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The use of 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives in syntheses of hyaluronic acid-related tetra-, hexa-, and octa-saccharides having a methyl β-D-glucopyranosiduronic acid at the reducing end

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Abstract

Expeditious and stereocontrolled syntheses are reported of β-D-Glc*p*NAc-(1 → 4)-[β-D-Glc*p*A-(1 → 3)-β-D-Glc*p*NAc-(1 → 4)]$_n$-β-D-Glc*p*A-(1 → OMe), where $n = 1$, 2, and 3, which represent structural elements of the extracellular polysaccharide hyaluronic acid. Condensation of 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl trichloroacetimidate with methyl (4-methoxyphenyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate gave the disaccharide derivative **9**, which was demethoxyphenylated and imidoylated to afford the pivotal disaccharide trichloroacetimidate **7**. Condensation of **7** with methanol followed by *O*-dechloroacetylation gave the acceptor **8**. Coupling of **7** with **8** gave the tetrasaccharide derivative **4**. *O*-Dechloroacetylation of **4** followed by condensation with imidate **7** afforded hexasaccharide **5**, which was transformed into octasaccharide **6** by a similar two-step procedure. Subsequent *O*-dechloroacetylation, transformation of the *N*-trichloroacetyl groups into *N*-acetyl, debenzylidenation, and saponification of **4**–**6** afforded the tetra- (**1**), hexa- (**2**), and octa-saccharide (**3**) derivatives in high yields, as their sodium salts. © 1996 Elsevier Science Ltd.

Keywords: Octasaccharide; Hyaluronic acid; 2-Deoxy-2-trichloroacetamido-D-glucopyranose derivatives

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1. Introduction

Hyaluronic acid (HA), first isolated [1] from bovine vitreous body of the eye, is a linear, high molecular weight glycosaminoglycan composed of disaccharide repeating units of 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid, namely [2], [4)-β-D-Glc*p*A-(1 → 3)-β-D-Glc*p*NAc-(1 →]$_n$. HA has been found in the extracellular matrix, connective tissues, synovial fluid in joints, and certain bacterial strains [3]. It is biosynthesized at the inner side of plasma membranes by a membrane-bound HA synthetase, and is then extruded to the cell surface [4]. The biological roles of HA are highly diversified, ranging from structural functions [5] to more intricate effects such as cell migration [6,7] or angiogenesis [8,9]. As a surgical aid, HA is used in eye surgery [10], for treatment of osteoarthritis [11], and as a template for nerve regeneration [12]. Due to its importance in biomedicine, various modifications of HA have been undertaken to improve its biological properties [13]. The glycoprotein CD44 is the major cell-surface receptor for HA, and the binding site coordinates at least a hexasaccharide sequence [5,14]. However, as is the case for most of the glycosaminoglycans, further biological studies are hampered because of the lack of oligosaccharide fragments of definite size and structure. Enzymatic synthesis of HA from UDP-sugars with HA synthetase has been reported [15], but lead to high molecular weight material (5×10^5 Da). Thus, chemical synthesis is an attractive alternative for defining the biological roles of different HA fragments.

