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A convenient access to β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl fluoride oligosaccharides and β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides by fluorolysis and fluorohydrolysis of chitosan [1]

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Abstract

β-(1 → 4)-Linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides, in the form of their α-glucopyranosyl fluorides at the reducing end, were obtained by fluorolysis of chitosan in anhydrous hydrogen fluoride at room temperature. The average dp depended on the reaction time and was conveniently monitored by ^{13}C NMR spectroscopy, using the signal ratios for β-(1 → 4) bonded C-1 at ~ 98.5 ppm and the C-1 doublet for the terminal glycosyl fluoride moiety at ~ 104 ppm. Preparative fractionation of dp 2–11 glycosyl fluoride oligosaccharides, obtained after 18 h of fluorolysis, was achieved by gel-permeation chromatography on Bio-Gel P-4 with aqueous acetic acid–ammonium acetate as eluent. Hydrolysis of the anomeric fluoride, with either aqueous perchloric acid, or by a sequence involving formation of the C-2 *N*-trifluoroacetate and subsequent simultaneous hydrolysis of the glycosyl fluoride and the amide substituent with aqueous methanol, yielded the free β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides which were separated, for dp 2–11, by the same gel-exclusion technique. Both oligosaccharide series, either free or in the form of their α-glycopyranosyl fluorides, were fully characterized.

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[1] For a review recording a preliminary account of these results, see ref 1. Part 17 in the Series Carbohydrate Reactivity in Anhydrous Hydrogen Fluoride. For part 16, see ref 2.

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Key words: Chitin; Hydrolysis; β-(1 → 4)-2-Amino-2-deoxy-D-glucopyranosyl oligosaccharides; Hydrogen fluoride

1. Introduction

β-(1 → 4)-Linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides were initially obtained by partial hydrolysis of chitosan, a commercial β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranan, with hydrochloric acid and separation of the oligosaccharide mixture by ion-exchange chromatography [3,4]. A recent improvment of this methodology involves the use of steric-exclusion chromatography for the separation of the oligosaccharide mixture [5]. Controlled nitrous acid deamination, followed by Sephadex gel filtration, has also been proposed [6] for their preparation, however, with the inherent limitation that it results in the 2,5-anhydro-D-mannose-terminated glycosides of the expected 2-amino-2-deoxy-D-glucose oligosaccharides. Depolymerization of chitosan to D-glucosamine oligosaccharides has also been achieved by enzymic hydrolysis using a *Trichoderma viride* cellulase [7]. Increased interest in chitosan oligosaccharides which have been found [8], *inter alia,* to play a significant role in eliciting defense-related responses to fungal parasites in various plants, has furthermore led to a recent stepwise synthesis of β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides, up to the dodecamer, from 2-amino-2-deoxy-D-glucose [9].

In a previous report in this series [10], β-(1 → 4)-linked oligosaccharides of 2-acetamido-2-deoxy-D-glucose were smoothly prepared by hydrogen fluoride (HF) fluorolysis of the commercial β-(1 → 4)-linked 2-acetamido-2-deoxy-D-glucopyranan, chitin. The main interest in this technique, as compared to methodologies involving more conventional mineral acids, is that it could be achieved at ambient or near sub-ambient temperature, without appreciable side-product formation, the average dp of the resulting oligosaccharide mixture being conveniently monitored by time and temperature control of the reaction. This concept is now extended to the preparation of β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides from the readily available 4-linked 2-amino-2-deoxy-D-glucopyranan, chitosan. Interestingly, this approach resulted in the initial preparation of the related, stable glycopyranosyl fluoride oligosaccharides, of interest as precursors in oligosaccharide synthesis [11].

