Carbohydrate Research, 205 (1990) 133–146
Elsevier Science Publishers B.V., Amsterdam

Synthesis of a tri- and a tetra-saccharide fragment of the capsular polysaccharide of type III Group B *Streptococcus**†

Vince Pozsgay, Jean-Robert Brisson, and Harold J. Jennings
National Research Council of Canada, Division of Biological Sciences, Ottawa, Ontario, K1A 0R6 (Canada)

(Received November 11th, 1989; accepted for publication, January 27th, 1990)

ABSTRACT

Syntheses of the propyl glycosides (**1–3**) of β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc, β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc, and β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc, respectively, are reported. Reaction of allyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide under $Hg(CN)_2$ catalysis, followed by oxidative removal of the 4-methoxybenzyl group, gave allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**10**) *O*-deacetylation of which, followed by hydrogenolysis/hydrogenation, gave **1**. Reaction of **10** with β-D-glucopyranose penta-acetate and β-lactose octa-acetate, under catalysis by trimethylsilyl trifluoromethanesulfonate, and treatment of the products as for **10** gave **2** and **3**, respectively. Attempted glycosylation of **10** with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bromide under catalysis by $Hg(CN)_2$ or silver trifluoromethanesulfonate gave an orthoester. Complete assignments of the ^1H- and ^{13}C-n.m.r. spectra of **1–3** are reported together with their carbon spin–lattice relaxation times which indicate that **3** assumes a compact instead of an extended shape.

INTRODUCTION

The five distinct serotypes[1] of human pathogenic Group B *Streptococci*[2] (GBS) share a highly complex, common polysaccharide antigen[3], and each of the serotypes features a specific, capsular polysaccharide having repeating units of 5–7 monosaccharide residues[2]. We have described[4–6] the synthesis of several oligosaccharides which form parts of the common polysaccharide antigen and used them in immunochemical studies[7] which led to the establishment of the immunodominant region of the common antigen.

In order to provide structurally well-defined carbohydrate probes for the study of antigen–antibody interactions of the individual serotypes, current work is directed towards the synthesis of oligosaccharides that correspond to the specific capsular polysaccharide of a particular serotype.

Of the five distinct serotypes of GBS, the most prevalent in human disease is type III which is, amongst others, the major cause of bacterial meningitis in newborn infants[2]. The capsular polysaccharide type III is composed of the following branched pentasaccharide repeating-unit[8]:

* Dedicated to Professor Leslie Hough in the year of his 65th birthday.
† Synthetic Oligosaccharides Related to Group B Streptococcal Polysaccharides, Part 4. For Part 3, see ref. 4. Issued as NRCC 00000.

0008-6215/90/$ 03.50

3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→
4
↑
1
α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*

The incomplete asialoantigen is identical in structure to the capsular polysaccharide of type 14 *Streptococcus pneumoniae*, represented by the following branched tetrasaccharide repeating-unit[9]:

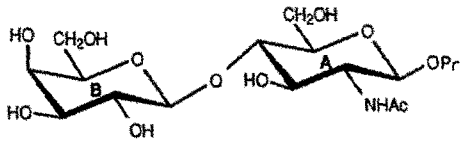
3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→
4
↑
1
β-D-Gal*p*

This tetrasaccharide has been synthesized in its reducing form[10]. Using the same strategy, the 7-methoxycarbonyl-3,6-dioxaheptyl and 8-acetamido-3,6-dioxaoctyl glycosides have also been synthesized[11]. The preparation of a fully protected, linear isomer has been described[12] and used in a polycondensation reaction. The syntheses of the propyl glycosides (**2** and **3**, respectively) of the branched tri- and tetra-saccharide fragments of type III GBS are now described together with the ^{1}H- and ^{13}C-n.m.r. data.

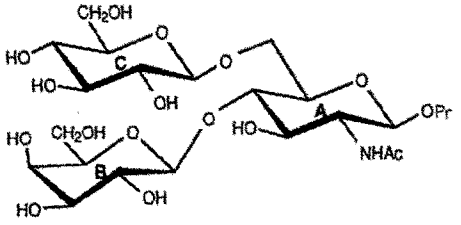
RESULTS AND DISCUSSION

The synthesis strategy involved allyl 2-acetamido-3-*O*-benzyl-2-deoxy 6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**7**) as the key intermediate, in which HO-4 was expected to be sufficiently nucleophilic to react with a galactopyranosyl donor. Removal of the 4-methoxybenzyl group would then expose HO-6 for reaction with a glucosyl and a lactosyl donor, respectively. This approach differs from that employed by other groups[10,11] which involved, as the first step, β-lactosylation at HO-6 of benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside[10] and the corresponding, 1,2-*trans* glycosides having 7-methoxycarbonyloctyl-3,6-dioxaheptyl[11] and 8-azido-3,6-dioxaoctyl aglycons[11]. The yields in this step varied[10,11] in the range 41–51%. The structures of the products were proved[10,11] by the fact that they failed to react with triphenylmethyl chloride in pyridine. As the second step, HO-4 was β-galactosylated, with[10] or without[11] prior activation, to provide the target tetrasaccharides in yields[10,11] of 31–61%.

