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Construction of linear GlcNAcβ1-6Galβ1-OR type oligosaccharides by partial cleavage of GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-OR sequences with jack bean β-*N*-acetylhexosaminidase

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Radiolabelled GlcNAcβ1-3(GlcNAcβ1-6)Gal (**1**), GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-OCH_3 (**4**), GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4Glc (**7**), and GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAc (**10**) were cleaved partially with jack bean β-*N*-acetylhexosaminidase (EC 3.2.1.30), and the digests were analysed chromatographically. All four oligosaccharides were hydrolysed faster at the (1-6) branch, than at the (1-3) branch, but a high branch specificity was observed only with the glycan **4**. The saccharides **1** and **7** resembled each other in the kinetics of the enzyme-catalysed release of their two non-reducing *N*-acetylglucosamine units, but the glycan **10** was rather different. The partial digestions made it possible to obtain radiolabelled GlcNAcβ1-6Gal, GlcNAcβ1-6Galβ1-OCH_3, GlcNAcβ1-6Galβ1-4Glc, and, in particular, GlcNAcβ1-6Galβ1-4GlcNAc.

Keywords: oligo-*N*-acetyllactosaminoglycans, enzymatic synthesis, *in vitro*, GlcNAcβ1-6Galβ1-4GlcNAc/Glc, GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAc|Glc, GlcNAcβ1-3(GlcNAcβ1-6)Gal, kinetics of β-*N*-acetylhexosaminidase cleavage, intramolecular interactions

Abbreviations: Lac, lactose; MT, maltotriose; MTet, maltotetraose.

Radiolabelled, linear oligosaccharides that contain a GlcNAcβ1-6Gal sequence have been obtained from partial acid hydrolysates of metabolically labelled poly-*N*-acetyllactosaminoglycans [1, 2], by catalytic tritium exchange at the anomeric carbon of chemically synthesized saccharides [1], and by enzyme catalysed transfer of radiolabelled monosaccharides from nucleotide sugars to chemically synthesized acceptors [2, 3]. These saccharides merit some attention because of their exceptional properties among the linear oligo-*N*-acetyllactosaminoglycans: They resist endo-β-galactosidase of *Escherichia freundii* [2], and they reveal unusually high affinity for immobilized wheat germ agglutinin [1–3]. The present report describes a general and relatively simple route for enzymatic *in vitro* construction of this type of glycans.

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Materials and methods

Chromatography

Prior to paper chromatography, the oligosaccharides were desalted by filtration through Dowex AG-1X8 (AcO^-) and Dowex AG-50-WX8 (H^+). The chromatograms were run on Whatman No 3 CHR paper as described [2], by using the upper phase of *n*-butanol:acetic acid:water, 4:1:5 by vol (solvent A), or *n*-butanol:ethanol:water, 10:1:2 by vol (solvent B). The runs were performed in a room where the temperature was not constant; in summer the saccharides migrated faster than in the winter. Radiolabelled saccharides were localized as described [2]; unlabelled glycans were stained with aniline phtalate or with silver nitrate.

^{1}H-NMR spectroscopy

Prior to ^{1}H-NMR spectroscopic analysis, the oligosaccharide samples (60–130 nmol) were repeatedly treated with

^2H_2O at room temperature. After each exchange treatment the sample was lyophilized. Finally, the sample was dissolved in ^2H_2O, and ^{1}H-NMR spectroscopy was performed at 500 MHz in a Bruker AM 500 spectrometer (BioCarb Chemicals, Lund, Sweden) operating in the Fourier transform mode. The probe temperature was kept at 300 K. Chemical shifts, expressed downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, were actually measured by reference to internal acetone ($\delta = 2.225$ ppm) with an accuracy of 0.002 ppm.

