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A novel synthesis of α-D-Gal*p*-(1→3)-β-D-Gal*p*-1-*O*-$(CH_2)_3$–NH_2, its linkage to activated matrices and absorption of anti-αGal xenoantibodies by affinity columns

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Abstract

Pig organs transplanted into primates are rapidly rejected because of the interaction between Galα(1→3)Gal epitopes carried by the graft and natural antibodies (anti-αGal antibodies) present in the blood of the recipient. This report describes a simplified synthesis of the xenogeneic disaccharide and its linkage to activated gel matrices. The digalactosides α-D-Gal*p*-(1→3)-α,β-D-Gal*p*-OAll were synthesized by the condensation of the trichloroacetimidoyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside donor with the 3,4-unprotected allyl 2,6-di-*O*-benzyl-α- or β-D-galactopyranoside acceptor precursor. Deallylation and hydrogenolysis led to the free digalactoside, whereas hydrogenolysis alone resulted in the 1-*O*-propyl digalactoside. Both products were tested by inhibition ELISA of natural anti-Galα(1→3)Gal antibodies. The α-D-Gal*p*-(1→3)-β-D-Gal*p*-OPr was found to be the best inhibitor. Thus, the allyl group of the partially benzylated α-D-Gal*p*-(1→3)-β-D-Gal*p*-OAll was engineered, via the hydroxy-, the tosyloxy- and the azidopropyl intermediates, into an aminopropyl group amenable to binding to *N*-hydroxysuccinimide-activated agarose gel matrices in order to obtain specific immunoabsorption columns. Columns made of gel substituted with 5 μmol of disaccharide per milliliter were found efficient for the immunoabsorption of anti-αGal antibodies from human plasma.

Keywords: Affinity column; 3,4-Unprotected β-D-galactopyranoside; (3-Amino)propyl glycoside; Immunoadsorption; Xenotransplantation

1. Introduction

Xenotransplantation in humans, especially of porcine organs, is presently being investigated as a possible means to alleviate the shortage of human organ donors. The first obstacle to be overcome is the hyperacute rejection, which occurs after recognition of antigens of the vascular endothelium of the grafted organ by natural antibodies of the host. Such a situation is identical with what is observed in human allotransplantation between non-concordant ABO blood group individuals [1], suggesting the involvement of anti-carbohydrate antibodies for the inter-species hyperacute rejection. It has been found

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that the major culprit of hyperacute rejection of pig organs by primates and man is the Galα(1→3)Gal epitope [2–4]. It is present in all mammals except man, apes, and Old World monkeys, which, in turn, express natural antibodies (anti-αGal antibodies) against it [5,6]. Therefore, it was suggested that ex vivo absorption of the plasma of a xenograft recipient on affinity columns carrying the proper synthetic oligosaccharide would prevent hyperacute rejection of the graft [1,2,7]. For this purpose, affinity columns bearing the xenogenic determinant Galα(1→3)Gal are required.

The digalactoside Galα(1→3)Gal was first synthesized as part of the human blood group B determinant, Galα(1→3)[Fucα(1→2)]-Galβ(1→4)GlcNAc, using bromide [8], imidate [9] or trichloroacetimidate [10] galactoside donor precursors. Recently, the stereoselective α(1→3) glycosylation was obtained using new donor galactoside derivatives [11–13]. Occasionally, the regioselective α-(1→3) linkage was obtained from 2,3-unprotected acceptors [10,14,15]. In this work, the α-D-Gal*p*-(1→3)-β-D-Gal*p*-OPrNH$_2$, which could easily bind to matrix support, was prepared from the α-D-Gal*p*-(1→3)-β-D-Gal*p*-OAllyl precursor, which was synthesized from a trichloroacetimidate-activated galactoside donor and a 3,4-unprotected allyl β-D-galactoside acceptor. The predominant reactivity of the equatorial over the axial hydroxyl was exploited to favor the (1→3) intergalactoside linkage.

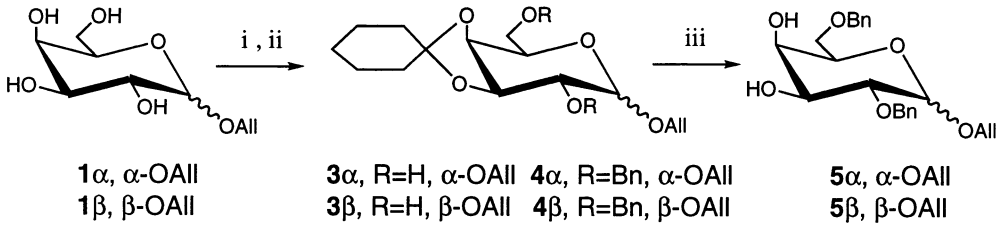
The allyl group was either removed to give reducing saccharides, hydrogenated to yield propyl glycosides, or easily functionalized into a small spacer arm for binding to matrices. Soluble propyl glycoside anomers were tested by inhibition ELISA on human plasma. The α-D-Gal*p*-(1→3)-β-D-Gal*p*-OPr–NH$_2$ was reacted with *N*-hydroxysuccinimide ester-activated agarose gels. The resulting affinity columns were evaluated for their capacity to retain anti-αGal antibodies from human plasma.

2. Results and discussion

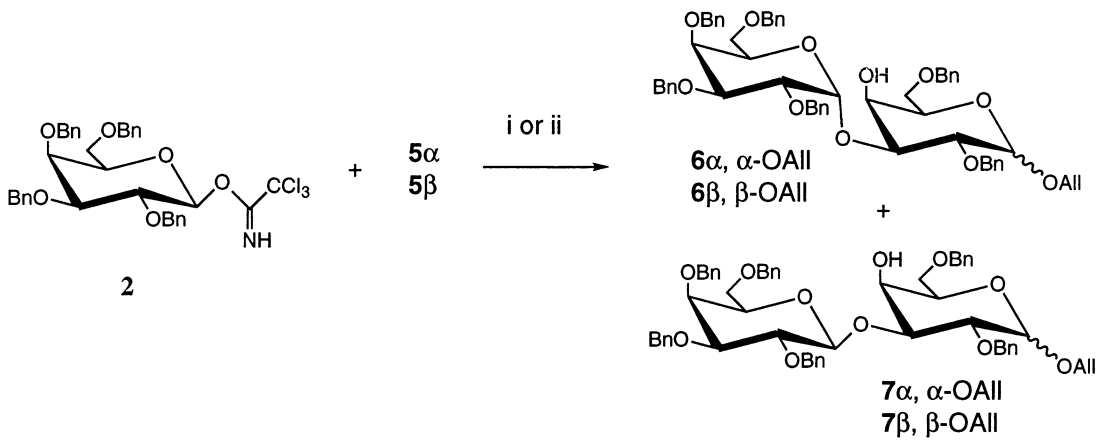
Synthesis of α-D-*Gal*p-*(1→3)*-α,β-D-*Gal*p-*OAll.*—Precursors **2** [10] and **5** [16] were prepared by modifications of previously described methods in order to simplify the procedures, improve their reproducibility, diminish the cost, and keep high yields in the scope of scaling up the synthesis. They were both synthesized starting from the allyl D-galactopyranosides. The allyl α-D-galactopyranoside **1**α was isolated in 35% yield by crystallization from the mixture resulting from the treatment of D-galactose with allylic alcohol in acidic conditions [17]. The allyl β-D-galactopyranoside **1**β [18] was synthesized in 80% overall yield from the readily available tetra-*O*-acetyl-α-D-galactopyranoside bromide [19] by treatment with allyl alcohol in the presence of silver trifluoromethanesulfonate [20], and subsequent potassium carbonate-catalyzed deacetylation [21].

The trichloroacetimidoyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (**2**) was synthesized in three steps: per-*O*-benzylation of **1**α [22], palladium(II) chloride-catalyzed anomeric deallylation [23,24], and reaction of the resulting protected D-galactopyranose with trichloroacetonitrile in the presence of potassium carbonate [25]. It was found that the per-*O*-benzylation of **1**α with benzyl bromide by a phase-transfer method, using powdered potassium hydroxide and tetraoctylammonium as a catalyst [22], was more efficient and easier to perform than the previously described method using sodium hydride [18]. The access to the tetra-*O*-benzyl-D-galactopyranose was originally obtained from the allyl tetra-*O*-benzyl-D-galactopyranosides via the prop-1-enyl intermediates [18,26]. The catalytic deallylation method of Corey and Suggs [23] was completed at room temperature in a few hours, and gave a more straightforward access to the tetra-*O*-benzyl-D-galactopyranose in 85% yield [24] (Scheme 1).

