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Binding studies on internal immunodeterminants: synthesis of β-(1 → 6)-linked oligosaccharide methyl glycosides having one to four internal D-galactopyranosyl residues flanked by gentiobiose residues

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ABSTRACT

The oligosaccharide glycosides β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-(1 → 6)-[β-D-Gal*p*-(1 → 6)]$_n$-β-D-Glc*p*-(1 → 6)-β-D-Glc*p*-1 → OMe ($n = 1$–4) were prepared by a convergent block synthesis. Haloacetyl, *tert*-butyldiphenylsilyl, and dimethylthexylsilyl groups were used as temporary protective groups for the preparation of the intermediate glycosyl donors and acceptors. The deoxygenated trisaccharide glycosides β-D-Glc*p*-(1 → 6)-β-D-Gal*p*-(1 → 6)-4-deoxy-β-D-*xylo*-Hex*p*-1 → OMe and β-D-Glc*p*-(1 → 6)-4-deoxy-β-D-*xylo*-Hex*p*-(1 → 6)-β-D-Gal*p*-1 → OMe were also synthesized. The binding of each glycoside to the monoclonal antigalactan antibody IgA J539 was studied and the results support the previous finding that J539 can bind to internal antigenic epitopes. The data are consistent with the interpretation that subsite C of that antibody binds glucose with a K_a of ~6 (cf. 10.9 for galactose).

INTRODUCTION

Antibodies of the gene family[1–4] X24 that bind to sequences of (1 → 6)-β-D-galacto-oligosaccharides have been studied[3–5] in detail. Of these galactan-specific antibodies, IgA J539 has been investigated the most extensively. Deductions on the mode of binding of this antibody were made from genetic studies and by measuring the affinity constants of numerous synthetic oligosaccharides, including deoxy and deoxyfluoro derivatives[3–8]. On the basis of the finding[4] that IgA J539 shows the highest affinity for (1 → 6)-β-D-galactotetraose and binds to a (1 → 6)-β-D-galactopyranan isolated from *Prototheca zopfi*[9], it was proposed that the antibody is capable of "reading" *internal* epitopes of four galactose residues of a sugar chain and, thus, belongs to the so-called "groove-binding" type[10]. That this is so has been

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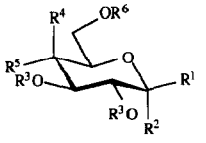
demonstrated[11] with a synthetic heptasaccharide glycoside that comprised a (1 → 3)-linked β-D-galactotriose terminus (by itself nonbinding) β-(1 → 6)-linked to the methyl β-glycoside of (1 → 6)-linked β-D-galactotetraose, which had a K_a similar to that of the corresponding (1 → 6)-linked methyl β-D-galactotetraoside. However, since this heptasaccharide glycoside did not present "true" *internal* sequences of (1 → 6)-linked-β-D-galactose residues to the antibody, we have now prepared (1 → 6)-linked oligosaccharide glycosides with 1–4 internal β-D-galactose residues flanked by a β-gentiobiose terminus and a methyl β-gentiobioside residue at the aglycon-bearing end, and measured their K_a values with IgA J539.

RESULTS AND DISCUSSION

Synthesis. — In order to reduce the number of steps in the synthesis, the desired β-(1 → 6)-linked oligosaccharide glycosides were synthesized by a blockwise approach which involved di- and tri-saccharide derivatives temporarily protected at O-6′ and O-6″, respectively. The haloacetyl group was used for this purpose since it can be cleaved selectively under mild conditions and has been used for the synthesis of higher β-(1 → 6)-linked oligosaccharides[11–13]. The β-gentiobiose derivatives **11** and **12**, which were needed to build the moieties flanking the inner β-(1 → 6)-galacto-oligosaccharide units, were prepared from amygdalin as described[14].

*β-D-Glc*p-*(1 → 6)-β-D-Glc*p-*(1 → 6)-β-D-Gal*p-*(1 → 6)-β-D-Glc*p-*(1 → 6)-β-D-Glc*p-*1 → OMe* (**34**). — For the construction of the benzoylated derivative **33** of **34**, the trisaccharide building block **22** was required and was synthesized as follows from methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**6**). Compound **6** has been obtained from methyl β-D-glucopyranoside (**1**) by selective 6-bromoacetylation, benzoylation, and debromoacetylation with thiourea[13]. In an alternative synthesis of **6**, the easily removable *tert*-butyldiphenylsilyl[8,14–17] and dimethylthexylsilyl[14,18] groups were used. Treatment of **1** with *tert*-butylchlorodiphenylsilane–imidazole or chlorodimethylthexylsilane–imidazole gave compounds **2** (70%) or **3** (43%), each of which was benzoylated, to give **4** (83%) or **5** (92%). Treatment of **4** or **5** with boron trifluoride in methanol[8,14] gave **6**. The conversion **5** → **6** proceeded significantly faster (2 h) and gave a higher yield (99%) than did the conversion **4** → **6** (2 days, 90%). This difference in the reactivity has been observed in the gentiobiose series[14]. Condensation of **6** and 2,3,4-tri-*O*-benzoyl-6-*O*-bromoacetyl-α-D-galactopyranosyl bromide[19] (**7**) gave methyl *O*-(2,3,4-tri-*O*-benzoyl-6-*O*-bromoacetyl-β-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**15**). Treatment of **15** with dichloromethyl methyl ether gave the glycosyl chloride **17** together with a small proportion of the methyl α-D-glycoside **16**. Similar side reactions have been found[12,20] with this reagent. Silver trifluoromethanesulfonate-promoted condensation of **6** and the disaccharide donor **17** then gave the trisaccharide derivative **21** (39%). The poor yield of **21** was due to the formation of an unidentified by-product which could not be removed easily by

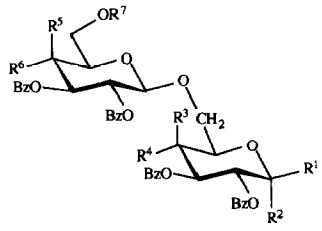


No.	R^1	R^2	R^3	R^4	R^5	R^6
1	OMe	H	H	H	OH	H
2	OMe	H	H	H	OH	$SiPh_2^tBu$
3	OMe	H	H	H	OH	$SiMe_2thex$
4	OMe	H	Bz	H	OBz	$SiPh_2^tBu$
5	OMe	H	Bz	H	OBz	$SiMe_2thex$
6	OMe	H	Bz	H	OBz	H
7	H	Br	Bz	OBz	H	BrAc
8	OMe	H	Bz	OBz	H	H
9	OMe	H	Bz	H	H	H
10	H	Br	Bz	H	OBz	Bz

chromatography. In an alternative approach, coupling of methyl *O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**11**) and 2,3,4-tri-*O*-benzoyl-6-*O*-bromoacetyl-α-D-galactopyranosyl bromide (**7**) gave **21** (98%). Finally, the 6″-*O*-bromoacetyl group in **21** was removed on treatment with thiourea, to give the target acceptor **22** (78%) that was condensed with hepta-*O*-benzoyl-β-gentiobiosyl chloride (**12**) to give **33** (50%). *O*-Deacetylation (Zemplén) of **33** then afforded **34** (55%)

β-D-*Glc*p-*(1 → 6)*-*β*-D-*Glc*p-*(1 → 6)*-*[β*-D-*Gal*p-*(1 → 6)]*$_2$-*β*-D-*Glc*p-*(1 → 6)*-*β*-D-*Glc*p-*1 → OMe* (**36**). — Originally, it was planned to synthesize oligosaccharide derivatives having 2–4 internal galactose residues from a tetrasaccharide nucleophile comprising two (1 → 6)-linked β-D-galactose units (1 → 6)-linked to methyl β-gentiobioside. Therefore, *O*-(2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-α-D-galactopyranosyl chloride (**14**) was prepared via an improved one-pot procedure of its known[11] synthesis (see Experimental Section). However, all attempts to couple **11** and **14**, were unsuccessful, and extensive decomposition of **14** occurred (TLC). In an alternative approach, **22** was reacted with the galactosyl donor **7**, to give, first, the 6‴-bromoacetylated tetrasaccharide derivative **29** (87%), treatment of which with thiourea gave **30** (95%). Condensation of **30** and **12** gave the hexasaccharide derivative **35** (68%), *O*-deacylation of which gave the target hexasaccharide glycoside **36** (78%).

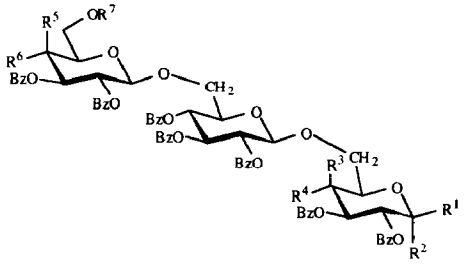
β-D-*Glc*p-*(1 → 6)*-*β*-D-*Glc*p-*(1 → 6)*-*[β*-D-*Gal*p-*(1 → 6)]*$_3$-*β*-D-*Glc*p-*(1 → 6)*-*β*-D-*Glc*p-*1 → OMe* (**38**). — Treatment of *O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-*O*-(2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-1,2,3,4-tetra-*O*-benzoyl-α-D-galactopyranose[14] (**23**) with dichloromethyl methyl ether gave the glycosyl chloride **24** (78%), which was coupled with **30** to give the heptasaccharide derivative **37** (69%). *O*-Deacylation of **37** then gave the target glycoside **38** (98%).



