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Carbohydrate Research 307 (1998) 233–242

CARBOHYDRATE RESEARCH

Synthesis of some amino and carboxy analogs of galabiose; evaluation as inhibitors of the pilus protein $PapG_{J96}$ from *Escherichia coli*

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Received 2 October 1997; accepted 20 December 1997

Abstract

The 2′-amino-2′-deoxy, 6-amino-6-deoxy, and 6-carboxy analogs of the reference inhibitor 2-(trimethylsilyl)ethyl (α-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside were synthesized and evaluated as inhibitors of the binding of the *Escherichia coli*-derived pilus protein $PapG_{J96}$, using an ELISA assay. The inhibitory efficiencies (K_{rel}; relative to the reference inhibitor) were: 157, 13, and <8, respectively. The results support the previously proposed combining site model, where the protein carries a negatively charged amino acid residue near HO-2′ and HO-6 of the galabioside.

Keywords: Galabiose analogs; *Escherichia coli* inhibitors; $PapG_{J96}$ adhesin; Combining site model

1. Introduction

We are currently interested in the development of improved inhibitors of the pathogens that utilize the galabiose moiety [Gal(α1–4)Gal; present in cell surface glycolipids of the globo series] as an anchoring point in the early stages of an infective process. The work is based on our previous investigations of the galabiose epitopes employed by uropathogenic *Escherichia coli* [1–5] (a Gram-negative species), the piglet pathogen *Streptococcus suis* [6,7] (a Gram-positive species), and *E. coli*-derived Verotoxin [8], for their adhesion to glycolipids on the surface of cells.

The epitope mappings of the *E. coli*-derived proteins $PapG_{J96}$ and Verotoxin indicated that HO-2′ and HO-6 of galabiose are situated in close proximity to charged amino acid residues in the respective protein binding sites [4,5,8]. Therefore, changing these hydroxyl groups for amino or carboxyl groups might improve the binding strength between the galabiose analogs and the protein, due to the possible formation of salt bridges.

We now report the synthesis of the three galabiose analogs **1–3** (Fig. 1) as well as an investigation of their inhibitory efficiency against adhesion of purified *E. coli*-derived $PapG_{J96}$ adhesin [9].

2. Results and discussion

The strategy used for the synthesis of compounds **1–3** (Fig. 1) was based on functionalization of monosaccharides, since such compounds would

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0008-6215/98/$19.00
PII S0008-6215(98)00020-2

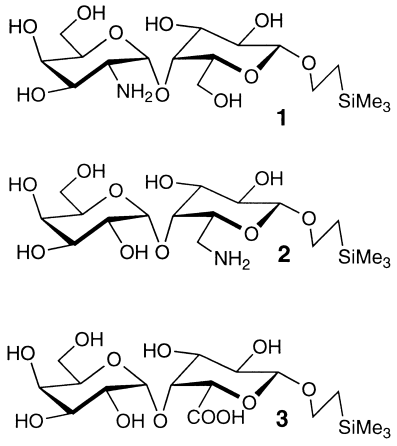
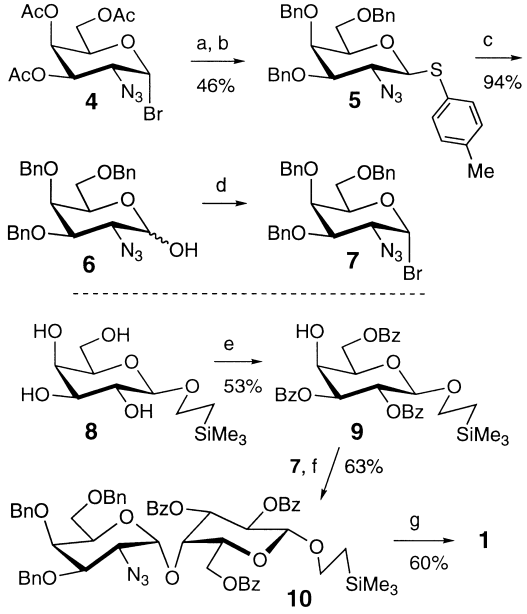


Fig. 1. Galabiose analogs **1–3**, tested as inhibitors of $PapG_{J96}$ adhesin

be more generally useful as building blocks than functionalized disaccharides.

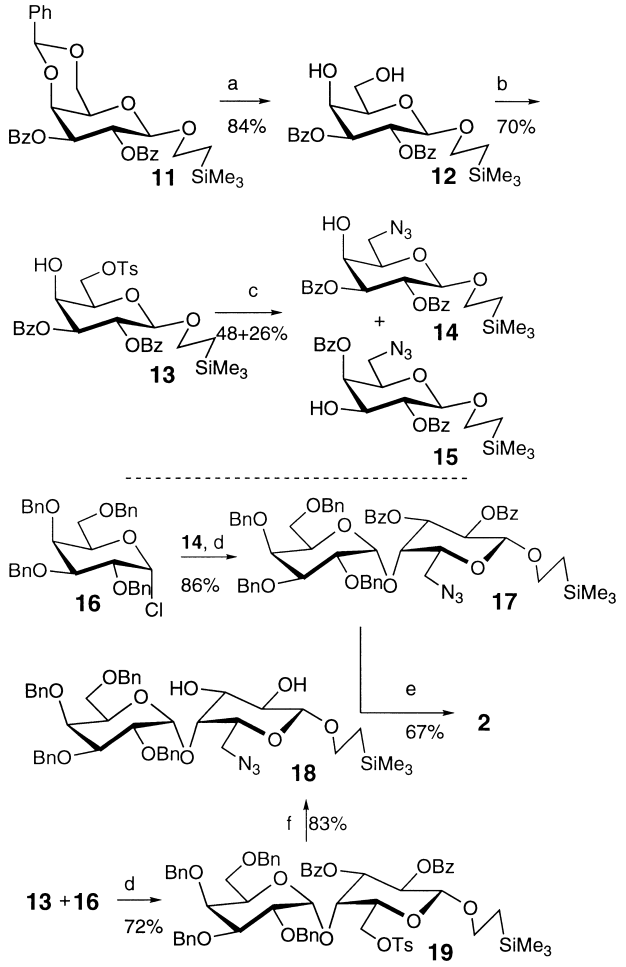
Treatment of the known [10] glycosyl bromide **4** (Scheme 1) with thiocresol and potassium hydroxide gave the corresponding thioglycoside [11], which was benzylated, using benzyl bromide and sodium hydride, to yield the thioglycoside **5** (46% overall). Removal of the thiocresyl group by treatment with *N*-iodosuccinimide gave the hemiacetal **6** (94%). Treatment of **6** with oxalyl bromide furnished the glycosyl bromide donor **7**, which was used for glycosylation without further purification.



Scheme 1. (a) HSPhMe, KOH, 4 h; (b) BnBr, NaH, 12 h; (c) NIS, 10:1 MeCN–H_2O, 5 min; (d) $(COBr)_2$, DMF, 200 min; (e) BzCl, 1:6 pyridine–Me_2CO; (f) AgOTf, TMU, −45→22 °C, 12 h; (g) MeONa–MeOH, 12 h, then H_2, Pd-C, EtOH–aq HCl, 12 h.

The glycosyl acceptor **9** [12] was prepared in 53% yield by partial benzoylation of the known [13] 2-(trimethylsilyl)ethyl (TMSEt) glycoside **8**. Although the yield is modest, the single step transformation is, in our hands, the most efficient route to compound **9**. Silver triflate-promoted glycosylation of **9** with the donor **7** gave the disaccharide **10** (63%). Compound **10** was de-*O*-benzoylated with sodium methoxide and the product was hydrogenated to remove the benzyl groups and reduce the azide group to an amino group, thus providing the galabioside analog **1** (60%).

