Syntheses of model oligosaccharides of biological significance. X. Syntheses and NMR and mass spectral analysis of trideuteriomethyl di-3,6-*O*-(4-*O*-β-D-galactopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside: the I antigen branchpoint penta- and tetrasaccharides and a related trisaccharide[1]

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As part of our studies of complex oligosaccharides, in particular their three-dimensional structure, we have synthesized the antigen I branchpoint penta- and tetrasaccharides. The unbranched trisaccharide **3***D* was also synthesized, and its derivative **3***B* served as the intermediate for the synthesis of higher oligosaccharides. NMR spectra of major intermediates as well as of the final oligosaccharides were completely assigned. Mass spectra of the synthetic intermediates and the final oligosaccharides were analyzed and compared with those of a similar group of oligosaccharides containing L-fucose, *N*-acetyl-D-glucosamine, and D-galactose. Certain observations were made that could be utilized in the interpretation of mass spectra for the structural determination of protected oligosaccharides during the synthesis.

Key words: oligosaccharide synthesis, I antigen, carbohydrate mass spectrometry, carbohydrate NMR spectrometry, Gal-GlcNAc oligomers.

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Dans le cadre de nos travaux sur les oligosaccharides complexes, en particulier en ce qui a trait à leur structure tridimensionnelle, on a synthétisé les penta- et tétra-saccharides des ramifications de l'antigène I. On a aussi synthétisé le trisaccharide linéaire **3***D* et on utilisé son dérivé **3***B* comme intermédiaire dans la synthèse d'oligosaccharides plus importants. On a attribué toutes les bandes des spectres RMN des intermédiaires principaux ainsi que des oligosaccharides finaux. On a analysé les spectres de masse des intermédiaires de synthèse ainsi que des oligosaccharides finaux et on les a comparés avec ceux d'un groupe similaire d'oligosaccharides contenant du L-fucose, de la *N*-acétyl-D-glucosamine et du D-galactose. On a fait certaines observations qui pourraient être utilisées durant les synthèses pour interpréter les spectres de masse et pour déterminer les structures d'oligosaccharides.

Mots clés : synthèse d'oligosaccharides, l'antigène I, spectrométrie de masse des hydrates de carbone, spectrométrie RMN des hydrates de carbone, oligomères Gal-GlcNAc.

[Traduit par la revue]

Introduction

Biological functions of the oligosaccharide moieties of glycoproteins depend on their three-dimensional structures, and of particular interest are the branching points, since these are areas of potential interactions between different chains (1). The more highly branched oligosaccharides have been consistently reported in both O-linked and N-linked oligosaccharides from glycoproteins obtained from tumour tissues and transformed or cancer cell lines (2). One such branching, resulting from the glycosylation of O-6 of the galactose residue of an "i" disaccharide fragment by another GlcNAc residue is responsible for the oncofetal differentiation from "i" to "I" structures (3). We have investigated by NMR spectroscopy and potential energy calculations the conformations of "minimal" structure of the "I" antigen class of compounds, GlcNAc(β1-3)[GlcNAc(β1-6)]-Galβ-, and in order to assess the influence of elongation of the oligosaccharide chains on the spatial arrangements of the chains, we have now synthesized tetra- and pentaoligosaccharides (the results of our conformational studies will be reported later).[4]

Synthesis

For our conformational analysis we required an NMR "silent" glycoside in order to avoid the overlap of OCH_n of an aglycon with ring hydrogens on the chemical shift scale and the ambiguity of anomerization (in the case of a free reducing sugar), and to reduce the number of relaxation pathways. Consequently, all our compounds are trideuteriomethyl glycosides.

Several groups have investigated methods for the formation of oligosaccharides at this branchpoint. Previously, Veyrières and co-workers prepared reducing oligosaccharides similar to **3***D* and **6***C* (5). They used an acid catalyzed oxazoline approach to prepare the GlcNAc(β1-3)Gal linkage in approximately 50% yield, and a similar yield is reported using silver promoted glycosylation with halides (6). These yields are comparable to those reported by Abbas and Matta (7) and Tejima and co-workers (8) for this linkage. Reports from both the Veyrières (6) and Ogawa (9) laboratories indicate yields in the 70–80% range using Lewis acid catalyzed glycosylation with fluorides or trichloroacetimidates. Enzymic synthesis also has been applied

[1]For part 9, see ref. 16.

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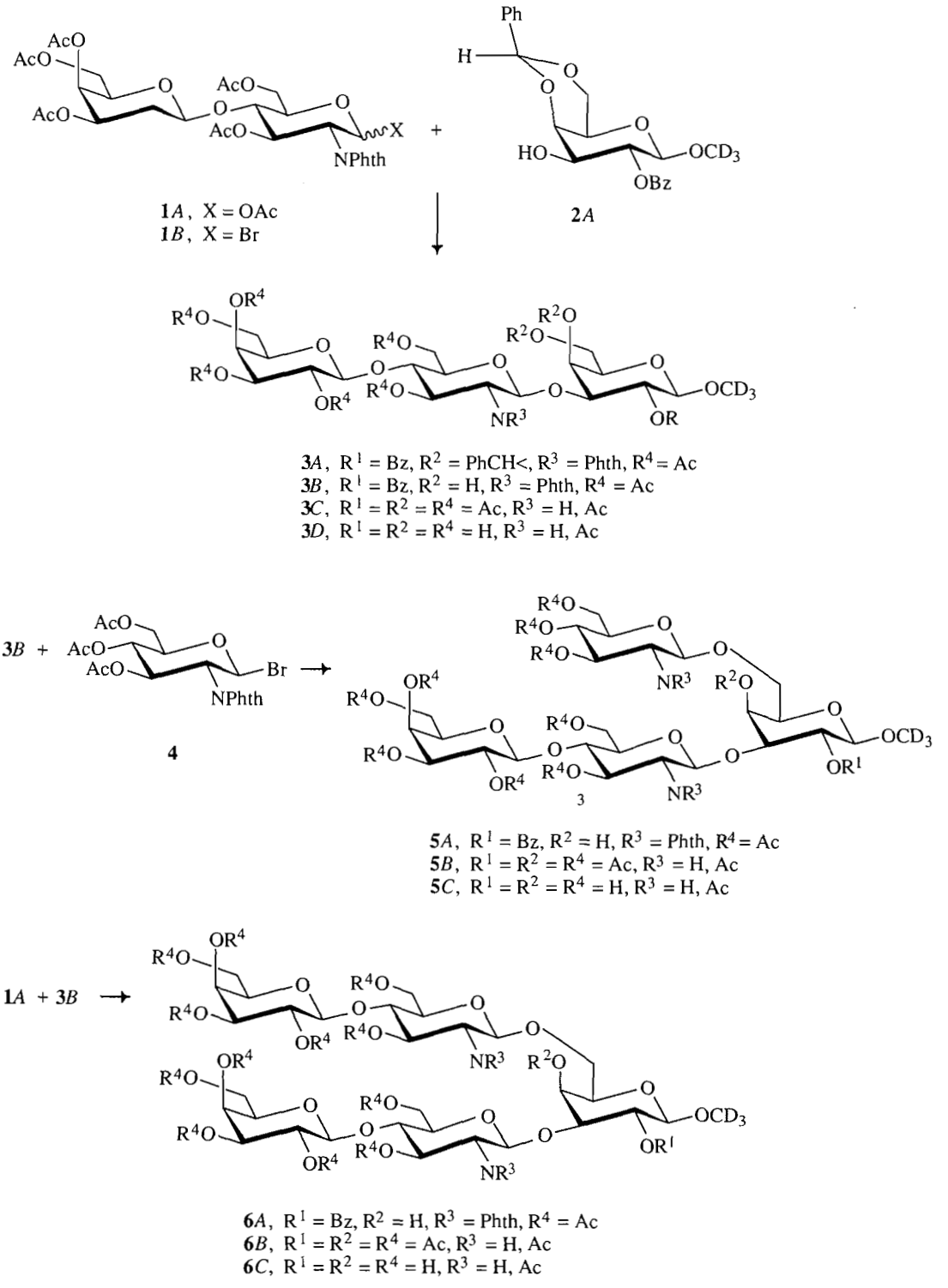

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to syntheses of oligosaccharides in this area (10). The approach described in this communication utilizes the non-nucleophilic base 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) to provide easily reproducible 72% yields. This base (DTBMP) was crucial to success of the silver triflate coupling, as the more often used bases such as sym-collidine (11) produced intractable mixtures from which only traces of the desired product **3***A* could be isolated after extensive purification by HPLC (16).