2. Results and discussion

Dissolution of chitosan in anhydrous HF was slow as compared to chitin [10]. Nevertheless, a clear, homogeneous solution could be obtained in the course of ~ 20–30 min, when the polysaccharide was stirred in ~ 12 parts of HF at room temperature. A ^{13}C NMR spectrum of this HF solution, recorded after 5 h, was in agreement with an almost unchanged β-(1 → 4)-linked 2-amino-2-deoxy-glucopyranan structure, with six well-resolved signals (Fig. 1A). After 18 h (Fig. 1B), a

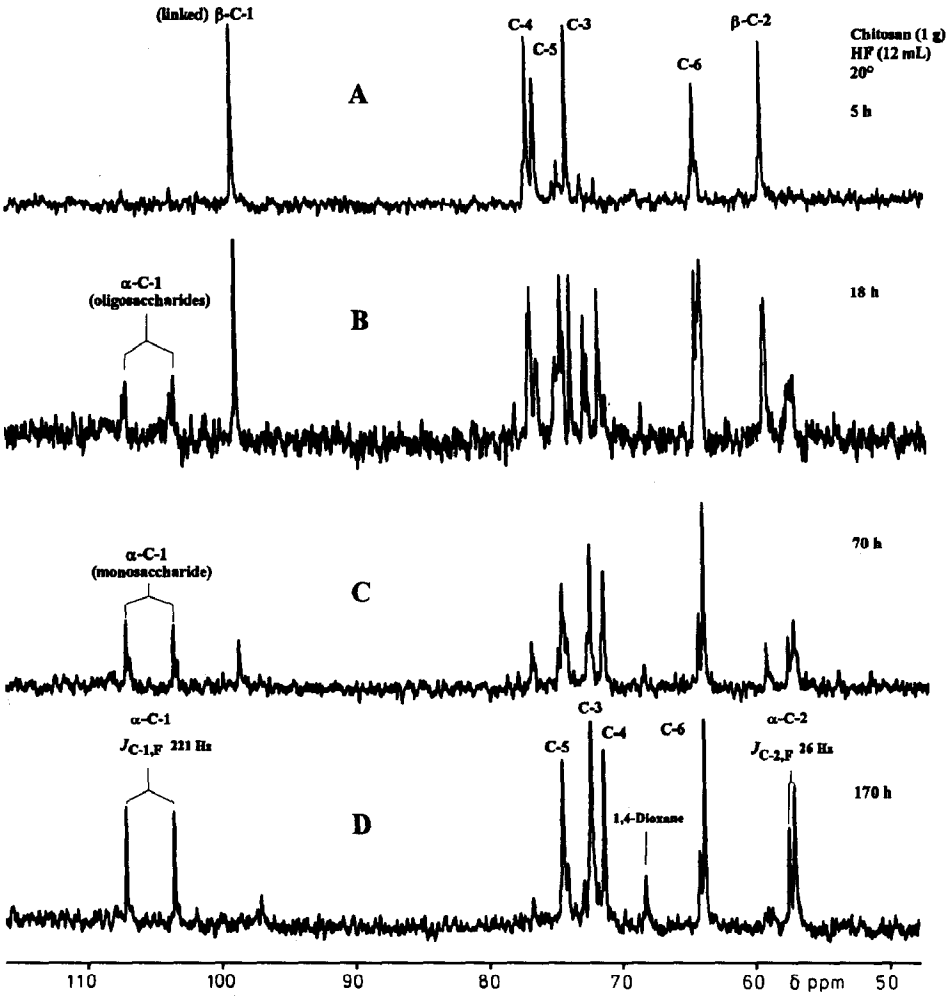


Fig. 1. Comparative ^{13}C NMR spectra at 22.9 MHz for solutions of chitosan in anhyd HF at 20°C. The spectra were measured after: A, 5; B, 18; C, 70; and D, 170 h with 1,4-dioxane as internal reference at δ 68.4. Numbers in brackets refer to Scheme 1.

large doublet for C-1 in the glycosyl fluoride at ~ 104 ppm ($J_{C-1,F}$ ~ 221 Hz) became apparent, together with a doublet at δ 54.7 ($J_{C-2,F}$ ~ 26 Hz), indicating [12] that some 2-amino-2-deoxy-α-D-glucopyranosyl fluoride residues had formed. After 70 h at 20°C, increasing signals at 54.7 and 104 ppm, together with signal splittings for C-4 and C-6 (Fig. 1C), confirmed that the glycosyl fluoride content of the solution increased gradually with time. A spectrum recorded after 170 h (Fig. 1D) was found to be identical to a spectrum of 2-amino-2-deoxy-D-glucose in HF solution, indicating that the fluorolysis of chitosan into 2-amino-2-deoxy-α-D-glucopyranosyl fluoride hydrofluoride was complete and, furthermore, that the reaction did not result in appreciable side-product formation.