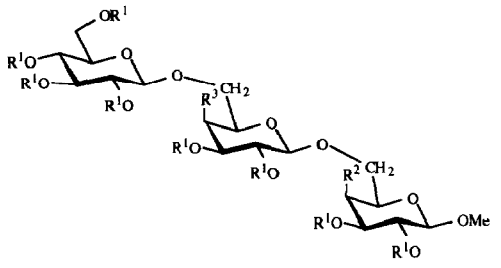
No.	R^1	R^2	R^3	R^4	R^5	R^6	R^7
11	OMe	H	H	OBz	H	OBz	H
12	H	Cl	H	OBz	H	OBz	OBz
13	H	OBz	OBz	H	OBz	H	ClAc
14	H	Cl	OBz	H	OBz	H	ClAc
15	OMe	H	H	OBz	OBz	H	BrAc
16	H	OMe	H	OBz	OBz	H	BrAc
17	H	Cl	H	OBz	OBz	H	BrAc
18	OMe	H	OBz	H	H	OBz	Bz
19	H	Cl	OBz	H	H	OBz	Bz
20	OMe	H	OBz	H	H	H	H

β-D-*Glc*p-(*1* → *6*)-*β*-D-*Glc*p-(*1* → *6*)-[*β*-D-*Gal*p-(*1* → *6*)]$_4$-*β*-D-*Glc*p-(*1* → *6*)-*β*-D-*Glc*p-*1* → *OMe* (**40**). — Condensation of **22** and **14** resulted in the 6″″-chloroacetylated pentasaccharide derivative **31** (63%), treatment of which with thiourea removed the chloroacetyl group to give **32** (80%). Silver trifluoromethanesulfonate-promoted condensation of **32** and **24** gave the octasaccharide derivative **39** (60%), *O*-deacylation of which afforded the target glycoside **40** (97%).

β-D-*Glc*p-(*1* → *6*)-*β*-D-*Gal*p-(*1* → *6*)-*4-deoxy*-*β*-D-xylo-*Hex*p-*1* → *OMe* (**26**). — Silver trifluoromethanesulfonate-promoted coupling of methyl 2,3,4-tri-*O*-benzoyl-

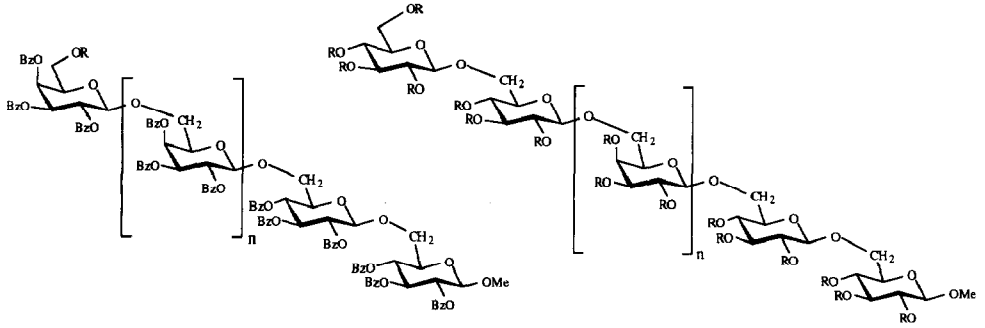


No.	R^1	R^2	R^3	R^4	R^5	R^6	R^7
21	OMe	H	H	OBz	OBz	H	BrAc
22	OMe	H	H	OBz	OBz	H	H
23	H	OBz	OBz	H	H	OBz	OBz
24	H	Cl	OBz	H	H	OBz	OBz



No.	R^1	R^2	R^3
25	Bz	H	OBz
26	H	H	OH
27	Bz	OBz	H
28	H	OH	H

β-D-galactopyranoside[13,21] (**8**) with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[22] (**10**) gave the disaccharide derivative **18** (91%), which was converted into the glycosyl chloride **19** (69%) by treatment with dichloromethyl methyl ether. Condensation of **19** and methyl 2,3-di-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranoside[8] (**9**) then resulted in the trisaccharide derivative **25** (73%), *O*-deacylation of which afforded the target glycoside **26** (87%).



No	n	R
29	1	BrAc
30	1	H
31	2	ClAc
32	2	H

No	n	R
33	1	Bz
34	1	H
35	2	Bz
36	2	H
37	3	Bz
38	3	H
39	4	Bz
40	4	H

TABLE I

Binding constants (K_a) and percent maximal ligand-induced fluorescence change ($LIFC_{max}$) for IgA J539

Compound [a]	Saccharide [b]	K_a (M^{-1})	$LIFC_{max}$
41	β-Gal-OMe	1.0×10^3	21
34	β1,6-Glc_2GalGlc_2-OMe	4.7×10^3	24
42	β1,6-Gal_2-OMe	4.7×10^4	41
36	β1,6-Glc_2-Gal_2-Glc_2-OMe	2.95×10^4	34
43	β1,6-Gal_3-OMe	4.8×10^5	43
38	β1,6-Glc_2-Gal_3-Glc_2-OMe	6.3×10^5	37
44	β1,6-Gal_4-OMe	5.8×10^5	38
40	β1,6-Glc_2-Gal_4-Glc_2-OMe	2.3×10^5	36
45	β1,6-Glc-Gal-OMe	6.2×10^3 [c]	23
46	β1,6-Glc-Glc-OMe	– [c]	0
47	β1,6-Gal-4dGal-OMe	1.3×10^4	36
48	β1,6-Gal-Gal-4dGal-OMe	1.8×10^5 [c]	38
26	β1,6-Glc-Gal-4dGal-OMe	8.8×10^4 [c]	39
49	β1,6-Gal-4dGal-Gal-OMe	4.7×10^4	35
28	β1,6-Glc-4dGal-Gal-OMe	6.9×10^3	36

[a] The binding constants of **41–45** and **47–49** have been reported[4,8]. [b] 4dGal, 4-deoxy-D-*xylo*-hexopyranose. [c] Measured with J539 Fab'.

*β-D-Glc*p-*(1 → 6)-4-deoxy-β-D*-xylo-*Hex*p-*(1 → 6)-β-D-Gal*p-*1 → OMe* (**28**). — Condensation of **10** with methyl *O*-(2,3-di-*O*-benzoyl-4-deoxy-β-D-*xylo*-hexopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside[8] (**20**) gave the trisaccharide derivative **27** (93%), *O*-deacylation of which gave the target glycoside **28** (98%).

Each oligosaccharide glycoside was purified by chromatography on silica gel, followed by additional elution from a column of Bio-Gel P2.

Observations on the binding to antibody J539. — Previous work[3,5] showed that the subsites A–D have "partial" affinity constants for their galactosyl 10^3, 47, 11, and 1.2, respectively, because the free energy of binding for the tetrasaccharide determinant is the sum of the partial free energies of binding for each component subsite[23]. The data in Table I show that the oligosaccharide glycosides **34**, **36**, **38**, and **40** bind to the antibody with affinities that are similar to those of galactosyl oligosaccarides (**41–44**) that do not have flanking gentiobiosyl residues. Thus, the antibody appears to bind its specific sugar(s) even if it is located between different sugars. The affinities of the oligosaccharide glycosides **36**, **38**, and **40**, which possess several inner galactosyl residues, have small differences from those of the corresponding saccharides not flanked by gentiobiosyl residues. However, **34** differs more substantially from **41**, which may be due to the weak binding ($K_a \sim 6$) of a β-glucosyl residue in subsite C. This view was suggested by the relative binding affinities of **45** and **41**, as well as of **26** and **47**, where the glucosyl-containing ligand binds some 6 times better (see Table II for the assignment of subsites). In both **45** and **26**, the glucose must be assigned subsite C. However, it is not clear

why a low affinity of the glucosyl residue for subsite C would not be reflected in an additional binding affinity of **36** when compared to that of **42**. It could be that the conformation of **36** may not allow ready contact of its glucosyl moiety with subsite C. Since the 4dGal residue should not bind[8] in subsite A, the glycoside **28** was expected to bind in subsites C (4dGal, subsite-K_a 11) and A (Gal-OMe, subsite-K_a 10^3) with a predicted K_a of $\sim 11 \times 10^3$, rather than in C (weakly with its Glc residue, subsite-K_a 6), A (where 4dGal can reside without affinity), and B (Gal-OMe, subsite-K_a 47) with a computed K_a of $\sim 2.82 \times 10^2$ (6×47; if the subsite K_a of 4dGal in A is 2 or 3, i.e., too small to notice individually, K_a would rise to near 10^3). In the former mode, it would be expected to perturb only one tryptophanyl (Trp) residue, and two Trp residues in the latter. The difference in K_a values should significantly favor the former (subsites C and A) mode. The measured K_a for **28** (6.9×10^3) is in reasonable agreement with one of the computed values, but the maximal change in fluorescence indicates that *two* Trp residues are perturbed. It is possible, since a rapid equilibrium between these two modes exists in the ratio of their respective affinities, that a time-averaged, enhanced fluorescence change, with a resulting intermediate K_a, would favor the higher value. A second, a priori possible explanation that would account for both the double perturbation, *and* the value found for the K_a (6.9×10^3), would be *if* the glucosyl residue of **28** could bind in the A-subsite with a K_a of 123, while 4dGal and Gal → OMe bound in the B and D subsites, respectively. However, careful titration of whole IgA J539 with methyl β-D-glucopyranoside failed to show any discernible binding. Although an affinity of 123 is low indeed, we would have expected a noticeable effect. Thus, the last explanation must be rejected.