The lactosamine unit protected as **1***A* was transformed into the bromide **1***B* (11) with which the galactosyl derivative **2***A* (8, 12) was glycosylated to give the protected trisaccharide **3***A* (previously the chloride (13) had also been used as a glycosylating agent). Both bromides **1***B* and **4** have been used for syntheses of larger oligosaccharides; e.g., refs. 9 and 11. The compound **3***A* was first subjected to hydrogenolysis to remove the benzylidine protection at the galactose, yielding **3***B* (it

TABLE 1. ^{1}H NMR spectra of synthetic intermediates: trisaccharides

Sugar	H1 (J_{12})	H2 (J_{23})	H3 (J_{34})	H4 (J_{45})	H5 (J_{56})	H6 ($J_{66'}$)	H6′ ($J_{56'}$)
				3A			
Gal-β	4.431 d	5.403 dd	3.888 dd	4.447 brd	3.443 brs	4.006 brd	3.807 brdd
OCD_3	(8.0)	(10.1)	(3.5)	(<0.5)	(<0.5)	(6.8)	—
GlcNPhth	5.591 d	4.239 dd	5.605 dd	3.873 dd	3.743 brdt	4.855 dd	4.019 dd
	(8.4)	(10.4)	(8.8)	(9.9)	(2.1)	(−12.0)	(3.6)
Gal-β-1,4-	4.559 d	5.112 dd	4.957 dd	5.303 brd	3.840 brs	4.340 dd	4.131 dd
	(7.9)	(10.5)	(3.4)	(<0.5)	(1.4)	(−12.2)	(1.0)
Acetates	2.117, 2.058, 2.048, 1.999, 1.961, 1.801 6× s(3)						
Aromatics	7.607 Bz_o dd(2), 7.454 Bz_p brt (1), 7.233 Bz_m brt(2)						
	(8.0, 1.4) (7.4)						
	7.548 $Phth_o$ dd(2), 7.3–7.4 m Benz + $Phth_m$ m(7)						
	(7.6, 1.9)						
Benzylidene	$PhCHO_2$ 5.588 s(1)						
				3B			
Gal-β	4.381 d	5.317 dd	3.790 dd	4.270 brd	3.628 brt	3.95[1] m	3.8[2] m
OCD_3	(8.0)	(9.8)	(3.3)	(<0.5)	(6.1)	—	—
GlcNPhth	5.608 d	4.293 dd	5.568 dd	3.95[1] m	3.8[2] m	4.749 dd	4.08[3] m
	(8.5)	(10.6)	(8.7)	—	(1.8)	(−11.8)	—
Gal-β-1,4-	4.650 d	5.112 dd	5.019 dd	5.302 dd	3.95[1]	3.95[1]	4.08[3] m
	(7.8)	(10.3)	(3.4)	(0.5)	—	—	—
Acetates	2.195, 2.118, 2.071, 2.008, 1.960, 1.813 6× s(3)						
Aromatics	7.570 Bz_o dd(2), 7.383 Bz_p brt(1), 7.139 Bz_m brt(2)						
	(7.1, 1.2) (7.4)						
	7.43–7.36 Phth brm(4)						
Hydroxyls	3.269 OH_4 brs(1), 2.945 OH_6 dd(1)						
	— (7.8, 5.6)						
				3C			
Gal-β	4.295 d	5.092 dd	3.8[4] m	5.35 brd[5]	3.875 brt	4.12[6] m	4.12[6] m
OCD_3	(8.0)	(9.9)	—	—	(7.2)	—	—
GlcNAc	4.782 d	3.43[7] m	5.227 dd	3.8[4] m	3.43[7] m	4.725 dd	3.990 dd
	(7.6)	(10.1)	(8.8)	—	(2.6)	(−12.0)	(3.6)
Gal-β-1,4-	4.533 d	5.111 dd	4.975 dd	5.35 brd[5]	3.8[4] m	4.12[6] m	4.12[6] m
	(7.9)	(10.4)	(3.4)				
Acetates	2.155, 2.148, 2.121, 2.096, 2.087, 2.066, 2.062, 2.046, 1.970, 1.905 10× s(3)						
N-H	5.418 brd (8.6)						

should be noted that an attempted removal with acetic acid led to splitting off of the complete galactose unit). Hydrazinolysis with hydrazine hydrate in ethanol effected the removal of the phthalimido protection in good yield and after acetylation gave **3***C*. Peracetylated oligosaccharides are usually more suitable for purification by chromatography than completely deprotected compounds; consequently **3***C* was subjected to chromatography and the acetyls were then removed by KCN-catalyzed methanolysis to give **3***D*. Potassium cyanide was superior to sodium methoxide (Zemplen reagent) in this situation since the latter afforded partially decomposed products only.

Protected tetrasaccharide **5***A* and pentasaccharide **6***A* were prepared by taking advantage of the differential reactivity in **3***B* of the primary (O*H*; δ = 2.945 ppm, dd, J = 7.8 and 5.6 Hz) and the secondary (O*H*; δ = 3.269 ppm, broad singlet) hydroxyls (cf. Table 1). After glycosylation only the secondary hydroxyls at δ = 2.863 ppm in **5***A* (cf. Table 2) and at δ = 2.866 ppm in **6***A* (cf. Table 3) were present in the spectra of the respective compounds.

Thus, glycosylation of **3***B* with the bromide **4**, using silver zeolite promoter (13), gave tetrasaccharide **5***A*, which was subjected to hydrazinolysis to remove both phthalimido protecting groups and the benzoyl group. Acetyls were lost during the hydrazinolysis as well. Complete reacetylation yielded **5***B*, from which *O*-acetyl groups were removed smoothly during KCN-catalyzed methanolysis to give the desired oligosaccharide **5***C*.

Protected pentasaccharide **6***A* was again prepared from the intermediate **3***B* using the bromide **1***B*. As in the case of the protected tetrasaccharide **5***A*, the hydroxyl on C-6 was substantially more reactive than the hydroxy group on C-4 and the pentasaccharide derivative **6***A* was obtained exclusively. Depro-

TABLE 2. ^{1}H NMR spectra of synthetic intermediates: tetrasaccharides

Sugar	H1 (J_{12})	H2 (J_{23})	H3 (J_{34})	H4 (J_{45})	H5 (J_{56})	H6 ($J_{66'}$)	H6′ ($J_{56'}$)
				5A			
Gal-β	4.263 d	5.223 dd	3.92 m[1]	4.02 m[2]	3.6 m[3]	4.2 m[4]	3.85 m[5]
OCD_3	(8.1)	(9.8)	—	—	—	—	—
GlcNAc-1,3	5.141 d	4.100 dd	5.386 dd	3.6 m[3]	3.9 m[1]	4.193 dd	3.85 m[5]
	(8.1)	(10.7)	(8.8)	—	(2.2)	(−12.3)	—
Gal-β-1,4	4.456 d	5.126 dd	4.964 dd	5.327 dd	3.6 m[3]	3.85 m[5]	3.85 m[5]
	(7.9)	(10.4)	(3.4)	(0.8)	—	—	—
GlcNAc-1,6	5.539 d	4.348 dd	5.730 dd	5.206 dd	3.006 ddd	4.351 m[2]	4.0 m[2]
	(8.5)	(9.7)	(9.1)	(9.7)	(1.8)	(−12.3)	(6.9)
Acetates	2.150, 2.145, 2.131, 2.114, 2.044, 2.016, 1.974, 1.870, 1.782, 9 × s(3)						
Aromatics	Phth 7.78 brm (4), Phth 7.42 brm (4) Bz_o 7.534 dd(2), Bz_p 7.313 brt(1), Bz_m 7.065 brt(2) (7.5, 1.3) (7.5)						
Hydroxyl	Gal_4 2.863 brd						
				5B			
Gal-β	4.263 d	5.081 dd	3.7 m[6]	5.328 brd	3.7 m[6]	4.1 m[7]	3.8 m
OCD_3	(8.1)	(9.9)	(3.5)	—	—	—	—
GlcNAc-1,3	4.735 d	3.52 m[8]	5.219 dd	3.75 m[6]	3.55 m[8]	4.791 dd	3.971 dd
	(7.8)	(10.3)	(8.9)	—	(2.2)	(−12.0)	(3.1)
N-H	5.540 (9.4)						
Gal-β-1,4	4.350 d	5.109 dd	4.980 dd	5.348 brd	3.75 m[6]	4.1 m[7]	4.1 m[7]
	(7.9)	(10.4)	(3.4)	—	—	—	—
GlcNAc-1,6	4.882 d	3.48 m[8]	5.537 dd	5.025 dd	3.55 m[8]	4.261 dd	4.1 m[7]
	(8.2)	(9.5)	(9.5)	(8.2)	(2.0)	(−12.0)	—
N-H	6.375 (8.6)						
Acetates	2.186, 2.147, 2.096, 2.091, 2.081, 2.044, 1.970, 1.936, 1.901, 9 × s(3) 2.066, 2.023 2 × s(6)						