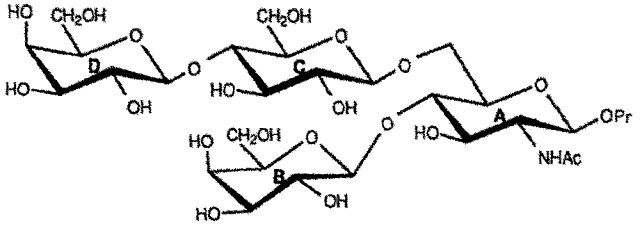
Compound **7** was obtained from allyl 2-acetamido-2-deoxy-β-D-glucopyranoside[13], which was treated with 4-methoxybenzaldehyde dimethyl acetal[14] in dry *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid to give **4** (92%). Reaction of **4** with benzyl bromide in the presence of barium oxide and barium hydroxide octahydrate in *N*,*N*-dimethylformamide gave **5** (98%). Treatment[15] of **5** for 10 min in



1



2

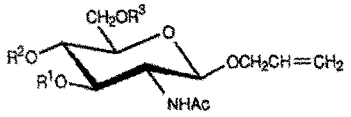


3

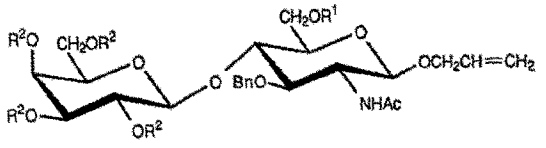
boiling methanol with pyridinium *p*-toluenesulfonate[16] removed the 4-methoxybenzylidene group to give the diol **6** (97%). Removal of the corresponding benzylidene acetal required[17] 24 h in 80% aqueous acetic acid at 50°. Compound **6** was regioselectively 4-methoxybenzylated at HO-6 with 4-methoxybenzyl chloride under the agency of dibutyltin oxide and tetrabutylammonium iodide[18] in benzene to provide **7** (75%) together with 5% of the regioisomer **8**.

Condensation of **7** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in nitromethane–toluene, under catalysis by mercury(II) cyanide, gave the expected, 1,2-*trans*-linked disaccharide **9** (65%). Selective removal of the 4-methoxybenzyl group by oxidation[19] with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in dichloromethane–water afforded allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**10**, 84%). The 1,2-*trans* configuration of the interglycosidic linkage in **10** was proved by the characteristic[20,21] $J_{H\text{-}1,C\text{-}1}$ values of 159 and 163 Hz for the C-1 resonances at 104.0 and 99.1 p.p.m., respectively.

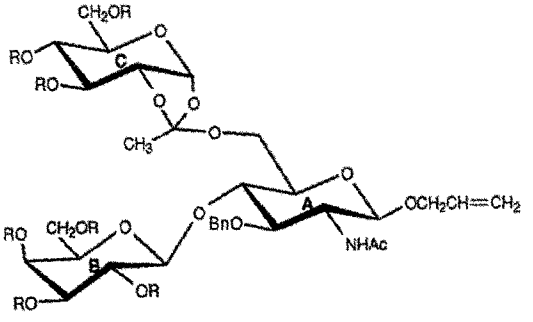
Unexpectedly, reaction of **10** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl



4 R^1=H, R^2,R^3=pMeOC_6H_4CH
5 R^1=Bn, R^2,R^3=pMeOC_6H_4CH
6 R^1=Bn, R^2=R^3=H
7 R^1=Bn, R^2=H, R^3=MBn
8 R^1=Bn, R^2=MBn, R^3=H



9 R^1=MBn, R^2=Ac
10 R^1=H, R^2=Ac
11 R^1=R^2=Ac
12 R^2=MBn, R^2=H



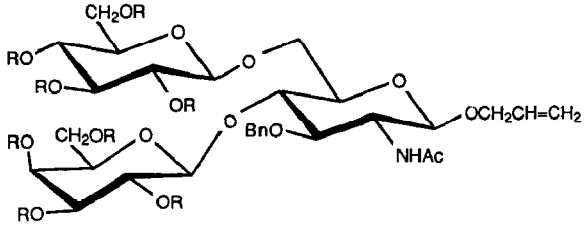
13 R=Ac
14 R=H

bromide in nitromethane–toluene in the presence of mercury(II) cyanide did not provide the trisaccharide derivative **15**, but gave the 6-acetate **11** and the orthoester **13** as the major products (experimental details not provided). Catalysis by silver trifluoromethanesulfonate, in the presence of tetramethylurea and 4 Å molecular sieves as acid scavengers in dichloromethane, also failed to promote the formation of **15**, and **13** was isolated (34%) as the major product. The structure of **13** was proved by the presence in its ^{13}C-n.m.r. spectrum of lines at 121.3 (CH_3CO_3), 97.0 ($J_{C\text{-}1,H\text{-}1}$ 183 Hz, C-1_B), and 21.5 (CH_3-CO_3), characteristic[22] of 1,2-cyclic orthoesters. Further confirmation was provid-

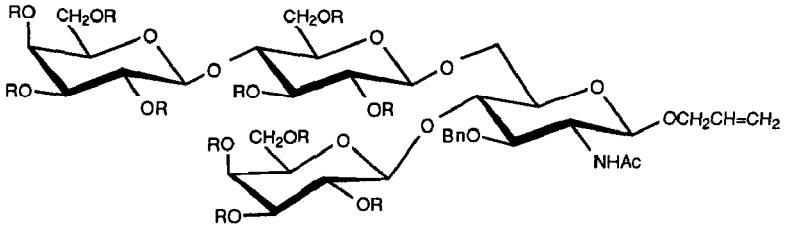
ed by the ^{13}C-n.m.r. spectrum of **14** (see Experimental) obtained from **13** by Zemplén *O*-deacetylation, and by its sensitivity to 0.01M hydrochloric acid. This finding was surprising, since the primary hydroxyl groups of several 2-acetamido-2-deoxy-D-glucosides were shown[10,11] to be sufficiently nucleophilic in glycosylation reactions using either the orthoester[10], the modified Koenigs–Knorr[11], or the trichloroacetimidate[11] methods.

The trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed reaction[23] between **10** and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose was examined next. It was assumed that an orthoester, even if formed initially, would be isomerized *in situ* by the Lewis acid-type catalyst and, indeed, D-glucopyranose penta-acetate reacted with **10** to provide the target trisaccharide **15** and no orthoester **13** could be isolated. In this reaction, the acetylated acceptor **11** was also formed as the major side-product (17% isolated). This type of side reaction in TMSOTf-catalyzed glycosylation reactions is well documented[5,24]. Similarly, the reaction of **10** with lactose octa-acetate under TMSOTf-catalysis gave the fully protected tetrasaccharide glycoside **17** and no orthoester was isolated. The yields of **15** (39%) and **17** (46%) in the above reactions are comparable to those (41% and 51%) reported[11] for lactosylation at HO-6 of 2-acetamido-2-deoxy-D-glucose derivatives having both HO-4 and HO-6 unsubstituted.

Conventional removal (NaOMe/MeOH then H_2–Pd/C) of the *O*-protecting groups from **9**, **15**, and **17** gave the propyl glycosides **1**–**3**, respectively, in which the



15 R=Ac
16 R=H



17 R=Ac
18 R=H

1,2-*trans* configuration of the interglycosidic linkages was proved by the characteristic $J_{H\text{-}1,H\text{-}2}$ values (7.8–8.0 Hz)[25] for **1–3** (Table I) and the $J_{C\text{-}1,H\text{-}1}$ values (160–163 Hz)[20,21] for **1** and **2** (Table II).

Combined use of 1D and 2D ^{1}H- and ^{13}C-n.m.r. spectroscopic methods [^{1}H–^{1}H COSY[26], relay-COSY[27], *J*-resolved spectroscopy, 1D, heteronuclear shift-correlation spectroscopy (CHORTLE[28])] permitted unambiguous assignment of ^{1}H- and ^{13}C-n.m.r. spectra of **1–3** (Tables I and II).

TABLE I

^{1}H-N.m.r. data[a] for **1–3**

Chemical shifts[b] *(p.p.m.)*				*Coupling constants*[c] *(Hz)*			
H atom[d]	**1**	**2**	**3**[e]	$J_{H,H}$[c]	**1**	**2**	**3**[e]
1_A	4.532	4.546	4.545	$1_A,2_A$	8.1	8.3	8.2
2_A	3.72	3.74	3.741	$2_A,3_A$	n.d.[d]	10.7	10.7
3_A	3.69	3.685	3.688	$3_A,4_A$	n.d.	8.4	8.6
4_A	3.70	3.828	3.83	$4_A,5_A$	n.d.	10.1	n.d.
5_A	3.580	3.720	3.72	$5_A,6_A$	2.2	1.8	2.0
6_A	3.825	4.289	4.287	$5_A,6'_A$	5.2	4.9	4.3
$6'_A$	3.980	3.953	3.959	$6_A,6'_A$	12.3	11.2	11.4
1_B	4.469	4.530	4.533	$1_B,2_B$	7.8	8.0	7.8
2_B	3.536	3.548	3.53	$2_B,3_B$	10.0	10.0	10.0
3_B	3.664	3.665	3.668	$3_B,4_B$	3.5	3.4	3.4
4_B	3.922	3.923	3.922	$4_B,5_B$	0.5	1.4	0.9
5_B	3.72	3.70	3.704	$5_B,6_B$	n.d.	n.d.	4.0
6_B		3.72	3.74	$5_B,6'_B$	n.d.	8.4	8.1
$6'_B$		3.78	3.79	$6_B,6'_B$	n.d.	12.0	12.3
1_C		4.527	4.556	$1_C,2_C$		7.9	8.0
2_C		3.332	3.370	$2_C,3_C$		9.4	9.6
3_C		3.505	3.655	$3_C,4_C$		8.6	n.d.
4_C		3.402	3.67	$4_C,5_C$		9.8	n.d.
5_C		3.462	3.595	$5_C,6_C$		2.4	2.4
6_C		3.921	3.984	$5_C,6'_C$		6.2	5.0
$6'_C$		3.73	3.820	$6_C,6'_C$		12.5	12.4
1_D			4.454	$1_D,2_D$			7.9
2_D			3.540	$2_D,3_D$			10.0
3_D			3.661	$3_D,4_D$			3.5
4_D			3.929	$4_D,5_D$			1.0
5_D			3.728	$5_D,6_D$			4.0
6_D			3.73	$5_D,6'_D$			8.2
$6'_D$			3.77	$6_D,6'_D$			11.6
CH_3CO	2.030	2.030	2.033				
$CH_3CH_2CH_2$	0.870	0.864	0.864				
$CH_3CH_2CH_2$	1.550	1.550	1.552				
$CH_2CH_2CH_2$	3.55	3.55	3.55				
	3.85	3.84	3.84				

[a] At 500 MHz, in D_2O, at 300 K; see Experimental for details. [b] Values obtained by first-order analysis. [c] A–D refer to the monosaccharide units as shown in the formulae. [d] Not determined. [e] Assignments for residues B and D may be interchanged.

Carbon spin–lattice relaxation time (T_1) values for **1–3** were measured in order to obtain information on the motional behaviour of the individual units in the glycoside **3**. Even though quantitative interpretation of carbon T_1 values is not without difficulty[29], such data can be used to characterize qualitatively the relative mobilities of sugar residues in oligosaccharides. For example, in a trisaccharide-containing steroid glycoside, the non-reducing, terminal glycosyl unit, farthest from the bulky, anchoring aglycon, had the longest T_1 relaxation times, indicating the least restricted motion among the sugar residues[30]. The non-reducing, terminal galactosyl units in pentasaccharides exhibit[31] carbon T_1 relaxation times longer than those in the middle of the chain, which was interpreted as an indication of their greater mobility.

