Carbohydrate Research, 193 (1989) 91–103
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS AND CHARACTERIZATION OF 1-*O*-α-LACTOSYL-(*R,S*)-GLYCEROLS AND 1-*O*-α-LACTOSYL-3-*O*-β-LACTOSYL-(*R,S*)-GLYCEROLS*

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(Received November 7th, 1988; accepted for publication, January 7th, 1989)

ABSTRACT

Coupling of 2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-lactosyl bromide with an equimolar amount of 1-*O*-acetyl-2-*O*-benzyl-(*R,S*)-glycerols in the presence of tetraethylammonium bromide and 4A molecular sieves in 1,2-dichloroethane afforded 3-*O*-acetyl-2-*O*-benzyl-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-lactosyl)-(*R,S*)-glycerols in 20.4% yield, which were deprotected to give 1-*O*-α-lactosyl-(*R,S*)-glycerols. 2-*O*-Benzyl-3-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-lactosyl)-(*R,S*)-glycerols were obtained in 61% yield by the reaction of hepta-*O*-acetyl-α-lactosyl bromide and 2-*O*-benzyl-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-lactosyl)-(*R,S*)-glycerols in the presence of mercury(II) cyanide in benzene–nitromethane; deprotection afforded 1-*O*-α-lactosyl-3-*O*-β-lactosyl-(*R,S*)-glycerols in an overall yield of 24%. The two target materials are useful in the assessment of the binding properties of D-galactosyl-terminated glyceryl glycosides to the asialoglycoprotein receptor of normal rabbit and human hepatocytes.

INTRODUCTION

The chemical formation of glycosidic linkages of defined stereochemistry has constituted one of the salient problems attacked by carbohydrate chemists in recent years[1–5]. Remarkable progress has been achieved and more efficient methods for the synthesis of complex oligosaccharides[1–8], including some found in Nature[5,7,9–13], have been devised. The synthetic difficulties are augmented when the oligosaccharide structure requires the introduction of 1,2-*cis*-glycopyranosyl

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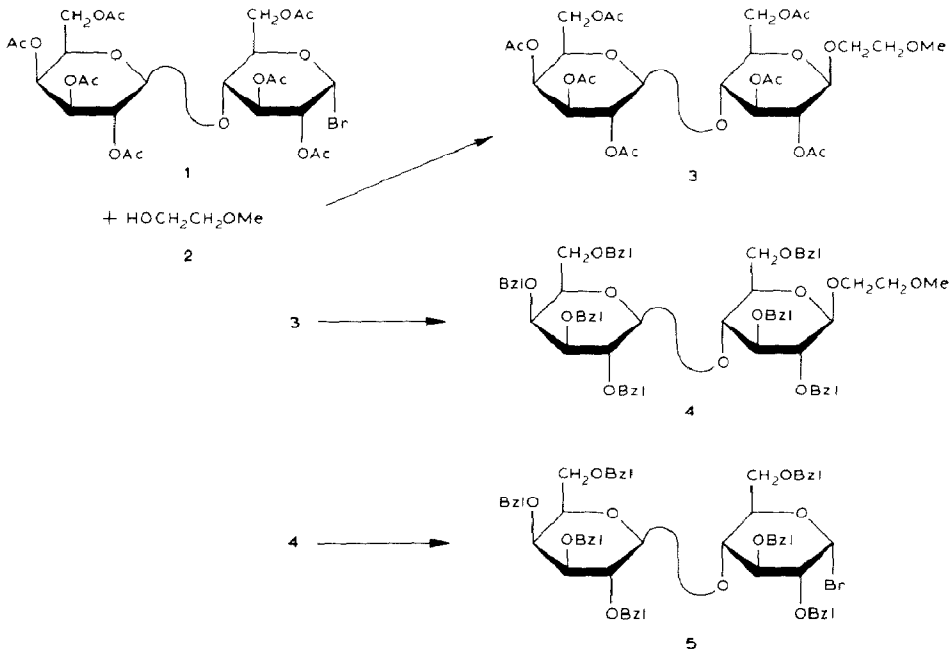
0008-6215/89/$ 03.50

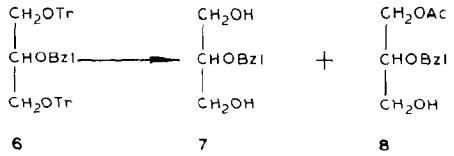
linkages[2,4,5,7,12,14,15], a configuration frequently encountered in the glycan components of glycoproteins[10,12]; the increased difficulty has been related to the important stereoelectronic factors[2,4,16] which must be present for such glycosides to be favored over the 1,2-*trans* isomers[2,4,7,12,16].

We have been engaged[17] in an investigation of the binding of a series of D-galactose-terminated oligosaccharides and glyceryl glycosides to the asialoglycoprotein receptor[18] of normal rabbit and human heptocytes. It was deemed necessary to assess the binding properties of some α-D-glucopyranosyl-linked members of the set of compounds, and as one aspect of the investigation we report herein the synthesis and characterization of 1-*O*-α-lactosyl-(*R*,*S*)-glycerols (**12**) and 1-*O*-α-lactosyl-3-*O*-β-lactosyl-(*R*,*S*)-glycerols (**18**).

