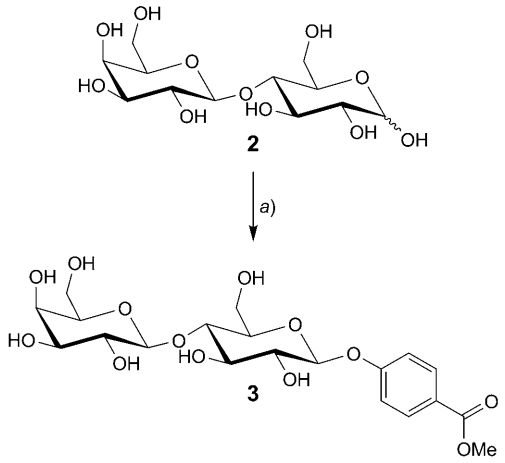
The treatment of lactoside **3** with a benzaldehyde–$ZnCl_2$ complex [9] gave acetal **6** in 76% yield. Regioselective replacement of the primary OH group at position 6 was achieved by reacting compound **6** with I_2 in the presence of Ph_3P and imidazole [10]. The corresponding 6-iodo-6-deoxy derivative **7** was obtained in 63% yield. Removal of the benzylidene acetal with HCl [11] furnished **8** in 75% yield (*Scheme 2*).

The hydrogenolysis of **8** (H_2, Pd/C) gave the 6-deoxy derivative **4a** in 73%. The reaction of **8** with NaN_3 in DMF furnished the corresponding 6-azido-6-deoxy derivative **4b** in 87% yield. Catalytic hydrogenation of **4b** afforded crude amine **4c** as an unstable, hygroscopic solid that resisted purification. Thus, it was converted, by reaction with PTSA, into the corresponding ammonium salt, which, although also hygroscopic, could be isolated in pure form in 58% yield from **4b**.

Treatment of crude **4c** with Ac_2O in MeOH [12] afforded the *N*-Ac derivative **4d**, in 61% yield (*Scheme 3*).

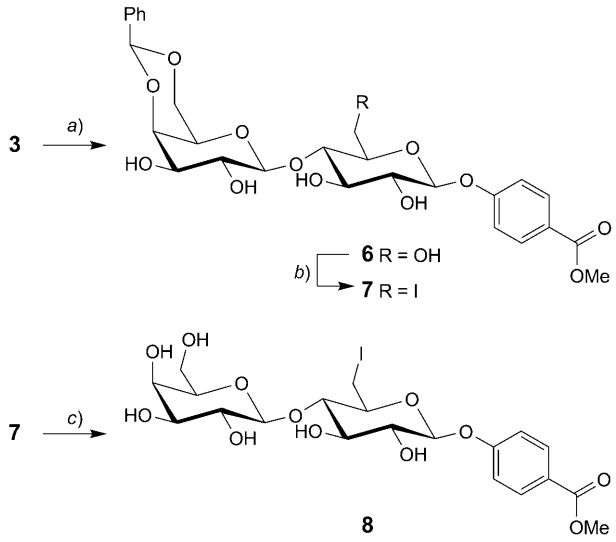
Next, the synthesis of compounds **5a**–**5d** was undertaken starting with the acetal **6**. Thus, treatment of **6** with BzCl in pyridine [13] furnished **9** in 84% yield. Reaction of **9** with NBS in CCl_4 afforded the expected 6′-bromo-6′-deoxy derivative **10** in 92% yield.

Scheme 1



a) 1. Ac_2O, AcONa, 100°; 2. HBr, AcOH; 3. Methyl 4-hydroxybenzoate, Na_2CO_3, H_2O, Me_2CO; 4. MeONa, MeOH.

Scheme 2

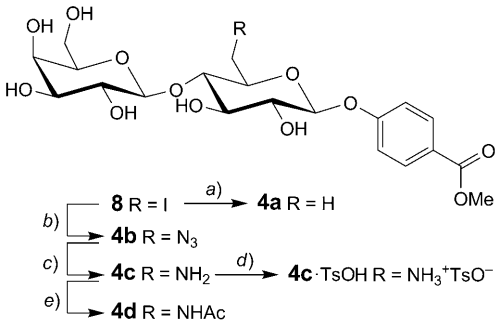


a) PhCHO, $ZnCl_2$; *b*) Ph_3P, I_2, imidazole, PhMe, MeCN, reflux; *c*) HCl, Me_2CO, H_2O, 75°.

Crude **10** was used in the next step without further purification. Treatment of **10** with NaN_3 in DMF at 80° afforded the corresponding azido derivative **11** along with the olefin **12**, in a ratio of *ca.* 2:1 (*Scheme 4*), according to ^{1}H-NMR data of the mixture.

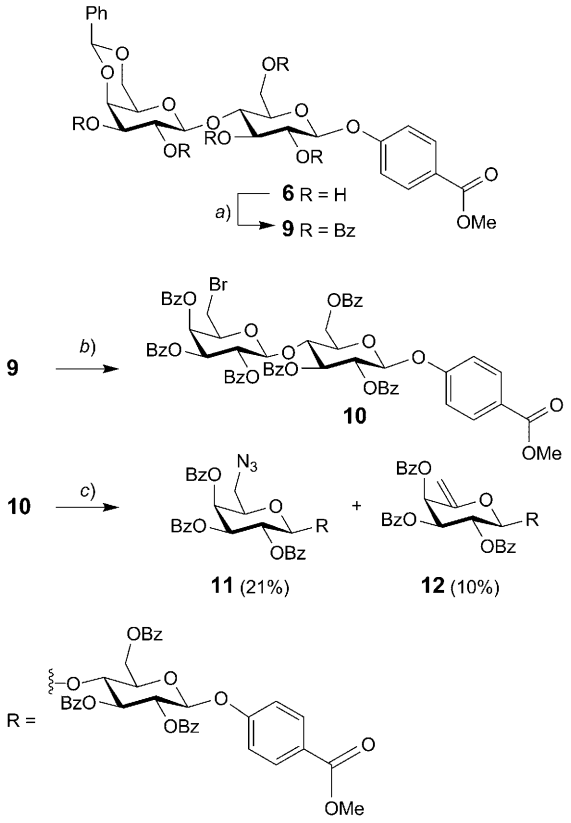
Separation of the compounds **11** and **12** by column chromatography was difficult, leading to pure **11** in low yield (21%). Several attempts to avoid the formation of the

Scheme 3



a) H_2, Pd/C, 4 Å molecular sieve, THF, MeOH, AcOEt; *b*) NaN_3, DMF, 50°; *c*) H_2, Pd/C, MeOH, THF; *d*) PTSA, MeOH; *e*) Ac_2O, MeOH.

Scheme 4



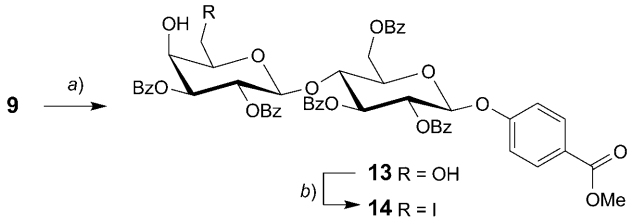
a) BzCl, Py; *b*) NBS, $BaCO_3$, CCl_4, reflux; *c*) NaN_3, DMF, 80°.

olefin **12** were made. For instance, performing the reaction in the presence of crown ether did not change the products ratio. The reaction did not go to completion when carried out at lower (60°) temperature, even after a week. Adding 10% H_2O to the mixture in order to enhance the solubility of NaN_3 led to the preferential formation of the olefin **12** (TLC). This procedure has previously been shown to be useful in the replacement of 6-*O*-Ts derivatives of D-galactose by azide [14].