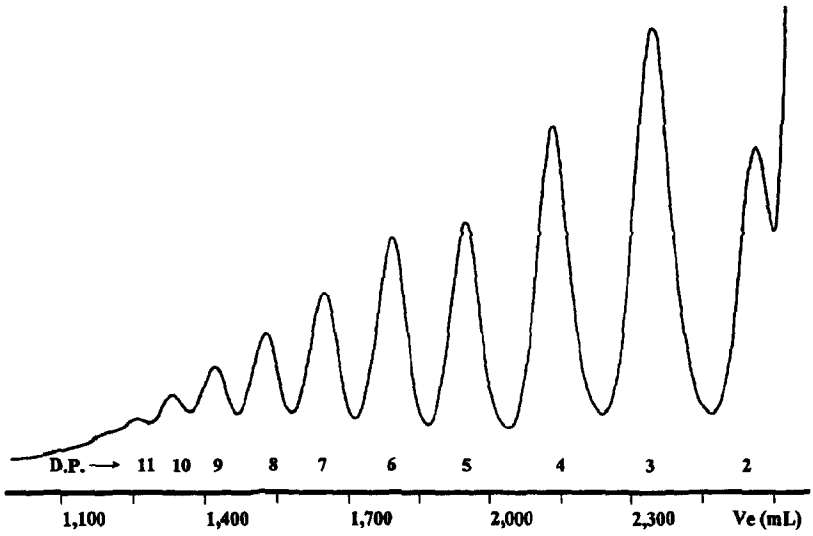


Fig. 2. Elution profile from Bio-Gel P-4 for β-(1 → 4)-linked (2-amino-2-deoxy-D-glucopyranosyl)$_{2-11}$-α-D-glucopyranosyl fluoride oligosaccharides.

The clear differentiation of the C-(1 → 4)-linked signal at 99.6 ppm, and the C-1 glycosyl fluoride doublet in the spectra shown in Figs. 1B–D, suggested that the ratio for the two sets of signals could be used for the estimation of the average degree of depolymerisation of chitosan in HF as a function of the reaction conditions. This approach was in fact correlated with the oligosaccharide composition of the mixtures according to the reaction time, as could be seen from their elution profiles obtained by steric-exclusion chromatography. The reaction products were readily recovered from their solution in HF by successive precipitations with ether, dissolution in water, and neutralization of the residual acidity with calcium carbonate. A Bio-Gel P-4 analytical chromatography column, previously used for chitin oligomers [10], was found convenient for the characterization of dp 2–11, using a slightly acidic pH in order to take into account the more polar nature of chitosan oligomers as compared to chitin. A typical elution profile using 0.25 M acetic acid–0.05 M ammonium acetate as eluent, as shown in Fig. 2 for an 18-h chitosan–HF mixture, confirmed that the average dp value was ~4 for the the mixture, which was also deduced from the ratio of the two sets of signals at 104 and 98.7 ppm in the spectrum shown in Fig. 1C.

Preparative fractionation of β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl fluoride oligosaccharides 1 was achieved using the same conditions as above, on 5-g batch experiments, resulting in an overall yield of 84% of dp 2–11 oligosaccharides with a maximum distribution for the dp 4–6 as shown in Table 1 when a fluorolysis time of ~20 h was used. All oligosaccharide samples showed the characteristic $J_{C\text{-}1,F}$ and $J_{C\text{-}2,F}$ couplings in their ^{13}C NMR spectra, with values of 221 and 25–26, Hz respectively, confirming the α-D anomeric configuration at the glycosyl fluoride site [12] (Table 2). As expected, homomorphous patterns were