TABLE II

^{13}C-N.m.r. chemical shifts[a,b] for **1–3** and $J_{C\text{-}1,H\text{-}1}$ values[c] for **1** and **2**

Carbon atom[d]	**1**	**2**	**3**[e]
1_A	101.83 (160)	101.97 (156)	101.98
2_A	55.95	55.94	55.96
3_A	73.34	73.32	73.20
4_A	79.33	78.74	78.72
5_A	75.60	74.35	74.34
6_A	60.91	68.2	68.27
1_B	103.71 (160)	103.59 (163)	103.79
2_B	71.80	71.78	71.78
3_B	73.29	73.28	73.33[f]
4_B	69.37	69.41	69.42[g]
5_B	76.17	76.1	76.18[h]
6_B	61.84	61.89	61.84
1_C		103.36 (160)	103.21
2_C		73.80	73.49
3_C		76.48	75.11
4_C		70.50	79.27
5_C		76.67	75.54
6_C		61.6	60.90
1_D			103.58
2_D			71.78
3_D			73.36[f]
4_D			69.39[g]
5_D			76.09[h]
6_D			61.84
CH_3CO	22.98	22.97	22.98
CH_3CO	175.34	175.36	175.34
$CH_3CH_2CH_2$	10.43	10.41	10.41
$CH_3CH_2CH_2$	22.90	22.92	22.92
$CH_3CH_2CH_2$	73.16	73.35	73.35

[a] At 300 K, at 125 MHz, in D_2O: see Experimental for details. [b] Assignments are based on 1D, ^{13}C–^{1}H correlation spectroscopy (CHORTLE[28]) using the ^{1}H-n.m.r. data in Table I: See Experimental for other details. [c] Values obtained by first-order analysis. [d] A–D refer to the monosaccharide units shown in the formulae. [e] Assignments for residues B and D may be interchanged. [f,g,h] Identical superscripts indicate interchangeable assignments.

TABLE III

^{13}C NT_1 values[a] for **1–3**

Carbon atom[b]	**1**	**2**	**3**[c]
1_A	0.43	0.35	0.31
2_A	0.40	0.34	0.29
3_A	0.46	0.40	0.38
4_A	0.39	0.33	0.25
5_A	0.40	0.31	0.27
6_A	0.44	0.35	0.27
1_B	0.45	0.41	0.34
2_B	0.43	0.41	–
3_B	0.41	0.34	0.35
4_B	0.40	0.34	0.31
5_B	0.38	0.38	0.32
6_B	0.62	0.54	0.49
1_C		0.39	0.30
2_C		0.43	0.31
3_C		0.46	0.32
4_C		0.41	0.28
5_C		0.40	0.31
6_C		0.44	0.32
1_D			0.34
2_D			–
3_D			0.29
4_D			0.30
5_D			0.31
6_D			0.49

[a] In s. [b] For designators A–D, see formulae **1–3**. [c] Values given for units B and D may be interchanged.

TABLE IV

Average ^{13}C NT_1 values[a] for **1–3**

Sugar unit[b]	**1**	**2**	**3**
A	0.42 (0.42)	0.34 (0.34)	0.30 (0.30)
B[c]	0.41 (0.45)	0.38 (0.40)	0.33 (0.36)
C		0.42 (0.42)	0.30 (0.31)
D[c]			0.31 (0.35)

[a] In s; the values represent averages for C-1/5; the values in parentheses represent averages for C-1/6. [b] For designators A–D, see formulae **1–3**. [c] The values for compound **3** may be interchanged.

The carbon T_1 values (Tables III and IV) for **1–3** indicate that the overall flexibility decreases with increasing molecular size. For **2**, the average NT_1 value (Table IV) for unit A is smaller than those for units B and C, indicating a relatively restricted segmental motion for the GlcNAc residue. In **2**, the motion of the Glc residue (unit C) is less restricted than that of the Gal residue (unit B) probably due to its (1→6) instead of

(1→4) linkage. The average NT_1 values (Table III) for **3** exhibit a further decrease relative to those for **2**, the decrease being most pronounced for the Glc residue (unit C), which constitutes further proof for the assignments in Table II. Although the average NT_1 values for the terminal, non-reducing residues (units B and D) are somewhat larger than those for units A and C, it is unlikely that the small differences (0.03 and 0.01 s, respectively) represent significant variations in the segmental motion of the individual units.

EXPERIMENTAL

Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at 20° with a Perkin–Elmer Type 141 polarimeter. Column chromatography was performed on Silica Gel 60 (Merck, 0.040–0.063 mm). The eluates in Sephadex chromatography were analyzed with a Waters R-403 differential refractometer. All reagents and solvents were of commercial grade. Solvents used for chromatography were distilled prior to use. Glycosylation reactions were performed under argon in anhydrous solvents. Molecular sieves were activated for 2 h at 150–200° prior to use. 2,3,4,6-Tetra-*O*-acetyl-α-D-galacto- and -gluco-pyranosyl bromide, D-glucose penta-acetate, and lactose octa-acetate were commercial products and were used without purification. The ^{1}H- (500 MHz) and ^{13}C-n.m.r. (125 MHz) spectra for **1–3** were recorded with a Bruker AM-500 instrument at 300 K and the ^{13}C-n.m.r. (50 MHz) spectra for **7–18** were recorded with a Bruker AM-200 instrument at 300 K. Internal references: CH_3 signal of acetone (2.225 for ^{1}H and 31.07 p.p.m. for ^{13}C for solutions in D_2O), $CDCl_3$ (77.0 p.p.m. for ^{13}C for solutions in $CDCl_3$), CD_3OD (49.9 p.p.m. for ^{13}C for solutions in CD_3OD). Proton homonuclear shift-correlated 2D-n.m.r. experiments (COSY[26], relay-COSY[27]) were performed by using standard pulse sequences provided by Bruker (DISB87). Heteronuclear ^{13}C–^{1}H shift-correlation spectroscopy was performed by the CHORTLE technique[28]. Solutions of **1–3** in 99.5% D_2O were freeze-dried twice before n.m.r. measurements in 99.95% D_2O.