EXPERIMENTAL

General. — NMR spectra were obtained for solutions in $CDCl_3$ (internal Me_4Si) at 25° with a Bruker CXP 300 or AC 250 F spectrometer. The ^1H assignments in Table III and in the Experimental were made on the basis of first-order analyses. For the magnetically non-equivalent geminal protons, that resonating at lower field is designated H-6a and the other as H-6b. The ^{13}C assignments in Table IV and in the Experimental were made by mutual comparison of the spectra and by comparison with spectra of related compounds[12,14,24]. The ^{13}C-NMR spectra of **26** and **28** (Table IV) and of **34**, **36**, **38**, and **40** (Experimental section) were measured for solutions in D_2O (internal MeOH, 49.3 ppm). Optical rotations were measured at 25° with a Perkin–Elmer model 241 automatic polarimeter. Melting points were measured with a Büchi apparatus, Model SMP-20. TLC was performed on Polygram SIL UV_{254} (Macherey–Nagel) with detection by UV light and/or by charring with H_2SO_4. Preparative chromatography was performed on Silica Gel 60 (Merck), using *A*, hexane–EtOAc; *B*, CCl_4–acetone; *C*, hexane–acetone; *D*, toluene–acetone; *E*, CH_2Cl_2–MeOH; *F*,

TABLE II

Binding mode [a] of several ligands to immunoglobulin J539

Compound	C	A	B	D
41		**Gal**→**Me**		
34	*Glc*→	**Gal**→	**Glc**→	**Glc**→**Me**
42		**Gal**→	**Gal**→	**Me**
36	*Glc*→	**Gal**→	**Gal**→	**Glc**→**Glc**→**Me**
43		**Gal**→	**Gal**→	**Gal**→**Me**
38	**Glc**→	**Gal**→	**Gal**→	**Glc**→**Glc**→**Me**
44		**Gal**→	**Gal**→	**Gal**→**Me**
40	**Glc**→	**Gal**→	**Gal**→	**Gal**→**Glc**→**Glc**→**Me**
45		*Glc*→	**Gal**→	**Me**
47			**Gal**→	**4dGal** [b] →**Me**
48			**Gal**→	**Gal**→**4dGal**→**Me**
26			*Glc*→	**Gal**→**4dGal**→**Me**
49				**Gal**→**4dGal**→**Gal**→**Me**
28		**Glc**→	**4dGal**→	**Gal**→**Me**
			Glc→	**4dGal**→**Gal**→**Me**

[a] A–D represent the galactosyl-binding substitutes. The approximate location of the two solvent-exposed tryptophan residues are in subsites A (33, heavy chain) and B (91, light chain). Specific (or partially specific) binding carbohydrate residues are shown in bold type and non-specific binding carbohydrate residues are shown in italics. [b] 4-Deoxy-D-*xylo*-hexopyranose.

2-propanol–EtOAc–water in the proportions designated. Solutions in organic solvents were dried with anhyd Na_2SO_4 and concentrated at 2 kPa and $\leq 40°$.

*Methyl 6-*O*-tert-butyldiphenylsilyl-β-*D*-glucopyranoside* (**2**). — A solution of **1** (2.72 g, 14.0 mmol), imidazole (2.10 g, 30.8 mmol), and *tert*-butylchlorodiphenylsilane (3.94 mL, 15.4 mmol) in *N*,*N*-dimethylformamide (40 mL) was stirred for 3 days at room temperature, then poured into water (~ 200 mL) with stirring, and extracted with CH_2Cl_2. The extract was washed with aq $NaHCO_3$ and concentrated, to give amorphous **2** (4.21 g, 70%), $[\alpha]_D$ −33.4° (*c* 0.6, $CHCl_3$).

Anal. Calcd for $C_{23}H_{32}O_6Si$: C, 63.68; H, 7.46. Found: C, 63.86; H, 7.56.

*Methyl 6-*O*-dimethylthexylsilyl-β-*D*-glucopyranoside* (**3**). — A solution of **1** (3.34 g, 17.2 mmol), imidazole (2.63 g, 38.6 mmol), and chlorodimethylthexylsilane (3.8 mL, 19.3 mmol) in *N*,*N*-dimethylformamide (50 mL) was stirred for 17 h at room temperature. TLC (solvent *A*, 5:1) showed incomplete conversion of **1**. More imidazole (2.02 g, 29.6 mmol) and chlorodimethylthexylsilane (2.9 mL, 14.8 mmol) were added and stirring was continued for 3 days. Work-up, as described for **2**, and column chromatography (solvent *A*, 3:1) gave **3** (2.45 g, 43%), mp 103–104° (from CCl_4), $[\alpha]_D$ −37.6° (*c* 1.1, $CHCl_3$).

Anal. Calcd for $C_{15}H_{32}O_6Si$: C, 53.54; H, 9.58. Found: C, 53.60; H, 9.66.

*Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-tert-butyldiphenylsilyl-β-*D*-glucopyranoside* (**4**). — Benzoyl chloride (4.0 mL, 34.4 mmol) was added at 0° to a solution of **2** (4.15 g, 9.8 mmol) in pyridine (35 mL) and the mixture was stirred at room temperature until TLC (solvent *B*, 5:1) indicated complete conversion of **2** (18 h). Water was added,

the solution was partitioned between CH_2Cl_2 and water, and the organic layer was washed successively with aq HCl and aq $NaHCO_3$, and then concentrated. Column chromatography (solvent *C*, 3:1) of the residue gave amorphous **4** (6.08 g, 83%), $[\alpha]_D$ + 6.8° (*c* 2.2, $CHCl_3$).

Anal. Calcd for $C_{44}H_{44}O_9Si$: C, 70.95; H, 5.95. Found: C, 70.78; H, 6.00.

Methyl 2,3,4-tri-O-benzoyl-6-O-dimethylthexylsilyl-β-D-glucopyranoside (**5**). — Benzoyl chloride (2.5 mL, 21.5 mmol) was added at 0° to a solution of **3** (2.45 g, 7.2 mmol) in pyridine (30 mL), and the mixture was stirred at room temperature until TLC (solvent *B*, 5:1) indicated complete conversion of **3** (20 h). Work-up, as described for **4**, gave **5** (4.30 g, 92%) as a glass, $[\alpha]_D$ + 2.7° (*c* 2.0, $CHCl_3$).

Anal. Calcd for $C_{36}H_{44}O_9Si$: C, 66.64; H, 6.84. Found: C, 66.87; H, 6.89.

Methyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**6**). — (*a*) A solution of **4** (6.08 g, 8.16 mmol) and BF_3 etherate (1.8 mL) in MeOH–CH_2Cl_2 (1:1, 80 mL) was stirred at room temperature until TLC (solvent *B*, 5:1) indicated complete conversion of **4** into a single, slower-moving product (2 days). Concentration of the mixture and column chromatography (solvent *B*, 5:1) of the residue gave amorphous **6** (3.68 g, 90%), $[\alpha]_D$ −3.4° (*c* 1.2, $CHCl_3$); lit.[13] $[\alpha]_D$ −6.6° ($CHCl_3$). The ^{1}H-NMR spectrum was identical to that reported[13].

(*b*) Compound **5** (5.29 g, 8.16 mmol) was treated as in (*a*) but for 2 h. Work-up, as described in (*a*), gave **6** (4.09 g, 99%).

O-(2,3,4-Tri-O-benzoyl-6-O-chloroacetyl-β-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzoyl-α-D-galactopyranosyl chloride (**14**). — A solution of 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-α-D-galactopyranosyl chloride[13] (1.36 g, 2.3 mmol) and 2,4,6-trimethylpyridine (266 mg, 2.2 mmol) in CH_2Cl_2 (5 mL) was added with stirring at 0° to a suspension of 1,2,3,4-tetra-*O*-benzoyl-α-D-galactopyranose[12] (1.31 g, 2.2 mmol) and silver trifluoromethanesulfonate (0.77 g, 3.0 mmol) in CH_2Cl_2 (10 mL). TLC (solvent *B*: 10:1) revealed complete conversion of the starting materials into one major product after 15 min. The mixture was washed successively with aq sodium thiosulfate, dil HCl and aq $NaHCO_3$, then concentrated, to give crude **13** (2.24 g) as a colorless foam. To a solution of **13** in dichloromethyl methyl ether (5 mL) and $CHCl_3$ (5 mL) was added $ZnCl_2$ (~ 10 mg), and the mixture was stirred at 60° until TLC (solvent *B*, 10:1) showed complete formation of a single faster-moving product (2 h). The mixture was concentrated, and toluene was evaporated from the residue. Column chromatography (solvent *C*, 5:1) of the residue and crystallisation from CCl_4–acetone gave **14** (1.9 g, 81%), mp 228°, $[\alpha]_D$ + 170° (*c* 1.0, $CHCl_3$); lit.[11] mp 229°, $[\alpha]_D$ + 173.5° ($CHCl_3$).