RESULTS AND DISCUSSION

The α-lactosyl bromide derivative to be used in the selective formation of an α-D-glucopyranosyl linkage by a Koenigs–Knorr-type condensation must have OH-2 protected by a substituent which would not afford neighboring-group assistance[1,2,4,5,12]. Therefore, 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-lactosyl bromide[17] (**1**) was converted into the hepta-*O*-benzyl-α-lactosyl bromide **5** as follows. 2-Methoxyethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactoside (**3**) was obtained from **1** by reaction with 2-methoxyethanol (**2**) in the presence of mercury(II) cyanide[19] in benzene–nitromethane at 40° for 9 h. The treatment of **3** with benzyl chloride and potassium hydroxide[20] effected the replacement of the acetyl groups by benzyl





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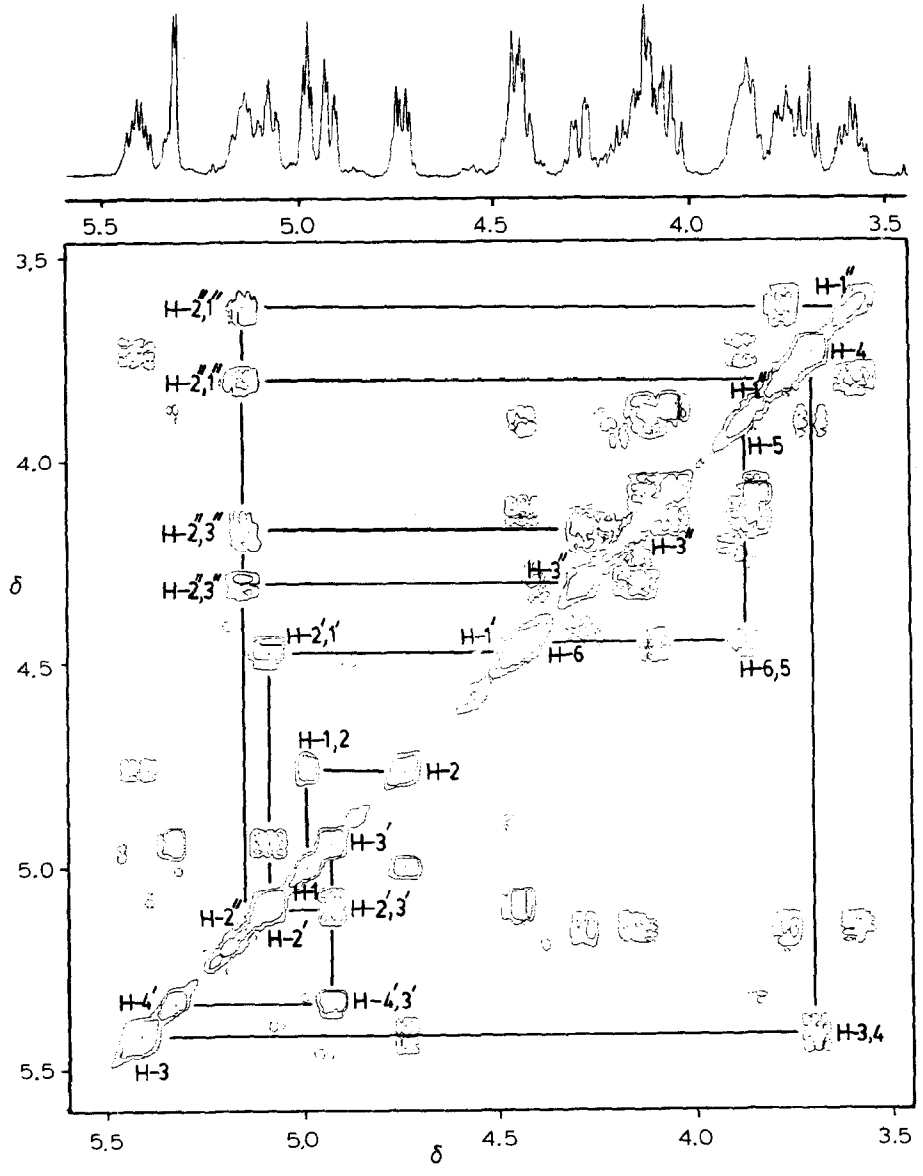


Fig. 1. 400-MHz ^{1}H-COSY spectrum of **13** in $CDCl_3$.

groups to give **4** as a white, crystalline solid. Compound **4** was treated with titanium(IV) bromide in dichloromethane to produce **5**, accompanied by small amounts of other products. The reaction product was used immediately, without further purification, for the syntheses of α-lactosides by application of the halide ion-catalyzed glycosidation method of Lemieux *et al.*[2,21].

The partially protected glycerol derivatives required for reaction with **5**, namely, 2-*O*-benzylglycerol (**7**) and 1-*O*-acetyl-2-*O*-benzyl-(*R,S*)-glycerols (**8**), were obtained by a one-step reaction from 2-*O*-benzyl-1,3-di-*O*-tritylglycerol[17] (**6**). A solution of **6** in 80% acetic acid was heated at reflux temperature for 20 min and the reaction products were separated by column chromatography to give **7** (61%) and **8** (25%). Compound **8** then was coupled to **5**, in 1,2-dichloroethane, by heating in the presence of tetraethylammonium bromide and molecular sieves 4Å to afford 3-*O*-acetyl-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-lactosyl)-(*R,S*)-glycerols (**9**) (20%) and glycal **10** (25%). The *O*-deacetylation of **9** was accomplished by stirring in 3:10:40 (v/v) triethylamine–water–methanol to give **11** in 72% yield. Compounds **10** and **11** also were obtained in yields of 18 and 39%, respectively, by coupling **5** with **7** in dichloromethane, in the presence of tetraethylammonium bromide and molecular sieves 4Å. 1-*O*-α-Lactosyl-(*R,S*)-glycerols (**12**) were obtained from **11** by *O*-debenzylation through catalytic, transfer hydrogenation[22] using 10% palladium-on-carbon in methanol containing 10% formic acid.

It had been observed earlier[17] that the ^{1}H-n.m.r. spectra of per-*O*-acetyl-lactosyl glycerides often were suitable for detailed analysis. Therefore, to characterize **9, 11**, and **12**, each of which afforded a very complex 400-MHz, ^{1}H-n.m.r.

