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HYDROGEN FLUORIDE-CATALYZED FORMATION OF GLYCOSIDES. PREPARATION OF METHYL 2-ACETAMIDO-2-DEOXY-β-D-GLUCO- AND -β-D-GALACTO-PYRANOSIDES, AND OF β-(1→6)-LINKED 2-ACETAMIDO-2-DEOXY-D-GLUCO- AND -D-GALACTO-PYRANOSYL OLIGOSACCHARIDES*†

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ABSTRACT

Dissolution of 2-acetamido-2-deoxy-D-glucose (**1**) or -D-galactose (**2**) in anhydrous hydrogen fluoride, followed by addition of methanol, gave stereospecifically the corresponding methyl β-D-glycopyranosides **7** and **8**. When solutions of **1** or **2** in hydrogen fluoride were slowly evaporated, mixtures of exclusively β-D-(1→6)-linked di- to hexa-saccharides containing 2-acetamido-2-deoxy-glucosyl (**9**) and -galactosyl (**10**) residues were obtained; these were separated by gel permeation chromatography to give pure products. Compounds **7** and **9** were also obtained when solutions of chitin were treated under appropriate conditions.

INTRODUCTION

In a previous paper, the behavior of chitin in anhydrous hydrogen fluoride was described[2]. It was found that chitin was readily soluble in 5 to 10 parts of hydrogen fluoride at 20°, and that it was slowly depolymerized to give, after a few h, a mixture of β-D-(1→4)-linked oligomers which could be separated by gel-permeation chromatography. After 16 h, a solution of chitin in hydrogen fluoride contained almost exclusively the monomeric pyranosyl **3** and furanosyl **5** oxazolinium ions, which yielded, after a workup involving precipitation with ether and dissolution in water, 2-acetamido-2-deoxy-D-glucose. A quite similar behavior was found[2] for the latter compound when dissolved in hydrogen fluoride. Oligosaccharides which could result from *trans*-acetalation, i.e., reversion reactions,

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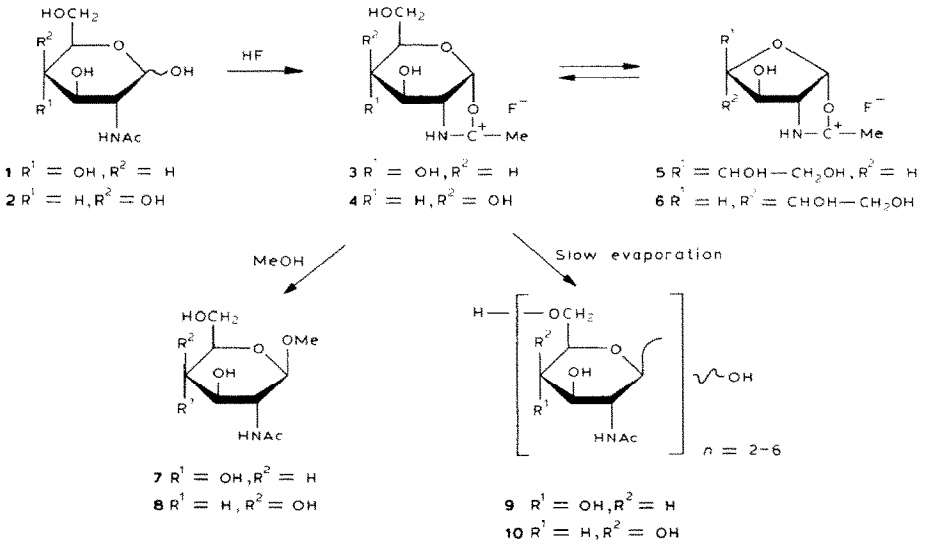
were not observed under these conditions and this behavior contrasts with that previously described for amylose and cellulose. In fact, when these neutral polysaccharides are dissolved in hydrogen fluoride, a much more rapid degradation takes place; however, the glycopyranosyl entities formed undergo simultaneous reversion to yield a complex mixture of oligosaccharides including positional isomers as well as higher homologs. D-Glucose behaved similarly when dissolved in hydrogen fluoride; the amount of reversion products formed were found to depend on the carbohydrate concentration and, when hydrogen fluoride solutions of amylose, cellulose, or D-glucose were evaporated, almost complete conversion into reversion products were observed[3]. This enhanced reactivity of carbohydrates towards reversion reactions in hydrogen fluoride, as compared to usual protonating agents, was ascribed to the expected enhanced stabilization of positively charged species, like oxocarbenium ions, in this solvent.

