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Synthesis and characterization of 2-*O*-β-lactosylglycerol, 1,2-di-*O*-β-lactosyl-(*R*,*S*)-glycerols, and 1,2,3-tri-*O*-β-lactosylglycerol*

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ABSTRACT

The reaction of 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-α-lactosyl bromide (**5**) and 1,3-di-*O*-benzylglycerol in the presence of mercury(II) cyanide in benzene–nitromethane afforded 1,3-di-*O*-benzyl-2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol (70%), which was converted into 2-*O*-β-lactosylglycerol. 1,2-Di-*O*-β-lactosyl-(*R*,*S*)-glycerols were obtained by way of the coupling of **5** to either 1-*O*-benzyl-(*R*,*S*)-glycerol or 1-*O*-benzyl-2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R*,*S*)-glycerols. The most efficient route to 1,2,3-tri-*O*-β-lactosylglycerol (**17**) involved treatment of 2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol with 3 mol. equiv. of **5** followed by removal of the blocking groups, to give **17** (47%).

INTRODUCTION

Glycosides having di-*O*-acylglycerol as the aglycon form one class of glycolipids[1] and, as such, are widely distributed in Nature. In plants[1,2] and some algae[3,4], the principal glycoglycerolipids are *O*-β-D-galactopyranosyl-(1→1′)-2′,3′-di-*O*-acyl-(*S*)-glycerols and *O*-β-D-galactopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→1′)-2′,3′-di-*O*-acyl-(*S*)-glycerols[1,4]. All photosynthetic organisms contain *O*-(6-deoxy-6-sulfo-α-D-glucopyranosyl)-(1→1′)-2′,3′-di-*O*-acyl-(*S*)-glycerols[1]. Digalactosyldiglycerides also have been found[5] in the human brain. Bacteria synthesize several glycosyldiglycerides[6,7], including *O*-α-D-galactopyranosyl-(1→2)-*O*-α-D-glucopyranosyl-(1→1′)-2′,3′-di-*O*-acylglycerols[6,8], *O*-α-D-mannopyranosyl-(1→1′)-2′,3′-di-*O*-acylglycerols, and *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→1′)-2′,3′-di-*O*-acylglycerols[9]. Glycosides of unesterified glycerol have been isolated from such diverse sources as algae[3,10–14], cyanobacteria[15], wheat flour[16], lilies[17], and human urine[18].

Despite the variety of di-[1,2,4,19,20] and oligo-saccharides[21–24] present in naturally occurring glycoglycerolipids, no lactosyldiglyceride or lactosylglycerol has been found, although lactosylceramides[25] are well known and alditolyl glycosides of *N*-acetyl-

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lactosamine-type oligosaccharides have been prepared[26]. Alditolyl glycosides occur in Nature[27], and a broad spectrum of such compounds has been obtained as products of the Smith degradation[28] of polysaccharides, by reduction of the products of the partial degradation of glycoproteins[29], and by a variety of synthetic methods[30–32]. However, Ogawa *et al.*[33,34] have described the synthesis of 1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-2,3-di-*O*-tetradecyl-(*R*)-glycerol, the corresponding hepta-*O*-acetyl-α-lactosyl stereoisomer[34], and the 1-*O*-α- and -β-lactosyl-2,3-di-*O*-tetradecylglycerols[34]. Hronowski *et al.*[35,36] have reported the synthesis of 1-*O*-β-lactosyl-(*R,S*)-glycerols, 1,3-di-*O*-β-lactosylglycerol[35], 1-*O*-α-lactosyl-(*R,S*)-glycerols, and 1-*O*-α-lactosyl-3-*O*-β-lactosyl-(*R,S*)-glycerols[36]. These compounds are of considerable interest for the study of the binding properties of the asialoglycoprotein receptor[37,38] of normal mammalian hepatocytes, a receptor which recognizes[39] and binds[40,41] D-galactopyranose-terminated glycoproteins and oligomers.

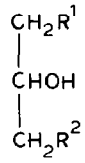
Apart from the work of Hronowski *et al.*[35,36], there are few examples of glycosylglycerols that have more than one hydroxyl group of the glycerol moiety involved in a glycosidic linkage. Wang *et al.*[18] obtained mass spectrometric evidence of the presence in urine of fucosyl- and galactosyl-glucosylglycerols in which one monosaccharide unit was attached to each of positions 1 and 2 of glycerol, but the unequivocal determination of the structures of these compounds has not been reported. We now describe the synthesis and characterization of 2-*O*-β-lactosylglycerol (**10**), 1,2-di-*O*-β-lactosyl-(*R,S*)-glycerols (**14**), and 1,2,3-tri-*O*-β-lactosylglycerol (**17**), thereby affording a significant extension of the range of lactosylated glycerols available for the elucidation of the binding properties of galactose-specific receptors.

RESULTS AND DISCUSSION

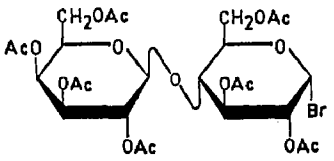
The synthesis of **10**, **14**, and **17** required the preparation of 1,3-di-*O*-benzylglycerol (**1**) and 1-*O*-benzyl-(*R,S*)-glycerol (**2**), which were obtained from 1,3-dichloro-2-propanol (**3**) and 3-chloro-1,2-propanediol (**4**), respectively, by the method of Fairbourne *et al.*[42]

1,3-Di-*O*-benzyl-2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol (**6**, 70% after chromatography) was synthesized by reaction of hepta-*O*-acetyl-α-lactosyl bromide[35] (**5**) with **1** in the presence of mercury(II) cyanide[43] in benzene–nitromethane for 16 h at 40°. *O*-Debenzylation of **6**, by catalytic transfer hydrogenation[44] over Pd–C in methanol–formic acid, gave 2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol (**7**, 59%) and 1-*O*-benzyl-2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R,S*)-glycerols (**8**, 17%); **8** consisted of a mixture of diastereomers. The yield of purified **8** could be increased to at least 41% by decreasing the time of reaction. The ^{1}H-n.m.r. spectrum of **8** indicated that the diastereomers were present in a ratio of 2:1. Treatment of **7** with acetic anhydride–pyridine gave 1,3-di-*O*-acetyl-2-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol (**9**). *O*-Deacetylation[45] of **7** with methanolic sodium methoxide afforded 2-*O*-β-lactosylglycerol (**10**, 97%).

^{1}H-N.m.r. spectroscopy is particularly convenient[35,36] for determining the config-



1 $R^1 = R^2 = OBzl$
2 $R^1 = OBzl$, $R^2 = OH$
3 $R^1 = R^2 = Cl$
4 $R^1 = Cl$, $R^2 = OH$



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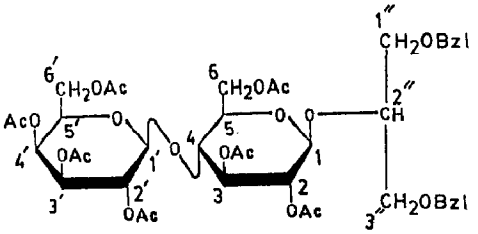

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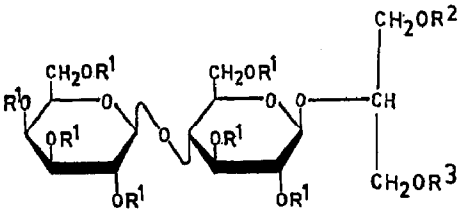


7 $R^1 = Ac$, $R^2 = R^3 = H$
8 $R^1 = Ac$, $R^2 = Bzl$, $R^3 = H$
9 $R^1 = R^2 = R^3 = Ac$
10 $R^1 = R^2 = R^3 = H$

uration of the glycosidic bond between lactose and glycerol. The ^1H resonances of **6**, **7**, and **9** were assigned on the basis of 2D, F.t., proton chemical-shift-correlation spectroscopy (COSY)[46,47] experiments. The $J_{1,2}$ values of the glucopyranosyl residues were in the range 7.9–8.1 Hz, which indicated[35,36] them to have the β configuration. A comparison of the chemical shifts of the resonances of the lactosyl protons of the two diastereomers, which comprised 1-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R*,*S*)-glycerols[35], with those of **9** (see Fig. 1), revealed differences of <0.02 p.p.m., except for H-1 of **9**, which resonated at δ 4.62, 0.11 p.p.m. downfield from that of the 1-*O*-isomer[35].