4-Methoxybenzaldehyde dimethyl acetal. — To an equimolar, stirred mixture of 4-methoxybenzaldehyde and trimethyl orthoformate was added a catalytic amount of *p*-toluenesulfonic acid. An exothermic reaction started immediately and the colour changed from yellow to violet. Stirring was continued under vacuum (30–50 mmHg) for 1 h. The product thus obtained was used without purification. For n.m.r. data, see ref. 14.

Allyl 2-acetamido-2-deoxy-4,6-O-(4-methoxybenzylidene)-β-D-glucopyranoside (**4**). — A mixture of allyl 2-acetamido-2-deoxy-β-D-glucopyranoside (26 g, 0.1 mol), 4-methoxybenzaldehyde dimethyl acetal (80 mL), *p*-toluenesulfonic acid (200 mg), and *N*,*N*-dimethylformamide (100 mL) was kept at room temperature for 24 h. Triethylamine (1 mL) was added, the solution was poured into a well-stirred mixture of water (1.5 L) and hexane (300 mL), and the precipitate was collected and washed several times with water and hexane to give **4** (34.8 g, 92.2%), m.p. 282–285° (dec), $[\alpha]_D$ −54° (*c* 1.5, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{19}H_{25}NO_7$ (379.39): C, 60.15; H, 6.59; N, 3.69. Found: C, 60.24; H, 6.49; N, 3.75.

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-*D*-glucopyranoside* (**5**). — A mixture of **4** (36.0 g, 95 mmol), benzyl bromide (25 mL, 36 g, 210 mmol), barium oxide (105 g), barium hydroxide octahydrate (32 g), and *N,N*-dimethylformamide (250 mL) was shaken vigorously for 12 h at room temperature, then filtered. The filter cake was extracted with 1,2-dichloroethane (2 × 600 mL) at reflux temperature, and the combined filtrate and washings were concentrated under vacuum to afford a solid which was washed thoroughly with hexane and water to give **5** (43.9 g, 98.5%), m.p. 262–264°, $[\alpha]_D$ −40° (*c* 1.2, *N,N*-dimethylformamide).

Anal. Calc. for $C_{26}H_{31}NO_7$ (469.51): C, 66.57; H, 6.65; N, 2.98. Found: C, 66.48; H, 6.75; N, 3.04.

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**6**). — A mixture of **5** (10.0 g, 21.3 mmol), pyridinium *p*-toluenesulfonate (1.0 g), and methanol (800 mL) was stirred under reflux until dissolution was complete (~10 min). T.l.c. (6:1 chloroform–methanol) showed complete conversion of **5** into the product. Concentration gave a solid which was washed with ether to give **6** (8.0 g, 97.2%), m.p. 213–214°, $[\alpha]_D$ −29° (*c* 0.5, ethanol); lit.[17] m.p. 188–189°, $[\alpha]_D$ −11.4° (methanol); lit.[32] m.p. 190–191°, $[\alpha]_D$ −4° (pyridine).

Anal. Calc. for $C_{18}H_{25}NO_6$ (351.38): C, 61.52; H, 7.11; N, 3.99. Found: C, 61.75; H, 7.16; N, 4.08.

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-* (**7**) *and -4-*O*-(4-methoxybenzyl)-β-*D*-glucopyranoside* (**8**). — A mixture of **6** (12.0 g, 34.2 mmol), dibutyltin oxide (8.55 g, 34.3 mmol), and benzene (500 mL) was stirred under reflux for 3 h, using a Dean–Stark trap, and ~200 mL of benzene was distilled. The stirred solution was cooled to ~50°, tetrabutylammonium iodide (14.5 g, 39.2 mmol) and 4-methoxybenzyl chloride (8.5 g, 7.35 mL, 54.3 mmol) were added, and stirring was continued for 6 h at 45–50°. The mixture was concentrated, and a solution of the syrupy residue in chloroform (200 mL) was extracted with water (3 × 100 mL), then concentrated. Chromatography (1:1 hexane–ethyl acetate, then ethyl acetate) of the residue gave **7** (12.0 g, 74.5%), m.p. 141–142°, $[\alpha]_D$ −10° (*c* 0.4, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.5 (C=O), 159.0 (C-4 MeOBn), 138.5 (C quat., Bn), 133.9 (CH= allyl), 129.7–127.7 (aromatic carbons), 117.3 (CH_2= allyl), 113.7 (C-3, 5MeOBn), 99.1 (C-1), 80.3 (C-3), 73.9, 73.7, 73.2 (2×), 70.2, 69.8 (C-4,5,6, CH_2 Bn, CH_2 MeOBn, CH_2 allyl), 56.6 (C-2), 55.2 (OCH_3), 23.5 (CH_3CO).