Taking into account that oxazolinium ion **5** was the main compound present in hydrogen fluoride solutions of chitin or 2-acetamido-2-deoxy-D-glucose[2], one could expect that, under appropriate conditions, such reactive entities could be converted into glycosides, or could result in the formation of reversion oligosaccharides. In the present paper, the hydrogen fluoride-catalyzed formation of methyl glycosides, and of reversion products from 2-acetamido-2-deoxy-D-glucose, chitin, and from 2-acetamido-2-deoxy-D-galactose as well, is described.

RESULTS AND DISCUSSION

When 2-acetamido-2-deoxy-D-glucose (**1**) or 2-acetamido-2-deoxy-D-galactose (**2**) were dissolved in hydrogen fluoride and the solution was subsequently treated with an excess of methanol, the corresponding methyl β-D-glycopyranosides **7** and **8** were obtained in good yield. The α-D anomers were not observed in the



^{13}C-n.m.r. spectra of the crude reaction mixtures. Such excellent stereospecificity is in agreement with a control of the glycosidation reaction involving, as anticipated, an oxazolinium intermediate as in **3** and **4**. It allows a more convenient access to **7** and **8** than the conventional acid–alcohol Fischer glycosidation technique which gives anomeric mixtures[4]. It is more specific and gives higher yields as compared to the diazomethane procedure[5], and is also much more convenient than the Koenigs–Knorr method which was also proposed for the preparation of **7** and **8** (refs. 6–8). It could then be considered as a general procedure for the preparation of alkyl 2-acetamido-2-deoxy-β-D-glycopyranosides. Readily available glycosaminoglycans can furthermore be used as starting materials for such syntheses, since addition of methanol to a solution of chitin kept in hydrogen fluoride at room temperature for 10 h led to **7** in a good yield.

These results let us to expect that appropriate reversion conditions might be selected in order to enhance the selectivity in the formation of specifically linked oligosaccharides, and this has been found. When 2-acetamido-2-deoxy-D-glucose (**1**) was dissolved in hydrogen fluoride and the solution was kept at 20° in an open bottle for 15 h, allowing the hydrogen fluoride to evaporate slowly, a product was obtained which contained starting material and considerable quantities of β-(1→6)-linked oligomers **9** with d.p. ranging up to 6. A ^{13}C-n.m.r. spectrum of the product