De-*O*-benzylidenation of **11** [13] (Scheme 2) gave the partially protected TMSEt galactoside **12** [14] (84%). Selective tosylation in the 6-position gave the monotosylate **13** (70%). Treatment of **13** with sodium azide in the presence of 15-crown-5 at 90 °C gave the desired 6-azido-6-deoxy derivative



Scheme 2. (a) aq HOAc, 90 °C, 1.5 h; (b) TsCl, pyridine, −45→22 °C, 22 h; (c) NaN_3, 15-crown-5, 90 °C, 5 h; (d) AgOTf, TMU, −45→22 °C, 12 h; (e) MeONa–MeOH, 20 h, then H_2, Pd-C, EtOH–aq HCl, 12 h; (f) NaN_3, 15-crown-5, 110 °C, 3 d, then MeONa–MeOH.

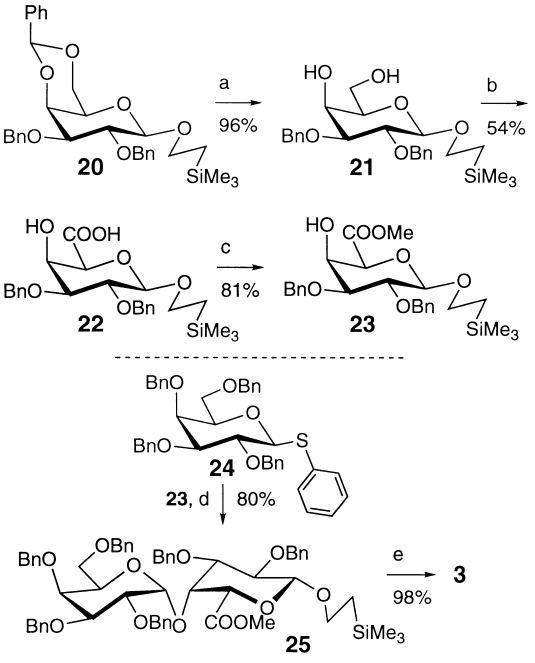
14 (48%) and the isomer **15** (26%; formed by benzoate migration in **13** and/or **14**). An analog of **13**, carrying a triflate instead of the tosylate group, was investigated in an attempt to raise the yield of **14**. Treatment with sodium azide was performed at room temperature, which gave **14** in approximately the same yield as above.

Silver triflate-promoted glycosylation of **14** with the known [15] galactosyl chloride **16** gave the disaccharide **17** (86%). De-*O*-benzoylation of **17** gave **18**, and hydrogenation of the crude product furnished the galabioside analog **2** (67% overall). An alternative route to **18** was also investigated. The tosylate **13** was glycosylated with **16**, using silver triflate as promoter, to provide the disaccharide tosylate **19** (72%). Substitution of the tosyl group of **19** with sodium azide required longer reaction time and higher temperature (3 days, 110 °C), which also caused some de-*O*-benzoylation to occur. Treatment of the crude product with sodium methoxide yielded **18** (83% overall).

Treatment of the known [15] acetal **20** (Scheme 3) with iodine in methanol [16] gave the partially benzylated galactoside **21** (96%). The hydroxymethyl group of **21** was oxidized by 2,2,6,6,-tetramethylpiperidinyloxy radical (TEMPO) and sodium hypochlorite [17] to give the galacturonic acid derivative **22** (54%). The corresponding methyl ester **23** was obtained in 81% yield by treatment of **22** with trimethylsilyldiazomethane [18]. *N*-Iodosuccinimide/triflic acid-induced glycosylation of **23** with the known [19] galactosyl donor **24** gave the disaccharide **25** (80%). Glycosylation of uronic acid acceptors with thioglycoside donors, giving high α-selectivity, was recently published [20]. De-*O*-benzylation of **25**, followed by ester hydrolysis, furnished the galabioside analog **3** (98%).

The conformations of galabiose and its analogs have been determined by NMR [21,22]. In addition to coupling constants and NOE's, the chemical shift of H-5′ is a sensitive conformational probe. In ordinary galabiosides, H-5′ is situated close in space to HO-3 (H-5′–O-3 distance: < 3 Å), which causes a strong (0.4–0.5 ppm relative to H-5′ of methyl α-D-galactopyranoside) deshielding of H-5′. In galabiose derivatives where O-3 is absent (as in the deoxy analog [21]) or where a distortion increases the H-5–O-3 distance [4], the deshielding of H-5′ is eliminated or reduced. In compounds **1–3**, the deshielding of H-5′ amounts to 0.46, 0.40, and 0.40 ppm, respectively, which clearly shows that the overall conformations are similar to those of other galabiosides.

The inhibitory efficiencies of compounds **1–3** were determined essentially as described in earlier investigations [2,4]. In short, a linker galabioside was covalently bound to a microtiter plate to give the glycoplate **26** (Fig. 2), and the inhibitors **1–3**, as well as the reference inhibitor **27**, were serially diluted in the wells of the plate. The purified $PapG_{J96}$ adhesin [9] (in complex with its chaperone



Scheme 3. (a) I_2/MeOH, 4 d; (b) TEMPO, KBr, Bu_4NBr, NaOCl, $NaHCO_3$, NaCl, 0 °C, 100 min; (c) Me_3SiCHN_2, 22 °C, 30 min; (d) NIS, F_3CSO_2OH, 1:2 CH_2Cl_2–Et_2O, −65→−60 °C, 4 h; (e) H_2, Pd-C, 12 h, then aq NaOH, 4 h.

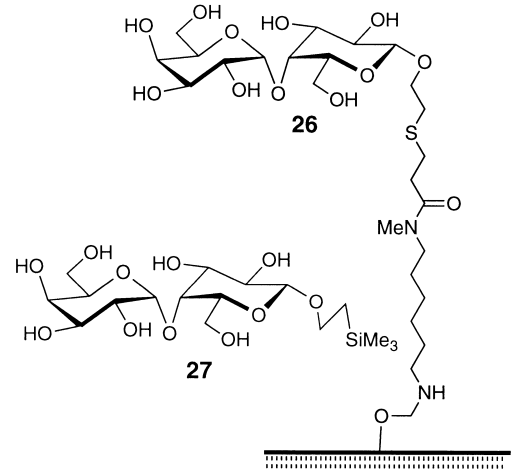


Fig. 2. Galabiose covalently linked to a microtiter plate (Glycoplate **26**) and the refererence inhibitor 2-(trimethylsilyl)ethyl galabioside **27**.

protein $PapD_{J96}$) was dissolved in PBS buffer and added to the wells. After incubation and washing of the plate, the amount of bound protein was determined by ELISA, using an anti-$PapG_{J96}$ antibody.

The inhibition data are depicted in Fig. 3. The 2′-amino-2′-deoxy compound **1** was ~50% more efficient than the reference compound **27**, whereas the 6-amino-6-deoxy compound **2** only retained 13% of the inhibitory efficiency. The uronic acid compound **3** was inefficient as inhibitor.

The inhibition data support the binding pattern suggested previously [1–5], where HO-2′ and HO-6 are involved in a cooperative hydrogen bonding to a carboxylic acid group in the $PapG_{J96}$ adhesin (Fig. 4a). It should be noted that experimental evidence for an intramolecular hydrogen bond between HO-2′ and HO-6 has been obtained by NMR [4,21]. The increased binding of **1** ($K_{rel} = 157$) is consistent with a salt bridge between the carboxylic acid group and protonated H_2N-2′ while maintaining the intramolecular hydrogen bond, as depicted in Fig. 4b. The geometrical requirements for intermolecular interactions are obviously strict, since compound **2** with its amino group in the 6-position has lost most of its binding efficiency ($K_{rel} = 13$). The lack of inhibitory efficiency of the carboxylic acid compound **3** strengthens the arguments for a carboxylic acid group in the protein, since both groups would be charged under the conditions used (pH 7.4) and would therefore repel each other.