Table 1

Yields, melting points, optical rotations, and analytical data for β-(1 → 4)-linked (2-amino-2-deoxy-D-glucopyranosyl)$_n$-β-(1 → 4)-2-amino-2-deoxy-α-D-glucopyranosyl fluoride oligosaccharides **1** resulting from the action of anhyd HF on chitosan for 19 h [a]

Compound (dp)	Yield (%)	Mp [b] (°C)	$[\alpha]_D^{20}$ (°) [c] (c)	Molecular formula	Analytical data (%)							
					Calcd				Found			
					C	H	F	N	C	H	F	N
2	3	247	+39.2 (0.98)	$C_{12}H_{23}FN_2O_8$, 2 HCl	34.46	6.02	4.58	6.75	34.35	6.30	4.90	6.80
3	9.9	245	+20.1 (0.92)	$C_{18}H_{34}FN_3O_{12}$, 3 HCl	35.26	6.04	3.10	6.86	35.40	6.01	3.90	6.84
4	14.3	245	+13 (1.01)	$C_{24}H_{45}FN_4O_{16}$, 4 HCl	35.55	6.05	2.35	6.91	35.60	6.00	2.00	6.80
5	15.9	260 (dec)	+10 (0.76)	$C_{30}H_{56}FN_5O_{20}$, 5 HCl	35.73	6.05	1.88	6.95	35.62	6.00	2.00	6.80
6	15.4	271 (dec)	+8 (0.52)	$C_{36}H_{67}FN_6O_{24}$, 6 HCl	35.85	6.06	1.58	6.97	35.70	6.01	> 1.0	7.06
7	12.0	> 271 (dec)	+7 (0.52)	$C_{42}H_{78}FN_7O_{28}$, 7 HCl	35.93	6.06	1.35	6.99	35.8	6.00	> 1.0	7.00
8	8.8	> 271 (dec)	+3 (0.45)	$C_{48}H_{89}FN_8O_{32}$, 8 HCl	36.00	6.06	1.19	7.00	35.82	6.00	> 1.1	6.78
9	4.7	> 271 (dec)	+2 (0.45)	$C_{54}H_{100}FN_9O_{36}$, 9 HCl	36.05	6.06	1.06	7.01	35.80	5.99	> 1.1	7.10
10	3.0	> 271 (dec)	+2 (0.33)	$C_{60}H_{111}FN_{10}O_{40}$, 10 HCl	36.09	6.07	0.95	7.02	35.91	6.03	0.9	7.12
11	1.0	> 271 (dec)	+2 (0.33)	$C_{66}H_{122}FN_{11}O_{44}$, 11 HCl	36.09	6.07	0.95	7.02	35.81	6.03	0.6	7.12

[a] From chitosan (5 g) treated with HF (100 mL) for 19 h, followed by a single chromatographic fractionation.

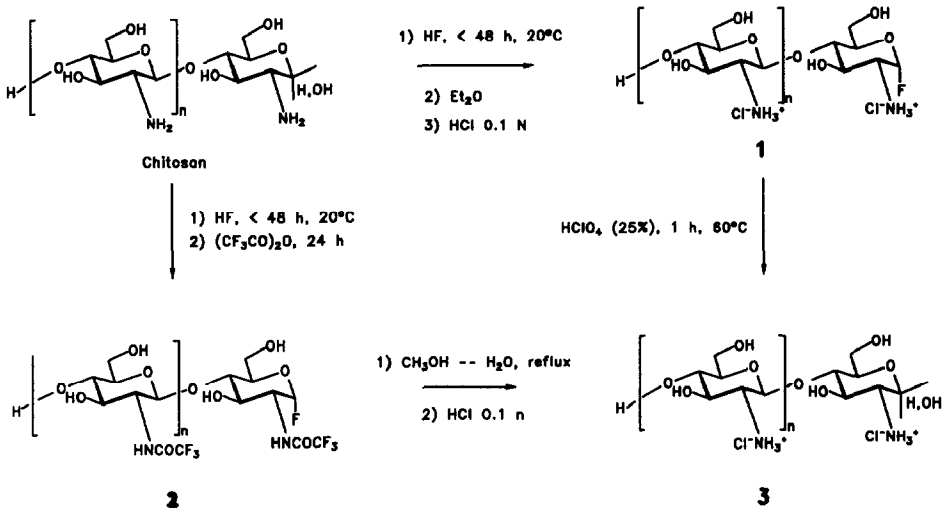
[b] Freeze-dried from aqueous solution. [c] In water.