Saccharides

Unlabelled *N*-acetylglucosamine, glucose, galactose, lactose, GlcNAcβ1-6Gal and GlcNAcβ1-6Galβ1-4Glc were purchased from Sigma (St Louis, MO, USA); the maltooligosaccharides were from Boehringer (Mannheim, Germany). Radiolabelled GlcNAcβ1-3Gal, GlcNAcβ1-6Gal, GlcNAcβ1-3Galβ1-4GlcNAc, and GlcNAcβ1-6Galβ1-4GlcNAc have been described previously [1]. The branched saccharide GlcNAcβ1-3(GlcNAcβ1-6)[U-^{14}C]Gal was synthesized enzymatically as described recently [4].

GlcNAcβ1-3Galβ1-OCH_3 was purchased from Sigma. It was β(1-6)-*N*-acetyl-[^{14}C]glucosaminylated [5] to GlcNAcβ1-3([^{14}C]GlcNAcβ1-6)Galβ1-OCH_3 with UDP-[^{14}C]GlcNAc (Amersham, UK) and β(1-6)-GlcNAc-transferase (EC 2.4.1.148) [6], obtained by isolating hog gastric mucosal microsomes according to [7]. The product was isolated by removing sucrose by gel filtration, and by purifying the crude product by paper chromatography ($R_{Lac} = 0.86$, $R_{MT} = 1.20$; solvent A); it migrated faster than the parent reducing saccharide GlcNAcβ1-3(GlcNAcβ1-6)[U-^{14}C]Gal [4].

[^{3}H]Galβ1-4Glc was obtained by incubating UDP-[^{3}H]Gal and glucose with bovine milk β(1-4)-galactosyltransferase (EC 2.4.1.90) (Sigma) otherwise as described [2], but α-lactalbumin (1 mg ml^{-1}) was also added [8]. The product was isolated by paper chromatography ($R_{Lac} = 1.00$; solvent A). Its β(1-3)-*N*-acetylglucosaminylation was carried out as described [9] to yield GlcNAcβ1-3[^{3}H]Galβ1-4Glc. The trisaccharide was isolated by paper chromatography ($R_{Lac} = 0.74$; $R_{MT} = 1.01$; solvent A). GlcNAcβ1-3(GlcNAcβ1-6)[^{3}H]Galβ1-4Glc was synthesized by β(1-6)-*N*-acetylglucosaminylation of GlcNAcβ1-3[^{3}H]Galβ1-4Glc with UDP-GlcNAc and the β(1-6)-GlcNAc-transferase of hog gastric mucosal microsomes. The tetrasaccharide was isolated from the reaction mixture by gel filtration, and subsequently by paper chromatography ($R_{MT} = 0.66$; $R_{MTet} = 1.06$; solvent A), where it migrated more slowly than the tetrasaccharide GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Galβ1-4GlcNAc [4].

The tetrasaccharide GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Galβ1-4GlcNAc was obtained as described [4]. Briefly, [^{14}C]Galβ1-4GlcNAc was constructed by incubating *N*-acetylglucosamine and UDP-[^{14}C]Gal with bovine milk β(1-4)-galactosyltransferase (EC 2.4.1.22) (Sigma); GlcNAcβ1-3[^{14}C]Galβ1-4GlcNAc was synthesized from [^{14}C]Galβ1-4GlcNAc by the method of Yates and Watkins [9]; the trisaccharide was then β(1-6)-*N*-acetylglucosaminylated using the hog stomach β(1-6)-GlcNAc-transferase (EC 2.4.1.148) as described [4]. The enzyme syntheses were scaled up to obtain ^{1}H-NMR spectra of the trisaccharide GlcNAcβ1-3[^{14}C]Galβ1-4GlcNAc and of the tetrasaccharide GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]-Galβ1-4GlcNAc. The spectrum of the latter is shown in Fig. 1, and the relevant data on the chemical shifts and coupling constants are collected in Table 1, together with analogous data reported for the same compound by Koenderman *et al.* [10]. The great similarity of the two sets of data establish that our GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Galβ1-4GlcNAc and the [^{14}C]GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAc of Koenderman *et al.* [10] are isotopic isomers of the same tetrasaccharide.