tection of **6*A*** by hydrazinolysis and KCN-catalyzed methanolysis subsequently gave **6*B*** and **6*C***.

NMR spectrometry

The ^{1}H NMR data of the synthetic intermediates are listed in the tables: the trisaccharide, Table 1, tetrasaccharide, Table 2, and pentasaccharide, Table 3. In most cases it was possible to assign also ^{1}H resonances by conventional decoupling and (or) ^{1}H–^{1}H COSY techniques. All coupling constants and chemical shifts are based on peak separations. Overlapping resonances are marked with the same superscript numbers. All spectra are consistent with the proposed structures. The ^{13}C and ^{1}H resonances of the unprotected oligosaccharides were assigned through heteronuclear ^{1}H–^{13}C COSY (cf. Tables 4 and 5).

The only ambiguity is in the assignment of the terminal galactose residues of the pentasaccharide derivatives. The appropriate connectivities were readily established within the respective arms, but which arm (6 or 3) remained ambiguous. The proposed assignments are based on a comparison with tetrasaccharides **5*A*–5*C*** where the terminal galactose is definitively on the 3 arm.

Several of these compounds contain a single benzoate group. Since its aromatic (*o, m, p*) resonances are very distinct, they provided a reliable marker allowing the assessment of the purity of individual preparations. All ^{1}H and ^{13}C phthalimido resonances were broad, and in the cases where these resonances overlapped with other aromatic resonances, their presence could only be ascertained by integration values. Fortunately, characteristic fragments for the phthalimido group were found in the mass spectra of the same samples (cf. below).

One surprising observation was made in connection with the upfield shift of the GlcNAcβ1,6 H-5 proton in compounds **5*A*** (3.006) and **6*A*** (2.823). The origin of this effect is not immediately apparent but it is suggestive of a juxtaposition of this hydrogen and an aromatic ring (such as the phthalimide).

Mass spectrometry

Fast atom bombardment mass spectrometry (FAB/MS) in positive ion mode was utilized to confirm the structures of the target substances and synthetic intermediates. In addition, a number of compounds previously synthesized in this laboratory (12, 16) (cf. formulae listed in Table 6) were also investigated by FAB/MS. We have often noted that, particularly in the case of more complicated protected oligosaccharides, it is difficult to interpret unequivocally their unidimensional NMR spectra because of the complexity and overlapping of their signals. In some such cases mass spectrometry can give a quick and definitive proof of the presence of certain structural elements

TABLE 3. ^{1}H NMR spectra of synthetic intermediates: pentasaccharides

Sugar	H1 (J_{12})	H2 (J_{23})	H3 (J_{34})	H4 (J_{45})	H5 (J_{56})	H6 ($J_{66'}$)	H6' ($J_{56'}$)
				6A			
Gal-β OCD_3	4.289 d (8.0)	5.230 dd (9.7)	3.604 dd (3.2)	3.85 m[1] —	3.85 m[1] —	4.05 m[2] —	3.85 m[1] —
GlcNAc-1,3	5.531 d (8.5)	4.195 dd (10.5)	5.681 dd (8.3)	3.903 dd (9.8)	3.85 m[1] (1.2)	4.307 dd (−11.8)	3.950 (7.3)
Gal-β-1,4	4.442 d (7.9)	5.128 dd (10.4)	4.966 m[3] (3.4)	5.325 m[4] (0.8)	3.6 m[5] —	4.05 m[2] —	4.05 m[1] —
GlcNAc-1,6	5.077 d (8.5)	4.066 dd (10.6)	5.350 dd (8.8)	3.85 m[1] (9.8)	2.823 ddd (1.4)	4.534 dd (−12.0)	4.185 dd (5.0)
Gal-β-1,4	4.551 d (8.1)	5.134 dd (10.4)	4.966 m[3] (3.4)	5.348 m[4] (0.8)	3.6 m[5] —	4.05 m[2] —	4.05 m[2] —
Acetates	2.179, 2.152, 2.139, 2.133, 2.091, 2.079, 2.045, 2.011, 1.975, 1.971, 1.914, 1.777 12 × s(3)						
Aromatics	Phth 8.147 brd (1), 7.909 m(1), 7.776 m(2), Phth 7.48 m(2), 7.38 m(2) Bz_o 7.541 dd(2), Bz_p 7.317 tt (1), Bz_m 7.059 brt(2)						
Hydroxyl	Gal_4 2.866 dd (2.6, 1.1)						
				6B			
Gal-β OCD_3	4.264 d (8.0)	5.057 dd (9.8)	3.775 dd (3.3)	5.308 brd (<0.5)	3.53 m[6] —	4.00 m[7] —	3.64 m[8] —
GlcNAc-1,3	4.683 d (7.7)	3.85 m[9] (10.1)	5.165 dd (8.3)	3.75 m[10] —	3.55 m[6] (1.9)	4.660 dd (−12.2)	3.998 dd (4.3)
N-H	6.161 (9.0)						
Gal-β-1,4	4.522 d (7.6)	5.109 dd (10.4)	4.979 dd (3.3)	5.356 brd (<0.5)	3.88 m[9] —	4.1 m[7] —	4.1 m[7] —
GlcNAc-1,6	4.681 d (7.6)	3.65 m[8] (10.1)	5.165 dd (8.3)	3.75 m[10] —	3.62 m[8] (1.2)	4.508 dd (−12.0)	4.08 m[7]
N-H	5.711 (8.7)						
Gal-β-1,4	4.516 d (7.6)	5.097 dd (10.4)	4.962 dd (3.4)	5.347 brd (<0.5)	3.88 m[9] —	4.1 m[7] —	4.1 m[7] —
Acetates	2.154, 2.152, 2.145, 2.122, 2.072, 2.063, 2.047, 1.972, 1.970, 1.932, 1.903 11 × s(3), 2.097 s(6), 2.057 s(9)						

TABLE 4. ^{13}C NMR spectra of free unprotected oligosaccharides

Sugar	C1	C2	C3	C4	C5	C6	C=O	CH_3
				Gal(β1-4)GlcNAc(β1-3)Gal(β-OCD_3) ***3D***				
Gal-OCD_3	104.64	70.53	83.11	68.52	75.51	61.74		
GlcNAc	103.49	56.08	73.04	78.95	75.41	60.67	175.76	23.02
Gal-1,4	103.67	71.79	73.32	67.51	76.60	61.86		
				Gal(β1-4)GlcNAc(β1-3)[GlcNAc(β1-6)]Gal(β-OCD_3) ***5C***				
Gal-OCD_3	104.48	71.79	82.95	69.34	74.22	70.14		
GlcNAc-1,3	103.45	56.08	73.03	78.92	75.40	60.68	175.77	23.01
Gal-1,4	103.68	70.45	73.32	69.34	76.17	61.85		
GlcNAc-1,6	102.31	56.30	74.56	70.72	76.64	61.52	175.28	23.01
				Gal(β1-4)GlcNAc(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β-OCD_3) ***6C***				
Gal-OCD_3	104.71	70.68	83.17	69.58	74.46	70.36		
GlcNAc-1,3	103.67	56.30	73.26	79.13	75.62	60.89	175.99	23.25
Gal-1,4(3)	103.90	72.01	73.32	69.59	76.41	62.08		
GlcNAc-1,6	102.46	56.06	73.40	79.44	75.76	61.05	175.46	23.25
Gal-1,4(6)	103.93	72.01	73.32	65.59	76.41	62.08		