The first approach to 1,2-di-*O*-β-lactosyl-(*R*,*S*)-glycerols (**14**) involved the reaction of **2** with **5** in the presence of mercury(II) cyanide[43] in benzene–nitromethane, which afforded 3-*O*-benzyl-1,2-bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R*,*S*)-glycerols (**11**, 34%) as a 1:1 mixture of the diastereomers. The second approach involved the partial *O*-debenzylation of **6** to produce **8**, which was coupled[43] with **5** to afford **11** (65% from **8**) as a 3:1 mixture of diastereomers. *O*-Debenzylation[44] of **11** (1:1 mixture of diastereomers) gave 1,2-bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-(*R*,*S*)-glycerols (**12**, 72%) as a 1:1 mixture of diastereomers. *O*-Debenzylation of **11** (3:1 mixture of diastereomers), and partial resolution of the product by column chromatography,

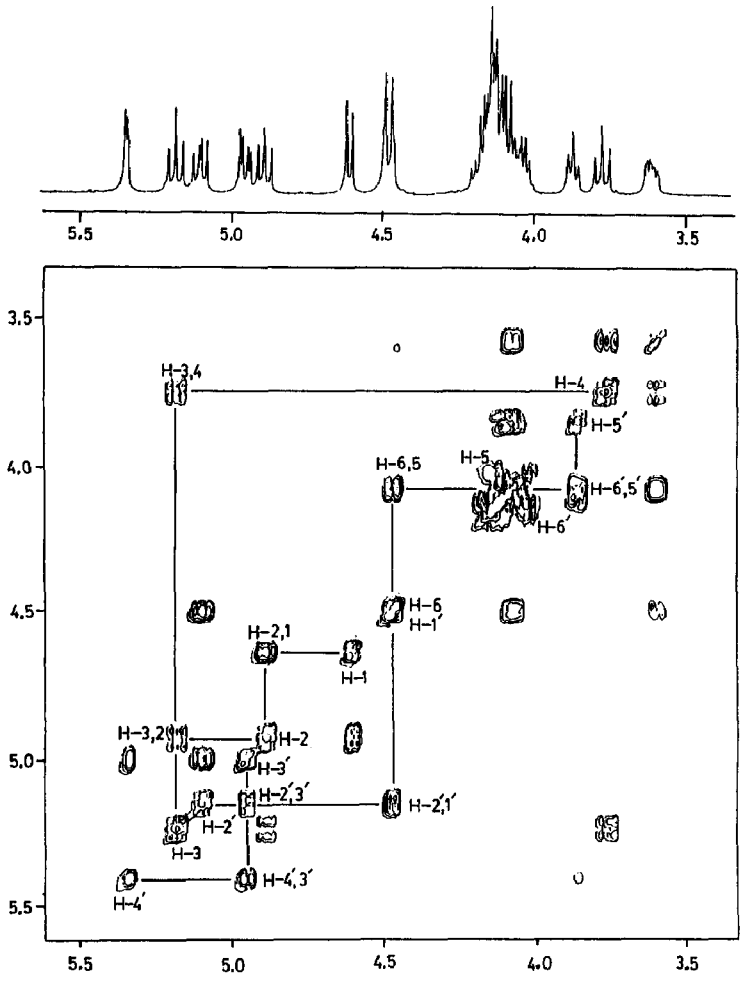
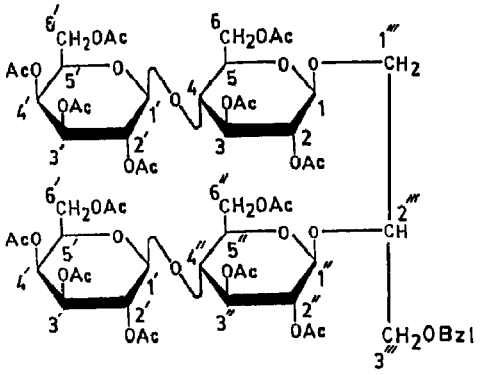


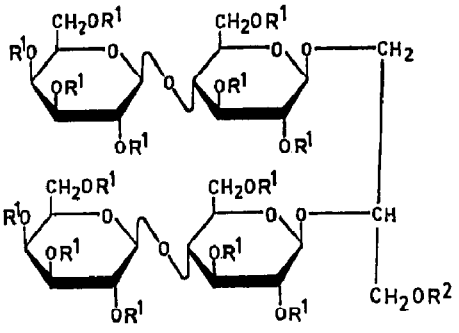
Fig. 1. 400-MHz ^{1}H-COSY spectrum of **9** in $CDCl_3$.

afforded **12** (48%) as a 7:1 mixture of the two diastereomers; in the solvent used (8:8:1 toluene–ethyl acetate–2-propanol), the R_F value of the major diastereomer was slightly lower than that of the minor isomer. The reaction of **12** (1:1 mixture) with acetic anhydride–pyridine gave 3-*O*-acetyl-1,2-bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-*β*-lactosyl)-(*R*,*S*)-glycerols (**13**, 94%). *O*-Deacetylation[45] of **12** or **13** then afforded 1,2-di-*O*-*β*-lactosyl-(*R*,*S*)-glycerols (**14**) in quantitative yield.

The ^{1}H resonances of **11**–**13** were assigned on the basis of COSY[46,47] experiments. The ^{1}H-COSY spectrum of **13** is given in Fig. 2. The chemical shifts of the H-1′ resonances of **11**–**13** were in the range δ 4.48–4.52 and $J_{1',2'}$ values were in the range 7.8–8.2 Hz. The H-1 resonances of these compounds exhibited a wider range (δ 4.44–4.54) of chemical shifts, but the $J_{1,2}$ values were of similar magnitude (7.8–8.1 Hz). The H-1″ resonances had chemical shifts in the range δ 4.60–4.71, 0.1–0.3 p.p.m. downfield



11 (mixture of diastereomers)



12 R^1 = Ac, R^2 = H
13 R^1 = R^2 = Ac
14 R^1 = R^2 = H

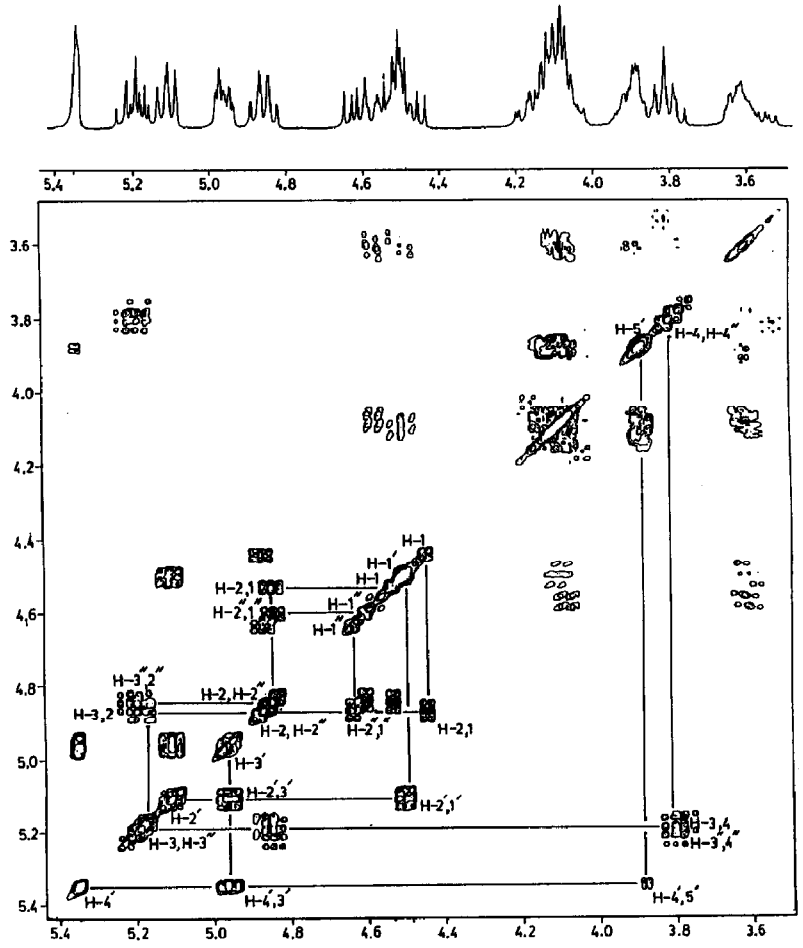
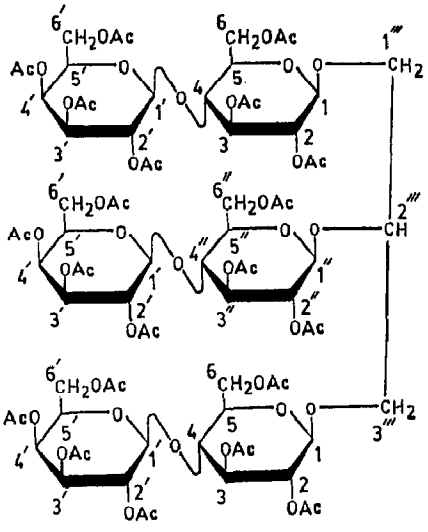


Fig. 2. 400-MHz ^{1}H-COSY spectrum of **13** in $CDCl_3$.

from those of the H-1′ resonances, and the $J_{1'',2''}$ values were 7.8–8.1 Hz. These data infer[35,36] that each of the lactosyl groups was β.