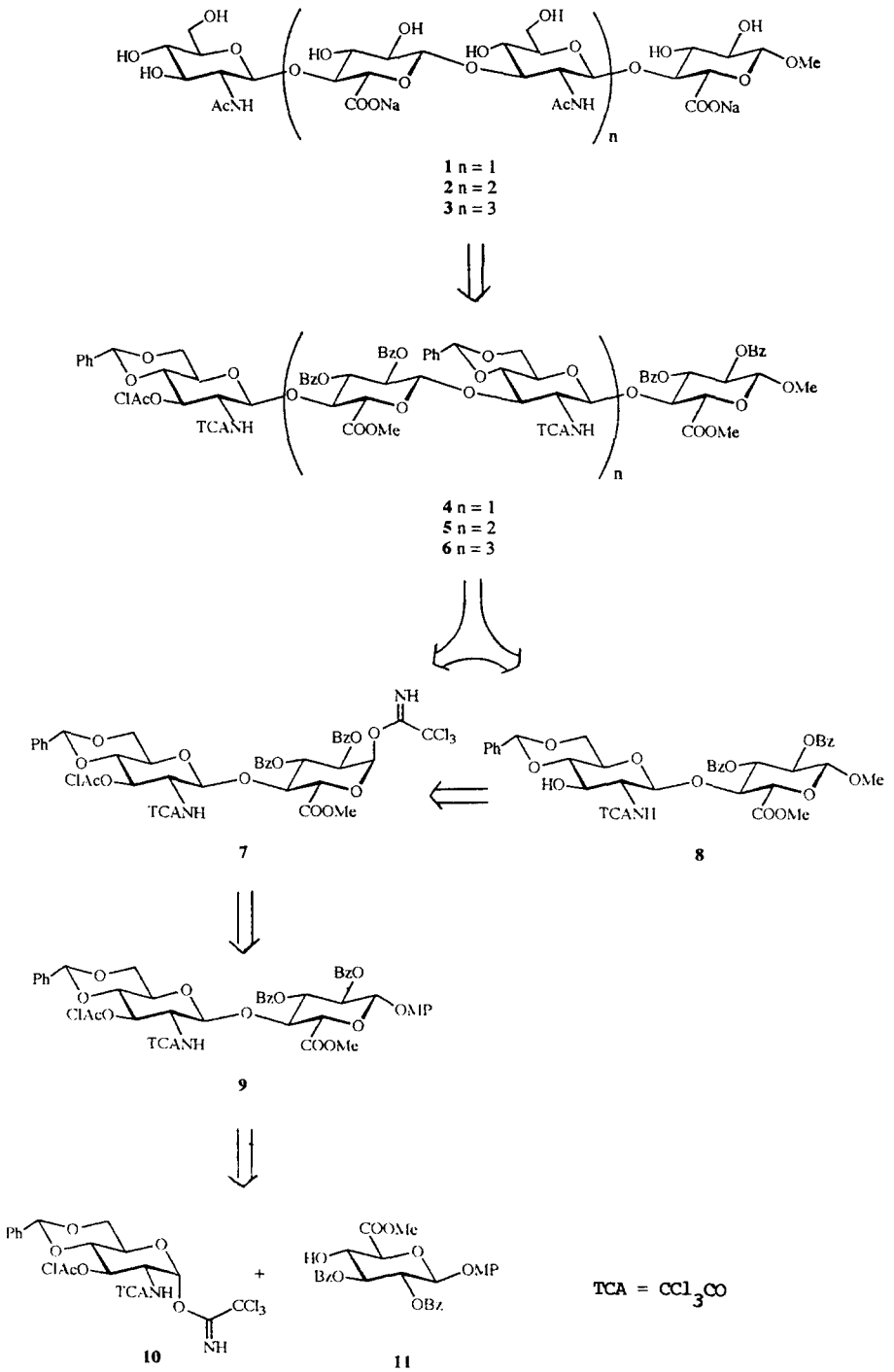
Several fragments of HA have been synthesized recently, such as a disaccharide methyl glycoside [16], and tetrasaccharide 4-methoxyphenyl glycosides having either a D-glucosamine [17] or D-glucuronic acid [18] residue at the reducing end. In all these syntheses, the D-glucuronic acid residue was obtained by selective oxidation at C-6 of a corresponding D-glucose residue, after construction of the oligosaccharide backbone.

As part of a programme devoted to the synthesis of proteoglycan fragments [19,20], we now report on expeditious and stereocontrolled preparations of HA tetra- (**1**), hexa- (**2**), and octa-saccharide (**3**) fragments having a methyl β-D-glucopyranosiduronic acid residue at the reducing end, by direct coupling of D-glucuronic acid derivatives with 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives [21].