TABLE 5. ^{1}H NMR spectra of synthetic unprotected oligosaccharides

Sugar	H1 (J_{12})	H2 (J_{23})	H3 (J_{34})	H4 (J_{45})	H5 (J_{56r})	H6r (J_{6r6s})	H6s' (J_{56s})
			Gal(β1-4)GlcNAc(β1-3)Gal(β-OCD_3) **3***D*				
Gal-OCD_3	4.299 d (8.0)	3.544 dd (9.9)	3.709 dd (3.2)	4.142 brd (<0.5)	3.684 m[7] —	3.772 m[8] —	3.754 m[8] —
GlcNAc	4.715 d (8.0)	3.789 dd (9.8)	3.731 m[8] —	3.736 m[8] —	3.578 m (2.2)	3.956 dd (−12.3)	3.844 dd (4.6)
$NHCOCH_3$	2.028						
Gal-1,4	4.475 d (7.8)	3.537 dd (10.0)	3.688 dd (3.3)	3.924 brd (<0.5)	3.719 m (3.6)	3.795 dd (−12.2)	3.75[8] m —
			Gal(β1-4)GlcNAc(β1-3)[GlcNAc(β1-6)]Gal(β-OCD_3) **5***C*				
Gal-OCD_3	4.273 d (8.0)	3.530 dd (9.9)	3.668 dd (3.4)	4.115 brd (<0.5)	3.765 m (7.0)	3.768 m (−12.0)	4.033 m (5.0)
GlcNAc-1,3	4.704 d (8.0)	3.790 dd (10.2)	3.715 m —	3.737 m —	3.582 ddd (2.2)	3.954 dd (−12.3)	3.845 dd (4.4)
$NHCOCH_3$	2.028						
GlcNAc-1,6	4.535 d (8.5)	3.710 dd (10.4)	3.541 dd (8.8)	3.443 dd —	3.450 m (1.40)	3.934 dd (−12.4)	3.735 m —
$NHCOCH_3$	2.024						
Gal-1,4	4.475 d (7.8)	3.536 dd (10.1)	3.694 dd (3.3)	3.923 brd (<0.5)	3.715 m (3.6)	3.744 m (−12.2)	3.744 m —
			Gal(β1-4)GlcNAc(β1-3)[Gal(β1-4)GlcNAc(β1-6)]Gal(β-OCD_3) **6***C*				
Gal-OCD_3	4.275 d (8.0)	3.536 dd (9.9)	3.676 dd (3.4)	4.115 brd (<0.5)	3.755 m (7.0)	3.750 (−12.0)	4.033 m (5.0)
GlcNAc-1,3	4.706 d (8.0)	3.771 dd (10.2)	3.708 —	3.715 —	3.597 ddd (2.2)	3.961 dd (−12.3)	3.835 dd (4.4)
Gal-1,4	4.472 d (7.8)	3.517 dd (10.1)	3.644 dd (3.3)	3.924 brd (<0.5)	3.708 m (3.6)	3.75 m (−12.2)	3.75 m —
GlcNAc-1,6	4.559 d (8.5)	3.755 dd (10.4)	3.700 m —	3.708 m —	3.515 m (1.40)	3.996 dd (−12.4)	3.827 m —
Gal-1,4	4.476 d (7.8)	3.517 dd (10.1)	3.644 dd (3.3)	3.924 brd (<0.5)	3.708 m (3.6)	3.75 m (−12.2)	3.75 m —
Acetate	2.026 s(6)						

in the molecule, in addition to the molecular mass of the compounds.

The mass spectra of the compounds investigated can be exemplified by that of compounds **6***A*, **6***C*, **8***A*, and **8***B* (shown in Figs. 1 and 2). Other mass spectrometric data are listed in Table 7. Relatively weak $[M+H]^+$ ions and $[M+Na]^+$ and $[M+NH_4]^+$ ions are observed in all the spectra. In compounds containing several benzyl and sometimes benzylidine protecting groups, $[M-H]^+$ ions become more dominant than $[M+H]^+$ ions; cf. for example, the more abundant m/z 804 $[M-H]^+$ ion than m/z 806 $[M+H]^+$ ion in Fig. 2a. Completely deprotected oligosaccharides usually display the $[M+H]^+$ ion with fair intensity: often it is one of the most abundant ions in the spectrum (with the exception of ions at very low m/z).

Fragmentation in the FAB mass spectra of peracetylated and permethylated oligosaccharides (4, 14, 17) occurs at the glycosidic bonds between the anomeric carbon and the charge-carrying oxygen (fragmentation pathway (a)). This fragmentation pathway gives rise to a series of fragment ions, for example, m/z 331, 706, and 1677 of compound **6***A* (Fig. 1a). The cleavage of the aglycon (loss of OCD_3 or OC_3D_7), however, is difficult in deprotected larger oligosaccharides and they consequently display very few $[M-OCD_3]^+$ or $[M-OC_3D_7]^+$ ions.

Gal- |-O-GN- |-O-Gal-| -O-Agl

(a)

In unprotected oligosaccharides (**14**, **3***D*, **5***C*, **6***C*), *N*-acetyllactosamine consistently gives rise to an abundant ion (m/z 366, fragmentation pathway (a), Fig. 1b). Terminal 2-acetamido-2-deoxyhexose (such as *N*-acetylglucosamine) gives rise to an ion at m/z 204 (**13***C*).

After the initial loss of the aglycon moiety through fragmentation pathway (a), further cleavage between the anomeric carbon and the oxygen, accompanied by hydrogen transfer to the oxygen and charge retention at the "reducing" end of the oligosaccharides, gives rise to m/z 972 in compound **6***A* (Fig. 1a), m/z 351 in compound **8***B* (Fig. 2b), and m/z 355 in compound **8***A* (Fig. 2a; cf. formula **15**, Table 6). The ion **15** (m/z 355, Table 6) indicates the presence of 2-*O*-benzoyl-4,6-*O*-benzylidene hexose in the molecule (e.g., monosaccharides **2***A* or **2***C*, or oligosaccharides **3***A*, **8***A*, **10**α, **10**β**A**). Transfer of

