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Carbohydrate Research 291 (1996) 63–83

CARBOHYDRATE RESEARCH

Synthesis of Hex *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ probes for exploration of the substrate specificity of glycosyltransferases: Part I, Hex = β-D-Gal, 4-deoxy-β-D-Gal, 4-*O*-methyl-β-D-Gal, 4-deoxy-4-fluoro-β-D-Gal, or β-D-Glc

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Received 4 March 1996; accepted 31 May 1996

Abstract

Five trisaccharide derivatives designed for detailed exploration of the acceptor specificity of glycosyltransferases involved in termination of *N*-acetyllactosamine-type structures have been synthesized: β-D-Gal *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ (**1**), 4-deoxy-β-D-Gal *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ (**2**), 4-*O*-methyl-β-D-Gal *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ (**3**), 4-deoxy-4-fluoro-β-D-Gal *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ (**4**), and β-D-Glc *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ (**5**). A general disaccharide acceptor octyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside was synthesized by condensation of 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl trichloroacetimidate with octyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside, followed by deacetylation. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate were used as the gly-

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PII S0008-6215(96)00154-1

cosyl donors in the syntheses of **1** and **5**. The modified galactosyl derivatives required subtle anomeric activation. Suitable donors for **2** turned out to be 2,3,6-tri-*O*-acetyl-4-deoxy-α-D-*xylo*-hexopyranosyl trichloroacetimidate and ethyl 2,3,6-tri-*O*-acetyl-4-deoxy-1-thio-α,β-D-*xylo*-hexopyranoside; for **3**, ethyl 2,3,6-tri-*O*-acetyl-4-*O*-methyl-1-thio-α,β-D-galactopyranoside; and for **4**, 2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-α-D-galactopyranosyl trichloroacetimidate. It was concluded that thioglycosides were most appropriate for stereoselective coupling of activated synthons (carrying deoxy or *O*-methyl groups), whereas trichloroacetimidates gave high yields with deactivated (fluorine-containing) synthons. © 1996 Elsevier Science Ltd.

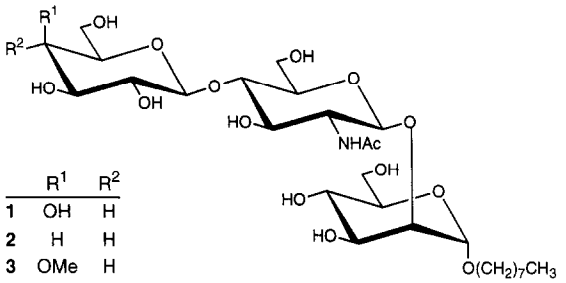
Keywords: Glycoproteins; Glycosyltransferases; *N*-Acetyllactosamine; Oligosaccharides; Substrate analogues

1. Introduction

Carbohydrate sequences located at the periphery of glycoconjugate glycans have been implicated in a variety of biological recognition phenomena, including cell–cell, receptor–ligand, and host–pathogen interactions, or tumour progression and metastasis [1–4]. The biosynthesis of these carbohydrate ligands requires the action of many distinct glycosyltransferases, which are highly specific for acceptor substrates and the type of linkage formed in the product [5]. A thorough analysis of the substrate specificity can be attained by using modified oligosaccharides, probing the contribution of individual hydroxyl groups in recognition and binding. Usually, the substrate specificity of glycosyltransferases is studied with the smallest active acceptor and their derivatives (see for example ref. [6]). However, a number of these enzymes have been shown to interact with parts of the acceptor structure remote from the site of glycosylation (see for example ref. [7]). These findings prompted a detailed exploration of the binding features of glycosyltransferases using modified acceptors beyond the minimal recognition structure, at the interface of a biologically relevant and synthetically feasible approach.

In a first approach, we focus on sialyltransferases, since sialic acids occur at the non-reducing termini of many glycans and are considered to be 'key' determinants in the regulation of many cell-surface recognition phenomena [8]. Recent studies with a purified rat-liver Gal-β-(1 → 4)-GlcNAc α-(2 → 6)-sialyltransferase revealed that the enzyme, though specific for the β-(1 → 4) linkage of the penultimate sugar in the *N*-acetyllactosamine epitope in glycoprotein N-glycans [9], tolerated some modifications in the accepting terminal monosaccharide, producing varying yields of oligosaccharides [10,11]. In β-D-Hex *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → O)CH_3 the enzyme transferred Neu5Ac from CMP-Neu5Ac to the primary hydroxyl group of Hex, where Hex was Gal, GalNAc, Glc, GlcNAc, or Man [10]. These results indicated that some of the hydroxyl groups of the terminal monosaccharide are of minor importance for effective sialylation, a phenomenon that has been further explored in the present study. To this end, a number of modified trisaccharides of the type D-Hex *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$, representing a complete branch of an *N*-acetyllactosamine glycan, have been synthesized chemically. These compounds all contain an octyl aglycon to facilitate the determination of kinetic parameters using the so-called Sep-Pak assay [12]. In this paper, the synthesis of β-D-Gal *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$ (**1**) and a first series of analogues is described, wherein

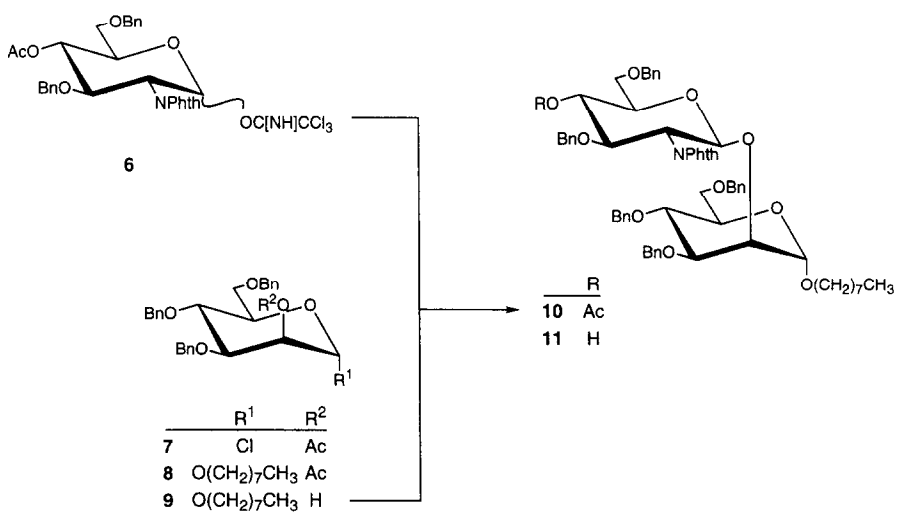
HO-4 of terminal galactopyranose was replaced by hydrogen (**2**), methoxy (**3**), or fluorine (**4**) functions, or epimerized (**5**).



	R^1	R^2
1	OH	H
2	H	H
3	OMe	H
4	F	H
5	H	OH

2. Results and discussion

For the syntheses of **1–5**, all containing the element → 4)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → O)$(CH_2)_7CH_3$, a general strategy was aimed at the preparation of the disaccharide acceptor octyl 3,4,6-tri-*O*-benzyl-2-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranoside (**11**), followed by elongation at HO-4′ using suitably modified glycosyl donors. This would allow the chemical modification (i.e., the 4-deoxygenation, the 4-*O*-methylation, and the 4-fluorination) to be carried out at the level of galactose, to minimize the number of protective groups that might interfere. Some of the modifications (particularly deoxygenation) turned out to give rise to an increase in reactivity at the anomeric center, necessitating the selection of a glycosyl donor of suitable reactivity.

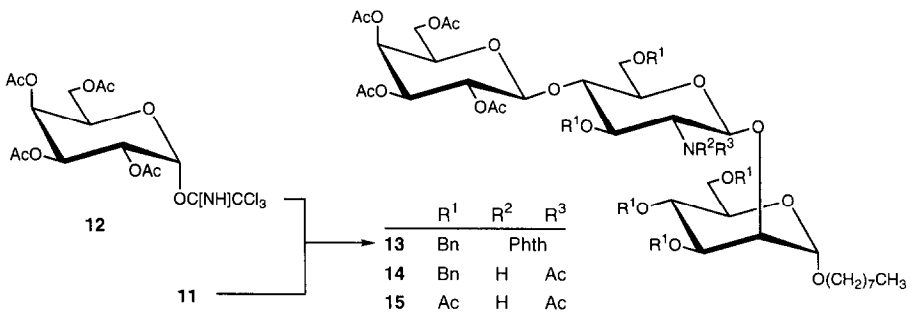


	R^1	R^2
7	Cl	Ac
8	$O(CH_2)_7CH_3$	Ac
9	$O(CH_2)_7CH_3$	H

	R
10	Ac
11	H

For the synthesis of the general disaccharide acceptor **11**, the mannosyl acceptor **9** was synthesized *via* a silver triflate promoted condensation of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**7**) [13] with 1-octanol in 1:1 nitromethane–toluene (→ **8**, 53%), followed by Zemplén deacetylation (→ **9**, 88%). Condensation of **9** with 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl trichloroacetimidate (**6**) [14] in CH_2Cl_2, using boron trifluoride etherate as a catalyst, gave **10** (90%), which was deacetylated to afford **11** (80%).

The synthesis of trisaccharide **1** involved galactosylation of **11** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**12**) [15] in CH_2Cl_2 as a first step, using trimethylsilyl triflate as a catalyst, to afford **13** (94%). Dephthaloylation of **13** with hydrazine monohydrate in aqueous ethanol, followed by *N*,*O*-acetylation, gave **14** (80%), but the use of ethylenediamine [16] in 1-butanol improved the yield of this step from 80% to 95%. Catalytic hydrogenation of **14** over 10% Pd–C and subsequent *O*-acetylation (→ **15**, 97%) and *O*-deacetylation afforded, after purification on Bio-Gel P-2 and lyophilization, **1** (94%). The *O*-acetylation step at the final stage of the deprotection sequence was carried out to allow a good chromatographic purification, ensuring a high purity of the deprotected structure. The ^{1}H NMR structural-reporter-group data of **1** are presented in Table 1. It should be noted that the propyl [17], aminohexyl [18], and 8-methoxycarbonyloctyl [11] analogues of **1** have been described previously.