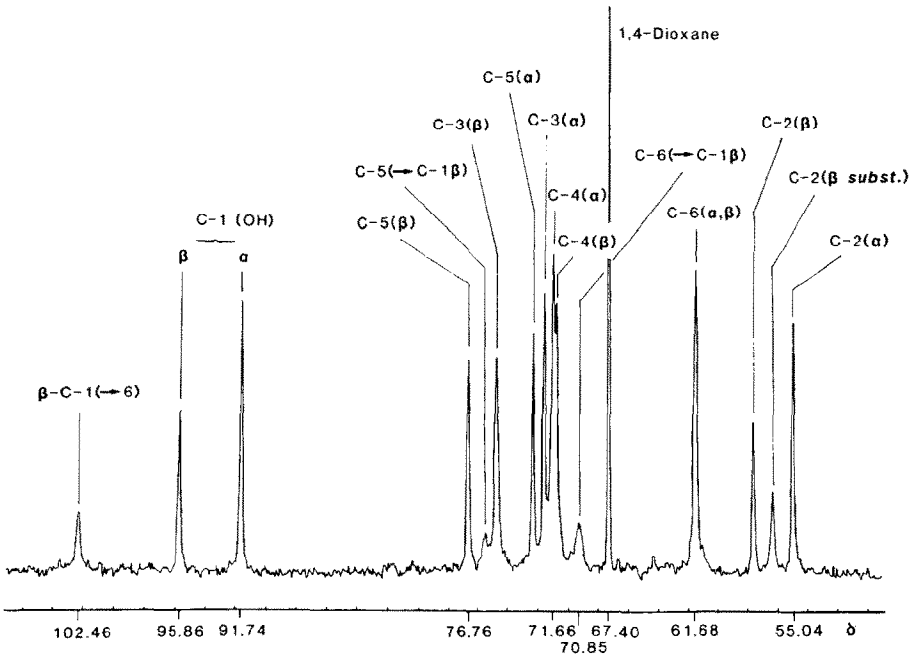


Fig. 1. ^{13}C-N.m.r. spectrum at 22.63 MHz of a solution in deuterium oxide of reaction products obtained by treatment of 2-acetamido-2-deoxy-D-glucose with hydrogen fluoride in an open bottle for 15 h at 20°.

TABLE I

YIELDS, MELTING POINTS, OPTICAL ROTATIONS, MASS SPECTROMETRY (F.A.B.), AND ANALYTICAL DATA FOR β-(1→6)-LINKED 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSYL OLIGOSACCHARIDES (D.P. 2–6) RESULTING FROM THE ACTION OF HYDROGEN FLUORIDE ON 2-ACETAMIDO-2-DEOXY-D-GLUCOSE[a] (1)

Oligosaccharide d.p.(n)	*Yields (%)*	*M.p. (degrees)*[b]	$[\alpha]_D^{25}$ *(degrees)*[c]	m/z $[M+H]^{+d}$	*Analytical data*[e]						*Lit.*		*Ref.*
					Calc.			*Found*			*M.p. (degrees)*	$[\alpha]_D$ *(degrees)*	
					C	*H*	*N*	*C*	*H*	*N*			
					For $C_{16}H_{28}N_2O_{11}$								
n = 2	30	163 (dec.)	+0.7 (4.55)	425	45.28	6.64	6.60	45.42	6.81	6.39	199–201	+10	9
											200 (dec.)	+1.2	10
											200	+8	11
					For $C_{24}H_{41}N_3O_{16}$								
n = 3	15	191–193 (dec.)	−4.0 (1.57)	628	45.93	6.57	6.69	45.73	7.01	6.45			
					For $C_{32}H_{54}N_4O_{21}$								
n = 4	6.6	195–197 (dec.)	−7.4 (1.62)	831	46.26	6.54	6.74	45.77	6.99	6.73			
					For $C_{40}H_{67}N_5O_{26}$								
n = 5	2	203–206 (dec.)	−14.0 (0.89)	1034	46.46	6.52	6.77	45.60	6.83	6.82			
					For $C_{48}H_{80}N_6O_{31}$								
n = 6	1.3	205–207 (dec.)	−20.0 (0.72)	1237	46.60	6.51	6.79	45.60	6.95	6.51			

[a]From 2-acetamido-2-deoxy-D-glucose (5 g), treated with hydrogen fluoride (10 mL) for 15 h, followed by a single chromatographic fractionation using water as eluent. [b]Freeze-dried from water. [c]For a solution in water at equilibrium, *c* in parentheses. [d]In f.a.b.-ionization mode; internal fragmentation not given. [e]Each sample dried for 24 h at 60° under vacuum.