The steric and polar effects due to the presence of an axial substituent at position 4 are invoked to explain, at least in part, the difficulties associated with the S_N2 reactions at position 6 in carbohydrates of the D-galacto series [14–16] and the formation of by-products as olefins and 3,6-anhydro derivatives [16]. In the case of compound **10**, the bulky benzoyl group at C(4′) was assumed to hamper the nucleophilic attack of azide and favor the elimination reaction.

Thus, a new approach to compounds **5a**–**5d** was attempted. The selective removal of acetal **9** with CF_3CO_2H gave the corresponding diol **13** in 64% yield. Treatment of **13** as for the preparation of **7** (*Scheme 2*) furnished the crude 6′-iodo derivative **14** in 77% yield (*Scheme 5*), which was used in the next steps without further purification. We sought that a better leaving group (iodide) at C(6′) and a smaller substituent at C(4′) (OH) would favor the displacement reaction over elimination.

Scheme 5



a) CF_3CO_2H, CH_2Cl_2, H_2O; *b*) Ph_3P, I_2, imidazole, PhMe, MeCN, reflux.

Treatment of **14** with NaN_3 in DMF, afforded two products having very close R_f values, as judged by TLC. It was thus rationalized that the two compounds could be the expected 6′-azido-6′-deoxy derivative along with the corresponding 2′,4′-di-*O*-benzoate resulting from benzoyl migration in the course of the reaction [17]. Debenzoylation of the crude mixture with MeONa/MeOH furnished **5b** in 36% yield from **13**.

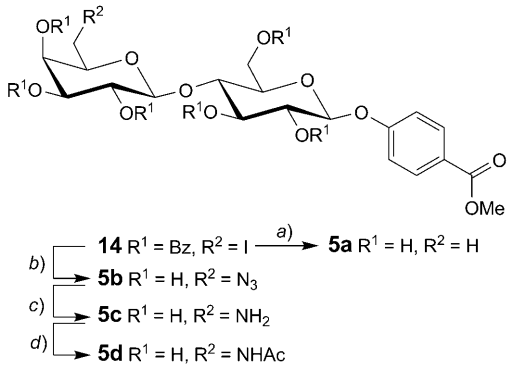
Catalytic hydrogenation of **5b** gave the corresponding 6′-amino-6′-deoxy derivative **5c** in 95% yield. Selective *N*-acetylation of **5c** with Ac_2O in MeOH [12] afforded **5d** in 70% yield from **5b**.

Catalytic hydrogenation of crude **14** (H_2, Pd/C, 1.4 bar) followed by debenzoylation (MeONa/MeOH) and purification by column chromatography afforded the 6′-deoxy derivative **5a** (*Scheme 6*) in 36% yield from **13**.

D-Galactose (**1**), lactose (**2**), lactoside **3**, compounds **4a**, **4b**, **4c** · TsOH, **4d**, and **5a**–**5d**, were evaluated in a hemagglutination inhibitory assay using ECL. The results are summarized in the *Table*.

The modifications at C(6) of lactoside **3** did not significantly influence the binding of compounds **4a**–**4d** to the lectin. Taking into account that in complexes of lactose

Scheme 6



a) 1. H_2, Pd/C, 4 Å molecular sieve, AcOEt; 2. MeONa, MeOH; *b*) 1. NaN_3, DMF, 60°; 2. MeONa, MeOH; *c*) H_2, Pd/C, MeOH, THF; *d*) Ac_2O, MeOH.

Table. *Inhibitory Potency of the Lactose Derivatives on the Agglutinating Activity of* Erythrina cristagalli *Relative to* D-*Galactose*

Lactose derivative	Relative Inhibitory Potency
1	1
2	4
3	8
4a	6
4b	4
4c · TsOH	4
4d	4
5a	1
5b	2
5c	< 0.1
5d	< 0.1

with *E. cristagalli* lectin [18], the OH group at C(6) is directed towards the outside of the site of interaction, it appears that the added substituents at this position were not able to make additional interactions that could be evidenced in the hemagglutination assay. On the other hand the relative potencies of compounds **5a**–**5d**, modified at position 6′, indicate that the replacement of the 6′-OH group had a deleterious effect on the interaction of these compounds with the lectin. The loss of affinity denotes the importance of the interaction of the OH group with the binding site of the lectin through a H_2O-mediated H-bond [18] that was lost with the replacement of the OH group in compounds **5a**–**5d**.

Experimental Part

General. Hydrogenation reactions that required controlled pressure of H_2 were carried out on a *Parr 3911 Hydrogenation Apparatus.* Column chromatography (CC): *Kieselgel 60* (SiO_2; 70–230 mesh or 230–400 mesh, *Merck*). Thin-layer chromatography (TLC): 0.25 mm thick SiO_2 *60G* (*Merck*, *7731*),

prepared on glass plates. M.p.: *Microquímica MQAs 301*, uncorrected. Optical rotations: *ADP220 Bellinghan + Stanley Ltd*. FT-IR Spectra: *Spectrum One* spectrometer, *Perkin-Elmer*, in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Advance DPX 200* and *Advance DRX 400* spectrometers, in $CDCl_3$ or $(CD_3)_2SO$; δ in ppm rel. to Me_4Si, J in Hz. HR-MS Spectra: *Q-TOF* spectrometer, *Waters*, ESI-MS in positive ion mode; samples diluted in MeOH/H_2O (1:1) with 1% HCO_2H; H_3PO_4 as reference mass. *E. cristagalli* lectin was purchased from *Sigma-Aldrich*.

*Methyl 4-{[4-*O*-(β-*D*-Galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**3**). Methyl 4-hydroxybenzoate (7.35 g, 48.31 mmol), Na_2CO_3 (10 g, 94.35 mmol), and H_2O (150 ml) were added to a soln. of α-lactosyl bromide peracetate [19] (15 g, 21.45 mmol) in Me_2CO (100 ml). The mixture was stirred for 3 h at r.t. Then, the Me_2CO was evaporated, and the aq. residue was extracted with CH_2Cl_2 (3 × 80 ml). The combined org. layers were washed with NaOH 0.5M soln. (4 × 80 ml), dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue was treated with MeONa in MeOH (0.05M, 80 ml). The mixture was stirred at r.t. for 3 h, and then neutralized with *Amberlite IRA 120* (H^+). The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from MeOH to afford **3** (5.63 g, 11.83 mmol; 55% yield from α-lactosyl bromide peracetate). White solid. M.p. 232.5–233°. $[\alpha]_D^{19} = -37.5$ ($c = 1.01$; DMSO). IR (neat): 3308, 1704, 1608, 1513, 1434, 847, 767. ^1H-NMR ($(CD_3)_2SO$): 7.92 (*d*, $J = 8.9$, H–C(3″), H–C(5″)); 7.14 (*d*, $J = 8.9$, H–C(2″), H–C(6″)); 5.52 (*d*, $J = 5.3$, OH); 5.11 (*d*, $J = 7.8$, H–C(1)); 5.08 (*d*, $J = 4.5$, OH); 4.81 (*d*, $J = 1.8$, OH); 4.78 (*d*, $J = 5.3$, OH); 4.66 (*t*, $J = 5.1$, OH); 4.61 (*t*, $J = 5.9$, OH); 4.51 (*d*, $J = 4.6$, OH); 4.25 (*d*, $J = 7.3$, H–C(1′)); 3.83 (*s*, MeO); 3.79–3.74 (*m*, H–C(6a) or H–C(6a′)); 3.68–3.28 (*m*, 11 sugar H, partially superimposed on the signal of H_2O of the solvent). ^{13}C-NMR ($(CD_3)_2SO$): 165.8 (C=O); 161.0 (C(1″)); 131.0 (C(3″), C(5″)); 123.0 (C(4″)); 116.0 (C(2″), C(6″)); 103.8 (C(1′)); 99.3 (C(1)); 79.9 (C(4)); 75.5, 75.0, 74.7 (C(2), C(3), C(5)); 73.2, 72.9 (C(3′), C(5′)); 70.5 (C(2′)); 68.1 (C(4′)); 60.4, 60.0 (C(6), C(6′)); 51.9 (MeO). HR-ESI-MS: 476.1614 ($C_{20}H_{28}O_{13}^+$; calc. 476.1530).

