Synthesis and structure–activity relationships of di- and trisaccharide inhibitors for *Shiga*-like toxin Type 1

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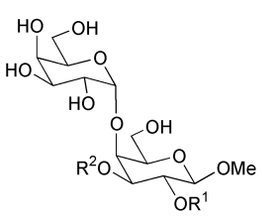
The syntheses of galabiose and P^k-trisaccharide analogues in which selected hydroxy groups are replaced by *O*-methyl, amino deoxy, acetamido deoxy, and carboxyalkyl groups are reported. The ability of these inhibitors to block *E. coli* verotoxin 1 binding to its mammalian cell-surface receptor are evaluated by a solid-phase competition assay. The synthesis of a biotinylated glycoconjugate for this assay is described, wherein a P^k-trisaccharide tether derivative **70** is constructed and covalently attached to bovine serum albumin followed by biotinylation. Galabiose derivatives **4** and **5** that contain a carboxymethyl or carboxyethyl substituent at *O*-2 of the β-galactose residue show 15–20-fold activity gains over the methyl glycoside of galabiose. This enhanced activity is not observed for the corresponding carboxymethyl-substituted P^k-trisaccharide analogue **13**. The inhibition data are rationalized with the solved crystal structure for verotoxin 1 complexed with a P^k-trisaccharide analogue and provide insight for the design of dimeric inhibitors that can exploit the unique binding-site distribution of the toxin's B subunit. This discussion provides a further example of the important role played by ordered water molecules in sugar–protein complexes.

Introduction

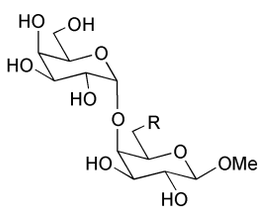
Infection by bacteria that produce *Shiga*-like toxins (SLTs), also known as verotoxins (VTs), results in serious gastrointestinal and urinary tract disorders.[1] Certain serotypes of *E. coli* that produce verotoxins are known to cause a potentially lethal disease, Hemolytic Uremic Syndrome (HUS), that may result in kidney failure. SLTs belong to a family of bacterial toxins that have a hexameric AB_5 structure,[2] where A denotes a cytotoxic enzyme and B_5 represents a homopentameric lectin-like carbohydrate recognizing complex that facilitates delivery and entry of the A component into the host's cell. Natural ligands for SLTs are glycolipids of the globo series Gb_3 and Gb_4 (ref. 3) that contain the P^k-trisaccharide, α-D-Gal(1→4)-β-D-Gal(1→4)-β-D-Glc. Since recognition and binding of the AB_5 toxin is an obligatory event that precedes entry of the enzyme A subunit into the cell, inhibitors that block the adherence of the B_5 subunit to the cell surface are of great interest.

A recently solved crystal structure for a VT-1 (B_5 subunit)–P^k analogue complex[4] revealed three different carbohydrate binding pockets (CBPs) per single B subunit, for a total of 15 bound trisaccharide units per pentamer. Each of the three sites exhibits a distinct set of sugar–protein interactions. Two distinct binding motifs corresponding to sites 1 and 2 were predicted on the basis of docking studies.[5] On the other hand, calorimetric studies[6] suggest only five identical, comparatively weak, binding sites per B_5 subunit. In order to probe the mode of action of the SLTs a set of inhibitors was designed that could potentially distinguish between individual CBPs.

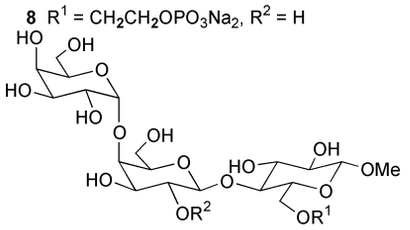
We present here the synthesis and biological activity of a series of these P^k-trisaccharide derivatives **12** and **13,** and disaccharide analogues of the terminal galabiose, **2**–**10**.



1 $R^1 = R^2 = H$
2 $R^1 = R^2 = CH_3$
3 $R^1 = H$, $R^2 = CH_3$
4 $R^1 = CH_2CO_2H$, $R^2 = H$
5 $R^1 = CH_2CH_2CO_2H$, $R^2 = H$
6 $R^1 = CH_2CH_2CH_2CO_2H$, $R^2 = H$
7 $R^1 = CH_2CH_2OSO_3Na$, $R^2 = H$
8 $R^1 = CH_2CH_2OPO_3Na_2$, $R^2 = H$



9 $R = NH_2$
10 R = NHAc



11 $R^1 = R^2 = H$
12 $R^1 = CH_2CO_2H$, $R^2 = H$
13 $R^1 = H$, $R^2 = CH_2CO_2H$

Results

Synthesis of di- and trisaccharide analogues of P^k-trisaccharide

For the synthesis of galabiose analogues **2**–**10** two strategies were chosen. The desired functionality at a certain position was introduced or prearranged either at the monosaccharide or disaccharide level. The galactosyl acceptor **15** was prepared by reductive opening of the benzylidene ring of the bis(methyl ether) derivative **14**.[7] Glycosylation of **15** by ethyl tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[8] **16**, promoted by *N*-iodosuccinimide–silver trifluoromethanesulfonate (NIS–AgOTf) in dichloromethane (DCM), gave disaccharide **17** in 69% yield. Hydrogenation of **17** furnished the target disaccharide **2** (Scheme 1).

The related monomethylated disaccharide **3** was synthesized starting from methyl 2,6-di-*O*-benzyl-β-D-galactopyranoside[9]

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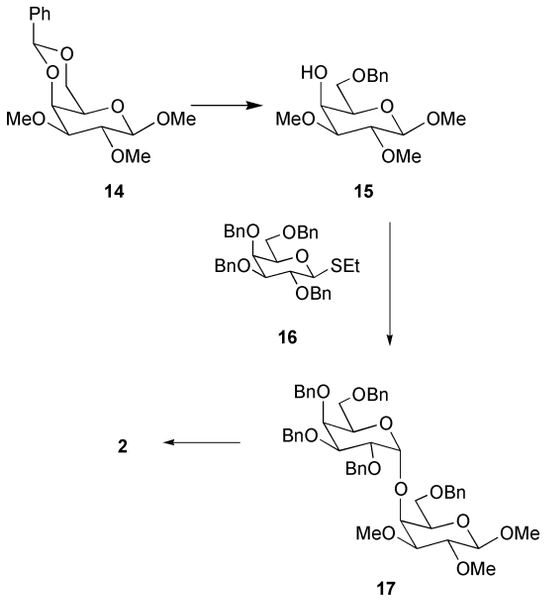
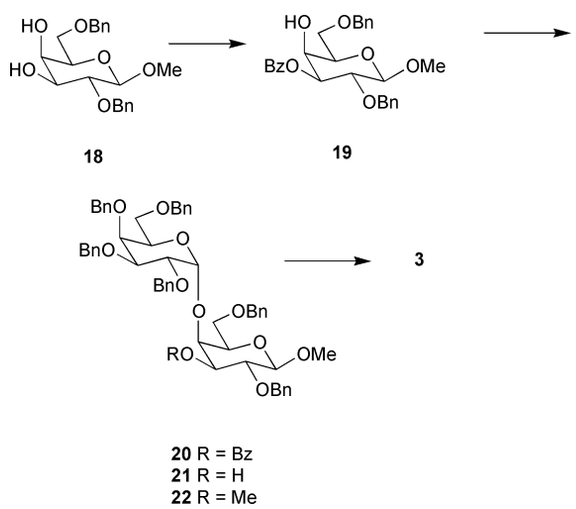


Scheme 1

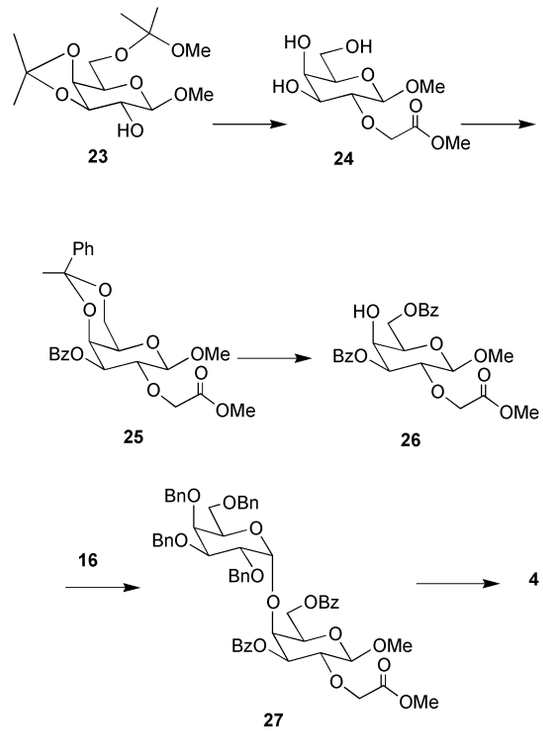
18. Selective benzoylation of **18** at *O*-3 followed by glycosylation of the resulting alcohol **19** with thioglycoside **16**[8] gave disaccharide **20** (74%). Saponification of the benzoyl group of **20** gave alcohol **21**, which was methylated to yield **22**. Hydrogenation of **22** gave disaccharide **3** (Scheme 2), the synthesis of which by a different route has been previously reported.[10]



Scheme 2

Synthesis of functionalized disaccharides **4**–**8** originates from a partially protected methyl galactoside **23**.[11] Alkylation of **23** with chloroacetic acid followed by methylation and acid hydrolysis gave the methoxycarbonylmethyl derivative **24**. Selective protection of the hydroxy groups by formation of a benzylidene acetal intermediate **25** followed by benzoylation provided glycosyl acceptor **26**. Glycosylation of **26** by thiogalactoside **16**[8] gave disaccharide **27**, and following deprotection the carboxymethyl-modified disaccharide **4** was obtained (Scheme 3).

Analogously, allylation of **23** followed by acid hydrolysis gave triol **28**.[11] Controlled benzoylation of **28** with benzoyl chloride in pyridine afforded a selectively protected alcohol **29** containing an unprotected hydroxy group at C-4. Glycosylation of alcohol **29** with the perbenzylated galactosyl chloride **30**[12] in the presence of AgOTf and a hindered base gave disaccharide **31** (85%). The key disaccharide **32** was obtained by replacing the benzoyl protective groups of **31** by benzyl groups.



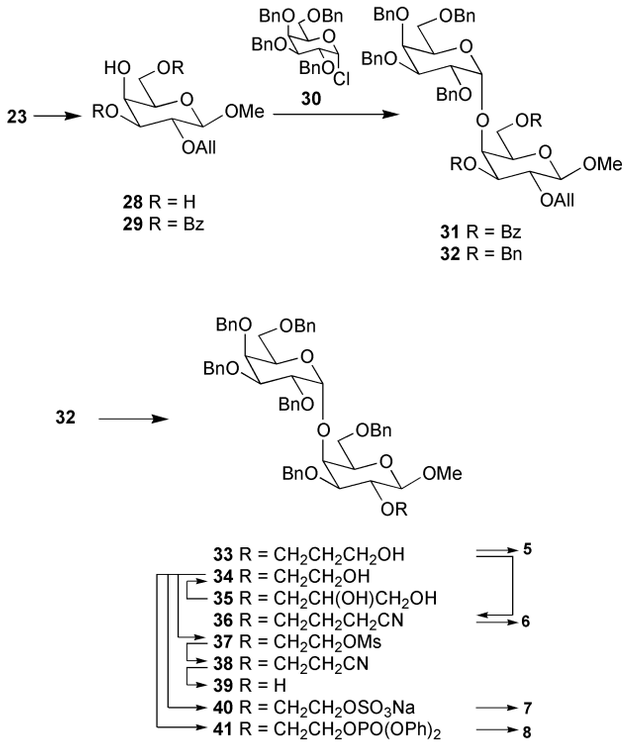
Scheme 3

The allyl group was converted into two homologous alcohols **33** and **34**. Oxidative hydroboration of **32** with 9-borabicyclo-[3.3.1]nonane (9-BBN), H_2O_2, and NaOH provided the propanol derivative **33**. Ozonolysis of **32** followed by reduction with $NaBH_4$ gave the ethanol derivative **34** in modest yield (61%). Alternatively, **34** was obtained *via* diol **35** by treatment of **32** with 4-methylmorpholine *N*-oxide in the presence of OsO_4, followed by $NaIO_4$ oxidation and $NaBH_4$ reduction of the resulting aldehyde.

Oxidation of the unprotected primary hydroxy group of compound **33** to a carboxylic acid by a mixture of CrO_3–Py–HOAc–*t*-BuOH in DCM followed by deprotection gave the propionic acid disaccharide derivative **5** (Scheme 4). Homologation of the hydroxypropyl substituent of **33**, *via* displacement of a methanesulfonate to give the nitrile **36**, followed by basic hydrolysis and hydrogenation yielded the target butyric acid derivative **6**.

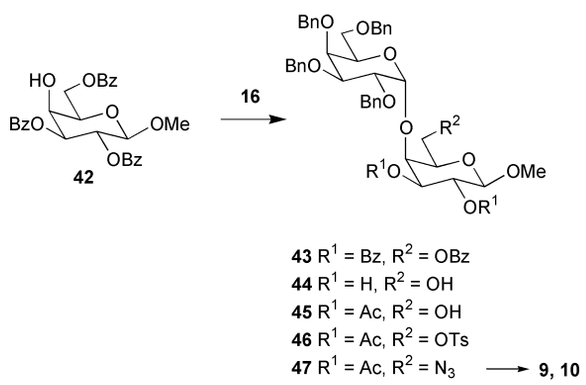
Owing to the comparatively low yield in the oxidation of **33** an attempt to obtain the propionic acid derivative **5** *via* cyanide **38** was undertaken. Alcohol **34** was esterified by MsCl in pyridine to give **37**. Reaction of the methanesulfonate with KCN provided the acrylonitrile derivative **38**. However, instead of hydrolysis under basic conditions the acrylonitrile **38** underwent β-elimination to form a product that was identical to the monohydroxy galabiose derivative **39**, which can also be obtained by removal of the allyl group of disaccharide derivative **32** with $PdCl_2$. Alcohol **34** was either sulfated or phosphorylated to give disaccharide derivatives **40** and **41** that upon hydrogenation furnished the corresponding sulfate **7** and phosphate **8** (Scheme 4).

Glycosylation of methyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside[13] **42** with the thioglycoside **16**[8] gave disaccharide **43**, which was used for the synthesis of 6-amino and 6-acetamido analogues **9** and **10**. Basic hydrolysis of **43** furnished the partially protected disaccharide **44**. Treatment of **44** with *t*-$BuMe_2SiCl$ followed by acetylation and acid hydrolysis gave the disaccharide **45** containing an unprotected 6-hydroxy group, which was esterified to give tosyl ester **46**. Displacement of the tosyloxy group by azide gave the 6-azido disaccharide **47**. Transesterification of **47** followed by hydrogenation furnished the target 6-amino-6-deoxygalabioside **9**, which may



Scheme 4

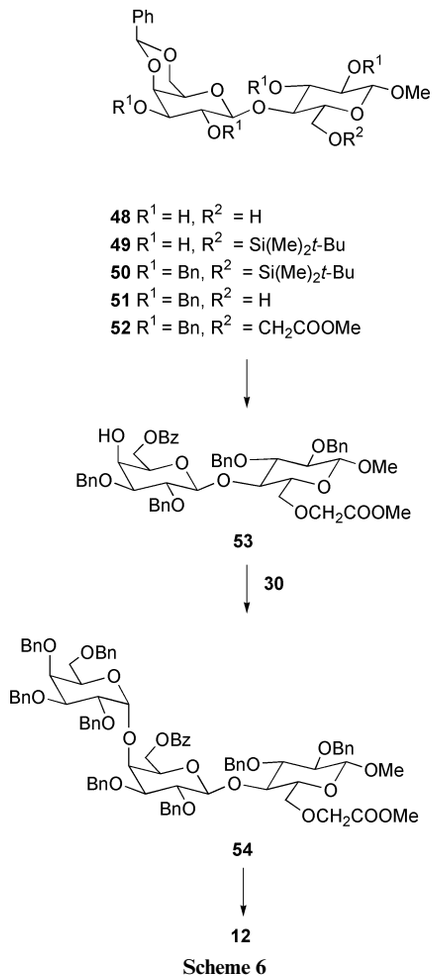
be selectively acetylated *in situ* to provide the 6-acetamido derivative **10** (Scheme 5).



Scheme 5

Analogues of the P^k-trisaccharide containing a carboxymethoxy group at the C-6 and C-2′ positions were also synthesized. Silylation of methyl 4′,6′-*O*-benzylidene lactoside[14] **48** with *t*-$BuMe_2SiCl$ followed by per benzylation of **49** and fluoride ion-promoted desilylation of the resulting disaccharide **50** gave a partially protected lactoside **51** with the 6-hydroxy group open for further manipulations. The methoxycarbonylmethyl group was introduced by successive treatment of **51** with NaH, chloroacetic acid, and MeI to give **52** in 95% yield. Acid hydrolysis with subsequent selective benzoylation converted lactoside **52** into the selectively protected disaccharide alcohol **53**. Glycosylation of **53** by the galactosyl chloride **30**[12] in the presence of AgOTf and 2,4,6-trimethylpyridine (2,4,6-collidine) afforded trisaccharide **54** (67%), and deprotection of **54** by basic hydrolysis and hydrogenation gave the P^k analogue **12** (Scheme 6).