The glycoside acceptors, allyl 2,6-*O*-benzyl-α,β-D-galactopyranosides **5**α [16] and **5**β [28], were prepared in three steps from **1**α and **1**β, respectively, following a procedure similar to that described by Nashed et al. [16]. However, cyclohexylidene derivatives **3** and **4** were preferred because their hydrophobicity made them easier to purify than homologous isopropylidene derivatives (Scheme 2).



Scheme 1. (i) 1,1-Dimethylcyclohexane, H_2SO_4, DMF, α 53%, β 71%; (ii) BnBr, KOH, Oct_4NBr, CH_2Cl_2, 94%; (iii) HCl aq, MeOH, quant.



Scheme 2. $BF_3{\cdot}Et_2O$, CH_2Cl_2; 6α, 33%, 7α, 15%; 6β, 25%, 25%, 7β, 20%; (ii) Me_3SiOTf, CH_2Cl_2, 6β, 60%, 7β, $< 5\%$.

The glycosylation reaction between **2** and either **5**α or **5**β was performed according to Schmidt and Michel [27]. Boron trifluoride etherate promoted the glycosylation of **5**α with the trichloroacetimidate **2** to give a mixture of disaccharides **6**α and **7**α in 33 and 15% yield, respectively. Starting from **5**β, the corresponding glycosides **6**β and **7**β were produced in 25 and 20% yields, respectively, under similar conditions. Trimethylsilyl trifluoromethanesulfonate appeared more effective for the stereocontrol of the glycosylation of compound **5**β, providing the α-(1→3) digalactoside **6**β in 60% yield. In terms of the regioselectivity of the reaction, the (1→3) linkage was largely favored, as compounds with the α- or β-(1→4) linkage were not detected in any of the above reactions, indicating that they were formed in less than 5% yield. This approach using 3,4-unprotected galactoside acceptor intermediates was also effective for the synthesis of Galα(1→3)-Galβ(1→3)Galα(1→1)All[1].

*Synthesis of α-*D*-Gal*p*-(1→3)-β-*D*-Gal*p-OPr–NH₂.*—Among various derivatives [29,30], allyl glycosides can readily lead to two types of end product: (i) a reducing saccharide, and (ii) a propyl glycoside. A third possibility was investigated, i.e., engineering of the allyl into an aminopropyl group that can be bound to a solid matrix (Scheme 3).

From the allyl glycosides **6**, the free Galα(1→3)Gal **8** was produced in two steps: palladium-catalyzed deallylation, prior to the hydrogenolysis of the benzyloxy groups (65% overall yield). Direct catalytic hydrogenation gave rise to both propyloxy isomers **9**α and **9**β, from the corresponding parents **6**α and **6**β, in quantitative yields.

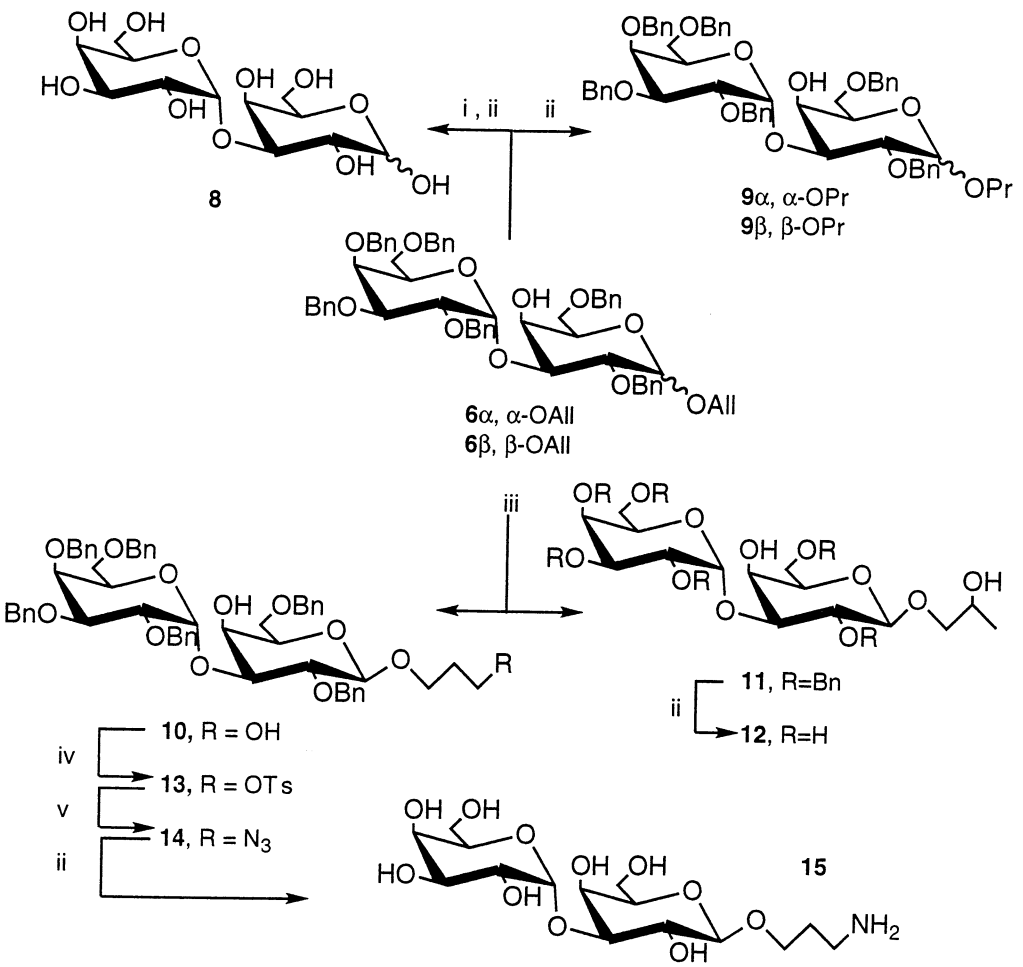
As the propyl glycoside **9**β was found to be a better inhibitor of anti-αGal antibodies than the anomer **9**α, the allyl glycoside **6**β was transformed into the aminopropyl glycoside **15** in order to make affinity columns. The allyl group of the allyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**6**β) was first converted into a hydroxypropyl group by a hydroboration–oxidation reaction [31–33], leading to

[1] Liaigre et al. unpublished.

the alcohols **10** and **11** with 40 and 36% yields, respectively. The use of a more voluminous reagent, such as 9-borabicyclo(3.3.1)nonane (9-BBN) in THF, favored the formation of the anti-Markovnikov products **10** and **11** in 60 and 5% yields, respectively. The regioselectivity of the reaction was improved, but a noticeable part of the products was degraded, probably because of the long time required for the reaction to proceed due to the weak reactivity of 9-BBN in solution in THF. A higher yield might be obtained as the hydroboration–oxidation of **1**β with solid 9-BBN gave the primary alcohol in a 81% yield.

The isomer **11** was deprotected by hydrogenolysis, and the resulting digalactoside **12** was tested as an inhibitor of the binding of anti-αGal antibodies to their substrate. The functionalized compound **10** could be modified in a number of ways in order to synthesize neoglycoconjugates. It was chosen here to obtain a primary amine aimed at forming amide bonds with spacer arms of available *N*-hydroxysuccinimide ester-activated agarose gels. For this purpose, the primary hydroxyl of **10** was converted into the tosylate **13**, which was displaced by sodium azide to give the azidopropyl digalactoside **14** (85% overall yield). Alternatively, the conversion of the primary hydroxyl into an azide function could be carried out using the Mitsunobu procedure [34]. Finally, the target Galα(1→3)Galβ(1→1)Pr–NH_2 (**15**) was generated by hydrogenolysis of the intermediate **14**.