*Methyl 4-{[4-*O*-(4,6-*O*-Benzylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**6**). A mixture of anh. $ZnCl_2$ (3.6 g, 26.41 mmol) and PhCHO (2.4 ml, 58.80 mmol) was stirred until a milky, gelatinous paste was formed. The lactoside **3** (6 g, 12.59 mmol) and more PhCHO (6 ml) were added, and the mixture was stirred for 20 h at r.t. Excess PhCHO was removed by extraction with petroleum ether (PE; 2 × 15 ml). Crushed ice and AcOEt were added to the paste resulted from decantation of the org. layer to promote the separation of a white solid, which was collected by filtration and washed with boiling H_2O. The acetal **6** (5.43 g, 9.61 mmol, 76% yield) thus obtained was directly used in the next steps. IR (neat): 3383, 1699, 1605, 1583, 1509, 1438, 770, 697. ^1H-NMR ($(CD_3)_2SO$): 7.92 (*d*, $J = 8.9$, H–C(3″), H–C(5″)); 7.48–7.34 (*m*, 5 arom. H); 7.15 (*d*, $J = 8.9$, H–C(2″), H–C(6″)); 5.58 (*s*, *H*CPh); 5.56 (*d*, $J = 5.4$, OH); 5.28 (*d*, $J = 4.5$, OH); 5.13 (*d*, $J = 7.8$, H–C(1)); 5.02 (*d*, $J = 5.8$, OH); 4.85 (*s*, OH); 4.60 (*t*, $J = 6.0$, OH); 4.45 (*d*, $J = 7.5$, H–C(1′)); 4.12–4.01 (*m*, H–C(4′), H–C(6a′), H–C(6b′)); 3.82 (*s*, MeO); 3.76–3.50 (*m*, 7 sugar H); 3.40–3.47 (*m*, H–C(2)); 3.40–3.35 (*m*, H–C(2′)). ^{13}C-NMR ($(CD_3)_2SO$): 165.8 (C=O); 161.0 (C(1″)); 138.5 (C(8′)); 131.1 (C(3″), C(5″)); 128.7 (H*C*Ph); 128.0, 126.3 (arom. C); 123.1 (C(4″)); 116.0 (C(2″), C(6″)); 103.0 (C(1′)); 99.8 (H*C*Ph); 99.4 (C(1)); 78.3 (C(4)); 75.8 (C(4′)); 75.1, 74.6 (C(3) or C(3′) or C(5′)); 73.1 (C(2)); 71.7 (C(3) or C(3′) or C(5′)); 69.9 (C(2′)); 68.5 (C(6′)); 66.3 (C(5′)); 59.8 (C(6)); 51.9 (MeO). HR-ESI-MS 564.1878 ($C_{27}H_{32}O_{13}^+$; calc. 564.1843).

*Methyl 4-{[4-*O*-(4,6-*O*-Benzylidene-β-*D*-galactopyranosyl)-6-deoxy-6-iodo-β-*D*-glucopyranosyl]oxy}-benzoate* (**7**). To a stirred soln. of Ph_3P (4.48 g, 17.08 mmol) and imidazole (2.32 g, 34.08 mmol) in PhMe/MeCN 2:1 (360 ml) was added I_2 (3.84 g, 15.13 mmol). After 10 min, **6** (2.40 g, 4.25 mmol) was added dropwise, and the resulting mixture was stirred at 60° for 90 min. Then, the mixture was concentrated under reduced pressure. The residue was partitioned between AcOEt and H_2O, and the aq. layer was extracted with AcOEt (4 × 100 ml). The combined org. layers were dried ($MgSO_4$) and concentrated under reduced pressure. The crude residue was purified by CC (SiO_2; CH_2Cl_2/MeOH 95:5) to afford **7** (1.82 g, 2.70 mmol, 63%). White solid. M.p. 222–223° (dec.). $[\alpha]_D^{19} = -100.0$ ($c = 1$, DMSO). IR (neat): 3403, 1687, 1604, 1583, 1507, 771, 686. ^1H-NMR ($(CD_3)_2SO$): 7.94 (*d*, $J = 8.9$, H–C(3″), H–C(5″)); 7.49–7.47, 7.42–7.36 (*m*, 5 arom. H); 7.22 (*d*, $J = 8.9$, H–C(2″), H–C(6″)); 5.63 (*d*, $J = 5.2$, OH); 5.59 (*s*, *H*CPh); 5.37 (*d*, $J = 4.7$, OH); 5.22 (*d*, $J = 7.8$, H–C(1)); 5.04 (*d*, $J = 5.5$, OH); 4.80 (*d*, $J = 1.7$, OH); 4.47 (*d*, $J = 7.5$, H–C(1′)); 4.12–4.02 (*m*, H–C(4′), H–C(6a′), H–C(6b′)); 3.88 (*dd*, $J = 1.6$, 10.6, H–C(6a)); 3.83 (*s*, MeO); 3.77–3.72 (*m*, H–C(3)); 3.66 (br. *s*, H–C(5′)); 3.59 (*td*, $J = 1.6$, 9.0,

H–C(5)); 3.53–3.50 (*m*, H–C(3′)); 3.48–3.45 (*m*, H–C(2′)); 3.44–3.33 (*m*, H–C(2), H–C(6b), H–C(4)). ^{13}C-NMR ((CD$_3$)$_2$SO): 165.7 (C=O); 160.7 (C(1″)); 138.5 (C(8′)); 130.9 (C(3″), C(5″)); 128.6 (arom. C); 127.9, 126.2 (arom. C); 123.2 (C(4″)); 116.2 (C(2″), C(6″)); 103.2 (C(1′)); 99.7 (H*C*Ph); 99.1 (C(1)); 82.8 (C(4)); 75.7 (C(4′)); 73.6 (C(3), C(5)); 73.0 (C(2)); 71.7 (C(3′)); 69.9 (C(2′)); 68.4 (C(6′)); 66.3 (C(5′)); 51.8 (MeO); 7.2 (C(6)). HR-ESI-MS: 674.0883 ($C_{27}H_{31}IO_{12}^{+}$; calc. 674.0860).