Three approaches to 1,2,3-tri-*O*-β-lactosylglycerol (**17**) are reported. The most efficient synthesis involved treatment[43] of **7** with 3 mol. equiv. of **5** which afforded 1,2,3-tris-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol (**15**, 48%). *O*-Deacetylation[45] of **15** gave **17** (97%). In contrast, the reaction[43] of 1,3-bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol[35] (**16**) with 1.7 mol. equiv. of **5** gave only 5% of **15**, and 52% of **16** was recovered. This finding indicates that the secondary hydroxyl group



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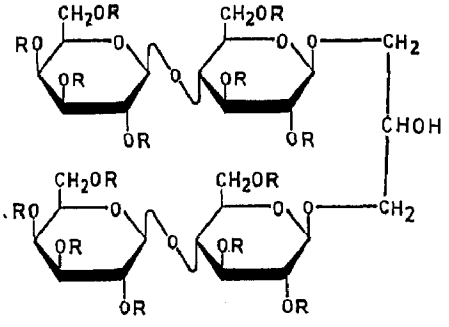
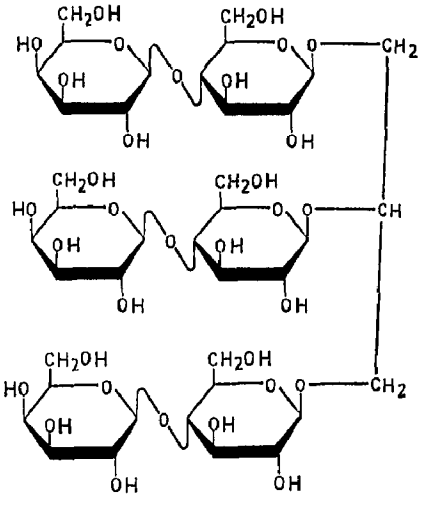


16 R = Ac



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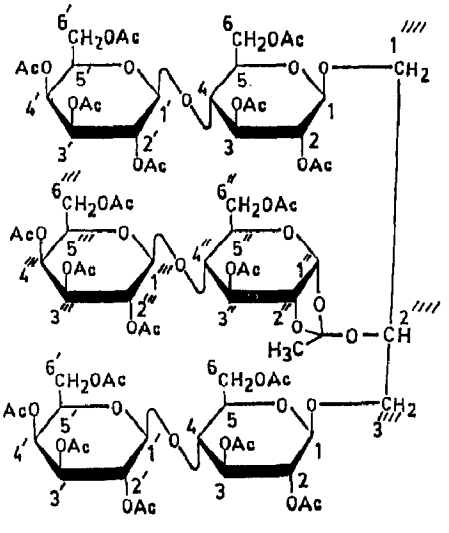


18

of **16** is so sterically hindered as to be almost unreactive under Helferich[43] glycosylation conditions, whereas the primary hydroxyl groups of **7** are reactive. The third route involved the reaction[48] of **16** with 2 mol. equiv. of **5** in the presence of silver trifluoromethanesulfonate and 2,4,6-trimethylpyridine in dichloromethane. Only one product was obtained, namely, 3,6,2′,3′,4′,6′-hexa-*O*-acetyl-α-lactose 1,2-[1,3-bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycer-2-yl orthoacetate] (**18**, 29%).

1,2-Orthoesters have been obtained as side-products during Helferich-type[43] glycosylation reactions. Normally, these ortho esters are separable easily from the desired glycosides, and these and other side-products will be discussed elsewhere.

The assignment of the ^{1}H resonances of **15** was deduced from the COSY spectrum (Fig. 3). The two H-1 atoms resonated at δ 4.39 and δ 4.53, whereas H-1″ resonated at δ 4.67. The $J_{1,2}$ and $J_{1'',2''}$ values were in the range 7.8–8.1 Hz, indicative[35,36] of β configurations.

The ^{1}H resonances of **18** were assigned on the basis of the COSY spectrum and by comparison with the data for **16** and 3,6,2′,3′,4′,6′-hexa-*O*-acetyl-α-lactose 1,2-(ethyl

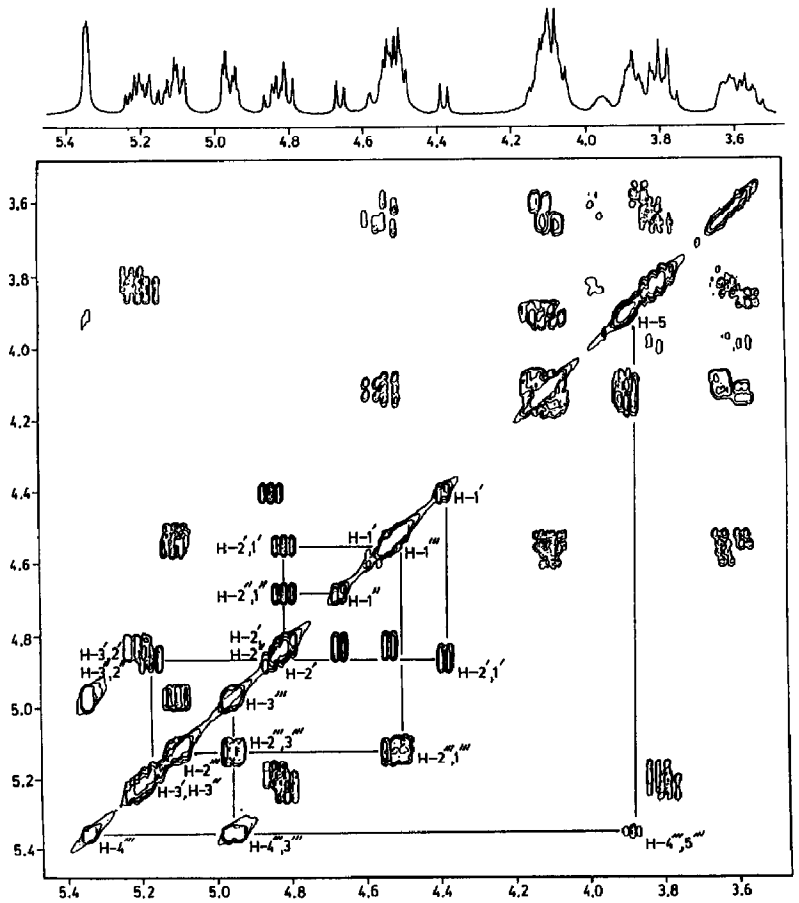


Fig. 3. 400-MHz ^{1}H-COSY spectrum of **15** in $CDCl_3$.

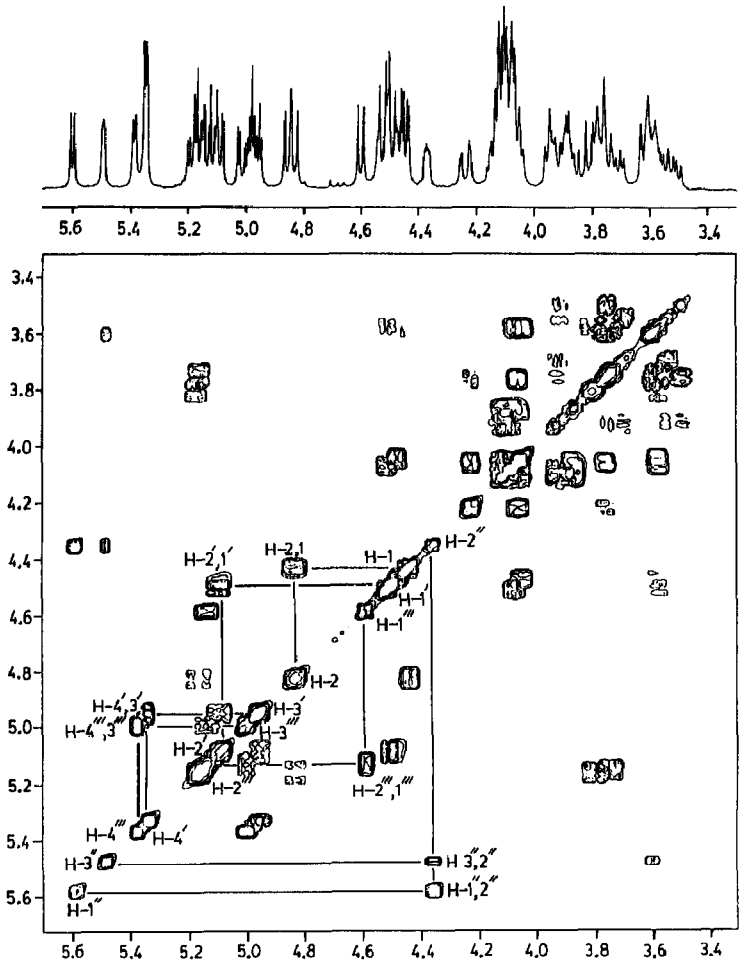


Fig. 4. 400-MHz ^{1}H-COSY spectrum of **18** in $CDCl_3$.

orthoacetate)[49]. The observation of a broad range of J values made it necessary to perform two COSY experiments (Figs. 4 and 5) to enable both the strong and the weak couplings to be measured (see ref. 50). The protons of the orthoacetate group gave a sharp singlet at δ 1.70. In addition, the lack of broadening or splitting of the individual peaks in the multiplets suggested that only one of the two possible diastereomeric ortho esters (see ref. 51) had been obtained.

Thus, the target compounds, **10**, **14**, and **17**, have been synthesized by reaction sequences in which the key intermediates have been characterized fully. The lactosylglycerols are useful in the assessment of the binding properties of galactose-terminated glyceryl glycosides to the asialoglycoprotein receptor of normal rabbit and human hepatocytes.