3. Experimental

General methods.—Melting points are uncorrected. NMR spectra were recorded on a 300 or a

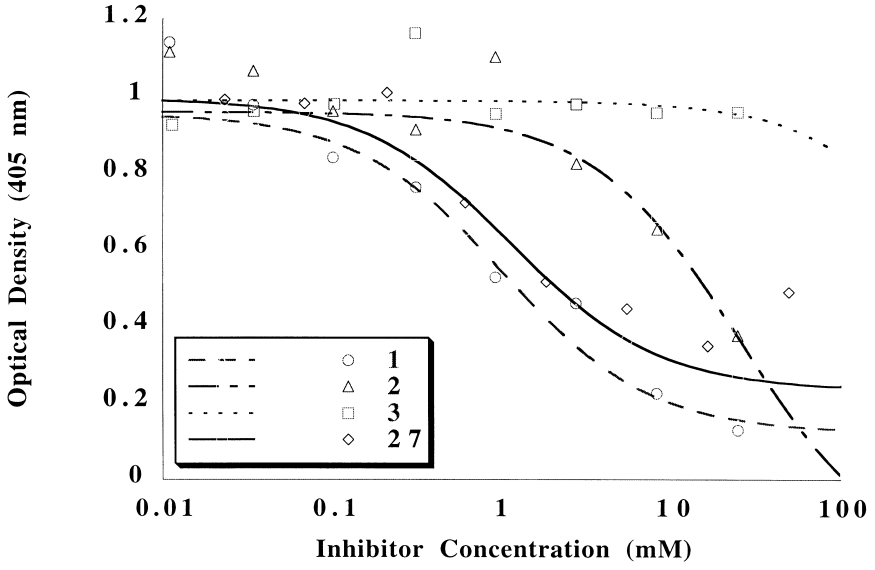


Fig. 3. Efficiency of compounds **1–3** and **27** as inhibitors of the binding of $PapG/PapD_{J96}$ complex to glycoplate **26**. IC_{50}, K_{rel} and $\Delta\Delta G$ values: **1** (1.4 mM, 157, −1.1 kJ mol^{-1}), **2** (17.2 mM, 13, +5.0 kJ mol^{-1}), **3** (> 24.9 mM, < 8, > +21.0 kJ mol^{-1}), **27** (2.2 mM, 0.0 kJ mol^{-1}).

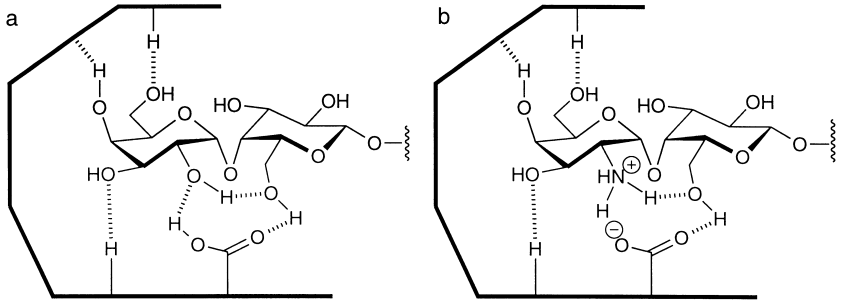


Fig. 4. (a) The hydrogen bonding pattern of galabioside—$PapG_{J96}$ recognition, based on previous investigations [1–5]. (b) Suggested hydrogen bonding and salt bridge formation in the recognition of compound **1** by $PapG_{J96}$.

400 MHz instrument. ^{1}H NMR spectral assignments were made by the double resonance technique (COSY) and chemical shifts for ^{1}H resonances are reported as though they were first order. Concentrations were made using rotary evaporation with a bath temperature at or below 40 °C. Anhydrous Na_2SO_4 was used as drying agent for the organic extracts in the workup procedures. Column chromatography was performed on SiO_2 (Matrex LC-gel: 60A, 35-70 MY, Grace), and TLC was performed on Kieselgel 60 F_{254} plates (Merck). Compounds **4** [10], **9** [12], **11** [13], **16** [15], **20** [15], **24** [19], **26** [4], and **27** [13] were prepared as described in the literature.

2-(Trimethylsilyl)ethyl (2-amino-2-deoxy-α-D-galactopyranosyl)-(1→4)-β-D-galactopyranoside (**1**).—Compound **10** was dissolved in MeOH (10 mL) and the mixture was treated with a catalytic amount of MeONa for 12 h, neutralized with Duolite C436 (H^+) resin, and concentrated. The resulting residue was chromatographed (3:1 EtOAc–heptane). The purified material was dissolved in a mixture of EtOH (10 mL) and 0.1 M aq HCl (0.780 mL, 0.078 mmol), and hydrogenated (H_2, Pd-C, 1 atm). After 12 h the mixture was filtered through Celite and concentrated. The residue was chromatographed (10:4:1 CH_2Cl_2–MeOH–Et_3N) to give **1** (20.7 mg, 60%); $[\alpha]^{24}_D$ +87° (*c* 0.6, H_2O); ^{1}H NMR (D_2O): δ 4.92 (d, 1 H, *J* 3.8 Hz, H-1′), 4.34 (d, 1 H, *J* 7.8 Hz, H-1), 4.24 (bt, 1 H, *J* 6.4 Hz, H-5′), 3.95 (bd, 1 H, *J* 2.7 Hz, H-4), 3.91 (m, 1 H, OC*H*$_2$CH$_2$Si), 3.87 (bd, 1 H, *J* 2.7 Hz, H-4′), 3.75 (dd, 1 H, *J* 3.1, 10.8 Hz, H-3′), 3.54–3.74 (m, 7 H, OC*H*$_2$CH$_2$Si, H-3,5,6,6′), 3.40 (dd, 1 H, *J* 7.8, 10.1 Hz, H-2), 3.04 (dd, 1 H, *J* 3.6, 10.9 Hz, H-2′), 1.78 (s, NH_2, partial exchange by D_2O), 0.90 (m, 2 H, CH_2Si), −0.11 (s, 9 H, $SiMe_3$); ^{13}C NMR (D_2O): δ 102.6, 99.3, 76.9, 75.2, 73.0, 71.6, 71.3, 69.3, 68.7, 68.6, 61.0, 60.7, 51.4, 18.0, −2.2; HRMS calcd for $C_{17}H_{35}O_{10}NSiNa$ (M + Na): 464.1928, found: 464.1927.