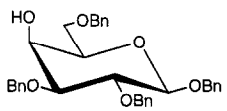
Since it is known that deoxyglycosyl donors are more reactive than the parent saccharides [19,20], several donors were examined for their suitability in the preparation of trisaccharide **2**, containing a 4-deoxy-D-galactosyl (systematic name: 4-deoxy-D-*xylo*-hexosyl) group. To this end, benzyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**16**) [21] was chosen as a precursor for the donors **22**, **23**, **24**, and **26**. As a first step, **16** was converted into the methyl xanthate **17**, which was reduced with tributyltin hydride (→ **18**, 82% from **16**), and then hydrogenolyzed over 10% Pd–C (→ **19**) and acetylated, to afford **20** (86% from **18**). Selective deacetylation of **20** (→ **21**) and treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave imidate **22** (63% from **20**). Treatment of **20** or **25**, obtained by conventional benzoylation (benzoyl chloride, pyridine) of **19**, with hydrogen bromide in acetic acid gave bromides **23** (93%) and **26** (82% from **19**). An anomeric mixture (α:β = 1:6) of ethyl thioglycosides (**24**, 70%) was obtained from **20** by a boron trifluoride etherate catalyzed

Table 1
500-MHz ^{1}H NMR data of trisaccharides **1–5** with the general formula β-D-Hex *p*-(1 → 4)-β-D-Glc *p*NAc-(1 → 2)-α-D-Man *p*-(1 → O)$(CH_2)_7CH_3$

Residue	Reporter group (J)	δ (ppm)/J (Hz)				
		1 Hex = Gal	**2** Hex = 4-deoxy-Gal	**3** Hex = 4-OMe-Gal	**4** Hex = 4-deoxy-4-fluoro-Gal	**5** Hex = Glc
α-D-Man *p*	H-1 ($J_{1,2}$)	4.861 (1.5)	4.860 (1.5)	4.860 (1.5)	4.861 (1.5)	4.860 (1.6)
	H-2 ($J_{2,3}$)	4.043 (3.5)	4.042 (3.5)	4.039 (3.4)	4.043 (3.5)	4.041 (3.5)
β-D-Glc *p*NAc	H-1 ($J_{1,2}$)	4.583 (7.6)	4.580 (7.8)	4.578 (7.7)	4.584 (7.8)	4.579 (7.7)
	NAc	2.050	2.050	2.050	2.051	2.051
β-D-Hex *p*	H-1 ($J_{1,2}$)	4.468 (7.9)	4.446 (7.9)	4.436 (7.8)	4.561 (8.4)	4.526 (8.0)
	H-2 ($J_{2,3}$)	n.d. [a]	3.210 (9.2)	n.d.	n.d.	3.309 (9.3)
	H-4eq ($J_{3,4eq}$) ($J_{4eq,F}$)	3.927 (3.4)	1.975 (5.2)	n.d.	4.847 (2.8) (50.3)	–
	H-4ax ($J_{3,4ax}$) ($J_{4eq,4ax}$)	–	1.448 (11.7) (12.9)	–	–	3.409 (9.6)
	CH_3O	–	–	3.516	–	–
Octyl	CH_3	0.860	0.861	0.860	0.861	0.860

[a] n.d. = not determined.

reaction with EtSH. The glycosylation behaviour of the bromides **23** and **26**, examined towards the corresponding allyl glycoside analogue of acceptor **11**, revealed that these donors produced mixtures (presumably of trisaccharide derivatives) which could not be separated (unpublished data). Therefore, **23** and **26** were not further investigated. The glycosylation of **11** with imidate **22** in CH_2Cl_2 at -30 °C, using trimethylsilyl triflate as a catalyst, resulted in the formation of pure **27** (74%). It should be noted that the donor reagent was added dropwise to a solution of acceptor (**11**) and the catalyst in this reaction, requiring a 2.5-fold excess of **22**. Alternatively, the use of thioglycosides **24** (1.3 equiv) in CH_2Cl_2 at 0 °C, with *N*-iodosuccinimide–triflic acid as a promoter, gave **27** in high yield (81%) without the necessity of a reversed addition of reagents. Deprotection of **27** (→ **28**, → **29**, → **2**), in an analogous way to that indicated for **13**, using hydrazine monohydrate as a dephthaloylation reagent, afforded **2** in an overall yield of 75%. For ^{1}H NMR data, see Table 1.



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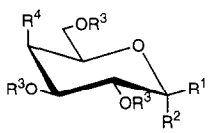
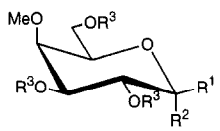


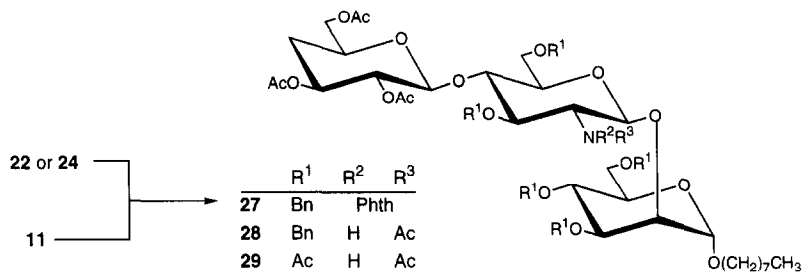


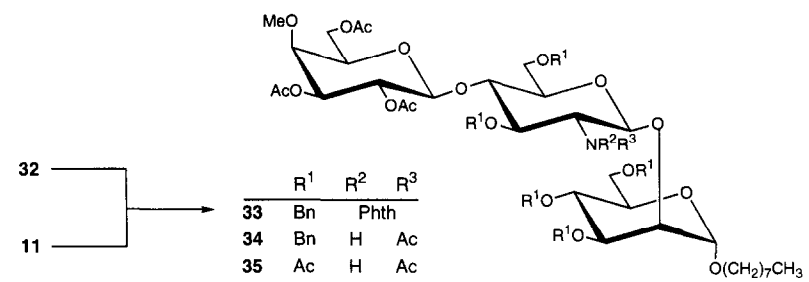
	R^1	R^2	R^3	R^4
17	OBn	H	Bn	OC[=S]SMe
18	OBn	H	Bn	H
19	H,OH		H	H
20	H,OAc		Ac	H
21	H,OH		Ac	H
22	H	$OC[=NH]CCl_3$	Ac	H
23	H	Br	Ac	H
24	H,SEt		Ac	H
25	H,OBz		Bz	H
26	H	Br	Bz	H

	R^1	R^2	R^3
30	OBn	H	Bn
31	OAc	H	Ac
32	H,SEt		Ac

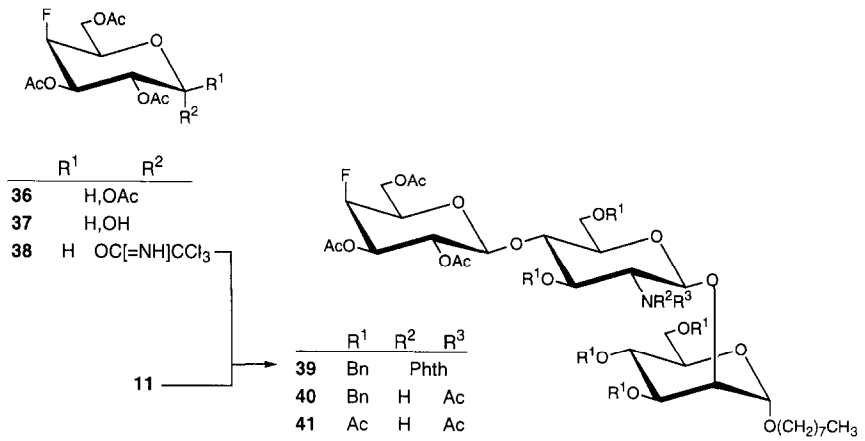
In the synthesis of trisaccharide derivative **3**, a 4-*O*-methyl-D-galactosyl donor was required for glycosylation of **11**. Preliminary experiments (data not shown) with the allyl glycoside analogue of **11** indicated that the presence of the methyl group enhanced the reactivity at the anomeric center of the galactose residue, leading to a loss of stereoselectivity. For example, the use of 2,3,6-tri-*O*-acetyl-4-*O*-methyl-α-D-galactopyranosyl trichloroacetimidate as a donor with either trimethylsilyl triflate or boron trifluoride etherate as a catalyst proved to be unsuitable for the exclusive formation of a β linkage.

A reversed addition of reagents was not attempted, since this would require a relatively large excess of the donor reagent. Instead, in view of the efficacy of thioglycosides **24** in the stereoselective glycosylation of **11**, the synthesis of thioglycosides **32** was planned, starting from compound **16**. Methylation (MeI, NaH) of **16** afforded crystalline **30** (86%), which was debenzylated and then acetylated to afford **31** (97%). Treatment of **31** with EtSH in the presence of boron trifluoride etherate gave an anomeric mixture (α:β = 1:2) of ethyl thioglycosides (**32**, 73%). Coupling of **11** with **32** in CH_2Cl_2 at 0 °C, using *N*-iodosuccinimide–triflic acid as a promoter, gave the expected β-linked trisaccharide **33** in high yield (85%). Deblocking of **33** (→ **34**, → **35**, → **3**) as indicated for **13**, using hydrazine monohydrate as the dephthaloylation reagent, yielded **3** in an overall yield of 62%. For ^{1}H NMR data, see Table 1.

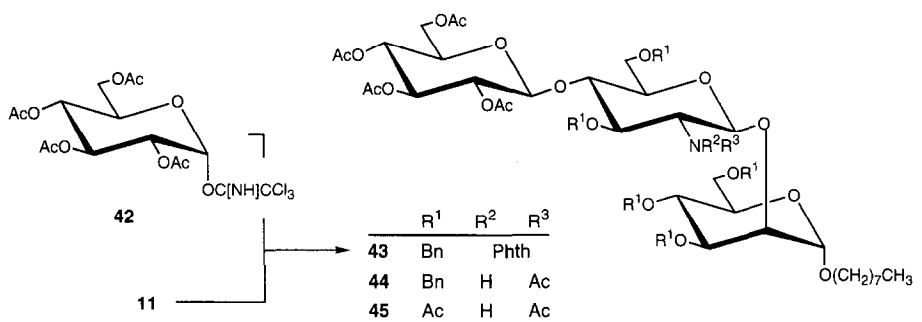




For the synthesis of the 4″-fluorinated trisaccharide **4**, galactosyl imidate **38** was prepared. Removal of the acetyl group at O-1 from 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-α,β-D-galactopyranose (**36**) [22] with hydrazine acetate (→ **37**) and imidation using trichloroacetonitrile and DBU yielded **38** (63% from **36**). Coupling of **11** and **38** in CH_2Cl_2 at 0 °C, using trimethylsilyl triflate as a catalyst, gave **39** (93%). After deprotection of **39** (→ **40**, → **41**, → **4**) as described for **13**, using hydrazine monohydrate as the dephthaloylation reagent, compound **4** was obtained in an overall yield of 59%. For ^{1}H NMR data, see Table 1.



The synthesis of the glucose-containing trisaccharide analogue **5** was carried out by condensation of **11** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**42**) [15] in CH_2Cl_2 at 0 °C, in the presence of trimethylsilyl triflate, affording **43** (76%). This trisaccharide derivative was deprotected in a similar way as described for **13** (→ **44**, → **45**, → **5**), using ethylenediamine as the dephthaloylation reagent, to give **5** in an overall yield of 65%. For ^{1}H NMR data, see Table 1.



Summarizing the various coupling results, it can be concluded that thioglycosides were most appropriate for stereoselective coupling of activated synthons carrying deoxy or *O*-methyl groups, whereas trichloroacetimidates gave high yields with deactivated fluorine-containing synthons. A kinetic study of the transfer of Neu5Ac from CMP-Neu5Ac to the trisaccharides **1**–**5** as well as to a series of additional synthesized trisaccharides wherein Hex = 3-*O*-methyl-β-D-Gal, 3-deoxy-β-D-Gal, 3-deoxy-3-fluoro-β-D-Gal, 3-amino-3-deoxy-β-D-Gal, β-D-Gul, α-L-Alt, or β-L-Gal, by several purified and recombinant α-(2 → 6)- and α-(2 → 3)-sialyltransferases, will be reported elsewhere.