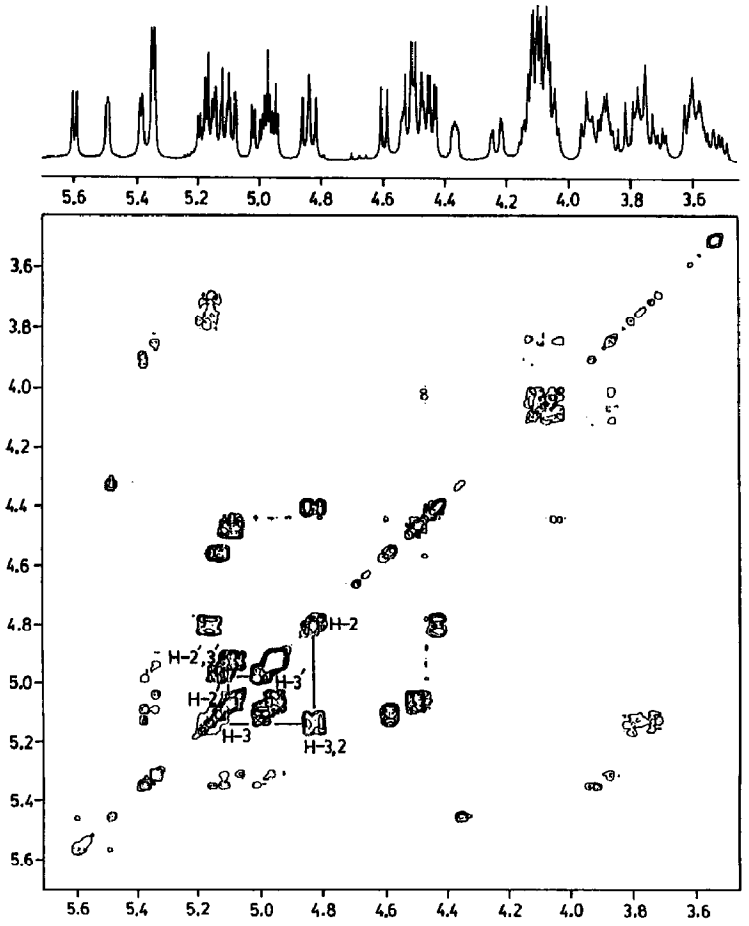


Fig. 5. 400-MHz ^{1}H-COSY spectrum of **18** in $CDCl_3$ with the spectrometer optimized for the visualization of the long-range couplings.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer model 241 automatic polarimeter (0.1-dm cell). ^{1}H-N.m.r. spectra (internal Me_4Si) were recorded with a Bruker AM-400 (400 MHz) spectrometer; singly and multiply primed numbers are assigned as shown in the formulae. T.l.c. was performed on Silica Gel $60F_{254}$ (Merck), using toluene–ethyl acetate *A*, 3:2; *B*, 2:3; *C*, 1:2; and *D*, 1:3; and *E*, 8:8:1 toluene–ethyl acetate–2-propanol; and detection with cerium(IV) sulfate (1%) and molybdic acid (1.5%) in aqueous 10% sulfuric acid at 150°. Column chromatography was performed on Silica Gel 60 (Merck, 7734, 70–230 mesh). Evaporations were performed under reduced pressure at $<40°$.

1,3-Di-O-benzylglycerol (**1**). — A stirred mixture of benzyl alcohol (26.3 g, 243 mmol), 1,3-dichloro-2-propanol (**3**; 10 g, 77.5 mmol), and potassium hydroxide (15 g,

267 mmol) was heated at 130–140° for 1 h, then cooled to room temperature and partitioned between dichloromethane (75 mL) and water (50 mL). The organic layer was extracted with water (2 × 50 mL), dried ($CaCl_2$), and concentrated, and **1** was fractionally distilled[42]. ^{1}H-N.m.r. data ($CDCl_3$): δ 2.6 (bs, 1 H, OH), 3.52 (dd, 2 H, J_{gem} 9.6, ^3J 6.2 Hz, H-1,3), 3.56 (dd, 2 H, J_{gem} 9.6, ^3J 4.4 Hz, H-1,3), 4.02 (m, 1 H, H-2), 4.54 (s, 4 H, 2 CH_2Ph), 7.2–7.4 (m, 10 H, 2 Ph); the OH proton was exchangeable with D_2O.

1-O-Benzyl-(R,S)*-glycerol* (**2**). — Compound **2** was prepared from **4** as described[42], except that potassium hydroxide was used instead of sodium hydroxide. ^{1}H-N.m.r. data ($CDCl_3$): δ 3.49 (dd, 1 H, J_{gem} 9.6, ^3J 6.0 Hz, H-1), 3.52 (dd, 1 H, J_{gem} 9.6, ^3J 4.2 Hz, H-1), 3.57 (dd, 1 H, J_{gem} 11.4, ^3J 5.9 Hz, H-3), 3.65 (dd, 1 H, J_{gem} 11.4, ^3J 3.7 Hz, H-3), 3.86 (m, 1 H, H-2), 4.53 (s, 2 H, CH_2Ph), 7.2–7.4 (m, 5 H, Ph).

1,3-Di-O-benzyl-2-O-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosyl)glycerol (**6**). — A mixture of **5** (4.48 g, 6.40 mmol), **1** (1.47 g, 5.40 mmol), and mercury(II) cyanide (1.92 g, 7.60 mmol) in benzene–nitromethane (1:1, 30 mL) was stirred at 40° for 16 h, then diluted with toluene (60 mL), washed sequentially with saturated aqueous sodium hydrogencarbonate (2 × 35 mL) and water (35 mL), dried ($CaCl_2$), and concentrated. Column chromatography (solvent *A*) of the residue gave **6** (3.39 g, 70%), isolated as a colorless, glassy solid, R_F 0.40, $[\alpha]_D^{24}$ −6.6° (*c* 1.75, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.92 (s, 3 H, OAc), 1.96 (s, 3 H, OAc), 2.031 (s, 3 H, OAc), 2.033 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.06 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 3.51–3.56 (m, 3 H, H-5,1″,3″), 3.63 (dd, 1 H, J_{gem} 10.7, ^3J 7.7 Hz, H-1″), 3.64 (dd, 1 H, J_{gem} 10.5, ^3J 6.2 Hz, H-3″), 3.78 (t, 1 H, ^3J 9.4 Hz, H-4), 3.86 (bt, 1 H, ^3J 6.8 Hz, H-5′), 4.01 (m, 1 H, H-2″), 4.04–4.15 (m, 3 H, H-6,6′,6′), 4.45 (dd, 1 H, J_{gem} 12.0, ^3J 2.2 Hz, H-6), 4.47 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.50 (s, 2 H, CH_2Ph), 4.51 (s, 2 H, CH_2Ph), 4.76 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.90 (dd, 1 H, $J_{2,3}$ 9.2, $J_{2,1}$ 8.1 Hz, H-2), 4.95 (dd, 1 H, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.3 Hz, H-3′), 5.10 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{2',1'}$ 8.0 Hz, H-2′), 5.18 (t, 1 H, ^3J 9.2 Hz, H-3), 5.34 (bd, 1 H, $J_{4',3'}$ 3.3 Hz, H-4′), 7.2–7.4 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{43}H_{54}O_{20}$: C, 57.97; H, 6.11. Found: C, 58.00; H, 6.06.

2-O-(2,3,6,2′,3′,4′,6′-Hepta-O-acetyl-β-lactosyl)glycerol (**7**) *and 1-O-benzyl-2-O-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosyl)-*(R,S)*-glycerols* (**8**). — A suspension of 10% Pd–C (2.3 g) in methanol (12 mL) was added to a solution of **6** (3.77 g, 4.22 mmol) in methanol (80 mL) containing 10% of formic acid[44]. The mixture was stirred at room temperature for 18 h, the solids were collected and washed with methanol (50 mL), and the combined filtrate and washings were concentrated. Column chromatography (solvent *E*) of the residue gave components with R_F 0.46 and 0.14. The latter component, **7** (1.77 g, 59%), was obtained as a colorless, glassy solid, $[\alpha]_D^{24}$ −0.8° (*c* 2.95, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.95 (m, 1 H, OH), 1.97 (s, 3 H, OAc), 2.059 (s, 6 H, 2 OAc), 2.064 (s, 3 H, OAc), 2.070 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 2.89 (m, 1 H, OH), 3.56–3.78 (m, 7 H, H-4,5,1″,1″,2″,3″,3″), 3.89 (m, 1 H, H-5′), 4.05 (dd, 1 H, J_{gem} 11.7, $J_{6,5}$ 6.1 Hz, H-6), 4.08 (dd, 1 H, J_{gem} 11.0, $J_{6',5'}$ 7.2 Hz, H-6′), 4.14 (dd, 1 H, J_{gem} 11.0, $J_{6',5'}$ 6.3 Hz, H-6′), 4.51 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.59 (dd, 1 H, J_{gem} 11.7, $J_{6,5}$ 2.2 Hz, H-6), 4.63 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.94 (dd, 1 H, $J_{2,3}$ 9.4, $J_{2,1}$ 7.9 Hz, H-2), 4.98 (dd, 1 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.3 Hz, H-3′), 5.12 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 8.0 Hz, H-2′), 5.24 (dd, 1 H, $J_{3,2}$

9.4, $J_{3,4}$ 8.9 Hz, H-3), 5.36 (bd, 1 H, $J_{4',3'}$ 3.3 Hz, H-4'); the protons giving the m at 1.95 and 2.89 p.p.m. were exchangeable with D_2O.

Anal. Calc. for $C_{29}H_{42}O_{20}$: C, 49.01; H, 5.96. Found: C, 48.41; H, 5.81.