2-(Trimethylsilyl)ethyl (α-D-galactopyranosyl)-(1→4)-6-amino-6-deoxy-β-D-galactopyranoside (**2**).—Compound **17** (55 mg, 0.053 mmol) was dissolved in MeOH (8 mL) and the mixture was treated with a catalytic amount of MeONa for 20 h, neutralized with Duolite C436 (H^+) resin, and concentrated. The resulting residue was chromatographed (1:1 EtOAc–heptane). The purified material was dissolved in a mixture of EtOH (6 mL) and 0.1 M aq HCl (0.530 mL, 0.053 mmol), and hydrogenated (H_2, Pd-C, 1 atm). After 12 h the mixture was filtered through Celite and concentrated. The residue was chromatographed (Varian Mega Bond Elut Column C18; 9:1→8:2→7:3→6:4→5:5 H_2O–MeOH, 6 mL of each) to give **2** (15.6 mg, 67%); $[\alpha]^{25}_D$ +142° (*c* 0.2, H_2O); ^{1}H NMR (D_2O): δ 4.85 (d, 1 H, *J* 3.7 Hz, H-1′), 4.36 (d, 1 H, *J* 7.8 Hz, H-1), 4.18 (bt, 1 H, *J* 6.5 Hz, H-5′), 3.93 (m, 1 H, OC*H*$_2$CH$_2$Si), 3.90 (m, 2 H, H-4′,4), 3.78 (dd, 1 H, *J* 3.1, 10.6 Hz, H-3′), 3.73 (dd, 1 H, *J* 3.6, 10.6 Hz, H-2′), 3.53–3.70 (m, 5 H, OC*H*$_2$CH$_2$Si, H-3,5,6′), 3.39 (dd, 1 H, *J* 7.9, 10.1 Hz, H-2), 3.09 (dd, 1 H, *J* 7.9, 13.1 Hz, H-6a), 2.94 (dd, 1 H, *J* 3.9, 13.3 Hz, H-6b), 1.81 (s, NH_2), 0.90 (m, 2 H, CH_2Si), −0.10 (s, 9 H, $SiMe_3$); ^{13}C NMR (D_2O): δ 102.5, 101.1, 79.3, 74.0, 72.9, 71.5, 71.3, 69.5, 69.3, 68.9, 68.7, 60.9, 40.8, 18.0, −2.2; HRMS calcd for $C_{17}H_{35}O_{10}NSiNa$ (M + Na): 464.1928, found: 464.1942.

2-(Trimethylsilyl)ethyl (α-D-galactopyranosyl)-(1→4)-β-D-galactopyranosiduronic acid (**3**).—Compound **25** (99 mg, 0.098 mmol) was dissolved in HOAc (10 mL) and hydrogenated (H_2, Pd-C, 1 atm). After 12 h the mixture was concentrated. The residue was dissolved in water (4 mL) and 2 M aq NaOH (0.14 mL, 0.28 mmol) was added at ambient temperature. After 4 h the mixture was neutralized with Duolite C436 (H^+) resin, filtered, and concentrated. The residue was chromatographed (10:5:1 CH_2Cl_2–MeOH–H_2O) to give **3** (44 mg, 98%); $[\alpha]^{22}_D$ +50° (*c* 1.0, H_2O); ^{1}H NMR data (D_2O): δ 4.92 (d, 1 H, *J* 3.3 Hz, H-1′), 4.34 (d, 1 H, *J* 7.8 Hz, H-1), 4.24 (d, 1 H, *J* 2.7 Hz, H-4), 4.18 (bt, 1 H, *J* 6.2 Hz, H-5′), 3.92–4.07 (m, 2 H, H-5 and OC*H*$_2$CH$_2$Si), 3.88 (bd, 1 H, *J* 3.0 Hz, H-4′), 3.76 (dd, 1 H, *J* 3.2, 10.4 Hz, H-3′), 3.53–3.69 (m, 4 H, H-2′,6′,3 and OC*H*$_2$CH$_2$Si), 3.41 (dd, 1 H, *J* 7.8, 9.9 Hz, H-2), 0.81–1.02 (m, 2 H, CH_2Si), −0.1 (s, 9 H, $SiMe_3$); ^{13}C NMR (D_2O): δ 179.0, 102.1, 100.0, 78.2, 72.8, 71.3, 70.9, 69.6, 69.4, 69.0, 68.7, 61.0, 17.9, −2.1; HRMS calcd for $C_{17}H_{31}O_{12}SiNa_2$ (M-H + 2Na): 501.1380, found: 501.1383.

4-Methylphenyl 2-azido-3,4,6-tri-O-benzyl-2-deoxy-1-thio-β-D-galactopyranoside (**5**).—3,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl bromide [10] (**4**; 500 mg, 1.27 mmol) was dissolved in $CHCl_3$ (3 mL) and the mixture was added dropwise to a mixture of KOH (120 mg, 2.14 mmol), thiocresol (190 mg, 1.53 mmol), and EtOH (3 mL) over 1 h, followed by stirring at 22 °C for 4 h. CH_2Cl_2 and sat aq $NaHCO_3$ were added and the organic layer was dried and concentrated. The residue was dissolved in DMF (2 mL) and benzyl

bromide (0.59 mL, 4.96 mmol) and NaH (80% in oil, 150 mg, 4.96 mmol) were added. After 12 h at 22 °C, MeOH (5 mL) was added. After 30 min, H_2O (15 mL) and toluene (40 mL) were added. The organic layer was washed with H_2O, dried, and concentrated. The residue was chromatographed (1:5→1:3 EtOAc–heptane) to give **5** (339 mg, 46%); $[\alpha]^{23}_D$ −14° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.00–7.60 (19 H, Ph), 4.91 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.76 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.69 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.56 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.52 (d, 1 H, *J* 13.2 Hz, CH_2Ph), 4.46 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.38 (d, 1 H, *J* 10.0 Hz, H-1), 3.98 (d, 1 H, *J* 2.4 Hz, H-4), 3.84 (t, 1 H, *J* 9.9 Hz, H-2), 3.45 (dd, 1 H, *J* 2.7, 9.8 Hz, H-3), 2.33 (s, 3 H, $PhCH_3$); ^{13}C NMR ($CDCl_3$): δ 138.5, 138.1, 137.8, 137.5, 133.5, 129.7, 128.6, 128.5, 128.2, 128.04, 128.00, 127.9, 127.7, 127.5, 86.6, 82.5, 76.7, 74.4, 73.6, 72.4, 72.1, 68.5, 61.5, 21.3; HRMS calcd for $C_{34}H_{35}O_4N_3SNa$ (M + Na): 604.2246, found: 604.2246.

2-Azido-3,4,6-tri-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl bromide* (**7**).—Compound **5** (50 mg, 0.086 mmol) was dissolved in 10:1 MeCN–H_2O (1.3 mL), and *N*-iodosuccinimide (21.4 mg, 0.095 mmol) was added. The mixture was stirred for 5 min at 22 °C and Et_3N (0.3 mL) and toluene (5 mL) were added. The mixture was concentrated and the residue was chromatographed (1:2 EtOAc–heptane) to give **6** (38.5 mg, 94%); HRMS calcd for $C_{27}H_{29}O_5N_3Na$ (M + Na): 498.2005, found: 498.1991. Compound **6** (223 mg, 0.469 mmol) was dissolved in CH_2Cl_2 (5 mL) and DMF (0.020 mL, 0.00026 mmol), and oxalyl bromide (0.050 mL, 0.534 mmol) was added. The mixture was stirred under Ar at 22 °C for 200 min. Cold toluene (30 mL) and cold sat aq $NaHCO_3$ (10 mL) were added. The organic layer was dried and concentrated to give crude **7**, which was used without further purification.