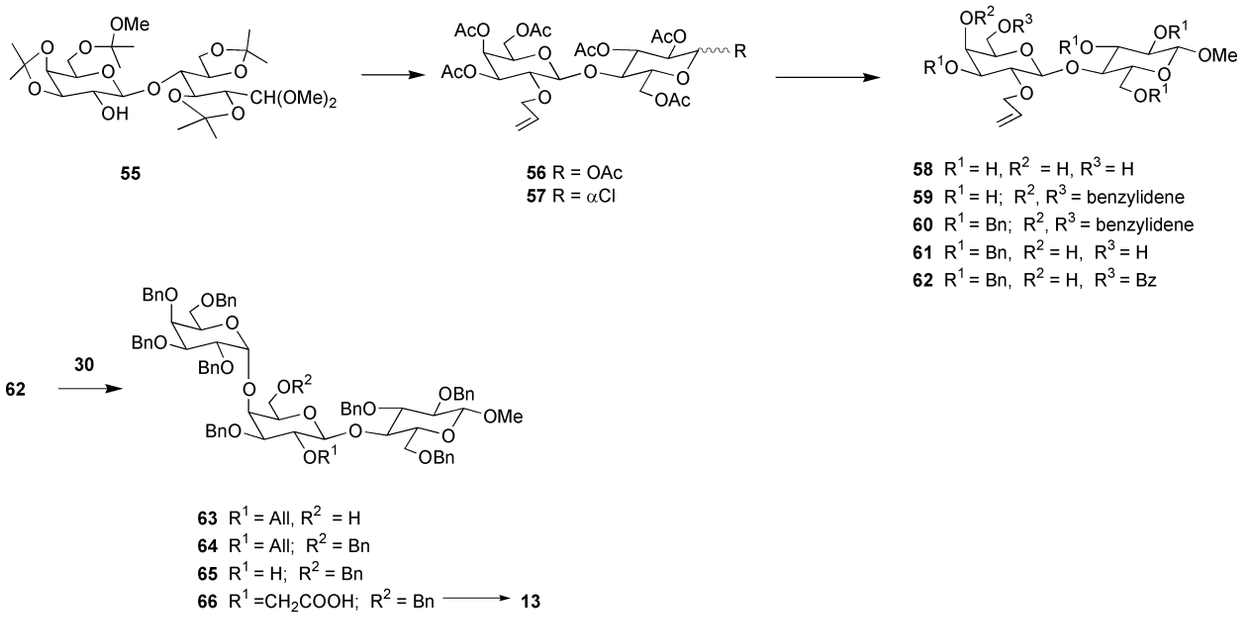
A general approach to the preparation of P^k-trisaccharide analogues modified at the C-2′ position of the central galactose residue was developed. A lactose acetonide derivative **55**[15] was allylated; the product was subjected to acid hydrolysis and acetylation to give a mixture of the anomeric heptaacetates **56**.



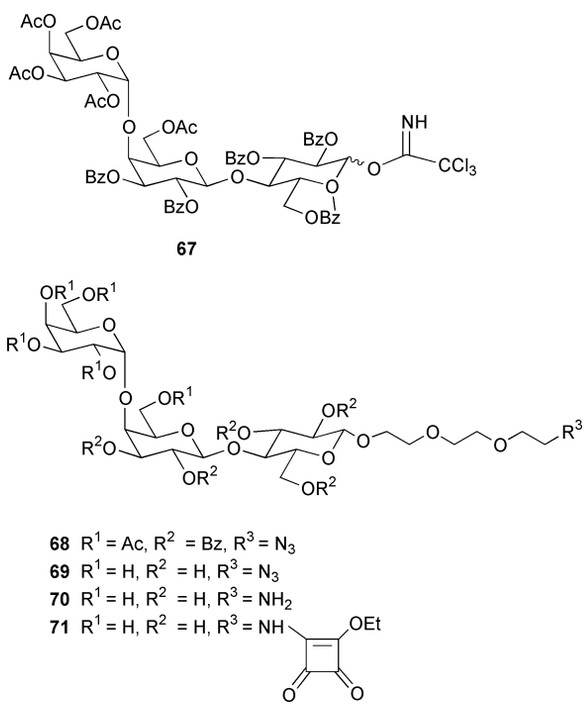
Scheme 6

Conversion of **56** into the lactosyl chloride **57** was achieved by treatment with dichloromethyl methyl ether in the presence of $ZnBr_2$. Glycosidation of chloride **57** with methanol with concurrent removal of acetates led to the methyl β-lactoside **58**. Reaction of the lactoside **58** with α,α-dimethoxytoluene gave the 4′,6′-*O*-benzylidene derivative **59**, which was perbenzylated to afford **60**. Acid hydrolysis of **60** followed by selective benzoylation of the primary hydroxy group of diol **61** with benzoyl chloride in pyridine afforded the glycosyl acceptor **62**. Glycosylation with chloride **30**[12] in toluene in the presence of AgOTf and a hindered pyridine base followed by saponification gave trisaccharide **63**. The trisaccharide **63** was benzylated to give the trisaccharide **64**, which is conveniently protected with temporary (allyl) and 'permanent' (benzyl) protecting groups. The allyl group was quantitatively removed upon treatment with $PdCl_2$, and the resulting alcohol **65** was alkylated with chloroacetic acid to furnish **66**. Hydrogenation of **66** completed the synthesis of the target 2′-*O*-carboxymethyl analogue **13** (Scheme 7).

In order to perform solid-phase competitive assays for evaluation of the synthetic galabiose and P^k-trisaccharide analogues **2**–**10**, **12**, and **13** a reporter molecule for monitoring SLT–oligosaccharide binding was required. Accordingly, a biotin-labelled P^k–bovine serum albumin (BSA) conjugate was synthesized. Imidate **67**[16] was glycosidated by 8-azido-3,6-dioxaoctan-1-ol[17] and the resulting glycoside **68** was transesterified to afford trisaccharide glycoside **69**. Reduction of the azido group afforded the ω-amino glycoside **70**, which was not isolated but allowed to react directly with excess of diethyl squarate at pH 8.[18] The activated compound **71** (Scheme 8) was coupled to BSA at pH 9,[18] and the product was derivatized with an *N*-hydroxysuccinimide–biotin derivative to provide the required biotinylated P^k–BSA glycoconjugate.



Scheme 7



Scheme 8

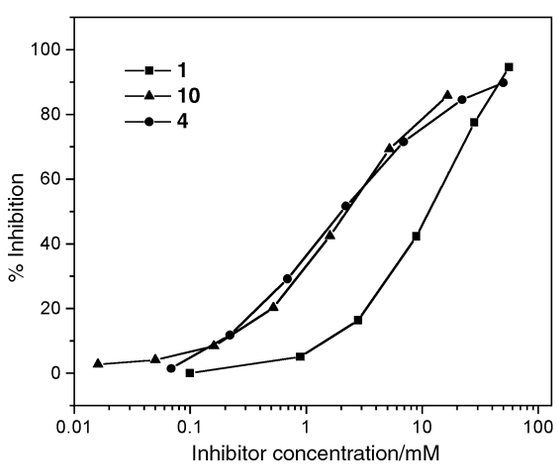


Fig. 1 Inhibition data for disaccharides **1**, **4** and **10** determined by solid-phase assay. Biotin-labelled P^k glycoconjugate binding to the B_5 subunit of verotoxin 1 absorbed to microtitre plates was inhibited by synthetic oligosaccharides.

Inhibition studies

Recombinant SLT B_5 homopentamer[19] was used to coat microtiter plates. The plates were incubated with a solution of a biotinylated P^k–BSA glycoconjugate in the presence of inhibitor, one of the modified analogues **2**–**10**, **12**, and **13**. Bound glycoconjugate was detected by a streptavidine–horseradish peroxidase conjugate after color reaction with 3,3′,5,5′-tetramethylbenzidine. Typical inhibition curves are shown in Fig. 1 and the results of inhibition studies are presented in Table 1.

Discussion

The protein–oligosaccharide contacts and the buried surface areas revealed for the three distinct trisaccharide binding sites in the crystal structure of the SLT-1 B_5 homopentamer complexed with a P^k-trisaccharide[4] suggest that the affinities of sites 1–3 for the P^k-trisaccharide should vary considerably. Controversy exits in the literature regarding the comparative significance of sites 1 and 2 in binding (see discussion in Ref. 4 and Ref. 5). While modelling studies correctly identified binding sites 1 and 2, they failed to identify site 3. Furthermore, docking studies[5] incorrectly predicted site 1, the cleft shaped site located between adjacent B subunits, as the highest affinity site. A second CBP, site 2, in which P^k-trisaccharide makes contacts within a single B subunit protein was predicted.[5] The position of site 2 corresponds topologically to the CBP found in the crystal structure of cholera toxin-GM_1 complex (PDB-2CHB).[2,4] Both sites 1 and 2 are found in the crystal structure of the verotoxin-P^k analogue,[4] in which carbohydrate bound to site 2 is involved in the most extensive network of hydrogen bonds and characterized by the best resolution and occupancy. This suggests that site 2 has the higher affinity, a conclusion that is also consistent with NMR studies.[20]

The design of SLT inhibitors was based on an examination of the crystal structure of the P^k-trisaccharide–SLT complex.[4] However, it has to be noted that interpretation of the inhibition assay could be complicated owing to both the presence of multiple non-equivalent CBPs on the B_5 homopentamer and multiple presentation of P^k-trisaccharide on the biotinylated reporting molecule, P^k–BSA glycoconjugate. It is not immediately clear whether observed IC_{50}-values would reflect the com-

Table 1 Inhibitory power of galabiose and P^k-trisaccharide analogues

Compound	Modification	IC_{50} (mM)
1[26]	None	30
2	2b,3b-Di-O-Me	110
3	3b-O-Me	410
4	2b-O-CH_2COOH	2
5	2b-O-CH_2CH_2COONa	1.6
6	2b-O-$CH_2CH_2CH_2COONa$	36
7	2b-O-$CH_2CH_2OSO_3Na$	3.6
8	2b-O-$CH_2CH_2OPO_3Na_2$	50
9	6b-Deoxy-6′-NH_2	100
10	6b-Deoxy-6′-NHAc	50
11[26]	None	2.1
12	2b-O-CH_2COOH	5.0
13	6a-O-CH_2COOH	4.5

bined effect of all 3 different binding sites or, most likely, only the interaction of inhibitors with the most avid CBP. Calorimetry,[6] NMR,[20] and mass spectrometry[21] data suggest the predominant importance of only one binding site, with the second site having an approximately 10-times weaker binding constant. This conclusion is corroborated by the verocell cytotoxicity of SLT-1 mutants, which can be correlated with specific amino acids and precise three-dimensional data from solved crystal structures of these recombinant proteins.[24]

When we started our study only a low-resolution crystal structure of the complex between SLT-1(B_5) and P^k-trisaccharide analogue was available, in which water molecules were not clearly defined. Later, when the refined structure was deposited in the Brookhaven Protein Data Bank (entry 1BOS) the role of water in binding of the sugar to protein could be better appreciated. Fig. 2 represents P^k/SLT-1 interactions, which were reconstructed by examination of the superimposed carbohydrate-binding domains corresponding to each binding site in the original crystal structure (entry 1BOS) and refined by comparison with high-resolution crystal structures of mutants with P^k-trisaccharide analogue (entries 1CQF, 1QOH, 2BOS, 4ULL, 1QNU).

It would originally appear that the hydroxy groups at C-2b and C-3b of the central β-galactose residue† are not involved in extensive networks of hydrogen bonds between carbohydrate and protein. However, blocking either one or both of them as methyl ethers leads to a substantial decrease in affinity for compounds **2** and **3**. This result is in accord with reported data on inhibition of SLT by deoxygenated galabiose analogues.[5] However, deoxygenation of C-2b had a greater effect than removal of the hydroxy at the C-3b position,[5] whereas the dimethylated galabioside **2** shows somewhat higher activity. The difference between alkylation and deoxygenation can only be explained if water-mediated hydrogen bonding is taken into account. The hydroxy group at C-3b forms a hydrogen bond with Gly 60 in site 1, whereas HO-2b is not involved in any direct interaction with the protein in any of the binding sites. In site 2, both HO-2b and HO-3b participate in a hydrogen bond with a water molecule H_2O-1, which can be also observed in the crystal structure of ligand-free protein (entry 1BOV).[22] We suggest that interaction with this water molecule constitutes the primary contribution of these two hydroxy groups for binding of galabiose disaccharide. The hydroxy group at C-3b donates while the hydroxy group at C-2b accepts an H-bond from this water molecule, which is firmly bound to the protein *via* a backbone N atom of Gly 62, as judged by the consistently high occupancy of this water molecule in the SLT-1 crystal structures both with and without bound sugar. Alkylation of HO-2b does not disrupt this interaction as can be observed in the crystal structure of the complex between SLT-1(B_5) and P^k-trisaccharide, which is derivatized at HO-2b (entry 1QNU).[23] Amino acid residue Gly 62 is known to be important for binding since the single mutation Gly 62/Thr 62 completely abolishes both binding to site 2 and toxicity,[24] due to steric clashes with the β-Gal moiety.

In this context, the results obtained for carboxyalkyl-galabiose analogues **4**–**8** are most interesting. Compounds **4**, **5**, and **7** show inhibitory power substantially higher than that of the unmodified galabioside **1**. The extended substituent at O-2b may replace a water molecule and provide a more entropically favorable framework for a new long-distance communication, not necessarily with the original partner. It is noteworthy that negatively charged species mediate an increase in affinity with the generally negatively charged protein surface of the receptor. It may be speculated that the anion-bearing substituents in compounds **4**, **5**, and **7** are able to span the distance of approximately 7.9 Å (site 1) or 7.2 Å (site 2) between O-2b and the imidazole ring of His 58 in order to establish a salt bridge. The efficiency of this interaction correlates with the length of the extension arm, thus, a spacer comprising two methylene groups is optimal for the distance, whereas a linker of three methylene groups is too long and the corresponding compound **6** does not differ in activity from that of unmodified galabioside. The lower activities of sulfate **7** and phosphate **8** may reflect the diminished hydrogen-bonding-ability of sulfates and phosphates compared with carboxylates.[25] Tentatively, the carboxymethyl and carboxyethyl derivatives represent a useful modification for developing better inhibitors with enhanced interactions with remote, positively charged side chains of the toxin.

Surprisingly, the affinity gain due to modification of the C-2b position in galabioside did not translate into increased activity at the trisaccharide level. In fact, the modified P^k-trisaccharide analogues **12** and **13** containing a carboxymethyl at the β-galactose C-2b position and at the C-6a position of glucose demonstrated slightly lower inhibitory power than did the unmodified P^k-glycoside. This despite the fact that the carboxymethyl substituents in both positions were expected to form a salt bridge with the neighboring His 58 side chain. This may be explained by considering complex intra- and inter-molecular interactions mediated by highly ordered water molecules. In site 2, the glucose moiety interacts with the protein mainly *via* a water-mediated H-bond between HO-3a and Asp 55 and an extensive network of H-bonds, in which HO-6a is involved. Ordered water molecules H_2O-2 and H_2O-3 also serve as conformational anchors that fix the mutual orientation of Gal and Glc in the lactose fragment, and their corresponding counterparts can be found in the first hydration sphere of the P^k-trisaccharide in sites 1 and 3. Although an alkyl substitution at HO-2b and HO-6a would not affect the important H-bond of O-2b with H_2O-1 in the case of the trisaccharide, they eliminate H_2O-3 and disrupt interglycosidic and glucose–protein water-mediated interactions. Apparently, the possible formation of a salt bridge with His 58 is unable to offset the combination of those unfavorable effects.

According to the crystal structure the HO-6b group of β-Gal is involved in a hydrogen bond with Asp 17 (carboxylate, site 1), Asn 55 (backbone nitrogen, site 2), Trp 34 (water-mediated, site 3) and Asn 32 (water-mediated, site 3). It was reasonable to expect that replacement of this sugar hydroxy group with an amino group would reinforce the interaction of the disaccharide with site 1 at the expense of electrostatic interactions, and, owing to the relative rotational freedom of the C5b–C6b bond, might also establish a salt bridge with the carboxy group of Asp 18 (site 3). On the other hand, this modification may disrupt binding with site 2 since the amino group, which is positively charged at physiological pH, is no longer able to accept a hydrogen bond from the amide nitrogen atom of Asn 55. The substantial decrease of inhibitory power that accompanies this modification (Table 1, compound **9**) strongly supports the

† The atoms associated with each of the sugar rings are labelled as shown in Fig 2: a, b and c refer respectively to β-Glu, β-Gal and α-Gal residues in the P^k-trisaccharide.

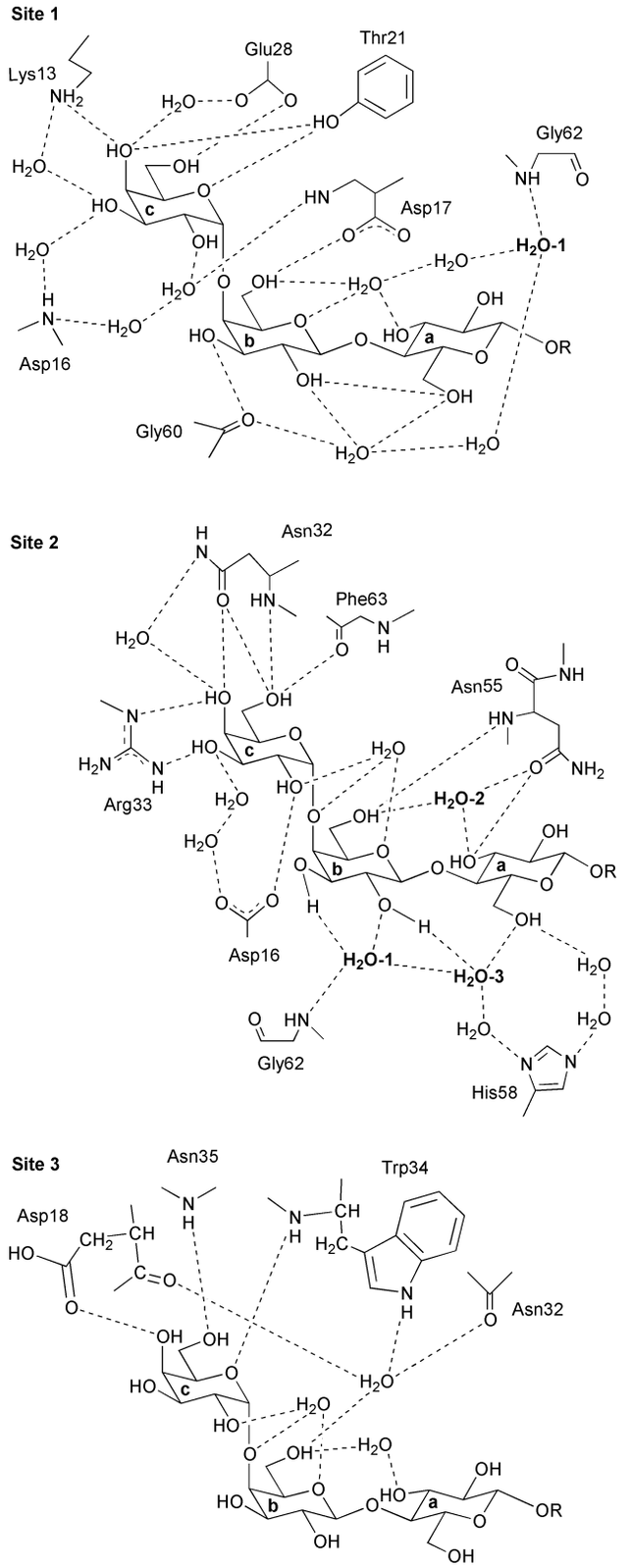


Fig. 2 Schematic representation of P^k-trisaccharide binding sites of the SLT-1(B_5) including water molecules, which are the best defined in the crystal structure. Dashed lines indicate putative hydrogen bonds.