The component, **8** (0.57 g, 17% crude yield), with R_F 0.46 was obtained as a colorless, glassy solid and was shown by ^{1}H-n.m.r. spectroscopy to be contaminated.

*1,3-Di-*O*-acetyl-2-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-lactosyl)glycerol* (**9**). — Compound **7** (0.180 g, 0.253 mmol) was treated conventionally with acetic anhydride (5 mL) in pyridine (15 mL) at room temperature for 3 days. Column chromatography (solvent *B*) of the product gave amorphous **9** (0.167 g, 83%), $[\alpha]_D^{24}$ −8.7° (*c* 2.4, chloroform), R_F 0.36. ^{1}H-N.m.r. data ($CDCl_3$): δ 1.97 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.047 (s, 3 H, OAc), 2.051 (s, 3 H, OAc), 2.059 (s, 3 H, OAc), 2.067 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 3.62 (ddd, 1 H, $J_{5,4}$ 9.5, $J_{5,6}$ 5.4, $J_{5,6}$ 1.9 Hz, H-5), 3.78 (t, 1 H, ^3J 9.3 Hz, H-4), 3.88 (bt, 1 H, ^3J 6.8 Hz, H-5'), 4.00–4.21 (m, 8 H, H-6,6',6',1'',1'',2'',3'',3''), 4.49 (m, 1 H, H-6), 4.49 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.62 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.90 (dd, 1 H, $J_{2,3}$ 9.2, $J_{2,1}$ 8.1 Hz, H-2), 4.96 (dd, 1 H, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.4 Hz, H-3'), 5.11 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{2',1'}$ 8.0 Hz, H-2'), 5.19 (t, 1 H, ^3J 9.3 Hz, H-3), 5.35 (bd, 1 H, $J_{4',3'}$ 3.4 Hz, H-4').

Anal. Calc. for $C_{33}H_{46}O_{22}$: C, 49.88; H, 5.83. Found: C, 49.90; H, 5.83.

*2-*O*-β-Lactosylglycerol* (**10**). — To a solution of **7** (0.244 g, 0.343 mmol) in methanol (15 mL) was added methanolic 0.1M sodium methoxide (10 mL). The solution was stirred at room temperature for 6 h, then treated with Amberlite IR-120 (H^+) resin (5 mL) for 20 min, the resin was collected and washed with methanol–water (1:1, 20 mL), the combined filtrate and washings were concentrated, and the residue was dried under vacuum to give **10** (0.139 g, 97%) as a hygroscopic, colorless, glassy solid, $[\alpha]_D^{24}$ +3° (*c* 0.55, water). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.01–3.78 (m, 17 H, CHO and CH_2O), 4.20 (d, 1 H, ^3J 7.3 Hz, H-1'), 4.35 (d, 1 H, ^3J 7.9 Hz, H-1), 4.40–4.45 (m, 2 H, 2 OH), 4.51 (d, 1 H, *J* 4.6 Hz, OH), 4.55 (t, 1 H, *J* 5.9 Hz, OH), 4.66 (t, 1 H, *J* 5.0 Hz, OH), 4.69 (bs, 1 H, OH), 4.78 (d, 1 H, *J* 5.3 Hz, OH), 5.09 (d, 1 H, *J* 4.3 Hz, OH), 5.18 (d, 1 H, *J* 3.9 Hz, OH); the protons responsible for the m's at 4.40–5.18 p.p.m. were exchangeable with D_2O.

Anal. Calc. for $C_{15}H_{28}O_{13}\cdot H_2O$: C, 41.47; H, 6.96. Found: C, 41.69; H, 6.81.

*3-*O*-Benzyl-1,2-bis-*O*-(2,3,6,2',3',4',6'-hepta-*O*-acetyl-β-lactosyl)-*(R,S)*-glycerols* (**11**). — (*a*) A mixture of **5** (4.46 g, 6.37 mmol), **2** (0.511 g, 2.80 mmol), and mercury(II) cyanide (2.00 g, 7.92 mmol) in benzene–nitromethane (1:1, 25 mL) was stirred at 40° for 18 h, then diluted with toluene (50 mL), and washed sequentially with saturated aqueous sodium hydrogencarbonate (2 × 30 mL) and water (30 mL), dried ($CaCl_2$), and concentrated. Column chromatography (solvent *B*) of the residue gave **11** (1.34 g, 34%) as a colorless, glassy solid, R_F 0.29, $[\alpha]_D^{24}$ −11° (*c* 2.25, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.945 (s, 3 H, OAc), 1.959 (s, 3 H, OAc), 1.968 (s, 12 H, 4 OAc), 2.035 (s, 9 H, 3 OAc), 2.045–2.053 (m, 18 H, 6 OAc), 2.060 (s, 12 H, 4 OAc), 2.083 (s, 3 H, OAc), 2.090 (s, 3 H, OAc), 2.105 (s, 6 H, 2 OAc), 2.132 (s, 3 H, OAc), 2.152 (s, 6 H, 2 OAc), 2.155 (s, 6 H, 2 OAc), 3.42 (dd, 1 H, J_{gem} 10.1, $J_{3''',2'''}$ 6.4 Hz, H-3'''), 3.49 (dd, 1 H, J_{gem} 10.6, 6.7 $J_{3''',2'''}$ H-3'''), 3.54–3.70 (m, 8 H, H-5,5,5'',5'',1''',1''',3''',3'''), 3.783 (t, 1 H,

^3J 9.9 Hz, H-4 or H-4″), 3.792 (t, 1 H, ^3J 9.1 Hz, H-4 or H-4″), 3.809 (t, 1 H, ^3J 9.6 Hz, H-4 or H-4″), 3.814 (t, 1 H, ^3J 9.3 Hz, H-4 or H-4″), 3.85–4.17 [m, 20 H, H-6(2),5′(4),6′(8),6″(2),1‴(2),2‴(2)], 4.44 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.46–4.56 [m, 12 H, H-6(2),1′(4),6″(2) and 2 CH_2Ph)], 4.54 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.69 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1″), 4.71 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1″), 4.835 (dd, 1 H, $J_{2,3}$ 9.4, $J_{2,1}$ 7.9 Hz, H-2), 4.859 (dd, 1 H, $J_{2'',3''}$ 9.6, $J_{2'',1''}$ 8.1 Hz, H-2″), 4.863 (dd, 1 H, $J_{2'',3''}$ 9.6, $J_{2'',1''}$ 8.1 Hz, H-2″), 4.872 (dd, 1 H, $J_{2,3}$ 9.5, $J_{2,1}$ 8.0 Hz, H-2), 4.955 (dd, 2 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.7 Hz, H-3′,3′), 4.964 (dd, 2 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.6 Hz, H-3′,3′), 5.110 (dd, 2 H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 8.0 Hz, H-2′,2′), 5.112 (dd, 2 H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 7.9 Hz, H-2′,2′), 5.184 (t, 1 H, ^3J 9.4 Hz, H-3 or H-3″), 5.193 (t, 2 H, ^3J 9.4 Hz, H-3 and/or H-3″), 5.217 (t, 1 H, ^3J 9.4 Hz, H-3), 5.35 [m, 4 H, H-4′ (4)], 7.2–7.4 (10 H, m, 2 Ph). The ^{1}H-n.m.r. spectrum showed that two diastereomers were present in approximately equal amounts.

Anal. Calc. for $C_{62}H_{82}O_{37}$: C, 52.47; H, 5.82. Found: C, 52.48, H, 6.00.

(*b*) *O*-Debenzylation[44] of **6** (3.15 g, 3.53 mmol), as described above for **7** and **8**, for 4 h, followed by column chromatography (solvent *E*) of the products, gave **8** (1.15 g, 41%) as a colorless, glassy solid. ^{1}H-N.m.r. spectroscopy indicated that **8** was a 2:1 mixture of diastereomers.

A mixture of **5** (0.82 g, 1.2 mmol), **8** (0.78 g, 0.97 mmol), and mercury(II) cyanide (0.33 g, 1.3 mmol) in benzene–nitromethane (1:1, 25 mL) was stirred at 40° for 15 h, then diluted with toluene (25 mL), washed with saturated aqueous sodium hydrogencarbonate (2 × 25 mL) and water (25 mL), dried ($CaCl_2$), and concentrated. Column chromatography (solvent *C*) of the residue gave **11** (0.90 g, 65%), which ^{1}H-n.m.r. spectroscopy indicated to be a 3:1 mixture of two diastereomers. In the major diastereomer, H-1″ resonated at δ 4.71 (in $CDCl_3$), and H-1″ of the minor isomer at δ 4.69.