Table 2
^{13}C NMR spectral data for a D_2O solution of β-(1 → 4)-linked (2-amino-2-deoxy-D-glucopyranosyl)$_n$-β-(1 → 4)-2-amino-2-deoxy-α-D-glucopyranosyl fluoride oligosaccharides **1**

[β-D-(1 → 4)-GlcNH$_2$]$_n$-β-(1 → 4)-α-D-GlcNH$_2$ fluoride	Chemical shifts (δ) [a]					
	C-1	C-2	C-3	C-4	C-5	C-6
$n = 1$	105.00	54.60	69.23	69.99	75.60	60.6
	$J_{C\,1,F}$ 221 Hz	$J_{C\text{-}2,F}$ 26 Hz				
$n = 2$	105.1	53.9	68.39	75.49	73.04	59.6
	$J_{C\text{-}1,F}$ 221 Hz	$J_{C\text{-}2,F}$ 25 Hz				
	98.41 (2) [b]	55.96 (2) [b]	72.4 (2) [b]	69.56 (2) [b]	76.30 (2) [b]	60.23 (2) [b]
$n = 3$	105.6	54.07	68.22	75.75	73.05	59.59
	$J_{C\text{-}1,F}$ 221 Hz	$J_{C\text{-}2,F}$ 25 Hz				
	99.17 (2,3) [b]	56.05 (2,3) [b]	71.11 (2) [b]	77.04 (2) [b]	74.75 (2) [b]	60.09 (2) [b]
			72.88 (3) [b]	69.59 (3) [b]	76.28 (3) [b]	60.40 (3) [b]
$n = 4$	105.6	54.31	69.62	76.35	74.29	59.66
	$J_{C\text{-}1,F}$ 221 Hz	$J_{C\text{-}2,F}$ 25 Hz				
	100.51 (2,3,4) [b]	56.28 (2,3,4) [b]	72.54 (2,3) [b]	77.55 (2,3) [b]	74.80 (2,3) [b]	60.11 (2) [b]
			73.05 (4) [b]	70.05 (4) [b]	76.25 (4) [b]	60.18 (3) [b]
						60.51 (4) [b]
$n = 11$	not apparent	54.27	69.63	76.30	73.11	59.64
		$J_{C\text{-}2F}$ 5 Hz				
	99.64 (2–10) [b]	55.95 (2–10) [b]	71.73 (2–10) [b]	77.16 (2–10) [b]	74.80 (2–10) [b]	60.12 (2–10) [b]
	99.64 (11) [b]	55.95 (11) [b]	73.71 (11) [b]	69.63 (11) [b]	76.30 (11) [b]	60.47 (11) [b]

[a] Relative to 1,4-dioxane (δ 67.4) as internal standard.

[b] Number(s) in parentheses indicate(s) the 2-amino-2-deoxy-D-glucopyranosyl unit, numbered from the reducing end, to which belongs the assigned carbon atom(s).



Scheme 1

displayed by the homologous series, with signals of both terminal units decreasing in intensity as the dp increased, allowing their unambiguous assignment and confirming the homogeneity of the oligosaccharide fraction. Yields and physicochemical data are reported in Table 1 for the dp 2–10 series of oligosaccharide fluorides, isolated as their hydrochloride amine salts **1** (Scheme 1).

The anomeric fluorine atom in β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl fluoride oligosaccharides proved to be much more stable than the corresponding 2-acetamido-2-deoxy derivatives [10] since boiling in water did not cause hydrolysis. In fact, this result is in agreement with the expected enhanced stabilization induced by the positive charge at the vicinal C-2 amino group. Two sets of experiments were thus devised in order to obtain the free β-(1 → 4)-glucosaminyl oligosaccharides **3**, involving either the use of a strong, but easily removable protonic reagent, or the temporary protection of the amino group by a readily cleavable substituent which would prevent protonation in protonic media.