Methyl 4-{[6-Deoxy-4-O-*(β*-D-*galactopyranosyl)-6-iodo-β*-D-*glucopyranosyl]oxy}benzoate* (**8**). A mixture of **7** (1.6 g, 2.37 mmol) and HCl 1M soln. (3.2 ml) in Me$_2$CO/H$_2$O 1:1 (64 ml) was stirred at 75° for 80 min and then neutralized with BaCO$_3$ (1 g, 5.07 mmol), which was removed by filtration. The filtrate was concentrated under reduced pressure. The residue was recrystallized from MeOH/H$_2$O 9:1 to afford **8** (1.04 g, 1.77 mmol, 75%). White solid. M.p. 216.5–217.5° (dec.). $[\alpha]_D^{19} = -78.9$ ($c = 1.01$, DMSO). IR (neat): 3352, 1702, 1606, 1509, 1436. ^{1}H-NMR ((CD$_3$)$_2$SO): 7.85 (*d*, $J = 8.9$, H–C(3″), H–C(5″)); 7.14 (*d*, $J = 8.9$, H–C(2″), H–C(6″)); 5.53 (*d*, $J = 5.2$, OH); 5.15–5.12 (*m*, H–C(1), OH); 4.80 (*d*, $J = 1.9$, OH); 4.73 (*t*, $J = 5.4$, OH); 4.60 (*t*, $J = 5.0$, OH); 4.45 (*d*, $J = 4.6$, OH); 4.19 (*d*, $J = 7.3$, H–C(1′)); 3.77–3.74 (*m*, H–C(6a)); 3.75 (*s*, MeO); 3.62 (*ddd*, $J = 2.1, 7.6, 9.8$, H–C(5)); 3.56 (br. *t*, $J = 3.8$, H–C(4′)); 3.51–3.40 (*m*, H–C(3), H–C(5′), H–C(6a′), H–C(6b′)); 3.32–3.21 (*m*, H–C(2), H–C(6b), H–C(2′), H–C(3′)); 3.19 (*t*, $J = 9.0$, H–C(4)). ^{13}C-NMR ((CD$_3$)$_2$SO): 165.7 (C=O); 160.7 (C(1″)); 130.9 (C(3″), C(5″)); 123.2 (C(4″)); 116.2 (C(2″), C(6″)); 104.0 (C(1′)); 99.0 (C(1)); 83.8 (C(4)); 75.6 (C(5′)); 74.1 (C(3)); 73.5 (C(5)); 73.2, 72.8 (C(2) or C(3′)); 70.4 (C(2′)); 68.1 (C(4′)); 60.4 (C(6′)); 51.9 (MeO); 7.6 (C(6)). HR-ESI-MS: 586.0529 ($C_{20}H_{27}IO_{12}^{+}$; calc. 586.0547).

Methyl 4-{[6-Deoxy-4-O-*(β*-D-*galactopyranosyl)-β*-D-*glucopyranosyl]oxy}benzoate* (**4a**). A soln. of **8** (100 mg, 0.17 mmol) in THF/MeOH/AcOEt 10:3:2 (9 ml) containing Pd/C 10% (200 mg), and 4-Å molecular sieves (1.3 g) was stirred under H_2 (1.4 bar) for 4 d. Then, the mixture was filtered and concentrated under reduced pressure. The residue was recrystallized from MeOH to afford **4a** (57 mg, 0.12 mmol, 73%). White solid. M.p. 250–251°. $[\alpha]_D^{24} = -32.5$ ($c = 0.51$, DMSO). IR (neat): 3359, 1706, 1245, 768. ^{1}H-NMR ((CD$_3$)$_2$SO): 7.94 (*d*, $J = 8.0$, H–C(3″), H–C(5″)); 7.14 (*d*, $J = 8.2$, H–C(2″), H–C(6″)); 5.54 (*d*, $J = 3.8$, OH)); 5.13–5.11 (*m*, 2 H–C, H–C(1), OH); 4.85 (*s*, OH); 4.80 (*s*, OH); 4.72 (*s*, OH); 4.54 (*s*, OH); 4.25 (*d*, $J = 5.6$, H–C(1′)); 3.84 (*s*, MeO); 3.71–3.30 (*m*, 11 sugar H, partially superimposed on the signal of H_2O of the solvent); 3.12 (*t*, $J = 8.5$, H–C(4)); 1.30 (*d*, $J = 5.1$, H–C(6)). ^{13}C-NMR ((CD$_3$)$_2$SO): 165.8 (C=O); 160.9 (C(1″)); 131.1 (C(3″), C(5″)); 123.0 (C(4″)); 115.9 (C(2″), C(6″)); 104.2 (C(1′)); 98.9 (C(1)); 85.5 (C(4)); 75.4 (C(5′)); 74.0 (C(3)); 73.2, 73.0 (C(2), C(3′)); 70.4 (C(2′)); 70.2 (C(5)); 68.1 (C(4′)); 60.4 (C(6′)); 51.9 (MeO); 17.4 (C(6)). HR-ESI-MS: 460.1472 ($C_{20}H_{28}O_{12}^{+}$; calc. 460.1581).

Methyl 4-{[6-Azido-6-deoxy-4-O-*(β*-D-*galactopyranosyl)-β*-D-*glucopyranosyl]oxy}benzoate* (**4b**). A mixture of **8** (800 mg, 1.36 mmol), NaN$_3$ (400 mg, 6.15 mmol), and anh. DMF (30 ml) was stirred for 24 h at 50°. Then, the solvent was evaporated, and the crude residue was subjected to CC (SiO_2, 1 × 3 cm, AcOEt/MeOH 1:1) to give a syrup which was recrystallized from MeOH to afford **4b** (592 mg, 1.18 mmol, 87%). White solid. M.p. 214.5–217° (dec.). $[\alpha]_D^{19} = -92.0$ ($c = 1$, DMSO). IR (neat): 3345, 2098, 1700, 1607, 1509, 1440. ^{1}H-NMR ((CD$_3$)$_2$SO): 7.92 (*d*, $J = 8.8$, H–C(3″), H–C(5″)); 7.16 (*d*, $J = 8.8$, H–C(2″), H–C(6″)); 5.62 (*d*, $J = 5.3$, OH); 5.23 (*d*, $J = 7.8$, H–C(1)); 5.17 (*d*, $J = 4.6$, OH); 4.91 (*d*, $J = 1.7$, OH); 4.80 (*d*, $J = 5.2$, OH); 4.67 (*t*, $J = 5.0$, OH); 4.51 (*d*, $J = 4.6$, OH); 4.19 (*d*, $J = 7.2$, H–C(1′)); 3.93–3.88 (*m*, H–C(5)); 3.82 (*s*, MeO); 3.72 (*dd*, $J = 1.8, 13.2$, H–C(6a)); 3.63 (br. *t*, $J = 3.3$, H–C(4′)); 3.51–3.40 (*m*, H–C(3), H–C(6b), H–C(5′), H–C(6a′), H–C(6b′)); 3.57–3.48 (*m*, H–C(2), H–C(4), H–C(2′), H–C(3′)). ^{13}C-NMR ((CD$_3$)$_2$SO): 165.7 (C=O); 160.6 (C(1″)); 130.9 (C(3″), C(5″)); 123.1 (C(4″)); 115.9 (C(2″), C(6″)); 103.9 (C(1′)); 98.8 (C(1)); 80.9 (C(4)); 75.5 (C(5′)); 74.3 (C(3)); 73.3 (C(5)); 73.1, 72.6 (C(2), C(3′)); 70.3 (C(2′)); 68.0 (C(4′)); 60.4 (C(6′)); 51.8 (MeO); 50.5 (C(6)). HR-ESI-MS: 501.1596 ($C_{20}H_{27}N_3O_{12}^{+}$; calc. 501.1595).