Partial hydrolyses with jack bean β-N-acetylhexosaminidase

Incubations with jack bean β-*N*-acetylhexosaminidase (EC 3.2.1.23) (Sigma) were carried out for specified times at 37°C in 0.05 M sodium citrate (pH 4.0), containing 55 mM γ-galactonolactone; the reaction volumes were 45 µl, containing 150 mU of the enzyme. The reactions were stopped by heating in a boiling water bath for 3 min and the digests desalted with ion exchange resins (see under Chromatography).

Results

The structures of key oligosaccharides are collected in Table 2, together with the chromatographic mobilities that were measured in the experiments described below.

The trisaccharide GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Gal (**1**) was treated with jack bean β-*N*-acetylhexosaminidase for 5 min. The digest revealed four components in paper chromatography (Fig. 2a). Peak 1 represented the unchanged substrate **1**, peak 2 the disaccharide GlcNAcβ1-6[^{14}C]Gal (**3**), peak 3 the disaccharide GlcNAcβ1-3[^{14}C]Gal (**2**); and peak 4 was [^{14}C]Gal, which obviously originated by a partial secondary cleavage of one or both of the disaccharides **2** and **3**. The amounts of radioactivity in the four peaks indicated that 37% of available *N*-acetylglucosaminidic linkages had been cleaved in the hydrolysis. The amount of disaccharide **2** in the digest was so much larger than that of disaccharide **3** that the GlcNAcβ1-6Gal linkage of **1** clearly had been cleaved much faster than the GlcNAcβ1-3Gal linkage; the amount of liberated [^{14}C]Gal was too small to have caused large changes in the primary cleavage products.

A 5 min hydrolysis with β-*N*-acetylhexosaminidase was carried out also with the saccharide **4**, GlcNAcβ1-3([^{14}C]-GlcNAcβ1-6)Galβ1-OCH_3, which is the β-methylglycoside of **1** (note that **4** contains the radiolabel in a position different from **1**). Paper chromatography of the digest revealed three labelled compounds (Fig. 2b). Peak 1 was the original substrate **4**, and peak 2 represented [^{14}C]-GlcNAcβ1-6Galβ1-OCH_3 (**6**). The identification of peak 2