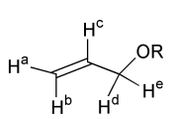
hypothesis that site 2 dominates solution binding. Since the P^k-trisaccharide binding constant for site 1 (the secondary binding site) was estimated to be only 15% of that of the dominant site any modification that compromises interaction with the most avid site 2 will result in a decreased overall activity. Acetylation restores the ability of the 6b-NH_2 towards hydrogen bonding, and the acetamido analogue **10** binds almost as tightly as the unmodified galabioside **1**. In contrast, deoxygenation of HO-6b of the β-Gal residue was shown to be the most detrimental for galabioside activity.

The most significant inferences to be drawn from the inhibition data presented here allowed us to identify a set of structural modifications on the central galactose residues of the P^k-trisaccharide that do not significantly change the ligand–protein interactions, but do influence the bound water molecules which are involved in the extended network of hydrogen bonds between the protein, water and ligand. For the galabiose disaccharide, substituents at O-2b of the β-galactose residue that direct a carboxylate group toward His 58 enhance binding. Extension of the substituent at this position avoids unfavorable contacts with the protein surface and although a carboxymethyl group at the corresponding O-2b position of the P^k-trisaccharide does not enhance binding in the expected manner, and produces a two-fold decrease of inhibitory power, this modification now offers the opportunity to tether two trisaccharides that could simultaneously occupy sites 1 and 2 within a single B subunit. Here, in contrast to most approaches toward oligovalent inhibitors, the optimum site for tethering ligands would not be the anomeric center of the terminal reducing sugar but rather the solvent-exposed HO-2b of the β-galactose residue.

Experimental

General methods

Optical rotations were measured on a Perkin-Elmer 241 polarimeter for samples in a 10 cm cell at ambient temperature (22 ± 2 °C). $[a]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical TLC was performed on silica gel 60-F_{254} (Merck) with detection by quenching of fluorescence and/or by charring with 10% H_2SO_4 in ethanol solution followed by heating at 180 °C. Column chromatography was performed on silica gel 60 (Merck, 40–60 μm), and solvents were distilled prior to use. Sep-Pak C_{18} cartridges (Waters) were conditioned prior to use by washing with methanol (10 mL) and water (20 mL). ^{1}H NMR spectra were recorded at 300 MHz (Varian Unity 300) in $CDCl_3$ (referenced to residual $CHCl_3$ at δ_H 7.24), CD_3OD (referenced to residual CD_2HOD at δ_H 3.3), or in D_2O (referenced to internal acetone at δ_H 2.225). *J*-Values are given in Hz. All commercial reagents were used as supplied; solvents were distilled from appropriate desiccants prior to use.[27] After extraction solutions in DCM were filtered through a cotton plug. Assignment of ^{1}H NMR signals for compounds containing an allyl group is as follows.



Methyl 6-*O*-benzyl-2,3-di-*O*-methyl-β-D-galactopyranoside 15

A mixture of **14**[7] (243 mg, 0.78 mmol), $BH_3{\cdot}NMe_3$ (343 mg, 4.7 mmol), and crushed mol. sieves (4Å; 1 g) in tetrahydrofuran (THF) (15 mL) was stirred for 30 min, then $AlCl_3$ (624 mg, 4.68 mmol) was added. After 3 h the mixture was filtered, diluted with ethyl acetate (50 mL), washed with aq. $NaHCO_3$, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (1 : 3) gave **15** (187 mg, 77%), $[a]_D$ −15.9 (*c* 0.2; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.33–7.27 (m, 5 H, arom), 4.58 (s, 2 H, CH_2Ph), 4.16 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.05 (ddd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0, $^2J_{4,OH}$ 2.7, H-4), 3.79 (dd, 1 H, $J_{6a,6b}$ 9.9, $J_{5,6a}$ 5.9, H-6a), 3.71 (dd, 1 H, H-6b), 3.55 (s, 3 H, OMe), 3.54 (m, 1 H, H-5), 3.52 (s, 3 H, OMe), 3.47 (s, 3 H, OMe), 3.24 (dd, 1 H, $J_{2,3}$ 9.3, H-2), 3.14 (dd, 1 H, H-3), 2.40 (dd, 1 H, $J_{5,OH}$ 0.8, OH) (Found: C, 61.3; H, 7.8. Calc. for $C_{16}H_{24}O_6$: C, 61.5; H, 7.7%).

Methyl 6-*O*-benzyl-2,3-di-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 17

A solution of **15** (165 mg. 0.53 mmol) and ethyl 2,3,4,6-tetra-*O*-

benzyl-1-thio-β-D-galactopyranoside[8] **16** (372 mg, 0.63 mmol) in DCM (5 mL) was stirred for 1 h in the presence of crushed mol. sieves (4Å; 0.5 g), then cooled at 0 °C and a mixture of NIS (171 mg, 0.76 mmol) and AgOTf (195 mg, 0.76 mmol) was added under argon. After 10 min the mixture was diluted with DCM, filtered through Celite, washed with saturated aq. $Na_2S_2O_3$ (Note: DCM is upper layer!) and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (3 : 1) gave **17** (304 mg, 69%), $[\alpha]_D$ +38.1 (*c* 0.8; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.34–7.15 (m, 25 H, arom), 4.96 (d, 1 H, $J_{1',2'}$ 2.6, H-1′), 4.93 (d, 1 H, ^2J 11.3, CH_2Ph), 4.89 (d, 1 H, ^2J 11.8, CH_2Ph), 4.75 (s, 2 H, CH_2Ph), 4.67 (d, 1 H, ^2J 11.8, CH_2Ph), 4.58 (d, 1 H, ^2J 11.3, CH_2Ph), 4.48–4.39 (m, 3 H, H-4′, CH_2Ph), 4.27 and 4.21 (2 d, 2 H, ^2J 11.8, CH_2Ph), 4.14 (d, 1 H, $J_{1,2}$ 7.5, H-1), 4.12–4.08 (m, 2 H, H-2′, H-3′), 4.04 (d, 1 H, $J_{3,4}$ 3.0, H-4), 3.97 (dd, 1 H, $J_{5,6a}$ 7.2, $J_{5,6b}$ 8.6, H-5), 3.66 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 8.8$, H-5′), 3.57 (s, 3 H, OMe), 3.53 (s, 3 H, OMe), 3.55–3.44 (m, 4 H, H-6a, H-6b, H-6′a, H-6′b), 3.33 (s, 3 H, OMe), 3.26 (dd, 1 H, $J_{2,3}$ 9.9, H-2), 3.04 (dd, 1 H, H-3) (Found: C, 72.1; H, 6.7. Calc. for $C_{50}H_{58}O_{11}$: C, 71.9; H, 7.0%).

Methyl 4-*O*-(α-D-galactopyranosyl)-2,3-di-*O*-methyl-β-D-galactopyranoside 2

A suspension of Pd/C (100 mg) in a solution of **17** (190 mg, 0.23 mmol) in acetic acid (5 mL) was stirred under H_2 for 24 h, then the catalyst was filtered off, and the mixture was concentrated, and chromatographed on silica gel with DCM–methanol (10 : 1 to 5 : 1) to give **2** (72 mg, 82%), $[\alpha]_D$ +108.1 (*c* 0.3, $CHCl_3$); ^{1}H NMR (D_2O) δ_H 4.95 (d, 1 H, $J_{1',2'}$ 3.8, H-1′), 4.45 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.29 (d, 1 H, $J_{3,4}$ 2.8, H-4), 4.24 (br t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6.7$, H-5), 4.06 (dd, 1 H, $J_{3',4'}$ 3.2, $J_{4',5'}$ 0.86, H-4′), 3.95–3.66 (m, 7 H, H-6a, H-6b, H-2′, H-3′, H-5, H-6′a, H-6′b), 3.59, 3.55 and 3.48 (3 s, 9 H, OMe), 3.42 (dd, 1 H, $J_{2,3}$ 10.3, H-3), 3.31 (dd, 1 H, H-2); *m*/*z* (EI) 407.1521 (MNa^+. $C_{15}H_{28}O_{11}$·Na requires *m*/*z*, 407.1529).

Methyl 3-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranoside 19

Benzoyl chloride (0.2 mL, 1.7 mmol) was added to a solution of methyl 2,6-di-*O*-benzyl-β-D-galactopyranoside[9] **18** (505 mg, 1.35 mmol) in pyridine (10 mL) at 0 °C. After 0.5 h water (2 mL) was added, and the mixture was concentrated, co-evaporated with toluene, and chromatographed on silica gel with pentane–ethyl acetate (1 : 1) to give **19** (450 mg, 69%), $[\alpha]_D$ +68.9 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.00–7.97 (m, 2 H, arom), 7.57–7.13 (m, 13 H, arom), 5.12 (dd, 1 H, $J_{3,4}$ 3.2, $J_{2,3}$ 10.0, H-3), 4.83 (d, 1 H, ^2J 11.6, CH_2Ph), 4.67 (d, 1 H, ^2J 11.6, CH_2Ph), 4.58 (d, 1 H, ^2J 12.1, CH_2Ph), 4.52 (d, 1 H, ^3J 12.1, CH_2Ph), 4.42 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.24 (d, 1 H, H-4), 3.87 (dd, 1 H, H-2), 3.80–3.70 (m, 3 H, H-5, H-6a, H-6b), 3.60 (s, 3 H, OMe) (Found: C, 70.4; H, 6.2. Calc. for $C_{28}H_{30}O_7$: C, 70.3; H, 6.3%).

Methyl 3-*O*-benzoyl-2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 20

A mixture of **19** (3.91 g, 8.17 mmol), ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[8] **16** (5.25 g, 8.98 mmol), and crushed mol. sieves (4 Å; 6 g) in DCM (50 mL) was stirred under an argon atmosphere for 1 h, then a mixture of NIS (2.6 g, 11.5 mmol) and AgOTf (0.42 g, 1.6 mmol) was added by portions. The mixture was filtered through Celite, supernatant was washed successively with aq. $Na_2S_2O_3$ and water, and concentrated. Chromatography of the residue on silica gel with toluene–ethyl acetate (95 : 5 to 90 : 10) afforded **20** (6.1 g, 74%), $[\alpha]_D$ +76.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.07–8.05 (m, 2 H, arom), 7.38–7.12 (m, 33 H, arom), 5.10 (dd, 1 H, $J_{3,4}$ 2.9, $J_{2,3}$ 10.3, H-3), 4.89–4.85 (m, 2 H, H-1′, CH_2Ph), 4.81–4.69 (m, 4 H, CH_2Ph), 4.59 (d, 2H, ^2J 11.4, CH_2Ph), 4.58 (d, 1 H, ^2J 11.7, CH_2Ph), 4.40 (d, 1 H, $J_{1,2}$ 7.5, H-1), 4.30 (s, 2 H, CH_2Ph), 4.25–4.21 (m, 2 H, H-4, H-6′a), 4.11 (s, 2 H, CH_2Ph), 4.05–3.95 (m, 3 H, H-2′, H-3′, -4′), 3.87 (dd, 1 H, $J_{5,6a}$ 5.1, $J_{6a,6b}$ 9.9, H-6a), 3.81 (dd, 1 H, H-2), 3.98 (br t, 1 H, H-5), 3.69 (dd, 1 H, $J_{5,6b}$ 6.5, H-6b), 3.61 (s, 3 H, OMe), 3.39 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 8.4$, H-5′), 3.08 (dd, 1 H, $J_{6'a,6'b}$ 5.5, H-6′b) (Found: C, 74.5; H, 6.3. Calc. for $C_{62}H_{64}O_{12}$: C, 74.4; H, 6.4%).

Methyl 2,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 21

A solution of **20** (4.55 g, 4.54 mmol) and NaOMe (100 mg) in a mixture of methanol (20 mL) and DCM (8 mL) was stored for 24 h at 50 °C. The mixture was neutralized with Dowex 50W (H^+), filtered and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (3 : 1) gave **21** (3.99 g, 98%), $[\alpha]_D$ +93.3 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.37–7.19 (m, 30 H, arom), 4.99 (d, 1 H, $J_{1',2'}$ 3.5, H-1′), 4.89 (d, 1 H, ^2J 11.6, CH_2Ph), 4.84–4.71 (m, 5 H, CH_2Ph), 4.64 (d, 1 H, ^2J 11.8, CH_2Ph), 4.52 (d, 1 H, ^2J 11.6, CH_2Ph), 4.42 and 4.35 (2 d, 2 H, ^2J 12.4, CH_2Ph), 4.30 (s, 2 H, CH_2Ph), 4.25 (d, 1 H, $J_{1,2}$ 7.5, H-1), 4.23 (br t, 1 H, H-5′), 4.07 (dd, 1 H, $J_{2',3'}$ 10.2, H-2′), 4.00 (dd, 1 H, $J_{3',4'}$ 2.6, H-3′), 3.90–3.83 (m, 3 H, H-4, H-4′, H-6′a), 3.78–3.65 (m, 3 H, H-2, H-3, OH), 3.60 (td, 1 H, $J_{4,5}$ 2.9, H-5), 3.56 (s, 3 H, OMe), 3.46 (dd, 1 H, $J_{5,6a}$ 9.5, $^2J_{6a,6b}$ 7.5, H-6a), 3.37 (dd, 1 H, $J_{5,6b}$ 9.9, H-6b), 3.30 (dd, 1 H, $J_{5',6'b}$ 9.5, $^2J_{6'a,6'b}$ 4.9, H-6′b) (Found: C, 73.7; H, 6.8. Calc. for $C_{55}H_{60}O_{11}$: C, 73.6; H, 6.7%).

Methyl 2,6-di-*O*-benzyl-3-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 22

To a solution of **21** (209 mg, 0.23 mmol) in *N*,*N*-dimethylformamide (DMF) (2 mL) was added 80% NaH (42 mg, 1.4 mmol). After 30 min of stirring, MeI (0.3 mL) was added. The reaction mixture was quenched by the addition of methanol after 1 h, diluted with water, and extracted with DCM. The extract was concentrated, and chromatographed on silica gel with pentane–ethyl acetate (4 : 1) to give **22** (197 mg, 92%), $[\alpha]_D$ +36 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.37–7.16 (m, 30 H, arom), 4.97 (d, 1 H, $J_{1',2'}$ 3.4, H-1′), 4.95 (d, 1 H, ^3J 11.3, CH_2Ph), 4.89 (d, 1 H, ^3J 11.9, CH_2Ph), 4.83 (d, 1 H, ^3J 11.4, CH_2Ph), 4.76–4.73 (m, 3 H, CH_2Ph), 4.68 (d, 1 H, ^3J 11.9, CH_2Ph), 4.60 (d, 1 H, ^3J 11.3, CH_2Ph), 4.48 (d, 1 H, ^3J 11.9, CH_2Ph), 4.44 (m, 1 H, H-5′), 4.43 (d, 1 H, ^3J 11.9, CH_2Ph), 4.27 (d, 1 H, ^3J 11.9, CH_2Ph), 4.24 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.23 (d, 1 H, ^3J 11.9, CH_2Ph), 4.32 (br s, 1 H, H-4′), 4.10 (dd, 1 H, $J_{2',3'}$ 10.3, H-2′), 4.05–3.94 (m, 3 H, H-4, H-3′, H-6′a), 3.66 (t, 1 H, $J_{5,6a} \approx J_{5,6b} \approx 8.8$, H-5), 3.57–3.45 (m, 7 H, OMe, H-2, H-6′b, H-6a, H-6b), 3.35 (s, 3 H, OMe), 3.14 (dd, 1 H, $J_{2,3}$ 9.9, H-3) (Found: C, 73.9; H, 7.0. Calc. for $C_{56}H_{62}O_{11}$: C, 73.8; H, 6.9%).

Methyl 4-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-β-D-galactopyranoside 3

A suspension of Pd/C (80 mg) in a solution of **22** (183 mg, 0.2 mmol) in acetic acid (4 mL) was stirred under a flow of H_2 for 4 h, then filtered and concentrated. A solution of the residue in water (5 mL) was passed through a Sep-Pak C_{18} cartridge. Concentration of the solution afforded **3** (59 mg, 79%), $[\alpha]_D$ +108.8 (*c* 0.6, H_2O); ^{1}H NMR (D_2O) δ_H 4.95 (d, 1 H, $J_{1',2'}$ 3.8, H-1′), 4.38 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.29 (d, 1 H, $J_{3,4}$ 2.9, H-4), 4.25 (ddd, ≈ 1H, $J_{4',5'}$ 1.1, $J_{5',6'a}$ 7.6, $J_{5',6'b}$ 5.5, H-5′), 4.06 (dd, 1 H, $J_{3',4'}$ 3.4, H-4′), 3.93 (dd, 1 H, $J_{5,6a}$ 7.8, $J_{6a,6b}$ 11.5, H-6a), 3.91 (dd, 1 H, $J_{2',3'}$ 10.7, H-3′), 3.85 (dd, 1 H, $J_{5,6b}$ 5.2, H-6b), 3.81 (dd, 1 H, H-2′), 3.77 (dd, 1 H, $J_{6'a,6'b}$ 11.3, H-6′a), 3.73 (dd, 1 H, H-5), 3.70 (dd, 1 H, H-6′b), 3.585 (s, 3 H, OMe), 3.58 (dd, 1 H, $J_{2,3}$ 10.2, H-2), 3.50 (s, 3 H, OMe), 3.36 (dd, 1 H, H-3); *m*/*z* (EI) 393.1374 (MNa^+. $C_{14}H_{26}O_{11}$·Na requires *m*/*z*, 393.1373).