Anal. Calc. for $C_{26}H_{33}NO_7$ (471.52): C, 66.22; H, 7.05; N, 2.97. Found: 66.32; H, 7.14; N, 3.04.

Subsequent elution gave **8** (0.85 g, 5.3%), m.p. 139–141°, $[\alpha]_D$ −2.5° (*c* 0.7, chloroform).

Anal. Found: C, 66.51, H, 7.12; N, 3.00.

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**9**). — A mixture of **7** (1.8 g, 3.8 mmol), 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (3.2 g, 7.8 mmol),

mercury(II) cyanide (1.8 g, 7.1 mmol), 4 Å molecular sieves (~2 g), nitromethane (10 mL), and toluene (10 mL) was stirred at room temperature for 4 h. T.l.c. (3:1 ethyl acetate–hexane) then revealed the complete disappearance of **7**. The mixture was filtered and concentrated, and a solution of the residue in chloroform (50 mL) was extracted with aqueous KI, then with water, and concentrated. Chromatography (2:1 ethyl acetate–hexane) of the residue gave **9**, isolated as a glass (1.98 g, 64.7%), $[\alpha]_D$ −11° (*c* 0.7, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.3–169.9 (C=O), 160.5 (C-4 MeOBn), 138.5 (C quat. Bn), 133.8 (CH= allyl), 129.5–127.5 (aromatic carbons), 117.4 (CH_2= allyl), 114.0 (C-3,5 MeOBn), 100.9, 99.6 (C-1_A, 1_B), 78.7 (C-3_A), 76.4 (C-4_A), 69.5 (C-6_A), 60.9 (C-6_B), 54.9 (C-2_A), 23.5 (CH_3CON), 20.7, 20.6, 20.3(2×) (4 CH_3CO).

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranoside* (**10**). — A mixture of **9** (1.9 g, 2.36 mmol), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.63 g, 2.77 mmol), dichloromethane (25 mL), and water (5 mL) was stirred for 3 h at room temperature, then extracted with aqueous $NaHCO_3$, dried, and concentrated. Chromatography (100:1 then 100:2 chloroform–methanol) of the residue gave **10** as an amorphous solid (1.35 g, 83.8%), $[\alpha]_D$ −10° (*c* 0.9, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.3, 170.2, 170.0, 169.9 (4 CH_3CO), 169.5 (CH_3CON), 138.5 (C quat. Bn), 133.8 [CH= allyl), 128.2–127.5 (aromatic carbons), 117.3 (CH_2= allyl), 104.0 ($J_{C\text{-}1,H\text{-}1}$ 159 Hz), 99.1 ($J_{C\text{-}1,H\text{-}1}$ 163 Hz) (C-1_A,1_B), 77.6 (C-3_A), 76.2 (C-4_A), 75.0, 73.7, 70.7 (C-5_A, CH_2 Bn, CH_2 allyl), 70.5 (C-5_B), 70.0 (C-3_B), 69.4 (C-2_B), 66.8 (C-4_B), 60.9, 60.6 (C-6_A,6_B), 55.3 (C-2_A), 20.3 (CH_3CON), 20.7, 20.5 (3×) (4 CH_3CO).

*3,4,6-Tri-*O*-acetyl-α-*D*-glucopyranose 1,2-{[allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosid-6-yl] orthoacetate}* (**13**). — A stirred mixture of **10** (50 mg, 0.073 mmol), 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide (40 mg, 0.097 mmol), tetramethylurea (58 mg, 60 mL; 0.5 mmol), 4 Å molecular sieves (200 mg), and dichloromethane (2 mL) was stirred for 1 h at 20°, then cooled to 0°, and treated with silver trifluoromethanesulfonate (30 mg, 0.11 mmol), and stirring was continued at 0° for 24 h. Solids were collected on Celite 545 and washed with dichloromethane, and the combined filtrate and washings were washed with ice-cold, aqueous $NaHCO_3$ and concentrated. Chromatography (1:1 ethyl acetate–hexane) of the residue gave syrupy **13** (25 mg, 34%) as the main product, $[\alpha]_D$ −11° (*c* 0.3, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.2–169.0 (C=O), 133.9 (CH= allyl), 128.3, 127.7 (aromatic carbons), 121.3 (C quat. orthoester), 117.1 (CH_2= allyl), 100.0 ($J_{C\text{-}1,H\text{-}1}$ 163 Hz, C-1_A), 99.0 ($J_{C\text{-}1,H\text{-}1}$ 156 Hz, C-1_B), 97.0 ($J_{C\text{-}1,H\text{-}1}$ 183 Hz, C-1_C), 63.0, 62.5, 60.8 (C-6_A,6_B,6_C), 50.9 (C-2_A), 23.3 (CH_3CON), 21.5 (CH_3 (orthoester), 20.8, 20.7 (CH_3CO).

*α-*D*-Glucopyranose 1,2-{[allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranosid-6-yl] orthoacetate}* (**14**). — A solution of **13** (20 mg) in anhydrous methanol (3 mL) was treated with a catalytic amount of sodium methoxide at room temperature for 24 h. The solution was neutralized with Dowex 50W (H^+) resin, filtered, and concentrated to give **14** as an unstable glassy solid (12 mg, 89.5%). ^{13}C-N.m.r. data (D_2O): δ 174.0 (C=O), 138.1 (C quat. Bn), 133.8 CH= allyl), 128.9,

128.5 (aromatic carbons), 121.6 (C quat. orthoester), 118.3 (CH_2= allyl), 103.3 ($C\text{-}1_B$), 100.3 ($C\text{-}1_A$), 97.8 ($C\text{-}1_C$), 80.6 ($C\text{-}3_A$), 77.8 ($C\text{-}4_A$), 62.3, 61.6 (2×) ($C\text{-}6_A$,6_B,6_C), 54.8 ($C\text{-}2_A$), 22.5 (CH_3CON), 21.9 (CH_3 orthoester).