TABLE I

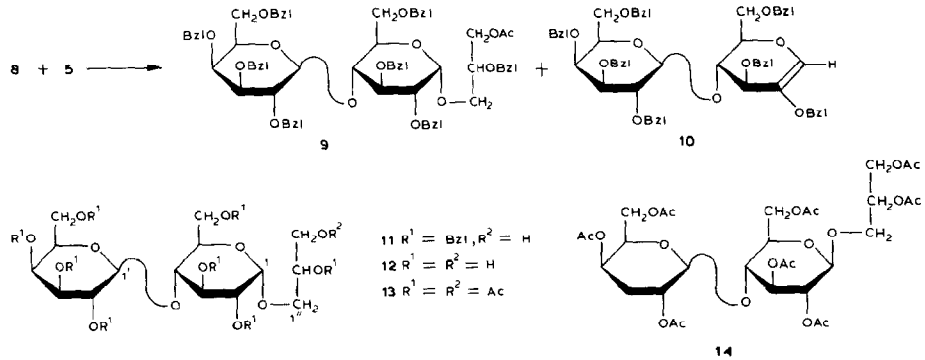
^{1}H-N.M.R. DATA[a] OF **13**[b] AND **14**[c]

Proton	*Chemical shifts (δ) and coupling constants (Hz)*[d]	
	13	**14**
H-1	5.014(4.4); 5.025(4.2)	4.51(7.9)
H-2	4.78	4.884; 4.896
H-3	5.438; 5.449	5.184; 5.190
H-4	3.74	3.794; 3.804
H-5	3.85–3.95	3.61
H-6a	4.06–4.23	4.06–4.16
H-6b	4.44–4.53	4.49
H-1′	4.476(8.1); 4.487(7.6)	4.49(8.0)
H-2′	5.115; 5.121	5.11
H-3′	4.96	4.96
H-4′	5.36	5.35
H-5′	3.85–3.95	3.88
H-6′a	4.06–4.23	4.06–4.16
H-6′b	4.06–4.23	4.06–4.16

[a]For a solution in $CDCl_3$. [b]More complete ^{1}H-n.m.r. data for **13** are given in the Experimental Section. [c]See compound **23** in ref. 17. More complete ^{1}H-n.m.r. data for **14** are given in ref. 17. [d]In parentheses.

spectrum, **12** was peracetylated with acetic anhydride in pyridine to give 2,3-di-*O*-acetyl-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-lactosyl)-(*R,S*)-glycerols (**13**). The signals of the protons of **13** were assigned on the basis of a two-dimensional, Fourier-transform, proton-chemical-shift-correlation spectroscopy[23,24] (COSY) experiment (Fig. 1). Table I summarizes the n.m.r. data, listing the coupling constants of the anomeric protons and the chemical shifts of the protons of the two diastereomers of which **13** is comprised, and those of 2,3-di-*O*-acetyl-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R,S*)-glycerols[17] (**14**). Those data pertaining to H-1 of **13** were of principal importance to the assignment of the α configuration to the D-glucopyranosidic bond. The chemical shifts of the H-1 protons of the diastereomers of **13** were found to be δ 5.014 and 5.025, approximately 0.5 p.p.m. downfield relative to those of the β-D-linked isomer **14**. The assignment of the α-D-glucosidic linkage to **13** was also supported by the significantly smaller coupling constants[21,24,25] exhibited by these protons of **13** (4.2–4.4 Hz) as compared to those of **14** (7.9 Hz) (ref. 17). It is noteworthy that whereas the chemical shifts of the correspondent protons of the D-galactosyl units (H-1′–H-6′) of **14** were virtually identical (Table I), the H-1′ and H-2′ protons of **13** each resonated at a measurably different field strength, although the chemical shifts of these correspondent protons of the D-galactosyl units of **13** and **14** did not differ by more than ±0.01 p.p.m. As expected, a comparison of the chemical shifts of the protons of the D-glucosyl residues of **13** and **14** revealed much larger differences, demonstrating thereby their dissimilar chemical environments. These data established the structure of **13** unequivocally and, hence, also attested to the structures assigned to **9, 11**, and **12**.

The synthesis of 1-*O*-α-lactosyl-3-*O*-β-lactosyl-(*R,S*)-glycerols (**18**) was commenced by the coupling of **11** with **1** in the presence of mercury(II) cyanide[19] in benzene–nitromethane to produce 2-*O*-benzyl-3-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-benzyl-α-lactosyl)-(*R,S*)-glycerols (**15**) as a 1:1 mixture of the two diastereomers. In order to obtain a 400-MHz ^{1}H-n.m.r. spectrum which was less complex than that of **15**, the compound was debenzylated[22] to give **16** which was then peracetylated in the usual manner to