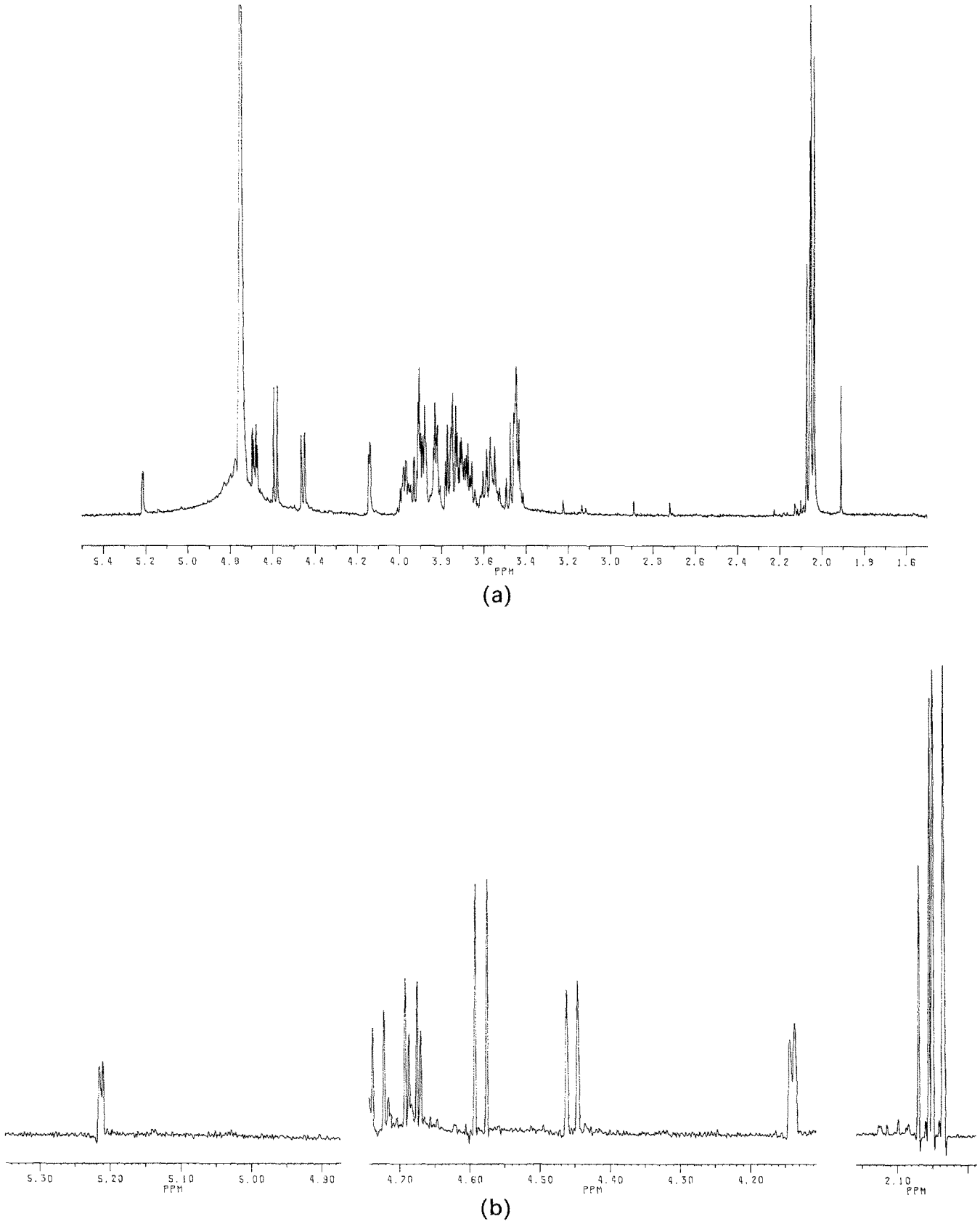

Figure 1. 500-MHz ^{1}H-NMR spectrum of GlcNAcβ1-3(GlcNAcβ1-6)Galβ1-4GlcNAc synthesized in the present experiments. (a) The overall spectrum; (b) expansions of the regions of structural reporter groups.

Table 1. Identification of glycan **10**, GlcNAcβ1-3(GlcNAcβ1-6)-Galβ1-4GlcNAc by 500-MHz ^{1}H-NMR.

Residue[a]	*Reporter group*	*Anomer of compound*	*Chemical shift (coupling constant*[b]*)*	
			Present study	*Ref. 10*
			ppm (Hz)	
GlcNAc	H-1	α	5.213 (2.7)	5.213 (2.5)
		β	4.731 (8.1)	4.731 (8.0)
	CH_3	α, β	2.051	2.051
Galβ4	H-1	α, β	4.455 (7.9)	4.455 (7.8)
	H-4	α, β	4.141 (3.6)	4.141 (3.2)
GlcNAcβ3	H-1	α	4.684 (8.4)	4.684 (8.3)
		β	4.679 (8.4)	4.679 (8.5)
	CH_3	α, β	2.036	2.036
GlcNAcβ6	H-1	α, β	4.585 (8.5)	4.584 (8.4)
	CH_3	α, β	2.056[c]	2.056[c]

[a] Labelling of monosaccharide residues is similar to that shown in [10].
[b] Coupling constant refers to $J_{1,2}$ in all H-1 resonances and to $J_{3,4}$ in galactose H-4.
[c] An additional singlet can be seen at 2.071 ppm. We suggest that this is due to the CH_3 group of GlcNAcβ6 of the β-anomer of this molecule, and the resonance at 2.056 to the α-anomer.

in panel B of Fig. 2 as compound **6** is based on the mode of its formation. It was formed from **4** which lost a β-linked *N*-acetylglucosamine unit. Since **6** was radioactive, it must have contained the (1-6)-linked [^{14}C]GlcNAcβ group. Since it migrated faster than lactose in solvent B, it must still have contained the methyl group; GlcNAcβ1-6Gal migrates more slowly than lactose in this solvent [4]. Peak 3 was [^{14}C]GlcNAc. Formation of **6** represents the cleavage of the GlcNAcβ1-3Gal linkage of **4**, whereas formation of [^{14}C]GlcNAc represents the cleavage of the [^{14}C]GlcNAc-β1-6Gal linkage of **4** and **6**. Assuming that the cleavage of **6** was insignificant in this experiment, the data imply that the [^{14}C]GlcNAcβ1-6Gal linkage of **4** was hydrolysed about five times faster than the GlcNAcβ1-3Gal linkage.