TABLE II

^{13}C-N.M.R. SPECTRAL DATA FOR β-(1→6)-LINKED 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSYL OLIGOSACCHARIDES (D.P. 2–5) IN DEUTERIUM OXIDE

$[\beta$-D-*(1→6)-GlcpNAc*$]_n$	*Chemical shifts (δ)*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
n = 2	91.70 (α) 95.82 (β) 102.49 (2α) 102.59 (2β)	54.86 (α) 57.54 (β) 56.32 (2)[b]	71.30 (α) 74.72 (β) 74.56 (2)[b]	70.92 (α) 70.74 (β) 70.74 (2)[b]	71.53 (α) 75.68 (β) 76.68 (2)[b]	69.37 (α) 69.62 (β) 61.56 (2)[b]
n = 2[c]	91.98 (α) 96.09 (β) 102.79 (2)	55.16 (α) 57.84 (β) 56.62 (2)	71.59 (α) 75.00 (β) 74.84 (2)	71.17 (α) 71.03 (β) 71.03 (2)	71.83 (α) 75.95 (β) 76.97 (2)	69.67 (α) 69.88 (β) 61.85 (2)
n = 3	91.70 (α) 85.83 (β) 102.47 (2α)[b] 102.56 (2β)[b] 102.33 (3)[b]	54.87 (α) 57.57 (β) 56.27, 56.31 (2,3)[b]	71.24 (α) 74.76 (β) 74.56, 74.51 (2,3)[b]	70.93 (α) 70.76 (β) 70.76, 70.84 (2,3)[b]	71.56 (α) 75.61 (β) 75.49 (2)[b] 76.69 (3)[b]	69.28 (α) 69.54 (β) 69.33 (2)[b] 61.55 (3)[b]
n = 4	91.70 (α) 95.83 (β) 102.45 (2α)[b] 102.53 (2β)[b] 102.36 (2C)	54.88 (α) 57.58 (β) 56.32 (1C) 56.27 (2C)	71.22 (α) 74.77 (β) 74.55 (3C)	70.95 (α) 70.76 (β) 70.76 (1C) 70.81 (2C)	71.57 (α) 75.58 (β) 75.46, 75.42 (2,3)[b] 76.70(4)[b]	69.25 (α) 69.51 (β) 69.33 (2C) 61.56 (4)[b]
n = 5	91.70 (α) 95.83 (β) 102.38 (4C)	54.88 (α) 57.58 (β) 56.32 (2C) 56.27 (2C)	71.22 (α) 74.56 (β) 74.56 (4C)	70.83 (α) 70.76 (β) 70.83 (2C) 70.76 (2C)	71.57 (α) 75.46 (β) 75.45 (1C) 75.38 (2C) 76.71 (5)[b]	69.29 (α) 69.76 (β) 69.30 (3C) 61.57 (5)[b]

[a]Relative to 1,4-dioxane (δ 67.4) as internal standard. [b]Number(s) in parentheses indicates the 2-acetamido-2-deoxy-D-glucopyranosyl unit, numbered from the reducing end, to which belongs the assigned carbon atom(s). [c]Ref. 12.

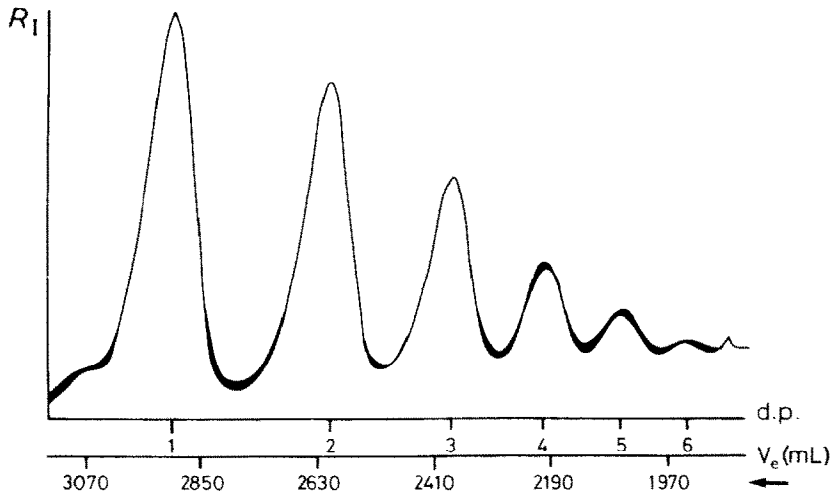


Fig. 2. Elution profile of a sample of β-(1→6)-linked 2-acetamido-2-deoxy-D-galactopyranosyl oligosaccharides (**10**, 2 g) in Bio-Gel P-4 (200–400 mesh, 350 g) with water as eluent at a flow rate of 110 mL/h.