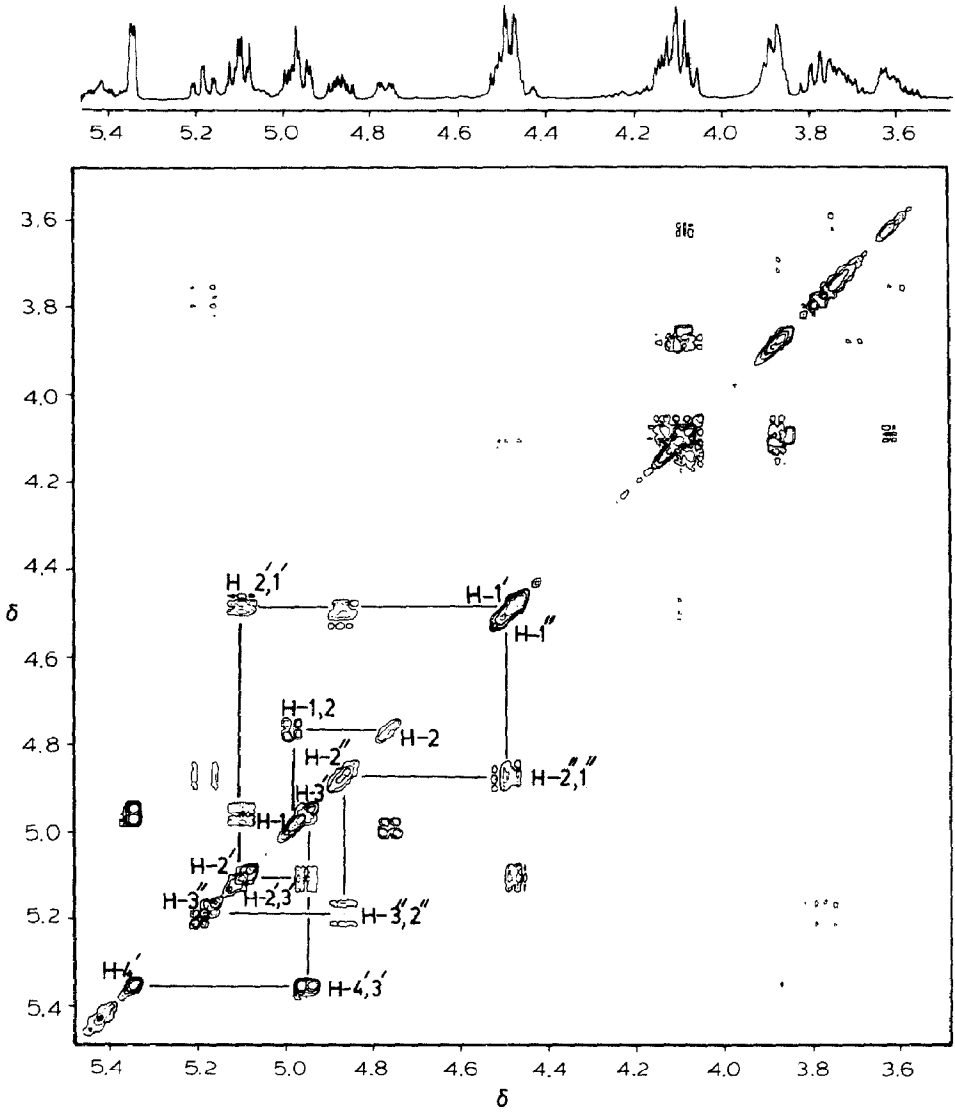
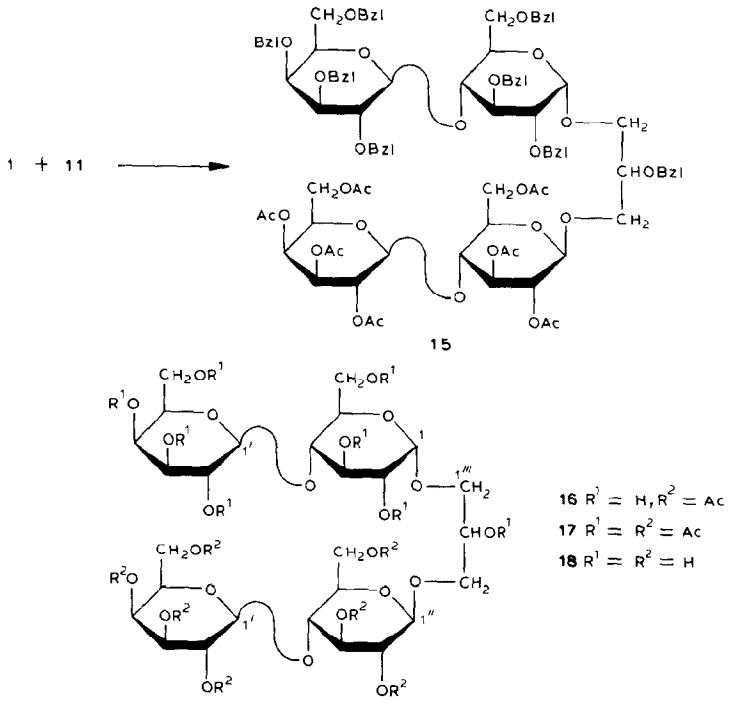


Fig. 2. 400-MHz ^{1}H-COSY spectrum of **17** in $CDCl_3$.

afford 2-*O*-acetyl-1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-lactosyl)-3-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R*,*S*)-glycerols (**17**), the COSY spectrum of which is shown in Fig. 2. Compound **17** was readily converted into **18** by *O*-deacetylation[26] using sodium methoxide in methanol.

Compound **17** contains a total of 33 OCH and OCH_2protons and 15 acetyl groups in each diastereomer and, even in the high-resolution ^{1}H-n.m.r. spectrum, not all of the multiplets present were sufficiently well-resolved to permit the measurement of every coupling constant. However, in the COSY spectrum of **17**,



the two doublets associated with the H-1 protons of the two diastereomers were readily identified at δ 4.981 and 4.998; these exhibited coupling constants of 4.0 and 3.5 Hz, respectively. The H-2″ protons were found to resonate at δ 4.868 and 4.879 with $J_{2'',1''}$ 8.1 and 7.8 Hz, respectively, thereby substantiating the assignment of the β-D configuration to the linkage of the second lactosyl unit to the glycerol compound. The chemical shifts of the H-1″ protons could be assigned only to the range δ 4.43–4.53. Thus, the target compounds, **12** and **18**, have been synthesized by reaction sequences in which the key intermediates, as well as the peracetates **13** and **17**, have been fully characterized.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 141 or 241 automatic polarimeter for solutions in a 0.1-dm cell. ^{1}H-N.m.r. spectra were recorded with a Bruker CXP-200 (200 MHz) or AM-400 (400 MHz) spectrometer, with tetramethylsilane (Me_4Si) as the internal standard, for solutions in (^{2}H)chloroform, unless otherwise stated. Chemical shifts (δ) are given downfield from the signal of Me_4Si. Analytical thin-layer chromatography (t.l.c.) was performed on glass plates precoated with Merck Silica Gel 60F-254 as the adsorbent (layer thickness: 0.25 mm). The following solvent systems (v/v) were

used: (*A*) 7:1, (*B*) 5:1, (*C*) 4:1, (*D*) 3:1, (*E*) 2:1, (*F*) 1:1, (*G*) 2:3, and (*H*) 1:2 toluene–ethyl acetate; (*I*) 9:9:1, (*J*) 8:8:1, and (*K*) 5:5:1 toluene–ethyl acetate–2-propanol; and (*L*) 3:5:1 ethanol–2-propanol–water. The developed plates were air-dried, sprayed with a solution of Cc_2SO_4 (1%) and H_2MoO_4 (1.5%) in 10% aqueous H_2SO_4, and heated at 150°. Column chromatography was performed with Merck 7734 Kieselgel 60 (70–230 mesh) as the solid phase. Solvents were evaporated under reduced pressure at <40°.