Methyl 2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside 24

A mixture of methyl 3,4-*O*-isopropylidene-6-*O*-(1-methoxy-1-methylethyl)-β-D-galactopyranoside[11] **23** (1.52 g, 4.96 mmol) and 80% NaH (360 mg, 12 mmol) in DMF (10 mL) was stirred for 0.5 h, then $ClCH_2COOH$ (470 mg, 4.97 mmol) was added. After 1 h, MeI (1 mL, 16 mmol) was added, and the mixture was stirred overnight then concentrated, diluted with water (50 mL), and extracted with ethyl acetate (6 × 30 mL). The combined organic fractions were concentrated, and co-evaporated with toluene. To a solution of the residue in methanol (5 mL) were added 2,2-dimethoxypropane (5 mL, 40.6 mmol) and toluene-*p*-sulfonic acid (PTSA) (30 mg). The mixture was stirred for 2 days, then concentrated. A solution of the residue in 80% aq. acetic acid (10 mL) was stored for 3 h at 50 °C then concentrated. Chromatography of the residue on silica gel with DCM–methanol (20 : 1) afforded **24** (794 mg, 60%), $[\alpha]_D$ +34.5 (*c* 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 5.04 (s, 1 H, OH), 4.38 (d, 1 H, ^2J 17.8, OCH_2CO$_2$Me), 4.30 (d, 1 H, OCH_2CO$_2$Me), 4.22 (d, 1 H, $J_{1,2}$ 7.7, H-1), 3.97 (d, 1 H, $J_{3,4}$ 3.4, H-4), 3.96 (m, 1 H, H-5), 3.83 (ddd, 1 H, $J_{5,6a}$ 4.7, $J_{6a,OH}$ 8.2, $J_{6a,6b}$ 12.6, H-6a), 3.75 (s, 3 H, OMe), 3.64 (dd, 1 H, $J_{2,3}$ 9.4, H-3), 3.51 (s, 3 H, OMe), 3.51 (m, 1 H, H-6b), 3.29 (dd, 1 H, H-2), 2.98 (s, 1 H, OH), 2.49 (dd, 1 H, $J_{5,6\text{-OH}}$ 3.9, 6-OH); ^{1}H NMR (CD_3OD) δ_H 4.33 (s, 2 H, CH_2CO$_2$Me), 4.23 (d, 1 H, $J_{1,2}$ 7.7, H-1), 3.84 (dd, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ 0.8, H-4), 3.74 (s, 3 H, OMe), 3.74–3.68 (m, 2 H, H-6a, H-6b), 3.58 (dd, 1 H, $J_{2,3}$ 9.6, H-3), 3.48 (td, 1 H, $J_{5,6a} \approx J_{5,6b} \approx 5.5$, H-5), 3.47 (s, 3 H, OMe) (Found: C, 45.1; H, 6.9. Calc. for $C_{10}H_{18}O_8$: C, 45.1; H, 6.8%).

Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside 25

A mixture of **24** (767 mg, 2.88 mmol) of α,α-dimethoxytoluene (0.56 mL, 3.73 mmol) and PTSA (20 mg) in acetonitrile (20 mL) was stored for 1 h, then Et_3N (0.3 mL) was added, and the mixture was concentrated and dried. To a solution of the residue in pyridine (10 mL) was added benzoyl chloride (0.8 mL, 6.9 mmol). After 1 h the reaction mixture was quenched with water, concentrated, co-evaporated with toluene, and chromatographed on silica gel with pentane–ethyl acetate (2 : 1 to 3 : 2) to give **25** (1.086 g, 82%), $[\alpha]_D$ +84.1 (*c* 0.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.08–8.06 (m, 2 H, arom), 7.55–7.29 (m, 8 H, arom), 5.49 (s, 1 H, C*H*Ph), 5.19 (dd, 1 H, $J_{3,4}$ 3.7, $J_{2,3}$ 10, H-3), 4.49 (dd, 1 H, $J_{4,5}$ 0.5, H-4), 4.45 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.40–4.30 (m, 4 H, H-5, H-6a, CH_2COOMe), 4.07 (dd, 1 H, $J_{5,6b}$ 1.7, $J_{6b,6a}$ 12.4, H-6b), 3.87 (dd, 1 H, H-2), 3.55 (s, 3 H, OMe), 3.53 (s, 3 H, OMe) (Found: C, 62.9; H, 5.9. Calc. for $C_{24}H_{26}O_9$: C, 62.9; H, 5.7%).

Methyl 3,6-di-*O*-benzoyl-2-*O*-methoxycarbonylmethyl-β-D-galactopyranoside 26

A solution of **25** (1.086 g, 2.37 mmol) in 80% aq. acetic acid (15 mL) was stored for 10 h at 60 °C, then concentrated and dried. To a solution of the residue in pyridine (10 mL) was added benzoyl chloride (0.375 mL, 3.23 mmol). After 1 h the reaction mixture was quenched by the addition of water, concentrated, and chromatographed on silica gel with pentane–ethyl acetate (2 : 1 to 3 : 2) to give **26** (709 mg, 63%), $[\alpha]_D$ +6.0 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.10–8.00 (m, 4 H, arom), 7.58–7.40 (m, 6 H, arom), 5.20 (dd, 1 H, $J_{3,4}$ 3.25, $J_{2,3}$ 10.0, H-3), 4.61 (dd, 1 H, $J_{5,6a}$ 6.9, $J_{6a,6b}$ 11.4, H-6a), 4.52 (dd, 1 H, $J_{5,6b}$ 6.3, H-6b), 4.45 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.33 (s, 2 H, CH_2COOMe), 4.21 (ddd, 1 H, $J_{4,OH}$ 5.2, $J_{4,5}$ 0.5, H-4), 3.92 (br t, 1 H, H-5), 3.79 (dd, 1 H, H-2), 3.55 (s, 3 H, OMe), 3.53 (s, 3 H, OMe), 2.34 (d, 1 H, OH) (Found: C, 60.2; H, 5.5. Calc. for $C_{24}H_{26}O_{10}$: C, 60.7; H, 5.5%).

Methyl 3,6-di-*O*-benzoyl-2-*O*-methoxycarbonylmethyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 27

A suspension of crushed mol. sieves (4 Å; 1 g), **26** (684 mg, 1.44 mmol) and **16**[8] (930 mg, 1.58 mmol) in DCM (20 mL) was stirred for 1 h, then a mixture of NIS (460 mg, 2.04 mmol) and AgOTf (74 mg, 0.28 mmol) was added under an argon atmosphere at 10 °C. After 30 min the solution was filtered through Celite, washed with aq. $Na_2S_2O_3$, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (7 : 3 to 1 : 1) afforded **27** (950 mg, 66%), contaminated with ≈ 5% of an unidentified monosaccharide impurity which could not be removed by several chromatographic separations.

Methyl 2-*O*-carboxymethyl-4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside 4

A suspension of Pd/C (400 mg) and **27** (521 mg, 0.52 mmol) in acetic acid (10 mL) was stirred for 2 days under H_2 flow, then filtered and concentrated. To a solution of the residue in methanol (10 mL) was added NaOH (0.6 g). After 6 h the mixture was neutralized with Dowex 50W (H^+) resin, filtered and concentrated. Chromatography of the residue on silica gel with DCM–MeOH–HOAc (70 : 30 : 1 to 60 : 40 : 1) afforded **4** (151 mg, 70%), $[\alpha]_D$ +67.0 (*c* 0.3, H_2O); ^{1}H NMR (D_2O) δ_H 5.16 (d, 1 H, $J_{1',2'}$ 3.8, H-1′), 4.69 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.59 (broad t, ≈ 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6.6$, H-5′), 4.42 (d, 1 H, ^2J 16.4, CH_2-COOH), 4.34 (d, 1 H, CH_2COOH), 4.28 (dd, 1 H, $J_{4,5} < 0.5$, $J_{3,4}$ 2.5, H-4), 4.26 (d, 1 H, $J_{3',4'}$ 3.1, H-4′), 4.16–3.95 (m, 5 H, H-3, H-3′, H-5′, H-6a, H-6b), 3.92 (d, 2 H, H-6′a, H-6′b), 3.79 (s, 3 H, OMe), 3.60 (dd, 1 H, $J_{2,3}$ 10.1, H-2); *m/z* (EI) 437.1270 (MNa^+. $C_{15}H_{26}O_{13}$•Na requires *m/z*, 437.1271).

Methyl 2-*O*-allyl-3,6-di-*O*-benzoyl-β-D-galactopyranoside 29

To a solution of **28**[11] (1.66 g, 7.08 mmol) in pyridine (40 mL) was added benzoyl chloride (1.65 mL, 14.2 mmol) at 0 °C. After 1 h the reaction mixture was quenched by the addition of methanol, concentrated, co-evaporated with toluene, the residue was dissolved in DCM, the solution washed with water and concentrated, and the product crystallized from ethyl acetate. Mother liquids were chromatographed on silica gel with pentane–ethyl acetate (8 : 2). The combined yield of **29** was 2.22 g (71%), $[\alpha]_D$ +5.8 (*c* 1.5; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.09–8.02 (m, 4 H, arom), 7.61–7.42 (m, 6 H, arom), 5.78 (1 H, H^c), 5.20 (1 H, H^b), 5.14 (dd, 1 H, $J_{3,4}$ 3.2, $J_{2,3}$ 10.0, H-3), 5.07 (1 H, H^a), 4.64 (dd, 1 H, $J_{5,6a}$ 6.8, $J_{6a,6b}$ 11.3, H-6a), 4.54 (dd, 1 H, $J_{5,6b}$ 6.3, H-6b), 4.42 (d, 1 H, $J_{1,2}$ 7.2, H-1), 4.32 (1 H, H^d), 4.21 (dd, 1 H, $J_{4,OH}$ 5.5, H-4), 4.15 (1 H, H^e), 3.94 (t, 1 H, H-5), 3.78 (dd, 1 H, H-2), 3.59 (s, 3 H, Me), 2.35 (d, 1 H, 4-OH) (Found: C, 65.1; H, 5.8. Calc. for $C_{24}H_{26}O_8$: C, 65.15; H, 5.9%).

Methyl 2-*O*-allyl-3,6-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 31

To a solution of **29** (2.73 g, 6.17 mmol), AgOTf (2.36 g, 9.18 mmol), and 2,4,6-collidine (1.1 mL, 8.34 mmol) in toluene (130 mL) was added a solution of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[12] **30** (4.66 g, 8.34 mmol) in toluene (15 mL). After 1 h the precipitate was filtered off and the solution was concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (8 : 2) gave **31** (5.07 g, 85%), $[\alpha]_D$ +33.6 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.1–7.99 (m, 4 H, arom), 7.45–7.10 (m, 26 H, arom), 5.75 (1 H, H^c), 5.13–4.97 (m, 3 H, H-3, H^b, H^a), 4.90–4.61 (m, 8 H, H-1′, H-6a, H-6b, CH_2Ph), 4.49 (d, 1 H, ^3J 11.2, CH_2Ph), 4.37 (d, 1 H, $J_{1,2}$ 7.5, H-1), 4.30–4.22 (m, 3 H, H-5′, H^d, H^e), 4.19–3.99 (m, 6 H, H-2′, H-3′, H-4′, H-4, CH_2Ph), 3.93 (t, 1 H, $J_{5,6a} \approx J_{5,6b} \approx 6.5$, H-5), 3.72 (dd, 1 H, $J_{2,3}$ 10.2, H-2), 3.58 (s, 3 H, Me), 3.39 (t, 1 H, $J_{5',6'a} \approx J_{6'a,6'b} \approx 8.4$, H-6′a), 3.07 (dd, 1 H, $J_{5',6'b}$ 5.4, H-6′b) (Found: C, 72.6; H, 6.3. Calc. for $C_{58}H_{60}O_{13}$: C, 72.2; H, 6.3%).

Methyl 2-*O*-allyl-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 32

A solution of **31** (1.2 g, 1.24 mmol) and MeONa (30 mg) in

methanol (5 mL) was stirred for 3 days, then neutralized by Dowex 50W (H^+), and concentrated. A solution of the residue in 5% of methanol in DCM was passed through a layer of silica gel. Appropriate fractions were collected and concentrated. To a solution of the residue in DMF (5 mL) were added NaH (90%; 132 mg, 4.95 mmol) and benzyl bromide (353 μL, 3.03 mmol). After 1 h the reaction mixture was quenched by methanol, diluted with ethyl acetate, washed with water, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (8 : 2) gave **32** (1.056 g, 86%), $[\alpha]_D$ +40.8 (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.37–7.15 (m, 30 H, arom), 5.94 (1 H, H^c), 5.26 (1 H, H^b), 5.12 (1 H, H^a), 5.00 (s, 1 H, H-1′), 4.87 (t, 2 H, ^2J 11.6, CH_2Ph), 4.76 (d, 1 H, ^2J 12.8, CH_2Ph), 4.74 (s, 2 H, CH_2Ph), 4.66 (d, 1 H, ^2J 11.9, CH_2Ph), 4.52 (d, 1 H, ^2J 12.8, CH_2Ph), 4.52 (d, 1 H, ^2J 11.3, CH_2Ph), 4.38 (dd, 1 H, $J_{5',6'b}$ 4.9, $J_{5'6'a}$ 9.1, H-5′), 4.33 (1 H, H^d), 4.24–4.19 (m, 5 H, H^e, CH_2Ph), 4.16 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.14–4.06 (m, 3 H, H-2′, H-3′, H-4′), 3.97 (d, 1 H, $J_{3,4}$ 2.9, H-4), 3.95 (dd, 1 H, $J_{5,6a}$ 7.3, $J_{6a,6b}$ 9.5, H-6a), 3.54–3.50 (m, 6 H, H-2, H-6b, H-6′a, Me), 3.45 (t, 1 H, H-5), 3.31 (dd, 1 H, $J_{2,3}$ 9.9, H-3), 3.20 (dd, 1 H, $J_{6'a,6'b}$ 8.4, H-6′b) (Found: C, 73.7; H, 7.1. Calc. for $C_{58}H_{64}O_{11}$·0.5H_2O: C, 73.6; H, 6.9%).

Methyl 3,6-di-*O*-benzyl-2-*O*-(3-hydroxypropyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 33

To a solution of **32** (863 mg, 0.92 mmol) in THF (5 mL) was added 9-BBN (0.5 M; 3.7 mL). The mixture was refluxed for 2 h, then 1 mL of 3 M NaOH and 2 mL of 30% H_2O_2 were added. After 0.5 h of stirring the solution was poured into water extracted with ethyl acetate, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (1 : 1) gave **33** (502 mg, 57%), $[\alpha]_D$ +41.4 (*c* 1.0; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.35–7.10 (m, 30 H, arom), 5.02 (s, 1 H, H-1′), 4.89 (d, 1 H, ^2J 11.2, CH_2Ph), 4.86 (d, 1 H, ^2J 11.7, CH_2Ph), 4.76 (d, 1 H, ^2J 12.5, CH_2Ph), 4.76 (s, 2 H, CH_2Ph), 4.67 (d, 1 H, ^3J 12.5, CH_2Ph), 4.53 (d, 1 H, ^3J 11.2, CH_2Ph), 4.51 (d, 1 H, ^3J 12.7, CH_2Ph), 4.37 (dd, 1 H, $J_{5',6'a}$ 9.0, $J_{5',6'b}$ 4.8, H-5′), 4.26 (d, 1 H, ^3J 11.8, CH_2Ph), 4.21 (d, 1 H, ^3J 11.8, CH_2Ph), 4.16–4.09 (m, 6 H, H-1, H-2′, H-3′, H-4′, CH_2Ph), 4.00–3.86 (m, 4 H, H-4, H-6a, $CH_2CH_2CH_2OH$), 3.74 (t, 2 H, ^2J 5.6, $CH_2CH_2CH_2OH$), 3.58–3.45 (m, 7 H, H-2, H-5, H-6b, H-6′a, Me), 3.29 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 2.7, H-3), 3.24 (dd, 1 H, $J_{6'a,6'b}$ 8.4, H-6b), 2.46 (br s, 1 H, OH), 1.79 (m, 2 H, CH_2CH_2-CH_2OH) (Found: C, 73.05; H, 6.9. Calc. for $C_{58}H_{66}O_{12}$: C, 72.9; H, 7.0%).

Methyl 3,6-di-*O*-benzyl-2-*O*-(2-hydroxyethyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 34

From 32. Ozone was passed through a solution of the allyl ether **32** (301 mg, 0.321 mmol) in DCM (3 mL)–methanol (5 mL) for 3 min at −30 °C, then $NaBH_4$ (100 mg) was added. After 0.5 h of stirring the mixture was concentrated, diluted with DCM, washed successively with 1 M HCl, water, and aq. $NaHCO_3$, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (3 : 2) gave **34** (185 mg, 61%).

From 32 *via* 35. A mixture of **32** (1.092 g, 1.12 mmol), 4-methylmorpholine *N*-oxide (327 mg), and OsO_4 (10 mg) in acetone (20 mL) was stirred overnight, then diluted with water, extracted with DCM, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (4 : 6) gave 1.05 mg (96%) of methyl 3,6-di-*O*-benzyl-2-*O*-[(*R*/*S*)-2,3-dihydroxypropyl)]-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galacto-pyranosyl)-β-D-galactopyranoside **35** (Found: C, 71.81; H, 7.02. Calc. for $C_{58}H_{66}O_{13}$: C, 71.73; H, 6.85%).

To a solution of **35** (1.84 g, 18.9 mmol) in THF (10 mL)–water (6 mL) was added $NaIO_4$ (607 mg, 1.5 eq.). The mixture was stirred for 1 h, then was partitioned between DCM and brine, and the organic layer was concentrated. To a solution of the residue in THF (10 mL) were added $NaBH_4$ (80 mg) and water (0.3 mL). After 10 min the mixture was diluted with DCM (50 mL), washed with brine, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (6 : 4 to 1 : 1) furnished **34** (1.45 g, 82%), $[\alpha]_D$ +41.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.35–7.17 (m, 30 H, arom), 5.29 (br d, 1 H, $J_{1,2}$ 1.8, H-1), 4.89 (d, 1 H, ^3J 11.2, CH_2Ph), 4.88 (d, 1 H, ^2J 11.9, CH_2Ph), 4.77 (d, 1 H, ^2J 12.4, CH_2Ph), 4.75 (s, 2 H, CH_2Ph), 4.67 (d, 1 H, ^2J 12.4, CH_2Ph), 4.54 (d, 1 H, ^2J 11.2, CH_2Ph), 4.48 (d, 1 H, ^2J 11.9, CH_2Ph), 4.34 (dd, 1 H, $J_{5',6'a}$ 8.5, $J_{5',6'b}$ 4.8, H-5′), 4.27–4.09 (m, 8 H, H-1, H-2′, H-3′, H-4′, CH_2Ph), 4.01 (d, 1 H, $J_{3,4}$ 2.8, H-4), 3.98 (dd, 1 H, $J_{5,6a}$ 7.1, $J_{6a,6b}$ 9.4, H-6a), 3.87–3.83 (m, 2 H, CH_2), 3.66–3.63 (m, 2 H, CH_2), 3.62–3.52 (m, 6 H, H-2, H-6b, H-6′a, Me), 3.47 (t, 1 H, H-5), 3.34 (dd, 1 H, $J_{2,3}$ 10.1, H-3), 3.26 (dd, 1 H, $J_{6'a,6'b}$ 8.3, H-6′b), 2.63 (br s, 1 H, OH) (Found: C, 72.7; H, 7.0. Calc. for $C_{37}H_{64}O_{12}$: C, 72.75; H, 6.85%).