Thus, when a mixture of glucosaminyl fluoride oligosaccharides resulting from the action of HF on chitosan for 18 h was heated at 60°C in aqueous 25% perchloric acid for 1 h, the signal at 104 ppm for the anomeric fluorides in the ^{13}C NMR spectrum disappeared and was replaced by two signals at 92.6 and 88.9 ppm corresponding, respectively, to the α and β anomers of the free glucosaminyl oligosaccharides. A comparable result was also obtained by methanolysis, involving evaporation with methanol under reduced pressure, of the related 2-deoxy-2-trifluoroacetamido glycosyl fluoride oligosaccharides **2**, which resulted in the simultaneous cleavage of the fluoro- and trifluoro-acetamido substituents. The latter *N*-trifluoroacetates were conveniently obtained by gently heating (~ 60°C) a solution in trifluoroacetic anhydride of the oligosaccharide mixture resulting from the precipitation with ether of the HF–chitosan mixture. An alternative, and even more

Table 3
Yields, melting points, optical rotations, and analytical data for β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides [a] **3**

Compound (dp)	Yield (%)	Mp [b] (°C)	$[\alpha]_D$ (°) (c)	Molecular formula [d]	Analytical data (%)						$[\alpha]_D$ (°)	Ref
					Calc.			Found				
					C	H	N	C	H	N		
											+43.5	3c
2	15.5	250–252	+43.1 (1)	$C_{12}H_{24}N_2O_9$, 2 HCl	34.86	6.30	6.78	35.01	6.00	7.01	+42.7	4
											+37.6	5
											+21.5	3c
3	23.8	255	+20.1 (0.9)	$C_{18}H_{35}N_3O_{13}$, 3 HCl	35.38	6.22	6.88	35.2	6.32	7.01	+26.4	4
											+22	5
											+16	3c
4	19.1	260	+18 (0.52)	$C_{24}H_{46}N_4O_{17}$, 4 HCl	35.64	6.19	6.93	35.49	5.95	7.00	+22.5	4
											+13.9	5
5	11.9	267	+10.3 (0.70)	$C_{30}H_{57}N_5O_{21}$, 5 HCl	35.80	6.16	6.96	35.73	5.95	7.10	16.4	4
											+9.6	5
6	11.3	268 (dec)	+7.2 (0.30)	$C_{36}H_{68}N_6O_{25}$, 6 HCl	35.91	6.15	6.98	35.75	6.04	6.55	+6.0	5
7	7.1	270 (dec)	+4.0 (0.30)	$C_{42}H_{79}N_7O_{29}$, 7 HCl	35.99	6.14	6.99	35.70	6.20	6.35	+2.9	5
8	6.0	270 (dec)	+1.7 (0.45)	$C_{48}H_{90}N_8O_{33}$, 8 HCl	36.05	6.13	7.01	35.68	6.04	6.34	+1.6	5
9	3.4	272 (dec)	+0.2 (0.25)	$C_{54}H_{101}N_9O_{37}$, 9 HCl	36.09	6.13	7.02	35.79	6.30	7.20	+0.0	5
10	1.8	272 (dec)	+0.2 (0.12)	$C_{60}H_{112}N_{10}O_{41}$, 10 HCl	36.13	6.12	7.02	35.69	5.90	7.20	−0.9	5

[a] From chitosan (5 g) treated with HF (30 mL) following Method *b*; trifluoroacetic anhydride (50 mL) was added to the cold mixture after 15 h.
[b] After freeze-drying from water.
[c] For a solution in water at 20°C.