2-Methoxyethyl 2,3,6,2',3',4',6'-hepta-O-acetyl-β-lactoside (**3**). — A mixture comprised of **1** (13.85 g, 19.8 mmol), **2** (2.0 g, 25.3 mmol), and $Hg(CN)_2$ (8.0 g, 32 mmol) in 1:1 (v/v) benzene–nitromethane (100 mL) was stirred for 9 h at 40°, diluted with toluene (150 mL), and washed sequentially with a saturated, aqueous solution of $NaHCO_3$ (2 × 75 mL) and water (75 mL). The organic phase was dried ($MgSO_4$), evaporated, and the residual mixture resolved by column chromatography to give **3** (9.5 g, 69%) as a white solid, $[\alpha]_D^{16}$ −14.4° (*c* 3.80, chloroform), R_F 0.40 (solvent *H*); ^{1}H-n.m.r. (400 MHz): δ 1.96 (3 H, s OAc), 2.041 (3 H, s, OAc), 2.045 (6 H, s, 2 × OAc), 2.06 (3 H, s, OAc), 2.12 (3 H, s, OAc), 2.15 (3 H, s, OAc), 3.35 (3 H, s, OMe), 3.47–3.56 (2 H, m, OCH_2CH_2O), 3.61 (1 H, ddd, $J_{5,4}$ 9.7, $J_{5,6}$ 5.0, $J_{5,6}$ 2.0 Hz, H-5), 3.70 (1 H, m, J_{gem} 11.0, ^3J 6.5, ^3J 4.0 Hz, OCH_2CH_2O), 3.80 (1 H, t, ^3J 9.5 Hz, H-4), 3.87 (1 H, br. t, ^3J 7.0 Hz, H-5'), 3.91 (1 H, m, J_{gem} 11.0, ^3J 4.8, ^3J 4.0 Hz, OCH_2CH_2O), 4.08 (1 H, dd, J_{gem} 11.0, $J_{6',5'}$ 7.6 Hz, H-6'), 4.10 (1 H, dd, J_{gem} 12.0, $J_{6,5}$ 5.0 Hz, H-6), 4.14 (1 H, dd, J_{gem} 11.0, $J_{6',5'}$ 6.4 Hz, H-6'), 4.48 (1 H, d, $J_{1',2'}$ 7.9 Hz, H-1'), 4.49 (1 H, dd, J_{gem} 12.0, $J_{6,5}$ 2.0 Hz, H-6), 4.56 (1 H, d, $J_{1,2}$ 8.0 Hz, H-1), 4.91 (1 H, dd, $J_{2,3}$ 9.5, $J_{2,1}$ 8.0 Hz, H-2), 4.95 (1 H, dd, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.4 Hz, H-3'), 5.11 (1 H, dd, $J_{2',3'}$ 10.4, $J_{2',1'}$ 7.9 Hz, H-2'), 5.20 (1 H, t, ^3J 9.3 Hz, H-3), and 5.35 (1 H, br. d, $J_{4',3'}$ 3.4 Hz, H-4').

Anal. Calc. for $C_{29}H_{42}O_{19}$: C, 50.14; H, 6.09. Found: C, 49.86; H, 5.92.

2-Methoxyethyl 2,3,6,2',3',4',6'-hepta-O-benzyl-β-lactoside (**4**). — A mixture of **3** (8.0 g, 11.5 mmol), benzyl chloride (30 g, 237 mmol), and KOH (30 g, 540 mmol) in toluene (100 mL) was heated at reflux temperature for 0.5 h, cooled to room temperature, diluted with toluene (50 mL), and extracted with water (120 mL). The organic phase was evaporated and the residual mixture resolved by column chromatography. The product having R_F 0.43 (solvent *B*) afforded **4** (7.82 g, 66%) as fine, white needles, from ethanol, m.p. 82–83°, $[\alpha]_D^{16}$ +10.2° (*c* 3.50, chloroform); ^{1}H-n.m.r. (400 MHz): δ 3.32–4.04 (16 H, a series of multiplets associated with the glycosyl CHO and CH_2O protons, and the OCH_2CH_2O protons), 3.37 (3 H, s, OMe), 4.23 and 4.34 (2 H, d's, J_{gem} 11.6 Hz, $CH_2C_6H_5$), 4.38 and 4.52 (2 H, d's, J_{gem} 12.1 Hz, $CH_2C_6H_5$), 4.42 (1 H, d, $J_{1,2}$ 7.6 Hz, H-1), 4.44 (1 H, d, $J_{1',2'}$ 8.0 Hz, H-1'), 4.55 and 4.96 (2 H, d's, J_{gem} 11.4 Hz, $CH_2C_6H_5$), 4.67 and 4.71 (2 H, d's, J_{gem} 12.2 Hz, $CH_2C_6H_5$), 4.72 and 4.92 (2 H, d's, J_{gem} 10.8 Hz, $CH_2C_6H_5$), 4.72 and 5.01 (2 H, d's, J_{gem} 10.8 Hz, $CH_2C_6H_5$), 4.75 and 4.81 (2 H, d's, J_{gem} 11.3 Hz, $CH_2C_6H_5$), and 7.10–7.43 (35 H, m, 7 × $CH_2C_6H_5$).

Anal. Calc. for $C_{64}H_{70}O_{12}$: C, 74.54; H, 6.84. Found: C, 74.57; H, 6.76.