3. Experimental

General methods.—All solvents were distilled from appropriate drying agents. Reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck) using solvent mixtures of appropriately adjusted polarity; solvent A = 4:2:2:1 1-butanol–EtOH–HOAc–H_2O. Compounds were visualized by charring with aq 50% sulfuric acid, after examination under UV light. In the workup procedures of reaction mixtures, organic solutions were washed with appropriate amounts of aqueous solutions as indicated, or with 8 mM phosphate buffer (pH 7.5), then dried ($MgSO_4$), and concentrated under reduced pressure at 20–40 °C. Column chromatography was performed on Kieselgel 60 F_{254} (70–230 mesh, Merck), unless otherwise stated. Optical rotations were determined for solutions in $CHCl_3$ unless otherwise stated, at 20 °C with a Perkin–Elmer 241 polarimeter, using a 10-cm 1-mL cell. ^{1}H NMR spectra were recorded with a Bruker AC 300 or AM 500 spectrometer; the values of δ_H are expressed in ppm relative to the signal for internal Me_4Si for solutions in $CDCl_3$, or by reference to acetone (δ 2.225) for solutions in D_2O. ^{13}C NMR spectra were recorded with a Bruker WP 200 (50 MHz) or a Varian Gemini-300 instrument (75 MHz); indicated values for δ_C are relative to the signal of $CDCl_3$ (δ 76.9). Microanalyses were carried out by the Mikroanalytisches Laboratorium of H. Kolbe (Mülheim an der Ruhr, Germany). Fast-atom-bombardment mass spectrometry (FABMS) was performed on a JEOL JMS SX/SX 102A four-sector mass spectrometer, operated at 10-kV accelerating voltage, equipped with a JEOL MS-FAB 10 D FAB gun operated at 10-mA emission current, producing a beam of 6-keV xenon atoms.

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**8**).—A solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride [13] (**7**; 6.5 g, 12.8 mmol) and 1-octanol (3 mL, 19.0 mmol) in 1:1 $MeNO_2$–toluene (30 mL), containing molecular sieves 3 Å (2 g), was cooled to −20 °C under Ar. After stirring for 30 min, a solution of silver triflate (4.6 g, 17.9 mmol) and *sym*-collidine (1.3 mL, 9.8 mmol) in 1:1 $MeNO_2$–toluene (12 mL) was added dropwise in the dark, and the mixture was allowed to attain room temperature. TLC (5:1 hexane–EtOAc) indicated the disappearance of **7** and the formation of a major new spot (**8**, R_f 0.33) as well as of some minor products, then the mixture was neutralized with Et_3N, and filtered through Celite. The filtrate was washed with aq 10% $Na_2S_2O_3$, H_2O, M HCl, aq 10% $NaHCO_3$, and H_2O, and then concentrated. Column chromatography (5:1 hexane–EtOAc) of the residue gave **8**, isolated as a syrup (4.1 g, 53%); $[\alpha]_D$ +16° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.38–7.12 (m, 15 H, 3 Ph), 5.362 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.850, 4.710, 4.690, 4.535, 4.512, and 4.466 (6 d, each 1 H, 3 PhCH_2O), 4.825 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 3.989 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 3.882 (dd, 1 H, $J_{4,5}$ 9.1 Hz, H-4), 3.656 and 3.391 (2 dt, each 1 H, octyl OCH_2), 2.150 (s, 3 H, Ac), 0.879 (t, 3 H, octyl CH_3); ^{13}C, δ 170.3 ($COCH_3$), 78.2, 74.4, 71.3, and 68.8 (C-2,3,4,5), 75.0, 73.3, 72.0, 68.9, and 67.9 (C-6, 3 PhCH_2O, and octyl OCH_2), 31.6, 29.2 (2 C), 29.0, 25.9, and 22.5 (6 octyl CH_2), 20.9 ($COCH_3$), 13.9 (octyl CH_3); ^{13}C (^{1}H coupled), δ 97.6 (d, $J_{C\text{-}1,H\text{-}1}$ 170.1 Hz, C-1). Anal. Calcd for $C_{37}H_{48}O_7$ (604.79): C, 73.48; H, 8.00. Found: C, 73.24; H, 7.99.

Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranoside (**9**).—To a solution of **8** (3.5 g, 5.8 mmol) in 1:1 CH_2Cl_2–MeOH (20 mL) was added NaOMe (pH 10), and the mixture

was stirred overnight, neutralized with Dowex-50 (H^+) resin, filtered, and then concentrated. Column chromatography (3:1 hexane–EtOAc) of the residue afforded **9**, isolated as a syrup (2.9 g, 88%); $[\alpha]_D$ +36° (*c* 1); R_f 0.41 (3:1 hexane–EtOAc); NMR ($CDCl_3$): ^1H, δ 7.45–7.15 (m, 15 H, 3 Ph), 4.885 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.818, 4.718, 4.674, 4.652, 4.531, and 4.502 (6 d, each 1 H, 3 PhCH_2O), 4.025 (dd, 1 H, $J_{2,OH}$ 2.8 Hz, H-2), 3.670 and 3.405 (2 dt, each 1 H, octyl OCH_2), 2.429 (d, 1 H, OH), 0.879 (t, 3 H, octyl CH_3); ^{13}C, δ 99.1 (C-1), 80.0, 74.1, 70.8, and 68.2 (C-2,3,4,5), 74.8, 74.1, 71.6, 68.9, and 67.5 (C-6, 3 PhCH_2O, and octyl OCH_2), 31.6, 29.1 (2 C), 28.9, 25.9, and 22.4 (6 octyl CH_2), 13.9 (octyl CH_3).

*Octyl 2-O-(4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-3,4,6-tri-O-benzyl-α-*D*-mannopyranoside* (**10**).—A solution of 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-α,β-D-glucopyranosyl trichloroacetimidate [14] (**6**; 1.7 g, 2.5 mmol) and **9** (1.1 g, 1.9 mmol) in CH_2Cl_2 (15 mL) was stirred under N_2 in the presence of molecular sieves 3 Å (1 g) for 30 min at −30 °C. Then $BF_3 \cdot Et_2O$ in CH_2Cl_2 (2 M, 0.3 mL) was added, and the mixture was stirred for 45 min at −30 → −15 °C. TLC (5:1 toluene–EtOAc) showed the disappearance of **9** and the formation of **10** (R_f 0.60), and the mixture was neutralized with Et_3N, diluted with CH_2Cl_2, washed with phosphate buffer and aq 5% NaCl, then concentrated. Column chromatography (3:1 hexane–EtOAc) of the residue yielded **10**, isolated as a colourless syrup (1.8 g, 90%); $[\alpha]_D$ +13° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.61–6.87 (m, 29 H, 5 Ph and Phth), 5.287 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 5.151 (dd, 1 H, $J_{3',4'}$ 8.7, $J_{4',5'}$ 9.8 Hz, H-4'), 3.169 (dt, 1 H, octyl OC*H*H), 1.946 (s, 3 H, Ac), 0.870 (t, 3 H, octyl CH_3); ^{13}C, δ 169.5 ($COCH_3$), 133.5, 131.5, and 122.9 (Phth), 96.7 (2 C, C-1,1'), 77.6, 76.5, 74.5, 73.6, 73.3, 72.4, and 71.4 (C-2,3,4,5,3',4',5'), 74.6, 73.5 (3 C), 72.6, 70.6, 69.8, and 67.5 (C-6,6', 5 PhCH_2O, and octyl OCH_2), 55.5 (C-2'), 31.6, 29.1 (2 C), 28.9, 25.8, and 22.4 (6 octyl CH_2), 20.6 ($CO\mathit{C}H_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{65}H_{73}NO_{13}$ (1076.31): C, 72.54; H, 6.84. Found: C, 72.43; H, 6.75.

*Octyl 3,4,6-tri-O-benzyl-2-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-α-*D*-mannopyranoside* (**11**).—To a solution of **10** (1.1 g, 1.0 mmol) in 4:1 MeOH–CH_2Cl_2 (25 mL) was added NaOMe (pH 9), and the mixture was stirred overnight, neutralized with Dowex-50 (H^+), filtered, and then concentrated. Column chromatography (3:1 hexane–EtOAc) of the residue gave **11**, isolated as a colourless syrup (0.82 g, 80%); $[\alpha]_D$ −6° (*c* 1); R_f 0.40 (2:1 hexane–EtOAc); NMR ($CDCl_3$): ^1H, δ 7.66–6.94 (m, 29 H, 5 Ph and Phth), 5.264 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.781, 4.735, 4.598, 4.553, 4.543, 4.503, 4.356, 4.077, and 3.997 (9 d, 1,1,1,1,1,2,1,1,1 H, 5 PhCH_2O), 4.453 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.323 (dd, 1 H, $J_{2',3'}$ 10.8 Hz, H-2'), 4.243 (dd, 1 H, $J_{3',4'}$ 7.8 Hz, H-3'), 4.023 (dd, 1 H, H-2), 3.167 (dt, 1 H, octyl OC*H*H), 0.869 (t, 3 H, octyl CH_3); ^{13}C, δ 133.4, 131.7, and 122.9 (Phth), 96.8 (2 C, C-1,1'), 78.4, 77.8, 74.6, 74.4 (2 C), 74.0, and 71.4 (C-2,3,4,5,3',4',5'), 74.7, 74.0, 73.8, 72.7, 71.1, 70.8, 69.9, and 67.6 (C-6,6', 5 PhCH_2O, and octyl OCH_2), 55.1 (C-2'), 31.6, 29.2 (2 C), 29.0, 25.9, and 22.5 (6 octyl CH_2), 13.9 (octyl CH_3). Anal. Calcd for $C_{63}H_{71}NO_{12} \cdot 0.5H_2O$ (1043.28): C, 72.53; H, 6.96. Found: C, 72.23; H, 6.89.

*Octyl (2,3,4,6-tetra-O-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-*D*-mannopyranoside* (**13**).—A solution of **11** (0.20 g, 0.19 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-

galactopyranosyl trichloroacetimidate [15] (**12**; 0.14 g, 0.28 mmol) in CH_2Cl_2 (5 mL), containing molecular sieves 4 Å (0.3 g), was stirred for 30 min under N_2 at 0 °C, and trimethylsilyl triflate in CH_2Cl_2 (0.1 M, 0.6 mL) was added. After stirring for 30 min, TLC showed the disappearance of both **11** and **12** and the presence of a new compound **13** (R_f 0.47, 3:1 toluene–EtOAc), and the mixture was neutralized by the addition of Et_3N, diluted with CH_2Cl_2, and filtered. The filtrate was washed twice with phosphate buffer and concentrated. Column chromatography (2:1 hexane–EtOAc) of the residue afforded **13**, isolated as a colourless syrup (0.25 g, 94%); $[\alpha]_D$ −1° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.65–6.85 (m, 29 H, 5 Ph and Phth), 5.271 (dd, 1 H, $J_{3'',4''}$ 3.5 Hz, H-4″), 5.239 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.159 (dd, 1 H, $J_{2'',3''}$ 10.4 Hz, H-2″), 4.864 (dd, 1 H, H-3″), 4.795, 4.788, 4.717, 4.473, 4.460, 4.396, 4.344, 4.077, and 3.994 (9 d, 1,1,2,1,1,1,1,1,1 H, 5 PhCH_2O), 4.635 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.476 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 3.165 (dt, 1 H, octyl OC*H*H), 2.064, 2.034, 2.010, and 1.970 (4 s, each 3 H, 4 Ac), 0.869 (t, 3 H, octyl CH_3); ^{13}C, δ 170.2, 170.1, 169.9, and 169.0 (4 *C*OCH$_3$), 133.3, 131.6, and 123.0 (Phth), 100.2 and 96.8 (2 C) (C-1,1′,1″), 78.0, 77.8, 74.8, 74.7, 74.6, 73.5, 71.6, 70.9, 70.5, 69.5, and 66.9 (C-2,3,4,5,3′,4′,5′,2″,3″,4″,5″), 74.7 (2 C), 74.1, 73.6, 72.7, 70.6, 68.4, 67.6, and 60.8 (C-6,6′,6″, 5 Ph*C*H$_2$O, and octyl OCH$_2$), 55.4 (C-2′), 31.7, 29.2 (2 C), 29.1, 25.9, and 22.5 (6 octyl CH_2), 20.4 (CO*C*H$_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{77}H_{89}NO_{21}$ (1364.57): C, 67.78; H, 6.57. Found: C, 67.83; H, 6.65.

*Octyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-acetyl-α-*D*-mannopyranoside* (**15**).—(*a*) *Dephthaloylation with hydrazine monohydrate.* To a solution of **13** (88.0 mg, 64.5 μmol) in 9:1 EtOH–H_2O (10 mL) was added hydrazine monohydrate (1.5 mL, 31 mmol), and the mixture was heated overnight at 90 °C. TLC (8:1 CH_2Cl_2–MeOH) then showed the absence of **13** and the formation of a single product (R_f 0.41), and the mixture was concentrated, and co-concentrated with toluene. The residue was dissolved in pyridine (3 mL) and acetylated overnight with Ac_2O (3 mL). After concentration, column chromatography (3:1 toluene–EtOAc) of the residue gave **14**, isolated as a colourless syrup (65.7 mg, 80%).

(*b*) *Dephthaloylation with ethylenediamine.* A solution of **13** (98.8 mg, 72.4 μmol) in 1-butanol (10 mL), containing molecular sieves 3 Å (0.3 g), was stirred for 30 min under Ar, and ethylenediamine (1 mL, 15 mmol) was added. The mixture was heated overnight at 90 °C, then concentrated, and further processed as described above, to yield **14** as a colourless syrup (87.9 mg, 95%); $[\alpha]_D$ +2° (*c* 1); R_f 0.31 (3:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.571 (d, 1 H, $J_{2',NH}$ 7.1 Hz, NH); ^{13}C, δ 23.2 (NHCO*C*H$_3$). To a solution of **14** (0.17 g, 0.13 mmol) in 1:1 EtOH–EtOAc (10 mL) were added HOAc (0.1 mL) and 10% Pd–C (55 mg), and the mixture was hydrogenated at atmospheric pressure for 30 min. TLC (solvent *A*) showed the complete conversion of **14** into a new product (R_f 0.73). After filtration through Celite and concentration of the solution, the residue was acetylated overnight with 1:1 Ac_2O–pyridine (10 mL). The latter solution was concentrated, then co-concentrated using toluene, and column chromatography (1:3 toluene–EtOAc) of the residue afforded **15**, isolated as a colourless glass (0.13 g, 97%); $[\alpha]_D$ −9° (*c* 1); R_f 0.21 (1:2 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.768 (d, 1 H, $J_{2',NH}$ 8.7 Hz, NH), 5.356 (dd, 1 H, $J_{3'',4''}$ 3.4, $J_{4'',5''}$ < 1 Hz, H-4″), 5.242 (dd, 1 H,

H-3′), 5.219 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-4), 5.102 (dd, 1 H, $J_{2'',3''}$ 10.4 Hz, H-2″), 5.090 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-3), 4.970 (dd, 1 H, H-3″), 4.719 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.670 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.484 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 3.418 (dt, 1 H, octyl OC*H*H), 2.149, 2.117, 2.089, 2.066, 2.042, 2.029, 1.994, 1.967, and 1.944 (9 s, 3,3,6,3,3,3,3,3,3 H, 10 Ac), 0.888 (t, 3 H, octyl CH_3); ^{13}C, δ 100.7, 99.2, and 97.2 (C-1,1′,1″), 75.8, 74.5, 72.5, 71.5, 70.6 (2 C), 70.1, 68.9, 68.4, 66.6, and 66.1 (C-2,3,4,5,3′,4′,5′,2″,3″,4″,5″), 68.2, 62.6, 62.4, and 60.7 (C-6,6′,6″ and octyl OCH_2), 53.8 (C-2′), 31.5, 29.1, 29.0, 28.9, 25.9, and 22.3 (6 octyl CH_2), 13.9 (octyl CH_3). Anal. Calcd for $C_{46}H_{69}NO_{25}$ (1036.06): C, 53.33; H, 6.71. Found: C, 53.48; H, 6.74.

*Octyl β-*D*-galactopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-α-*D*-mannopyranoside* (**1**).—To a solution of **15** (0.13 g, 0.13 mmol) in 4:1 MeOH–CH_2Cl_2 (10 mL) was added NaOMe (pH 8), and the mixture was stirred for 2 h. After neutralization with Dowex-50 (H^+) resin and filtration, the solution was concentrated. Gel filtration of the residue on a Bio-Gel P-2 column, eluted with H_2O, and subsequent lyophilization gave **1** as a white powder (77.3 mg, 94%); $[\alpha]_D$ −6° (*c* 1.3, MeOH); R_f 0.33 (solvent *A*); NMR (D_2O): ^1H, see Table 1. FABMS: *m*/*z* 658 $[M + H]^+$, 680 $[M + Na]^+$.

*Benzyl 2,3,6-tri-*O*-benzyl-4-deoxy-β-*D*-xylo-hexopyranoside* (**18**).—To a solution of benzyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside [21] (**16**; 2.0 g, 3.7 mmol) in tetrahydrofuran (15 mL) under N_2 were added imidazole (11.3 mg, 0.17 mmol) and NaH (0.24 g, 10.0 mmol), and the suspension was stirred for 1 h, then CS_2 (2 mL, 33 mmol) was added. The stirring was continued for 1 h, MeI (0.53 mL, 8.5 mmol) was added, and after 30 min TLC (7:3 hexane–EtOAc) showed the formation of **17** (R_f 0.59). The mixture was diluted with CH_2Cl_2, washed with phosphate buffer, 0.2 M HCl, aq 10% $NaHCO_3$, and H_2O, then concentrated. The crude residue was dissolved in toluene (30 mL) and heated to 80 °C under Ar. Tributyltin hydride (12.5 mL, 46.5 mmol) and a catalytic amount of 2,2-azobisisobutyronitrile were added, and the mixture was stirred for 30 min, when TLC (95:5 toluene–EtOAc) showed the conversion of **17** (R_f 0.47) into **18** (R_f 0.34). The mixture was concentrated, and a solution of the residue in MeCN was washed twice with hexane, then concentrated. Column chromatography (95:5 toluene–EtOAc) of the residue yielded **18**, isolated as a colourless syrup (1.6 g, 82% from **16**); $[\alpha]_D$ −21° (*c* 1, CH_2Cl_2); NMR ($CDCl_3$): ^1H, δ 7.40–7.27 (m, 20 H, 4 Ph), 4.957, 4.930, 4.767, 4.665, 4.513, and 4.567 (6 d, each 1 H, 3 PhCH_2O), 4.671 (s, 2 H, PhCH_2O), 4.467 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.395 (dd, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 2.123 (ddd, 1 H, $J_{3,4eq}$ 5.3, $J_{4eq,4ax}$ 12.8, $J_{4eq,5}$ 1.6 Hz, H-4eq), 1.487 (ddd, 1 H, $J_{3,4ax}$ 11.3, $J_{4ax,5}$ 11.3 Hz, H-4ax); ^{13}C, δ 102.6 (C-1), 82.7, 78.0, and 70.9 (C-2,3,5), 74.8, 73.3 (2 C), 72.3, and 72.1 (C-6 and 4 Ph*C*H_2O), 33.7 (C-4).

*1,2,3,6-Tetra-*O*-acetyl-4-deoxy-α,β-*D*-xylo-hexopyranose* (**20**).—A solution of **18** (0.19 g, 0.36 mmol) in 1:1 EtOH–EtOAc (20 mL), containing HOAc (0.5 mL) and 10% Pd–C (100 mg), was hydrogenolyzed at atmospheric pressure for 2 h. Because of incomplete debenzylation, the hydrogenolysis was repeated with intermediate filtration through Celite and addition of new catalyst. After 5 h, when TLC (solvent *A*) showed the presence of one new product (**19**, R_f 0.34), the mixture was filtered through Celite and concentrated. The crude residue (**19**) was acetylated overnight in 1:2 Ac_2O–pyridine (7.5 mL). After concentration, and co-concentration with toluene, column chro-

matography (4:1 toluene–EtOAc) of the residue gave **20**, isolated as a syrup (103 mg, 86%, $\alpha:\beta = 2:3$); R_f 0.45 (2:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H δ 6.336 (d, 0.4 H, $J_{1,2}$ 3.6 Hz, H-1α), 5.653 (d, 0.6 H, $J_{1,2}$ 8.0 Hz, H-1β), 2.149, 2.092, 2.054, and 2.025 (4 s, each 1.2 H, 4 α-Ac), 2.105, 2.086, and 2.045 (3 s, 1.8,1.8,3.6 H, 4 β-Ac); ^{13}C, δ 170.5, 170.0, 169.3, and 168.5 (4 $COCH_3$), 92.0 (C-1β), 89.9 (C-1α), 64.9 (C-6), 32.3 (C-4). Anal. Calcd for $C_{14}H_{20}O_9$ (332.31): C, 50.60; H, 6.07. Found: C, 50.35; H, 6.15.

2,3,6-Tri-O-*acetyl-4-deoxy-α*-D-xylo-*hexopyranosyl trichloroacetimidate* (**22**).—A solution of **20** (34.9 mg, 105 μmol) and hydrazine acetate (10.7 mg, 116 μmol) in DMF (2 mL) was heated for 30 min at 50 °C. Since TLC (3:2 toluene–EtOAc) showed that ~ 25% of the starting material (R_f 0.49) was still present, an additional amount of hydrazine acetate (2.5 mg, 27 μmol) was added. After 30 min, when TLC indicated a complete conversion of **20** into **21** (R_f 0.32), the mixture was diluted with EtOAc, washed with aq 5% NaCl (2 ×) and H_2O, concentrated, and co-concentrated with toluene. To a solution of the residue (**21**) in CH_2Cl_2 (2 mL) was added trichloroacetonitrile (0.1 mL, 1.0 mmol), and the mixture was cooled to 0 °C. Then, 0.2 M DBU in CH_2Cl_2 (0.25 mL) was added and the mixture was stirred overnight. Column chromatography (3:1 CH_2Cl_2–EtOAc) of the solution afforded **22**, isolated as a colourless syrup (28.7 mg, 63% from **20**); $[\alpha]_D$ +97° (*c* 1); R_f 0.48 (3:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 8.610 (s, 1 H, NH), 6.535 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.361 (ddd, 1 H, $J_{2,3}$ 10.2, $J_{3,4eq}$ 5.2, $J_{3,4ax}$ 11.3 Hz, H-3), 5.064 (dd, 1 H, H-2), 4.31 (m, 1 H, H-5), 2.301 (ddd, 1 H, $J_{4eq,4ax}$ 12.8, $J_{4eq,5}$ 2.3 Hz, H-4eq), 2.057, 2.051, and 2.018 (3 s, each 3 H, 3 Ac), 1.688 (ddd, 1 H, $J_{4ax,5}$ 8.2 Hz, H-4ax). FABMS: *m/z* 456:458:460 (9:9:3) $[M + Na]^+$.