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**15**). — A mixture of **10** (200 mg, 0.29 mmol), 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (200 mg, 09.51 mmol), 4 Å molecular sieves (~500 mg), and anhydrous dichloromethane (5 mL) was stirred for 1 h at room temperature. Trimethylsilyl trifluoromethanesulfonate (200 μL) was added and stirring was continued for 24 h. Triethylamine (0.5 mL) was added, solids were collected and washed with dichloromethane, and the combined filtrate and washings were concentrated *in vacuo*. Chromatography (1:1 ethyl acetate–hexane) of the residue gave, first, allyl 2-acetamido-6-*O*-acetyl-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (**11**), isolated as a syrup (35 mg, 16.5%), $[\alpha]_D$ −2°, (*c* 0.2, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.1 (C=O), 130.7 (CH= allyl), 128.3, 127.6 (aromatic carbons), 117.3 (CH_2= allyl), 100.2, 98.9 ($C\text{-}1_A$,1_B), 76.0, 75.6 ($C\text{-}3_A$,4_A), 72.9, 72.5, 70.9, 70.5, 69.5, 69.1, 66.7 ($C\text{-}5_A$,$C\text{-}2_B$,3_B,4_B,5_B, CH_2 Bn, CH_2= allyl), 63.7 ($C\text{-}6_A$), 60.8 ($C\text{-}6_B$), 51.3 ($C\text{-}2_A$), 23.2 (CH_3CON), 20.7, 20.6 (4×) (5 CH_3CO).

Eluted second was **15** (115 mg, 38.7%), $[\alpha]_D$ −29° (*c* 0.3, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 171–169 (C=O), 133.8 (CH= allyl), 128.3, 127.6 (aromatic carbons), 116.9 (CH_2= allyl), 101.1, 99.8, 99.0 ($C\text{-}1_A$,1_B,1_C), 68.3 ($C\text{-}6_A$), 61.7, 60.7 ($C\text{-}6_B$,6_C), 49.6 ($C\text{-}2_A$), 23.1 (CH_3CON), 20.6 (CH_3CO).

*Allyl 2-acetamido-3-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-6-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**17**). — A mixture of **10** (400 mg, 0.58 mmol), lactose octa-acetate (600 mg, 0.88 mmol), 4 Å molecular sieves (1.5 g), and dichloromethane (10 mL) was stirred at room temperature for 1 h. Trimethylsilyl trifluoromethanesulfonate (300 μL) was added and stirring was continued for 24 h. Work-up, as described for **15**, gave **17** as an amorphous solid (350 mg, 45.8%), $[\alpha]_D$ −24° (*c* 0.9, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.0–168.8 (C=O), 138.0 (C quat. Bn), 133.6 (CH= allyl), 128.0, 127.4 (aromatic carbons), 116.7 (CH_2= allyl), 100.8 ($J_{C\text{-}1,H\text{-}1}$ 160 Hz), 100.5 ($J_{C\text{-}1,H\text{-}1}$ 161 Hz), 99.6 ($J_{C\text{-}1,H\text{-}1}$ 161 Hz), 98.8 ($J_{C\text{-}1,H\text{-}1}$ 164 Hz) ($C\text{-}1_A$,1_B,1_C,1_D), 68.9 ($C\text{-}6_A$), 61.7, 60.6, 60.4 ($C\text{-}6_B$,6_C,6_D), 50.3 ($C\text{-}2_A$), 22.9 (CH_3CON), 20.7–20.3 (CH_3CO).

*Propyl 2-acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**1**). — A solution of **9** (170 mg, 0.21 mmol) in anhydrous methanol (10 mL) was treated with a catalytic amount of sodium methoxide at room temperature for 24 h. The solution was neutralized with Dowex 50W (H^+) resin, filtered, and concentrated to give **12** (128 mg, 95%) as a syrup. ^{13}C-N.m.r. data ($CDCl_3$): δ 160.2 (C-4 MBn), 135.5 (CH= allyl), 130.6–128.7 (aromatic carbons), 117.1 (CH_2= allyl), 114.8 (C-3,5 MeOBn), 104.1, 101.7 ($C\text{-}1_A$, $C\text{-}1_B$), 82.3 ($C\text{-}3_A$), 77.3 ($C\text{-}4_A$), 76.4, 74.8, 70.8 (3 CH_2 allyl, Bn, MeOBn), 76.1, 74.7, 73.0 (2×), 70.3 ($C\text{-}5_A$,2_B,3_B,4_B,5_B), 67.0 ($C\text{-}6_A$), 56.2 (CH_3O), 55.2 ($C\text{-}2_A$), 23.0 (CH_3CON).

A mixture of **12** (110 mg), ethanol (10 mL), water (3 mL), glacial acetic acid (0.5 mL), and 10% Pd/C (200 mg) was stirred for 24 h under hydrogen at 20° under atmospheric pressure, then filtered, and concentrated. The amorphous residue was eluted from Sephadex G-10 with water. Fractions that contained **1** were combined and freeze-dried to give **1** as an amorphous solid (53 mg, 72%), $[\alpha]_D$ −9° (*c* 0.2, water). For the n.m.r. data, see Tables I–IV.