2,3,6,2',3',4',6'-Hepta-O-benzyl-α-lactosyl bromide (**5**). — A solution of

$TiBr_4$ (1.31 g, 3.56 mmol) in dichloromethane (10 mL) was added slowly (3 min) to a solution of **4** (4.99 g, 4.83 mmol) in dichloromethane (40 mL) at room temperature. The reaction solution was stirred for 5 min, cooled in an ice bath for 13 min, and treated with an ice-cold saturated, aqueous solution of $NaHCO_3$ (30 mL) for 5 min. The organic phase was dried ($CaCl_2$) and evaporated to give **5** as an oil. The product was shown by t.l.c. to consist of a major component and several minor components. Solutions of **5** in dichloromethane or 1,2-dichloroethane were used immediately, without further purification, in the subsequent reactions.

*2-*O*-Benzylglycerol* (**7**) *and 1-*O*-acetyl-2-*O*-benzyl-*(R,S)*-glycerols* (**8**). — A mixture of 2-*O*-benzyl-1,3-di-*O*-tritylglycerol[17] (**6**) (57.3 g, 85.9 mmol) in 80% acetic acid (250 mL) was heated at reflux temperature for 20 min with stirring, cooled to room temperature, stored at 4° overnight, and the precipitated triphenylmethanol removed by filtration. The filtrate was evaporated and the residual mixture was resolved by column chromatography to give **7** (9.49 g, 60.6%), R_F 0.39 (solvent *K*), as a white solid[17], and **8** (4.77 g, 24.8%), R_F 0.67, as a yellow oil; for compound **8**, ^{1}H-n.m.r. (400 MHz): δ 2.06 (3 H, s, OAc), 2.34 (1 H, br. s, OH), 3.59–3.71 (3 H, m, 2 H-3, H-2), 4.18–4.25 (2 H, m, 2 H-1), 4.60 (1 H, d, J_{gem} 11.9 Hz, $CH_2C_6H_5$), 4.69 (1 H, d, J_{gem} 11.9 Hz, $CH_2C_6H_5$), and 7.2–7.4 (5 H, m, $CH_2C_6H_5$).

*3-*O*-Acetyl-2-*O*-benzyl-1-*O*-(2,3,6,2',3',4',6'-hepta-*O*-benzyl-α-lactosyl)-(R,S)-glycerols* (**9**) *and 1,5-anhydro-2,3,6-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-*D*-galactopyranosyl)-*D*-arabino-hex-1-enitol* (**10**). — A mixture of **5** (4.93 g, 4.83 mmol theoretical), **8** (1.106 g, 4.93 mmol), tetraethylammonium bromide (1.02 g, 4.83 mmol), and 4Å molecular sieves (5 g) in 1,2-dichloroethane (40 mL) was stirred at room temperature for 3 h, at 75–80° for 41 h, and at room temperature for 48 h. The solids were removed by filtration and washed with dichloromethane (40 mL). The combined filtrate and wash solution was evaporated and the residual mixture resolved by column chromatography to afford two products, namely, **9** (1.16 g, 20.4%) as a colorless oil, R_F 0.35 (solvent *A*), and **10** (1.17 g, 25.3%) as a yellow oil, R_F 0.60.

For compound **9**, the ^{1}H-n.m.r. (400 MHz) spectrum exhibited: δ 2.05 (3 H, s, OAc), 2.09 (3 H, s, OAc), and 3.30–5.07 (70 H, a series of multiplets associated with the CHO and CH_2O protons), 7.13–7.43 (80 H, m, 16 × C_6H_5). These data indicated that **9** was a 1:1 mixture of two diastereomers.

Anal. Calc. for $C_{73}H_{78}O_{14}$: C, 74.34; H, 6.67. Found: C, 74.13; H, 6.80.

For compound **10**, the ^{1}H-n.m.r. spectrum exhibited: δ 3.46 (1 H, dd, $J_{3',2'}$ 9.8, $J_{3',4'}$ 3.1 Hz, H-3'), 3.44–3.56 (3 H, m, H-5' and 2 H-6'), 3.61 (1 H, dd, J_{gem} 10.6, $J_{6,5}$ 4.2 Hz, H-6), 3.79 (1 H, dd, $J_{2',1'}$ 7.8, $J_{2',3'}$ 9.8 Hz, H-2'), 3.82 (1 H, dd, J_{gem} 10.6, $J_{6,5}$ 7.3 Hz, H-6), 3.79 (1 H, dd, $J_{2',1'}$ 7.8, $J_{2',3'}$ 9.8 Hz, H-2'), 3.82 (1 H, dd, J_{gem} 10.6, $J_{6,5}$ 7.3 Hz, H-6), 3.87 (1 H, m, H-4'), 4.18 (1 H, t, ^3J 3.4 Hz, H-4), 4.27 (1 H, dd, $J_{3,4}$ 3.4, $J_{3,5}$ 1.0 Hz, H-3), 4.31 (1 H, m, H-5), 4.33 and 4.37 (2 H, d's, J_{gem} 11.6 Hz, $CH_2C_6H_5$), 4.44 and 4.50 (2 H, d's, J_{gem} 12.2 Hz, $CH_2C_6H_5$), 4.49 (1 H, d, $J_{1',2'}$ 7.8 Hz, H-1'), 4.60 and 4.94 (2 H, d's, J_{gem} 11.6 Hz, $CH_2C_6H_5$), 4.64

and 4.71 (2 H, d's, J_{gem} 12.1 Hz, $CH_2C_6H_5$), 4.65 and 4.68 (2 H, d's, J_{gem} 11.4 Hz, $CH_2C_6H_5$), 4.67 and 4.84 (2 H, d's, J_{gem} 10.8 Hz, $CH_2C_6H_5$), 4.69 and 4.75 (2 H, d's, $CH_2C_6H_5$), 6.29 (1 H, s, H-1), and 7.22–7.38 (35 H, m. 7 $CH_2C_6H_5$).