4-(Methoxycarbonyl)phenyl 6-Ammonio-6-deoxy-4-O-*β*-D-*galactopyranosyl-β*-D-*glucopyranoside 4-Methylbenzenesulfonate* (**4c**·TsOH). A soln. of **4b** (200 mg, 0.4 mmol) in MeOH/THF 3:2 (25 ml) containing Pd/C 10% (50 mg) was stirred under a H_2 atmosphere for 5 h. Then, the mixture was filtered and concentrated under reduced pressure. The residue (184 mg, 0.39 mmol) was dissolved in hot MeOH, and to this soln. was added monohydrated PTSA (120 mg, 0.62 mmol). The ammonium salt **4c**·TsOH was precipitated by the addition of Et$_2$O as a hygroscopic solid (128 mg, 0.20 mmol, 58% yield from **4b**). M.p. 131–133°. $[\alpha]_D^{24} = -9.9$ ($c = 0.51$, DMSO). IR (neat): 3363, 1717, 1606, 1509, 1434, 768. ^{1}H-NMR

((CD$_3$)$_2$SO): 7.94 (*d*, *J* = 8.4, H–C(3″), H–C(5″)); 7.88 (*s*, 3 NH); 7.51 (*d*, *J* = 7.6, 2 arom. H); 7.19 (*d*, *J* = 8.4, H–C(2″), H–C(6″)); 7.14 (*d*, *J* = 7.6, 2 arom. H); 5.16 (*d*, *J* = 7.6, H–C(1)); 4.34 (*d*, *J* = 7.5, H–C(1′)); 4.24–3.42 (*m*, 11 sugar H, partially superimposed on the H_2O signal from the solvent); 3.84 (*s*, MeO); 3.05–3.02 (*m*, sugar H); 2.31 (*s*, Me). ^{13}C-NMR ((CD$_3$)$_2$SO): 165.7 (C=O); 160.9 (C(1″)); 145.4 (arom. C); 138.0 (arom. C); 131.3 (C(3″), C(5″)); 128.8 (arom. C); 125.6 (arom. C); 123.4 (C(4″)); 116.2 (C(2″), C(6″)); 103.5 (C(1′)); 99.4 (C(1)); 80.7 (C(4)); 75.9 (C(5′)); 74.4, 73.2 (C(3), C(5)); 72.6, 70.7 (C(2), C(3′)); 70.4 (C(2′)); 68.3 (C(4′)); 60.6 (C(6′)); 52.1 (MeO); 39.8 (C(6)); 20.9 (Me). HR-ESI-MS: 475.1405 ($C_{20}H_{29}NO_{12}^{+}$; calc. 475.1690).

*Methyl 4-{[6-(Acetylamino)-6-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**4d**). A soln. of **4b** (280 mg, 0.56 mmol) in MeOH/THF 3 : 2 (30 ml) containing Pd/C 10% (70 mg) was stirred under a H_2 atmosphere for 5 h. Then, the mixture was filtered and concentrated under reduced pressure. The residue was dissolved in anh. MeOH (5 ml). The soln. was cooled on an ice bath while Ac_2O (0.14 ml, 1.48 mmol) was added. The mixture was stirred for 20 min at r.t. Then, H_2O was added (0.5 ml), and the solvent was evaporated. The crude residue was recrystallized from MeOH/H_2O 3 : 1 to afford **4d** (177 mg, 0.34 mmol, 61% yield from **4b**). White solid. M.p. 233.5–234°. $[\alpha]_D^{24} = -17.6$ (*c* = 0.51, DMSO). IR (neat): 3327, 1716, 1646, 1607, 1070, 678. ^{1}H-NMR ((CD$_3$)$_2$SO): 7.92 (*d*, *J* = 8.9, H–C(3″), H–C(5″)); 7.88 (*dd*, *J* = 4.5, 6.8, NH); 7.12 (*d*, *J* = 8.9, H–C(2″), H–C(6″)); 5.55 (*d*, *J* = 5.4, OH); 5.11 (*d*, *J* = 4.9, OH); 5.04 (*d*, *J* = 7.8, H–C(1)); 4.97 (*d*, *J* = 1.8, OH); 4.81 (*d*, *J* = 5.4, OH); 4.70 (*t*, *J* = 4.9, OH); 4.53 (*d*, *J* = 4.7, OH); 4.30 (*d*, *J* = 7.4, H–C(1′)); 3.82 (*s*, MeO); 3.69–3.62 (*m*, H–C(5), H–C(4′)); 3.57–3.45 (*m*, H–C(6a), H–C(6a′), H–C(6b′), H–C(5′), H–C(3) or H–C(3′)); 3.41–3.29 (*m*, H–C(2), H–C(4), H–C(6b), H–C(2′), H–C(3) or H–C(3′), superimposed on the signal of H_2O of the solvent); 1.81 (*s*, Me). ^{13}C-NMR ((CD$_3$)$_2$SO): 169.4 (NHC=O); 165.7 (C=O); 160.8 (C(1″)); 130.9 (C(3″), C(5″)); 123.1 (C(4″)); 115.9 (C(2″), C(6″)); 103.6 (C(1′)); 99.2 (C(1)); 82.0 (C(4)); 75.6 (C(5)); 74.7, 73.3, 72.6, 72.5 (C(2), C(3), C(3′), C(5′)); 70.5 (C(2′)); 68.1 (C(4′)); 60.4 (C(6′)); 51.8 (MeO); 39.8 (C(6)); 22.5 (Me). HR-ESI-MS: 517.1813 ($C_{22}H_{31}NO_{13}^{+}$; calc. 517.1795).

*Methyl 4-{[2,3,6-Tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**9**). A stirred suspension of **6** (5 g, 8.86 mmol) in anh. pyridine (70 ml) was cooled in an ice bath while BzCl (15.5 ml, 132.32 mmol) was added dropwise. The resulting mixture was stirred for 4 h at r.t. Then, H_2O (60 ml) was added, and, after 30 min, the mixture was partitioned between CH_2Cl_2 and H_2O, and the aq. layer was extracted with CH_2Cl_2 (2 × 60 ml). The combined org. layers were washed with 2M aq. H_2SO_4 soln. (5 × 60 ml), sat. aq. $NaHCO_3$ soln. (4 × 60 ml), and H_2O (2 × 60 ml). The org. phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was recrystallized from Me_2CO/PE 1 : 1 to afford **9** (8.05 g, 7.42 mmol, 84%). White solid. M.p. 263.5–265°. $[\alpha]_D^{22} = +35.9$ (*c* = 1, $CHCl_3$). IR (neat): 1716, 1263, 1241, 705. ^{1}H-NMR ($CDCl_3$): 8.15 (*d*, *J* = 7.2, 2 arom. H); 7.96–7.89 (*m*, 6 arom. H); 7.81 (*d*, *J* = 7.2, 2 arom. H); 7.77 (*d*, *J* = 8.8, H–C(3″), H–C(5″)); 7.56–7.25 (*m*, 18 arom. H); 7.19 (*t*, *J* = 7.6, 2 arom. H); 6.90 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 5.92 (*t*, *J* = 7.8, H–C(3)); 5.82 (*dd*, *J* = 8.0, 10.4, H–C(2′)); 5.62 (*t*, *J* = 7.8, H–C(2)); 5.39 (*d*, *J* = 7.8, H–C(1)); 5.31 (*s*, *H*CPh); 5.23 (*dd*, *J* = 3.5, 10.4, H–C(3′)); 4.89 (*d*, *J* = 8.0, H–C(1′)); 4.67 (*dd*, *J* = 1.4, 11.7, H–C(6a)); 4.39–4.31 (*m*, H–C(4, H–C(6b), H–C(4′)); 4.12–4.09 (*m*, H–C(5)); 3.83 (*s*, MeO); 3.80 (*d*, *J* = 12.3, H–C(6a′)); 3.61 (*d*, *J* = 12.3, H–C(6b′)); 3.05 (*s*, H–C(5′)). ^{13}C-NMR ($CDCl_3$): 166.3–164.8 (6 C=O); 160.0 (C(1″)); 137.4–124.7 (arom. C); 116.2 (C(2″), C(6″)); 101.7 (C(1′)); 100.6 (H*C*Ph); 97.9 (C(1)); 77.2 (C(4)); 74.0 (C(3)); 73.1, 73.0 (C(5), C(4′)); 72.5 (C(3′)); 71.9 (C(2)); 69.4 (C(2′)); 68.0 (C(6′)); 66.6 (C(5′)); 62.3 (C(6)); 51.9 (MeO). HR-ESI-MS: 1084.3020 ($C_{62}H_{52}O_{18}^{+}$; calc. 1084.3154).