2-(Trimethylsilyl)ethyl 2,3,6-tri-O-*benzoyl*-β-D-*galactopyranoside* (**9**) [12].—To a solution of 2-(trimethylsilyl)ethyl-β-D-galactopyranoside [13] (**8**; 116 mg, 0.414 mg) in dry acetone (0.8 mL) and dry pyridine (0.132 mL) was added benzoyl chloride (0.193 mL, 1.65 mmol) dropwise at −78 °C. The reaction was carefully monitored by TLC (1:2 EtOAc–heptane). When the amount of **9** (R_f 0.34) was at its optimum, MeOH (0.1 mL) was added and the temperature was allowed to reach ~22 °C. The mixture was filtered, diluted with CH_2Cl_2, washed with H_2O, dried, and concentrated. The residue was chromatographed (1:50 EtOAc-toluene) to give **9** (129 mg, 53%); mp 88–89 °C (from EtOAc–heptane); $[\alpha]^{25}_D$ +49° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.96–8.07 (6 H, Ph), 7.32–7.62 (9 H, Ph), 5.78 (dd, 1 H, *J* 7.9, 10.3 Hz, H-2), 5.37 (dd, 1 H, *J* 3.2, 10.3 Hz, H-3), 4.77 (d, 1 H, *J* 7.9 Hz, H-1), 4.71 (dd, 1 H, *J* 6.3, 11.3 Hz, H-6a), 4.63 (dd, 1 H, *J* 6.7, 11.4 Hz, H-6b), 4.38 (bs, 1 H, H-4), 4.10 (bt, 1 H, *J* 6.7 Hz, H-5), 4.00–4.07 (m, 1 H, CH_2CH_2O), 3.64 (m, 1 H, CH_2CH_2O), 2.76 (bd, 1 H, *J* 4.9 Hz, HO-4), 0.82–1.02 (m, 2 H, CH_2Si), −0.07 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.9, 166.4, 165.8, 128.7–133.0, 101.3, 74.8, 72.7, 70.2, 67.9, 67.8, 63.3, 18.4, −1.1 Anal. Calcd for $C_{32}H_{36}O_9Si$: C, 64.8; H, 6.1. Found: C, 64.5; H, 6.0.

2-(Trimethylsilyl)ethyl (2-azido-3,4,6-tri-O-*benzyl-2-deoxy*-α-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*benzoyl*-β-D-*galactopyranoside* (**10**).—A mixture of compound **9** [12] (152 mg, 0.256 mmol), tetramethylurea (0.090 mL, 0.75 mmol), silver triflate (145 mg, 0.564 mmol), molecular sieves (4Å), and toluene (4 mL) was cooled to −45 °C, and a solution of **7** (253 mg, 0.469 mmol) in toluene (5 mL) was added. The mixture was stirred for 12 h, while the temperature was allowed to slowly reach ~22 °C. The mixture was filtered through Celite and concentrated, and the residue was chromatographed (1:5 EtOAc–heptane) to give **10** (169 mg, 63%); $[\alpha]^{24}_D$ +72° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.93–8.10 (6 H, Ph), 7.15–7.64 (24 H, Ph), 5.76 (dd, 1 H, *J* 7.7, 10.6 Hz, H-2), 5.27 (dd, 1 H, *J* 2.9, 10.6 Hz, H-3), 5.00 (d, 1 H, *J* 3.5 Hz, H-1′), 4.77 (d, 1 H, *J* 7.7 Hz, H-l), 4.47 (d, 1 H, *J* 2.8 Hz, H-4), 4.44 (d, 1 H, *J* 11.0 Hz, CH_2Ph), 4.34 (dd, 1 H, *J* 5.0, 9.3 Hz, H-5), 4.17 (dd, 1 H, *J* 2.5, 10.6 Hz, H-3′), 4.04 (dd, 1 H, *J* 3.5, 10.6 Hz, H-2′), 3.66 (dt, 1 H, *J* 6.6, 10.2 Hz, OCH_2CH_2Si), 3.39 (t, 1 H, *J* 8.5 Hz, H-6′a), 2.91 (dd, 1 H, *J* 5.1, 8.4 Hz, H-6′b), 0.94 (m, 2 H, CH_2Si), −0.05 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.4, 166.1, 165.4, 138.5, 138.1, 137.7, 133.4, 133.3, 133.1, 127.5–129.9 (Ar), 100.9, 99.9, 75.0, 74.9, 74.2, 73.2, 72.9, 72.3, 72.1, 69.73, 69.66, 67.5, 67.2, 62.0, 60.5, 18.0, −1.5; HRMS calcd for $C_{59}H_{63}O_{13}N_3SiNa$ (M + Na): 1072.4028, found: 1072.4022.

2-(Trimethylsilyl)ethyl 2,3-di-O-*benzoyl*-β-D-*galactopyranoside* (**12**).—2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside [13] (**11**; 900 mg, 1.56 mmol) was treated with aq 80% HOAc (10 mL) at 90 °C for 90 min. The mixture was cooled to ~22 °C and CH_2Cl_2

(30 mL) was added. The organic layer was washed with sat aq $NaHCO_3$, dried, and concentrated. The residue was chromatographed (1:1 EtOAc–heptane) to give **12** (641 mg, 84%); mp 117–120 °C; $[\alpha]^{23}_D$ +77° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.90–8.00 (m, 4 H, Ph), 7.22–7.54 (m, 6 H, Ph), 5.76 (dd, 1 H, *J* 8.0, 10.3 Hz, H-2), 5.29 (dd, 1 H, *J* 3.2, 10.3 Hz, H-3), 4.74 (d, 1 H, *J* 8.1 Hz, H-1), 4.42 (bd, 1 H, *J* 3.2 Hz, H-4), 3.78 (t, 1 H, *J* 5.4 Hz, H-5), 3.59 (dt, 1 H, *J* 6.6, 10.0 Hz, OC*H*$_2$CH$_2$Si), 3.31 (bs, 1 H, OH), 2.82 (bs, 1 H, OH), 0.90 (m, 2 H, CH_2Si), −1.0 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.1, 165.5, 133.4, 133.1, 129.9, 129.8, 129.7, 129.1, 128.4, 128.3, 101.0, 74.7, 74.2, 69.9, 68.0, 67.7, 62.2, 18.0, −1.5; HRMS calcd for $C_{25}H_{32}O_8SiNa$ (M + Na): 511.1764, found: 511.1774. The data were in good agreement with those published [14].

2-(Trimethylsilyl)ethyl 2,3-di-O-*benzoyl-6*-O-*tosyl*-β-D-*galactopyranoside* (**13**).—Compound **12** (640 mg, 1.31 mmol) was dissolved in dry pyridine (12 mL), and the mixture was cooled to −45 °C under Ar. *p*-Toluenesulfonyl chloride (tosyl chloride; 315 mg, 1.65 mmol) was added, and the mixture was stirred for 22 h, while slowly raising the temperature to ~22°C. CH_2Cl_2 (30 mL) and sat aq $NaHCO_3$ (10 mL) were added, and the organic layer was dried and concentrated. The residue was chromatographed (1:2 EtOAc–heptane) to give **13** (589 mg, 70%); $[\alpha]^{23}_D$ +50° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.32–8.00 (14 H, Ph), 5.66 (dd, 1 H, *J* 8.0, 10.3 Hz, H-2), 5.27 (dd, 1 H, *J* 3.2, 10.3 Hz, H-3), 4.68 (d, 1 H, *J* 7.9 Hz, H-1), 3.56 (dt, 1 H, *J* 6.8, 10.0 Hz, OC*H*$_2$CH$_2$Si), 2.42 (s, 3 H, $PhCH_3$), 2.24 (d, 1 H, *J* 5.9 Hz, HO-4), 0.87 (m, 2 H, CH_2Si), −0.08 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.3, 165.8, 145.6, 133.9, 133.5, 132.8, 130.4, 130.3, 130.2, 130.0, 129.3, 128.9, 128.7, 128.5, 101.1, 74.6, 72.5, 70.0, 68.3, 68.0, 67.3, 22.1, 18.4, −1.0; HRMS calcd for $C_{32}H_{38}O_{10}SSiNa$ (M + Na): 665.1853, found: 665.1866.