Inhibition of anti-αGal antibodies by synthesized carbohydrates.—The digalactosides were tested for their capacity to inhibit anti-αGal antibodies of human plasma (Fig. 1). The best inhibitor was Galα(1→3)Galβ(1→1)(2-OH)Pr



Scheme 3. (i) $PdCl_2$, MeOH; (ii) H_2/Pd–C, EtOH; (iii) 9-BBN, THF, then H_2O_2, NaOH; (iv) TsCl, Et_3N, CH_2Cl_2; (v) NaN_3, DMF.

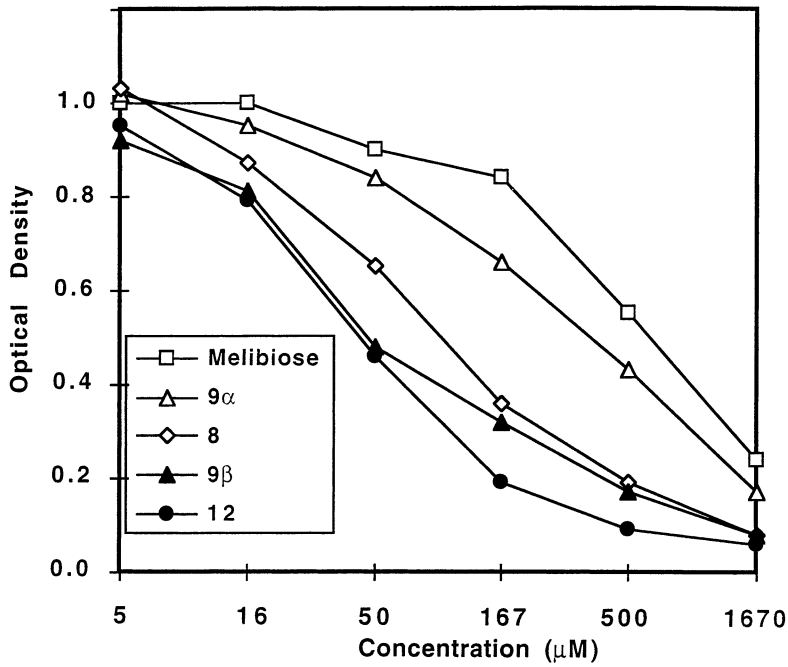


Fig. 1. Inhibition ELISA of anti-Gal(α1→3)Gal human IgG by various alpha galactoside disaccharides. A human plasma was diluted 1/16 and incubated for 30 min with the saccharides at the indicated concentrations before ELISA on rabbit erythrocytes glycolipids. The secondary antibody was a peroxidase-conjugated anti-human γ chain.

(**12**), followed by Galα(1→3)Galβ(1→1)Pr (**9**β). The disaccharide Galα(1→3)-Galα-(1→1)Pr (**9**α) was much less inhibitory than its anomer **9**β (390 μM versus 50 μM for OD = 0.5), and the free disaccharide Galα(1→3)Gal **8** had an intermediary inhibitory activity. Melibiose, Galα(1→6)Glc, which was formerly used as inhibitor of anti-αGal antibodies [5], was by far the worst inhibitor (700 μM for OD = 0.5). The largest discriminating effect on inhibition of IgG was seen with saccharide concentrations ranging from 0.05 to 0.5 mM. These concentrations were insufficient to inhibit IgM. Using concentrations of inhibitors ten times higher than for IgG, anti-αGal IgM was inhibited by each saccharide in the same order as for IgG. These results were taken into account to select the protected Galα(1→3)Galβ(1→1)All (**6**β) as precursor of Galα(1→3)Galβ(1→1)PrNH_2 (**15**) for binding to NHS-activated agarose gels.

Affinity column with the (3-amino)propyl 3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside.—An aliquot (2 mL) of Affi-Gel 10 (Bio-Rad) was poured in a small column, washed with 5 vol of anhyd 2-propanol, and equilibrated with anhyd dimethyl sulfoxide. The aminopropyl digalactoside **15** dissolved in one vol of dimethyl sulfoxide was added to the gel. The column was capped and stirred end over end at room temperature overnight. The column was washed with water, blocked with a 1 M ethanolamine solution (pH 8), and equilibrated with PBS pH 7.4. It was found that the coupling efficiency was 80% by measuring the amount of sugar exposed to the gel and the residual concentration after the covalent binding. The gel final vol was 1.3 mL. The column was stored with NaN_3 0.05% in PBS.

Disaccharide load of affinity columns.—Four columns were prepared. Column A was a control column without bound saccharide. Columns B, C, and D were loaded with 2, 4, and 15 μmol of disaccharide, respectively. Then, 20 mL of fresh plasma were applied on each column and the amount of anti-αGal antibodies was measured by ELISA. It was found that the unsubstituted gel did not retain any antibody, and that the more important the sugar load, the more efficient was the column at removing IgG and IgM (Fig. 2). It was also found that none of the columns was overloaded; the titer of anti-αGal antibodies was the same in the first and the last milliliters

coming out of the column. From these experiments, it was concluded that the optimal load of digalactoside was 5 μmol/mL of gel. A similar digalactoside load was also found to be optimal for the capacity of a glass beads-based matrix at retaining both anti-αGal of human plasma in vitro [35] and baboon plasma ex vivo [36].

These absorptions reflected the ability of the columns to bind the antibodies under a flow

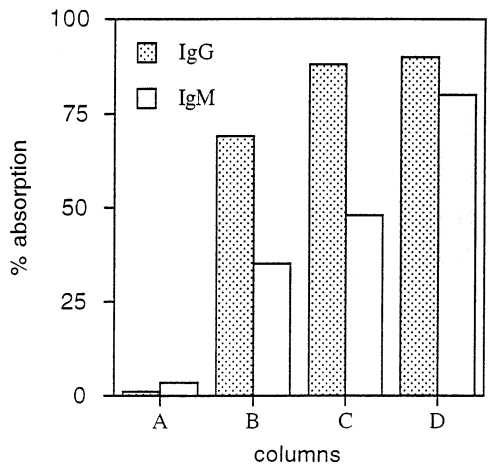


Fig. 2. Absorption of anti-Galα(1→3)Gal antibodies on columns with various loads of Galα(1→3)Galβ(1→1)-Pr–NH_2. Undiluted plasma (20 mL) was applied to columns of Affigel (1.3 mL) loaded with 0 (A), 2 (B), 4 (C), and 15 μmol (D) of Galα(1→3)Galβ(1→1)Pr–NH_2. The non-retained fractions were assayed for anti-Galα(1→3)Gal by ELISA using anti IgG or anti-IgM as secondary antibodies, and compared with the unabsorbed plasma (0% absorption, 100% activity).

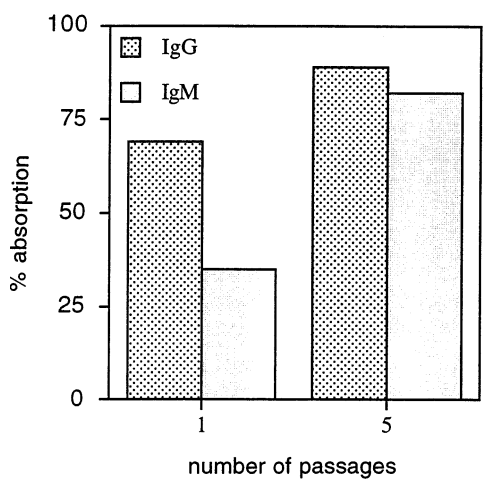


Fig. 3. Improvement of the absorption of anti-Galα(1→3)Gal antibodies by recirculation of the plasma on the affinity column. Plasma (20 mL) was applied one to five times on column B (Fig. 2) without intermediary elutions. The anti-Galα(1→3)Gal antibodies of the non-retained fraction were assayed by ELISA.