*Methyl 4-{[4-*O*-(6-Azido-2,3,4-tri-*O*-benzoyl-6-deoxy-β-*D*-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-*D*-glucopyranosyl]oxy}benzoate* (**11**) *and Methyl 4-{[2,3,6-Tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzoyl-6-deoxy-α-*L*-arabino-hex-5-enopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**12**). To a refluxing mixture of **9** (2 g, 1.84 mmol), $BaCO_3$ (760 mg, 3.86 mmol), and CCl_4 (60 ml), with stirring, NBS (660 mg, 3.62 mmol) was added in portions. After 5 h, the solvent was removed under reduced pressure. The residue was suspended in CH_2Cl_2, and the $BaCO_3$ was eliminated by filtration. The filtrate was washed with H_2O (3 × 20 ml), and the org. phase was dried (Na_2SO_4) and concentrated *in vacuo* to afford *methyl 4-{[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4-tri-*O*-benzoyl-6-bromo-6-deoxy-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**10**) as a syrup which was directly used in the next step. To a soln. of crude **10** in anh.

DMF (30 ml) NaN_3 (700 mg, 10.77 mmol) was added. The mixture was stirred for 17 h at 80°. Then, the solvent was eliminated, and the residue was purified by CC (SiO_2; $CHCl_3$) to give **11** (441 mg, 0.39 mmol, 21% yield from **9**) and **12** (210 mg, 0.19 mmol, 10%).

Data of **11**: M.p. 104.5–106.5°. $[\alpha]_D^{22} = +33.1$ ($c = 1$, $CHCl_3$). IR (neat): 2104, 1722, 1259, 1089, 1066, 1025. ^{1}H-NMR ($CDCl_3$,): 7.99–7.93 (*m*, 10 arom. H); 7.84 (*d*, *J* = 8.8, H–C(3″), H–C(5″)); 7.73 (*d*, *J* = 7.5, 2 arom. H); 7.67–7.60 (*m*, 2 arom. H); 7.54–7.47 (*m*, 5 arom. H); 7.42–7.29 (*m*, 7 arom. H); 7.25–7.17 (*m*, 4 arom. H); 6.95 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 5.86 (*t*, *J* = 9.4, H–C(3)); 5.76–5.71 (*m*, H–C(2), H–C(2′)); 5.61 (*d*, *J* = 2.9, H–C(4′)); 5.38–5.34 (*m*, H–C(1), H–C(3′)); 4.89 (*d*, *J* = 7.8, H–C(1′)); 4.66 (*d*, *J* = 11.9, H–C(6a)); 4.48 (*dd*, *J* = 4.8, 11.9, H–C(6b)); 4.34 (*t*, *J* = 9.4, H–C(4)); 4.07 (*dd*, *J* = 4.8, 9.4, H–C(5)); 3.85 (*s*, MeO); 3.69 (*t*, H–C(5′)); 2.73 (*dd*, *J* = 5.3, 12.9, H–C(6a′)); 2.64 (*dd*, *J* = 7.2, 12.9, H–C(6b′)). ^{13}C-NMR ($CDCl_3$): 166.2–164.6 (6 C=O); 159.9 (C(1″)); 133.5–116.1 (arom. C); 100.6 (C(1′)); 98.2 (C(1)); 75.5 (C(4′)); 73.1 (C(5)); 72.8 (C(5′)); 72.5 (C(3)); 71.5 (C(3′)); 71.2, 69.6 (C(2), C(2′)); 67.9 (C(4)); 62.2 (C(6)); 51.7 (MeO); 49.2 (C(6′)). HR-ESI-MS: 1125.3054 ($C_{62}H_{51}N_3O_{18}^+$; calc. 1125.3168).

Data of **12**: M.p. 191–192°. $[\alpha]_D^{22} = +33.9$ ($c = 1$, $CHCl_3$). IR (neat): 1718, 1267, 703. ^{1}H-NMR ($CDCl_3$): 7.99–7.92 (*m*, 8 arom. H); 7.83 (*d*, *J* = 7.9, 5 arom. H); 7.62–7.56 (*m*, 2 arom. H); 7.52–7.25 (*m*, 15 arom. H); 7.11 (*t*, *J* = 7.7, 2 arom. H); 6.95 (*d*, *J* = 8.6, H–C(2″), H–C(6″)); 5.90–5.85 (*m*, H–C(3), H–C(2′), H–C(4′)); 5.72 (*t*, *J* = 7.9, H–C(2)); 5.43 (*dd*, *J* = 3.2, 9.7, H–C(3′)); 5.38 (*d*, *J* = 7.9, H–C(1)); 5.01 (*d*, *J* = 6.7, H–C(1′)); 4.65 (*d*, *J* = 12.0, H–C(6a)); 4.63 (*s*, H–C(6a′)); 4.58 (*s*, H–C(6b′)); 4.49 (*dd*, *J* = 5.2, 12.0, H–C(6b)); 4.35 (*t*, *J* = 9.2, H–C(4)); 4.08 (*dd*, *J* = 5.2, 9.2, H–C(5)); 3.85 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 165.6–164.9 (6 C=O); 160.0 (C(1″)); 149.2–124.9 (arom. C); 116.3 (C(2″), C(6″)); 102.9 (C(6′)); 101.7 (C(1′)); 98.4 (C(1)); 76.8 (C(4)); 73.3 (C(5)); 73.0 (C(3), C(2′) or C(4′)); 71.5 (C(2)); 70.2 (C(3′)); 69.9, 68.8 (C(3), C(2′) or C(4′)); 62.5 (C(6)); 51.9 (MeO). HR-ESI-MS: 1082.2991 ($C_{62}H_{50}O_{18}^+$; 1082.2997).