(Fig. 1) showed clearly the formation of β-D-glycosidic linkages from the signal at δ ~102.4 and the substitution at C-6 from the shift to δ 70.8 of the C-6 signal (δ 61.68) of the starting material. The products were separated by gel-permeation chromatography on Bio-Gel P-4 (Pharmacia) using water as eluent, to give each of the pure β-(1→6)-linked oligosaccharides which were characterized through their analyses, optical rotations, and mass (Table I) and ^{13}C-n.m.r. spectra (Table II). A similar treatment of 2-acetamido-2-deoxy-D-galactose (**2**) yielded an analogous series of β-(1→6)-linked oligosaccharides (**10**) which were separated in the same way (Fig. 2) and characterized as shown in Tables III and IV. In both the reactions mentioned above, only β-(1→6)-linked oligomers were found.

As shown above for the preparation of methyl 2-acetamido-2-deoxy-D-glucose, readily available chitin may be used as well for the one step preparation of the β-(1→6)-linked oligosaccharides of 2-acetamido-2-deoxy-D-glucose listed in Table I. Slow evaporation of a hydrogen fluoride solution of chitin, previously kept for ~10 h at room temperature, led to a crude mixture which contained almost exclusively these oligomers. Since interferences with *N*-deacetylated 2-amino-2-deoxy-D-glucosyl constituents of the native chitin might be expected in the gel exclusion chromatography of this crude mixture, as previously shown for the preparation of β-(1→4)-linked 2-acetamido-2-deoxy-D-glucopyranosyl oligosaccharides[2], a two-step fractionation on Bio-Gel P-4 using successively 50mM aqueous ammonium acetate and then water was carried out. An almost comparable yield, as reported in Table I for reversion products from **1**, was obtained. This procedure may, thus, be used for the large-scale preparation of β-(1→4)-linked 2-acetamido-

TABLE III

YIELDS, MELTING POINTS, OPTICAL ROTATIONS, MASS SPECTROMETRY (F.A.B.), AND ANALYTICAL DATA FOR β-(1→6)-LINKED 2-ACETAMIDO-2-DEOXY-D-GALACTOPYRANOSYL OLIGOSACCHARIDES (D.P. 2–6) RESULTING FROM THE ACTION OF HYDROGEN FLUORIDE ON 2-ACETAMIDO-2-DEOXY-D-GALACTOSE[a] (2)

Oligosaccharide d.p.(n)	*Yields (%)*	*M.p. (degrees)*[b]	$[\alpha]_D^{25}$ *(degrees)*[c]	m/z $[M+H]^{+d}$	*Analytical data*[e]						*Lit.*		*Ref.*
					Calc.			*Found*			*M.p. (degrees)*	$[\alpha]_D$ *(degrees)*	
					C	*H*	*N*	*C*	*H*	*N*			
					For $C_{16}H_{28}N_2O_{11}$								
n = 2	28	182	+46.0 (2.05)	425	45.3	6.6	6.6	45.92	6.81	6.39	syrup	+42.5	13
					For $C_{24}H_{41}N_3O_{16}$								
n = 3	21	202	+32.5 (0.73)	628	45.9	6.6	6.7	45.73	7.01	6.45			
					For $C_{32}H_{54}N_4O_{21}$								
n = 4	11	178–179	+31.5 (0.9)	831	46.3	6.5	6.7	45.77	6.99	6.73			
					For $C_{40}H_{67}N_5O_{26}$								
n = 5	6	201	+30.5 (0.75)	1034	46.5	6.5	6.8	45.60	6.83	6.82			
					For $C_{48}H_{80}N_6O_{31}$								
n = 6	2.6	215–216	+28.5 (0.4)	1237	46.6	6.5	6.8	45.1	6.95	6.51			

[a]From 2-acetamido-2-deoxy-D-galactose (2 g), treated with hydrogen fluoride (10 mL) for 15 h, followed by a single chromatographic fractionation using water as eluent. [b]Freeze-dried from water. [c]For a solution in water at equilibrium, *c* in parentheses. [d]In f.a.b.-ionization mode; internal fragmentation not given. [e]Each sample dried for 24 h at 60° under vacuum.