*1,2-Bis-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)-*(R,S)*-glycerols* (**12**). — *(a) From the 1:1 mixture of the two diastereomers of* **11**. Compound **11** (1.20 g, 0.85 mmol) was *O*-debenzylated as described for the preparation of **7**and **8** . After 4.5 h, the solids were collected and washed with methanol (40 mL), and the combined filtrate and washings were concentrated. Column chromatography (solvent *E*) of the residue gave **12** (0.804 g, 72%), isolated as a colorless, glassy solid, R_F 0.37, $[\alpha]_D^{24}$ −6° (*c* 2.1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.97 (s, 12 H, 4 OAc), 2.05–2.07 (m, 45 H, 15 OAc), 2.076 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 2.16 (s, 15 H, 5 OAc), 3.44–3.95 [m, 22 H, H-4(2),5(2),5′(4),4″(2),5″(2),1‴(4),2‴(2),3‴(4)], 4.01–4.19 [m, 12 H, H-6(2),6′(8),6″(2)], 4.45 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.46–4.61 (m, 4 H, H-6,6,6″,6″), 4.505 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.512 (d, 2 H, $J_{1',2'}$ 8.2 Hz, H-1′,1′), 4.518 (d, 2 H, $J_{1',2'}$ 8.0 Hz, H-1′,1′), 4.61 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.65 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.852 (dd, 1 H, $J_{2,3}$ 9.4, $J_{2,1}$ 8.1 Hz, H-2), 4.857 (dd, 1 H, ^3J 9.9, ^3J 7.8 Hz, H-2 or H-2″), 4.863 (dd, 1 H, $J_{2'',3''}$ 9.3, $J_{2'',1''}$ 8.2 Hz, H-2″), 4.884 (dd, 1 H, ^3J 9.3, ^3J 8.4 Hz, H-2 or H-2″), 4.964 (dd, 2 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.2 Hz, H-3′,3′), 4.972 (dd, 2 H, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.1 Hz, H-3′,3′), 5.12 [m, 4 H, H-2′(4)], 5.183 (t, 1 H, ^3J 9.3 Hz, H-3 or H-3″), 5.205 (t, 1 H, ^3J 9.0 Hz, H-3 or H-3″), 5.216 (t, 1 H, ^3J 9.4 Hz, H-3 or H-3″), 5.225 (t, 1 H, ^3J 9.2 Hz, H-3 or H-3″), 5.35 [m, 4 H, H-4′(4)].^{1}H-N.m.r. spectroscopy revealed that **12** was a 1:1 mixture of diastereomers.

Anal. Calc. for $C_{55}H_{76}O_{37}$: C, 49.70; H, 5.76. Found: C, 49.78; H, 6.06.

(b) From the 3:1 mixture of the two diastereomers of **11**. *O*-Debenzylation of **11** (0.80 g, 0.56 mmol) was performed for 2 h as described for the preparation of **7** and **8**. Column chromatography (solvent *E*) of the products gave **12** (0.36 g, 48%) isolated as a colorless, glassy solid, R_F 0.33, $[\alpha]_D^{24}$ −7° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): major diastereomer, δ 1.97 (s, 6 H, 2 OAc), 2.05–2.06 (m, 24 H, 8 OAc), 2.13 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 2.16 (s, 6 H, 2 OAc), 3.55–3.69 (m, 5 H, H-5,5″ and 3 glyceryl H's), 3.805 (t, 1 H, ^3J 9.5 Hz, H-4 or H-4″), 3.816 (t, 1 H, ^3J 9.5 Hz, H-4 or H-4″), 3.76–3.84 (m, 1 H, glyceryl H), 3.89 (m, 2 H, H-5′,5′), 3.88–3.95 (m, 1 H, glyceryl H), 4.05–4.18 [m, 6 H, H-6,6′(4),6″], 4.508 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.514 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.521 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.583 (dd, 1 H, J_{gem} 12.0, ^3J 2.0 Hz, H-6 or H-6″), 4.595 (dd, 1 H, J_{gem} 11.9, ^3J 2.0 Hz, H-6 or H-6″), 4.66 (d, 1 H, $J_{1'',2''}$ 8.1 Hz, H-1″), 4.853 (dd, 1 H, $J_{2,3}$ 9.6, $J_{2,1}$ 7.8 Hz, H-2), 4.865 (dd, 1 H, $J_{2'',3''}$ 9.6, $J_{2'',1''}$ 8.1 Hz, H-2″), 4.966 (dd, 1 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.3 Hz, H-3′), 4.974 (dd, 1 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.1 Hz, H-3′), 5.12 (dd, 2 H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 7.8 Hz, H-2′,2′), 5.205 (t, 1 H, ^3J 9.3 Hz, H-3 or H-3″), 5.217 (t, 1 H, ^3J 9.4 Hz, H-3 or H-3″), 5.35 (bd, 2 H, ^3J 3.3 Hz, H-4′,4′). ^{1}H-N.m.r. spectroscopy revealed that **12** was a 7:1 mixture of diastereomers.

Anal. Calc. for $C_{55}H_{76}O_{37}$: C, 49.70; H, 5.76. Found: C, 49.57; H, 5.62.

3-O-Acetyl-1,2-bis-O-(2,3,6,2′,3′,4′,6′-hepta-O-acetyl-β-lactosyl)-(R,S)*-glycerols* (**13**). — Compounds **12** (1:1 mixture of diastereomers) (0.743 g, 0.56 mmol) were treated conventionally with acetic anhydride (5 mL) in pyridine (15 mL) at room temperature for 22 h. Column chromatography (solvent *C*) of the product gave **13** (0.725 g, 94%), isolated as a colorless, glassy solid, $[\alpha]_D^{24}$ −12° (*c* 2.25, chloroform); R_F 0.28. ^{1}H-N.m.r. data ($CDCl_3$): δ 1.97 (s, 12 H, 4 OAc), 2.04–2.06 (m, 51 H, 17 OAc), 2.10 (s, 3 H, OAc), 2.121 (s, 3 H, OAc), 2.127 (s, 3 H, OAc), 2.137 (s, 3 H, OAc), 2.139 (s, 3 H, OAc), 2.154 (s, 12 H, 4 OAc), 3.54 (dd, 1 H, J_{gem} 11.1, $J_{1''',2'''}$ 7.1 Hz, H-1‴), 3.56–3.66 (m, 5 H, H-5,5,5″,5″,1‴), 3.78 (t, 1 H, ^3J 9.5 Hz, H-4 or H-4″), 3.81 (t, 3 H, ^3J 9.6 Hz, 1 or 2 × H-4 and 1 or 2 × H-4″), 3.81–3.95 [m, 6 H, H-5′(4),1‴(2)], 4.01–4.21 (m, 18 H, H-6(2),6′(8), 6″(2),2‴(2),3‴(4)], 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.47–4.53 (m, 2 H, H-6,6″, or H-6,6, or H-6″,6″), 4.501 (d, 2 H, $J_{1',2'}$ 7.9 Hz, H-1′,1′), 4.511 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.513 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.53 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.55 (dd, 1 H, J_{gem} 12.3, ^3J 2.1 Hz, H-6 or H-6″), 4.57 (dd, 1 H, J_{gem} 11.7, ^3J 1.8 Hz, H-6 or H-6″), 4.60 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.64 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.841 (dd, 1 H, $J_{2'',3''}$ 9.6, $J_{2'',1''}$ 7.9 Hz, H-2″), 4.846 (dd, 1 H, $J_{2,3}$ 9.6, $J_{2,1}$ 8.0 Hz, H-2), 4.867 (dd, 1 H, $J_{2'',3''}$ 9.5, $J_{2'',1''}$ 7.9 Hz, H-2″), 4.870 (dd, 1 H, $J_{2,3}$ 9.5, $J_{2,1}$ 8.0 Hz, H-2), 4.955 (dd, 1 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.9 Hz, H-3′), 4.958 (dd, 1 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.2 Hz, H-3′), 4.966 (dd, 1 H, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.1 Hz, H-3′), 4.969 (dd, 1 H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 4.1 Hz, H-3′), 5.109 (dd, 2H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 7.9 Hz, H-2′,2′), 5.112 (dd, 2 H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 7.9 Hz, H-2′,2′), 5.180 (t, 1 H, ^3J 9.4 Hz, H-3 or H-3″), 5.191 (t, 2 H, ^3J 9.6 Hz, H-3″,3″, or H-3,3″), 5.215 (t, 1 H, ^3J 9.6 Hz, H-3), 5.35 [m, 4 H, H-4′(4)]. ^{1}H-N.m.r. spectroscopy revealed that **13** was a 1:1 mixture of diastereomers.

1,2-Di-O-β-lactosyl-(R,S)*-glycerols* (**14**). — *(a) From the 1:1 mixture of the two diastereomers of* **13**. Compound **13** (0.638 g, 0.465 mmol) was *O*-deacetylated, as described for the preparation of **10**, to give **14** (0.332 g, 99%) as a colorless, glassy solid,

$[\alpha]_D^{24}$ +5° (*c* 2, water). ^{1}H-N.m.r. [$(CD_3)_2SO$]: δ 2.98–3.91 (m, 58 H, CHO and CH_2O), 4.19–4.21 (m, 4 H, 4 H-1, H-1′, or H-1″), 4.23 (d, 1 H, *J* 8.0 Hz, H-1, H-1′, or H-1″), 4.31 (d, 1 H, *J* 8.0 Hz, H-1, H-1′, or H-1″), 4.39 (d, 1 H, *J* 8.0 Hz, H-1, H-1′, or H-1″), 4.47(d, 1 H, *J* 7.7 Hz, H-1, H-1′, or H-1″), 4.44–4.59 (m, 10 H, 10 OH), 4.64–4.69 (m, 8 H, 8 OH), 4.76–4.79 (m, 4 H, 4 OH), 5.07–5.10 (m, 6 H, 6 OH), 5.12 (d, 1 H, *J* 5.0 Hz, OH), 5.16 (d, 1 H, *J* 4.3 Hz, OH). All of the OH protons were exchangeable with D_2O. ^{1}H-N.m.r. spectroscopy showed **14** to be a 1:1 mixture of diastereomers.