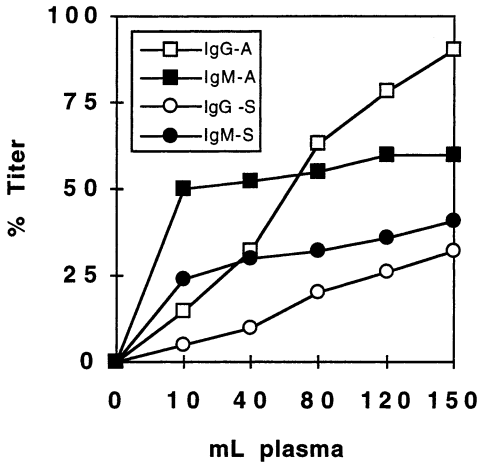


Fig. 4. Capacity of affinity columns. Undiluted plasma (150 mL) was applied to each of the columns (1 mL) of either Affigel (A) or Sepharose Fast Flow (S) loaded with 5 μmol of Galα(1→3)Galβ(1→1)Pr–NH_2. The plasma was collected by 10 ml fractions that were assayed for anti-Galα(1→3)Gal antibodies by ELISA. The titer was defined as the reciprocal of the dilution giving the same absorbance in ELISA than the original plasma at the dilution 1/100.

of 1 mL/min. During ex vivo immunoabsorption, the plasma is recirculated several times through the column. Therefore, plasma (20 mL) was applied five times onto column B, the least efficient column (Fig. 3). It is obvious that the column capacity was better used under these conditions, i.e., on one passage the column absorbed 50% of anti-αGal IgG and 20% of IgM, whereas after five passages the plasma was depleted from 90% of anti-αGal IgG and 70% of IgM.

Large-size columns.—It was decided to make a 25 mL affinity column with Affigel-10 (Bio-Rad) loaded with 50 mg (5 μmol/mL) of **15**. A 1 mL-aliquot of gel was poured into a small (5 mm diameter) column, and tested with 150 mL of plasma. The plasma was collected after the column by 10 mL fractions, which were assayed for anti-αGal activity (Fig. 4). The column appeared saturated with anti-αGal IgG after 150 mL of plasma had run through it, i.e., 50–60% of anti-αGal IgM was retained, without great variation during the process.

Therefore, another 25 mL column was made of Sepharose Fast Flow (Pharmacia Biotech), also loaded with 50 mg of **15**. The performance of the Sepharose column was tested on a 1 mL minicolumn as for the Affigel column. It retained antibodies better

than the Affigel column. In the 70–80 mL fraction, 80% of the anti-αGal IgG and 68% of the IgM were retained. In the 140–150 mL fraction, 68% of the anti-αGal IgG and 59% of the IgM were still retained. It was concluded that a 25 mL column would be able to deplete more than 68% of anti-αGal antibodies of 2 L of plasma, and more than 59% of 4 L of plasma, after only one passage through the column.

In the most recent report on anti-αGal immunoabsorption [37], the authors found that a 50 mL column of silica particles linked to the type 6 trisaccharide maximally absorbed anti-αGal antibodies from 1.25 L of pooled human plasma. This type of column was able to delay the rejection of a pig kidney transplanted into a cynomolgus monkey by more than 48 h [37]. By comparison, the 25 mL Sepharose Gal-α(1→3)Galβ(1→1)Pr–NH–CO–R column that was built in the present work had a larger capacity and a smaller volume, both important properties for an efficient immunoabsorption operation. Furthermore, its capacity might be even greater ex vivo than in vitro, as increasing the number of passages through the column improves the antibody depletion. Both large columns, Sepharose- and Affigel-based, are presently being tested for baboon-specific anti-αGal ex vivo immunoabsorption.

3. Experimental

Materials.—Chemical reagents were purchased from Sigma–Aldrich–Fluka (F-38297 St. Quentin Fallavier, France) and Acros Organics (F-93166 Noisy-le-Grand, France). Anhydrous solvents were purchased and kept under Ar on 4 Å molecular sieves. Solvents for chromatography were distilled in the laboratory. *N*-Hydroxysuccinimide-activated agarose gels were purchased from BioRad (F-94200 Ivry-sur-Seine, France) and from Amersham Pharmacia Biotech (F-91940 Les Ulis, France).

Carbohydrate quantitation.—Digalactoside concentrations were assayed in microtiter plates by a modification of the method of Dubois et al. [38]. To each well were added 35 μL of solution containing 0.5–15 μg of carbohydrate, 35 μL of a 5% aq phenol solution, and 180 μL of concd H_2SO_4. Each well content was thoroughly mixed. The reaction was allowed to proceed for 30 min. Plates were read at 490 nm with a Dynatech MRX microplate reader. Concentrations were calculated from a standard curve established with a titrated galactose solution.

Inhibition ELISA.—For inhibition experiments the plasma, diluted in phosphate-buffered saline (PBS) pH 7.4, was incubated for 1 h in the presence of various concentrations of sugars. Residual activities were measured by ELISA using microtiter plates Immulon I (Dynatech, Poly Labo, F-67 Strasbourg, France) coated with purified neutral glycosphingolipids from rabbit erythrocyte membranes [39]. Anti-αGal antibodies bound to glycolipids were detected with peroxidase-conjugated anti-IgG or anti-IgM (Dako S.A., F-78190 Trappes, France). Enzyme activity was detected with ABTS (Boehringer Mannheim, F-38243 Meylan, France) as substrate.

Absorption experiments.—For testing the absorption of anti-αGal antibodies on columns, undiluted plasma was applied to columns and recovered as indicated. Anti-αGal antibodies were measured by ELISA on serial dilution of plasma before and after absorption. The titer was defined as the dilution for which half maximal OD of the undiluted plasma was obtained.

General methods.—^{1}H and ^{13}C NMR spectra were recorded with a Bruker ARX-400 spectrometer. Coupling constants (*J*) are given in Hertz (Hz). Multiplicities are recorded as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet or complex). Mass spectra were recorded on a low-resolution MS Engine (Hewlett–Packard, CH-1217 Meyrin, Switzerland) with NH_3-chemical ionization in a thermospray source. Alternatively, mass spectra were obtained in the electrospray ionisation (ESMS) mode with an ESQUIRE-LC ion-trap mass spectrometer (Bruker Daltonik, Bremen, Germany) at 0.6 mass unit resolution. Thin-layer chromatography (TLC) was performed on Silica Gel 60 plates (E. Merck, D-64293 Darmstadt, Germany). Column chromatography was carried out as indicated using SiO_2 unless otherwise specified.

Allyl 2,3,4,6-tetra-O-*benzyl*-α-D-*galactopyranoside*.—Allyl α-D-galactopyranoside **1**α (2.5 g, 11.3 mmol) was dissolved in benzyl bromide (8.5 mL, 72.2 mmol) and CH_2Cl_2 (3 mL). Powdered KOH (3.8 g, 67.8 mmol) and catalytic amounts of tetraoctylammonium bromide were added. The mixture was vigorously stirred for 6 days at room temperature (rt). Stirring was maintained for 3 h after addition of MeOH (10 mL). The mixture was filtered through Celite with CH_2Cl_2 and the filtrate was evaporated to dryness. Column chromatography (1:19 EtOAc–petroleum ether) of the residue gave syrupy allyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (4.4 g, 70%). R_f 0.55 (1:4 EtOAc–petroleum ether). CIMS (NH_3): m/z 598 $[M + NH_4]^+$, 540, 458.

2,3,4,6-Tetra-O-*benzyl*-D-*galactopyranose*.—Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (3.0 g, 5.2 mmol) was dissolved in MeOH (50 mL) and $PdCl_2$ (305 mg, 1.7 mmol) was added. Competitive formation of methyl glycosides was prevented by the addition of a few drops of water. The mixture was stirred for 3.5 h at rt, filtered through Celite with CH_2Cl_2 and the filtrate was evaporated to dryness. Column chromatography (3:17 EtOAc–petroleum ether) of the residue gave syrupy 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose (2.4 g, 86%). R_f 0.10 (1:4 EtOAc–petroleum ether). CIMS (NH_3): m/z 558 $[M + NH_4]^+$, 450, 342.