2-(Trimethylsilyl)ethyl 6-azido-2,3-di-O-*benzoyl-6-deoxy*-β-D-*galactopyranoside* (**14**) *and 2-(trimethylsilyl)ethyl 6-azido-2,4-di*-O-*benzoyl-6-deoxy*-β-D-*galactopyranoside* (**15**).—To a solution of compound **13** (174 mg, 0.271 mmol) in DMF (4 mL) and 15-crown-5 (0.055 mL, 0.278 mmol) was added NaN_3 (110 mg, 1.69 mmol). The mixture was stirred at 90 °C for 5 h, and cooled to 22 °C. H_2O and CH_2Cl_2 were added. The organic layer was dried and concentrated, and the residue was chromatographed (1:5→1:3 EtOAc–heptane) to give **15** (36 mg, 26%) and **14** (67 mg, 48%). Compound **14** had: $[\alpha]^{22}_D$ +43° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.25–8.05 (10 H, Ph), 5.73 (dd, 1 H, *J* 7.9, 10.3 Hz, H-2), 5.32 (dd, 1 H, *J* 3.2, 10.3 Hz, H-3), 4.76 (d, 1 H, *J* 7.9 Hz, H-1), 4.26 (m, 1 H, H-4), 4.08 (m, 1 H, OC*H*$_2$CH$_2$Si), 3.90 (ddd, 1 H, *J* 1.0, 4.2, 8.2 Hz, H-5), 3.81 (dd, 1 H, *J* 8.0, 12.8 Hz, H-6a), 3.64 (dt, 1 H, *J* 6.6, 10.2 Hz, OC*H*$_2$CH$_2$Si), 3.36 (dd, 1 H, *J* 4.3, 12.8 Hz, H-6b), 2.40 (d, 1 H, *J* 5.5 Hz, HO-4), 0.82–1.02 (m, 2 H, CH_2Si), −0.06 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.3, 165.8, 134.0, 133.6, 130.3, 130.2, 130.0, 129.3, 128.9, 128.7, 101.2, 74.8, 74.5, 70.0, 68.4, 68.0, 51.4, 18.3, −1.1; HRMS calcd for $C_{25}H_{31}O_7N_3SiNa$ (M + Na): 536.1829, found: 536.1843. Compound **15** had: ^1H NMR ($CDCl_3$): δ 7.41–8.22 (10 H, Ph), 5.56 (d, 1 H, *J* 3.6 Hz, H-4), 5.34 (dd, 1 H, *J* 7.8, 10.0 Hz, H-2), 4.74 (d,1 H, *J* 7.9 Hz, H-1), 3.93 (dd, 1 H, *J* 3.3, 8.6 Hz, H-5), 3.24 (dd, 1 H, *J* 3.4, 13.2 Hz, H-6), 2.78 (bd, 1 H, *J* 6.6 Hz, HO-3), 0.80–1.08 (m, 2 H, CH_2Si), −0.05 (s, 9 H, $SiMe_3$).

2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-O-*benzyl*-α-D-*galactopyranosyl)-(1→4)-6-azido-2,3-di*-O-*benzoyl-6-deoxy*-β-D-*galactopyranoside* (**17**).—To a mixture of compound **14** (109 mg, 0.212 mmol), *N,N,N′,N′*-tetramethylurea (0.039 mL, 0.325 mmol), molecular sieves (4Å), and dry toluene (4 mL) was added a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride [15] (**16**;160 mg, 0.296 mmol) in dry toluene (7 mL). The mixture was cooled to −45 °C and silver triflate (82 mg, 0.319 mmol) was added. The mixture was stirred under Ar for 12 h, while slowly raising the temperature to ~22 °C. The mixture was filtered through Celite and concentrated, and the residue was chromatographed (1:4 EtOAc–heptane) to give **17** (189 mg, 86%); $[\alpha]^{22}_D$ +89° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.73 (dd, 1 H, *J* 7.8, 10.6 Hz, H-2), 5.19 (dd, 1 H, *J* 2.9, 10.6 Hz, H-3), 4.92 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 4.90 (d, 1 H, *J* 11.1 Hz, CH_2Ph), 4.84 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.81 (d, 1 H, *J* 3.6 Hz, H-1′), 4.80 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.73 (d, 1 H, *J* 7.8 Hz, H-1), 4.62 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 4.55 (d, 1 H, *J* 11.2 Hz, CH_2Ph), 4.36 (bdd, 1 H, *J* 5.2, 9.4 Hz, H-5′), 4.25 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.24 (d, 1 H, *J* 2.9 Hz, H-4), 4.21 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.16 (bs, 1 H, H-4′), 4.12 (m, 1 H, OC*H*$_2$CH$_2$Si), 4.09 (dd, 1 H, *J* 5.5, 9.7 Hz, H-3′), 4.04 (dd, 1 H, *J* 3.4, 10.1 Hz, H-2′), 3.76–3.85 (m, 2 H, H-5,6a), 3.64 (dt, 1 H, *J* 6.7, 10.2 Hz, OC*H*$_2$CH$_2$Si), 3.49 (t, 1 H, *J* 8.7 Hz, H-6′a), 3.21 (dd, 1 H, *J* 8.9, 18.2 Hz, H-6b), 3.13 (dd,

1 H, *J* 5.0, 8.5 Hz, H-6′b), 0.96 (m, 2 H, CH_2Si), −0.03 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 167.0, 165.7, 127.8–139.3, 101.6, 101.2, 79.0, 75.5, 75.4, 74.8, 74.75, 73.5, 72.5, 70.2, 70.1, 68.0, 67.9, 51.6, 18.3, −1.0; HRMS calcd for $C_{59}H_{65}O_{12}N_3SiNa$ (M + Na): 1058.4235, found: 1058.4243.