A 5 min hydrolysis with β-*N*-acetylhexosaminidase was carried out also with the saccharide **7**, GlcNAcβ1-3(GlcNAcβ1-6)[^{3}H]Galβ1-4Glc. Paper chromatography of the digest revealed four labelled compounds (Fig. 2c). Peak 1 was the original substrate **7**, and peak 2 represented GlcNAcβ1-6[^{3}H]Galβ1-4Glc (**9**). The peak 2 was identified as compound **9** chromatographically. It co-chromatographed with unlabelled GlcNAcβ1-6Galβ1-4Glc in the experiment of panel C. When re-chromatographed in solvent A, the radiolabelled material co-chromatographed again with the unlabelled GlcNAcβ1-6Galβ1-4Glc marker (solvent A). Peak 3 represented GlcNAcβ1-3[^{3}H]Galβ1-4Glc (**8**); peak 4 was

Table 2. The structures and paper chromatographic mobilities[a] of key saccharides of the present experiments.

No.	*Saccharide*	R_{Gal}	R_{Lac}	R_{MT}	R_{MTet}	R_{MP}
1	GlcNAcβ1 → 6,3Gal ← GlcNAcβ1			0.67		
2	3Gal ← GlcNAcβ1	0.40	1.44			
3	GlcNAcβ1 → 6Gal		0.90	1.90		
4	GlcNAcβ1 → 6,3Galβ1-OCH_3 ← GlcNAcβ1		0.53	1.19		
5	3Galβ1-OCH_3 ← GlcNAcβ1	ND[b]	ND	ND		
6	GlcNAcβ1 → 6Galβ1-OCH_3	0.47	1.47	3.30		

(*continued*)

Table 2 (*continued*)

No.	*Saccharide*	R_{Gal}	R_{Lac}	R_{MT}	R_{MTet}	R_{MP}
7	GlcNAcβ1 $^{6}_{3}$Galβ1-4Glc GlcNAcβ1				0.81	2.58
8	$_{3}$Galβ1-4Glc GlcNAcβ1			0.89	2.65	
9	GlcNAcβ1 ^{6}Galβ1-4Glc			0.64	1.91	
10	GlcNAcβ1 $^{6}_{3}$Galβ1-4GlcNAc GlcNAcβ1			0.44	1.34	
11	$_{3}$Galβ1-4GlcNAc GlcNAcβ1		0.74	1.54		
12	GlcNAcβ1 ^{6}Galβ1-4GlcNAc		0.50	1.05	3.23	

[a] R_{Gal}, R_{Lac}, R_{MT}, R_{MTet} and R_{MP} give the mobilities of the saccharides in relation to galactose, lactose, maltotriose, -tetraose, and -pentaose, respectively, in solvent B, as measured in the present experiments.
[b] ND, not determined.