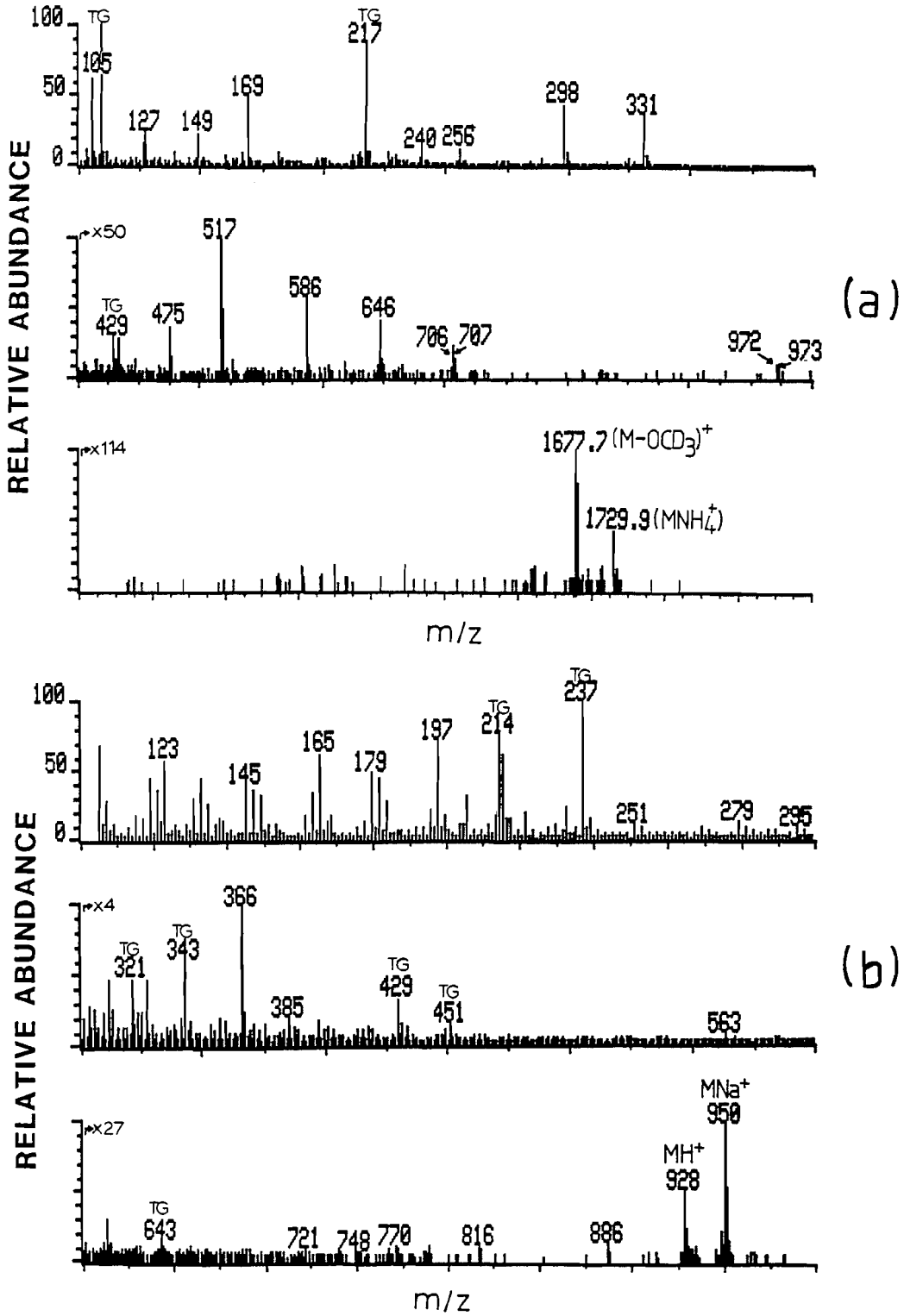


FIG. 1. FAB mass spectra of (a) compound **6***A*, and (b) compound **6***C*. TG indicates ions originating from thioglycerol matrix.

groups other than hydrogen, such as benzyl or acetyl, to the oxygen has occasionally been observed.

Certain protecting groups also can be identified. For instance, benzoyls give rise to an ion at m/z 105 in every compound studied (**7***A*–**7***C*, **8***A*, **8***B*; cf. spectra of **8***A* and **8***B*, Fig. 2). Benzyl groups can be identified through the loss of 90 mass units (benzyl group and hydrogen transfer). The phthalimido group used frequently for the protection of the NH_2 functionality reveals itself by the presence of an ion at m/z 418 (2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetylhexose, at a non-reducing terminus), which subsequently loses two acetyl groups to give an abundant m/z 298 ion. The m/z 298 ion is also formed from an internal unit bearing an *N*-phthalimido group (**3***A*, **3***B*, **9***A*, **10**α, **10**β*A*, **10**β*B*, **11***A*, **13***A*).

As an example of a quick analysis utilizing the above principles, the mass spectrum of **6***A* (Fig. 1) reveals the presence of benzoyl (m/z 105), peracetylated *N*-phthalimido-lactosamine (m/z 706, 331, 298), and the remaining portion of the molecule after the loss of one arm from $[M-OCD_3]^+$ at m/z 1677. The spectrum of **6***B* confirms that the phthalimido group

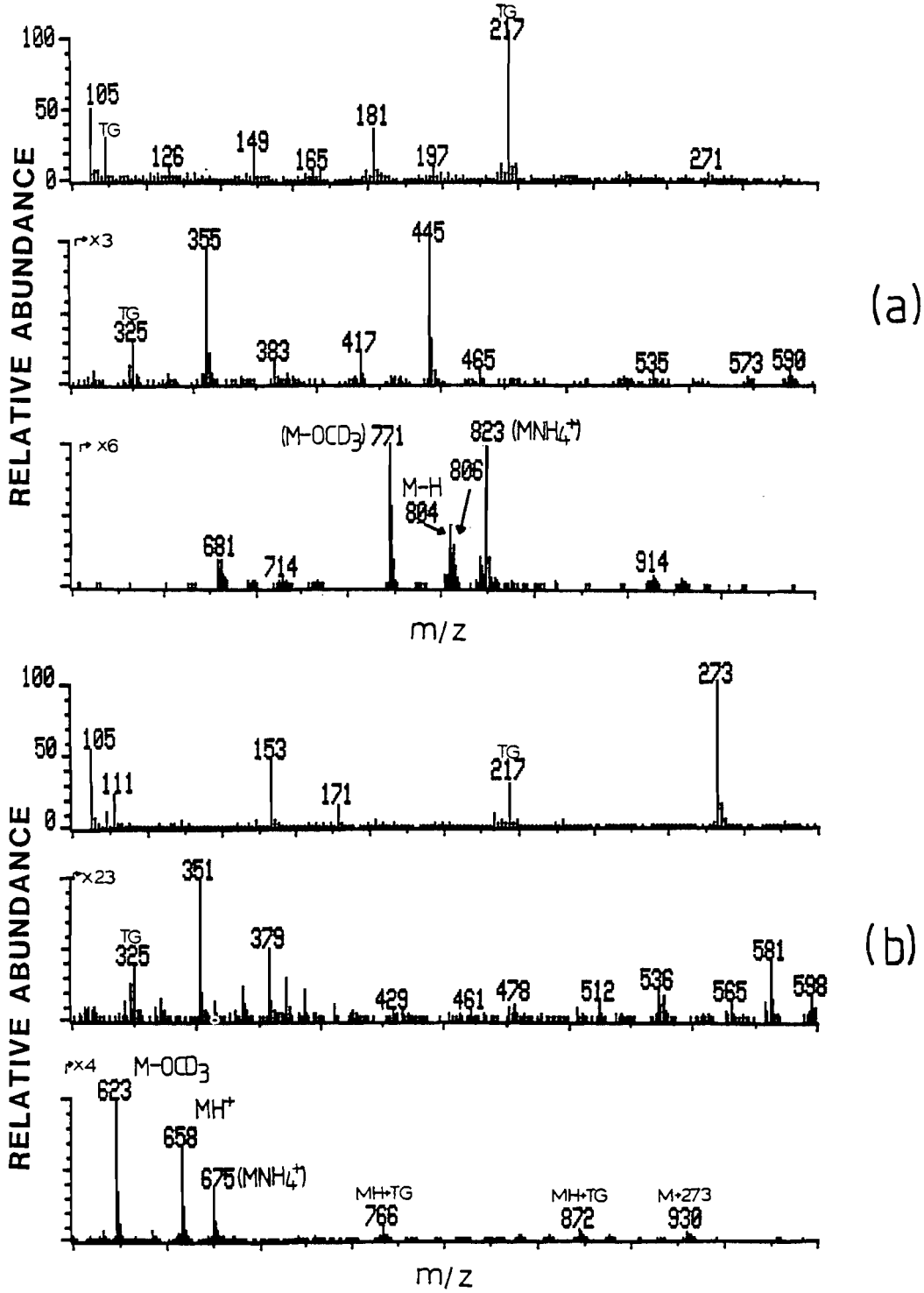


FIG. 2. FAB mass spectra of (a) compound **8***A*, and (b) compound **8***B*. TG indicates ions originating from thioglycerol matrix.

was completely replaced by *N*-acetyl (ions at *m/z* 618, 331; the other part of the molecule after the loss of one arm from $[M+H]^+$ gives rise to an ion at *m/z* 899).

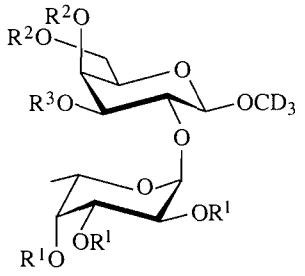
Experimental

General methods

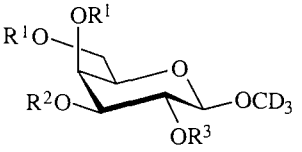
Melting points were determined on a Reichert Thermovar melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer polarimeter (model 243B) at 26 ± 1°C. Microanalyses were performed by the Micro Analytical Laboratory Ltd., Markham, Ontario. IR spectra were recorded on a Perkin–Elmer (model 1430) spectrometer using thin film on NaCl plates.