*2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzyl-*α*-*D*-galactopyranosyl)-(1→4)-6-azido-6-deoxy-*β*-*D*-galactopyranoside* (**18**).—Compound **19** (191 mg, 0.164 mmol) was dissolved in a mixture of dry DMF (8 mL) and 15-crown-5 (0.035 mL, 0.164 mmol). NaN_3 (75 mg, 1.15 mmol) was added and the mixture was stirred at 110 °C for 3 days, then cooled to ∼22 °C. H_2O (10 mL) and CH_2Cl_2 (25 mL) were added and the organic layer was isolated, dried, and concentrated. The crude material was dissolved in MeOH and a catalytic amount of MeONa was added. After 12 h, the mixture was neutralized with Duolite 436 (H^+) resin and concentrated. The residue was chromatographed (1:2 EtOAc–heptane) to give **18** (113 mg, 83%); $[\alpha]^{22}_D$ +8° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.15–7.45 (20 H, Ph), 4.94 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.89 (d, 1 H, *J* 4.2 Hz, H-l′), 4.87 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.83 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.79 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.63 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.56 (d, 1 H, *J* 11.6 Hz, CH_2Ph), 4.47 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.39 (d, 1 H, *J* 11.4 Hz, CH_2Ph), 4.27 (d, 1 H, *J* 7.5 Hz, H-1), 4.16 (bdd, 1 H, *J* 3.5, 8.6 Hz, H-5′), 3.93–4.12 (m, 3 H, H-2′,3′ and OCH_2CH_2Si), 3.90 (bd, 1 H, *J* 1.9 Hz, H-4′), 3.78 (dd, 1 H, *J* 8.2, 13.1 Hz, H-6a), 3.68 (bd, 1 H, *J* 1.7 Hz, H-4), 3.54–3.69 (m, 3 H, H-5, 6′a and OCH_2CH_2Si), 3.48 (dd, 1 H, *J* 7.6, 10.1 Hz, H-2), 3.37 (bt, 1 H, *J* 10.6 Hz, H-3), 3.30 (dd, 1 H, *J* 3.9, 9.6 Hz, H-6′b), 3.15 (dd, 1 H, *J* 3.8, 13.2 Hz, H-6b), 2.38 (s, 1 H, OH), 2.12 (bs, 1 H, OH), 0.97–1.16 (m, 2 H, CH_2Si), 0.03 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 138.80, 138.78, 138.6, 138.0, 128.93, 128.9, 128.8, 128.7, 128.6, 128.4, 128.3, 128.25, 128.1, 127.9, 103.2, 101.3, 82.0, 79.2, 76.8, 75.2, 75.1, 75.0, 74.54, 74.5, 74.2, 73.3, 72.4, 71.7, 70.2, 68.0, 51.9, 18.6, −1.0; HRMS calcd for $C_{45}H_{57}O_{10}N_3SiNa$ (M + Na): 850.3711, found: 850.3723. An analytical sample was acetylated in 1:1 pyridine–Ac_2O (4 mL) overnight. The mixture was concentrated and the residue was chromatographed (1:3 EtOAc–heptane) to give 2-(trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzyl-*α*-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-6-azido-6-deoxy-*β*-D-galactopyranoside; ^1H NMR ($CDCl_3$): δ 7.24–7.44 (m, 20 H, Ph), 5.23 (dd, 1 H, *J* 7.8, 10.6 Hz, H-2), 4.93 (d, 1 H, *J* 11.2 Hz, CH_2Ph), 4.90 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.82 (dd, 1 H, *J* 3.0, 10.6 Hz, H-3), 4.81 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.77 (d, 1 H, *J* 11.7 Hz, CH_2Ph), 4.70 (d, 1 H, *J* 2.9 Hz, H-1′), 4.62 (d, 1 H, *J* 11.5 Hz, CH_2Ph), 4.60 (d, 1 H, *J* 11.2 Hz, CH_2Ph), 4.48 (s, 2 H, CH_2Ph), 4.46 (d, 1 H, *J* 7.8 Hz, H-1), 4.30 (bdd, 1 H, *J* 5.2, 8.5 Hz, H-5′), 4.15 (bs, 1 H, H-4′), 3.99–4.09 (m, 3 H, H-2′,3′ and OCH_2CH_2Si), 3.95 (d, 1 H, *J* 2.6 Hz, H-4), 3.47-3.77 (m, 5 H, H-6′,6a,5 and OCH_2CH_2Si), 3.13 (dd, 1 H, *J* 4.1, 12.9 Hz, H-6b), 2.06 (s, 3 H, Ac), 1.92 (s, 3 H, Ac), 0.85–1.05 (m, 2 H, CH_2Si), 0.02 (s, 9 H, $SiMe_3$).

*2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-benzyl-*α*-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-6-O-tosyl-*β*-D-galactopyranoside* (**19**).—To a mixture of compound **13** (300 mg, 0.467 mmol), compound **16** [15] (313 mg, 0.56 mmol), *N,N,N′,N′*-tetramethylurea (0.069 mL, 0.575 mmol), molecular sieves (4Å), and dry toluene (14 mL), was added silver triflate (145 mg, 0.564 mmol) at −45 °C under Ar. The temperature was slowly raised to ∼22 °C. After 12 h, the mixture was filtered through Celite and concentrated, and the residue was chromatographed (1:3 EtOAc–heptane) to give **19** (390 mg, 72%); $[\alpha]^{24}_D$ +69° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.19–7.99 (34 H, Ph), 5.70 (dd, 1 H, *J* 7.7, 10.5 Hz, H-2), 5.19 (dd, 1 H, *J* 2.9, 10.5 Hz, H-3), 4.85 (d, 1 H, *J* 11.1 Hz, CH_2Ph), 4.72–4.78 (m, 3 H, CH_2Ph), 4.76 (d, 1 H, *J* 3.4 Hz, H-1′), 4.69 (d, 1 H, *J* 7.8 Hz, H-1), 4.61 (d, 1 H, *J* 12.1 Hz, CH_2Ph), 4.61 (dd, 1 H, *J* 5.4, 10.9 Hz, H-6a), 4.50 (d, 1 H, *J* 11.1 Hz, CH_2Ph), 4.41 (dd, 1 H, *J* 6.9, 11.0 Hz, H-6b), 4.29–4.36 (m, 2 H, H-4,5′), 4.20 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.15 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.10 (bs, 1 H, H-4′), 3.99–4.08 (m, 3 H, H-3′,5 and OCH_2CH_2Si), 3.97 (dd, 1 H, *J* 3.3, 10.2, H-2′), 3.61 (dt, 1 H, *J* 6.8, 9.9 Hz, OCH_2CH_2Si), 3.44 (t, 1 H, *J* 8.8 Hz, H-6′a), 3.06 (dd, 1 H, *J* 5.0, 8.5 Hz, H-6′b), 2.4 (s, 3 H, $PhCH_3$), 0.93 (m, 2 H, CH_2Si), −0.04 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 166.8, 165.7, 145.3, 127.8–139.3, 101.4, 101.2, 79.4, 76.6, 76.2, 75.4, 75.1, 74.4, 74.1, 73.4, 73.0, 70.2, 70.0, 69.0, 68.0, 67.9, 22.0, 18.4, −1.0; HRMS calcd for $C_{66}H_{72}O_{15}SSiNa$ (M + Na): 1187.4259, found: 1187.4250.

*2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzyl-*β*-D-galactopyranoside* (**21**).—2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*β*-D-galactopyranoside [15] (**20**; 30 mg, 0.055 mmol) was dissolved in CH_2Cl_2 (1 mL) and a solution of iodine (10 mg) in MeOH (2 mL) was added. After 4 days, aq 10%

$Na_2S_2O_7$ was added. The organic layer was dried and concentrated and the residue was chromatograped (2:1 EtOAc–heptane) to give **21** (24 mg, 96%); $[\alpha]^{21}_D$ +2° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.25–7.42 (10 H, Ph), 4.95 (d, 1 H, *J* 11.1 Hz, CH_2Ph), 4.70–4.79 (m, 3 H, CH_2Ph), 4.40 (d, 1 H, *J* 7.7 Hz, H-1), 3.94–4.09 (m, 3 H, H-4,6 and OCH_2CH_2Si), 3.79–3.89 (m, 1 H, H-6), 3.66 (dd, 1 H, *J* 7.8, 9.3 Hz, H-2), 3.58–3.66 (m, 1 H, OCH_2CH_2Si), 3.52 (dd, 1 H, *J* 3.5, 9.4 Hz, H-3), 3.47 (bt, 1 H, *J* 5.7 Hz, H-5), 2.65 (s, 1 H, HO-4), 2.20 (bs, 1 H, HO-6), 1.02–1.10 (m, 2 H, CH_2Si), 0.04 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 139.1, 138.2, 128.9, 128.7, 128.5, 128.4, 128.3, 128.1, 103.8, 80.9, 79.4, 75.6, 74.3, 73.0, 68.0, 67.95, 63.0, 19.0, −1.0; HRMS calcd for $C_{25}H_{36}O_6SiNa$ (M + Na): 483.2179, found: 483.2173.