Methyl O-(2,3,4-tri-O-benzoyl-6-O-bromoacetyl-β-D-galactopyranosyl)-(1 → 6)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**15**). — A solution of **7** (1.69 g, 2.67 mmol) and 2,4,6-trimethylpyridine (0.28 g, 2.3 mmol) in CH_2Cl_2 (8 mL) was added with stirring at 0° to a suspension of **6** (1.16 g, 2.3 mmol) and silver trifluoromethanesulfonate (0.77 g, 3.0 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at room temperature until TLC (solvent *B*, 5:1) showed the complete conversion of **6** and **7** into a single product (30 min). Work-up, as described for the preparation of **13**

TABLE III

^{1}H-NMR data [a]

Compound	Chemical shifts (δ), multiplicities, and coupling constants (Hz)							
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 $J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)	Others
2 [b]	4.19d	3.38–3.30m	3.38–3.30m	3.38–3.30m	3.19bt	4.01dd	3.87dd	3.54s OMe
	(7.7)	(–)	(–)	(–)	(1.6)	(4.8)	(11.0)	
3 [b]	4.13d	3.37–3.20m	3.37–3.20m	3.37–3.20m	3.13ddd	3.95dd	3.76dd	3.50s OMe
	(7.8)	(–)	(–)	(–)	(1.9)	(5.1)	(11.2)	
4	4.72d	5.50dd	5.85t	5.65t	3.82–3.78m	3.82–3.78m	3.82–3.78m	3.56s OMe
	(7.8)	(9.7)	(9.7)	(9.4)	(–)	(–)	(–)	
5	4.71d	5.45dd	5.85t	5.49t	3.86–3.82m	3.86–3.82m	3.86–3.82m	3.55s OMe
	(7.8)	(9.7)	(9.7)	(9.6)	(–)	(–)	(–)	
15	4.55d	5.37dd	5.83t	5.33t	4.04bdd	4.38–4.20m	4.38–4.20m	3.12s OMe
	(7.9)	(9.8)	(9.6)	(9.7)	(8.0)	(9.0)	(–)	
	4.95d	5.80dd	5.52dd	5.88bd	4.38–4.20m	4.14dd	3.85dd	3.73s CH_2Br
	(7.9)	(9.5)	(3.4)	(< 1)	(1.4)	(8.4)	(11.1)	
16	4.91d	5.08dd	6.09t	5.35t	4.43–4.22m	4.43–4.22m	4.43–4.22m	3.10s OMe
	(3.5)	(10.1)	(9.9)	(9.9)	(–)	(–)	(–)	
	4.93d	5.83dd	5.60dd	5.89bd	4.43–4.22m	4.17dd	3.79dd	3.76s CH_2Br
	(7.9)	(10.4)	(3.3)	(< 1)	(1.3)	(7.3)	(11.4)	
17	6.28d	5.21dd	6.13t	5.47t	5.56m	4.37dd	4.31dd	
	(3.9)	(10.1)	(9.8)	(10.1)	(7.0)	(5.3)	(11.4)	
	4.92d	5.81dd	5.59dd	5.88bd	4.26–4.20m	4.17dd	3.86dd	3.10s CH_2Br
	(7.8)	(10.0)	(3.3)	(< 1)	(1.5)	(6.0)	(12.0)	
18	4.55d	5.69dd	5.53dd	5.86bd	4.19–4.07m	4.19–4.07m	3.88dd	3.24s OMe
	(7.9)	(10.2)	(3.4)	(< 1)	(–)	(6.5)	(10.6)	
	4.95d	5.50dd	5.90t	5.70t	4.19–4.07m	4.63dd	4.35dd	
	(7.9)	(9.7)	(9.7)	(9.8)	(2.9)	(4.8)	(12.2)	
19	6.44d	5.71dd	5.94dd	5.97bd	4.72bt	4.12–4.05m	3.90dd	
	(3.9)	(10.0)	(3.3)	(< 1)	(–)	(7.2)	(11.1)	
	4.94d	5.52dd	5.89t	5.63t	4.12–4.05m	4.50dd	4.29dd	
	(7.8)	(9.6)	(9.6)	(9.6)	(3.1)	(4.9)	(12.1)	
21	4.56d	5.26dd	5.73t [c]	5.46t	4.67–4.02m [c]	4.67–4.02m [d]	3.96–3.81m [d]	3.20s OMe
	(7.9)	(9.7)	(9.6)	(9.8)	(–)	(–)	(–)	

	4.70d	5.48dd	5.85t [c]	5.18t	3.96–3.81m [c]	3.96–3.81m	3.66dd	
	(7.8)	(9.7)	(9.6)	(9.7)	(–)	(6.1)	(11.2)	
	5.06d	5.77dd	5.79dd	5.89bd	4.30m	4.33dd	4.29dd	3.77s CH_2Br
	(7.3)	(10.0)	(3.3)	(< 1)	(2.2)	(4.3)	(11.3)	
22	4.54d	5.33dd	5.84t [c]	5.30t	4.07–4.03m [c]	3.96–3.89m [d]	4.07–4.03m [d]	3.76s OMe
	(7.9)	(9.6)	(9.7)	(9.6)	(–)	(–)	(–)	
	4.75d	5.45dd	5.74t [c]	5.43t	3.96–3.89m	4.07–4.03m	3.67–3.55m	
	(7.7)	(9.7)	(9.7)	(9.7)	(–)	(–)	(–)	
	5.02d	5.82dd	5.77dd	5.80bd	3.96–3.89m	3.96–3.89m [d]	4.07–4.03m [d]	
	(7.4)	(10.0)	(3.3)	(< 1)	(–)	(–)	(–)	
24	6.42d	5.68dd	4.62bdd	5.95bd	4.03–3.87m	4.03–3.87m	3.51dd	
	(3.8)	(10.1)	(3.3)	(< 1)	(–)	(7.2)	(12.0)	
	4.73d [c]	5.38dd [c]	5.84t [c]	5.28t	4.03–3.87m	4.03–3.87m	3.69dd	
	(7.8)	(10.4)	(9.8)	(9.6)	(–)	(7.1)	(10.4)	
	4.67d [c]	5.35dd [c]	5.77t [c]	5.43t	4.03–3.87m	4.56dd	4.38dd	
	(7.8)	(9.8)	(9.6)	(9.7)	(3.0)	(5.1)	(12.3)	
25	4.36–4.31m	5.24–5.19m	5.24–5.19m	2.05m [e], 1.41m [f]	3.71–3.67m	4.19–4.07m	3.89dd	3.18s OMe
	(–)	(–)	(–)	(–)	(–)	(7.4)	(10.7)	
	4.94d	3.73dd	5.55dd	5.88bd	4.19–4.07m	3.77dd	3.50dd	
	(7.8)	(10.9)	(3.5)	(< 1)	(2.9)	(6.7)	(10.6)	
	4.77d	5.54bt	5.87t	5.69t	4.23–4.21m	4.65dd	4.36–4.31m	
	(7.9)	(9.6)	(9.6)	(9.6)	(2.9)	(–)	(12.3)	
27	4.82d	5.66dd	5.51dd	5.83bd	4.08bdd	3.83dd	3.64dd	3.26s OMe
	(7.8)	(10.3)	(3.4)	(< 1)	(5.1)	(4.8)	(10.0)	
	4.59–4.49m	5.31–5.21m	5.31–5.21m	2.26m [e], 1.67m [f]	4.17–4.13m	3.96dd	3.74bdd	
	(–)	(–)	(–)	(–)	(4.2)	(–)	(10.6)	
	4.59–4.49m	5.50dd	5.90t	5.69t	4.59–4.49m	4.66dd	3.74bdd	
	(7.7)	(9.5)	(9.5)	(9.6)	(3.3)	(–)	(12.3)	
29	4.52d	5.31dd	5.83t [c]	5.42t	4.34–4.10m	4.08dd	3.91–3.82m	3.65s OMe
	(7.9)	(9.7)	(9.6)	(9.7)	(5.3)	(–)	(12.4)	
	4.69d	5.44dd	5.71t [c]	5.24t	3.91–3.82m	4.34–4.10m	3.73–3.56m	
	(7.8)	(9.8)	(9.6)	(9.8)	(–)	(–)	(–)	
	4.89d	5.69dd	5.64dd	5.83bd	3.73–3.56m	4.34–4.10m	4.34–4.10m [c]	
	(7.6)	(9.6)	(3.3)	(< 1)	(–)	(–)	(–)	
	4.79d	5.73dd	5.68dd	5.89bd	4.34–4.10m	4.01dd	3.91–3.82m [c]	3.27s CH_2Br
	(7.1)	(10.4)	(3.5)	(< 1)	(1.4)	(–)	(11.4)	

(continued)

TABLE III (continued)