Table 4
^{13}C NMR spectral data for solutions of β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides [a] 3 in D_2O

Compound (dp)	Chemical shifts (δ) relative to 1,4-dioxane (δ 67.4)					
	C-1	C-2	C-3	C-4	C-5	C-6
$n = 2$	89.73 (1α)	55.00 (1α)	68.80 (1α)	77.41 (1α)	70.85 (1α)	60.61 (1α)
	93.42 (1β)	57.53 (1β)	71.15 (1β)	77.52 (1β)	75.41 (1β)	61.11 (1β)
	98.56 (2)	56.77 (2)	72.78 (2)	70.40 (2)	77.20 (2)	61.23 (2)
$n = 3$	89.72 (1α)	55.03 (1α)	68.70 (1α)	77.37 (1α)	70.77 (1α)	61.07 (1α)
	93.42 (1β)	57.50 (1β)	71.05 (1β)	77.52 (1β)	75.35 (1β)	61.15 (1β)
	98.22 (2α)	56.73 (2α)	70.90 (2α)	77.52 (2α)	75.60 (2α)	60.96 (2α)
	98.29 (2β)	56.73 (2β)	70.90 (2β)	77.52 (2β)	75.60 (2β)	60.96 (2β)
	98.51 (3)	56.80 (3)	72.56 (3)	70.48 (3)	77.19 (3)	61.19 (3)
$n = 6$	89.72 (1α)	55.03 (1α)	68.70 (1α)	77.31 (1α)	70.78 (1α)	61.06 (1α)
	93.42 (1β)	57.45 (1β)	71.05 (1β)	77.51 (1β)	75.35 (1β)	61.14 (1β)
	98.26 (2–5)	56.67 (2–5)	70.88 (2–5)	77.25 (6)	75.59 (2–5)	60.93 (2–5)
	98.51 (6)	56.79 (6)	72.57 (6)	70.47 (6)	77.20 (6)	61.18 (6)
$n = 10$	89.72 (1α)	55.02 (1α)	68.71 (1α)	77.30 (1α)	70.76 (1α)	61.06 (1α)
	93.42 (1β)	57.48 (1β)	71.05 (1β)	77.51 (1β)	75.39 (1β)	61.14 (1β)
	98.34 (2–9)	56.66 (2–9)	71.87 (2–9)	77.25 (2–9)	75.59 (2–9)	60.95 (2–9)
	98.54 (10)	56.72 (10)	72.58 (10)	70.46 (10)	77.22 (10)	61.17 (10)

[a] Number(s) in parentheses indicate(s) the 2-amino-2-deoxy-D-glucopyranosyl unit numbered from the reducing unit end to which belongs the assigned carbon atom(s).

direct *N*-trifluoroacetylation technique involves the addition of trifluoroacetic anhydride to the HF–chitosan oligomer solution, after the required depolymerization time, resulting in simultaneous quenching and *N*-acylation of the oligoglucosaminyl components.

β-(1 → 4)-Linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides of dp 2–10 were found to be conveniently separated by gel-exclusion chromatography, using Bio-Gel P-4, with aqueous acetic acid–ammonium acetate as eluent (Fig. 3). Yields

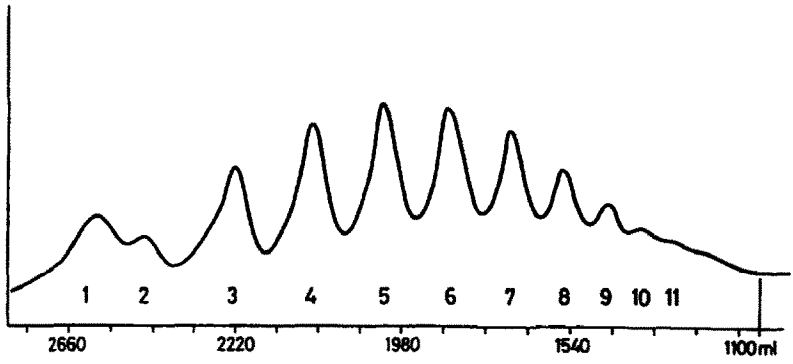


Fig. 3. Elution profile from Bio-Gel P-4 for β-(1 → 4)-linked (2-amino-2-deoxy-D-glucopyranosyl)$_{2-10}$ oligosaccharides.

and physicochemical data for such a homologous series in the form of their hydrochloride salts **3** are reported in Table 3 for the entire series of oligosaccharides.