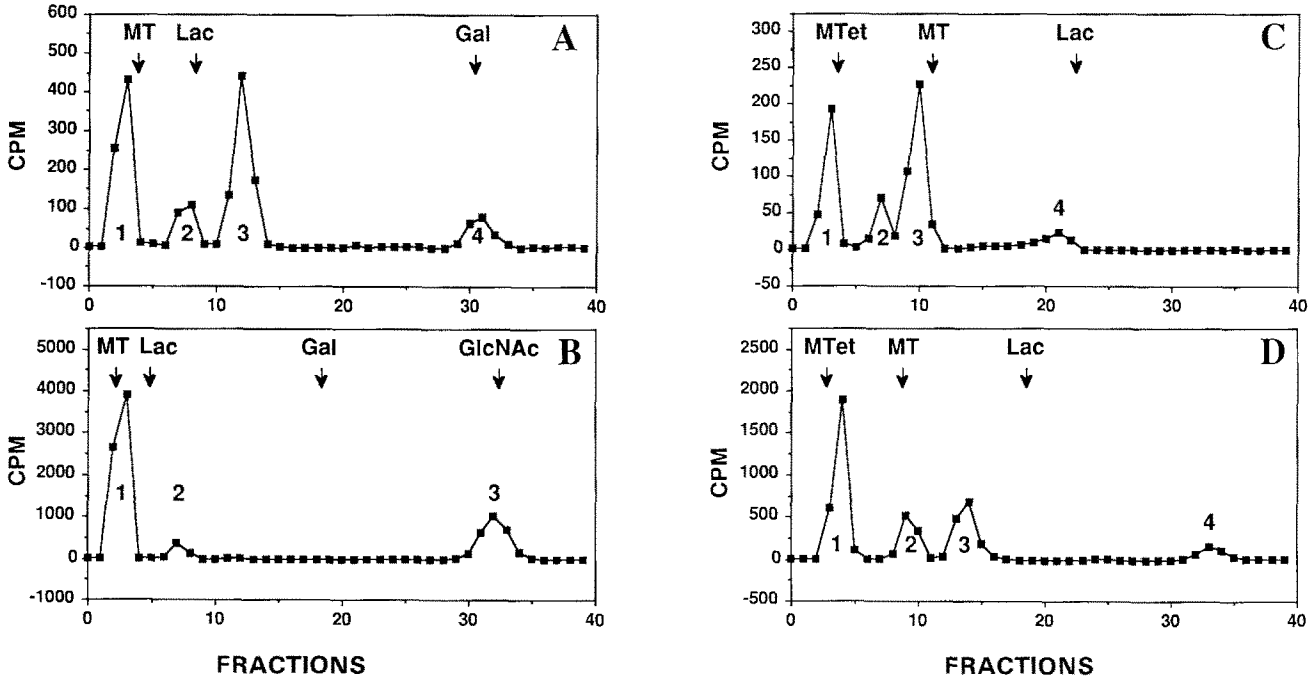

Figure 2. Paper chromatograms (solvent B) of β-*N*-acetylhexosaminidase digests. A. Digest from GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Gal (**1**); running time of the chromatogram 122 h. B. Digest from GlcNAcβ1-3([^{14}C]GlcNAcβ1-6)Galβ1-OCH_3 (**4**); running time of the chromatogram 65 h. C. Digest from GlcNAcβ1-3(GlcNAcβ1-6)[^{3}H]Galβ1-4Glc (**7**); running time of the chromatogram 241 h. D. Digest from GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Galβ1-4GlcNAc (**10**); running time of the chromatogram 256 h. The arrows marked GlcNAc, Gal, Lac, MT, and MTet show peak positions of *N*-acetylglucosamine, galactose, lactose, maltotriose and maltotetraose, respectively.

Table 3. Kinetics of β-*N*-acetylhexosaminidase-catalysed cleavage of bi-antennary oligosaccharides.

	GlcNAcβ1 → 6,3 Gal ← GlcNAcβ1 **(1)**	GlcNAcβ1 → 6,3 Galβ1-4Glc ← GlcNAcβ1 **(7)**	GlcNAcβ1 → 6,3 Galβ1-4GlcNAc ← GlcNAcβ1 **(10)**
2 min hydrolysis			
Degree of hydrolysis effected[a]	15%	20%	15%
GlcNAcβ1 → 6 Galβ1-OR formed	5.8%	9.7%	11%
GlcNAcβ1 → 3 Galβ1-OR formed	18%	27%	16%
Apparent branch specificity of the cleavage[b]	3.0	2.7	1.5
5 min hydrolysis			
Degree of hydrolysis effected[a]	37%	38%	29%
GlcNAcβ1 → 6 Galβ1-OR formed	12%	13%	17%
GlcNAcβ1 → 3 Galβ1-OR formed	41%	47%	27%
Apparent branch specificity of the cleavage[b]	3.5	3.5	1.5
10 min hydrolysis			
Degree of hydrolysis effected[a]	55%		49%
GlcNAcβ1 → 6 Galβ1-OR formed	9%		21%
GlcNAcβ1 → 3 Galβ1-OR formed	50%		32%
Apparent branch specificity of the cleavage[b]	5.3		1.5