Trichloroacetimidoyl 2,3,4,6-tetra-O-*benzyl*-β-D-*galactopyranoside* (**2**).—2,3,4,6-Tetra-*O*-benzyl-D-galactopyranose (2.3 g, 4.2 mmol) was dissolved in CH_2Cl_2 (20 mL) under Ar. Potassium carbonate (2.3 g, 17.0 mmol) and trichloroacetonitrile (2 mL, 20.0 mmol) were added. The mixture was vigorously stirred for 5 h at rt and filtered through Celite with CH_2Cl_2 (30 mL). The filtrate was washed with satd aq $NaHCO_3$, dried over $MgSO_4$ and concentrated under reduced pressure. Column chromatography (1:4 EtOAc–petroleum ether containing 1% Et_3N) of the residue gave syrupy **2** (2.3 g, 80%). R_f 0.65 (3:7 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 8.62 (s, 1 H, N*H*), 7.34–7.25 (m, 20 H, aromatics), 5.75 (d, 1 H, ^3J 8.0 Hz, H-1), 4.94 and 4.63 (2 d, 2 H, ^2J 11.5 Hz, OCH_2Ph), 4.90 and 4.81 (2 d, 2 H, ^2J 10.7 Hz, OCH_2Ph), 4.73 (s, 2 H, OCH_2Ph), 4.47 and 4.42 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.09 (dd, 1 H, ^3J 8.0, 9.6 Hz, H-2), 3.98 (de, 1 H, ^3J 3.3 Hz, H-4), 3.73 (te, 1 H, ^3J 6.1 Hz, H-5), 3.63 (m, 3 H, H-3, H-6, H-6′). ^{13}C NMR data ($CDCl_3$): δ 161.5 (*C*NH), 138.5, 138.2, 137.8, 128.4, 128.2, 127.9, 127.8, 127.6, 127.5 (aromatics), 98.7 (C-1), 82.2 (C-3), 78.1 (C-2), 75.2, 74.8 (2 O*C*H_2Ph), 74.4 (C-5), 73.4 (C-4), 73.4, 73.0 (2 O*C*H_2Ph), 68.1 (C-6).

Allyl 3,4-O-*cyclohexylidene*-α-D-*galactopyranoside* (**3**α).—Compound **1**α (2.0 g, 9.1 mmol) was dissolved in DMF (15 mL). Dimethoxycyclohexane (2.5 mL, 18.2 mmol) and concd H_2SO_4 (0.2 mL) were added. The mixture was stirred for 17 h at rt, then diluted in CH_2Cl_2 (100 mL), washed with satd aq $NaHCO_3$, dried over $MgSO_4$ and concentrated under reduced pressure. Column chromatography (1:1 EtOAc–petroleum ether) of the residue gave syrupy **3**α (1.4 g, 53%). R_f 0.60 (EtOAc). ^{1}H NMR data ($CDCl_3$): δ 5.95 (m, 1 H, CH_2CHCH_2), 5.32 (dd, 1 H, ^2J 1.4, ^3J 17.2 Hz, CH_2CHCH_2), 5.24 (dd, 1 H, ^2J 1.4 Hz, ^3J 10.4 Hz, CH_2CHCH_2), 4.96 (d, 1 H, ^3J 3.8 Hz, H-1), 4.30–4.25 (m, 3 H, H-3, H-4, CH_2CHCH_2), 4.13–4.07 (m, 2 H, H-5, CH_2CHCH_2), 3.99 (dd, 1 H, ^3J 6.1, ^2J 11.8 Hz, H-6), 3.89–3.80 (m, 2 H, H-2, H-6′), 1.67–1.40 (m, 10 H, cyclohexyl). ^{13}C NMR data ($CDCl_3$): δ 133.6 (CH_2CHCH_2), 117.8 (CH_2CHCH_2), 96.9 (C-1), 75.9 (C-3), 73.4 (C-4), 69.9 (C-2), 68.7 (CH_2CHCH_2), 68.3 (C-5), 62.6 (C-6), 37.5, 35.1, 24.9, 24.0, 23.6 (cyclohexyl). Calcd for $C_{15}H_{24}O_6$: M_w 300.3; CIMS (NH_3): m/z 318 $[M + NH_4]^+$, 301 $[M + H]^+$, 243.

Allyl 3,4-O-*cyclohexylidene*-β-D-*galactopyranoside* (**3**β).—The same experimental procedure as above but starting from **1**β gave syrupy **3**β (2.1 g, 71%). R_f 0.65 (EtOAc). ^{1}H NMR data ($CDCl_3$): δ 5.94 (m, 1 H, CH_2CHCH_2), 5.32 (dd, 1 H, ^2J 1.4, ^3J 17.2 Hz, CH_2CHCH_2), 5.22 (dd, 1 H, ^2J 1.4, ^3J 10.4 Hz, CH_2CHCH_2), 4.39 (dd, 1 H, ^3J 5.4, ^2J 12.6 Hz, CH_2CHCH_2), 4.25 (d, 1 H, ^3J 8.2 Hz, H-1), 4.16–4.08 (m, 3 H, H-3, H-4, CH_2CHCH_2), 4.02 (dd, 1 H, ^3J 8.1, ^2J 12.7 Hz, H-6), 3.86 (m, 2 H, H-5, H-6′), 3.58 (dd, 1 H, ^3J 7.2, 8.2 Hz, H-2), 1.74–1.35 (m, 10 H, cyclohexyl). ^{13}C NMR data ($CDCl_3$): δ 133.7

(CH_2CHCH_2), 118.0 (CH_2CHCH_2), 111.0 (CIV cyclohexyl), 101.3 (C-1), 78.5 (C-3), 73.9 (C-2), 73.5 (C-5), 73.5 (C-4), 70.2 (CH_2CHCH_2), 62.4 (C-6), 37.8, 35.4, 24.8, 23.9, 23.6 (cyclohexyl). CIMS (NH_3): m/z 318 $[M + NH_4]^+$, 301 $[M + H]^+$, 243.

*Allyl 3,4-*O*-cyclohexylidene-2,6-di-*O*-benzyl-α-*D*-galactopyranoside* (**4**α).—Compound **3**α (850 mg, 2.8 mmol) was dissolved in CH_2Cl_2 (5 mL) and benzyl bromide (1.3 mL, 11.3 mmol). Powdered KOH (640 mg, 11.3 mmol) and catalytic amounts of tetraoctylammonium bromide were added. The mixture was vigorously stirred for 3 days at rt. Stirring was maintained for 2 h after the addition of MeOH (5 mL). The mixture was filtered through Celite and evaporated to dryness. Column chromatography (1:9 EtOAc–petroleum ether) of the residue gave **4**α (1.1 g, 81%). R_f 0.60 (1:4 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.36–7.27 (m, 10 H, aromatics), 5.93 (m, 1 H, CH_2CHCH_2), 5.32 (dd, 1 H, ^2J 1.4, ^3J 17.2 Hz, CH_2CHCH_2), 5.20 (dd, 1 H, ^2J 1.4, ^3J 10.4 Hz, CH_2CHCH_2), 4.83 (d, 1 H, ^3J 3.5 Hz, H-1), 4.80 and 4.71 (2 d, 2 H, ^2J 12.6 Hz, OCH_2Ph), 4.64 and 4.54 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.36 (dd, 1 H, ^3J 5.4, 7.9 Hz, H-3), 4.22–4.15 (m, 3 H, H-4, H-5, CH_2CHCH_2), 4.01 (dd, 1 H, ^3J 6.2, ^2J 13.0 Hz, CH_2CHCH_2), 3.76 (dd, 1 H, ^3J 5.3, ^2J 10.1 Hz, H-6), 3.71 (dd, 1 H, ^3J 6.9, ^2J 10.1 Hz, H-6′), 3.52 (dd, 1 H, ^3J 3.5, 7.9 Hz, H-2), 1.65–1.37 (m, 10 H, cyclohexyl). ^{13}C NMR data ($CDCl_3$): δ 133.7 (CH_2CHCH_2), 128.3, 128.3, 127.7, 127.6, 127.5, 127.4 (aromatics), 117.7 (CH_2CHCH_2), 96.0 (C-1), 76.7 (C-2), 75.5 (C-3), 73.4 (OCH_2Ph), 73.4 (C-4), 72.2 (OCH_2Ph), 69.6 (C-6), 68.3 (CH_2CHCH_2), 66.8 (C-5), 38.0, 35.5, 25.0, 24.1, 23.7 (cyclohexyl). CIMS (NH_3): m/z 498 $[M + NH_4]^+$, 243.