2-O-Benzyl-1-O-(2,3,6,2',3',4',6'-hepta-O-benzyl-α-lactosyl)-(R,S)-glycerols (**11**). — A suspension of **9** (1.11 g, 0.94 mmol) in 3:10:40 (v/v) triethylamine–water–methanol (53 mL) was stirred for 24 h at room temperature, diluted with ethanol (20 mL), stirred for 48 h at room temperature, evaporated, and the residual mixture resolved by column chromatography to give **11** (0.82 g, 77%) as a viscous, colorless oil, R_F 0.32 (solvent *C*); ^{1}H-n.m.r. (400 MHz): δ 2.3 (1 H, m, OH), 2.5 (1 H, m, OH), 3.30–5.06 (70 H, a series of complex overlapping multiplets associated with the CHO and CH_2O protons), and 7.13–7.38 (80 H, m, 16 C_6H_5).

Anal. Calc. for $C_{71}H_{76}O_{13}$: C, 74.98; H, 6.73. Found: C, 74.70; H, 6.83.

Preparation of **10** *and* **11** *from* **5** *and* **7**. — A mixture of **5** (2.1 g, 2.05 mmol theoretical), **7** (0.128 g, 0.70 mmol), tetraethylammonium bromide (0.206 g, 1.0 mmol), and 4Å molecular sieves (6 g) in dichloromethane (30 mL) was stirred for 9 days at room temperature. T.l.c. indicated that a small amount of **5** was present. The solids were removed by filtration and washed with dichloromethane (40 mL). The combined filtrate and wash solution was evaporated and the residual mixture resolved by column chromatography to give **11** (0.308 g, 39%), R_F 0.41 (solvent *D*), and **10** (0.355 g, 18%), R_F 0.85.

1-O-α-Lactosyl-(R,S)-glycerols (**12**). — A suspension of 10% (w/w) Pd–C (1.0 g) in methanol (10 mL) was added to a solution of **11** (0.388 g, 0.341 mmol) in methanol (40 mL) containing 10% formic acid, and the mixture was stirred for 5.5 h at room temperature. The solids were removed by filtration and washed with 67% (v/v) aqueous methanol (50 mL). The combined filtrate and wash solution was evaporated and the residue dried under vacuum to give impure **12** (0.195 g).

A solution of **12** (0.11 g, 0.255 mmol theoretical) and acetic anhydride (5 mL) in pyridine (15 mL) was stirred for 3 days at room temperature, evaporated, and the residual mixture resolved by column chromatography to give 2,3-di-*O*-acetyl-1-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-α-lactosyl)-(*R,S*)-glycerols (**13**) (0.133 g, 66%) as a colorless, glassy solid, R_F 0.36 (solvent *G*); ^{1}H-n.m.r. (400 MHz): δ 1.94 (6 H, s, 2 OAc), 2.02–2.05 (33 H, m, 11 OAc), 2.07 (3 H, s, OAc), 2.11 (6 H, br. s, 2 OAc), 2.14 (6 H, s, 2 OAc), 3.609 (1 H, dd J_{gem} 11.3, $J_{1'',2''}$ 5.1 Hz, H-1''), 3.637 (1 H, dd, J_{gem} 11.3, $J_{1'',2''}$ 5.4 Hz, H-1''), 3.74 (2 H, t, ^3J 9.7 Hz, 2 H-4), 3.800 (1 H, dd, J_{gem} 11.2, $J_{1'',2''}$ 5.3 Hz, H-1''), 3.806 (1 H, dd, J_{gem} 11.3, $J_{1'',2''}$ 4.2 Hz, H-1''), 3.85–3.98 (4 H, m, 2 H-5 and 2 H-5'), 4.06–4.23 (8 H, m, 2 H-6, 4 H-6', and 2 H-3''), 4.316 (1 H, dd, J_{gem} 12.1, $J_{3'',2''}$ 3.8 Hz, H-3''), 4.321 (1 H, dd, J_{gem} 12.0, $J_{3'',2''}$ 4.4 Hz, H-3''), 4.44–4.53 (2 H, m, 2 H-6), 4.476 (1 H, d, $J_{1',2'}$ 8.1 Hz, H-1'), 4.487 (1 H, d, $J_{1',2'}$ 7.6 Hz, H-1'), 4.78 (2 H, dd, $J_{2,1}$ 3.7, $J_{2,3}$ 10.3 Hz, 2 H-2), 4.96 (2 H, dd, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.4 Hz, 2 H-3'), 5.014 (1 H, d, $J_{1,2}$ 4.4 Hz, H-1), 5.025 (1 H, d, $J_{1,2}$ 4.2 Hz, H-1), 5.115 (1 H, dd, $J_{2',1'}$ 8.0, $J_{2',3'}$ 10.4 Hz, H-2'), 5.121 (1 H, dd, $J_{2',1'}$ 8.0, $J_{2',3'}$ 10.4 Hz, H-2'), 5.18 (2 H, m, 2 H-2''), 5.36 (2 H, br. d, $J_{4',3'}$ 3.4 Hz, 2 H-4'), 5.438 (1 H, dd, $J_{3,2}$ 10.2, $J_{3,4}$ 9.5 Hz, H-3), and 5.449 (1 H, dd, $J_{3,2}$ 10.0, $J_{3,4}$

9.2 Hz, H-3); the ^{1}H-n.m.r. spectrum showed the presence of traces of impurities.

A solution of **13** (81 mg, 0.102 mmol) in absolute methanol (15 mL) was treated with 0.1M sodium methoxide in methanol (7 mL) for 2.25 h at room temperature, with stirring. The reaction solution was stirred with Amberlite IR-120 (H^+) cation-exchange resin (5 mL) for 20 min. The resin was removed by filtration, washed with methanol (25 mL), and the combined filtrate and wash solution evaporated. The residue was dried under vacuum to give **12** (42 mg, 100%) as a very hygroscopic, white crystalline solid (from absolute methanol).

Anal. Calc. for $C_{15}H_{28}O_{13}\cdot 0.5\ H_2O$: C, 42.35; H, 6.87. Found: C, 42.41; H, 7.12.