TABLE IV

^{13}C-N.M.R. SPECTRAL DATA FOR β-(1→6)-LINKED 2-ACETAMIDO-2-DEOXY-D-GALACTOPYRANOSYL OLIGOSACCHARIDES (D.P. 2–4) IN DEUTERIUM OXIDE

$[\beta$-D-*(1→6)-Gal*p*NAc*$]_n$	*Chemical shifts (δ)*[a]					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
n = 2	91.89 (α)	51.11 (α)	68.08 (α)	69.34 (α)	69.88 (α)	69.88 (α)
	96.20 (β)	54.60 (β)	71.80 (β)	68.63 (β)	74.67 (β)	69.80 (β)
	102.90 (2)[b]	53.22 (2)[b]	71.80 (2)[b]	68.63 (2)[b]	75.96 (2)[b]	61.83 (2)[b]
	103.01 (2)					
n = 2[c]	92.2 (α)	51.5 (α)	68.5 (α)	69.7 (α)	70.3 (α)	69.0 (α)
	96.6 (β)	55.0 (β)	72.2 (β)	69.0 (β)	75.1 (β)	69.0 (β)
	103.3 (2)[b]	53.6 (2)[b]	72.2 (2)[b]	69.0 (2)[b]	76.3 (2)[b]	62.2 (2)[b]
n = 3	91.89 (α)	51.11 (α)	68.09 (α)	69.34 (α)	69.82 (α)	69.82 (α)
	96.25 (β)	54.61 (β)	71.78 (β)	68.65 (β)	74.44 (β)	69.82 (β)
	102.77 (2C)	53.21 (2C)	71.78 (2C)	68.65 (2C)	75.96 (2C)	69.82 (2)[b]
						61.86 (3)[b]
n = 4	91.89 (α)	51.12 (α)	68.09 (α)	69.34 (α)	69.71 (α)	69.82 (α)
	96.25 (β)	54.61 (β)	71.79 (β)	68.65 (β)	74.40 (β)	69.82 (β)
	102.83 (3C)	53.22 (2C)	71.79 (2C)	68.64 (3C)	75.97 (3C)	69.71 (3C)
		53.15 (1C)	71.64 (1C)			61.86 (4)[b]

[a]Relative to 1,4-dioxane (δ 67.4) as internal standard. [b]Number(s) in parentheses indicates the 2-acetamido-2-deoxy-D-galactopyranosyl unit, numbered from the reducing end, to which belongs the assigned carbon atom(s). [c]Ref. 14.

2-deoxy-D-glucopyranosyl oligosaccharides, of interest as building blocks for various biopolymers[9,15] and analogs.

Acid reversion of 2-acetamido-2-deoxy-D-glucose has already been reported to occur in the presence of moist hydrogen chloride vapor for four weeks, leading preponderantly, although in low yield, to an anomeric mixture of (1→6)-linked 2-acetamido-2-deoxy-D-glucopyranosyl disaccharides[10]. The high stereospecificity obtained in the present work, with the exclusive formation of β-(1→6) linkages in the D-*gluco* and D-*galacto* series as well, and the enhanced formation of higher d.p. oligomers, is obviously connected with an increase in the ease of formation of oxazolinium ions related with an enhanced stabilization of such species in hydrogen fluoride. It should, however, be noticed that, although a solution of *N*-acetamido-2-deoxy-D-glucose (**1**) in hydrogen fluoride contains a substantial proportion of the pyranosyl oxazolinium ion **3** in admixture with a major proportion of the corresponding furanosyl ion **5**, a ^{13}C-n.m.r. spectrum of 2-acetamido-2-deoxy-D-galactose (**2**) in hydrogen fluoride solution showed virtually one product only (C-1 and C-4 at δ 116 and 91, respectively) assumed to be the furanose-oxazolinium ion **6**. The corresponding pyranosyl ion **4** could not be observed with certainty. The formation of the β-(1→6)-linked oligomers and the methyl β-pyranosides probably takes place *via* oxazolinium ions **3** and **4**. Hence, it can be assumed that although the furanose ions **5** and **6** are the main species present in hydrogen fluoride solution, the pyranose ions **3** and **4** are probably the reactive entities. Another possibility would be that β-(1→6)-linked glycofuranosyl oligosaccharides, which could be formed initially through the ions **5** and **6**, could rearrange through protonation at O-4 into the thermodynamically stable pyranosyl oligomers, possibly due to the enhanced driving force of the exo-anomeric effect in pyranosyl structures.