[a] The fraction of available *N*-acetylglucosaminidic linkages cleaved.
[b] The ratio of GlcNAcβ1-3Galβ1-OR to GlcNAcβ1-6Galβ1-OR in the digest.

[^{3}H]Galβ1-4Glc. The relative amounts of the four peaks indicated that 38% of available *N*-acetylglucosaminidic linkages had been cleaved in the hydrolysis. The amount of trisaccharide **8** formed was much larger than that of trisaccharide **9**, which implies that the GlcNAcβ1-6Gal linkage of **7** had been cleaved much faster than its GlcNAcβ1-3Gal linkage.

A 5 min hydrolysis with jack bean β-*N*-acetylhexosaminidase was carried out also with the saccharide **10**, GlcNAcβ1-3(GlcNAcβ1-6)[^{14}C]Galβ1-4GlcNAc. Paper chromatography of the digest revealed four labelled compounds (Fig. 2(d)). Peak 1 was the original substrate **10**, peak 2 represented GlcNAcβ1-6[^{14}C]Galβ1-4GlcNAc (**12**), and peak 3 represented GlcNAcβ1-3[^{14}C]Galβ1-4GlcNAc (**11**); peak 4 was [^{14}C]Galβ1-4GlcNAc. The relative amounts of the four peaks indicated that 29% of available *N*-acetylglucosaminidic linkages had been cleaved. The amount of trisaccharide **11** was distinctly larger than that of trisaccharide **12**, suggesting that the GlcNAcβ1-6[^{14}C]Gal linkage of **10** had been cleaved faster than the GlcNAcβ1-3Gal linkage.

The glycan **1** was hydrolysed with β-*N*-acetylhexosaminidase also using other incubation periods. It released slightly increased relative amounts of GlcNAcβ1-6Gal in the 2 min digestion (Table 3). The 2 min experiment is believed to reflect rather precisely the primary cleavage of the original substrate, because the secondary cleavages had little opportunity to distort the distribution of the primary products. A 10 min hydrolysis of **1** gave a much decreased relative amount of GlcNAcβ1-6Gal (Table 3), implying that

the secondary cleavages were already distorting the primary picture considerably; GlcNAcβ1-6Gal (**3**) appeared to be hydrolysed much more rapidly than GlcNAcβ1-3Gal (**2**), despite the concentration difference in favour of the glycan **2**. This observation was confirmed in several longer incubations with the trisaccharide **1**, which gave even smaller relative amounts of **3** (not shown). In a 1 min experiment, where only 9% of available *N*-acetylglucosaminidic linkages were cleaved, the ratio of liberated GlcNAcβ1-3[^{14}C]Gal to GlcNAcβ1-6[^{14}C]Gal remained the same (3.0) as in the 2 min experiment. This establishes the rates of the primary cleavages in **1** with fair accuracy.

The saccharide **7** behaved rather like **1** in the 2 min hydrolysis (Table 3). Compared to the 5 min experiment, there was a distinct change in the ratio of GlcNAcβ1-3Galβ1-4Glc to GlcNAcβ1-6Galβ1-4Glc in the digest, suggesting that the secondary cleavages were distorting the ratio in the 5 min hydrolysis. Lack of material prevented 1 min and 10 min experiments with **7**, at this stage, but the evidence of Table 3 suggests that the secondary cleavage of GlcNAcβ1-6Galβ1-4Glc (**9**) was faster than that of GlcNAcβ1-3Galβ1-4GlcNAc (**8**), despite the concentration difference in favour of **8**.