Methyl 4-{[2,3,6-Tri-O-*benzoyl-4*-O-*(2,3-di*-O-*benzoyl-β*-D-*galactopyranosyl)-β*-D-*glucopyranosyl]-oxy}benzoate* (**13**). To a soln. of **9** (8 g, 7.37 mmol) in CH_2Cl_2 (50 ml) were added CF_3CO_2H (8 ml, 108.05 mmol) and H_2O (0.4 ml). The mixture was stirred vigorously for 10 h at r.t. Then, it was diluted with CH_2Cl_2 (60 ml) and washed with H_2O (2 × 40 ml), sat. aq. $NaHCO_3$ soln. (2 × 40 ml), and again with H_2O (3 × 30 ml). The org. phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by CC (SiO_2; CH_2Cl_2/MeOH 95:5) to afford **13** (4.7 g, 4.71 mmol, 64%). White solid. M. p. 237–238°. $[\alpha]_D^{23} = +34.9$ ($c = 1$, DMSO). IR (neat): 1717, 1265, 704. ^{1}H-NMR ($(CD_3)_2SO$): 7.96–7.90 (*m*, 4 arom. H); 7.86–7.81 (*m*, 6 arom. H); 7.71–7.36 (*m*, 15 arom. H); 7.30 (*t*, *J* = 7.5, 2 arom. H); 7.01 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 5.91 (*d*, *J* = 7.9, H–C(1)); 5.84–5.79 (*m*, H–C(3)); 5.51–5.45 (*m*, H–C(2), H–C(2′)); 5.22 (*d*, *J* = 5.4, HO–C(4′)); 5.18 (*dd*, *J* = 3.0, 10.4, H–C(3′)); 5.02 (*d*, *J* = 8.0, H–C(1′)); 4.57–4.47 (*m*, H–C(6a), H–C(6b)); 4.48 (*t*, *J* = 5.0, HO–C(6′)); 4.38–4.34 (*m*, H–C(4), H–C(5)); 4.06 (br. *t*, H–C(4′)); 3.78 (*s*, MeO); 3.55 (br. *t*, H–C(5′)); 3.09–3.06 (*m*, H–C(6a′)); 2.95 (*td*, *J* = 5.0, 10.0, H–C(6b′)). ^{13}C-NMR ($(CD_3)_2SO$): 165.6–165.6 (6 C=O); 159.6 (C(1″)); 133.8–133.3 (arom. C); 130.9 (C(3″), C(5″)); 129.4–123.7 (arom. C); 115.9 (C(2″), C(6″)); 100.5 (C(1′)); 96.0 (C(1)); 76.0 (C(4)); 74.8 (C(3′)); 74.6 (C(5′)); 72.9 (C(3)); 72.6 (C(5)); 71.7, 70.2 (C(2), C(2′)); 64.6 (C(4′)); 62.9 (C(6)); 58.2 (C(6′)); 51.9 (MeO). HR-ESI-MS: 996.2760 ($C_{55}H_{48}O_{18}^+$; calc. 996.2840).

Methyl 4-{[2,3,6-Tri-O-*benzoyl-4*-O-*(2,3-di*-O-*benzoyl-6-deoxy-6-iodo-β*-D-*galactopyranosyl)-β*-D-*glucopyranosyl]oxy}benzoate* (**14**). To a stirred soln. of Ph_3P (3.2 g, 12.2 mmol) and 1*H*-imidazole (1.66 g, 24.44 mmol) in PhMe/MeCN 2:1 (380 ml), I_2 (2.5 g, 12.21 mmol) was added. After 10 min, **13** (4.8 g, 4.81 mmol) was added dropwise, and the resulting mixture was stirred at 60° for 9 h. Then, the mixture was concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and H_2O, and the aq. layer was extracted with CH_2Cl_2 (2 × 150 ml). The combined org. layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was washed with Et_2O to afford **14** (4.03 g, 3.71 mmol, 77% crude yield) that was used in subsequent reactions without further purification. ^{1}H-NMR ($CDCl_3$): 8.08 (*d*, *J* = 7.0, 2 arom. H); 7.95–7.87 (*m*, arom. H); 7.81 (*d*, *J* = 8.8, H–C(3″), H–C(5″)); 7.64–7.20 (*m*, arom. H); 6.93 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 5.89 (*t*, *J* = 8.8, H–C(3)); 5.76–5.66 (*m*, H–C(2), H–C(2′)); 5.41 (*d*, *J* = 7.4, H–C(1)); 5.15 (*dd*, *J* = 3.1, 10.3, H–C(3′)); 4.82 (*d*, *J* = 7.8, H–C(1′)); 4.68 (br. *d*, *J* = 11.0, H–C(6a)); 4.47–4.26 (*m*, H–C(4), H–C(4′), H–C(6b)); 4.15 (*m*, H–C(5)); 3.84 (*s*, MeO); 3.56 (*t*, *J* = 6.8, H–C(5′)); 2.88 (*dd*, *J* = 6.8, 10.1,

H–C(6a′)); 2.58 (*dd*, *J* = 6.8, 10.1, H–C(6b′)); 2.42 (*d*, *J* = 7.0, HO–C(4′)). ^{13}C-NMR ($CDCl_3$): 133.5–128.4 (arom. C); 116.2 (C(2″), C(6″)); 100.6 (C(1′)); 98.1 (C(1)); 75.7 (C(4)); 74.1 (C(3)); 73.2, 73.1, 71.6, 69.4, 67.6 (C(2), C(5), C(2′), C(3′), C(4′), C(5′)); 62.4 (C(6)); 51.9 (MeO); 0.5 (C(6′)).

*Methyl 4-{[4-*O*-(6-Deoxy-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**5a**). A soln. of crude **14** (772 mg, 0.77 mmol) in AcOEt (35 ml) containing Pd/C 10% (1.64 g), and 4-Å molecular sieves (5.4 g) was stirred under a H_2 atmosphere (1.4 bar) for 8 d. The mixture was filtered and concentrated under reduced pressure. The residue was subjected to the same reaction conditions for others 2 d. After filtration and concentration under reduced pressure, the residue was treated with MeONa in MeOH. (0.05M, 80 ml). The mixture was stirred at r.t. for 5 h, and then neutralized with *Amberlite IRA 120* (H^+). The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by CC (SiO_2; CH_2Cl_2/MeOH 88:12) to afford **5a** (160 mg, 0.35 mmol, 37% yield from **13**). White solid. M.p. 230–232°. $[\alpha]_D^{24} = +10.0$ (*c* = 0.50, MeOH). IR (neat): 3393, 1704, 1607, 1509, 1436, 769. ^{1}H-NMR ($(CD_3)_2SO$): 7.93 (*d*, *J* = 8.6, H–C(3″), H–C(5″)); 7.15 (*d*, *J* = 8.6, H–C(2″), H–C(6″)); 5.58 (*d*, *J* = 5.2, OH); 5.12–5.10 (*m*, H–C(1), OH); 4.80 (*s*, OH); 4.77 (*s*, OH); 4.66 (*t*, *J* = 5.5, OH); 4.62 (*d*, *J* = 4.6, OH); 4.25 (*m*, H–C(1′)); 3.83 (*s*, MeO); 3.78–3.74 (*m*, sugar H); 3.68–3.61 (*m*, 3 sugar H); 3.60–3.40 (*m*, 5 sugar H, superimposed on the signal of H_2O of the solvent); 3.38–3.34 (*m*, 3 sugar H); 1.17 (*d*, *J* = 6.2, H–C(6′)). ^{13}C-NMR ($(CD_3)_2SO$): 166.0 (C=O); 161.1 (C(1″)); 131.2 (C(3″), C(5″)); 123.2 (C(4″)); 116.1 (C(2″), C(6″)); 103.5 (C(1′)); 99.4 (C(1)); 79.7 (C(4)); 75.1, 74.7, 73.4, 73.0, 71.0, 70.6, 70.2 (7 C); 59.9 (C(6)); 52.0 (MeO); 16.4 (C(6′)). HR-ESI-MS: 460.1730 ($C_{20}H_{28}O_{12}^+$; calc. 460.1581).