Anal. Calc. for $C_{27}H_{48}O_{23}\cdot 2H_2O$: C, 41.75; H, 6.75. Found: C, 42.08; H, 6.85.

(b) From the 7:1 mixture of the two diastereomers of **12**. Compound **12** (0.265 g, 0.2 mmol) was *O*-deacetylated, as described for the preparation of **10**, to give **14** (0.146 g, 99%), as a colorless solid, $[\alpha]_D^{24}$ +4° (*c* 2, water). ^{1}H-N.m.r. spectroscopy showed **14** to be a 7:1 mixture of diastereomers; for the major isomer, H-1, H-1′, and H-1″ resonated at δ 4.20 (2 H), 4.23, and 4.39, respectively.

Anal. Calc. for $C_{27}H_{48}O_{23}\cdot 2H_2O$: C, 41.75; H, 6.75. Found: C, 42.23; H, 6.85.

*1,2,3-Tris-*O*-(2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-lactosyl)glycerol* (**15**). — (*a*) A mixture of **5** (2.7 g, 3.9 mmol), **7** (0.91 g, 1.3 mmol), and mercury(II) cyanide (1.04 g, 4.12 mmol) in benzene–nitromethane (1:1, 27 mL) was stirred at 40° for 10 h, then at room temperature overnight, diluted with toluene (60 mL), washed with saturated aqueous sodium hydrogencarbonate (2 × 30 mL) and water (30 mL), dried ($CaCl_2$), and concentrated. Column chromatography (solvent *C*) of the residue gave **15** (1.19 g, 48%) as a colorless, glassy solid, R_F 0.29, R_F 0.31 (solvent *D*), $[\alpha]_D^{24}$ −10° (*c* 2.85, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.97 (s, 9 H, 3 OAc), 2.04–2.06 (m, 33 H, 11 OAc), 2.11 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.14 (s, 3 H, OAc), 2.150 (s, 3 H, OAc), 2.154 (s, 9 H, 3 OAc), 3.5–4.2 (m, 5 H, glyceryl H), 3.53–3.66 (m, 3 H, H-5,5,5″), 3.76–3.93 [m, 6 H, H-4(2),5′(3),4″], 4.06–4.17 [m, 9 H, H-6(2),6′(6),6″], 4.39 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.50–4.60 (m, 3 H, H-6,6,6″), 4.50 (d, 1 H, $J_{1',2}$ 7.9 Hz, H-1′), 4.51 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1′), 4.53 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.54 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.67 (d, 1 H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.82 (dd, 2 H, ^3J 9.8, ^3J 7.9 Hz, H-2,2″), 4.85 (dd, 1 H, ^3J 9.7, ^3J 8.1 Hz, H-2), 4.96 (dd, 1 H, ^3J 10.3, ^3J 2.9 Hz, H-3′), 4.97 (dd, 2 H, ^3J 10.4, ^3J 3.2 Hz, H-3′,3′), 5.11 (dd, 2 H, ^3J 10.6, ^3J 7.9 Hz, H-2′,2′), 5.12 (dd, 1 H, ^3J 10.3, ^3J 7.9 Hz, H-2′), 5.18 (t, 1 H, ^3J 9.4 Hz, H-3 or H-3″), 5.21 (t, 1 H, ^3J 9.4 Hz, H-3 or H-3″), 5.22 (t, 1 H, ^3J 9.5 Hz, H-3 or H-3″), 5.35 (m, 3 H, H-4′,4′,4′).

Anal. Calc. for $C_{81}H_{110}O_{54}$: C, 49.95; H, 5.69. Found: C, 49.77; H, 6.01.

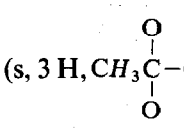
(*b*) A mixture of **5** (0.49 g, 0.70 mmol), **16** (ref. 35) (0.54 g, 0.41 mmol), and mercury(II) cyanide (0.23 g, 0.89 mmol) in benzene–nitromethane (1:1, 20 mL) was stirred at 40° for 15.5 h, then diluted with toluene (25 mL), washed with saturated aqueous sodium hydrogencarbonate (2 × 25 mL) and water (25 mL), dried($CaCl_2$), and concentrated. Column chromatography (solvent *D*) of the residue gave **15** (0.043 g, 5%) and **16** (0.28 g, 52%).

*1,2,3-Tri-*O*-β-lactosylglycerol* (**17**). — Compound **15** (0.439 g, 0.225 mmol) was *O*-deacetylated, as described for the preparation of **10**, to give **17** (0.234 g, 97%) as a colorless, glassy solid, $[\alpha]_D^{24}$ +3° (*c* 1.8, water). ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 2.96–3.66 (m, 35 H, CHO and CH_2O), 3.73–3.78 (m, 3 H, CHO and/or CH_2O), 3.88–3.97 (m, 3 H,

CHO and/or CH_2O), 4.18–4.23 (m, 3 H, H-1, H-1′, or H-1″), 4.27 (d, 1 H, *J* 7.9 Hz, H-1, H-1′, or H-1″), 4.35 (d, 1 H, *J* 7.8 Hz, H-1, H-1′, or H-1″), 4.50 (d, 1 H, *J* 7.5 Hz, H-1, H-1′, or H-1″), 4.47–4.57 (m, 6 H, 6 OH), 4.63–4.68 (m, 6 H, 6 OH), 4.75–4.77 (m, 3 H, 3 OH), 5.06–5.11 (m, 5 H, 5 OH), 5.13 (d, 1 H, *J* 5.0 Hz, OH). All of the OH protons were exchangeable with D_2O.

Anal. Calc. for $C_{39}H_{68}O_{33}\cdot 3H_2O$: C, 41.86; H, 6.67. Found: C, 42.05; H, 6.76.

3,6,2′,3′,4′,6′-Hexa-O-*acetyl-α-lactose 1,2-[1,3-bis*-O-*(2,3,6,2′,3′,4′,6′-hepta*-O-*acetyl-β-lactosyl)glycer-2-yl orthoacetate]* (**18**). — To a solution of **16** (0.711 g, 0.535 mmol) in dichloromethane (20 mL) was added 2,4,6-trimethylpyridine (0.137 g, 1.13 mmol) and silver trifluoromethanesulfonate (0.298 g, 1.16 mmol). The mixture was cooled to −40° and a solution of **5** (0.782 g, 1.12 mmol) in dichloromethane (10 mL) was added. After 30 min, the mixture was stirred at room temperature overnight, then diluted with dichloromethane (60 mL), washed with saturated aqueous sodium hydrogencarbonate (30 mL) and water (30 mL), dried ($CaCl_2$), and concentrated. Column chromatography (solvent *C*) of the residue gave compounds having R_F 0.14 and 0.22, corresponding to **16** and **18**, respectively. Compound **18** (0.303 g, 29%) was obtained as a colorless, glassy solid, $[\alpha]_D^{24}$ −7.7° (*c* 2.4, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.70



(s, 3 H, $CH_3C(=O)$), 1.97 (s, 6 H, 2 OAc), 1.98 (s, 3 H, OAc), 2.03–2.06 (m, 30 H, 10 OAc), 2.11 (s, 3 H, OAc), 2.135–2.152 (m, 15 H, 5 OAc), 2.18 (s, 3 H, OAc), 3.50–3.97 [m, 14 H, H-4(2),5(2),5′(2),4″,5″,5‴,1⁗(2),2⁗,3⁗(2)], 4.05–4.17 [m, 9 H, H-6(2),6′(4),6″,6‴(2)], 4.24 (dd, 1H, J_{gem} 11.8, $J_{6'',5''}$ 1.6 Hz, H-6″), 4.37 (dd, 1 H, $J_{2'',1''}$ 5.1, $J_{2'',3''}$ 1.8 Hz, H-2″), 4.447 (d, 1 H, ^3J 7.9 Hz, H-1), 4.454 (d, 1 H, ^3J 8.1 Hz, H-1), 4.47–4.55 (m, 2 H, H-6,6), 4.50 (d, 1 H, ^3J 8.1 Hz, H-1′), 4.53 (d, 1 H, ^3J 8.0 Hz, H-1′), 4.60 (d, 1 H, $J_{1''',2'''}$ 8.1 Hz, H-1‴), 4.85 (dd, 2 H, $J_{2,1}$ 7.9, $J_{2,3}$ 9.4 Hz, H-2,2), 4.966 (dd, 1 H, $J_{3',2'}$ 10.2, $J_{3',4'}$ 3.3 Hz, H-3′), 4.975 (dd, 1 H, $J_{3',2'}$ 10.4, $J_{3',4'}$ 3.6 Hz, H-3′), 5.016 (dd, 1 H, $J_{3''',2'''}$ 10.4, $J_{3''',4'''}$ 3.5 Hz, H-3‴), 5.107 (m, 1 H, H-2′), 5.110 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{2',1'}$ 8.1 Hz, H-2′), 5.15 (m, 1 H, H-2‴), 5.173 (t, 1 H, ^3J 9.4 Hz, H-3), 5.183 (t, 1 H, ^3J 9.4 Hz, H-3), 5.35 (bd, 2 H, *J* 3.3 Hz, H-4′,4′), 5.39 (bd, 1 H, *J* 3.2 Hz, H-4‴), 5.49 (m, 1 H, H-3″), 5.60 (d, 1 H, $J_{1'',2''}$ 5.1 Hz, H-1″).