Compound	Chemical shifts (δ), multiplicities, and coupling constants (Hz)							
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 $J_{5,6a}$)	H-6a ($J_{5,6b}$)	H-6b ($J_{6a,6b}$)	Others
30	4.54d	5.32dd	5.84t [c]	5.42t	4.06–3.84m	4.06–3.84m	3.65dd	3.28s OMe
	(7.9)	(9.7)	(9.6)	(9.7)	(–)	(5.8)	(12.2)	
	4.69d	5.44dd	5.70t [c]	5.24t	3.78bt	4.06–3.84m	3.54dd	
	(7.9)	(9.7)	(9.8)	(9.6)	(6.0)	(5.6)	(11.9)	
	4.86d	5.74dd	5.63dd	5.69bd	3.41–3.33m	4.06–3.84m	4.06–3.84m	
	(7.9)	(9.6)	(3.3)	(<1)	(–)	(–)	(–)	
	4.83d	5.80dd	5.73dd	5.97bd	4.14bt	4.06–3.84m	3.67dd	
	(8.0)	(10.4)	(3.3)	(<1)	(1.2)	(5.6)	(12.2)	

[a] For solutions in $CDCl_3$ unless otherwise indicated. Data in the 1st row of each entry refer to sugar residue 1; data in the 2nd, 3rd, and 4th rows, if present, refer to the sugar residues 2, 3, and 4, respectively. [b] For a solution in CD_3OD. [c] Assignments in the same column may be reversed. [d] Assignments in the same row may be reversed. [e] H-4a. [f] H-4b.

followed by column chromatography (solvent *B*, 10:1), gave amorphous **15** (2.13 g, 84%), $[\alpha]_D$ + 64.3° (*c* 0.7, $CHCl_3$).

Anal. Calcd for $C_{57}H_{49}BrO_{18}$: C, 62.13; H, 4.48; Br, 7.25. Found: C, 62.10; H, 4.49; Br, 7.13.

Methyl O-*(2,3,4-tri-*O*-benzoyl-6-*O*-bromoacetyl-β-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**16**) *and* O-*(2,3,4-tri-*O*-benzoyl-6-*O*-bromoacetyl-β-*D*-galactopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-α-*D*-glucopyranosyl chloride* (**17**). — $ZnCl_2$ (~ 10 mg) was added to a solution of **15** (1.59 g, 1.4 mmol) and dichloromethyl methyl ether (4 mL) in $CHCl_3$ (3 mL), and the mixture was stirred at 65–70° until TLC (solvent *B*, 5:1) showed complete disappearance of **15** (4 h). Work-up, as described for **14**, and column chromatography (solvent *B*, 10:1) first gave amorphous **17** (1.12 g, 70%), $[\alpha]_D$ + 105.5° (*c* 0.9, $CHCl_3$).

Anal. Calcd for $C_{56}H_{46}BrClO_{17}$: C, 60.80; H, 4.19; Cl, 3.20; Br, 7.22. Found: C, 60.90; H, 4.20; Cl, 3.00; Br, 7.10.

Eluted next was amorphous **16** (0.25 g, 16%), $[\alpha]_D$ + 92.8° (*c* 0.3, $CHCl_3$).

Anal. Calcd for $C_{57}H_{49}BrO_{18}$: C, 62.13; H, 4.48; Br 7.25. Found: C, 62.13; H, 4.29; Br, 7.73.

Methyl O-*(2,3,4,6-tetra-*O*-benzoyl-β-*D*-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-*D*-galactopyranoside* (**18**). — A solution of **8** (1.01 g, 2.0 mmol), **10**[22] (1.45 g, 2.2 mmol), and 2,4,6-trimethylpyridine (0.24 g, 2.0 mmol) in CH_2Cl_2 (10 mL) was added at 0° to a suspension of silver trifluoromethanesulfonate (0.77 g, 3.3 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred at room temperature until TLC (solvent *B*, 5:1) showed complete conversion of **8** and **10**. The mixture was neutralized with 2,4,6-trimethylpyridine, filtered, and washed with aq sodium thiosulfate, and the solvent was evaporated. Crystallisation of the residue from acetone–hexane gave **18** (1.97 g, 91%), mp 181.5–182°, $[\alpha]_D$ + 86.8° (*c* 0.4, $CHCl_3$).

Anal. Calcd for $C_{62}H_{52}O_{18}$: C, 68.63; H, 4.83. Found: C, 68.42; H, 4.76.

O-*(2,3,4,6-Tetra-*O*-benzoyl-β-*D*-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-α-*D*-galactopyranosyl chloride* (**19**). — $ZnCl_2$ (~ 10 mg) was added to a solution of **18** (2.0 g, 1.84 mmol) and dichloromethyl methyl ether (5 mL) in $CHCl_3$ (10 mL), and the mixture was stirred at 65° until TLC (solvent *B*, 5:1) showed complete conversion of **18** into a single faster-moving product (3 h). Work-up, as described for **14**, and column chromatography (solvent *D*, 40:1) gave amorphous **19** (1.38 g, 69%), $[\alpha]_D$ + 111.1° (*c* 0.3, $CHCl_3$).

Anal. Calcd for $C_{61}H_{49}ClO_{17}$: C, 67.25; H, 4.53. Found: C, 66.97; H, 4.58.

Methyl O-*(2,3,4-tri-*O*-benzoyl-6-*O*-bromoacetyl-β-*D*-galactopyranosyl)-(1 → 6)-*O*-*(2,3,4-tri-*O*-benzoyl-β-*D*-glucopyranosyl)-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-*D*-glucopyranoside* (**21**). — (*a*) A solution of **7** (1.22 g, 1.8 mmol) and 2,4,6-trimethylpyridine (169 mg, 1.4 mmol) in CH_2Cl_2 (5 mL) was added at 0° to a suspension of **11** (1.37 g, 1.4 mmol) and silver trifluoromethanesulfonate (0.77 g, 3.0 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **7** and **11** (15 min). Work-up, as described for **14**, and column chromatography (solvent *B*, 7:1) gave amorphous **21** (2.16 g, 98%), $[\alpha]_D$ + 36.1° (*c* 0.3, $CHCl_3$).

TABLE IV

Compound	Chemical shifts (δ)						
	C-1	C-2	C-3	C-4	C-5	C-6	Others
2 [b]	105.4	75.2	78.3 [c]	71.6	78.2 [c]	64.8	57.2 [f]
3 [b]	105.3	75.1	78.2	71.6	78.2	63.9	57.2 [f]
4	101.9	72.1	73.4	69.3	75.2	62.8	56.7 [f]
5	101.9	72.0	73.4	69.8	75.4	62.4	58.8 [f]
15	101.8 [c]	73.9	72.9	71.2 [d]	71.8 [e]	68.9	56.6 [f]
	101.7 [c]	69.9 [d]	71.6 [e]	68.0	69.7 [d]	63.4	25.1 [g]
16	96.5	71.9	70.3	69.6	69.6	68.7	55.0 [f]
	102.2	69.2	71.5	68.0	71.1	63.3	25.1 [g]
17	90.1	72.3	71.7 [c]	69.7	68.3	67.6	
	102.0	69.4	71.6 [c]	68.0	71.2	63.4	25.2 [g]
18	102.1	69.8	71.8	68.8	73.2	68.7	56.9 [f]
	101.3	72.8	72.2	69.4	71.7	62.6	
19	91.3	68.0	69.0	68.6	71.83 [c]	67.5	
	101.3	72.4	72.9	69.6	71.77 [c]	62.5	
21	101.9	73.7	72.9	70.1	71.8	68.7	56.7 [f]
	100.9	74.5	72.7	70.6	71.8	68.3	
	101.5	69.4	71.4	67.9	71.2	63.6	25.2 [g]
22	101.8	73.8	72.8 [c]	70.4	71.8	67.5	56.8 [f]
	101.1	74.3	72.7 [c]	69.5	71.8	69.2	
	101.4	70.1	71.7	68.7	74.2	60.7	
24	91.4	68.0	69.1	68.6	71.8 [c]	66.4	
	100.6 [d]	72.8	72.8	70.0	71.4	68.4	
	101.2 [d]	73.6	72.3	69.6	71.7 [c]	63.0	
25	101.9	73.3 [c]	72.0 [c]	32.6	71.7 [c]	71.3	56.4 [f]
	101.6	69.9	71.6 [c]	68.8	72.4	68.6	
	101.3	72.7	72.9	69.5	70.9 [c]	62.7	
26 [h]	103.7	74.9	69.3	34.4	72.7	69.8	57.4 [f]
	103.4	70.8	73.2	68.8	71.5	71.7	
	102.9	73.9	76.0 [c]	70.4	75.9 [c]	60.9	
27	102.2	70.0	70.5	68.8	72.9 [c]	68.0	56.8 [f]
	101.3	73.2 [d]	71.9 [d]	33.1	71.8 [d]	71.0	
	101.3	72.4	72.8 [c]	69.8	71.4 [d]	63.1	
28 [h]	103.9	70.7	72.7	68.8	73.2	69.8	57.4 [f]
	103.2	74.9	69.3	34.3	71.5	71.7	
	102.9	73.9	76.0 [c]	70.3	75.8 [c]	60.9	
29	101.8 [c]	74.1 [d]	73.2	70.2	72.8	68.6	56.8 [f]
	101.1	74.0 [d]	72.9	69.6	72.8	68.4	
	101.5 [c]	70.0 [e]	71.5	68.1	71.8	67.6	
	101.1	69.9 [e]	71.5	67.7	71.0	63.2	25.2 [g]
30	101.8	74.24 [c]	73.0 [d]	70.4	72.9 [d]	69.0	56.8 [f]
	101.6	74.15 [c]	72.9 [d]	69.5	72.9 [d]	68.4	
	101.3	70.2	71.8	67.8 [e]	71.8	67.7 [e]	
	101.1	70.2	71.5	68.6	74.0 [c]	60.7	
31	101.8	74.2 [d]	72.7	69.7	72.8	68.5	56.7 [f]
	101.6 [c]	74.1 [d]	72.4	69.4	72.8	68.4	
	101.1 [c]	70.1	71.6	67.9 [e]	71.8	66.4	
	100.9 [c]	70.1	71.6	67.7 [e]	71.8	67.1	
	100.7 [c]	70.1	71.6	67.6 [c]	70.8	62.9	40.5 [i]
32	101.8 [c]	74.2 [d]	72.7	70.0	72.9	68.7	56.7 [f]
	101.6 [c]	74.1 [d]	72.2	69.5	72.9	67.6	