Methyl 2-*O*-(2-carboxyethyl)-4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside sodium salt 5

To a solution of CrO_3 (188 mg, 1.88 mmol), pyridine (0.3 mL), 1.4 g of *t*-BuOH, and acetic acid (1 mL) in DCM (10 mL) was added the alcohol **33** (450 mg, 0.47 mmol). The mixture was stirred for 16 h at room temperature and refluxed for 2 h. The solution was applied to a silica gel column, eluted with ethyl acetate, and concentrated. A solution of the residue in DCM (2 mL) and 80% trifluoroacetic acid (TFA) (2 mL) was stirred for 2 h, then concentrated. The residue was hydrogenated in acetic acid (10 mL) in the presence of Pd/C (100 mg) for 18 h, filtered through Celite, and the filtrate was chromatographed on IATROBEADS with DCM–MeOH–HOAc (800 : 200 : 5), and passed through Dowex 50W (Na^+) to give **5** (115 mg, 54%), $[\alpha]_D$ +75.8 (*c* 1.0, H_2O); ^{1}H NMR ($CDCl_3$) δ_H 4.95 (d, 1 H, $J_{1',2'}$ 4.0, H-1′), 4.42 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.40 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx$ 6.6, H-5′), 4.07–4.03 (m, 3 H, H-4, H-4′, CH_2O), 3.93 (dd, 1 H, $J_{2',3'}$ 10.7, $J_{3',4'}$ 3.2, H-3′), 3.90–3.81 (m, 4 H, H-6a, H-6b, H-2′, CH_2O), 3.76–3.70 (m, 4 H, H-3, H-5, H-6′a, H-6′b), 3.59 (s, 3 H, Me), 3.37 (dd, 1 H, $J_{2,3}$ 10.1, H-2), 2.47 (t, 2 H, ^2J 6.4, CH_2COONa); *m*/*z* (EI) 451.1424 (MNa^+. $C_{16}H_{28}O_{13}$·Na requires *m*/*z*, 451.1427).

Methyl 3,6-di-*O*-benzyl-2-*O*-(3-cyanopropyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 36

MsCl (63 μL, 0.81 mmol) was added to a solution of **33** (429 mg, 0.45 mmol) in pyridine (5 mL). After 4 h the reaction mixture was quenched by the addition of water, diluted with DCM, washed successively with 1 M HCl and aq. $NaHCO_3$, concentrated, and dried. To a solution of the residue in dry DMF (4 mL) was added KCN (58 mg, 0.89 mmol). The mixture was sonicated and stirred at 80 °C for 3 h. The solution was diluted with ethyl acetate, washed with brine and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (80 : 20 to 70 : 20) gave **36** (295 mg, 70%), $[\alpha]_D$ +40.1 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.37–7.15 (m, 30 H, arom), 5.02 (s, 1 H, H-1′), 4.90 (d, 1 H, ^2J 11.2, CH_2Ph), 4.88 (d, 1 H, ^2J 11.8, CH_2Ph), 4.77 (d, 1 H, ^2J 12.5, CH_2Ph), 4.76 (s, 2 H, CH_2Ph), 4.67 (d, 1 H, ^2J 11.8, CH_2Ph), 4.53 (d, 1 H, ^2J 11.2, CH_2Ph), 4.47 (d, 1 H, ^2J 12.5, CH_2Ph), 4.37 (dd, 1 H, $J_{5',6'a}$ 5.0, $J_{5',6'b}$ 9.0, H-5′), 4.26 (d, 1 H, ^2J 11.8, CH_2Ph), 4.21 (d, 1 H, ^2J 11.8, CH_2Ph), 4.14–4.07 (m, 6 H, H-1, H-2′, H-3′, H-4′, CH_2Ph), 4.00 (d, 1 H, $J_{3,4}$ 2.7, H-4), 4.97 (dd, 1 H, $J_{5,6a}$ 7.3, $J_{6a,6b}$ 9.4, H-6a), 3.86–3.71 (m, 2 H, CH_2CN), 3.57–3.52 (m, 5 H, H-6b, H-6′a, Me), 3.46 (t, 1 H, $J_{5,6a} \approx J_{5,6b} \approx$ 7.0, H-5), 3.40 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9.9, H-2), 3.28 (dd, 1 H, H-3), 3.24 (dd, 1 H, $J_{6'a,6'b}$ 8.4, H-6′b), 2.44–2.28 (m, 2 H, $CH_2CH_2CH_2CN$), 1.84–1.77 (m, 2 H, CH_2-CH_2CN) (Found: C, 73.4; H, 7.0; N, 1.4. Calc. for $C_{59}H_{65}NO_{11}$: C, 73.5; H, 6.8; N, 1.45%).

Methyl 2-*O*-(3-carboxypropyl)-4-*O*-(-α-D-galactopyranosyl)-β-D-galactopyranoside sodium salt 6

To a solution of **36** (279 mg, 0.29 mmol) in EtOH (8 mL) was added aq. NaOH (0.5 g in 1 mL). The mixture was refluxed for 2 days, then neutralized by HCl, diluted with brine, extracted with DCM, and the organic layer was concentrated. The residue was hydrogenated in acetic acid (10 mL) for 6 h in the presence of Pd/C (100 mg). Catalyst was filtered off, the supernatant was concentrated, and the residue was chromatographed on IATROBEADS with DCM–MeOH–HOAc (800 : 200 : 0 to 700 : 300 : 5); the product was dissolved in water and passed through Dowex 50W (Na^+-form) to give **6** (130 mg, 96%), $[\alpha]_D$ +75.0 (*c* 1.0, H_2O); ^1H NMR (D_2O) δ_H 4.96 (d, 1 H, $J_{1',2'}$ 4.0, H-1′), 4.42 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.35 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6.4$, H-5′), 4.04–4.03 (m, 2 H, H-4, H-4′), 3.92 (dd, 1 H, $J_{2',3'}$ 7.3, $J_{3',4'}$ 3.2, H-3′), 3.90–3.84 (m, 3 H, H-6a, H-6b, CH_2CO_2Na), 3.82 (dd, 1 H, H-2′), 3.75 (dd, 1 H, $J_{2,3}$ 9.9, $J_{3,4}$ 3.0, H-3), 3.73–3.66 (m, 4 H, H-5, H-6′a, , CH_2CO_2Na), 3.58 (s, 3 H, Me), 3.33 (dd, 1 H, H-2), 2.29–2.19 (m, 2 H, $CH_2CH_2CH_2CO_2Na$), 1.87–1.79 (m, 2 H, $CH_2CH_2CO_2Na$); *m*/*z* (EI) 465.1893 (MH^+. $C_{17}H_{30}O_{13}$·Na requires *m*/*z*, 465.1584).

Methyl 3,6-di-*O*-benzyl-2-*O*-[2-(methylsulfonyloxy)ethyl]-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 37

To a solution of **34** (920 mg, 0.67 mmol) in pyridine (5 mL) was added MsCl (1.5 eq.). After 2 h at 0–15 °C the reaction mixture was quenched with water, taken up into DCM, washed with water, and concentrated. Chromatography of the residue on silica gel in hexane–ethyl acetate (8 : 2 to 6 : 4) gave **37** (976 mg, 98%), $[\alpha]_D$ +39.1 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ_H 7.4–7.1 (m, 30 H, arom), 5.03 (d, 1 H, $J_{1',2'}$ 2.1, H-1′), 4.91 (d, 1 H, ^2J 11.1, CH_2Ph), 4.88 (d, 1 H, ^2J 11.4, CH_2Ph), 4.78 (d, 1 H, ^2J 11.5, CH_2Ph), 4.67 (d, 1 H, ^2J 11.8, CH_2Ph), 4.76 (s, 2 H, CH_2Ph), 4.54 (d, 1 H, ^2J 11.2, CH_2Ph), 4.52 (d, 1 H, ^2J 12.7, CH_2Ph), 4.38 (dd, 1 H, $J_{5',6'a}$ 5.0, $J_{5',6'b}$ 8.6, H-5′), 4.29–4.20 (m, 4 H, CH_2Ph, CH_2OMs), 4.14 (d, 1 H, $J_{1,2}$ 7.5, H-1), 4.14 (s, 2 H, CH_2Ph), 4.09 (m, 3 H, H-2′, H-3′, H-4′), 4.04–3.91 (m, 4 H, H-4, H-6a, CH_2), 3.56–3.44 (m, 4 H, H-2, H-5, H-6b, H-6′a), 3.52 (s, 3 H, Me), 3.31 (dd, 1 H, $J_{3,4}$ 2.9, $J_{2,3}$ 9.9, H-3), 3.26 (dd, 1 H, $J_{6a',6'b}$ 8.4, H-6′b), 2.88 (s, 3 H, Ms) (Found: C, 68.3; H, 6.6; S, 3.3. Calc. for $C_{58}H_{66}O_{14}S$: C, 68.35; H, 6.5; S, 3.15%).

Methyl 3,6-di-*O*-benzyl-2-*O*-(2-cyanoethyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 38

A solution of **37** (310 mg, 0.304 mmol) and KCN (292 mg, 4.5 mmol) in DMF (5 ml) was stirred for 3 h at 80 °C. DMF was evaporated off and a solution of the residue in DCM was washed with aq. sodium chloride and concentrated. Column chromatography (hexane–ethyl acetate, 3 : 1) of the residue gave **38** (236 mg, 82.9%), $[\alpha]_D$ +33.9 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ_H 7.4–7.1 (m, 30 H, arom), 5.00 (s, 1 H, H-1′), 4.88 (d, 1 H, ^2J 11.4, CH_2Ph), 4.84 (d, 1 H, ^2J 12.3, CH_2Ph), 4.73 (d, 1 H, ^2J 12.3, CH_2Ph), 4.72 (s, 2 H, CH_2Ph), 4.64 (d, 1 H, ^2J 11.7, CH_2Ph), 4.51 (d, 1 H, ^2J 11.2, CH_2Ph), 4.50 (d, 1 H, ^2J 12.7, CH_2Ph), 4.34 (dd, 1 H, $J_{5',6'a}$ 4.7, $J_{5',6'b}$ 8.0, H-5′), 4.24 (d, 1 H, ^2J 12.0, CH_2Ph), 4.18 (d, 1 H, ^2J 12.7, CH_2Ph), 4.14 (s, 2 H, CH_2Ph), 4.12 (d, 1 H, $J_{1,2}$ 7.4, H-1), 4.05 (m, 3 H, H-2′, H-3′, H-4′), 3.95–3.76 (m, 4 H, H-4, H-6a, CH_2), 3.56–3.50 (m, 2 H, H-6b, H-6′a), 3.52 (s, 3 H, Me), 3.44–3.34 (m, 2 H, H-2, H-5), 3.29–3.24 (m, 2 H, H-3, H-6′b), 2.52–2.35 (m, 2 H, CH_2) (Found: C, 73.25; H, 6.8; N, 1.4. Calc. for $C_{58}H_{63}NO_{11}$: C, 73.3; H, 6.6; N, 1.5%).

Methyl 3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 39

From 32. To a solution of **32** (978 mg, 1.04 mmol) in acetic acid (5 mL)–methanol (8 mL) was added $PdCl_2$ (200 mg). The mixture was sonicated for 5 min then stirred for 5 h, filtered through Celite, and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (70 : 30 to 60 : 40) gave **39** (762 mg, 81%).

From 38. A solution of **38** (204 mg, 0.214 mmol) in a mixture of methanol (10 ml), sodium hydroxide (200 mg), ethanol (4 ml) and water (1 ml) was stirred for 2 h at 80 °C. The mixture was acidified with 0.1 M hydrochloric acid, concentrated, and a solution of the residue in DCM was washed with water. Concentration of the organic layer and column chromatography of the residue in hexane–ethyl acetate (7 : 3) gave **39** (185 mg, 96%), $[\alpha]_D$ +49.7 (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ_H 7.35–7.10 (m, 30 H, arom), 5.00 (d, 1 H, $J_{1,2}$ 1.8, H-1′), 4.88 (d, 1 H, ^2J 11.2, CH_2Ph), 4.86 (d, 1 H, ^2J 11.9, CH_2Ph), 4.79 (d, 1 H, ^2J 12.6, CH_2Ph), 4.72 (s, 2 H, CH_2Ph), 4.65 (d, 1 H, ^2J 11.8, CH_2Ph), 4.50 (d, 1 H, ^2J 11.6, CH_2Ph), 4.48 (d, 1 H, ^2J 13.0, CH_2Ph), 4.38 (dd, 1 H, $J_{5',6'a}$ 4.8, $J_{5',6'b}$ 8.8, H-5′), 4.25 (d, 1 H, ^2J 11.8, CH_2Ph), 4.20 (d, 1 H, ^2J 11.8, CH_2Ph), 4.16 (s, 2 H, CH_2Ph), 4.12 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.08–3.95 (m, 5 H, H-2′, H-3′, H-4′, H-4, H-6a), 3.79 (dd, 1 H, $J_{2,3}$ 9.8, H-2), 3.58–3.46 (m, 6 H, H-5, H-6b, H-6′a, OMe), 3.28–3.23 (m, 2 H, H-3, H-6′b) (Found: C, 73.6; H, 6.8. Calc. for $C_{55}H_{60}O_{11}$: C, 73.6; H, 6.7%).

Methyl 3,6-di-*O*-benzyl-2-*O*-(2-sulfoxyethyl)-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside sodium salt 40

A mixture of **34** (207 mg, 0.22 mmol) and SO_3–pyridine complex (140 mg, 0.88 mmol) in pyridine (2 mL) was stirred at 60 °C for 2 h, then aq. $NaHCO_3$ (1 mL) was added. The mixture was concentrated, extracted with DCM, and the extract was chromatographed on IATROBEADS with DCM–MeOH (9 : 1) to give **40** (218 mg, 95%), $[\alpha]_D$ +23.4 (*c* 0.4, $CHCl_3$); ^1H NMR (CD_3OD) δ_H 7.40–7.15 (m, 30 H, arom), 5.00 (d, 1 H, $J_{1',2'}$ 3.5, H-1′), 4.8–4.7 (m, 5 H, CH_2Ph), 4.62 (d, 1 H, ^2J 11.6, CH_2Ph), 4.60 (d, 1 H, ^2J 12.5, CH_2Ph), 4.45 (d, 1 H, ^2J 11.2, CH_2Ph), 4.19 (dd, 1 H, $J_{5',6'a}$ 5.7, $J_{5',6'b}$ 8.3, H-5′), 4.27 (s, 2 H, CH_2Ph), 4.23–3.83 (m, 10 H, H-1, H-4, H-6a, H-2′, H-3′, H-4′, CH_2Ph, CH_2), 3.62–3.40 (m, 10 H, H-2, H-3, H-5, H-6b, H-6′a, CH_2, OMe), 3.22 (dd, 1 H, $J_{6'a,6'b}$ 8.7, H-6′b); *m*/*z* (EI) 1065.3678 (MNa^+. $C_{57}H_{63}Na_2O_{15}S$ requires *m*/*z*, 1065.3683).

Methyl 4-*O*-(α-D-galactopyranosyl)-2-*O*-(2-sulfoxyethyl)-β-D-galactopyranoside sodium salt 7

To a solution of **40** (190 mg, 0.182 mmol) in EtOH (5 mL) was added Pd/C (10%; 50 mg). The mixture was stirred overnight under a flow of H_2. The suspension was concentrated, diluted with methanol (5 mL), additional Pd/C (50 mg) was added, and the mixture was stirred again for 24 h under a flow of H_2. The catalyst was filtered off, the supernatant was concentrated, and the residue was dissolved in water and passed through a Sep-Pak C_{18} cartridge. Passage through Dowex 50W (Na^+) and purification on a BIOGEL P-2 column gave **7** (87.8 mg, 96%), $[\alpha]_D$ +78.2 (*c* 0.9, H_2O); ^1H NMR (D_2O) δ_H 4.96 (d, 1 H, $J_{1',2'}$ 4.0, H-1′), 4.45 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.38 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6.6$, H-5′), 4.23–4.17 (m, 2 H, $CH_2CH_2OSO_3Na$), 4.12–4.08 (m, 1 H, $CH_2CH_2OSO_3Na$), 4.05–4.03 (m, 2 H, H-4, H-4′), 3.99–3.89 (m, 3 H, H-3′, H-6′a, $CH_2CH_2OSO_3Na$), 3.85–3.81 (m, 2 H, H-2′, H-6b), 3.78 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.0, H-3), 3.74 (dd, 1 H, $J_{5,6a}$ 5.2, $J_{5,6b}$ 7.5, H-5), 3.71 (d, 2 H, H-6′a, H-6′b), 3.60 (s, 3 H, Me), 3.41 (dd, 1 H, H-2); *m*/*z* (EI) 525.0866 (MNa^+. $C_{15}H_{27}Na_2O_{15}S$ requires *m*/*z*, 525.0866).

Methyl 3,6-di-*O*-benzyl-2-*O*-[2-(diphenoxyphosphonyl)ethyl]-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 41

A mixture of **34** (325 mg, 0.345 mmol) and diphenyl chlorophosphate (279 mg, 1.03 mmol) in pyridine (4 mL) was stirred for 1 h, then the reaction mixture was quenched by the addition

of methanol, concentrated, extracted with DCM, and the extract chromatographed on silica with pentane–ethyl acetate (8 : 2) to give **41** (372 mg, 91%), $[\alpha]_D$ +33.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.33–7.15 (m, 40 H, arom), 5.02 (d, 1 H, $J_{1',2'}$ 2.4, H-1′), 4.90 (d, 1 H, ^2J 11.1, CH_2Ph), 4.86 (d, 1 H, ^2J 11.4, CH_2Ph), 4.75 (d, 1 H, ^2J 11.8, CH_2Ph), 4.73 (s, 2 H, CH_2Ph), 4.66 (d, 1 H, ^2J 11.8, CH_2Ph), 4.53 (d, 1 H, ^2J 11.4, CH_2Ph), 4.53 (d, 1 H, ^2J 11.1, CH_2Ph), 4.42–4.31 (m, 3 H, H-5, CH_2), 4.26 (d, 1 H, ^2J 11.8, CH_2Ph), 4.21 (d, 1 H,^2J 11.8, CH_2Ph), 4.15 (s, 2 H, CH_2Ph), 4.11–3.88 (m, 8 H, H-1, H-4, H-6a, H-2′, H-3′, H-4′, CH_2), 3.56–3.40 (m, 7 H, H-2, H-5, H-6b, H-6′a, H-6′b, Me), 3.28–3.24 (m, 2 H, H-3, H-6′b) (Found: C, 70.7; H, 6.3. Calc. for $C_{69}H_{73}O_{15}P$: C, 70.6; H, 6.3%).