2-O-Benzyl-3-O-(2,3,6,2',3',4',6'-hepta-O-acetyl-β-lactosyl)-1-O-(2,3,6, 2',3',4',6'-hepta-O-benzyl-α-lactosyl)-(R,S)*-glycerols* (**15**). — A mixture of **1** (0.666 g, 0.95 mmol), **11** (0.159 g, 0.46 mmol), and mercury(II) cyanide (0.272 g, 1.08 mmol) in 1:1 (v/v) benzene–nitromethane (20 mL) was stirred at 40° for 12 h. Compound **1** (0.2 g, 0.3 mmol) and mercury(II) cyanide (0.079 g, 0.313 mmol) were added to the reaction mixture; stirring was continued at 40° for 11 h, and at room temperature for 12 h. The mixture was diluted with toluene (30 mL), washed sequentially with saturated, aqueous $NaHCO_3$ (2 × 25 mL) and water (25 mL), and the organic phase dried ($CaCl_2$) and concentrated. The residual mixture was separated by column chromatography to give **15** (0.493 g, 61%) as a colorless, glassy solid, R_F 0.37 (solvent *E*), which consisted of a 1:1 mixture of two diastereomers; ^{1}H-n.m.r. (400 MHz): δ 1.91 (3 H, s, OAc), 1.95 (3 H, s, OAc), 1.97 (6 H, s, 2 OAc), 2.03 (6 H, s, 2 OAc), 2.040 (3 H, s, OAc), 2.045 (6 H, s, 2 OAc), 2.060 (6 H, s, 2 OAc), 2.065 (3 H, s, OAc), 2.16 (6 H, s, 2 OAc), 3.3–5.21 (96 H, a series of complex overlapping multiplets corresponding to the CHO and CH_2O protons), 5.35 (2 H, br. d, ^3J 2.7 Hz, 2 H-4'), and 7.11–7.40 (80 H, m, 16 C_6H_5).

Anal. Calc. for $C_{97}H_{110}O_{30}$: C, 66.35; H, 6.31. Found: C, 66.28; H, 6.46.

3-O-(2,3,6,2',3',4',6'-Hepta-O-acetyl-β-lactosyl)-1-O-α-lactosyl-(R,S)*-glycerols* (**16**). — Compound **15** (0.459 g, 0.26 mol) was debenzylated in the manner described above for **11**. After 16 h, the solids were removed by filtration, washed with methanol (100 mL), the combined filtrate and wash solution concentrated, and the residue dried under vacuum to give **15** (0.248 g, 92%) as a gray, glassy solid, R_F 0.63 (solvent *L*); ^{1}H-n.m.r. [400 MHz, $(CD_3)_2SO$]: δ 1.90 (6 H, s, 2 OAc), 1.97 (6 H, s, 2 OAc), 1.99 (6 H, s, 2 OAc), 2.01 (12 H, s, 4 OAc), 2.09 (6 H, s, 2 OAc), 2.10 (6 H, s, 2 OAc), and 3.17–5.23 (82 H, a series of multiplets corresponding to CHO, CH_2O, and OH protons).

2-O-Acetyl-1-O-(2,3,6,2',3',4',6'-hepta-O-acetyl-α-lactosyl)-3-O-(2,3,6, 2',3',4',6'-hepta-O-acetyl-β-lactosyl)-(R,S)*-glycerols* (**17**). — A mixture of **16** (0.240 g, 0.232 mmol) in acetic anhydride (8 mL, 85 mmol) and pyridine (30 mL) was stirred for 3.5 days at room temperature. The liquids were removed by coevaporation with toluene (3 × 20 mL) and the residue was resolved by column chromatography to afford **17** (0.149 g, 47%), R_F 0.38 (solvent *H*), as a colorless,

glassy solid which was a 1:1 mixture of two diastereomers; ^{1}H-n.m.r. (400 MHz): δ 1.97 (12 H, s, 4 OAc), 2.05–2.08 (54 H, m, 18 OAc), 2.13–2.17 (24 H, m, 8 OAc), 3.54–3.95 (20 H, m, 2 H-4, 2 H-4″, 2 H-5, 4 H-5′, 2 H-5″, 4 H-1‴, and 4 H-3‴), 4.06–4.16 (12 H, m, 2 H-6, 8 H-6′, and 2 H-6″), 4.43–4.53 (10 H, m, 4 H-1′, 2 H-1″, 2 H-6, and 2 H-6″), 4.77 (2 H, m, 2 H-2), 4.868 (1 H, dd, $J_{2'',1''}$ 8.1, $J_{2'',3''}$ 9.3 Hz, H-2″), 4.879 (1 H, dd, $J_{2'',1''}$ 7.8, $J_{2'',3''}$ 9.6 Hz, H-2″), 4.96 (4 H, dd, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.5 Hz, 4 H-3′), 4.981 (1 H, d, $J_{1,2}$ 4.0 Hz, H-1), 4.998 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1), 5.04–5.14 (2 H, m, 2 H-2‴), 5.11 (4 H, $J_{2',1'}$ 7.9, $J_{2',3'}$ 10.4 Hz, 4 H-2′), 5.188 (1 H, t, ^3J 9.3 Hz, H-3″), 5.191 (1 H, t, 9.4 Hz, H-3″), 5.35 (4 H, br. d, ^3J 3.0 Hz, 4 H-4′), and 5.43 (2 H, m, 2 H-3).

Anal. Calc. for $C_{57}H_{78}O_{38}$: C, 49.93; H, 5.73. Found: C, 49.74, H, 5.72.

1-O-α-Lactosyl-3-O-β-lactosyl-(R,S)*-glycerols* (**18**). — Compound **17** (0.112 g, 0.082 mmol) was *O*-deacetylated in the manner described for the preparation of **12** from **13**, to give **18** (0.0565 g, 93%) as a colorless, glassy solid.

Anal. Calc. for $C_{27}H_{48}O_{23} \cdot H_2O$: C, 42.74; H, 6.64. Found: C, 42.58: H, 6.70.

ACKNOWLEDGMENTS

The authors are grateful to the Natural Sciences and Engineering Research Council Canada for partial support of the research in the form of a grant (to W.A.S.).

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