TABLE IV (continued)

Compound	Chemical shifts (δ)						
	C-1	C-2	C-3	C-4	C-5	C-6	Others
32	101.1 [c]	70.1	71.8	68.4	71.7 [e]	66.7	
	101.0 [c]	70.1	71.8	68.4	71.6 [e]	68.0	
	100.7 [c]	70.1	71.8	67.0	73.9 [d]	60.8	

[a] For solutions in $CDCl_3$ unless otherwise indicated. Data in the 1st row of each entry refer to sugar residue 1; data in the 2nd, 3rd, and 4th rows, if present, refer to the sugar residues 2, 3, and 4, respectively. [b] For a solution in CD_3OD. [c,d,e] Assignments may be reversed. [f] OCH_3. [g] CH_2Br. [h] For a solution in D_2O. [i] CH_2Cl.

Anal. Calcd for $C_{84}H_{71}BrO_{26}$: C, 64.00; H, 4.54; Br, 5.07. Found: C, 63.81; H, 4.74; Br, 5.04.

(*b*) A solution of **17** (0.65 g, 0.59 mmol) and 2,4,6-trimethylpyridine (60.6 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **6** (0.51 g, 1.0 mmol) and silver trifluoromethanesulfonate (0.39 g, 1.5 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **6** and **17** (1 h). Work-up, as described in (*a*), column chromatography (solvent *B*, 10:1), and then rechromatography (solvent *D*, 20:1) gave **21** (0.37 g, 39%).

Methyl O-(2,3,4-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**22**). —A solution of **21** (3.09 g, 1.96 mmol) and thiourea (0.19 g, 2.5 mmol) in CH_2Cl_2–MeOH (2:1, 20 mL) was stirred at room temperature until TLC (solvent *B*, 5:1) showed complete formation of a single slower-moving product (20 h). The mixture was diluted with CH_2Cl_2 (~ 30 mL), washed successively with dil HCl and aq $NaHCO_3$, and concentrated. Column chromatography (solvent *B*, 5:1) then gave amorphous **22** (2.23 g, 78%), $[\alpha]_D$ +37.1° (*c* 1.1, $CHCl_3$).

Anal. Calcd for $C_{82}H_{70}O_{25}$: C, 67.67; H, 4.85. Found: C, 67.71; H, 4.90.

O-(2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-Obenzoyl-α-D-galacopyranosyl chloride (**24**). — $ZnCl_2$ (~ 2 mg) was added to a solution of **23**[14] (1.32 g, 0.8 mmol) and dichloromethyl methyl ether (4 mL) in $CHCl_3$ (4 mL), and the mixture was stirred at 60° for 3 h. Work-up, as described for **14**, and column chromatography (solvent *B*, 10:1) gave amorphous **24** (0.98 g, 78%), $[\alpha]_D$ + 76.6° (*c* 0.5, $CHCl_3$).

Anal. Calcd for $C_{88}H_{71}ClO_{25}$: C, 67.58; H, 4.58; Cl, 2.27. Found: C, 67.30; H, 4.70; Cl, 2.21.

Methyl O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 6)-2,3-di-O-benzoyl-4-deoxy-β-D-xylo-hexopyranoside (**25**). — A solution of **19** (1.2 g, 1.1 mmol) and 2,4,6-trimethylpyridine (0.11 g, 0.9 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **9**[8] (0.35 g, 0.9 mmol), silver trifluoromethanesulfonate (0.39 g, 1.5 mmol), and molecular sieves (4A, 1 g) in CH_2Cl_2 (15 mL). The mixture was stirred until TLC

(solvent *B*, 5:1) showed complete conversion of **9** and **19**. Work-up, as described for **14**, and column chromatography (solvent *B*, 15:1) gave amorphous **25** (0.94 g, 73%), $[\alpha]_D$ + 75.1° (*c* 0.9, $CHCl_3$).

Anal. Calcd for $C_{82}H_{70}O_{24}$: C, 68.42; H, 4.90; Found: C, 68.19; H, 4.95.

Methyl O-β-D-*glucopyranosyl-(1 → 6)*-O-β-D-*galactopyranosyl-(1 → 6)-4-deoxy-β*-D-xylo-*hexopyranoside* (**26**). — A solution of **25** (288 mg, 0.2 mmol) and a catalytic amount of sodium methoxide in toluene–MeOH (1:1, 20 mL) was kept at room temperature with occasional shaking for 20 h, then neutralized with Dowex (H^+) resin, and the solvent was evaporated. Column chromatography (solvent *E*, 1:1) of the residue gave amorphous **26** (87.8 mg, 87%), $[\alpha]_D$ − 25.7° (*c* 0.6, H_2O).

Anal. Calcd for $C_{19}H_{34}O_{15}$: C, 45.42; H, 6.82. Found: C, 44.51; H, 6.45.

Methyl O-*(2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3-di*-O-*benzoyl-4-deoxy*-β-D-xylo-*hexopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl*-β-D-*galactopyranoside* (**27**). — A solution of **10** (264 mg, 0.4 mmol) and 2,4,6-trimethylpyridine (36.7 mg, 0.3 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **20**[8] (0.26 g, 0.3 mmol), silver trifluoromethanesulfonate (0.39 g, 1.5 mmol), and molecular sieves (4A, 0.5 g) in CH_2Cl_2 (10 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **10** and **20**. Work-up, as described for **25**, gave amorphous **27** (0.4 g, 93%), $[\alpha]_D$ + 75.2° (*c* 0.8, $CHCl_3$).

Anal. Calcd for $C_{82}H_{70}O_{24}$: C, 68.42; H, 4.90. Found: C, 68.18; H, 4.97.

Methyl O-β-D-*glucopyranosyl-(1 → 6)*-O-*(4-deoxy*-β-D-xylo-*hexopyranosyl)-(1 → 6)*-β-D-*galactopyranoside* (**28**). — A solution of **27** (143.9 mg, 0.1 mmol) and a catalytic amount of sodium methoxide in toluene–MeOH (1:1, 10 mL) was kept at room temperature with occasional shaking for 20 h. Work-up, as described for **26**, gave amorphous **28** (49.2 mg, 98%), $[\alpha]_D$ − 35.3° (*c* 0.4, H_2O).

Anal. Calcd for $C_{19}H_{34}O_{15} \cdot H_2O$: C, 43.85; H, 6.97. Found: C, 43.40; H, 7.00.

Methyl O-*(2,3,4-tri*-O-*benzoyl-6*-O-*bromoacetyl*-β-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl*-β-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranoside* (**29**). — A solution of **7** (0.37 g, 0.55 mmol) and 2,4,6-trimethylpyridine (48 mg, 0.4 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **22** (0.64 g, 0.46 mmol), silver trifluoromethanesulfonate (0.26 g, 1.0 mmol), and molecular sieves (3A, 0.5 g) in CH_2Cl_2 (5 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **7** and **22** (15 min). Work-up, as described for **25**, and column chromatography (solvent *B*, 6:1) gave amorphous **29** (0.82 g, 87%), $[\alpha]_D$ + 65.9° (*c* 0.3, $CHCl_3$).

Anal. Calcd for $C_{111}H_{93}BrO_{34}$: C, 65.00; H, 4.57; Br, 3.90. Found: C, 65.07; H, 4.71; Br, 4.14.

Methyl O-*(2,3,4-tri*-O-*benzoyl*-β-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl*-β-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl*-β-D-*glucopyranoside* (**30**). — A solution of **29** (0.64 g, 0.31 mmol) and thiourea (53 mg, 0.7 mmol) in CH_2Cl_2–MeOH (1:1, 10 mL) was stirred at room temperature until TLC (solvent *B*, 5:1) showed complete conversion of **29**

into a single slower-moving product (3 h). Work-up, as described for **22**, and column chromatography (solvent *B*, 3:1) gave amorphous **30** (0.57 g, 95%), $[\alpha]_D$ + 75.7° (*c* 0.3, $CHCl_3$).