*Allyl 3,4-*O*-cyclohexylidene-2,6-di-*O*-benzyl-β-*D*-galactopyranoside* (**4**β).—The same experimental procedure as above but starting from **3**β gave syrupy **4**β (3.1 g, 94%). R_f 0.50 (1:4 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.45 (m, 10 H, aromatics), 5.95 (m, 1 H, CH_2CHCH_2), 5.40 (dd, 1 H, ^2J 1.0, ^3J 17.2 Hz, CH_2CHCH_2), 5.25 (dd, 1 H, ^2J 1.0, ^3J 10.4 Hz, CH_2CHCH_2), 4.91 and 4.87 (2 d, 2 H, ^2J 11.9 Hz, OCH_2Ph), 4.70 and 4.62 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.47 (dd, 1 H, ^3J 5.1, ^2J 13.0 Hz, CH_2CHCH_2), 4.41 (d, 1 H, ^3J 8.1 Hz, H-1), 4.19 (m, 3 H, H-3, H-4, CH_2CHCH_2), 3.96 (te, 1 H, H-5), 3.86 (m, 2 H, H-6, H-6′), 3.48 (dd, 1 H, ^3J 6.1, 8.1 Hz, H-2), 1.66–1.39 (m, 10 H, cyclohexyl). ^{13}C NMR data ($CDCl_3$): δ 138.2 (aromatics), 134.0 (CH_2CHCH_2), 128.3, 128.1, 127.6, 127.5, 127.4 (aromatics), 117.1 (CH_2CHCH_2), 110.4 (CIV cyclohexyl), 101.8 (C-1), 80.0 (C-2), 78.7 (C-3), 73.6 (OCH_2Ph), 73.6 (OCH_2Ph), 73.3 (C-4), 72.3 (C-5), 69.7 (CH_2CHCH_2), 69.6 (C-6), 37.6, 35.5, 25.0, 24.0, 23.7 (cyclohexyl). CIMS (NH_3): m/z 498 $[M + NH_4]^+$, 440.

*Allyl 2,6-di-*O*-benzyl-α-*D*-galactopyranoside* (**5**α).—Compound **4**α (1.2 g, 2.5 mmol) was dissolved in MeOH (32 mL) and 1 M aq HCl (8 mL). The mixture was stirred for 1 h at 60 °C. After cooling, satd aq $NaHCO_3$ (10 mL) was added and MeOH was evaporated. The residue was diluted in CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$, dried over $MgSO_4$ and the filtrate was evaporated to dryness to give syrupy **5**α (920 mg, 92%). R_f 0.15 (3:7 EtOAc–petroleum ether). CIMS (NH_3): m/z 418 $[M + NH_4]^+$, 360.

*Allyl 2,6-di-*O*-benzyl-β-*D*-galactopyranoside* (**5**β).—The same experimental procedure as above but starting from **4**β gave syrupy **5**β (2.5 g, 98%). R_f 0.20 (3:7 EtOAc–petroleum ether). CIMS (NH_3): m/z 418 $[M + NH_4]^+$, 360.

*Allyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**6**α).—Compounds **2** (910 mg, 1.3 mmol) and **5**α (800 mg, 2.0 mmol) were dissolved in dry CH_2Cl_2 (20 mL) and activated 4 Å molecular sieves (2 g) were added under Ar. The mixture was stirred for 30 min at −30 °C. Then boron trifluoride diethyl etherate (50 μL, 0.4 mmol) was added, and stirring was maintained for 24 h at −30 °C. The mixture was filtered through Celite, washed with satd aq $NaHCO_3$, dried over $MgSO_4$ and the filtrate was concentrated under reduced pressure. Column chromatography (1:9 EtOAc–petroleum ether) of the residue gave syrupy **6**α (400 mg, 33%). R_f 0.60 (3:7 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.41–7.22 (m, 30 H, aromatics),

5.94 (m, 1 H, CH_2CHCH_2), 5.30 (dd, 1 H, ^3J 17.2 Hz, CH_2CHCH_2), 5.18 (dd, 1 H, ^3J 10.9 Hz, CH_2CHCH_2), 4.85 (d, 1 H, ^3J 3.9 Hz, H-1^I), 4.84 (d, 1 H, ^3J 3.8 Hz, H-1^{II}), 4.88 and 4.54 (2 d, 2 H, ^2J 11.7 Hz, OCH_2Ph), 4.78 and 4.74 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.63 and 4.57 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.57 (d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.37 and 4.33 (2 d, 2 H, ^2J 11.7 Hz, OCH_2Ph), 4.20 (te, 1 H, H-5^{II}), 4.14 (dd, 1 H, ^3J 5.2, ^2J 12.8 Hz, CH_2CHCH_2), 4.12–3.93 (m, 6 H, H-2^{II}, H-3^{II}, H-4^I, H-4^{II}, H-5^I, CH_2CHCH_2), 3.82 (dd, 1 H, ^3J 3.6, 9.8 Hz, H-3^I), 3.74 (dd, 1 H, ^3J 5.7, ^2J 10.0 Hz, H-6^I), 3.68 (dd, 1 H, ^3J 6.9, ^2J 10.0 Hz, H-6′^{I}), 3.53 (m, 3 H, H-2^I, H-6^{II}, H-6′^{II}). ^{13}C NMR data ($CDCl_3$): δ 138.3, 138.1, 137.9 (aromatics) 134.4 (CH_2CHCH_2), 128.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.5, 127.3 (aromatics), 117.8 (CH_2CHCH_2), 95.9 (C-1^I), 94.9 (C-1^{II}), 79.9 (C-3^{II}), 77.0 (C-3^I), 75.7 (C-2^I), 75.0 (C-2^{II}), 74.9, 74.8 (2 OCH_2Ph), 74.6 (C-4^I), 73.5, 73.4 (2 OCH_2Ph), 73.1 (C-5^I), 72.9, 72.3 (2 OCH_2Ph), 69.7 (CH_2CHCH_2), 69.8 (C-5^{II}), 68.2 (C-6^I), 68.1 (C-6^{II}), 65.9 (C-4^{II}). ESMS: m/z 945.4 $[M + Na]^+$.

*Allyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**6**β).—Compounds **2** (4.7 g, 6.9 mmol) and **5**β (2.3 g, 5.7 mmol) were dissolved in dry CH_2Cl_2 (60 mL) and activated 4 Å molecular sieves (5 g) were added under Ar. Tetramethylurea (0.8 mL, 6.9 mmol) was added and the mixture was stirred for 30 min at −10 °C. Then trimethylsilyl trifluoromethanesulfonate (0.1 mL, 0.55 mmol) was added and stirring was maintained for 48 h at −10 °C and for 24 h at rt. The mixture was filtered through Celite, washed with satd aq $NaHCO_3$, dried over $MgSO_4$ and the filtrate was concentrated under reduced pressure. Column chromatography (1:4 EtOAc–petroleum ether) of the residue gave syrupy **6**β (3.2 g, 60%). R_f 0.60 (3:7 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.35–7.22 (m, 30 H, aromatics), 5.94 (m, 1 H, CH_2CHCH_2), 5.31 (dd, 1 H, ^2J 1.6, ^3J 17.2 Hz, CH_2CHCH_2), 5.16 (dd, 1 H, ^2J 1.4, ^3J 10.4 Hz, CH_2CHCH_2), 4.89 and 4.53 (2 d, 2 H, ^2J 11.3 Hz, OCH_2Ph), 4.87 and 4.85 (2 d, 2 H, ^2J 10.9 Hz, OCH_2Ph), 4.80 (d, 1 H, ^3J 3.7 Hz, H-1^{II}), 4.74 (s, 2 H, OCH_2Ph), 4.65 and 4.64 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.62 and 4.58 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.42 (ddt, 1 H, ^4J 1.4, ^3J 5.0, ^2J 12.9 Hz, CH_2CHCH_2), 4.37 (d, 1 H, ^3J 6.0 Hz, H-1^I), 4.31 and 4.24 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.13 (t, 1 H, H-5^{II}), 4.12 (ddt, 1 H, ^4J 1.4, ^3J 6.0, ^2J 12.9 Hz, CH_2CHCH_2), 4.01 (dd, 1 H, ^3J 3.7, 9.7 Hz, H-2^{II}), 3.87 (m, 3 H, H-3^{II}, H-4^I, H-4^{II}), 3.80 (dd, 1 H, ^3J 5.9, ^2J 10.0 Hz, H-6^I), 3.74 (dd, 1 H, ^3J 6.2, ^2J 10.0 Hz, H-6′^{I}), 3.64 (d, 1 H, ^3J 5.3 Hz, H-3^I), 3.55 (t, 1 H, ^3J 6.0 Hz, H-5^I), 3.41 (m, 3 H, H-2^I, H-6^{II}, H-6′^{II}). ^{13}C NMR data ($CDCl_3$): δ 138.6, 138.5, 138.4, 138.1, 138.0, 137.8 (aromatics), 134.0 (CH_2CHCH_2), 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3 (aromatics), 116.9 (CH_2CHCH_2), 102.6 (C-1^I), 94.8 (C-1^{II}), 78.9 (C-3^{II}), 77.6 (C-3^I), 77.4 (C-2^I), 75.5 (C-2^{II}), 74.9, 74.7 (2 OCH_2Ph), 74.6 (C-4^I), 74.2, 73.5 (2 OCH_2Ph), 73.0 (C-5^I), 72.9, 72.4 (2 OCH_2Ph), 69.8 (CH_2CHCH_2), 69.3 (C-5^{II}), 69.2 (C-6^I), 68.3 (C-6^{II}), 64.9 (C-4^{II}). ESMS: m/z 945.4 $[M + Na]^+$. Anal. Calcd for $C_{57}H_{62}O_{11}$: C, 74.17; H, 6.77. Found: C, 74.00; H, 6.76.