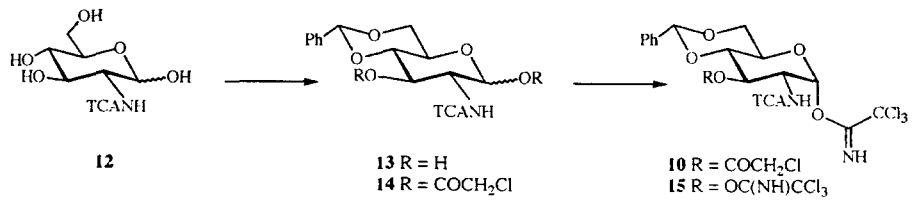
2. Results and discussion

For the syntheses of the target oligosaccharides **1**–**3**, a series of key intermediates, namely **4**–**6**, were designed, which may in turn be bond-disconnected into a glycosyl donor (**7**), to be used in an iterative way, and a glycosyl acceptor (**8**), both compounds being prepared from a common precursor (**9**). The disaccharide **9** may, in turn, be obtained by direct coupling of a glycosyl donor (**10**) with a glycosyl acceptor (**11**) (Scheme 1).

We recently demonstrated [19,21] that variously *O*-protected and activated 2-deoxy-2-trichloroacetamido-D-glucopyranose derivatives are powerful glycosyl donors for the stereoselective synthesis of 1,2-*trans*-2-amino-2-deoxy-D-glucosides, and that the *N*-trichloroacetyl group in the oligosaccharide products could be easily transformed into



Scheme 1. Retrosynthetic analysis of oligosaccharide structures **1**–**3**.



12 **13** R = H, **14** R = $COCH_2Cl$ **10** R = $COCH_2Cl$, **15** R = $OC(NH)CCl_3$

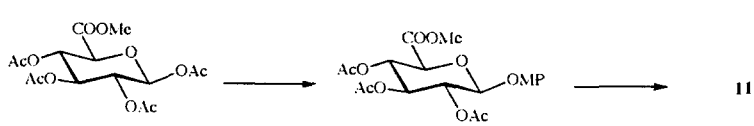
Scheme 2.

N-acetyl under neutral conditions using tributylstannane. This method allows the direct and high-yielding glycosylation of the low-reactive [22] 4-OH group of D-glucuronic acid derivatives, thus avoiding the difficult oxidation of the synthetic oligosaccharides.

Preparation of the glycosyl donor **10** was achieved as follows (Scheme 2). 2-Deoxy-2-trichloroacetamido-D-glucopyranose (**12**) [21] was treated with benzaldehyde and zinc chloride to give the crystalline 4,6-*O*-benzylidene derivative **13** in 74% yield. Chloroacetylation of **13** afforded crystalline **14**, as a ~ 2:1 mixture of anomers, in 93% yield. Several conditions were examined for the selective *O*-deprotection at the anomeric center. The use of benzylamine [23], piperidine [24], or hydrazine acetate [25] led to concomitant extensive *O*-deprotection at O-3. However, the silica-gel-mediated anomeric *O*-deacetylation in MeOH [26] allowed the reproducible preparation of the corresponding free hemiacetal, which was directly treated with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) to afford the crystalline α-trichloroacetimidate **10** in 82% overall yield. The 1,3-di-*O*-trichloroacetimidoyl derivative **15** was also isolated in ~ 10% yield, and was the result of some unavoidable *O*-dechloroacetylation at O-3 during the deprotection step at the anomeric center.

The D-glucuronic acid derivative **11** was prepared as follows (Scheme 3). Fusion [27] of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**16**) [28] with 4-methoxyphenol under acid catalysis (*p*-toluenesulfonic acid) afforded crystalline **17** in 71% yield. Conventional *O*-deacetylation of **17** gave the corresponding triol, that was directly submitted to the tin procedure [29] (dibutyltin oxide in refluxing benzene). Treatment [19] of the intermediary stannylene acetal with benzoyl chloride (2.1 equiv) and triethylamine (1.5 equiv) afforded the crystalline 2,3-di-*O*-benzoyl derivative **11** as the major product in 63% overall yield. The 2,4- and 3,4-di-*O*-benzoylated isomers (18%) were also isolated, but directly *O*-debenzoylated and recycled. The regioselectivity observed for the di-*O*-benzoylation of this triol was in agreement with that reported [19] for the analogous methyl glycoside.