Anal. Calcd for $C_{109}H_{92}O_{33}$: C, 67.84; H, 4.81. Found: C, 67.74; H, 4.87.

Methyl O-*(2,3,4-tri*-O-*benzoyl-6*-O-*chloroacetyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranoside* (**31**). — A solution of **14** (0.93 g, 0.88 mmol) and 2,4,6-trimethylpyridine (6 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **22** (1.31 g, 0.9 mmol), silver trifluoromethanesulfonate (0.39 g, 1.5 mmol), and molecular sieves (3A, 0.5 g) in CH_2Cl_2 (5 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **14** and **22** (45 min). Work-up, as described for **25**, and column chromatography (solvent *B*, 6:1) gave amorphous **31** (1.37 g, 63%), $[\alpha]_D$ + 60.8° (*c* 1.0, $CHCl_3$). ^{1}H-NMR data ($CDCl_3$): δ 4.81 (bd, 1 H, $J_{1''',2'''}$ 7.7 Hz, H-1'''), 4.73, 4.68, 4.58 (3 d, 3 H, *J* 7.7, 7.8, and 7.9 Hz, H-1',1'',1'''), 4.50 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.26 (s, 3 H, OMe), 2.47 (s, 2 H, CH_2Cl).

Anal. Calcd for $C_{138}H_{113}ClO_{42}$: C, 66.87; H, 4.60; Cl, 1.43. Found: C, 66.54; H, 4.78; Cl, 1.56.

Methyl O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranoside* (**32**). — A solution of **31** (1.21 g, 0.49 mmol) and thiourea (76 mg, 1.0 mmol) in CH_2Cl_2–MeOH (1:1, 10 mL) was stirred at 40° until TLC (solvent *B*, 5:1) showed complete conversion of **31** into a single slower-moving product (24 h). Work-up, as described for **22**, and column chromatography (solvent *B*, 3:1) gave amorphous **32** (0.95 g, 80%), $[\alpha]_D$ + 62.6° (*c* 0.5, $CHCl_3$), ^{1}H-NMR data ($CDCl_3$): δ 4.82 (bd, 1 H, $J_{1''',2'''}$ 7.5 Hz, H-1'''), 4.75, 4.70, 4.57 (3 d, 3 H, *J* 7.5, 7.8, and 7.9 Hz, H-1',1'',1'''), 4.53 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.27 (s, 3 H, OMe).

Anal. Calcd for $C_{136}H_{114}O_{41}$: C, 68.00; H, 4.70. Found: C, 67.96; H, 4.84.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl-β*-D-*glucopyranoside* (**33**). — A solution of **12** (272.4 mg, 0.25 mmol) and 2,4,6-trimethylpyridine (18.2 mg, 0.15 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **22** (282.8 mg, 0.19 mmol), silver trifluoromethanesulfonate (77 mg, 0.3 mmol), and molecular sieves (3A, 0.5 g) in CH_2Cl_2 (5 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **12** and **22** (30 min). Work-up, as described for **25**, and column chromatography (solvent *B*, 7:1) gave amorphous **33** (241.8 mg, 50%), $[\alpha]_D$ + 14.2° (*c* 0.1, $CHCl_3$). ^{13}C-NMR data: δ 101.7, 101.5, 101.4, 101.1, 100.9 (C-1,1',1'',1''',1''''), 74.0, 73.9, 73.5 (C-2',2'''',3), 73.0, 72.9 (each 3 C, C-2,2''',3',3''',3'''',5'), 72.3, 72.0, 71.9, 71.8 (1 C, 1 C, 1 C, and 2 C, C-3'',5,5',5''',5''''), 70.05, 69.98, 69.7 (each 2 C, C-2'',4,4',4''',6,6'), 68.8, 68.2, 67.9, 67.8 (C-4'',4'''',6'',6'''), 63.0 (C-6''''), 56.7 (OMe).

Anal. Calcd for $C_{143}H_{118}O_{42}$: C, 68.47; H, 4.74. Found: C, 68.41; H, 4.85.

Methyl O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**34**). — A solution of **33** (141.8 mg, 0.06 mmol) and a catalytic amount of sodium methoxide in CH_2Cl_2–MeOH (1:1, 10 mL) was kept at room temperature with occasional shaking for 24 h, then neutralized with Dowex (H^+) resin, and the solvent was evaporated. Column chromatography (solvent *F*, 5:3:3) of the residue, concentration of the sugar-containing fractions, and rechromatography (water) of the residue on Bio-Gel P2 gave amorphous **34** (26.9 mg, 55%), $[\alpha]_D$ – 16.0° (*c* 0.1, H_2O). ^{13}C-NMR data (D_2O): δ 103.82, 103.76, 103.3 (1 C, 1 C, and 3 C, C-1,1′,1″,1‴,1⁗), 76.4, 76.3, 76.1, 76.0 (C-3′,3‴,3⁗,5⁗), 75.3 (3 C, C-5,5′,5‴), 74.3 (C-5″), 73.5 (4 C, C-2,2′,2‴,2⁗), 73.0 (C-3″), 71.1 (C-2″), 70.1, 69.8 (2 C and 4 C, C-3,4,4′,4‴,4⁗,6′), 69.2, 69.1, 69.07 (1 C, 2 C and 1 C, C-4″,6,6″,6‴), 61.2 (C-6⁗, 57.9 (OMe).

Anal. Calcd for $C_{31}H_{54}O_{26}$: C, 44.18; H, 6.46. Found: C, 43.90; H, 6.60.

Methyl O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-galactopyranosyl)-(1 → 6)-O-(2,3,4-tri-O-benzoyl-β-D-glucopyranosyl)-(1 → 6)-2,3,4-tri-O-benzoyl-β-D-glucopyranoside (**35**). — A solution of **12** (220.7 mg, 0.2 mmol) and 2,4,6-trimethylpyridine (12.1 mg, 0.1 mmol) in CH_2Cl_2 (5 mL) was added at room temperature to a suspension of **30** (322.7 mg, 0.17 mmol), silver trifluoromethanesulfonate (51.4 mg, 0.2 mmol), and molecular sieves (3A, 0.5 g) in CH_2Cl_2 (10 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **12** and **30** (30 min). Work-up, as described for **25**, and column chromatography (solvent *B*, 5:1) gave amorphous **35** (339.2 mg, 68%), $[\alpha]_D$ + 19.4° (*c* 0.3, $CHCl_3$). ^{13}C-NMR data: δ 101.7, 101.5, 101.1, 100.9 (1 C, 2 C, 1 C, and 2 C, C-1,1′,1″,1‴,1⁗,1⁗′), 74.1, 73.9, 73.3 (C-2′,2⁗′,3), 72.91, 72.86, 72.6, 72.4, 72.3 (3 C, 1 C 1 C, 1 C, 1 C, C-2,2⁗′,3′,3⁗′,3⁗′,5″,5‴), 71.9, 71.85, 71.8, 71.76, 71.72, 71.6 (C-3″,3‴,5,5′,5⁗′,5⁗′), 70.2, 70.1, 69.8, 69.5, 69.2 (2 C, 2 C, 1 C, 1 C, and 1 C, C-2″,2‴,4,4′,4⁗′,6,6′), 68.5, 68.3, 67.6, 67.3, 67.2 (2 C, 1 C, 1 C, 1 C, and 1 C, C-4″,4‴,4⁗′,6″,6‴,6⁗′), 63.1 (C-6⁗′), 56.7 (OMe).

Anal. Calcd for $C_{170}H_{140}O_{50}$: C, 68.45; H, 4.73. Found: C, 68.06; H, 4.75.

Methyl O-β-D-glucopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 6)-O-β-D-galactopyranosyl-(1 → 6)-O-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**36**). — A solution of **35** (225 mg, 0.075 mmol) and a catalytic amount of sodium methoxide in CH_2Cl_2–MeOH (1:1, 20 mL) was kept at room temperature with occasional shaking for 24 h. Work-up, as described for **34**, gave amorphous **36** (55.4 mg, 78%), $[\alpha]_D$ – 20.6° (*c* 0.2, H_2O). ^{13}C-NMR data (D_2O): δ 103.89, 103.78, 103.4 (1 C, 2 C, and 3 C, C-1,1′,1″,1‴,1⁗,1⁗′), 76.4, 76.2, 76.1, 76.0 (C-3′,3⁗′,3⁗′,5⁗′), 75.3 (3 C, C-5,5′,5⁗′), 74.2 (2 C, C-5″,5‴), 73.5 (4 C, C-2,2′,2⁗′,2⁗′), 73.0 (2 C, C-3″,3‴), 71.2 (2 C, C-2″,2‴), 70.1, 70.0, 69.89, 67.77 (1 C, 1 C, 4 C, and 1 C, C-3,4,4′,4⁗′,4⁗′,6′,6‴), 69.3, 69.1 (1 C and 3 C, C-4″,4‴,6″,6⁗′), 61.2 (C-6⁗′), 57.9 (OMe).