*Propyl 3-*O*-(α-*D*-galactopyranosyl)-α-*D*-galactopyranoside* (**9**α).—Palladium 10% on activated charcoal (200 mg) was suspended in EtOH (30 mL). Compound **6**α (140 mg, 0.15 mmol) dissolved in CH_2Cl_2 (2 mL) was added and the mixture was stirred at rt for 16 h under H_2 (2 atm). The mixture was filtered on Celite and the filtrate was concentrated under reduced pressure. Lyophilization of the residue gave syrupy **9**α (58 mg, 99%). ^{1}H NMR data (Me_2SO-d_6): δ 4.98 (d, 1 H, ^3J 3.7 Hz, H-1^I), 4.81 (d, 1 H, ^3J 2.9 Hz, H-1^{II}), 4.06 (s, 1 H, H-3^I), 4.00 (t, 1 H, ^3J 6.1 Hz, H-3^{II}), 3.85–3.76 (m, 5 H, H-2^{II}, H-4^I, H-4^{II}, H-5^I, H-5^{II}), 3.69 (dd, 1 H, ^3J 3.7, 10.2 Hz, H-2^I), 3.58 (m, 4 H, H-6^I, H-6′^{I}, H-6^{II}, H-6′^{II}), 3.51 (dt, 1 H, ^3J 7.0, ^2J 9.6 Hz, $CH_2CH_2CH_3$), 3.33 (dt, 1 H, ^3J 6.2, ^2J 9.6 Hz, $CH_2CH_2CH_3$), 1.46 (m, 2 H, $CH_2CH_2CH_3$), 0.75 (t, 3 H, ^3J 7.4 Hz, $CH_2CH_2CH_3$). ESMS: m/z 407.2 $[M + Na]^+$.

*Propyl 3-*O*-(α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**9**β).—The same experimental procedure as above but starting from **6**β gave **9**β (98%). ^{1}H NMR data (Me_2SO-d_6):

δ 4.81 (d, 1 H, ^3J 3.1 Hz, H-1^{II}), 4.15 (d, 1 H, ^3J 7.3 Hz, H-1^{I}), 4.00 (t, 1 H, ^3J 5.8 Hz, H-3^{II}), 3.83–3.33 (m, 14 H, H-2^{I}, H-2^{II}, H-3^{I}, H-4^{I}, H-4^{II}, H-5^{I}, H-5^{II}, H-6^{I}, H-6′^{I}, H-6^{II}, H-6′^{II}, CH_2CH$_2$CH$_3$), 1.44 (m, 2 H, CH$_2$CH_2CH$_3$), 0.74 (t, 3 H, ^3J 7.4 Hz, CH$_2$CH$_2$CH_3). ESIMS: m/z 407.2 $[M + Na]^+$.

*(3-Hydroxy)propyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**10**).—Compound **6**β (4.1 g, 4.4 mmol) was dissolved in THF (60 mL) under Ar at 0 °C. A 0.5 M solution of 9-BBN in THF (20 mL, 10.0 mmol) was added and the mixture was stirred for 24 h during which time the temperature was allowed to reach rt. As TLC control showed residual presence of precursor, an additional 20 mL of the 0.05 M solution of 9-BBN in THF was added and stirring was maintained for 24 h at rt. The mixture was cooled to 0 °C. Then 60 mL of a cooled aq 2 M NaOH soln and 15 mL of cooled H_2O_2 35% were added dropwise simultaneously. After 30 min of stirring, the mixture was diluted with EtOAc (100 mL), washed with satd aq NH_4Cl, dried over $MgSO_4$, and the organic layer was concentrated under reduced pressure. Column chromatography (1:4 EtOAc–petroleum ether) of the residue gave syrupy **10** (2.5 g, 60%). R_f 0.15 (3:7 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.36–7.19 (m, 30 H, aromatics), 4.89 and 4.53 (2 d, 2 H, ^2J 11.3 Hz, OCH_2Ph), 4.85 and 4.82 (2 d, 2 H, ^2J 10.9 Hz, OCH_2Ph), 4.79 (d, 1 H, ^3J 3.7 Hz, H-1^{II}), 4.73 (s, 2 H, OCH_2Ph), 4.65 and 4.62 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.64 and 4.56 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.32 (d, 1 H, ^3J 7.6 Hz, H-1^{I}), 4.31 and 4.24 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.11 (te, 1 H, ^3J 6.8 Hz, H-5^{II}), 4.02 (m, 2 H, H-2^{II}, CH_2CH$_2$CH$_2$OH), 3.88–3.70 (m, 7 H, H-3^{II}, H-4^{I}, H-4^{II}, H-6^{I}, H-6′^{I}, CH_2CH$_2$CH$_2$OH, CH$_2$CH$_2$CH_2OH), 3.65–3.55 (m, 4 H, H-2^{I}, H-3^{I}, H-5^{I}, CH$_2$-CH$_2$CH_2OH), 3.42 (dd, 1 H, ^3J 7.4, ^2J 9.2 Hz, H-6^{II}), 3.40 (dd, 1 H, ^3J 6.2, ^2J 9.2 Hz, H-6′^{II}), 1.83 (m, 2 H, CH$_2$CH_2CH$_2$OH). ^{13}C NMR data ($CDCl_3$): δ 138.6, 138.4, 138.1, 138.0, 137.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4 (aromatics), 103.7 (C-1^{I}), 94.9 (C-1^{II}), 79.0 (C-3^{II}), 77.8 (C-3^{I}), 77.5 (C-2^{I}), 75.6 (C-2^{II}), 75.0, 74.8 (2 OCH$_2$Ph), 74.6 (C-4^{I}), 74.4, 73.6, 73.1 (3 OCH$_2$Ph), 73.0 (C-5^{I}), 72.5 (OCH$_2$Ph), 69.4 (C-5^{II}), 69.3 (C-6^{I}), 68.3 (C-6^{II}), 67.3 (CH$_2$CH$_2$CH$_2$OH), 65.0 (C-4^{II}), 59.9 (CH$_2$-CH$_2$$CH_2$OH), 32.4 (CH$_2$$CH_2CH_2$OH). ESIMS: m/z 963.4 $[M + Na]^+$.