EXPERIMENTAL

General methods. — Melting points were determined with a Zeiss microscope hot-stage, and are corrected. Optical rotation were measured with a Perkin–Elmer 241 instrument. The ^{13}C-n.m.r. spectra were recorded with Bruker WH-90, WP-100, and AM-500 instruments. Spectra of hydrogen fluoride solutions were obtained in Teflon tubes that fitted tightly inside a 10-mm glass sample tube [($^2H_6$)acetone was used as lock substance and internal reference taken at δ 29.2]. Spectra of isolated oligosaccharides (Tables II and IV) were recorded for solutions in D_2O (internal 1,4-dioxane, δ 67.40). Mass spectra in the f.a.b. (+)-ionisation mode (Xe, glycerol matrix) were recorded for oligosaccharides of d.p. 2–6 with a double-focusing Kratos-AEI MS-50 apparatus (Manchester), fitted with a 1.2-T magnet, and operating at the full accelerating potential (6 kV); an f.a.b. 11 NF, Ion Tech atom-gun; and an MAT SS 200 Finnigan (DEC-PDP 11-34) computer.

Anhydrous hydrogen fluoride was a commercial product obtained in steel cylinders. Prior to use, it was kept in polyethylene bottles at −20°. All reactions with hydrogen fluoride were conducted in polyethylene bottles. Chitin was pur-

chased from Sigma Chemical Co. (St. Louis, MO). It originated from crab shells and was practical grade. The *N*-acetyl content was higher than 90% as determined by i.r. spectroscopy. Ultrafiltration was performed with Amicon equipment on YCO5 6.2-cm (diam.) membranes and 200-mL cells. Microanalyses were obtained on compounds dried under vacuum (60°, 70 Pa, 24 h).

The equipment used in the gel-permeation chromatography of β-(1→6)-linked oligosaccharides consisted of two 5 × 100 cm (preparative K50/100) glass columns (Pharmacia, Uppsala), filled with Bio-gel P-4 (200–400 mesh; Pharmacia), fitted to a Milton–Roy controlled-volume pump (minipump A, 35 MPa; Dosapro, Pont St Pierre, France) operating at a flow rate of 110 mL/h. A refractive-index detector (Waters, model R401) was used on line as concentration detector. The sample (2 g), dissolved in the eluent (5 mL), was introduced on the top of the column by means of an injection loop (Rheodyne, Cotati, CA; model 7010). Fractions were collected with an LKB (Bromma, Sweden) 2112 Redirac fraction collector with a 10-min spacing time between each collection. Fractions of similar hydrodynamic volume (corresponding to base-peak width) were pooled, concentrated, and freeze-dried.

*Methyl 2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**7**). — (*a*) *From 2-acetamido-2-deoxy-*D*-glucose.* 2-Acetamido-2-deoxy-D-glucose (**1**; 2.5 g) was dissolved in hydrogen fluoride (10 mL) at 0° and the temperature of the solution was allowed to rise to 20°. After 1 h, the solution was cooled to 0°; methanol (50 mL) was added, and the mixture kept for 45 min at 20°. It was then cooled to −70° (dry ice) and poured into cold ether (600 mL) to give a precipitate which was filtered off, washed with ether, and dried. A ^{13}C-n.m.r. spectrum of this product (2.5 g) showed only the methyl β-glycoside[16] **7**. Recrystallization from water–ethanol gave 1.9 g (71%), m.p. 204–205°, $[\alpha]_D^{20}$ −47.5° (*c* 2.0, water); lit.[5] m.p. 196–197°, $[\alpha]_D^{20}$ −43° (water); lit.[6] m.p. 204–204.5°, $[\alpha]_D^{20}$ −44.3° (water); lit.[7] m.p. 204°, $[\alpha]_D^{19}$ −47° (water).