Ethyl 2,3,6-tri-O-*acetyl-4-deoxy-1-thio-α,β*-D-xylo-*hexopyranoside* (**24**).—A solution of **20** (36.4 mg, 110 μmol) in CH_2Cl_2 (3 mL), containing molecular sieves 3 Å (0.3 g), was stirred for 30 min, and EtSH (0.1 mL, 1.4 mmol) and $BF_3 \cdot Et_2O$ (40 μL, 0.32 mmol) were added. After 2 h, when TLC (2:1 toluene–EtOAc) showed the conversion of **20** into a major (**24β**, R_f 0.54) and a minor (**24α**, R_f 0.58) product, the mixture was neutralized with Et_3N, filtered, and concentrated. Column chromatography (3:1 toluene–EtOAc) of the residue afforded **24**, isolated as a syrup (25.5 mg, 70%, $\alpha:\beta = 1:6$); $[\alpha]_D$ −22° (*c* 1, β anomer); NMR ($CDCl_3$): ^{1}H (α), δ 5.676 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1), 5.194 (ddd, 1 H, $J_{2,3}$ 10.2, $J_{3,4eq}$ 5.3, $J_{3,4ax}$ 11.2 Hz, H-3), 4.990 (dd, 1 H, H-2), 4.46 (m, 1 H, H-5), 4.159 (dd, 1 H, $J_{5,6b}$ 5.8, $J_{6a,6b}$ 11.7 Hz, H-6b), 4.089 (dd, 1 H, $J_{5,6a}$ 3.9 Hz, H-6a), 2.64–2.46 (m, 2 H, SCH_2CH$_3$), 2.187 (ddd, 1 H, $J_{4eq,4ax}$ 12.5, $J_{4eq,5}$ 2.2 Hz, H-4eq), 2.079, 2.076, and 2.037 (3 s, each 3 H, 3 Ac), 1.564 (ddd, 1 H, $J_{4ax,5}$ 10.0 Hz, H-4ax), 1.266 (t, 3 H, SCH$_2$CH_3); ^{1}H (β), δ 5.020 (ddd, 1 H, $J_{2,3}$ 9.4, $J_{3,4eq}$ 4.9, $J_{3,4ax}$ 11.2 Hz, H-3), 4.938 (dd, 1 H, H-2), 4.430 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.171 (dd, 1 H, $J_{5,6b}$ 6.0, $J_{6a,6b}$ 11.7 Hz, H-6b), 4.095 (dd, 1 H, $J_{5,6a}$ 4.3 Hz, H-6a), 3.771 (m, 1 H, H-5), 2.80–2.61 (m, 2 H, SCH_2CH$_3$), 2.176 (ddd, 1 H, $J_{4eq,4ax}$ 12.7, $J_{4eq,5}$ 2.0 Hz, H-4eq), 2.076, 2.070, and 2.033 (3 s, each 3 H, 3 Ac), 1.619 (ddd, 1 H, $J_{4ax,5}$ 12.0 Hz, H-4ax), 1.268 (t, 3 H, SCH$_2$CH_3); ^{13}C (β), δ 170.3, 169.9, and 169.5 (3 $COCH_3$), 83.4 (C-1), 73.1, 71.5, and 70.5 (C-2,3,5), 65.3 (C-6), 32.7 (C-4), 23.9 (SCH_2CH$_3$), 20.7, 20.6, and 20.5 (3 COCH_3), 14.7 (SCH$_2$$CH_3$). Anal. Calcd for $C_{14}H_{22}O_7S$ (334.40): C, 50.29; H, 6.63. Found: C, 50.53; H, 6.86.

Octyl (2,3,6-tri-O-*acetyl-4-deoxy-β*-D-xylo-*hexopyranosyl)-(1 → 4)-(3,6-di*-O-*benzyl-*

2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**27**).—(*a*) *via Trichloroacetimidate*. A solution of **11** (27.0 mg, 26.1 μmol) and trimethylsilyl triflate (1.3 μmol) in CH_2Cl_2 (3 mL), containing molecular sieves 4 Å (0.2 g), was stirred for 30 min under N_2 at −30 °C, after which a solution of **22** (28.0 mg, 64.4 μmol) in CH_2Cl_2 (2 mL) was added dropwise during 30 min. TLC (3:2 hexane–EtOAc) showed the disappearance of **11** and the presence of a new compound **27** (R_f 0.37), then the mixture was neutralized with Et_3N, filtered, washed with phosphate buffer and H_2O, and concentrated. Column chromatography (Kieselgel, 2:1 hexane–EtOAc; then Sephadex LH-20, 1:1 CH_2Cl_2–MeOH) of the residue gave **27**, isolated as a colourless syrup (25.2 mg, 74%).

(*b*) *via Thioglycoside*. To a solution of **11** (55.0 mg, 53.2 μmol) and **24** (23.0 mg, 68.8 μmol) in CH_2Cl_2 (5 mL), containing molecular sieves 4 Å (0.2 g), stirred for 1 h under N_2 at 0 °C, was added dropwise during 10 min a solution of *N*-iodosuccinimide (91.1 μmol) and triflic acid (11.3 μmol) in CH_2Cl_2 (2 mL). TLC (3:2 hexane–EtOAc) showed the disappearance of **11** and the formation of **27** (R_f 0.37). The mixture was neutralized with Et_3N, filtered, washed with aq 5% $NaHSO_3$, aq 10% $NaHCO_3$, and H_2O, and concentrated. Column chromatography (2:1 hexane–EtOAc) of the residue yielded **27**, isolated as a syrup (55.6 mg, 81%); $[\alpha]_D$ −2° (*c* 0.5); NMR ($CDCl_3$): ^1H, δ 7.62–6.79 (m, 29 H, 5 Ph and Phth), 5.230 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.604 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, H-1''), 4.467 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 3.161 (dt, 1 H, octyl OC*H*H), 2.023 and 2.019 (2 s, 6,3 H, 3 Ac), 1.525 (ddd, 1 H, H-4''ax), 0.867 (t, 3 H, octyl CH_3); ^{13}C, δ 170.5, 170.1, and 169.3 (3 *C*OCH$_3$), 133.2, 131.6, and 122.9 (Phth), 100.2 and 96.7 (2 C) (C-1,1',1''), 78.0, 77.7, 76.5, 74.9, 74.6, 73.4, 72.8, 71.5, 70.7, and 69.0 (C-2,3,4,5,3',4',5',2'',3'',5''), 74.6, 74.0, 73.5, 72.7, 70.5, 69.8, 68.3, 67.5, and 64.9 (C-6,6',6'', 5 Ph*C*H$_2$O and octyl OCH$_2$), 55.4 (C-2'), 32.5 (C-4''), 31.6, 29.5, 29.2, 29.0, 25.9, and 22.5 (6 octyl CH_2), 20.7, 20.6, and 20.5 (3 CO*C*H$_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{75}H_{87}NO_{19}$ (1306.53): C, 68.95; H, 6.71. Found: C, 69.02; H, 6.78.

Octyl (2,3,6-tri-O-acetyl-4-deoxy-β-D-xylo-hexopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3,4,6-tri-O-acetyl-α-D-mannopyranoside (**29**).—A solution of **27** (115 mg, 87.9 μmol) and hydrazine monohydrate (1.1 mL) in 9:1 EtOH–H_2O (10 mL) was boiled under reflux for 16 h, when TLC (9:1 CH_2Cl_2–MeOH) showed the conversion of the starting material into an intermediate amino compound. The mixture was concentrated, then co-concentrated with toluene, and a solution of the residue in 1:1 pyridine–Ac_2O (10 mL) was stirred overnight. After concentration, column chromatography (3:2 hexane–EtOAc) of the residue afforded **28**, isolated as a syrup (94.0 mg, 88%); $[\alpha]_D$ +3° (*c* 1); R_f 0.21 (3:1 toluene–EtOAc); NMR ($CDCl_3$): ^1H, δ 5.653 (d, 1 H, $J_{2',NH}$ 7.2 Hz, NH); ^{13}C, δ 32.5 (C-4''), 23.1 (NHCO*C*H$_3$). To a solution of **28** (91.3 mg, 74.9 μmol) in 1:1 EtOH–EtOAc (10 mL) were added 10% Pd–C (40 mg) and HOAc (0.1 mL), and the suspension was hydrogenolyzed at atmospheric pressure for 45 min. TLC (solvent *A*) showed the complete disappearance of **28** and the presence of a new compound (R_f 0.65), and after filtration through Celite, the filtrate was concentrated. A solution of the residue in 1:1 pyridine–Ac_2O (10 mL) was kept for 16 h at room temperature, then concentrated. Column chromatography (1:3 toluene–EtOAc) of the residue gave **29**, isolated as a colourless glass (67.3 mg, 89%); $[\alpha]_D$ −27° (*c* 2); R_f 0.29 (1:3 toluene–EtOAc); NMR

($CDCl_3$): ^1H, δ 5.613 (d, 1 H, $J_{2',NH}$ 7.4 Hz, NH), 5.225 (dd, 1 H, H-3'), 5.222 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-4), 5.093 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-3), 4.939 (ddd, 1 H, $J_{2'',3''}$ 9.6, $J_{3'',4eq''}$ 5.2, $J_{3'',4ax''}$ 11.3 Hz, H-3''), 4.813 (dd, 1 H, H-2''), 4.708 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.664 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.392 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, H-1''), 3.417 (dt, 1 H, octyl OC*H*H), 2.11 (m, 1 H, H-4''eq), 2.111, 2.099, 2.089, 2.067, 2.041, 2.027, 2.011, 1.992, and 1.938 (9 s, each 3 H, 9 Ac), 0.888 (t, 3 H, octyl CH_3); ^{13}C, δ 170.6–169.3 ($COCH_3$), 100.7, 99.2, and 97.3 (C-1,1',1''), 75.8, 74.4, 72.6, 72.2, 71.4, 70.3, 70.1, 69.4, 68.4, and 66.0 (C-2,3,4,5,3',4',5',2'',3'',5''), 68.2, 64.9, 62.6, and 62.4 (C-6,6',6'' and octyl OCH_2), 53.8 (C-2'), 32.2 (C-4''), 31.6, 29.2, 29.1, 29.0, 25.9, and 22.4 (6 octyl CH_2), 22.9 ($NHCOCH_3$), 20.5 ($OCOCH_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{44}H_{67}NO_{23}$ (978.02): C, 54.04; H, 6.91. Found: C, 53.97; H, 6.97.

*Octyl (4-deoxy-β-*D*-xylo-hexopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-α-*D*-mannopyranoside* (**2**).—A solution of **29** (57.0 mg, 58.3 μmol) in 4:1 MeOH–CH_2Cl_2 (5 mL) was stirred with NaOMe (pH 8) for 2 h, then neutralized with Dowex-50 (H^+), filtered, and concentrated. Gel filtration of the residue on Bio-Gel P-2 (H_2O) yielded **2**, isolated after lyophilization as a white powder (36.0 mg, 96%); $[\alpha]_D$ −7° (*c* 0.8, MeOH); R_f 0.38 (solvent *A*); NMR (D_2O): ^1H, see Table 1; FABMS: *m/z* 642 $[M + H]^+$, 664 $[M + Na]^+$.

*Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-methyl-β-*D*-galactopyranoside* (**30**).—A solution of benzyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside [21] (**16**; 2.5 g, 4.6 mmol) in DMF (40 mL) was added to NaH (0.22 g, 9.3 mmol), and the mixture was stirred for 30 min under N_2. Then, MeI (0.58 mL, 9.3 mmol) was added, and the stirring was continued for another 30 min. After dilution with CH_2Cl_2, the solution was washed twice with phosphate buffer and H_2O, and concentrated. The residue was crystallized from EtOH to give **30** (2.2 g, 86%); mp 87–88 °C; $[\alpha]_D$ −22° (*c* 1, CH_2Cl_2); R_f 0.43 (97:3 CH_2Cl_2–EtOAc); NMR ($CDCl_3$): ^1H, δ 7.37–7.25 (m, 20 H, 4 Ph), 4.937, 4.903, 4.619, 4.599, 4.549, and 4.378 (6 d, each 1 H, 3 PhCH_2O), 4.737 (s, 2 H, PhCH_2O), 4.441 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 3.786 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 3.755 (dd, 1 H, $J_{5,6b}$ 7.6, $J_{6a,6b}$ 9.2 Hz, H-6b), 3.666 (dd, 1 H, $J_{5,6a}$ 5.5 Hz, H-6a), 3.648 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.7 Hz, H-4), 3.576 (s, 3 H, OCH_3), 3.531 (ddd, 1 H, H-5), 3.475 (dd, 1 H, H-3); ^{13}C, δ 102.6 (C-1), 81.9, 79.6, 76.0, and 73.2 (C-2,3,4,5), 75.2, 73.6, 72.8, 70.7, and 68.4 (C-6 and 4 PhCH_2O), 61.2 (OCH_3). Anal. Calcd for $C_{35}H_{38}O_6$ (554.69): C, 75.78; H, 6.91. Found: C, 75.66; H, 6.86.

*1,2,3,6-Tetra-*O*-acetyl-4-*O*-methyl-β-*D*-galactopyranose* (**31**).—To a solution of **30** (0.59 g, 1.1 mmol) in 1:1 EtOH–EtOAc (50 mL), containing HOAc (0.2 mL), was added 10% Pd–C (0.3 g), and the mixture was hydrogenolyzed at atmospheric pressure for 2.5 h. Then TLC (solvent *A*) showed the debenzylation to be complete. After filtration through Celite, the mixture was concentrated and the residue was treated overnight with 1:1 Ac_2O–pyridine (20 mL). Concentration, and co-concentration with toluene, followed by column chromatography (2:1 toluene–EtOAc) of the residue yielded **31**, isolated as a colourless glass (0.37 g, 97%); $[\alpha]_D$ +14° (*c* 1); R_f 0.28 (2:1 toluene–EtOAc); NMR ($CDCl_3$): ^1H, δ 5.653 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.409 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 4.995 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.279 (dd, 1 H, $J_{5,6b}$ 8.9, $J_{6a,6b}$ 11.3 Hz, H-6b), 4.229 (dd, 1 H, $J_{5,6a}$ 6.1 Hz, H-6a), 3.866 (ddd, 1 H, H-5), 3.707 (dd, 1 H, $J_{4,5}$ 1.1 Hz, H-4), 3.519 (s, 3 H, OCH_3), 2.106, 2.092, 2.080, and 2.034 (4 s, each 3

H, 4 Ac); ^{13}C, δ 170.1, 169.9, 169.0, and 168.8 (4 $COCH_3$), 91.5 (C-1), 75.9, 73.5, 72.9, and 68.3 (C-2,3,4,5), 61.9 (C-6), 61.2 (OCH_3). Anal. Calcd for $C_{15}H_{22}O_{10}$ (362.34): C, 49.72; H, 6.12. Found: C, 49.84; H, 6.16.

*Ethyl 2,3,6-tri-*O*-acetyl-4-*O*-methyl-1-thio-α,β-*D*-galactopyranoside* (**32**).—A solution of **31** (100 mg, 0.28 mmol) in CH_2Cl_2 (5 mL) was stirred for 1 h under N_2 in the presence of molecular sieves 3 Å (0.3 g), then EtSH (0.2 mL, 2.7 mmol) and $BF_3 \cdot Et_2O$ (0.1 mL, 0.81 mmol) were added. The mixture was stirred overnight, after which TLC (2:1 toluene–EtOAc) showed the conversion of **31** into a major (**32β**, R_f 0.48) and a minor (**32α**, R_f 0.54) product. The mixture was neutralized with Et_3N and concentrated. Column chromatography (4:1 toluene–EtOAc) of the residue afforded **32**, isolated as a syrup (73 mg, 73%, $\alpha:\beta = 1:2$); $[\alpha]_D$ +51° (*c* 0.25, α anomer) and +2° (*c* 0.33, β anomer); NMR ($CDCl_3$): ^1H (α), δ 5.713 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1), 5.362 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 5.147 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 4.405 (ddd, 1 H, $J_{4,5}$ 1.2 Hz, H-5), 4.259 (dd, 1 H, $J_{5,6b}$ 6.5, $J_{6a,6b}$ 11.2 Hz, H-6b), 4.207 (dd, 1 H, $J_{5,6a}$ 6.1 Hz, H-6a), 3.739 (dd, 1 H, H-4), 3.493 (s, 3 H, OCH_3), 2.64–2.45 (m, 2 H, SCH_2CH_3), 2.108, 2.071, and 2.062 (3 s, each 3 H, 3 Ac), 1.261 (t, 3 H, SCH_2CH_3); ^1H (β), δ 5.334 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 4.962 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.402 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.301 (dd, 1 H, $J_{5,6b}$ 6.5, $J_{6a,6b}$ 11.2 Hz, H-6b), 4.188 (dd, 1 H, $J_{5,6a}$ 6.3 Hz, H-6a), 3.739 (ddd, 1 H, $J_{4,5}$ 1.2 Hz, H-5), 3.685 (dd, 1 H, H-4), 3.496 (s, 3 H, OCH_3), 2.80–2.61 (m, 2 H, SCH_2CH_3), 2.097, 2.080, and 2.062 (3 s, each 3 H, 3 Ac), 1.255 (t, 3 H, SCH_2CH_3); ^{13}C (β), δ 170.4, 170.1, and 169.4 (3 $COCH_3$), 83.5 (C-1), 76.4, 75.9, 75.0, and 67.7 (C-2,3,4,5), 62.4 (C-6), 61.3 (OCH_3), 23.7 (SCH_2CH_3), 20.7 ($COCH_3$), 14.7 (SCH_2CH_3). Anal. Calcd for $C_{15}H_{24}O_8S$ (364.42): C, 49.44; H, 6.64. Found: C, 49.84; H, 6.67.

*Octyl (2,3,6-tri-*O*-acetyl-4-*O*-methyl-β-*D*-galactopyranosyl)-(1 → 4)-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**33**).—A solution of **11** (63.8 mg, 61.7 μmol) and **32** (33.7 mg, 92.5 μmol) in CH_2Cl_2 (5 mL) was stirred for 1 h under N_2 in the presence of molecular sieves 3 Å (0.3 g), then cooled to 0 °C. To the mixture was added dropwise during 10 min a solution of *N*-iodosuccinimide (0.12 mmol) and triflic acid (14 μmol) in CH_2Cl_2 (2 mL). TLC (3:1 toluene–EtOAc) indicated the formation of **33** (R_f 0.41), and the mixture was neutralized (Et_3N), filtered, washed with aq 5% $NaHSO_3$, aq 10% $NaHCO_3$, and H_2O, and concentrated. Column chromatography (3:2 hexane–EtOAc) of the residue afforded **33**, isolated as a colourless syrup (70.4 mg, 85%); $[\alpha]_D$ +1° (*c* 0.5); NMR ($CDCl_3$): ^1H, δ 7.61–6.80 (m, 29 H, 5 Ph and Phth), 5.261 (dd, 1 H, $J_{2'',3''}$ 10.4 Hz, H-2″), 5.226 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.801 (dd, 1 H, H-3″), 4.794, 4.775, 4.757, 4.687, 4.463, 4.454, 4.414, 4.340, 4.068, and 3.980 (10 d, each 1 H, 5 $PhCH_2O$), 4.599 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.471 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 3.431 (s, 3 H, OCH_3), 3.163 (dt, 1 H, octyl OCHH), 2.082, 2.060, and 2.002 (3 s, each 3 H, 3 Ac), 0.867 (t, 3 H, octyl CH_3); ^{13}C, δ 170.3, 170.1, and 169.0 (3 $COCH_3$), 133.2, 131.7, and 122.9 (Phth), 100.3 and 96.8 (2 C) (C-1,1′,1″), 78.0, 77.7, 76.6, 76.1, 74.9, 74.7, 74.6, 73.9, 71.9, 71.6, and 70.1 (C-2,3,4,5,3′,4′,5′,2″,3″,4″,5″), 74.6, 74.2, 73.9, 73.6, 72.7, 70.5, 69.9, 68.4, and 67.5 (C-6,6′,6″, 5 $PhCH_2O$, and octyl OCH_2), 61.2 (OCH_3), 55.5 (C-2′), 31.6, 29.1 (2 C), 29.0, 25.9, and 22.4 (6 octyl CH_2), 20.6 ($COCH_3$), 13.9 (octyl

CH_3). Anal. Calcd for $C_{76}H_{89}NO_{20}$ (1336.56): C, 68.30; H, 6.71. Found: C, 68.40; H, 6.50.

*Octyl (2,3,6-tri-*O*-acetyl-4-*O*-methyl-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-acetyl-α-*D*-mannopyranoside* (**35**).—A mixture of **33** (106 mg, 79.3 μmol) and hydrazine monohydrate (1.0 mL) in 9:1 EtOH–H_2O (10 mL) was boiled under reflux overnight, when TLC (9:1 CH_2Cl_2–MeOH) showed the dephthaloylation to be complete, and the mixture was concentrated, and co-concentrated with toluene. The residue was dissolved in 1:1 Ac_2O–pyridine (10 mL), stirred overnight at room temperature, and concentrated. Column chromatography of the residue gave **34**, isolated as a colourless syrup (82.2 mg, 83%); $[\alpha]_D$ +2° (*c* 1); R_f 0.22 (3:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.748 (d, 1 H, $J_{2',NH}$ 7.4 Hz, NH), 3.464 (s, 3 H, OCH_3); ^{13}C, δ 61.2 (OCH_3), 23.1 ($NHCOCH_3$). A solution of **34** (80.2 mg, 64.2 μmol) in 1:1 EtOH–EtOAc (10 mL), containing HOAc (0.1 mL) and 10% Pd–C (40 mg), was hydrogenated at atmospheric pressure for 60 min, when TLC (solvent *A*) showed the presence of a single product (R_f 0.56). The mixture was filtered through Celite and concentrated. The resulting syrup was treated overnight with 1:1 Ac_2O–pyridine (10 mL), and concentrated. Column chromatography (1:2 toluene–EtOAc) of the residue afforded **35**, isolated as a colourless glass (51.1 mg, 77%); $[\alpha]_D$ −10° (*c* 1); R_f 0.28 (1:3 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.634 (d, 1 H, $J_{2',NH}$ 8.7 Hz, NH), 5.222 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-4), 5.219 (dd, 1 H, H-3′), 5.186 (dd, 1 H, $J_{2'',3''}$ 10.4 Hz, H-2″), 5.102 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 4.878 (dd, 1 H, $J_{3'',4''}$ 3.0 Hz, H-3″), 4.720 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.620 (d, 1 H, $J_{1',2'}$ 7.2 Hz, H-1′), 4.406 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 3.477 (s, 3 H, OCH_3), 3.420 (dt, 1 H, octyl OC*H*H), 2.103, 2.100, 2.089, 2.075, 2.032, 2.022, 1.991, and 1.941 (8 s, 3,3,6,3,3,3,3,3 H, 9 Ac), 0.879 (t, 3 H, octyl CH_3); ^{13}C, δ 170.6–169.2 ($COCH_3$), 100.7, 99.3, and 97.3 (C-1,1′,1″), 75.8, 75.4, 74.6, 73.6, 72.6, 72.1, 71.4, 70.2, 69.6, 68.5, and 66.2 (C-2,3,4,5,3′,4′,5′,2″,3″,4″,5″), 68.3, 62.7, 62.6, and 61.8 (C-6,6′,6″ and octyl OCH_2), 61.3 (OCH_3), 53.5 (C-2′), 31.6, 29.3, 29.2, 29.0, 26.0, and 22.5 (6 octyl CH_2), 23.0 ($NHCOCH_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{45}H_{69}NO_{24}$ (1008.05): C, 53.62; H, 6.90. Found: C, 53.75; H, 7.13.