Anal. Calcd for $C_{37}H_{64}O_{31}$: C, 44.22; H, 6.42. Found: C, 44.15; H, 6.61.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β-*D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl-β-*D-*glucopyranoside* (**37**). — A solution of **24** (196.8 mg, 0.13 mmol) and 2,4,6-trimethylpyridine (9.7 mg, 0.08 mmol) in CH_2Cl_2 (3 mL) was added at room temperature to a suspension of **30** (289.5 mg, 0.15 mmol), silver trifluoromethanesulfonate (64.2 mg, 0.25 mmol), and molecular sieves (3A, 0.25 g) in CH_2Cl_2 (8 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **24** and **30** (30 min). Work-up, as described for **25**, and column chromatography (solvent *B*, 7:1) gave amorphous **37** (302 mg, 69%), $[\alpha]_D$ +25.9° (*c* 0.5, $CHCl_3$). ^{13}C-NMR data: δ 101.8, 101.6, 101.5, 100.2, 101.1, 100.7 (1 C, 1 C, 1 C, 1 C, 2 C, and 1 C, C-1,1′,1″,1‴,1⁗,1⁗′,1⁗″), 74.1, 73.9, 73.4 (C-2′,2⁗″,3), 72.9, 72.5, 72.4, 72.3, 72.2 (4 C, 1 C, 1 C, 1 C, and 1 C, C-2,2⁗′,3′,3⁗⁗,3⁗′,5″,5‴,5⁗′), 71.99, 71.81, 71.7, 71.6 (1 C, 3 C, 2 C, and 1 C, C-3″,3‴,3⁗′,5,5′,5⁗⁗,5⁗″), 70.3, 70.2, 70.1, 70.0, 69.8, 69.5, 69.4 (1 C, 1 C, 2 C, 1C, 1 C, 1 C, and 1 C, C-2″,2‴,2⁗′,4,4′,4⁗″,6,6′), 68.3, 68.1, 67.5, 67.3, 67.0, 66.7 (2 C, 2 C, 1 C, 1 C, 1 C, and 1 C, C-4″,4‴,4⁗′,4⁗″,6″,6‴,6⁗′,6⁗″), 63.1 (C-6⁗″), 56.7 (OMe).

Anal. Calcd for $C_{197}H_{162}O_{58}$: C, 68.44; H, 4.72. Found: C, 68.45; H, 4.73.

Methyl O-*β-*D-*glucopyranosyl-(1 → 6)*-O-*β-*D-*glucopyranosyl-(1 → 6)*-O-*β-*D-*galactopyranosyl-(1 → 6)*-O-*β-*D-*galactopyranosyl-(1 → 6)-O-β-*D-*galactopyranosyl-(1 → 6)*-O-*β-*D-*glucopyranosyl-(1 → 6)-β-*D-*glucopyranoside* (**38**). — A solution of **37** (212 mg, 0.06 mmol) and a catalytic amount of sodium methoxide in CH_2Cl_2–MeOH (1:1, 5 mL) was kept at room temperature with occasional shaking for 24 h. Work-up, as described for **34**, gave amorphous **38** (68.8 mg, 98%), $[\alpha]_D$ −6.2° (*c* 0.2, H_2O). ^{13}C-NMR data (D_2O): δ 103.89, 103.82, 103.77, 103.4 (1 C, 3 C, 2 C, and 1 C, C-1,1′,1″,1‴,1⁗′,1⁗″,1⁗‴), 76.1, 76.0 (2 C and 2 C, C-3′,3⁗″,3⁗‴,5⁗‴), 75.3 (3 C, C-5,5′,5⁗′), 74.2, 74.18, (1 C and 2 C, C-5″,5‴,5⁗′), 73.5 (4 C, C-2,2′,2⁗″,2⁗‴), 73.2, 73.02, 73.0 (C-3″,3‴,3⁗′), 71.2 (3 C, C-2″,2‴,2⁗′), 69.9, 69.6 (3 C and 3 C, C-3,4,4′,4⁗′,4⁗‴,6′), 69.23, 69.1 (2 C and 6 C, C-4″,4‴,4⁗′,6,6″,6‴,6⁗′,6⁗″), 61.5 (C-6⁗‴), 57.9 (OMe).

Anal. Calcd for $C_{43}H_{74}O_{36} \cdot H_2O$: C, 43.58; H, 6.46. Found: C, 43.57; H, 6.32.

Methyl O-*(2,3,4,6-tetra*-O-*benzoyl-β-*D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*galactopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*benzoyl-β-*D-*glucopyranosyl)-(1 → 6)-2,3,4-tri*-O-*benzoyl-β-*D-*glucopyranoside* (**39**). — A solution of **24** (547.4 mg, 0.35 mmol) and 2,4,6-trimethylpyridine (30.3 mg, 0.25 mmol) in CH_2Cl_2 (4 mL) was added at room temperature to a suspension of **32** (793.4 mg, 0.33 mmol), silver trifluoromethanesulfonate

(256.9 mg, 1.0 mmol), and molecular sieves (3A, 0.5 g) in CH_2Cl_2 (10 mL). The mixture was stirred until TLC (solvent *B*, 5:1) showed complete conversion of **24** and **32** (30 min). Work-up, as described for **25**, and column chromatography (solvent *B*, 5:1) gave material which was rechromatographed (solvent *D*, 10:1) to give amorphous **39** (771.7 mg, 69% relative to the consumed amount of **32**), $[\alpha]_D + 27.9°$ (*c* 0.7, $CHCl_3$). ^{13}C-NMR data: δ 101.83, 101.76, 101.56, 101.33, 101.25, 101.1, 100.8, 100.6 (C-1,1′,1″,1‴,1⁗,1⁗′,1⁗″1⁗‴′), 74.2, 73.9, 73.5 (C-2′,2⁗‴′,3), 72.99, 72.89, 72.58, 72.5, 72.2, 72.1 (1 C, 4 C, 1 C, 1 C, 1 C, and 1 C, C-2,2⁗‴,3′,3⁗‴,3⁗‴′,5″,5‴,5⁗″,5⁗⁗), 71.97, 71.8, 71.7, 71.5 (2 C, 3 C, 2 C, and 1 C, C-3″,3‴,3⁗″,3⁗⁗,5,5′,5⁗⁗,5⁗‴′), 70.4, 70.2, 70.1, 70.0, 69.8, 69.4 (1 C, 2 C, 2 C, 2 C, 1 C, and 1 C, C-2″,2‴,2⁗″,2⁗⁗,4,4′,4⁗⁗,6,6′), 68.4, 68.3, 68.1, 67.9, 67.8, 67.6, 67.5, 67.1, 66.8, 66.0 (C-4″,4‴,4⁗″,4⁗⁗,4⁗‴′,6″,6‴,6⁗″,6⁗⁗,6⁗⁗), 63.1 (C-6⁗‴′), 56.7 (OMe).

Anal. Calcd for $C_{224}H_{184}O_{66}$: C, 68.43; H, 4.72. Found: C, 68.44; H, 4.84.

Methyl O-β-D-*glucopyranosyl-(1 → 6)*-O-β-D-*glucopyranosyl-(1 → 6)*-O-β-D-*galactopyranosyl-(1 → 6)*-O-β-D-*galactopyranosyl-(1 → 6)*-O-β-D-*galactopyranosyl-(1 → 6)*-O-β-D-*galactopyranosyl-(1 → 6)*-O-β-D-*glucopyranosyl-(1 → 6)*-β-D-*glucopyranoside* (**40**). — A solution of **39** (698.3 mg, 0.18 mmol) and a catalytic amount of sodium methoxide in CH_2Cl_2–MeOH (1:1, 12 mL) was kept at room temperature with occasional shaking for 24 h. Work-up, as described for **34**, gave amorphous **40** (232.6 mg, 97%), $[\alpha]_D - 17.0°$ (*c* 0.1, H_2O). ^{13}C-NMR data (D_2O): δ 103.8, 103.5, 103.4 (5 C, 1 C, and 2 C, C-1,1′,1″,1‴,1⁗,1⁗′,1⁗‴,1⁗⁗,1⁗⁗′), 76.3, 76.1 (1 C and 3 C, C-3′,3⁗‴,3⁗⁗′,5⁗⁗′), 75.3 (3 C, C-5,5′,5⁗⁗), 74.18 (4 C, C-5″,5‴,5⁗″,5⁗⁗), 73.5 (4 C, C-2,2′,2⁗⁗,2⁗⁗′), 73.06, 73.0 (2 C and 2 C, C-3″,3‴,3⁗″,3⁗⁗), 71.1 (4 C, C-2″,2‴,2⁗″,2⁗⁗), 70.1 69.9, 69.7 (2 C, 4 C, and 1 C, C-3,4,4′,4⁗⁗,4⁗⁗′,6′,6‴), 69.23, 69.1 (2 C and 7 C, C-4″,4‴,4⁗″,4⁗⁗,6,6″,6⁗″,6⁗⁗,6⁗⁗), 61.2 (C-6⁗⁗′), 57.9 (OMe).

Anal. Calcd for $C_{49}H_{84}O_{41} \cdot 5\ H_2O$: C, 41.47; H, 6.67. Found: C, 41.37; H, 6.57.

Binding studies. — Affinity constants were measured on solutions of antibody (3.6×10^{-7} M) in phosphate-buffered saline (pH 7.4), using ligand-induced protein fluorescence change[4,6]. The excitation and emission wavelengths were 295 and 334 nm, respectively, and the temperature was 25°.

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