Methyl 4-*O*-(α-D-galactopyranosyl)-2-*O*-(2-phosphonyloxyethyl)-β-D-galactopyranoside disodium salt 8

To a solution of **41** (345 mg, 0.294 mmol) in EtOH (5 mL) was added Pd/C (10%; 50 mg). The mixture was stirred overnight under a flow of H_2. Then PtO_2 (20 mg) was added and hydrogenation was continued for 5 h. The catalyst was filtered off, the supernatant was concentrated and the residue was chromatographed on IATROBEADS with DCM–MeOH–water (800 : 200 : 0 to 700 : 300 : 5). The product was dissolved in water and passed through Dowex 50W (Na^+) to give **8** (87.8 mg, 96%), $[\alpha]_D$ +74.9 (*c* 0.6, H_2O); ^{1}H NMR (D_2O) δ_H 4.96 (d, 1 H, $J_{1',2'}$ 4.0, H-1′), 4.45 (d, 1 H, $J_{1,2}$ 7.6, H-1), 4.37 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6.2$, H-5′), 4.07–4.03 (m, 3 H, H-4, H-4′, CH_2), 4.00–3.97 (m, 2 H, CH_2), 3.94–3.81 (m, 5 H, H-6a, H-6b, H-2′, H-3′, CH_2), 3.79 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.0, H-3), 3.74 (dd, 1 H, $J_{5,6a}$ 5.0, $J_{5,6b}$ 7.5, H-5), 3.70 (d, 2 H, H-6′a, H-6′b), 3.60 (s, 3 H, Me), 3.40 (dd, 1 H, H-2); *m*/*z* (EI) 503.1145 [$(MH_2 - Na)^+$. $C_{15}H_{29}NaO_{15}P$ requires *m*/*z*, 503.1142].

Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 43

A mixture of methyl 2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside[13] **42** (5.2 g, 10.2 mmol), **16**[8] (6.3 g, 10.77 mmol), and mol. sieves (4 Å, 10 g) in DCM (50 mL) was stirred for 1 h under an argon atmosphere. The mixture was cooled at −15 °C, and a mixture of NIS (2.35 mg, 10.44 mmol) and AgOTf (378 mg, 1.47 mmol) was added in 3 portions. The precipitate was filtered off, and the solution was washed successively with aq. $Na_2S_2O_3$ and water, and concentrated. Chromatography of the residue on silica gel in pentane–ethyl acetate (6 : 1 to 4 : 1) gave **43** (10.18 g, 95%), $[\alpha]_D$ +57.1 (*c* 0.7; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.00–7.88 (m, 6 H, arom), 7.45–7.11 (m, 29 H, arom), 5.77 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 10.6, H-2), 5.22 (dd, 1 H, $J_{3,4}$ 2.9, H-3), 4.91 (d, 1 H, $J_{1',2'}$ 3.4, H-1′), 4.85–4.67 (m, 7 H, CH_2Ph), 4.61 (d, 1 H, H-1), 4.44 (d, 1 H, ^2J 11.1, CH_2Ph), 4.39 (d, 1 H, H-4), 4.32 (dd, 1 H, $J_{5,6a}$ 5.0, $J_{5,6b}$ 9.4, H-5), 4.16 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3',4'}$ 2.5, H-3′), 4.15 (br s, 1 H, H-4′), 4.10–4.00 (m, 4 H, H-2′, H-6a, H-6b, H-6′a), 3.53 (m, 3 H, OMe), 3.35 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 9.0$, H-5′), 2.87 (dd, 1 H, $J_{6'a,6'b}$ 4.9, H-6′b) (Found: C, 72.4; H, 5.8. Calc. for $C_{62}H_{60}O_{14}$: C, 72.4; H, 5.9%).

Methyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 44

A solution of **43** (2.89 g, 2.8 mmol) in methanol (40 mL) was refluxed for 5 h in the presence of MeONa (300 mg), then neutralized with Dowex 50W (H^+) and concentrated. Chromatography of the residue on silica gel in DCM–MeOH (20 : 1) gave **44** (1.96 g, 97%), $[\alpha]_D$ −7.8 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.37–7.25 (m, 20 H, arom), 4.90 (d, 2 H, ^3J 11.7, CH_2Ph), 4.80 (d, 1 H, $J_{1',2'}$ 3.7, H-1′), 4.74 (s, 2 H, CH_2Ph), 4.67 (d, 1 H, CH_2Ph), 4.52 (d, 1 H, CH_2Ph), 4.42 (d, 1 H, ^3J 11.3, CH_2Ph), 4.35 (d, 1 H, CH_2Ph), 4.14–4.07 (m, 3 H, H-1, H-2′, H-5′), 3.99 (dd, 1 H, $J_{3',4'}$ 2.6, $J_{2',3'}$ 10.1, H-3′), 3.93–3.96 (m, 3 H, H-3, H-4, H-4′), 3.75–3.70 (br m, 1 H, H-6a), 3.66–3.62 (br m, 1 H, H-5), 3.54 (t, 1 H, $J_{5',6'a} \approx J_{6'a,6'b} \approx 9.4$, H-6′a), 3.51 (s, 3 H, OMe), 3.41–3.30 (m, 2 H, H-2, H-6b), 3.27 (dd, 1 H, $J_{5',6'b}$ 3.7, H-6′b), 2.03 (br s, 1 H, OH), 1.53 (br s, 2 H, OH) (Found: C, 68.5; H, 6.8. Calc. for $C_{41}H_{48}O_{11}$: C, 68.7; H, 6.75%).

Methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 45

A solution of **44** (1.723 g, 2.4 mmol) and *t*-$BuMe_2SiCl$ (0.55 g, 3.65 mmol) in pyridine (15 mL) was stirred for 3 h, then Ac_2O (3 mL) was added. After 2 days the reaction mixture was quenched by the addition of methanol, concentrated, and co-evaporated with toluene. A solution of the residue in 80% aq. acetic acid (20 mL) was stored for 1 h at 60 °C, then was concentrated, and chromatographed on silica gel with pentane–ethyl acetate (7 : 3 to 1 : 1) to give **45** (1.364 g, 70%), $[\alpha]_D$ +31.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.38–7.26 (m, 20 H, arom), 5.19 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.7, H-2), 4.90 (d, 1 H, ^2J 11.7, CH_2Ph), 4.89 (d, 1 H, ^2J 11.1, CH_2Ph), 4.82 (dd, 1 H, $J_{3,4}$ 2.9, H-3), 4.78–4.46 (m, 6 H, CH_2Ph), 4.60 (d, 1 H, $J_{1',2'}$ 1.9, H-1′), 4.34 (d, 1 H, H-1), 4.29 (br dd, 1 H, $J_{5',6'a}$ 9.0, $J_{6'a,6'b}$ 3.3, H-6′a), 4.17 (d, 1 H, H-4), 4.14 (br s, 1 H, H-4′), 4.04 (m, 2 H, H-2′, H-3′), 3.80–3.55 (m, 6 H, H-5, H-6a, H-6b, H-5′, H-6′b, OH), 4.18 (s, 3 H, OMe), 2.04 and 1.93 (2 s, 6 H, 2Ac) (Found: C, 67.5; H, 6.4. Calc. for $C_{45}H_{52}O_{13}$: C, 67.5; H, 6.5%).

Methyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-6-*O*-(*p*-tolylsulfonyl)-β-D-galactopyranoside 46

To a solution of **45** (2.62 g, 3.27 mmol) in pyridine (20 mL) was added TsCl (1.87 g, 9.8 mmol). After 2 days of stirring at room temperature the mixture was diluted with DCM, washed successively with water, 1 M HCl, water, and aq. $NaHCO_3$, and the extract was concentrated, and chromatographed on the silica gel with pentane–ethyl acetate (4 : 1 to 3 : 2) to give **46** (2.07 g, 66%), $[\alpha]_D$ +40.6 (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.67–7.64 (m, 2 H, arom), 7.35–7.20 (m, 22 H, arom), 5.19 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 10.5, H-2), 4.88 (d, 1 H, ^2J 11.2, CH_2Ph), 4.82 (dd, 1 H, $J_{3,4}$ 3.0, H-3), 4.73 (d, 1 H, ^3J 12.1, CH_2Ph), 4.70 (s, 2 H, CH_2Ph), 4.65 (br s, 1 H, H-1′), 4.59 (d, 1 H, ^3J 12.1, CH_2Ph), 4.53 (d, 1 H, ^3J 11.2, CH_2Ph), 4.47 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 11.0, H-6a), 4.43 (s, 2 H, CH_2Ph), 4.33 (d, 1 H, H-1), 4.29 (dd, 1 H, $J_{5,6b}$ 6.8, H-6b), 4.23 (dd, 1 H, $J_{6'a,6'b}$ 5.4, $J_{5',6'a}$ 8.7, H-6′a), 4.07 (br s, 1 H, H-4′), 4.04 (br d, 1 H, H-4), 3.95 (m, 2 H, H-2′, H-3′), 3.80 (br t, 1 H, H-5), 3.61 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 8.8$, H-5′), 3.45 (s, 3 H, OMe), 3.45 (dd, 1 H, H-6′b), 2.35 (s, 3 H, Ar*Me*), 2.05 and 2.04 (2 s, 6 H, Ac) (Found: C, 65.4; H, 6.15; S, 3.1. Calc. for $C_{52}H_{58}O_{15}S$: C, 65.4; H, 6.1; S, 3.4%).

Methyl 2,3-di-*O*-acetyl-6-azido-6-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 47

A suspension of NaN_3 (3.57 mg, 55 mmol) in a solution of **46** (1.052 g, 1.1 mmol) and Bu_4NI (50 mg) in DMF (5 mL) was stirred for 24 h at 110 °C. The mixture was diluted with water, extracted with DCM, and the organic layer was concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (2 : 1) afforded **47** (595 mg, 65%), $[\alpha]_D$ +57.2 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.4–7.24 (m, 20 H, arom), 5.22 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.6, H-2), 4.94–4.88 (m, 2 H, CH_2Ph), 4.81 (dd, 1 H, $J_{3,4}$ 2.9, H-3), 4.79 (d, 1 H, ^2J 13, CH_2Ph), 4.75 (d, 1 H, ^2J 13.0, CH_2Ph), 4.69 (d, 1 H, $J_{1',2'} \approx 1$, H-1′), 4.60 (d, 1 H, ^3J 11.5, CH_2Ph), 4.58 (d, 1 H, ^2J 11.2, CH_2Ph), 4.46 (s, 2 H, CH_2Ph), 4.36 (d, 1 H, H-1), 4.27 (br dd, 1 H, $J_{5',6'a}$ 8.2, $J_{6'a,6'b}$ 5.7, H-6′a), 4.13 (broad s, 1 H, H-4′), 4.03 (m, 2 H, H-2′, H-3′), 3.95 (d, 1 H, H-4), 3.71 (dd, 1 H, $J_{6a,6b}$ 12.6, $J_{5,6a}$ 8.1, H-6a), 3.68–3.60 (m, 2 H, H-5, H-5′), 3.51 (s, 3 H, OMe), 3.47 (dd, 1 H, $J_{5',6'b}$ 8.0, H-6′b), 3.14 (dd, 1 H, $J_{5,6b}$ 4.0, H-6b), 2.05 and 2.02 (2 s, 6 H, Ac) (Found: C, 65.20; H, 6.31; N, 4.72. Calc. for $C_{45}H_{51}N_3O_{12}$: C, 65.44; H, 6.22; N, 5.09%).

Methyl 6-amino-6-deoxy-4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside 9

A solution of **47** (200 mg, 0.24 mmol) and MeONa (20 mg) in DCM (1 mL)–methanol (3 mL) was stirred overnight and neutralized by Dowex 50W (H^+) before being filtered and concentrated. To a solution of the residue in toluene (1 mL)–EtOH (3 mL) was added $Pd(OH)_2$/C (100 mg), and the suspension was stirred under a flow of H_2 for 2 h. The mixture was concentrated, dissolved in acetic acid (5 mL), to which solution Pd/C (100 mg) was added, and the mixture was stirred under a flow of H_2 for 18 h. The mixture was filtered through Celite and concentrated. Chromatography of the residue on IATROBEADS with MeOH–DCM–water–HOAc (500 : 500 : 10 : 10) afforded **9** (50 mg, 61%), $[\alpha]_D$ +89.6 (*c* 0.5, H_2O); ^{1}H NMR (D_2O) δ_H 5.02 (d, 1 H, $J_{1',2'}$ 3.4, H-1′), 4.43 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.24 (br t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6$, H-5′), 4.08 (d, 1 H, $J_{3,4}$ 2.9, H-4), 4.04 (d, 1 H, $J_{3',4'}$ 1.4, H-4′), 4.02 (dd, 1 H, $J_{5,6a}$ 7.2, $J_{5,6b}$ 3.8, H-5), 3.93–3.87 (m, 2 H, H-2′, H-3′), 3.76–3.69 (m, 3 H, H-3, H-6′a, H-6′b), 3.60 (s, 3 H, OMe), 3.54 (dd, 1 H, $J_{2,3}$ 8.4, H-2), 3.52 (dd, 1 H, $J_{6a,6b}$ 13.4, H-6a), 3.38 (dd, 1 H, H-6b); *m*/*z* (EI) 356.1559 (MH^+. $C_{13}H_{26}NO_{10}$ requires *m*/*z*, 356.1557).

Methyl 6-acetamido-6-deoxy-4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranoside 10

A solution of **47** (100 mg, 0.12 mmol) and NaOMe (10 mg) in methanol (5 mL) was stored overnight, then neutralized with Dowex 50W (H^+) resin and concentrated. To a solution of the residue in methanol (5 mL) was added $Pd(OH)_2$/C (50 mg), and the suspension was stirred under a flow of H_2 for 2 h. Acetic anhydride (0.3 mL) was added, and after 1 h the mixture was filtered through Celite and concentrated. The residue was dissolved in acetic acid (5 mL), Pd/C (30 mg) was added, and the mixture was stirred under a flow of H_2 overnight. The mixture was filtered through Celite, and the filtrate was concentrated and chromatographed on silica gel with MeOH–DCM–water (100 : 400 : 5) to give **10** (36 mg, 75%), $[\alpha]_D$ +95.5 (*c* 0.3, H_2O); ^{1}H NMR (D_2O) δ_H 5.01 (d, 1 H, $J_{1',2'}$ 3.9, H-1′), 4.35 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.32 (t, 1 H, $J_{5',6'a} \approx J_{5',6'b} \approx 6.6$, H-5′), 4.03 (br d, 1 H, $J_{3',4'}$ 3.2, H-4′), 3.99 (d, 1 H, $J_{3,4}$ 3.0, H-4), 3.93 (dd, 1 H, $J_{2',3'}$ 10.5, H-3′), 3.87 (dd, 1 H, H-2′), 3.80 (dd, 1 H, $J_{5,6a}$ 4.9, $J_{5,6b}$ 8.1, H-5), 3.75–3.54 (m, 3 H, H-6′a, H-6′b, H-3), 3.60–3.54 (m, 2 H, H-6a, H-6b), 3.56 (s, 3 H, OMe), 3.51 (dd, 1 H, $J_{2,3}$ 10.2, H-2), 2.01 (s, 3 H, OAc); *m*/*z* (EI) 420.1485 (MNa^+. $C_{15}H_{27}NO_{11}$·Na requires *m*/*z*, 420.1482).

Methyl 4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-6-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside 49

To a solution of methyl 4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside[14] **48** (3.1 g, 6.97 mmol) in dry pyridine (10 mL) was added *t*-$BuMe_2SiCl$ (1.26 g, 1.2 eq.). After being stirred overnight, the mixture was concentrated, and chromatographed on silica gel in DCM–MeOH (10 : 1) to give **49** (3.15 g, 82%), $[\alpha]_D$ −16.7 (*c* 1.3, MeOH); ^{1}H NMR (CD_3OD) δ_H 7.55–7.30 (m, 5 H, arom), 5.62 (s, 1 H, C*H*Ph), 4.48 (d, 1 H, $J_{1',2'}$ 7.2, H-1′), 4.24–4.13 (m, 4 H, H-1, H-4′, H-6′a, H-6′b), 4.05 (dd, 1 H, $J_{5,6a}$ 3.8, H-6a), 3.99 (dd, 1 H, $J_{5,6b}$ 1.9, H-6b), 3.69–3.50 (m, 5 H, H-3, H-4, H-2′, H-3′, H-5′), 3.50 (s, 3 H, OMe), 3.39 (ddd, 1 H, $J_{4,5}$ 9.3, H-5), 3.21 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.0, H-2), 0.92 (s, 9 H, *t*-Bu), 0.10 (s, 6 H, Me) [Found: C, 55.9; H, 7.6. Calc. for $C_{26}H_{42}O_{11}Si$ (558.69): C, 55.9; H, 7.6%].

Methyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-6-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside 50

A mixture of **49** (3.07 g, 5.61 mmol) and NaH (80%, 0.87 g, 29 mmol) in DMF (10 mL) was stirred for 30 min, then benzyl bromide (3.2 mL, 26.9 mmol) was added. After 1 h, the reaction was quenched by methanol. The mixture was taken up in ethyl acetate, washed with water, and concentrated. Chromatography of the residue on silica gel in pentane–ethyl acetate (8 : 2) gave **50** (3.98 g, 77%), $[\alpha]_D$ +12.2 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.55–7.15 (m, 25 H, arom), 5.48 (s, 1 H, C*H*Ph), 5.18 (d, 1 H, ^2J 10.5, CH_2Ph), 4.87–4.69 (m, 8 H, H-1′, CH_2Ph), 4.30 (dd, 1 H, $J_{5',6'a}$ 1.2, $J_{6'a,6'b}$ 12.3, H-6′a), 4.27 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.10 (br d, 1 H, $J_{3',4'}$ 3.6, H-4′), 4.05 (dd, 1 H, $J_{6a,6b}$ 11.8, $J_{5,6a}$ 2.9, H-6a), 3.97 (t, 1 H, $J_{3,4} \approx J_{4,5} \approx 9.4$, H-4), 3.92 (dd, 1 H, $J_{5',6'b}$ 1.7, H-6′b), 3.80 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.7, H-2′), 3.72 (dd, 1 H, $J_{5,6a}$ 1.5, H-6b), 3.60 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx 9.1$, H-3), 3.51 (dd, 1 H, H-3′), 3.50 (s, 3 H, OMe), 3.34 (dd. 1 H, H-2), 3.20 (br s, 1 H, H-5′), 3.11 (br ddd, 1 H, H-5), 0.81 (s, 9 H, *t*-Bu), −0.01 and −0.02 (2 s, 6 H, Me) (Found: C, 70.75; H, 7.2. Calc. for $C_{54}H_{66}SiO_{11}$: C, 70.6; H, 7.2%).

Methyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside 51

To a solution of **50** (3.92 g, 4.2 mmol) in dry THF (10 mL) was added Bu_4NF (1 M in THF; 8.4 mL), after 1 h the mixture was poured in water and extracted with DCM, and the extract was concentrated. Chromatography of the residue on silica gel in pentane–ethyl acetate (1 : 1 to 4 : 6) gave **51** (3.26 g, 96%), $[\alpha]_D$ +2.2 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.5–7.2 (m, 25 H, arom), 5.45 (s, 1 H, C*H*Ph), 5.16 (d, 1 H, ^2J 10.7, CH_2Ph), 4.88–4.70 (m, 7 H, CH_2Ph), 4.55 (d, 1 H, $J_{1',2'}$ 7.8, H-1′), 4.33 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.40 (d, 1 H, $J_{6a,6b}$ 12.4, H-6a), 4.06 (d, 1 H, $J_{3',4'}$ 3.6, H-4′), 3.96–3.75 (m, 5 H, H-4, H-6b, H-2′, H-6′a, H-6′b), 3.62 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx 9.1$, H-3), 3.55 (dd, 1 H, $J_{2',3'}$ 9.7, H-3′), 3.54 (s, 3 H, OMe), 3.35 (t, 1 H, H-2), 3.27–3.20 (m, 2 H, H-5, H-5′), 1.86 (dd, 1 H, $J_{6a,OH}$ 9.3, $J_{6b,OH}$ 4.7, OH) (Found: C, 71.65; H, 6.5. Calc. for $C_{48}H_{52}O_{11}$: C, 71.6; H, 6.5%).

Methyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-6-*O*-methoxycarbonylmethyl-β-D-glucopyranoside 52

A mixture of **51** (2.92 g, 3.62 mmol) and NaH (80%; 326 mg, 10.8 mmol) in dry DMF (10 mL) was stirred for 30 min at 60 °C, then $ClCH_2COOH$ (580 mg, 6.1 mmol) was added. Stirring at 90 °C was continued for 2 h, then MeI (≈2 mL) was added. After 2 h the reaction was quenched by methanol, and the mixture was taken up in ethyl acetate, washed with brine, and concentrated. Chromatography of the residue on silica gel in pentane–ethyl acetate (8 : 2) gave **52** (3.01 g, 95%), $[\alpha]_D$ +14.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.50–7.15 (m, 25 H, arom), 5.45 (s, 1 H, C*H*Ph), 5.16 (d, 1 H, ^2J 10.6, CH_2Ph), 487–4.68 (m, 10 H, H-1′, CH_2Ph, CH_2COO), 4.25 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.22 (br d, 1 H, H-6′a), 4.11–4.06 (m, 2 H, H-6a, H-4′), 4.01–3.86 (m, 3 H, H-4, H-6b, H-6′b), 4.78 (dd, 1 H, $J_{1',2'}$ 8.0, H-2′), 3.63–3.56 (m, 5 H, H-3, H-3′, OMe), 3.50 (s, 3 H, OMe), 3.36 (dd, 1 H, $J_{2,3}$ 8.8, H-2), 3.36–3.25 (br m, 2 H, H-5, H-5′) (Found: C, 69.8; H, 6.5. Calc. for $C_{51}H_{56}O_{13}$: C, 69.8; H, 6.4%).

Methyl 2,3-di-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-6-*O*-benzoyl-β-D-galactopyranosyl)-6-*O*-methoxycarbonylmethyl-β-D-glucopyranoside 53

A solution of **52** (2.31 g, 2.6 mmol) in acetic acid (80%, 15 mL) was stirred for 2 h at 80 °C, concentrated, co-evaporated with toluene, and dried. To a solution of the residue in DCM (10 mL)–pyridine (10 mL) was added benzoyl chloride (0.3 mL, 2.6 mmol) at 0 °C. After 2 h the mixture was concentrated, and chromatographed on silica gel in pentane–ethyl acetate (6 : 4) to give **53** (1.53 g, 66%), $[\alpha]_D$ +16.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.00–7.90 (m, 2 H, C_6H_5CO), 7.51–7.20 (m, 23 H, arom), 4.99 (d, 1 H, ^2J 10.8, CH_2Ph), 4.83–4.65 (m, 8 H, H-1′, CH_2Ph), 4.52 (dd, 1 H, $J_{6'a,6'b}$ 11.2, $J_{5',6'a}$ 6.5, H-6′a), 4.30 (dd, 1 H, $J_{5',6'b}$ 6.5, H-6′b), 4.24 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.14–3.93 (m, 5 H, H-4, -4′, H-6a, CH_2COO), 3.72–3.49 (m, 5 H, H-3, H-6b, H-2′, H-3′, H-5′), 3.62 (s, 3 H, OMe), 3.50 (s, 3 H, OMe),

3.37–3.30 (m, 2 H, H-2, H-5) (Found: C, 68.9; H, 6.4. Calc. for $C_{51}H_{56}O_{14}$: C, 68.6; H, 6.3%).

Methyl 2,3-di-*O*-benzyl-6-*O*-benzoyl-4-*O*-[2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-6-*O*-methoxycarbonylmethyl-β-D-glucopyranoside 54

To a solution of **53** (1.02 g, 1.14 mmol), AgOTf (373 mg, 1.45 mmol), and 2,4,6-collidine (205 μL, 1.55 mmol) in dry toluene (30 mL) was added a solution of freshly prepared 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[12] **30** (0.765 g, 1.2 eq.) at 5 °C. The mixture was allowed to warm to room temperature, then taken up in DCM, washed successively with aq. $Na_2S_2O_3$ and water, and concentrated. Chromatography of the residue on silica gel in pentane–ethyl acetate (8 : 2 to 6 : 4) gave **54** (1.08 g, 67%), $[\alpha]_D$ +21.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.95–7.03 (m, 45 H, arom), 5.09 (d, 1 H, ^2J 10.6, CH_2Ph), 4.98 (d, 1 H, $J_{1'',2''}$ 3.3, H-1″), 4.88–4.63 (m, 13 H, H-6′a, H-6′b, CH_2Ph), 4.54–4.42 (m, 4 H, CH_2Ph), 4.32 (dd, 1 H, $J_{5'',6''b}$ 4.6, $J_{5'',6''a}$ 8.7, H-5″), 4.24 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.14–3.91 (m, 10 H, H-4, H-6a, H-6b, H-1′, H-4′, H-2″, H-3″, H-4″, CH_2COO), 3.68–3.44 (m, 5 H, H-3, H-2′, H-3′, H-5′, H-6″a), 3.62 (s, 3 H, OMe), 3.50 (s, 3 H, OMe), 3.32 (dd, 1 H, $J_{2,3}$ 10.6, H-2), 3.30 (m, 1 H, H-5), 3.17 (dd, 1 H, $J_{6''a,6''b} \approx$ 5.0, H-6″b) (Found: C, 72.1; H, 6.45. Calc. for $C_{85}H_{90}O_{19}$: C, 72.1; H, 6.4%).

Methyl 6-*O*-carboxymethyl-4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 12

A solution of **54** (200 mg, 0.14 mmol) and NaOH (≈ 200 mg) in methanol (5 mL) was stirred overnight then neutralized with acetic acid, taken up in DCM, washed with water, and concentrated. The residue was hydrogenated in acetic acid in the presence of Pd/C for 18 h. Catalyst was filtered off, the supernatant was concentrated and the residue was chromatographed on silica gel in DCM–MeOH–HOAc (50 : 50 : 1) and gave **12** (78.5 g, 96%), $[\alpha]_D$ +46.8 (*c* 0.2, $CHCl_3$); ^{1}H NMR (D_2O) δ_H 4.96 (d, 1 H, $J_{1'',2''}$ 3.9, H-1″), 4.55 (d, 1 H, $J_{1',2'}$ 7.9, H-1′), 4.42 (d, 1 H, $J_{1,2}$ 8.0, H-1), 4.37 (br t, 1 H, $J_{5'',6''a} \approx J_{5'',6''b} \approx$ 6.7, H-5″), 4.06–4.04 (m, 2 H, H-4′, H-4″), 3.99 (s, 2 H, CH_2COO), 3.96–3.54 (m, 14 H, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-5′, H-6′a, H-6′b, H-2″, H-3″, H-6″a, H-6″b), 3.58 (s, 3 H, OMe), 3.32 (dd, 1 H, $J_{2,3}$ 9.2, H-2); *m*/*z* (EI) 599.1790 (MNa^+. $C_{21}H_{36}O_{18}$·Na requires *m*/*z*, 599.1799).

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-allyl-β-D-galactopyranosyl)-α,β-D-glucopyranose 56

A solution of **55**[15] (6.81 g, 11.7 mmol), NaH (95%, 0.885 g, 35.2 mmol), and allyl bromide (2.03 mL, 23.4 mmol) was refluxed for 3 h until alkylation was complete. Then methanol was added and the mixture was neutralized by acetic acid. The mixture was filtered through Celite and the filtrate was concentrated. The residue was dissolved in 60% aq. acetic acid, refluxed for 2 h, and concentrated. The residue was crystallized from EtOH, and the crystals were collected and dried in a desiccator over P_2O_5. A mixture of the residue in pyridine (30 ml) and Ac_2O (30 ml) was stirred at 60 °C for 1 h under an argon atmosphere. The mixture was concentrated, co-evaporated three times with toluene, and the residue was chromatographed on silica gel with pentane–ethyl acetate (7 : 3) to give **56** (5.79 g, 73%); ^{1}H NMR ($CDCl_3$) δ_H (*inter alia*) 6.26 (d, $J_{1,2}$ 3.7, H-1α), 5.78 (H^e), 5.68 (d, $J_{1,2}$ 8.3, H-1β), 5.44 (dd, *J* 9.3, *J* 10.2, H-3α) (Found: C, 51.4; H, 5.8. Calc. for $C_{29}H_{40}O_{18}$: C, 51.5; H, 6.0%).

2,3,6-Tri-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-allyl-β-D-galactopyranosyl)-α-D-glucopyranosyl chloride 57

To a solution of **56** (3.5 g, 5.17 mmol) in DCM (10 mL) were added Cl_2CHOCH_3 (0.56 mL, 6.2 mmol) and $ZnBr_2$ (100 mg) under an argon atmosphere. The mixture was stirred for 1 h, then concentrated, and the residue was chromatographed on silica gel with pentane–ethyl acetate (6 : 4) to give **57** (396 mg, 85%), $[\alpha]_D$ +93.4 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 6.22 (s, 1 H, $J_{1,2}$ 3.9, H-1), 5.80 (m, 1 H, H^e), 5.56 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 9.7, H-3), 5.31 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.8, H-4′), 5.20 (1 H, H^b), 5.12 (1 H, H^a), 4.93 (dd, 1 H, H-2), 4.86 (dd, 1 H, $J_{2',3'}$ 10.2, H-3′), 4.58 (dd, 1 H, $J_{6a,6b}$ 12.1, $J_{5,6a}$ 0.7, H-6a), 4.34 (d, 1 H, $J_{1',2'}$ 7.7, H-1′), 4.29 (dd, 1 H, $J_{5,6b}$ 4.2, H-6b), 4.25–4.01 (m, 5 H, H^d, H^e, H-5, H-6′a, H-6′b), 3.85–3.78 (m, 2 H, H-4, H-5′), 3.42 (dd, 1 H, H-2′), 2.09, 2.10, 2.11 (6 × 3 H, OAc) (Found: C, 49.7; H, 5.7; Cl, 5.7. Calc. for $C_{27}H_{37}ClO_{16}$: C, 49.7; H, 5.7; Cl, 5.4%).

Methyl 4-*O*-(2-*O*-allyl-β-D-galactopyranosyl)-β-D-glucopyranoside 58

Chloride **57** (4.4 g, 6.74 mmol) was dissolved in dry methanol (20 mL) and left at room temperature for two days. The mixture was neutralized with Dowex 50W (H^+) resin, the suspension was filtered and the filtrate was crystallized from EtOH to give **58** (1.8 g, 67%), $[\alpha]_D$ −1.8 (*c* 0.2, H_2O); mp 222–223 °C; ^{1}H NMR (D_2O) δ_H 5.99 (m, 1 H, H^e), 5.36 (m, 1 H, H^b), 5.28 (m, 1 H, H^a), 4.49 (d, 1 H, $J_{1',2'}$ 7.9, H-1′), 4.41 (d, 1 H, $J_{1,2}$ 8.0, H-1), 4.34 (m, 1 H, H^d), 4.26 (m, 1 H, H^e), 3.99 (br d, 1 H, H-6a), 3.93 (d, 1 H, $J_{3',4'}$ 3.3, H-4′), 3.83–3.62 (m, 8 H, H-3, H-4, H-5, H-6b, H-3′, H-5′, H-6′a, H-6′b), 3.58 (s, 3 H, Me), 3.4 (dd, 1 H, $J_{2',3'}$ 10.0, H-2′), 3.32 (m, 1 H, H-2) (Found: C, 49.7; H, 5.9; Cl, 5.7. Calc. for $C_{16}H_{28}O_{11}$: C, 49.7; H, 5.7; Cl, 5.4%).

Methyl 4-*O*-(2-*O*-allyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside 59

Methyl β-lactoside **58** (1.56 g, 3.93 mmol) was lyophilized from water (20 mL). To a mixture of the residue and α,α-dimethoxytoluene (1.2 mL, 2 eq.) in dry acetonitrile (15 mL) was added PTSA (150 mg). The mixture was refluxed for 3 min, then was neutralized with pyridine and concentrated. Chromatography of the residue on silica gel with DCM–MeOH (20 : 1) gave **59** (1.25 g, 64%), $[\alpha]_D$ −23.1 (*c* 0.4; MeOH); mp 212–213 °C; ^{1}H NMR (CD_3OD) δ_H 7.55–7.33 (m, 5 H, arom), 5.96 (m, 1 H, H^e), 5.63 (s, 1 H, C*H*Ph), 5.27 (m, 1 H, H^b), 5.12 (m, 1 H, H^a), 4.52 (d, 1 H, $J_{1',2'}$ 7.8, H-1′), 4.29–4.12 (m, 6 H, H^d, H^e, H-1, H-4′, H-6′a, H-6′b), 3.88 (m, 2 H, H-6a, H-6b), 3.70 (dd, 1 H, $J_{3',4'}$ 3.6, $J_{2',3'}$ 9.7, H-3′), 3.62–3.54 (m, 3 H, H-3, H-4, H-5′), 3.52 (s, 3 H, Me), 3.45 (dd, 1 H, H-2′), 3.42–3.37 (m, 1 H, H-5), 3.23 (m, 1 H, H-2) (Found: C, 56.8; H, 6.7. Calc. for $C_{23}H_{32}O_{11}$: C, 57.0; H, 6.7%).

Methyl 4-*O*-(2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 60

Benzyl bromide (1.1 mL, 9.33 mmol) was added to a mixture of **59** (780 mg, 1.79 mmol) and 95% NaH (235 mg, 9.33 mmol) in dry DMF (5 mL). After 1 h the reaction was quenched with methanol and the mixture was diluted with ethyl acetate. The solution was washed with brine, and then concentrated. Chromatography of the residue on silica gel with hexane–ethyl acetate (7 : 3) gave **60** (1.35 g, 89%), $[\alpha]_D$ +23.0 (*c* 0.3, $CHCl_3$); mp 128–129 °C; ^{1}H NMR ($CDCl_3$) δ_H 7.50–7.12 (m, 25 H, arom), 5.91 (m, 1 H, H^e), 5.42 (s, 1 H, C*H*Ph), 5.24 (1 H, H^b), 5.13 (d, 1 H, ^2J 10.5, CH_2Ph), 5.11 (1 H, H^a), 4.86 (d, 1 H, ^2J 11.0, CH_2Ph), 4.78–4.65 (m, 4 H, CH_2Ph), 4.41 (d, 1 H, $J_{1',2'}$ 7.8, H-1′), 4.29 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.29–4.19 (m, 2 H, H^d, H^e), 4.15 (dd, 1 H, $J_{6'a,6'b}$ 12.2, $J_{5',6'a} <$ 1, H-6′a), 4.02–4.92 (m, 3 H, H-4, H-6a, H-4′), 3.84 (dd, 1 H, $J_{6a,6b}$ 9.5, $J_{5,6b}$ 1.4, H-6b), 3.80 (dd, 1 H, $J_{5',6'b}$ 1.8, H-6′b), 3.63–3.55 (m, 2 H, H-2′, H-3), 3.55 (s, 3 H, Me), 3.44–3.38 (m, 2 H, H-2, H-5), 3.28 (dd, 1 H, $J_{3',4'}$ 3.7, $J_{2',3'}$ 9.6, H-3′), 2.85 (br s, 1 H, H-5′) (Found: C, 72.4; H, 6.6. Calc. for $C_{51}H_{56}O_{11}$: C, 72.5; H, 6.7%).

Methyl 4-*O*-(2-*O*-allyl-3-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 61

Disaccharide **60** (2.13 g, 2.52 mmol) was dissolved in 80% aq. acetic acid (30 mL) and the solution was stirred at 80 °C for 2 h. Solvents were evaporated off, and the residue was co-

evaporated with toluene (3 ×) and then chromatographed on silica gel with hexane–ethyl acetate (4 : 1) to give **61** (1.6 g, 85.6%), $[\alpha]_D$ +31.0 (*c* 0.6, $CHCl_3$); mp 119–120 °C; ^{1}H NMR ($CDCl_3$) δ_H 7.20–7.10 (m, 20 H, arom), 5.90 (1 H, H^c), 5.25 (1 H, H^b), 5.13 (1 H, H^a), 4.97 (d, 1 H, ^2J 10.9, CH_2Ph), 4.87 (d, 1 H, ^2J 11.0, CH_2Ph), 4.77 (d, 1 H, ^2J 10.9, CH_2Ph), 4.72 (d, 1 H, ^2J 11.1, CH_2Ph), 4.68 (s, 2 H, CH_2Ph), 4.66 (d, 1 H, ^2J 12.1, CH_2Ph), 4.50 (d, 1 H, ^2J 12.1, CH_2Ph), 4.34 (d, 1 H, $J_{1',2'}$ 7.9, H-1′), 4.30 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.30–4.18 (m, 2 H, H^c, H^d), 3.95–3.80 (m, 4 H, H-4, H-6a, H-6b, H-4′), 3.62–3.38 (m, 9 H, H-2, H-3, H-5, H-2′, H-6′a, H-6′b, Me), 3.24 (dd, 1 H, $J_{2',3'}$ 9.3, $J_{3',4'}$ 3.4, H-3′), 3.09 (m, 1 H, H-5′), 2.58 (d, 1 H, $J_{4',OH}$ 1.9, 4′-OH), 2.02 (dd, 1 H, $J_{6'a,OH}$ 4.7, $J_{6'b,OH}$ 8.3, 6′-OH) (Found: C, 69.8; H, 7.0. Calc. for $C_{44}H_{52}O_{11}$: C, 69.8; H, 6.9%).