*Octyl (4-*O*-methyl-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-α-*D*-mannopyranoside* (**3**).—A solution of **35** (44.2 mg, 43.8 μmol) and NaOMe (pH 8) in 4:1 CH_2Cl_2–MeOH (5 mL) was stirred for 2 h at room temperature. After neutralization with Dowex-50 (H^+), the mixture was filtered and concentrated. Gel filtration of the resulting syrup on a Bio-Gel P-2 column, eluted with H_2O, and subsequent lyophilization gave **3** as a white powder (28.5 mg, 97%); $[\alpha]_D$ −7° (*c* 0.7, MeOH); R_f 0.31 (solvent *A*); NMR (D_2O): ^{1}H, see Table 1; FABMS: *m*/*z* 672 $[M + H]^+$, 694 $[M + Na]^+$.

*2,3,6-Tri-*O*-acetyl-4-deoxy-4-fluoro-α-*D*-galactopyranosyl trichloroacetimidate* (**38**). —A solution of 1,2,3,6-tetra-*O*-acetyl-4-deoxy-4-fluoro-α,β-D-galactopyranose [22] (**36**; 0.21 g, 0.61 mmol) and hydrazine acetate (61.7 mg, 0.67 mmol) in DMF (3 mL) was stirred for 30 min at 50 °C, when TLC (1:1 toluene–EtOAc) showed the complete disappearance of **36** (R_f 0.50) and the presence of a new product (**37**, R_f 0.40). The mixture was diluted with EtOAc, washed with aq 5% NaCl (3 ×), and concentrated. To a solution of the crude residue (**37**) in CH_2Cl_2 (5 mL) were added trichloroacetonitrile

(0.58 mL, 5.8 mmol) and 0.2 M DBU in CH_2Cl_2 (1.5 mL), and the mixture was stirred overnight, when TLC (2:1 toluene–EtOAc) showed a complete reaction (**38**, R_f 0.60). Column chromatography (3:1 toluene–EtOAc) of the solution gave **38**, isolated as a pale-yellow amorphous solid (0.17 g, 63% from **36**); $[\alpha]_D$ +118° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 8.685 (s, 1 H, NH), 6.615 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.433 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 5.348 (ddd, 1 H, $J_{3,4}$ 2.5, $J_{3,F}$ 25.3 Hz, H-3), 5.039 (dd, 1 H, $J_{4,F}$ 50.4, $J_{4,5}$ < 1 Hz, H-4), 2.148, 2.058, and 2.035 (3 s, each 3 H, 3 Ac); ^{13}C, δ 170.0 (2 C) and 169.6 (3 $COCH_3$), 160.6 (OC[NH]CCl_3), 93.3 (C-1), 90.6 (OC[NH]CCl_3), 86.1 (d, $J_{C-4,F}$ 185.7 Hz, C-4), 69.1 (d, $J_{C-5,F}$ 18.3 Hz, C-5), 67.9 (d, $J_{C-3,F}$ 17.8 Hz, C-3), 66.5 (C-2), 61.2 (d, $J_{C-6,F}$ 5.9 Hz, C-6), 20.6, 20.4, and 20.3 (3 $COCH_3$).

*Octyl (2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-β-*D*-galactopyranosyl)-(1 → 4)-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**39**).—A solution of **11** (0.13 g, 0.12 mmol) and **38** (77.0 mg, 0.17 mmol) in CH_2Cl_2 (5 mL), containing molecular sieves 4 Å (0.2 g), was stirred for 30 min under N_2 at 0 °C, and trimethylsilyl triflate in CH_2Cl_2 (0.1 M, 61 μL) was added. TLC (3:2 hexane–EtOAc) showed that **39** (R_f 0.42) was formed within 30 min. The mixture was neutralized with Et_3N, diluted with CH_2Cl_2, filtered, and concentrated. Column chromatography (6:1 toluene–EtOAc) of the residue afforded **39**, isolated as a colourless syrup (0.15 g, 93%); $[\alpha]_D$ +4° (*c* 0.8); NMR ($CDCl_3$): ^{1}H, δ 7.65–6.82 (m, 29 H, 5 Ph and Phth), 5.244 (dd, 1 H, $J_{2'',3''}$ 10.4 Hz, H-2″), 5.232 (d, 1 H, $J_{1',2'}$ 8.5 Hz, H-1′), 4.787 (ddd, 1 H, $J_{3'',F}$ 27.4, $J_{3'',4''}$ 2.7 Hz, H-3″), 4.758, 4.722, 4.476, 4.433, 4.393, 4.346, 4.066, and 3.989 (8 d, 3,1,1,1,1,1,1,1 H, 5 PhCH_2O), 4.730 (ddd, 1 H, $J_{4'',F}$ 49.2, $J_{4'',5''}$ < 1 Hz, H-4″), 4.632 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, H-1″), 3.162 (dt, 1 H, octyl OCHH), 2.103, 2.079, and 2.027 (3 s, each 3 H, 3 Ac), 0.869 (t, 3 H, octyl CH_3); ^{13}C, δ 170.2, 170.0, and 168.8 (3 $COCH_3$), 133.3, 131.6, and 122.9 (Phth), 100.0 and 96.7 (2 C) (C-1,1′,1″), 85.6 (d, $J_{C-4'',F}$ 186.3 Hz, C-4″), 78.1, 77.8, 74.7, 74.6, 73.5, 71.6 (2 C), and 69.3 (C-2,3,4,5,3′,4′,5′,2″), 71.4 and 70.7 (2 d, C-3″ and C-5″), 74.6, 74.3, 73.6, 72.7, 70.6, 69.8, 68.1, and 67.6 (C-6,6′, 5 PhCH_2O, and octyl OCH_2), 61.0 (d, $J_{C-6'',F}$ 5.5 Hz, C-6″), 55.4 (C-2′), 31.6, 29.1 (2 C), 29.0, 25.9, and 22.4 (6 octyl CH_2), 20.5 ($COCH_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{75}H_{86}FNO_{19}$ (1324.52): C, 68.01; H, 6.54. Found: C, 67.83; H, 6.55.

*Octyl (2,3,6-tri-*O*-acetyl-4-deoxy-4-fluoro-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-acetyl-α-*D*-mannopyranoside* (**41**).—A solution of **39** (0.13 g, 96.6 μmol) and hydrazine monohydrate (1.1 mL) in 9:1 EtOH–H_2O (15 mL) was boiled under reflux overnight, when TLC (9:1 CH_2Cl_2–MeOH) showed the disappearance of **39** and the formation of a new compound (R_f 0.50). Then, the mixture was concentrated, and co-concentrated with toluene. To a solution of the residue in pyridine (5 mL) was added Ac_2O (5 mL), and the mixture was stirred overnight, then concentrated. Column chromatography (3:2 hexane–EtOAc) of the residue gave **40**, isolated as a syrup (90.8 mg, 76%); $[\alpha]_D$ +11° (*c* 1); R_f 0.16 (3:2 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.655 (d, 1 H, $J_{2',NH}$ 7.1 Hz, NH); ^{13}C, δ 85.5 (d, $J_{C-4'',F}$ 186.2 Hz, C-4″), 23.1 (NHCOCH_3). A solution of **40** (90.0 mg, 72.8 μmol) in 1:1 EtOH–EtOAc (10 mL), containing HOAc (0.1 mL) and 10% Pd–C (50 mg), was hydrogenated at room temperature at atmospheric pressure. After 90 min, when TLC (solvent *A*) showed the conversion of **40** into a new compound

(R_f 0.67), the mixture was filtered through Celite, and the filtrate was concentrated. The residue was acetylated overnight in 1:1 pyridine–Ac_2O (10 mL), and concentrated. Column chromatography (1:3 toluene–EtOAc) of the residue yielded **41**, isolated as a colourless glass (59.8 mg, 82%); $[\alpha]_D$ −5° (*c* 1); R_f 0.27 (1:3 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.665 (bd, 1 H, NH), 5.223 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.169 (dd, $J_{2'',3''}$ 10.3 Hz, H-2″), 5.093 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-3), 4.904 (ddd, 1 H, $J_{3'',F}$ 27.4, $J_{3'',4''}$ 2.7 Hz, H-3″), 4.812 (ddd, $J_{4'',F}$ 50.1, $J_{4'',5''} < 1$ Hz, H-4″), 4.715 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.653 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.478 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 3.418 (dt, 1 H, octyl OC*H*H), 2.114, 2.107, 2.094, 2.091, 2.082, 2.046, 2.026, 1.993, and 1.938 (9 s, each 3 H, 9 Ac), 0.887 (t, 3 H, octyl CH_3); ^{13}C, δ 170.6–168.9 ($COCH_3$), 100.5, 99.4, and 97.3 (C-1,1′,1″), 85.4 (d, $J_{C\text{-}4'',F}$ 187.2 Hz, C-4″), 75.7, 74.6, 72.7, 71.3, 70.2, 68.9, 68.5, and 66.2 (C-2,3,4,5,3′,4′,5′,2″), 71.2 and 70.9 (2 d, $J_{C\text{-}3'',F} = J_{C\text{-}5'',F} = 18.0$ Hz, C-3″ and C-5″), 68.3, 62.7, and 62.4 (C-6,6′ and octyl OCH_2), 61.0 (d, $J_{C\text{-}6'',F}$ 5.3 Hz, C-6″), 53.7 (C-2′), 31.6, 29.5, 29.3, 29.0, 26.0, and 22.5 (6 octyl CH_2), 23.0 ($NHCOCH_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{44}H_{66}FNO_{23}$ (996.01): C, 53.06; H, 6.68. Found: C, 53.08; H, 6.74.

*Octyl (4-deoxy-4-fluoro-β-*D*-galactopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-α-*D*-mannopyranoside* (**4**).—A solution of **41** (55.9 mg, 56.1 μmol) and NaOMe (pH 8) in 4:1 MeOH–CH_2Cl_2 (5 mL) was stirred for 2 h, when TLC (solvent *A*) showed the *O*-deacetylation to be complete. After neutralization with Dowex-50 (H^+), the mixture was filtered, and the filtrate was concentrated. Gel filtration on Bio-Gel P-2 (H_2O) of the residue and subsequent lyophilization yielded **4** as a white powder (35.2 mg, 95%); $[\alpha]_D$ −3° (*c* 1, MeOH); R_f 0.40 (solvent *A*); NMR (D_2O): ^{1}H, see Table 1; FABMS: *m*/*z* 660 $[M + H]^+$, 682 $[M + Na]^+$.

*Octyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**43**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate [15] (**42**; 19.3 mg, 39.2 μmol) and **11** (24.9 mg, 24.1 mmol) in CH_2Cl_2 (3 mL), containing molecular sieves 4 Å (0.2 g), was added trimethylsilyl triflate in CH_2Cl_2 (0.2 M, 39 μL) at 0 °C. When TLC (4:3 hexane–EtOAc) showed the disappearance of **11** and the formation of a new product (R_f 0.46), the mixture was neutralized (Et_3N), filtered, washed with phosphate buffer, and concentrated. Column chromatography (4:3 hexane–EtOAc) of the residue gave **43**, isolated as a colourless syrup (25.0 mg, 76%); $[\alpha]_D$ −5.4° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.62–6.78 (m, 29 H, 5 Ph and Phth), 5.221 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.792, 4.771, 4.755, 4.730, 4.477, 4.429, 4.410, 4.378, 4.067, and 3.980 (10 d, each 1 H, 5 PhCH_2O), 4.671 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.457 (bs, 1 H, H-1), 3.155 (dt, 1 H, octyl OC*H*H), 2.011, 1.997, and 1.982 (3 s, 6,3,3 H, 4 Ac), 0.867 (t, 3 H, octyl CH_3); ^{13}C, δ 170.6, 170.1, 169.3, and 169.0 (4 $COCH_3$), 133.3, 131.6, and 122.9 (Phth), 99.9 and 96.8 (2 C) (C-1,1′,1″), 78.3, 77.8, 76.4, 74.7, 74.6, 73.5, 73.0, 71.8, 71.6, 71.4, and 68.2 (C-2,3,4,5,3′,4′,5′,2″,3″,4″,5″), 74.3, 73.6, 72.7 (2 C), 70.6, 69.8, 68.1, 67.6, and 61.6 (C-6,6′,6″, 5 PhCH_2O, and octyl OCH_2), 55.4 (C-2′), 31.7, 29.2 (2 C), 29.1, 25.9, and 22.5 (6 octyl CH_2), 20.5 ($COCH_3$), 14.0 (octyl CH_3).

*Octyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-(1 → 4)-(2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-3,4,6-tri-*O*-acetyl-α-*D*-mannopyranoside*

(**45**).—A solution of **43** (21.5 mg, 15.8 μmol) and ethylenediamine (0.5 mL) in 1-butanol (6 mL) was boiled under reflux overnight, when TLC (8:1 CH_2Cl_2–MeOH) showed the formation of a new compound (R_f 0.45). After filtration and concentration, a solution of the residue in 3:2 pyridine–Ac_2O (5 mL) was stirred for 16 h at room temperature, then concentrated, and co-concentrated with toluene. Column chromatography (3:1 toluene–EtOAc) of the residue afforded **44**, isolated as a colourless syrup (15.4 mg, 76%); $[\alpha]_D$ −2.8° (*c* 1); R_f 0.20 (4:1 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.622 (d, 1 H, $J_{2',NH}$ 7.1 Hz, NH); ^{13}C, δ 23.2 ($NHCOCH_3$). A solution of **44** (15.0 mg, 15.0 μmol) in 1:1 EtOH–EtOAc (8 mL), containing 10% Pd–C (25 mg) and HOAc (0.1 mL), was hydrogenated at atmospheric pressure for 40 min, when TLC (solvent *A*) showed the presence of a single new compound (R_f 0.64). The mixture was filtered through Celite and the filtrate was concentrated. To a solution of the residue in pyridine (3 mL) was added Ac_2O (2 mL), and the mixture was stirred for 16 h, then concentrated, and co-concentrated with toluene. Column chromatography (1:3 toluene–EtOAc) of the residue gave **45**, isolated as a syrup (12.1 mg, 99%); $[\alpha]_D$ −45° (*c* 3); R_f 0.25 (1:3 toluene–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 5.690 (d, 1 H, $J_{2',NH}$ 8.6 Hz, NH), 5.233 (dd, 1 H, $J_{2',3'}$ 8.3, $J_{3',4'}$ 10.0 Hz, H-3′), 5.218 (dd, 1 H, $J_{2'',3''}$ 9.2 Hz, H-3″), 5.150 (dd, 1 H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 5.082 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.060 (dd, 1 H, $J_{3'',4''} = J_{4'',5''}$ = 9.4 Hz, H-4″), 4.910 (dd, 1 H, H-2″), 4.702 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.663 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.505 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 3.412 (dt, 1 H, octyl OC*H*H), 2.121, 2.092, 2.058, 2.029, 2.024, 2.013, 1.991, 1.980, and 1.934 (9 s, 3,6,3,3,3,3,3,3,3 H, 10 Ac), 0.887 (t, 3 H, octyl CH_3); ^{13}C, δ 170.6–167.0 ($COCH_3$), 100.5, 99.3, and 97.4 (C-1,1′,1″), 76.1, 74.6, 72.7, 71.9, 71.5, 71.3, 71.2, 70.2, 68.6, 68.3, and 66.2 (C-2,3,4,5,3′,4′,5′,2″,3″,4″,5″), 68.0, 62.7, 62.3, and 61.7 (C-6,6′,6″ and octyl OCH_2), 54.1 (C-2′), 31.7, 29.5, 29.3, 29.2, 25.9, and 22.5 (6 octyl CH_2), 23.0 ($NHCOCH_3$), 13.9 (octyl CH_3). Anal. Calcd for $C_{46}H_{69}NO_{25}$ (1036.06): C, 53.33; H, 6.71. Found: C, 53.31; H, 6.74.

*Octyl β-*D*-glucopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-α-*D*-mannopyranoside* (**5**).—A solution of **45** (39.0 mg, 37.6 μmol) in 4:1 MeOH–CH_2Cl_2 (5 mL) was treated with NaOMe (pH 8) for 2 h, then neutralized with Dowex-50 (H^+), filtered, and concentrated. Gel filtration of the residue on a Bio-Gel P-2 column (H_2O) and subsequent lyophilization gave **5** as a white solid (21.6 mg, 87%); $[\alpha]_D$ −5° (*c* 0.7, MeOH); R_f 0.43 (solvent *A*); NMR (D_2O): ^{1}H, see Table 1; FABMS: *m/z* 658 $[M + H]^+$, 680 $[M + Na]^+$.

Acknowledgements

The authors thank Dr. P.H. Kruiskamp and Ms. Dr. J.J. Kettenes-van den Bosch for recording NMR spectra, and Ms. A. van der Kerk-van Hoof for recording FAB-mass spectra.

References

[1] A. Kobata, *Eur. J. Biochem.*, 209 (1992) 483–501.
[2] A. Varki, *Glycobiology*, 3 (1993) 97–130.

[3] H. Lis and N. Sharon, *Eur. J. Biochem.*, 218 (1993) 1–27.
[4] S. Hakomori, *Adv. Cancer Res.*, 52 (1989) 257–331.
[5] T.A. Beyer, J.E. Sadler, J.I. Rearick, J.C. Paulson, and R.L. Hill, *Adv. Enzymol.*, 52 (1981) 23–175.
[6] See, for example G. Möller, F. Reck, H. Paulsen, K.J. Kaur, M. Sarkar, H. Schachter, and I. Brockhausen, *Glycoconjugate J.*, 9 (1992) 180–190; G.J. Vella, H. Paulsen, and H. Schachter, *Can. J. Biochem. Cell Biol.*, 62 (1984) 409–417; F. Reck, E. Meinjohanns, M. Springer, R. Wilkens, J.A.L.M. van Dorst, H. Paulsen, G. Möller, I. Brockhausen, and H. Schachter, *Glycoconjugate J.*, 11 (1994) 210–216; H. Paulsen, F. Reck, E. Meinjohanns, M. Springer, I. Brockhausen, and H. Schachter, in K. Bock and H. Clausen (Eds.), *Complex Carbohydrates in Drug Research*, Alfred Benzon Symposium 36, 1994, Munksgaard, Copenhagen, Denmark, pp 78–86; I. Lindh and O. Hindsgaul, *J. Am. Chem. Soc.*, 113 (1991) 216–223; C.H. Wong, Y. Ichikawa, T. Krach, C. Gautheron-Le Narvor, D.P. Dumas, and G.C. Look, *J. Am. Chem. Soc.*, 113 (1991) 8137–8145; T. Linker, S.C. Crawley, and O. Hindsgaul, *Carbohydr. Res.*, 245 (1993) 323–331.
[7] See, for example M.R. Pâquet, S. Narasimhan, H. Schachter, and M.A. Moscarello, *J. Biol. Chem.*, 259 (1984) 4716–4721; W.M. Blanken, A. van Vliet, and D.H. van den Eijnden, *J. Biol. Chem.*, 259 (1984) 15131–15135; S.C. Ats, J. Lehmann, and S. Petry, *Carbohydr. Res.*, 252 (1994) 325–332; D.H. Joziasse, W.E.C.M. Schiphorst, D.H. van den Eijnden, J.A. van Kuik, H. van Halbeek, and J.F.G. Vliegenthart, *J. Biol. Chem.*, 260 (1985) 714–719; M. Nemansky, W.E.C.M. Schiphorst, and D.H. van den Eijnden, *FEBS Lett.*, 363 (1995) 280–284; M.J. Elices and I.J. Goldstein, *J. Biol. Chem.*, 264 (1989) 1375–1380.
[8] R. Schauer and J.P. Kamerling, in J. Montreuil, J.F.G. Vliegenthart, and H. Schachter, (Eds.), *Glycoproteins, New Comprehensive Biochemistry*, Vol. 298, Elsevier, Amsterdam, 1996, in press.
[9] J. Weinstein, U. De Souza-e-Silva, and J.C. Paulson, *J. Biol. Chem.*, 257 (1982) 13845–13853.
[10] C.H. Hokke, J.G.M. van der Ven, J.P. Kamerling, and J.F.G. Vliegenthart, *Glycoconjugate J.*, 10 (1993) 82–90.
[11] K.B. Wlasichuk, M.A. Kashem, P.V. Nikrad, P. Bird, C. Jiang, and A.P. Venot, *J. Biol. Chem.*, 268 (1993) 13971–13977.
[12] M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconjugate J.*, 5 (1988) 49–63.
[13] T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, 64 (1978) C3–C9.
[14] F. Yamazaki, T. Kitajima, T. Nukada, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 201 (1990) 15–30.
[15] R.R. Schmidt and M. Stumpp, *Liebigs Ann. Chem.*, (1983) 1249–1256.
[16] O. Kanie, S.C. Crawley, M.M. Palcic, and O. Hindsgaul, *Carbohydr. Res.*, 243 (1993) 139–164.
[17] K.K. Sadozai, T. Kitajima, Y. Nakahara, T. Ogawa, and A. Kobata, *Carbohydr. Res.*, 152 (1986) 173–182.
[18] H. Ammann and G. Dupuis, *Can. J. Chem.*, 66 (1987) 1651–1655.
[19] Z. Zhiyuan and G. Magnusson, *Carbohydr. Res.*, 262 (1994) 79–101.
[20] H. Paulsen, V. Rutz, and I. Brockhausen, *Liebigs Ann. Chem.*, (1992) 747–758.
[21] S. David, A. Thieffry, and A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, (1981) 1796–1801.
[22] K. Koch and R.J. Chambers, *Carbohydr. Res.*, 241 (1993) 295–299.