3. Experimental

Generals methods.—The methods were as described in ref 10. Chitosan was purchased from Sigma (St Louis, Mo).

β-(1 → 4)-Linked (2-amino-2-deoxy-D-glucopyranosyl)$_n$-β-(1 → 4)-2-amino-2-deoxy-α-D-glucopyranosyl fluoride oligosaccharides (**1**).—Fully deacetylated chitosan [13] (5 g) was suspended in anhyd HF (60 mL) at 0°C, and the suspension was stirred while the temperature was allowed to rise to ~20°C. The clear solution, obtained within 30 min, was kept at this temperature for <200 h, depending on the desired oligosaccharide dp distribution and taking into account that, after 170 h, 2-amino-2-deoxy-D-glucopyranosyl fluoride hydrofluoride was the main species present in the solution. The chilled solution (0°C) was then poured into cold stirred diethyl ether (2 L) which was cooled in Dry Ice. The resulting precipitate was washed with ether (1 L) and dried. It was dissolved in water (0.5 L) and powdered $CaCO_3$ was added to neutralize any residual acidity. The filtrate containing the chitosan oligomers, in the form of their glucosyl fluoride derivatives **1**, was freeze-dried (4.5 g). The volume was adjusted to ~20 mL and the resulting solution was used for the gel-permeation separation. When a fluorolysis time of 18 h was chosen, Bio-Gel P-4 (Pharmacia, 200–400 mesh) was found to give a convenient separation for dp 2–11 using 1:1 0.25 M aq AcOH–0.05 M ammonium acetate as eluent, with the elution profile reported in Fig. 2. The recovered glucosaminyl fluoride oligosaccharides were ultrafiltered (24 h), made neutral with HCl (0.1 M), and recovered as their hydrochloride amine salts. Physicochemical characterization and NMR data for individual oligosaccharide derivatives are reported in Tables 1 and 2, respectively.

β-(1 → 4)-Linked 2-amino-2-deoxy-D-glucopyranosyl oligosaccharides (**3**).—Method *a*. The glucosaminyl fluoride oligosaccharide mixture was suspended in 1.5:5 70% $HClO_4$–water (65 mL) and heated for 1 h at 60°C. The volume was then reduced under diminished pressure to ~20 mL and the resulting solution was used for the gel-permeation chromatographic separation.

Method *b*. The chitosan oligomers solution in HF (5 g, 60 mL), kept for <170 h at 20°C, was evaporated in a stream of air, and trifluoroacetic anhydride (50 mL) was added to the residue. After 24 h at 60°C, the solution was evaporated under reduced pressure and MeOH (3 × 100 mL) was evaporated from the residue. Alternatively, in a separate experiment, trifluoroacetic anhydride (50 mL) was added to the HF–chitosan oligomers solution, and the mixture was kept for 24 h at room temperature with stirring. After cooling at 0°C, it was poured into cold diethyl ether and the resulting precipitate, washed with ether (1 L) and dried, was dissolved in 1:1 MeOH–water (200 mL) and refluxed for ~12 h, then concentrated and used as in Method *a* for the gel-permeation chromatography. As for the

corresponding fluorinated oligomers, Bio-Gel P-4 (Pharmacia, 200–400 mesh) was found to give an optimal separation for dp 2–10 oligosaccharides resulting from a 15-h treatment of chitosan with HF, using 1:1 0.25 M AcOH–0.05 M ammonium acetate as eluent. A typical elution pattern is given in Fig. 3 for dp 2–10 oligosaccharides. A further purification using the same parameters was eventually used for each separated oligosaccharide. After ultrafiltration, the final glucosaminyl oligosaccharide solutions were neutralized with 0.1 M HCl, then freeze-dried. Yields, physicochemical, and ^{13}C NMR data are reported in Tables 3 and 4, respectively, for dp 2–10 oligosaccharides obtained by quenching a solution of chitosan in HF after 15 h with trifluoroacetic anhydride, followed by the above processing.

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