The saccharide **10** behaved unlike **1** and **7**. Its cleavage in the 2 min and 10 min experiments of Table 3 gave GlcNAcβ1-3Galβ1-4GlcNAc and GlcNAcβ1-6Galβ1-4GlcNAc in the same ratio as in the 5 min experiment. This was true also in several longer incubations of **10** with the β-*N*-acetylhexosaminidase (not shown). These findings suggest that the secondary cleavages of GlcNAcβ1-3Galβ1-4GlcNAc (**11**) and GlcNAcβ1-6Galβ1-4GlcNAc (**12**) proceeded at approximately the same rates in terms of pmol min^{-1}. As the concentration favoured **11** only slightly over **12**, the rate constant of the cleavage of **12** can be only slightly larger than that of the cleavage of **11**.

The paper chromatography data of Table 2 show that prolonged runs in solvent B give remarkable separations, allowing, for instance, simultaneous separation of the saccharides **7**–**12** from each other.

Discussion

The present data enabled us to construct linear oligosaccharides of the type R_1-4GlcNAcβ1-6Galβ1-OR_2. A particularly valuable finding was that the GlcNAcβ1-6Gal bond of the tetrasaccharide **10**, and of the trisaccharide **12**, were rather slowly cleaved, making it possible to isolate the trisaccharide **12** in reasonably good yields from the partial β-*N*-acetylhexosaminidase digests of **10**. The trisaccharide **12** is a valuable primer for construction of marker oligosaccharides related to teratocarcinoma poly-*N*-acetyllactosaminoglycans; partial acid hydrolysates of these polysaccharides contain large amounts of oligosaccharides carrying the linear sequence GlcNAcβ1-6Galβ1-4GlcNAc at the reducing end [1, 2].

Our data suggest that there may be two discernible modes of cleavage among the saccharides studied. The glycans **1**–**3** and **7**–**9** were cleaved in the β-*N*-acetylhexosaminidase-catalysed reaction so that in the di-antennary saccharides, the (1-6)-linked branches were attacked with a high preference, and among the mono-antennary glycans, the isomers containing the (1-6)-linkages were hydrolysed much faster than the isomers containing the (1-3)-linkages. In contrast, the saccharides **10**–**12** were cleaved so that in the di-antennary **10**, the (1-6)-linked branch was attacked with only a modest preference over the (1-3)-linked branch, and among the mono-antennary saccharides, **12**, which contains the (1-6)-linkage, was hydrolysed only slightly faster than **11**, which contains the (1-3)-linkage.

It is noteworthy that the fully β(1-4)-galactosylated forms of **1** [11] and **7** [O. Renkonen *et al.*, unpublished] are degalactosylated rapidly at the (1-6)-branch, and slowly at the (1-3)-branch by *E. coli* β-galactosidase, while the fully β(1-4)-galactosylated form of **10** is degalactosylated equally rapidly at both branches [12]. This difference appears to be due to an exceptionally slow degalactosylation reaction of the (1-6)-linked branch, at least in the galactosylated form of **10** [12]. The saccharides **1**, **7**, and **10** differ markedly even in their enzymatic β(1-4)-galactosylation: all are galactosylated preferentially at the (1-6)-linked *N*-acetylglucosamine, but **1** [13] and **7** [O. Renkonen *et al.*, unpublished] react with higher branch specificities than **10** [O. Renkonen *et al.*, unpublished]. A unifying hypothesis may explain all these observations: we believe that the reducing end *N*-acetylglucosamine of **10**, **12**, and the galactosylated derivative of **10**, may interact with the *N*-acetylglucosamine unit of the (1-6)-linked branch, thereby reducing its "mobility" and reactivity in de-*N*-acetylglucosaminylation, in de-galactosylation, and also in galactosylation. The *N*-acetyl group of the reducing end *N*-acetylglucosamine unit appears to be involved in this interaction; in the glucose-containing analogues, the reducing end obviously fails to inhibit the reactivity of the (1-6)-linked branch. In **1**, **3**, and the galactosylated derivative of **1**, the reducing end also fails to inhibit the reactivity of the (1-6)-linked branch.

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