^{1}H NMR spectra were recoreded at 360.06 MHz with a Nicolet spectrometer at the NMR Spectroscopy Laboratory of the Carbohydrate Research Centre, University of Toronto. ^{13}C NMR spectra were recorded at 100.566 MHz with a Varian XL-400 spectrometer at the Chemistry Department, University of Toronto. Spectra were obtained at 19 ± 2°C either in $CDCl_3$ containing a trace of TMS (0 ppm, ^{1}H and ^{13}C) as the internal standard or in D_2O (99.98%, Aldrich) containing a trace of acetone (2.225 ppm relative to internal DSS, ^{1}H) as the internal standard. ^{13}C spectra in D_2O were referenced to external dioxane (67.4 ppm relative to TMS). Samples for NMR analyses in D_2O were passed through a Chelex 100 (Bio-Rad) ion-exchange resin in doubly distilled H_2O to remove paramagnetic impurities, lyophilized, and then lyophilized three times with D_2O. In the ^{1}H NMR tables, overlapping

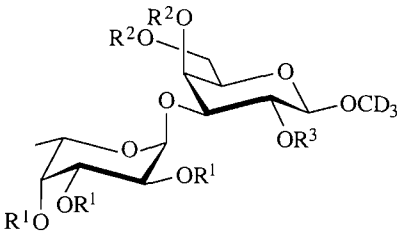
TABLE 6. Structures of compounds used for mass spectrometric studies



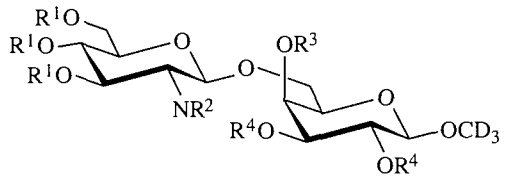
7*A*, R^1 = Bn, R^2 = PhCH<, R^3 = Bz
7*B*, $R^1 = R^2$ = Ac, R^3 = Bz
7*C*, $R^1 = R^2 = R^3$ = H



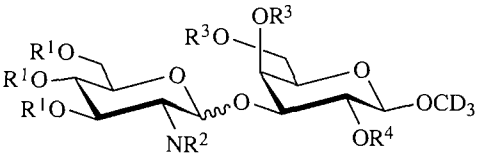
2*A*, R^1 = PhCH<, R^2 = H, R^3 = Bz
2*B*, R^1 = PhCH<, $R^2 = R_3$ = Bz
2*C*, R^1 = PhCH<, R^2 = Bz, R^3 = H
2*D*, R^1 = H, $R^2 = R^3$ = Bz



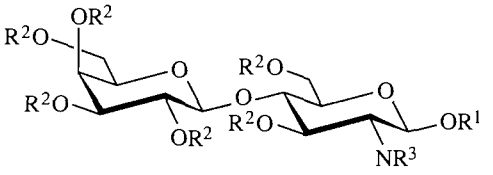
8*A*, R^1 = Bn, R^2 = PhCH<, R^3 = Bz
8*B*, $R^1 = R^2$ = Ac, R^3 = Bz
8*C*, $R^1 = R^2 = R^3$ = H



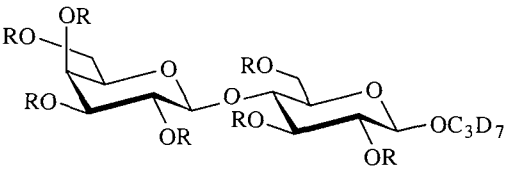
9*A*, R^1 = Ac, R^2 = Phth, R^3 = H, R^4 = Bz
9*B*, $R^1 = R^3 = R^4$ = Ac, R^2 = H, Ac
9*C*, $R^1 = R^3 = R^4$ = H, R^2 = H, Ac



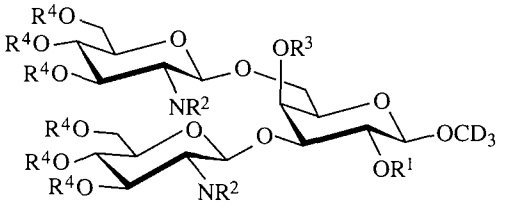
10α, R^1 = Ac, R^2 = Phth, R^3 = PhCH<, R^4 = Bz
10β*A*, R^1 = Ac, R^2 = Phth, R^3 = PhCH<, R^4 = Bz
10β*B*, R^1 = Ac, R^2 = Phth, R^3 = H, R^4 = Bz
10β*C*, $R^1 = R^3 = R^4$ = Ac, R^2 = H, Ac



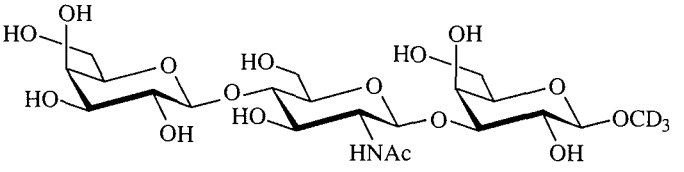
10*A*, $R^1 = C_3D_7$, R^2 = Ac, R^3 = Phth
10*B*, $R^1 = C_3D_7$, R^2 = H, R^3 = H, Ac
10*C*, $R^1 = CD_3$, R^2 = Ac, R^3 = H, Ac
10*D*, $R^1 = CD_3$, R^2 = H, R^3 = H, Ac



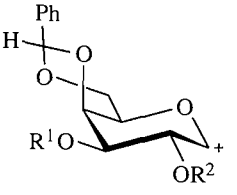
12*A*, R = Ac
13*B*, R = H



13*A*, R^1 = Bz, R^2 = Phth, R^3 = H, R^4 = Ac
13*B*, $R^1 = R^3 = R^4$ = Ac, R^2 = H, Ac
13*C*, $R^1 = R^3 = R^4$ = H, R^2 = H, Ac



14



15

R^1 = H, R^2 = Bz
R^1 = Bz, R^2 = H

TABLE 7. Mass spectrometric data of unprotected oligosaccharides and synthetic intermediates

Compounds	*M*	*M*−aglycon	Diagnostic fragment(s)
2*A*	389	355	105
2*B*	493	459	105
2*C*	389	355	—
2*D*	405	371	105
3*A*	1094	1060	298, 331, 355, 706
3*B*	1006	972	105, 298, 331, 706
3*C*	940	—	331, 618
3*D*	562	—	366
5*A*	1423	1389	298, 331, 706, 684, 972
5*B*	1227	—	330, 331, 618
5*C*	765	—	366
6*B*	1515	—	331, 618
6*C*	927	—	366
7*A*	805	771	105, 355, 417, 445
7*B*	657	623	105, 273
7*C*	343	309	147
9*A*	822	788	105, 298
9*B*	652	618	330
9*C*	400	366	—
10α	806	772	105, 298, 355, 418
10β*A*	806	772	105, 298, 355, 418
10β*B*	718	684	105, 298, 418
10β*C*	652	618	330
11*A*	772	706	331
11*B*	432	366	—
11*D*	400	366	—
12*A*	685	619	331
12*B*	391	—	163
13*A*	1135	1101	105, 298, 418, 684
13*B*	939	—	330
13*C*	603	—	204
14	562	—	366

resonances are marked with the same superscript numbers. Throughout the experimental section, ^{13}C NMR resonances whose resonances may be interchanged are marked with the same superscript numbers.

Fast Atom Bombardment mass spectras (FAB-MS) were recorded with a VG Analytical ZAB-SE mass spectrometer at the Mass Spectrometry Laboratory of the Carbohydrate Research Centre, University of Toronto. The samples in thioglycerol were bombarded by neutral xenon atoms (8 keV and 1 mA anode current) generated by Ion-Tech Saddle Field Ion Gun.

Thin-layer chromatography (TLC) was performed on silica gel $^{60}F_{254}$ (Merck) plastic plates and visualized by spraying with 50% sulfuric acid and (or) 1% orcinol acidic ferric chloride (15) and heating at 200°C. Silica gel (230–400 mesh; Toronto Research Chemicals) was used for flash chromatography. All starting materials were dried overnight under vacuum (10^{-3} Torr; 1 Torr = 133.3 Pa) prior to use and the solvents were distilled from appropriate drying agents. Solutions were concentrated at water aspirator pressure.

*Trideuteriomethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-4-*O*-β-D-galactopyranosyl-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (3*A*)*

The crude bromide **1***B* was prepared by saturating a mixture of dichloromethane (30 mL) and acetic anhydride (2 mL) with HBr gas at 5°C, adding acetate **1***A* (1.86 g; 2.4 mmol) as a solid, and rinsing the residue into the flask with dichloromethane (10 mL). HBr was bubbled through this mixture for 45 min and then the flask was tightly stoppered and the temperature allowed to rise for 1.5 h. Excess HBr was removed by flushing the flask with argon gas for 20 min. The remaining solvents were removed by evaporation on a rotary evaporator at high vacuum without heating. Dry dichloromethane (5 mL) and toluene (10 mL) were added to the residue and evaporated at high vacuum (twice), to remove residual acetic anhydride. The residue was dried under vacuum overnight, then dissolved in dry dichloromethane (15 mL) and added to a stirred mixture of alcohol **2***A* (0.46 g; 1.2 mmol), silver trifluoromethanesulphonate (0.6 g; 2.4 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.48 g; 2.4 mmol), flame dried molecular sieves 3 Å (5 g), and dry dichloromethane (20 mL) at 0–5°C under an atmosphere of argon. The stirring was continued for 16 h and the temperature was allowed to rise to room temperature. The solids were removed by filtration through a Celite bed, washed with dichloromethane, and the combined filtrates evaporated to dryness. The residue was purified by flash chromatography on silica gel (ethyl acetate:toluene:hexane 60:30:10) to yield **3***A* as a mixture (1.1 g; 85:15; 72%) with an unidentified impurity. A small sample of this mixture was purified to homogeneity by preparative HPLC (μ Porasil, hexane:dichloromethane:ethyl acetate 40:20:40); mp 268–272°C; $[\alpha]_D$ +29.9° (*c* 0.68, $CHCl_3$); IR (neat): 1780 sh, 1750, 1725 (CO) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ: 169.1–170.3 (6 × CH_3CO), 167.5 (br, CONCO), 167.3 (PhCO), 101.7[1] (Cl-Gal-β-OCD_3), 101.0[1] (Cl-Gal-β-1,4-), 100.8[1] ($PhCO_2$), 99.5 (Cl-Glc). Anal. calcd. for $C_{53}H_{54}D_3NO_{24}$ (1095.002): C 58.14, H 4.97, N 1.28; found: C 57.92, H 4.91, N 1.28.

*Trideuteriomethyl 2-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-4-*O*-β-D-galactopyranosyl-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (3*B*)*

Compound **3***A* (1.0 g, 0.78 mmol), prepared as described above and without purification, was dissolved in acetic acid (40 mL) and hydrogenated at room temperature and atmospheric pressure for 16 h with Palladium black (Strem, 700 mg) as catalyst. The solids were removed by filtration through acetic acid wetted Celite, rinsed with 95% ethanol, and the solvents were evaporated at high vacuum. The residual acetic acid and water were removed by azeotropic distillation with toluene (2 × 50 mL). The residue was purified by flash chromatography on silica gel (isopropanol:dichloromethane 8:92) to yield pure **3***B* (0.68 g, 87%); mp 153–154°C; $[\alpha]_D$ +36.7° (*c* 1.8, $CHCl_3$); IR (neat):3250 (br OH), 1780 sh, 1750, 1725 (CO) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ: 169.2–171.0 (6 × CH_3CO), 167.8 (br, CONCO), 164.7 (PhCO), 101.9[2] (Cl-Gal-β-OCD_3, $^2J_{C-H}$ 160), 101.1[2] (Cl-Gal-β-1,4-, $^2J_{C-H}$ 162), 98.5 (Cl-Glc, $^2J_{C-H}$ 160). Anal. calcd, for $C_{46}H_{50}D_3NO_{24}$ (1006.893): C 54.87, H 5.00, N 1.39; found: C 55.45, H 5.03, N 1.36

*Trideuteriomethyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-4-*O*-β-D-galactopyranosyl-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (3*C*)*

Diol (**3***B*) (0.35 g, 0.35 mmol) was dissolved in ethanol (6 mL), hydrazine hydrate (Aldrich 85%, 3 mL) was added, and the mixture heated at 70°C for 30 min. The liquids were removed by evaporation at high vacuum and the residual water removed by coevaporation with toluene (2 × 25 mL). The residue was cooled in an ice bath under an atmosphere of argon, then dry pyridine (8 mL) and dry acetic anhydride (5 mL) were added. The stirring was continued for 60 h and the temperature allowed to rise to room temperature. The liquids were removed by evaporation at high vacuum followed by coevaporation with toluene (3 × 20 mL). The residue was purified by chromatography on silica gel (isopropanol:dichloromethane 6:94) to yield **3***C* (0.28 g, 85%); mp 124–127°C; $[\alpha]_D$ +7.8° (*c* 1.04, $CHCl_3$); IR (neat): 3365 br (OH), 1750 (COO), 1680, 1550 (CON) cm^{-1}. Anal. calcd. for $C_{39}H_{52}D_3NO_{25}$ (940.8315): C 49.79, H 5.57, N 1.49; found: C 50.10, H 5.61, N 1.53.

*Trideuteriomethyl 3-*O*-(2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosyl)-β-D-galactopyranoside (3*D*)*

Peracetate (**3***C*) (0.15 g, 0.16 mmol) was dissolved in methanol (6 mL) and KCN (70 mg) was added under argon; the mixture was left to stir for 16 h under an argon atmosphere. To this mixture was added methanol (10 mL), water (5 mL), and mixed bed resin Ag 501-X8 (5 g). After 30 min of stirring, the solids were removed by filtration, washed with methanol:water (1:1, 50 mL), and methanol (50 mL). The filtrate was concentrated at high vacuum and taken up in a small volume of water, filtered through a disposable millipore filter, and lyophilized to yield pure **3***D* (0.82 g, 91%); mp 181–186°C; $[\alpha]_D$ −8.3° (*c* 1.21,

$CH_3OH:H_2O$ 4:1); IR (neat): 3350 (br, OH, NH), 1665, 1550 (CON), cm^{-1}. Anal. calcd. for $C_{21}H_{34}D_3NO_{16}$ (562.4967): C 44.84, H 6.09, N 2.49; found: C 45.16, H 5.83, N 2.43.

Trideuteriomethyl 2-O-benzoyl-3-O-(2,3,4,6-tetra-O-acetyl-4-O-β-D-galactopyranosyl-3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-galactopyranoside (5A)

Bromide **4** (Toronto Research Chemicals, 85 mg; 0.17 mmol) dissolved in dichloromethane (1 mL) was added to a stirred mixture of diol **3*B*** (115 mg; 0.11 mmol), silver zeolite 13X (2 g), and dichloromethane (6 mL) under an atmosphere of argon. The stirring was continued for 2.5 h and the solids removed by filtration through Celite and rinsed with dichloromethane. The filtrates were evaporated to dryness and the residue was purified by flash chromatography (silica gel, dichloromethane:isopropanol 25:1) to yield tetrasaccharide **5*A*** (112 mg; 69%; mp 170–173°C; $[\alpha]_D$ +32.7° (*c* 0.97, $CHCl_3$); IR (neat): 3400 (OH), 1780 sh, 1750, 1720 (CO) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ: 169.4–170.8 (9 × CH_3CO), 167.5 (br, CONCO), 164.4 (PhCO), 101.4[3] (Cl-Gal-β-OCD_3), 101.3[3], (Cl-Gal-β-1,4-) 97.7[4], (Cl, 3-Glc), 97.6[4], (Cl, 6-Glc). Anal. calcd. for $C_{66}H_{69}D_3N_2O_{33}$ (1424.265): C 55.66, H 4.88, N 1.97; found: C 55.82, H 5.03, N 1.92.