Methyl 4-*O*-(2-*O*-allyl-6-*O*-benzoyl-3-*O*-benzyl-β-D-galacto-pyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 62

To a solution of **61** (1.53 g, 2.024 mmol) in dry pyridine (10 mL) was added benzoyl chloride (0.235 mL, 2.024 mmol) dropwise at 0 °C under an argon atmosphere. After 3 h a few drops of water were added to the mixture and all solvents were evaporated off. Chromatography of the residue on silica gel with hexane–ethyl acetate (4 : 1) gave **62** (1.57 g, 90.2%), $[\alpha]_D$ +19.7 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.0–7.1 (m, 25 H, arom), 5.90 (m, 1 H, H^c), 5.24 (m, 1 H, H^b), 5.13 (m, 1 H, H^a), 4.98 (d, 1 H, ^2J 10.8, CH_2Ph), 4.84 (d, 1 H, ^2J 11.0, CH_2Ph), 4.76–4.64 (m, 5 H, CH_2Ph), 4.48 (dd, 1 H, $J_{5',6'a}$ 6.6, $J_{6'a,6'b}$ 11.2, H-6′a), 4.46 (d, 1 H, ^2J 12.1, CH_2Ph), 4.39 (d, 1 H, $J_{1',2'}$ 7.8, H-1′), 4.28 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.28–4.16 (m, 3 H, H^d, H^e, H-6′b), 3.98 (t, 1 H, $J_{3,4} \approx J_{4,5} \approx$ 9.1, H-4), 3.91 (dd, 1 H, $J_{5,6a}$ 4.0, $J_{6a,6b}$ 10.9, H-6a), 3.84–3.78 (m, 2 H, H-4′, H-6b), 3.58 (t, 1 H, $J_{2,3}$ 9.1, H-3), 3.54 (s, 3 H, Me), 3.46–3.33 (m, 4 H, H-2, H-2′, H-5, H-5′), 3.26 (dd, 1 H, $J_{2',3'}$ 9.3, $J_{3',4'}$ 3.5, H-3′), 2.38 (br s, 1 H, 4′-OH) (Found: C, 71.0; H, 6.7. Calc. for $C_{51}H_{56}O_{12}$: C, 71.1; H, 6.6%).

Methyl 4-*O*-[2-*O*-allyl-3-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 63

A solution of freshly prepared 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[12] **30** (5.63 g, 10.07 mmol) in dry toluene (30 mL) was added dropwise to a stirred mixture of **62** (5.45 g, 6.34 mmol), silver triflate (3.08 g, 12 mmol) and 2,4,6-collidine (1.6 mL, 12 mmol) in dry toluene (100 mL) at −40 °C. The reaction mixture was allowed to warm to room temperature. After 2 h the mixture was transferred to a separatory funnel, washed with aq. $Na_2S_2O_3$, and extracted with toluene. All solvents were evaporated off and the crude product was used for the next step without purification. The product was treated with a catalytic amount of NaOMe in MeOH–THF at room temperature overnight until the benzoyl group was removed. The reaction mixture was neutralized with acetic acid, concentrated, taken up into DCM, washed with water, and concentrated. Column chromatography (hexane–ethyl acetate, 4 : 1) yielded **63** (6.02 g, 74.3%), $[\alpha]_D$ +29.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.35–7.10 (m, 40 H, arom), 5.82 (1 H, H^c), 5.16 (1 H, H^b), 5.08–5.03 (m, 2 H, H^a, CH_2Ph), 4.96 (d, 1 H, $J_{1'',2''}$ 3.3, H-1″), 4.86–4.78 (m, 3 H, CH_2Ph), 4.69–4.43 (m, 10 H, CH_2Ph), 4.37 (d, 1 H, $J_{1',2'}$ 7.7, H-1′), 4.31–4.21 (m, 3 H, H-1, CH_2Ph), 4.15–4.08 (m, 3 H, H-5″, H^c, H^d), 4.02 (dd, 1 H, $J_{2'',3''}$ 10.1, H-2″), 3.93–3.79 (m, 6 H, H-4, H-6a, H-6b, H-4′, H-3″, H-4″), 3.70–3.57 (m, 2 H, H-6′a, H-6′b), 3.54 (s, 3 H, Me), 3.53 (t, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0, H-3), 3.45–3.32 (m, 4 H, H-2, H-5, H-2″, H-6″a), 3.28 (dd, 1 H, $J_{6''a,6''b}$ 8.9, $J_{5'',6''b}$ 5.4, H-6″b), 3.22–3.16 (m, 2 H, H-3′, H-5′) (Found: C, 73.3; H, 6.9. Calc. for $C_{78}H_{86}O_{16}$: C, 73.2; H, 6.8%).

Methyl 4-*O*-[2-*O*-allyl-3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 64

Benzyl bromide (0.84 mL, 7.07 mmol) was added with stirring to a solution of **63** (6.02 g, 4.71 mmol) in DMF (50 mL) containing a suspension of 95% NaH (180 mg, 7.07 mmol) at room temperature. After 3 h, excess of sodium hydride was decomposed by adding a few drops of methanol. Solvents were evaporated off, the residue was dissolved in ethyl acetate, and the solution was washed with brine and concentrated. Column chromatography (hexane–ethyl acetate, 9 : 1) of the residue gave **64** (5.4 g, 83.8%), $[\alpha]_D$ +34.2 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.38–7.10 (m, 45 H, arom), 5.91 (1 H, H^c), 5.23 (1 H, H^b), 5.11 (1 H, H^a), 5.05–5.00 (m, 2 H, H-1″, CH_2Ph), 4.83 (d, 1 H, ^2J 11.2, CH_2Ph), 4.81 (d, 1 H, ^2J 11.0, CH_2Ph), 4.75–4.62 (m, 6 H, CH_2Ph), 4.52–4.40 (m, 6 H, H-1′, CH_2Ph), 4.34–3.89 (m, 10 H, H-1, H-4, H-6a, H-4′, H-5′, H-2″, H-3″, H-4″, H-5″, CH_2Ph, H^d, H^e), 3.83 (dd, 1 H, $J_{5,6b}$ 1.5, $J_{6a,6b}$ 10.8, H-6b), 3.55 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 8.0, H-3), 3.53 (s, 3 H, Me), 3.50–3.38 (m, 4 H, H-5, H-2′, H-6′a, H-6″a), 3.35 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.1, H-2), 3.24 (dd, 1 H, $J_{5',6'b}$ 5.3, $J_{6'a,6'b}$ 8.4, H-6′b), 3.19 (dd, 1 H, $J_{3',4'}$ 2.7, $J_{2',3'}$ 10.0, H-3′), 3.12 (dd, 1 H, $J_{5'',6''b}$ 4.6, $J_{6''a,6''b}$ 8.2, H-6″b) (Found: C, 74.4; H, 6.8. Calc. for $C_{85}H_{92}O_{16}$: C, 74.5; H, 6.8%).

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[3,6-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 65

A solution of **64** (95 mg, 0.069 mmol) in a mixture of acetic acid (1 mL) and methanol (1.5 mL) was stirred in the presence of $PdCl_2$ (20 mg) overnight at room temperature. The reaction mixture was diluted with methanol, filtered through Celite and concentrated. Chromatography of the residue in hexane–ethyl acetate (7 : 3) gave **65** (90 mg, 97%), $[\alpha]_D$ +36.7 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.35–7.10 (m, 45 H, arom), 4.98–4.94 (m, 2 H, H-1″, CH_2Ph), 4.86–4.41 (m, 16 H, CH_2Ph), 4.52 (d, 1 H, $J_{1',2'}$ 7.5, H-1′), 4.35 (ddd, 1 H, $J_{5'',6''a}$ 4.6, $J_{5'',6''b}$ 8.5, $J_{4'',5''}$ 0.6, H-5″), 4.27 (d, 1 H, $J_{1,2}$ 7.7, H-1), 4.12–3.93 (m, 10 H, H-4, H-6a, H-4′, H-6′a, H-6′b, H-2″, H-3″, H-4″, CH_2Ph), 3.82 (dd, 1 H, $J_{5,6b}$ 1.7, $J_{6a,6b}$ 11.5, H-6b), 3.73 (dd, 1 H, $J_{2',3'}$ 10.1, H-2′), 3.62 (t, 1 H, $J_{2,3} \approx J_{3,4}$ 9.0, H-3), 3.54 (s, 3 H, Me), 3.50–3.44 (m, 2 H, H-5, H-6″a), 3.36 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 8.9, H-2), 3.27–3.12 (m, 3 H, H-3′, H-5′, H-6″b) (Found: C, 73.9; H, 6.7. Calc. for $C_{82}H_{88}O_{16}$: C, 74.1; H, 6.7%).

Methyl 2,3,6-tri-*O*-benzyl-4-*O*-[3,6-di-*O*-benzyl-2-*O*-carboxy-methyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 66

To a solution of **65** (146 mg, 110 μmol) in DMF were added (1 mL) NaH (95%; 27 mg, 10 eq.) and chloroacetic acid (31.2 mg, 3 eq). After 3 h at 80 °C the reaction was quenched by methanol. The mixture was acidified with 1 M HCl, taken up in ethyl acetate, washed with water, and the organic layer was concentrated. Chromatography of the residue in hexane–ethyl acetate (7 : 3) gave **66** (130 mg, 85%), $[\alpha]_D$ +36.8 (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 7.35–7.10 (m, 45 H, arom), 5.00 (d, 1 H, $J_{1'',2''}$ 3.4, H-1″), 4.93 (d, 1 H, ^2J 10.7, CH_2Ph), 4.84–4.62 (m, 8 H, CH_2Ph), 4.51–4.13 (m, 14 H, H-1, H-1′, H-5″, CH_2COO, CH_2Ph), 4.09–4.97 (m, 4 H, H-4, H-4′, H-2″, H-4″), 3.91 (dd, 1 H, $J_{2'',3''}$ 10.3, $J_{3'',4''}$ 2.6, H-3), 3.77 (d, 2 H, $J_{5,6a} \approx J_{5,6b} \approx$ 2.5, H-6a, H-6b), 3.54 (s, 3 H, Me), 3.52–3.35 (m, 6 H, H-3, H-5, H-2′, H-6′a, H-6′b, H-6″a), 3.35 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.0, H-2), 3.26–3.16 (m, 3 H, H-3′, H-5′, H-6″b) (Found: C, 73.0; H, 6.7. Calc. for $C_{84}H_{90}O_{18}$: C, 72.7; H, 6.5%).

Methyl 4-*O*-[2-*O*-carboxymethyl-4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 13

A solution of **66** (42 mg, 30.2 μmol) in acetic acid (5 mL) was stirred under a flow of H_2 in the presence of Pd/C (30 mg) overnight. Catalyst was filtered off, the supernatant was concentrated and the residue was taken up in water and passed through Sep-Pak C_{18} to give **13** (16 mg, 92%), $[\alpha]_D$ +60.0 (*c* 0.2, H_2O); ^{1}H NMR (D_2O) δ_H 4.94 (d, 1 H, $J_{1'',2''}$ 3.5, H-1″), 4.60

(d, 1 H, $J_{1',2'}$ 7.8, H-1′), 4.44–4.29 (m, 4 H, H-1, H-5″, CH_2COO), 4.05 (br s, 2 H, H-4′, H-4″), 4.00–3.62 (m, 13 H, H-3, H-4, H-5, H-6a, H-6b, H-3′, H-5′, H-6′a, H-6′b, H-2″, H-3″, H-6″a, H-6″b), 3.57 (s, 3 H, OMe), 3.48 (dd, 1 H, $J_{2',3'}$ 10.1, H-2′), 3.29 (t, 1 H, $J_{1,2} \approx J_{2,3} \approx 8.2$, H-2); *m/z* (EI) 599.1802 (MNa^+. $C_{21}H_{36}O_{18}$·Na requires *m/z*, 599.1799).

8-Azido-3,6-dioxaoctyl 4-*O*-[6-*O*-acetyl-2,3-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside 68

A mixture of imidate **67**[16] (396 mg, 0.287 mmol), 8-azido-3,6-dioxaoctan-1-ol[17] (62 mg, 1.5 eq.), and mol. sieves (4 Å, 1 g) in DCM (5 mL) was stirred under an argon atmosphere for 1 h, then TMSOTf (10 μL) was added. After 30 min the solution was filtered through Celite and concentrated. Chromatography of the residue on silica gel with pentane–ethyl acetate (1 : 1) gave **68** (340 mg, 85%), $[\alpha]_D$ +92 (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ_H 8.04–7.80 (m, 25 H, arom), 5.76 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx 9.2$, H-3), 5.64 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.8, H-2′), 5.47 (dd, 1 H, $J_{4'',5''} < 1$, $J_{3'',4''}$ 2.7, H-4″), 5.37 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.5, H-2), 5.30 (dd, 1 H, $J_{2'',3''}$ 11.0, H-3″), 5.10 (dd, 1 H, $J_{1'',2''}$ 3.6, H-2″), 5.04 (dd, 1 H, $J_{3',4'}$ 2.7, H-3′), 4.82 (d, 1 H, H-1′), 4.79 (d, 1 H, H-1), 4.61 (dd, 1 H, $J_{5,6a}$ 1.9, $J_{6a,6b}$ 12.0, H-6a), 4.49–4.41 (m, 2 H, H-6b, H-5″), 4.18 (t, 1 H, H-4), 4.14 (d, 1 H, H-4′), 3.90–3.27 (m, 18 H, H-5, H-5′, H-6′a, H-6′b, H-6″a, H-6″b, CH_2), 2.04, 1.99, 1.97, 1.94 and 1.93 (5 s, 15 H, 5 × Ac) (Found: C, 59.4; H, 5.05; N, 2.9. Calc. for $C_{69}H_{73}N_3O_{28}$: C, 59.5; H, 5.3; N, 3.0%).

8-Azido-3,6-dioxaoctyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 69

A solution of **68** (320 mg, 0.23 mmol) and MeONa (30 mg) in methanol (5 mL) was refluxed for 2 h, then neutralized with Dowex 50W (H^+), and concentrated. An aqueous solution of the residue was passed through a Sep-Pak C_{18} cartridge and concentrated to give **69** (143 mg, 94%), $[\alpha]_D$ +67.4 (*c* 0.5, H_2O); ^{1}H NMR (D_2O) δ_H 4.95 (d, 1 H, $J_{1'',2''}$ 3.8, H-1″), 4.52 (d, 1 H, $J_{1,2}$ 8.0, H-1), 4.51 (d, 1 H, $J_{1',2'}$ 7.7, H-1′), 4.35 (t, 1 H, $J_{5'',6''a} \approx J_{5',6''b} \approx 6.3$, H-5″), 4.10–4.57 (m, 26 H, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6′a, H-6′b, H-2″, H-3″, H-4″, H-6″a, H-6″b, OCH_2), 3.51 (t, 2 H, ^3J 4.6, CH_2N), 2.34 (t, 1 H, H-2); *m/z* (EI) 662.2545 (MH^+. $C_{24}H_{44}N_3O_{18}$ requires *m/z* 662.2620).

8-[(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]-3,6-dioxaoctyl 4-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranoside 71

A mixture of **69** (30 mg, 45 μmol) and $Pd(OH)_2$/C (25 mg) in methanol (5 mL) was stirred under a flow of H_2 for 3 h. The mixture was filtered through Celite and concentrated. To a solution of the residue **70** in EtOH (1 mL)–water (2 mL) were added diethyl squarate (10 μL, 67 μmol) and Et_3N (6 μL). After 16 h the mixture was concentrated, and chromatographed on silica gel with DCM–MeOH–water (300 : 300 : 5) to give **71** (25 mg, 70%), $[\alpha]_D$ +39.6 (*c* 0.3, H_2O); ^{1}H NMR (D_2O) δ_H 4.95 (d, 1 H, $J_{1'',2''}$ 3.8, H-1″), 4.76–4.71 (m, 2 H, CH_2CH_3), 4.51 (d, 1 H, $J_{1,2}$ 7.8, H-1), 4.50 (d, 1 H, $J_{1',2'}$ 7.9, H-1′), 4.36 (t, 1 H, $J_{5'',6''a} \approx J_{5'',6''b} \approx 6.6$, H-5″), 4.06–3.56 (m, 28 H, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-5′, H-6′a, H-6′b, H-2″, H-3″, H-4″, H-6″a, H-6″b, OCH_2), 3.34 (t, 1 H, H-2), 1.45 (dd, 3 H, ^3J 7.1, ^3J 14, CH_2CH_3); *m/z* (EI) 782.2693 (MNa^+. $C_{30}H_{49}NO_{21}$·Na requires 782.2695).

Synthesis of the biotinylated P^k–BSA glycoconjugate

A solution of BSA (5 mg) and trisaccharide **71** (2.87 mg, 50 eq.) in borate buffered potassium bicarbonate pH 9 (0.35 M, $KHCO_3$–$Na_2B_4O_7$ 0.07 M) was stirred for 24 h, then biotin-NHS (2.58 mg, 100 eq.) was added, and the mixture was stirred for 48 h, then dialyzed and lyophilized.

Inhibition ELISA assay

PVC microtiter plates (Gibco BRL Inc.) were incubated with *Shiga*-like toxin Type 1 (2.5 μg mL^{-1}, 100 μL $well^{-1}$) at room temperature overnight, then washed (6 ×) with PBST (0.05% Tween 20 in phosphate buffer saline, PBS). Blocking solution (2.5% skimmed milk in PBS, 100 μL $well^{-1}$) was added and the plates were incubated for 1 h at room temperature, then washed (6 ×) with PBST. An inhibitor at decreasing concentrations (starting from ≈50 mg mL^{-1}, 3.16 dilution factor; 50 μL $well^{-1}$) was mixed with biotinylated P^k–BSA glycoconjugate (2 μg mL^{-1}; 50 μL $well^{-1}$) and the mixtures were applied to the plates in triplicate. After incubation at room temperature for 18 h, the plates were washed (6 ×) with PBST, incubated with streptavidine–horseradish peroxidase in PBST (1 μg mL^{-1}; 100 μL $well^{-1}$) for 1 h at room temperature, then washed (6 ×) with PBST. 3,3′,5,5′-Tetramethylbenzidine (TMB) peroxidase substrate was added (both the streptavidine–horseradish peroxidase and its TMB substrate were purchased from Gibco BRL Inc.). After 2–5 min the reaction was quenched by addition of H_3PO_4 (100 μL $well^{-1}$). The plates were read at 450 nm.

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