*Methyl 4-{[4-*O*-(6-Azido-6-deoxy-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**5b**). A mixture of crude **14** (3.7 g, 3.7 mmol), NaN_3 (1.17 mg, 18 mmol), and anh. DMF (40 ml) was stirred for 20 h at 60°. Then, the solvent was eliminated, and the crude residue was treated with MeONa in MeOH (0.05M, 120 ml). The mixture was stirred at r.t. for 3 h and then neutralized with *Amberlite IRA 120* (H^+). The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was subjected to CC (SiO_2; CH_2Cl_2/MeOH 85:15) to afford **5b** (897 mg, 1.79 mmol, 36% yield from **13**). White solid. M.p. 112–115°. $[\alpha]_D^{24} = -22.3$ (*c* = 0.94, MeOH). IR (neat): 3376, 2105, 1704, 1306, 1506, 1436, 770. ^{1}H-NMR (CD_3OD): 8.01 (*d*, *J* = 8.8, H–C(3″), H–C(5″)); 7.19 (*d*, *J* = 8.8, H–C(2″), H–C(6″)); 5.12 (*d*, *J* = 7.7, H–C(1)); 4.47 (*d*, H–C(1′)); 3.97–3.90 (*m*, H–C(6a), H–C(6b)); 3.91 (*s*, MeO); 3.81–3.53 (*m*, 10 sugar H). ^{13}C-NMR (CD_3OD): 168.4 (C=O); 162.8 (C(1″)); 132.5 (C(3″), C(5″)); 125.2 (C(4″)); 117.3 (C(2″), C(6″)); 105.0 (C(1′)); 101.4 (C(1)); 80.2 (C(4)); 76.7, 76.2, 74.9, 74.6, 74.5, 72.3 (C(2), C(3), C(5), C(3′), C(4′), C(5′)); 70.5 (C(2′)); 61.7 (C(6)); 52.5 (C(6′)); 52.4 (MeO). HR-ESI-MS: 501.1596 ($C_{20}H_{27}N_3O_{12}^+$; calc. 501.1595).

*Methyl 4-{[4-*O*-(6-Amino-6-deoxy-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]oxy}benzoate* (**5c**). A soln. of **5b** (130 mg, 0.26 mmol) in anh. MeOH (15 ml) containing Pd/C 10% (32 mg) was stirred under a H_2 atmosphere for 3 h. The mixture was filtered and concentrated under reduced pressure to give **5c** (118 mg, 0.10 mmol, 95%). White solid. M.p. 123–127.5°. $[\alpha]_D^{23} = -21.1$ (*c* = 0.80, MeOH). IR (neat): 3359, 1737, 1716, 1605, 1609, 1435, 769. ^{1}H-NMR ($(CD_3)_2SO$): 7.91 (*d*, *J* = 8.6, H–C(3″), H–C(5″)); 7.13 (*d*, *J* = 8.6, H–C(2″), H–C(6″)); 5.11 (*d*, *J* = 7.6, H–C(1)); 4.24 (*d*, *J* = 6.6, H–C(1′)); 3.81 (*s*, MeO); 3.72–3.17 (*m*, 11 sugar H, partially superimposed on the signal of H_2O of the solvent); 2.72 (*d*, *J* = 5.6, 2 NH). ^{13}C-NMR ($(CD_3)_2SO$): 132.0 (C(3″), C(5″)); 116.9 (C(2″), C(6″)); 104.4 (C(1′)); 100.2 (C(1)); 80.0 (C(4)); 77.0, 76.0, 74.3, 73.9, 71.4, (C(2), C(3), C(5), C(3′), C(4′), C(5′)); 69.6 (C(2′)); 60.7 (C(6)); 52.8 (MeO); 42.9 (C(6′)). HR-ESI-MS: 475.1660 ($C_{20}H_{29}NO_{12}^+$; calc. 475.1690).

*Methyl 4-({4-*O*-[6-(Acetylamino)-6-deoxy-β-*D*-galactopyranosyl]-β-*D*-glucopyranosyl}oxy)benzoate* (**5d**). A soln. of **5b** (200 mg, 0.4 mmol) in anh. MeOH (5 ml) containing Pd/C 10% (50 mg) was stirred under a H_2 atmosphere for 2 h. Then, the mixture was cooled in an ice bath while Ac_2O (0.1 ml, 0.99 mmol) was added. The mixture was stirred for 20 min at r.t., H_2O was added (0.5 ml), the catalyst removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from EtOH to afford **5d** (143 mg, 0.28 mmol, 70% yield from **5b**). White solid. M.p. 211–211.5°. $[\alpha]_D^{23} = -6.0$ (*c* = 1, MeOH). IR (neat): 3600, 3396, 1738, 1693, 1629, 772. ^{1}H-NMR ($(CD_3)_2SO$): 7.93 (*d*, *J* = 8.4, H–C(3″), H–C(5″)); 7.86 (br. *s*, NH)); 7.15 (*d*, *J* = 8.4, H–C(2″), H–C(6″)); 5.56 (*d*, *J* = 5.0, OH); 5.14–5.10 (*m*, H–C(1), OH); 4.81 (*d*, *J* = 4.4, OH); 4.69 (*t*, *J* = 5.5, OH); 4.66–4.63 (*m*, OH(2); 4.27 (*d*, *J* = 6.6, H–C(1′)); 3.84 (*s*, MeO); 3.80–3.76 (*m*, H–C(6a)); 3.69–3.29 (*m*, 10 sugar H,

partially superimposed on the signal of H_2O of the solvent); 3.21–3.17 (*m*, H–C(6b′)); 1.86 (*s*, Me). ^{13}C-NMR ($(CD_3)_2SO$): 169.9 (NHC=O); 165.8 (C=O); 160.9 (C(1″)); 131.0 (C(3″), C(5″)); 123.0 (C(4″)); 116.0 (C(2″), C(6″)); 103.3 (C(1′)); 99.3 (C(1)); 79.1 (C(4)); 75.0, 74.3, 73.0, 73.0, 72.8 (C(2), C(3), C(5), C(3′), C(5′)); 70.4 (C(2′)); 68.4 (C(4′)); 59.8 (C(6)); 51.9 (MeO); 39.3 (C(6′)); 22.5 (Me). HR-ESI-MS: 517.1813 ($C_{22}H_{31}NO_{12}^{+}$; calc. 517.1795).

Hemagglutination Inhibition Assay. The experiments were performed as described earlier [3d]. In short, 50 µl of human type O erythrocytes (4% hematocrit) were mixed with 50 µl of a ECL soln., preincubated with 50 µl of the serially diluted compounds **1**, **2**, **3**, **4a**, **4b**, **4c** · TsOH, **4d**, and **5a**–**5d**, and incubated at r.t. for 1 h. The lowest concentrations giving complete inhibition of the hemagglutination were recorded and expressed by their inhibitory potency relative to D-galactose (*Table*). ECL and carbohydrates solns. were prepared using a saline containing Ca^{2+} and Mn^{2+} (0.15M NaCl, $5 \cdot 10^{-3}$ M $CaCl_2$, and $5 \cdot 10^{-3}$ M $MnCl_2$).

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Received June 18, 2008