Trideuteriomethyl 2,4-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-4-O-β-D-galactopyranosyl-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-β-D-galactopyranoside (5B)

Tetrasaccharide **5*A*** (112 mg; 0.079 mmol) was treated with the same procedure described for the peracetate **3*C***. The product was purified by flash chromatography (silica gel, isopropanol:dichloromethane 1:10) to yield **5*B*** (77 mg; 80%); mp 269–273°C; $[\alpha]_D$ −19.3° (*c* 0.23, $CHCl_3$); IR (neat): 3300 (br, NH), 1750 (COO), 1660, 1550 (CON) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ: 169.0–171.2 (13 × CH_3CO), 101.7[5] (Cl-Gal-β-OCD_3, $^2J_{C-H}$ 160), 101.0[5] (Cl-Gal-β-1,4-, $^2J_{C-H}$ 160), 100.3[6] (Cl, 3-Glc; $^2J_{C-H}$ 160), 99.9[6] (Cl, 6-Glc, $^2J_{C-H}$ 160. Anal. calcd. for $C_{51}H_{69}D_3N_2O_{32}$ (1228.10): C 49.88, H 5.66, N 2.28; found: C 49.98, H 5.62, N 2.26

Trideuteriomethyl 3-O-(4-O-β-D-galactopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (5C)

The acetyls of **5*B*** (20 mg; 0.016 mmol) were removed following the procedure used for compound **3*D*** to yield pure tetrasaccharide **5*C*** (8 mg; 65% (**5*C***)); mp 238–243°C; $[\alpha]_D$ −17.4° (*c* 0.47, $CH_3OH:H_2O$ 5:1); IR (neat): 3320 (br, OH, NH), 1650, 1550 (CON) cm^{-1}. Anal. calcd. for $C_{29}H_{47}D_3N_2O_{21}$ (765.6911): C 45.49, H 6.19, N 3.66; found: C 45.42, H 6.28, N 3.76.

Trideuteriomethyl 2-O-benzoyl-3,6-di-O-(2,3,4,6-tetra-O-acetyl-4-O-β-D-galactopyranosyl-3,6-di-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-β-D-galactopyranoside (6A)

Compound **6*A*** was prepared as described for tetrasaccharide **5*A*** from diol **3*B*** (464 mg; 0.46 mmol) except that bromide **1*B*** generated from acetate **1*A*** (545 mg; 0.69 mmol), as described above, was used as the donor. The pentasaccharide **6*A*** was purified by flash chromatography (silica gel, dichloromethane:isopropanol 25:1) to yield pure **6*A*** (361 mg; 46%); mp 169–173°C; $[\alpha]_D$ +25.4 (*c* 1.7, $CHCl_3$); IR (neat): 3560 (OH), 1780 sh, 1750, 1720 (CO) cm^{-1}; ^{13}C NMR ($CDCl_3$) δ: 169.1–170.8 (12 × CH_3CO), 168, 167.5 (br, CONCO), 164.4 (PhCO), 101.4[7], (Cl-Gal-β-OCD_3, $^2J_{C-H}$ 160), 101.4[7] (Cl-Gal-β-1,4 (6), $^2J_{C-H}$ 160), 101.2[7] (Cl-Gal-β,4 (3), $^2J_{C-H}$ 160), 97.6[8] (Cl,3-Glc, $^2J_{C-H}$ 160), 97.3[8] (Cl,6-Glc, $^2J_{C-H}$ 160). Anal. calcd. for $C_{78}H_{85}D_3N_2O_{41}$ (1712.518); C 54.71, H 5.00, N 1.64; found: C 54.71, H 4.84, N 1.42.

Trideuteriomethyl 2,4-di-O-acetyl-3,6-di-O-(2,3,4,6-tetra-O-acetyl-4-O-β-D-galactopyranosyl-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (6B)

Pentasaccharide **6*A*** (279 mg; 0.16 mmol) was treated with the same procedure described for the peracetate **3*C***. The product was purified by flash chromatography (silica gel, isopropanol:dichloromethane 1:10) to yield pure **6*B*** (173 mg; 70%); mp 135–142°C; $[\alpha]_D$ −3.5° (*c* 1.5, $CHCl_3$); IR (neat): 3340, 3220 (NH), 1750 (COO), 1660, 1545 (CON) cm^{-1}. Anal. calcd. for $C_{63}H_{85}D_3N_2O_{40}$ (1516. 354): C 49.90, H 5.65, N 1.85; found: C 50.06, H 5.62, N 1.83.

Trideuteriomethyl 3,6-di-O-(4-O-β-D-galactopyranosyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside (6C)

The acetates of **6*B*** (112 mg; 0.074 mmol) were removed, following the procedure used for compound **3*D***, to yield pure pentasaccharide **6*C*** (50 mg; 73%); mp 204–207°C; $[\alpha]_D$ −7.67° (*c* 0.30, $CH_3OH:H_2O$ 1:1); IR (neat): 3300 (br, OH, NH), 1650, 1550 (CON) cm^{-1}. Anal. calcd. for $C_{35}H_{57}D_3N_2O_{26}$ (927.8331): C 45.31, H 6.19, N 3.02; found: C 45.41, H 6.18, N 2.92.

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1. J. P. Carver, S. W. Michnik, A. Imberty, and D. A. Cumming. Glycoproteins in biological recognition. *Edited by* G. Bock and S. Harnett. CIBA Foundation, Wiley, Chichester 1989. pp. 6–18.
2. J. W. Dennis, S. Laferte, C. Waghorne, M. L. Breitman, and R. S. Kerbel. Science, **236**, 582 (1987).
3. T. Feizi. Trends Biochem. Sci. **6**, 333 (1981).
4. H. Egge, A. Dell, and H. U. Nicolai. Arch. Biochem. Biophys. **224**, 235 (1983).
5. C. Auge, S. David, and A. Veyrières. Nouv. J. Chim. **3**, 491 (1979); M. A. Nashed, M. Kisco, C. W. Slife, and L. Anderson. Carbohydr. Res. **90**, 71 (1981).
6. A. Maranduba and S. Veyrières. Carbohydr. Res. **151**, 105 (1986).
7. S. A. Abbas and K. L. Matta. Carbohydr. Res. **123**, 53 (1983); **124**, 115 (1983).
8. T. Takamura, T. Chiba, and S. Tejima. Chem. Pharm. Bull. **29**, 1027 (1981).
9. Y. Ito, S. Sato, M. Mori, and T. Ogawa. J. Carbohydr. Chem. **7**, 359 (1988).
10. C. Augé, C. Mathieu, and C. Merienne. Carbohydr. Res. **151**, 147 (1986); C. Augé, S. David, C. Mathieu, and C. Gautheron. Tetrahedron Lett. **25**, 1467 (1984).
11. R. U. Lemieux, T. Takeda, and B. Y. Chung. *In* Synthetic methods for carbohydrates. *Edited by* H. S. El Khadem. ACS Symposium Series 39, Am. Chem. Soc., Washington, DC, 1976; J. Arnarp, M. Haraldsson, and J. Longgren. J. Chem. Soc. Perkin Trans. 1, 535 (1985); H. Baumann, H. Lönn, and J. Lönggren. Carbohydr. Res. **114**, 317 (1983).
12. D. M. Whitfield, J. P. Carver, and J. J. Krepinsky. J. Carbohydr. Chem. **4**, 369 (1985); A. Stoffyn and P. Stoffyn. J. Org. Chem. **32**, 4001 (1967).
13. R. U. Lemieux, S. Z. Abbas, B. Y. Chung. Can. J. Chem. **60**, 58 (1981); **60**, 68 (1981); R. U. Lemieux, S. Z. Abbas, M. H. Burczynska, and R. M. Ratcliffe. Can. J. Chem. **60**, 63 (1981); J. Alais and A. Veyrières. Tetrahedron Lett. **24**, 5223 (1983).
14. H. Egge and J. Peter-Katalinic. Mass Spectrom. Rev. **6**, 331 (1987).
15. J. C. Touchstone and M. F. Dobbins. Practice of thin layer chromatography. Wiley, New York. 1983.
16. D. M. Whitfield, C. J. Ruzicka, J. P. Carver, and J. J. Krepinsky. Can. J. Chem. **65**, 693 (1987).
17. A. Dell and M. Panico. *In* Mass spectrometry in biomedical research. *Edited by* S. Gaskel. Wiley, New York. 1986. pp. 149–180.