2-(Trimethylsilyl)ethyl 2,3-di-O-*benzyl*-β-D-*galactopyranosiduronic acid* (**22**).—To a solution of compound **21** (235 mg, 0.51 mmol) in CH_2Cl_2 (1.5 mL) was added 2,2,6,6,-tetramethylpiperidinyloxy radical (TEMPO; 2 mg, 0.013 mmol), followed by a solution of KBr (5.6 mg) and tetrabutylammonium bromide (8.6 mg) in sat aq $NaHCO_3$ (1.1 mL). The mixture was cooled to 0 °C and 7:3:6 aq NaOCl (tech.)–sat aq $NaHCO_3$–sat aq NaCl (4.5 mL) was added dropwise until the reaction was completed (100 min). The organic layer was dried and concentrated and the residue was chromatographed (15:5:1 EtOAc–heptane–HOAc) to give **22** (130 mg, 54%); $[\alpha]^{21}_D$ −7° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.27–7.41 (m, 10 H, Ph), 4.93 (d, 1 H, *J* 11.1 Hz, CH_2Ph), 4.69–4.79 (m, 3 H, CH_2Ph), 4.45 (d, 1 H, *J* 7.7 Hz, H-1), 4.36 (dd, 1 H, *J* 1.3, 3.3 Hz, H-4), 4.02–4.13 (m, 2 H, H-5 and OCH_2CH_2Si), 3.60–3.77 (m, 2 H, H-2 and OCH_2CH_2Si), 3.58 (dd, 1 H, *J* 3.3, 9.3 Hz, H-3), 1.01–1.10 (m, 2 H, CH_2Si), 0.04 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 170.6, 138.8, 137.8, 129.0, 128.8, 128.5, 128.4, 128.2, 103.3, 80.0, 78.6, 75.7, 74.0, 73.0, 68.7, 68.0, 18.9, −1.0; HRMS calcd for $C_{25}H_{34}O_7SiNa$ (M + Na): 497.1972, found: 497.1962.

Methyl [2-(Trimethylsilyl)ethyl 2,3-di-O-*benzyl*-β-D-*galactopyranosid]uronate* (**23**).—Compound **22** (91 mg, 0.192 mmol) was dissolved in a mixture of MeOH (1 mL) and benzene (3.5 mL). Trimethylsilyldiazomethane [18] (2 M in hexane; 0.125 mL, 0.250 mmol) was added and the mixture was stirred for 30 min. HOAc was added until the yellow color disappeared. Toluene (10 mL) was added and the mixture was concentrated. The residue was chromatographed (1:3 EtOAc–heptane) to give **23** (77 mg, 81%); $[\alpha]^{21}_D$ −2° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.28–7.41 (m, 10 H, Ph), 4.95 (d, 1 H, *J* 11.1 Hz, CH_2Ph), 4.71–4.79 (m, 3 H, CH_2Ph), 4.39 (d, 1 H, *J* 7.7 Hz, H-1), 4.34 (bs, 1 H, H-4), 4.07–4.16 (m, 1 H, OCH_2CH_2Si), 4.07 (bs, 1 H, H-5), 3.84 (s, 3 H, COOMe), 3.69 (dd, 1 H, *J* 7.7, 9.4 Hz, H-2), 3.58 (dd, 1 H, *J* 3.4, 9.3 Hz, H-3), 3.57–3.65 (m, 1 H, OCH_2CH_2Si), 2.57 (bs, 1 H, HO-4), 1.00–1.13 (m, 2 H, CH_2Si), 0.04 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 169.0, 139.0, 138.0, 129.0, 128.7, 128.5, 128.4, 128.3, 128.1, 103.3, 80.2, 78.8, 75.6, 74.1, 72.9, 68.4, 68.1, 53.0, 18.9, −1.0; HRMS calcd for $C_{26}H_{36}O_7SiNa$ (M + Na): 511.2128, found: 511.2125.

Methyl [(2,3,4,6-tetra-O-*benzyl*-α-D-*galactopyranosyl)-(1→4)-2,3-di*-O-*benzyl*-β-D-*galactopyranosid]uronate* (**25**).—A mixture of phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**24**) [19] (124 mg, 0.197 mmol), compound **23** (60 mg, 0.123 mmol), *N*-iodosuccinimide (48 mg, 0.213 mmol), molecular sieves (4Å, 1 g), CH_2Cl_2 (3 mL) and Et_2O (6 mL) was cooled to −78 °C under N_2, and triflic acid (0.005 mL, 0.00006 mmol) was added. The temperature was kept between −60 and −65 °C. After 4 h, Et_3N (1 mL) was added and the mixture was allowed to reach ~22 °C. The mixture was filtered through Celite, and Et_2O (20 mL) was added. The mixture was washed with sat aq $NaHCO_3$, the organic layer was dried and concentrated, and the residue was chromatographed (1:7 EtOAc–heptane) to give **25** (99 mg, 80%); $[\alpha]^{22}_D$ +30° (*c* 3.1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.20–7.45 (m, 30 H, Ph), 5.14 (d, 1 H, *J* 2.3 Hz, H-1′), 4.92 (d, 2 H, *J* 11.2 Hz, CH_2Ph), 4.69–4.83 (m, 6 H, CH_2Ph), 4.64 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.56 (d, 1 H, *J* 11.3 Hz, CH_2Ph), 4.47 (d, 1 H, *J* 2.6 Hz, H-4), 4.39 (d, 1 H, *J* 7.6 Hz, H-1), 4.35–4.42 (m, 1 H, H-5′), 4.32 (d, 1 H, *J* 11.9 Hz, CH_2Ph), 4.28 (d, 1 H, *J* 11.8 Hz, CH_2Ph), 4.12–4.21 (m, 1 H, OCH_2CH_2Si), 3.98–4.08 (m, 4 H, H-5,2′,3′,4′), 3.77 (dd, 1 H, *J* 7.7, 9.8 Hz, H-2), 3.59–3.69 (m, 1 H, OCH_2CH_2Si), 3.55 (s, 3 H, COOMe), 3.46–3.57 (m, 2 H, H-3,6′a), 3.39 (dd, 1 H, *J* 5.4, 8.8 Hz, H-6′b), 1.03–1.19 (m, 2 H, CH_2Si), 0.06 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 169.0, 139.4, 127.7, 104.0, 99.6, 80.9, 79.2, 79.0, 76.6, 75.7, 75.6, 75.4, 75.3, 74.0, 73.7, 73.3, 73.1, 73.0, 70.2, 68.9, 68.4, 52.6, 19.0, −0.9; HRMS calcd for $C_{60}H_{70}O_{12}SiNa$ (M + Na): 1033.4534, found: 1033.4530.

Covalent coupling of galabioside to microtiter plates.—Microtiter plates functionalized with secondary amino groups (CovaLink®) microtiter

plates, A/S Nunc, P.O. Box 280, Kamstrup, DK-4000 Roskilde, Denmark) were used for covalent coupling of a carboxylic acid-functionalized galabioside, as previously described [4,5], furnishing the glycoplate **26** (Fig. 2).

*ELISA assay. Competitive inhibition of the PapG/PapD$_{J96}$ complex by **1**, **2**, and **3**.*—The PapG/PapD$_{J96}$ complex [9] (0.1 mg mL^{-1} in 2% BSA/PBS, 0.050 mL microtiter well^{-1}) was added to the glycoplate, containing 0.050 mL of the inhibitors **1–3** and the reference inhibitor **27**, serially diluted three times between each well. Every inhibitor was investigated two or three times in order to obtain mean values. After 45 min incubation, the plates were washed three times with PBS and then blocked for nonspecific binding by a 1 h incubation with 0.4 mL 2% BSA in PBS. The PapG/PapD$_{J96}$ complex was detected by incubation with primary anti-PapG/PapD$_{J96}$ rabbit antiserum (diluted 1/500 in 2% BSA in PBS, 100 μL well^{-1}) for 1 h, followed by washing (three times) with Covabuffer and addition of alkaline phosphatase-conjugated antirabbit IgG (Sigma A7539, diluted 1/5000) and phosphatase substrate 104 (Sigma 104). The optical density was read at 405 nm. Incubations were at 24 °C unless otherwise stated. The results are shown in Fig. 3.

Acknowledgements

We are grateful to Professor Scott Hultgren for a generous gift of PapG/PapD$_{J96}$ protein. This work was supported by grants from the Swedish Natural Science Research Council and the National Institutes of Health (NIH, USA).

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