(*b*) *From chitin.* Chitin (5 g) was suspended in hydrogen fluoride (20 mL) at 0° and the suspension stirred while the temperature was allowed to rise to 20°. The clear solution, obtained after 10 min, was kept overnight at 20°. It was then cooled to 0°, methanol (50 mL) was added, and the mixture was kept for 45 min at room temperature, and then cooled to −70° and poured in cold ether. Work-up was then performed as in (*a*) to yield 4.5 g of product. Recrystallization from water–ethanol gave 3 g (52%) of **7**.

*Methyl 2-acetamido-2-deoxy-β-*D*-galactopyranoside* (**8**). — The technique described for the preparation of **7** (*a*) was followed, starting from 2-acetamido-2-deoxy-D-galactose **2** (4 g). A ^{13}C-n.m.r. spectrum of the crude product, obtained after the ether precipitation step (3.8 g), showed that it contained only the methyl β-glycoside **8**. Recrystallization from water–ethanol gave 2 g (47%), m.p. 188–189°, $[\alpha]_D^{20}$ −12° (*c* 1, water); lit.[8] m.p. 191–193°, $[\alpha]_D^{23}$ −12° (*c* 1.05, methanol); ^{13}C-n.m.r. (25.182 MHz, D_2O): δ 103.2 (C-1), 75.9 (C-5), 72.0 (C-4), 61.8 (C-6), 57.7 (OCH_3), 53.1 (C-2), and 23.0 ($COCH_3$).

Preparation of β-(1→6)-linked 2-acetamido-2-deoxy-D-glucopyranosyl oligosaccharides (**9**). — (*a*) *From 2-acetamido-2-deoxy-D-glucose*. 2-Acetamido-2-deoxy-D-glucose (**1**; 5 g) was dissolved in anhydrous hydrogen fluoride (10 mL) at 0°. The solution was then stirred at 20° in an open bottle, allowing the hydrogen fluoride to evaporate slowly. After 15 h, a sticky residue was obtained, which was dissolved in water (10 mL) and made neutral with $CaCO_3$. Filtration and freeze-drying of the aqueous solution gave a crude product (~5 g) which was separated by gel-exclusion chromatography using the equipment described in the general methods and water as eluent. Yields and data for the products thus obtained are given in Tables I and II.

(*b*) *From chitin*. Chitin (10 g) was suspended in hydrogen fluoride (50 mL) at 0° and the suspension stirred while the temperature was allowed to rise to 20°. The clear solution, obtained after 10 min, was kept for 8–10 h at room temperature in a closed bottle, which was then opened to allow the hydrogen fluoride to evaporate slowly. The sticky residue obtained after 15 h was then dissolved in water (20 mL) and made neutral with $CaCO_3$. Filtration and freeze-drying gave a crude product (12 g) which was separated by gel-exclusion chromatography using the above equipment and a two-steps separation technique[2]. Firstly, the mixtures were fractionated in 50mM aqueous ammonium acetate at pH 4.5, and the recovered fractions were then rechromatographed on the same column with water as eluent. The resulting oligosaccharide distribution is similar to that described in Table I.

Preparation of β-(1→6)-linked 2-acetamido-2-deoxy-D-galactopyranosyl oligosaccharides (**10**). — The technique is identical to that used for the preparation of the β-(1→6)-linked 2-acetamido-2-deoxy-D-glucopyranosyl oligosaccharides but starts from 2-acetamido-2-deoxy-D-galactose (5 g). Elution profile, yields, and data are given in Fig. 2 and Tables III and IV.

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