*Propyl 2-acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-6-*O*-(β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**2**). — Compound **15** was *O*-deacetylated, as described for **9**, to give **16** as a glass (36 mg, 93%). ^{13}C-N.m.r. data (D_2O): δ 174.9 (C=O), 138.4 (C quat. Bn), 134.1 (CH= allyl), 129.4, 129.1 (aromatic carbons), 119.0 (CH_2= allyl), 103.7, 103.3, 100.9 (C-1_A,1_B,1_C), 80.9 (C-3_A), 67.7 (C-6_A), 62.1, 61.6 (C-6_B,6_C), 55.3 (C-2_A), 23.0 (CH_3CO).

Hydrogenolysis of **16**, as described for **12**, followed by purification on Sephadex G-15, gave **2** as an amorphous solid (77%), $[\alpha]_D$ −12° (*c* 0.2, water). For the n.m.r. data, see Tables IIV.

*Propyl 2-acetamido-2-deoxy-4-*O*-(β-*D*-galactopyranosyl)-6-*O*-[4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-glucopyranoside* (**3**). — Compound **17** was *O*-deacetylated, as described for **9**, to give **18** (95%). ^{13}C-N.m.r. data (D_2O): δ 171.8 (C=O), 138.3 (C quat. Bn), 134.0 (CH= allyl), 129.3, 129.0 (aromatic carbons), 118.9 (CH_2= allyl), 103.7, 103.6, 103.0, 100.8 (C-1_A,1_B,1_C,1_D), 80.0 (C-3_A), 67.7 (C-6_A), 62.0, 61.8, 60.8 (C-6_B,6_C,6_D), 55.2 (C-2_A), 22.9 (CH_3CO).

Hydrogenolysis of **18**, as described for **12**, gave **3** as an amorphous solid, $[\alpha]_D$ −9° (*c* 0.1, water). For the n.m.r. data, see Tables I–IV.

REFERENCES

1 J. L. DiFabio, F. Michon, J.-R. Brisson, H. J. Jennings, M. R. Wessels, V.-J. Benedi, and D. L. Kasper, *Can. J. Chem.*, 67 (1989) 877–882.
2 H. J. Jennings, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 155–208.
3 F. Michon, E. Katzenellenbogen, D. L. Kasper, and H. J. Jennings, *Biochemistry*, 26 (1987) 476–486.
4 V. Pozsgay and H. J. Jennings, *J. Org. Chem.*, 53 (1988) 4042–4052.
5 V. Pozsgay, J.-R. Brisson, and H. J. Jennings, *Can. J. Chem.*, 65 (1987) 2764–2769.
6 V. Pozsgay and H. J. Jennings, *Carbohydr. Res.*, 179 (1988) 61–75.
7 F. Michon, R. Charlifour, A. Gamian, R. Feldman, D. L. Kasper, V. Pozsgay, M. Wessels and H. J. Jennings, unpublished work.
8 M. R. Wessels, V. Pozsgay, D. L. Kasper, and H. J. Jennings, *J. Biol. Chem.*, 262 (1987) 8262–8267.
9 B. Lindberg, J. Lönngren, and D. A. Powell, *Carbohydr. Res.*, 58 (1977) 177–186.
10 S. E. Zurabyan, V. A. Nesmeyanov, and A. Ya. Khorlin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1976) 1421–1428.
11 P.-H. Avram-Zollo and P. Sinaÿ, *Carbohydr. Res.*, 150 (1986) 199–212.
12 N. K. Kochetkov, N. E. Nifant'ev, and L. V. Backinowsky, *Tetrahedron*, 43 (1987) 3109–3121.
13 R. T. Lee and Y. C. Lee, *Carbohydr. Res.*, 37 (1974) 193–201.
14 M. Kloosterman, T. Slaghek, J. P. G. Hermans, and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 103 (1984) 335–341.
15 R. Sterzycki, *Synthesis*, (1979) 724-725.
16 N. Miyashita, A. Yoshikoshi, and P. A. Grieco, *J. Org. Chem.*, 42 (1977) 3772–3774.
17 P. Rollin and P. Sinaÿ. *J. Chem. Soc., Perkin Trans. 1*, (1977) 2513–2517.
18 S. David, A. Thieffry, and A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, (1981) 1796–1801.
19 Y. Oikawa, T. Yoshioko, and O. Yonemitsu, *Tetrahedron Lett.*, 23 (1982) 885–888.

20 A. S. Perlin and B. Casu, *Tetrahedron Lett.*, (1969) 2921–2924.
21 K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
22 V. Pozsgay and A. Neszmelyi, *Carbohydr. Res.*, 80 (1980) 196–202.
23 H. Paulsen and M. Paal, *Carbohydr. Res.*, 135 (1984) 53–69.
24 P. Kovac, *Carbohydr. Res.*, 153 (1986) 237–251.
25 K. Bock and H. Thøgersen, *Annu. Rep. NMR Spectrosc.*, 13 (1983) 1–57.
26 W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
27 G. Eich, G. Bodenhausen, and R. R. Ernst, *J. Am. Chem. Soc.*, 104 (1982) 3731–3732.
28 G. A. Pearson, *J. Magn. Reson.*, 64 (1985) 487–500.
29 P. Dais, *Carbohydr. Res.*, 160 (1987) 73–93.
30 A. Neszmelyi, K. Tori, and G. Lukacs, *J. Chem. Soc., Chem. Commun.*, (1977) 613–614.
31 H. Paulsen, T. Peters, V. Sinnwell, R. Lebuhn, and B. Meyer, *Liebigs Ann. Chem.*, (1985) 489–509.
32 M. A. E. Shaban, V. N. Reinhold, and R. W. Jeanloz, *Carbohydr. Res.*, 59 (1977) 213–233.