Anal. Calc. for $C_{81}H_{110}O_{54}$: C, 49.95; H, 5.69. Found: C, 50.33; H, 5.72.

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REFERENCES

1 A. D. Elbein, in J. Preiss (Ed.), *The Biochemistry of Plants. A Comprehensive Treatise*, Vol. 3, Academic Press, New York, 1980, pp. 571–587.

2 J. L. Harwood, in P. K. Stumpf (Ed.), *The Biochemistry of Plants. A Comprehensive Treatise*, Vol. 4, Academic Press, New York, 1980, pp. 1–55; R. Douce and J. Joyard, *ibid.*, pp. 321–362; R. K. Murray, R. Narasimhan, M. Levine, M. Shirley, C. Lingwood, and H. Schachter, in C. C. Sweeley (Ed.), *Cell Surface Glycolipids*, American Chemical Society, Washington, D.C., 1980, pp. 105–125.
3 B. Wickberg, *Acta Chem. Scand.*, 12 (1958) 1183–1186.
4 N. Kataoka and A. Misaki, *Agric. Biol. Chem.*, 47 (1983) 2349–2355.
5 D. A. Wenger, J. W. Petipas, and R. A. Pieringer, *Biochemistry*, 7 (1968) 3700–3707; D. A. Wenger, K. Subba Rao, and R. A. Pieringer, *J. Biol. Chem.*, 245 (1970) 2513–2519.
6 D. E. Brundish, N. Shaw, and J. Baddiley, *Biochem. J.*, 95 (1965) 21c–22c; 97 (1965) 158–165.
7 A. D. Elbein, *Annu. Rev. Plant Physiol.*, 30 (1979) 239–272.
8 J. J. Distler and S. Roseman, *Proc. Natl. Acad. Sci. U.S.A.*, 51 (1964) 897–905.
9 W. J. Lennarz and B. Talamo, *J. Biol. Chem.*, 241 (1966) 2707–2719.
10 B. Lindberg, *Acta Chem. Scand.*, 9 (1955) 1093–1096, 1097–1099.
11 V. H. Kollman, J. L. Hanners, R. E. London, E. G. Adame, and T. E. Walker, *Carbohydr. Res.*, 73 (1979) 193–202.
12 R. H. Reed, J. C. Collins, and G. Russell, *J. Exp. Bot.*, 31 (1980) 1539–1554.
13 L. J. Borowitzka, S. Demmerle, M. A. Mackay, and R. S. Norton, *Science*, 210 (1980) 650–651.
14 R. H. Reed, *Phycologia*, 22 (1983) 351–354; *Br. Phycol. J.*, 20 (1985) 211–218.
15 D. L. Richardson, R. H. Reed, and W. D. P. Stewart, *FEMS Microbiol. Lett.*, 18 (1983) 99–102.
16 H. E. Carter, R. H. McCluer, and E. D. Slifer, *J. Am. Chem. Soc.*, 78 (1956) 3735–3738.
17 M. Kaneda, K. Mizutani, Y. Takahashi, G. Kurono, and Y. Nishikawa, *Tetrahedron Lett.*, (1974) 3937–3940; H. Shimomura, Y. Sashida, Y. Mimaki, and Y. Iitaka, *Chem. Pharm. Bull.*, 36 (1988) 2430–2446; H. Shimomura, Y. Sashida, Y. Mimaki, Y. Kudo, and K. Maeda, *ibid.*, 4841–4848.
18 W. T. Wang, F. Matsuura, H. Nunez, N. Ledonne, Jr., B. Baltzer, and C. C. Sweeley, *Glycoconjugate J.*, 1 (1984) 17–35.
19 T. Endo, K. Inoue, and S. Nojima, *J. Biochem. (Tokyo)*, 92 (1982) 953–960.
20 A. J. N. Al-Shammari and P. F. Smith, *J. Bacteriol.*, 112 (1979) 356–361.
21 T. Galliard, *Biochem. J.*, 115 (1969) 335–339.
22 D. E. Webster and S. B. Chang, *Plant Physiol.*, 44 (1969) 1523–1527; G. E. Kenny and R. M. Newton, *Ann. N.Y. Acad. Sci.*, 225 (1973) 54–61.
23 M. Kates and P. W. Deroo, *J. Lipid Res.*, 14 (1973) 438–445.
24 W. Fischer, D. Schuster, and R. A. Laine, *Biochim. Biophys. Acta*, 575 (1979) 389–398.
25 J. Thurin, T. Brodin, B. Bechtel, P.-Å. Jovall, H. Karlsson, N. Strömberg, S. Teneberg, H. O. Sjögren, and K.-A. Karlsson, *Biochim. Biophys. Acta*, 1002 (1989) 267–272, and references therein.
26 P. Scudder, A. M. Lawson, E. F. Hounsell, R. A. Carruthers, R. A. Childs, and T. Feizi, *Eur. J. Biochem.*, 168 (1987) 585–593; E. F. Hounsell, J. Feeney, and P. Scudder, *Biochem. J.*, 250 (1988) 9–13.
27 B. Lindberg, B.-G. Silvander, and C. A. Wachtmeister, *Acta Chem. Scand.*, 18 (1964) 213–216, and references therein; T. Kobayashi, S. Ito, and K. Okomoto, *Agric. Biol. Chem.*, 51 (1987) 1715–1716.
28 I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, *Abstr. Pap. Am. Chem. Soc. Meet.*, 135 (1959) 3D; *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
29 M. L. E. Bergh, G. J. M. Hooghwinkel, and D. H. van den Eijnden, *J. Biol. Chem.*, 258 (1983) 7430–7436; A. M. Wu, E. A. Kabat, B. Nilsson, D. A. Zopf, F. G. Gruezo, and J. Liao, *ibid.*, 259 (1984) 7178–7186.
30 N. K. Kochetkov, A. J. Khorlin, and A. F. Bochkov, *Tetrahedron*, 23 (1967) 693–707; L. V. Backinowsky, S. A. Nepogod'ev, A. S. Shashkov, and N. K. Kochetkov, *Carbohydr. Res.*, 138 (1985) 41–54.
31 M. Horisberger, B. A. Lewis, and F. Smith, *Carbohydr. Res.*, 23 (1972) 175–182.
32 V. Pozsgay and H. J. Jennings, *J. Org. Chem.*, 53 (1988) 4042–4052.
33 T. Ogawa, K. Beppu, and S. Nakabayashi, *Carbohydr. Res.*, 93 (1981) c6–c9.
34 T. Ogawa and K. Beppu, *Agric. Biol. Chem.*, 46 (1982) 255–262.
35 L. J. J. Hronowski, W. A. Szarek, G. W. Hay, A. Krebs, and W. T. Depew, *Carbohydr. Res.*, 190 (1989) 203–218.
36 L. J. J. Hronowski, W. A. Szarek, G. W. Hay, A. Krebs, and W. T. Depew, *Carbohydr. Res.*, 193 (1989) 91–103.
37 G. Ashwell and J. Harford, *Annu. Rev. Biochem.*, 51 (1982) 531–554.
38 R. J. Stockert and A. G. Morell, *Hepatology*, 3 (1983) 750–757; D. A. Wall, G. Wilson, and A. L. Hubbard, *Cell*, 21 (1980) 79–93.
39 G. Ashwell and A. G. Morell, *Adv. Enzymol.*, 41 (1974) 99–128.

40 Y. C. Lee, R. R. Townsend, M. R. Hardy, J. Lönngren, J. Arnap, M. Haraldsson, and H. Lönn, *J. Biol. Chem.*, 258 (1983) 199–202.
41 M. C. S. Herzig and P. H. Weigel, *Biochemistry*, 28 (1989) 600–610.
42 A. Fairbourne, G. P. Gibson, and D. W. Stephens, *J. Chem. Soc.*, (1931) 445–458.
43 B. Helferich and K. Weis, *Chem. Ber.*, 89 (1956) 314–321.
44 V. S. Rao and A. S. Perlin, *Carbohydr. Res.*, 83 (1980) 175–177.
45 A. Thompson and M. L. Wolfrom, *Methods Carbohydr. Chem.*, 2 (1963) 215–220, and references therein.
46 A. Bax, R. Freeman, and G. Morris, *J. Magn. Reson.*, 42 (1981) 164–168; R. Benn and H. Günther, *Angew. Chem. Int. Ed. Engl.*, 22 (1983) 350–380; S. L. Patt, *J. Carbohydr. Chem.*, 3 (1984) 493–511.
47 A. Bax, W. Egan, and P. Kováč, *J. Carbohydr. Chem.*, 3 (1984) 593–611.
48 J. Arnap, M. Haraldsson, and J. Lönngren, *Carbohydr. Res.*, 97 (1981) 307–313.
48 L. J. J. Hronowski, W. A. Szarek, G. W. Hay, A. Krebs, and W. T. Depew, unpublished results.
50 G. Batta and A. Liptak, *J. Am. Chem. Soc.*, 106 (1984) 248–250, and references therein.
51 R. U. Lemieux and A. R. Morgan, *Can. J. Chem.*, 43 (1965) 2199–2204.