*(3-Tosyloxy)propyl 2,6-di-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranoside* (**13**).—Compound **10** (2.4 g, 2.5 mmol) was dissolved in CH_2Cl_2 (60 mL) under Ar. Triethylamine (2 mL, 14.4 mmol) and tosyl chloride (2.1 g, 11.0 mmol) were added and the mixture was stirred for 4 days at rt. The mixture was washed with satd aq NH_4Cl, dried over $MgSO_4$ and the organic layer was concentrated under reduced pressure. Column chromatography (1:9 EtOAc–petroleum ether) of the residue gave syrupy **13** (2.5 g, 94%). R_f 0.40 (3:7 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.75 (d, 2 H, aromatics), 7.34–7.18 (m, 32 H, aromatics), 4.89 and 4.53 (2 d, 2 H, ^2J 11.3 Hz, OCH_2Ph), 4.85 and 4.65 (2 d, 2 H, ^2J 11.7 Hz, OCH_2Ph), 4.78 (d, 1 H, ^3J 3.7 Hz, H-1^{II}), 4.73 (s, 2 H, OCH_2Ph), 4.67 and 4.52 (2 d, 2 H, ^2J 11.1 Hz, OCH_2Ph), 4.60 and 4.56 (2 d, 2 H, ^2J 12.1 Hz, OCH_2Ph), 4.32 and 4.26 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.21 (d, 1 H, ^3J 7.7 Hz, H-1^{I}), 4.17–4.06 (m, 3 H, H-5^{II}, CH_2CH$_2$CH$_2$OTs), 4.01 (dd, 1 H, ^3J 3.7, 9.9 Hz, H-2^{II}), 3.92 (ddd, 1 H, ^3J 5.6, 6.2, ^2J 10.0 Hz, CH$_2$CH$_2$CH_2OTs), 3.86–3.81 (m, 3 H, H-3^{II}, H-4^{I}, H-4^{II}), 3.76 (dd, 1 H, ^3J 5.9, ^2J 9.9 Hz, H-6^{I}), 3.70 (dd, 1 H, ^3J 6.2, ^2J 9.9 Hz, H-6′^{I}), 3.59 (dd, 1 H, ^3J 3.3, 9.4 Hz, H-3^{I}), 3.56–3.48 (m, 3 H, H-2^{I}, H-5^{I}, CH$_2$CH$_2$CH_2OTs), 3.41 (m, 2 H, H-6^{II}, H-6′^{II}), 2.36 (s, 3 H, CH_3PhS), 1.94 (m, 2 H, CH$_2$CH_2CH$_2$OTs). ^{13}C NMR data ($CDCl_3$): δ 144.5, 138.6, 138.4, 138.3, 138.0, 137.7, 132.9, 129.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.3 (aromatics), 103.6 (C-1^{I}), 94.9 (C-1^{II}), 79.1 (C-3^{II}), 77.7 (C-3^{I}), 77.4 (C-2^{I}), 75.6 (C-2^{II}), 75.0, 74.8 (2 OCH$_2$Ph), 74.6 (C-4^{I}), 74.4, 73.6, 73.1 (3 OCH$_2$Ph), 73.0 (C-5^{I}), 72.5 (OCH$_2$Ph), 69.4 (C-5^{II}), 69.2 (C-6^{I}), 68.4 (C-6^{II}), 67.7 (CH$_2$CH$_2$CH$_2$OTs), 65.2 (CH$_2$CH$_2$-CH$_2$OTs), 64.9 (C-4^{II}), 29.5 (CH$_2$$CH_2CH_2$-OTs), 21.5 ($CH_3$PhS). ESMS: m/z 1117.3 $[M + Na]^+$.

(3-Azido)propyl 2,6-di-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside (**14**).—Compound **13** (2.4 g, 2.1 mmol) was dissolved in DMF (60 mL) under Ar. Sodium azide (280 mg, 4.3 mmol) was added and the mixture was stirred for 1 day at rt. The mixture was diluted with CH_2Cl_2 (50 mL), washed with satd aq $NaHCO_3$, dried over $MgSO_4$ and the organic layer was concentrated under reduced pressure. Column chromatography (1:4 EtOAc–petroleum ether) of the residue gave syrupy **14** (1.9 g, 90%). R_f 0.65 (3:7 EtOAc–petroleum ether). ^{1}H NMR data ($CDCl_3$): δ 7.34–7.18 (m, 30 H, aromatics), 4.89 and 4.53 (2 d, 2 H, ^2J 11.3 Hz, OCH_2Ph), 4.86 and 4.80 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.79 (d, 1 H, ^3J 3.7 Hz, H-1^{II}), 4.74 (s, 2 H, OCH_2Ph), 4.65 and 4.63 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.62 and 4.58 (2 d, 2 H, ^2J 12.0 Hz, OCH_2Ph), 4.31 and 4.24 (2 d, 2 H, ^2J 11.8 Hz, OCH_2Ph), 4.30 (d, 1 H, ^3J 7.6 Hz, H-1^{I}), 4.11 (te, 1 H, ^3J 6.8 Hz, H-5^{II}), 4.00 (m, 2 H, H-2^{II}, CH_2CH$_2$CH$_2$N$_3$), 3.88 (m, 3 H, H-3^{II}, H-4^{I}, H-4^{II}), 3.78 (dd, 1 H, ^3J 5.8, ^2J 9.9 Hz, H-6^{I}), 3.72 (dd, 1 H, ^3J 6.2, ^2J 9.9 Hz, H-6′^{I}), 3.67–3.56 (m, 4 H, H-2^{I}, H-3^{I}, H-5^{I}, CH_2CH$_2$CH$_2$N$_3$), 3.41 (m, 2 H, H-6^{II}, H-6′^{II}), 3.36 (t, 2 H, ^3J 6.8 Hz, CH$_2$CH$_2$CH_2N$_3$), 1.86 (m, 2 H, CH$_2$CH_2-CH$_2$N$_3$). ^{13}C NMR data ($CDCl_3$): δ 138.6, 138.5, 138.1, 137.8, 128.5, 128.4, 128.4, 128.2, 128.2, 128.0, 128.0, 127.9, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4 (aromatics), 103.6 (C-1^{I}), 94.9 (C-1^{II}), 79.0 (C-3^{II}), 77.8 (C-3^{I}), 77.5 (C-2^{I}), 75.6 (C-2^{II}), 75.1, 74.8 (2 OCH$_2$Ph), 74.6 (C-4^{I}), 74.4, 73.6, 73.1 (3 OCH$_2$Ph), 73.1 (C-5^{I}), 72.5 (OCH$_2$Ph), 69.4 (C-5^{II}), 69.3 (C-6^{I}), 68.3 (C-6^{II}), 66.7 (CH$_2$CH$_2$CH$_2$N$_3$), 65.0 (C-4^{II}), 48.3 (CH$_2$CH$_2$CH$_2$N$_3$), 29.2 (CH$_2$CH$_2$-CH$_2$N$_3$). ESIMS: m/z 988.4 $[M + Na]^+$.

(3-Amino)propyl 3-O-(α-D-galactopyranosyl)-β-D-galactopyranoside (**15**).—Palladium 10% on activated charcoal (300 mg) was suspended in EtOH (30 mL). Compound **14** (300 mg, 0.31 mmol) dissolved in CH_2Cl_2 (2 mL) was added and the mixture was stirred at rt for 16 h under H_2 (2 atm). The mixture was filtered on Celite and the filtrate was concentrated. Lyophilization of the residue gave syrupy **15** (120 mg, 97%) for which MS data were not available (note of the editor). ^{1}H NMR data (Me_2SO-d_6): δ 4.82 (d, 1 H, ^3J 3.1 Hz, H-1^{II}), 4.16 (d, 1 H, ^3J 7.6 Hz, H-1^{I}), 3.99 (te, 1 H, ^3J 6.4 Hz, H-3^{II}), 3.85–3.30 (m, 13 H, H-2^{I}, H-2^{II}, H-3^{I}, H-4^{I}, H-4^{II}, H-5^{I}, H-5^{II}, H-6^{I}, H-6′^{I}, H-6^{II}, H-6′^{II}, CH_2CH$_2$CH$_2$NH$_2$), 2.85 (t, 1 H, ^3J 7.1 Hz, CH$_2$CH$_2$CH$_2$NH_2), 2.79 (t, 1 H, CH$_2$CH$_2$CH_2NH$_2$), 2.71 (q, 1 H, CH$_2$CH$_2$CH_2NH$_2$), 1.77 (m, 2 H, CH$_2$CH_2-CH$_2$NH$_2$, 1.09 (t, 1 H, ^3J 7.1 Hz, CH$_2$CH$_2$-CH$_2$NH_2)).

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