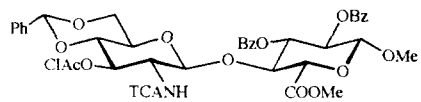
As a first step in the construction of the target oligosaccharides, imidate **10** (1.2



16 **17** **11**

Scheme 3.

equiv) was condensed with alcohol **11** (1 equiv) in dichloromethane at room temperature, in the presence of trimethylsilyl triflate (15% based on **10**), to give the crystalline disaccharide derivative **9** in 89% yield. Oxidative removal [30] of the 4-methoxyphenyl group in **9** with ceric ammonium nitrate, followed by imidoylation, as described for the preparation of **10**, afforded the crystalline imidate **7** in 78% overall yield, the anomeric configuration of which was deduced from its ^{1}H NMR spectrum ($J_{1,2}$ 3.5 Hz). Condensation of imidate **7** with methanol (5 equiv), as described above, afforded the crystalline disaccharide methyl glycoside **18**, but in a non-reproducible manner (60–90% yield), as is often the case in glycosylation reactions with methanol. However, condensation of imidate **10** with methyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate (**19**) [19], as described above, readily afforded **18** in 91% yield. Treatment of **18** with thiourea gave the crystalline alcohol **8** in 96% yield.



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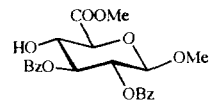

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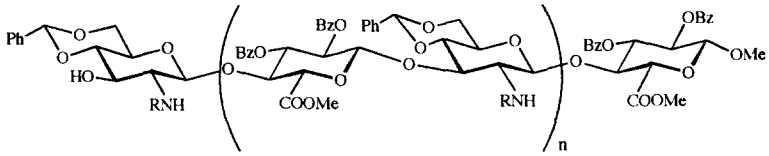
Following the retrosynthetic analysis depicted in Scheme 1, the glycosyl imidate **7** (1.3 equiv) was condensed with alcohol **8** (1 equiv), as described for the preparation of **9**, to give the crystalline tetrasaccharide derivative **4** in 87% yield. The ^{1}H NMR spectrum of **4** showed, inter alia, the presence of four anomeric protons at δ 5.07, 4.99, 4.94, and 4.64, with J values ($J_{1,2}$ 7.0–8.0 Hz) characteristic of 1,2-*trans* linkages in the D-*gluco* series. As a general rule, the J values ($J_{1,2}$ 7.0 Hz) observed for the anomeric protons of β-D-glucuronic acid residues are slightly smaller than those observed for the amino sugar units [19]. *O*-Dechloroacetylation of **4**, as described above, afforded the crystalline tetrasaccharide acceptor **20** in 95% yield. The ^{1}H NMR spectrum of **20**, recorded in deuteriochloroform, showed a signal at δ 2.99 (d, J 3.5 Hz) attributed, by spin-decoupling experiments and exchange with D_2O, to the 3-OH group in the terminal non-reducing amino sugar moiety.

Second condensation of imidate **7** (1.5 equiv), now with alcohol **20** (1 equiv), as described for the preparation of **9**, gave the crystalline hexasaccharide derivative **5** in 93% yield. Conventional *O*-dechloroacetylation of **5** afforded the crystalline alcohol **21** in 83% yield.

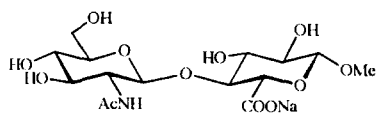
Third coupling of imidate **7** (1.7 equiv) with alcohol **21** (1 equiv), as described above, gave the crystalline octasaccharide derivative **6** in 93% yield. *O*-Dechloroacetylation of **6** gave the crystalline alcohol **22** in 86% yield, which is available for further chain extension at the non-reducing end.

Complete deprotection of **20–22** and **8** (which possesses the structure of the basic repeating unit of our synthetic oligosaccharides) was achieved as follows. The *N*-trichloroacetyl groups in **8** and **20–22** were readily transformed into *N*-acetyl by treatment with tributylstannane (4.5 mol equiv for each NHTCA group) and azoisobutyronitrile (AIBN) in benzene–*N*,*N*-dimethylacetamide to give the crystalline acetamides **23–26** in

81, 88, 91, and 92% yields, respectively. Treatment of **23–26** with aq acetic acid at 100 °C, followed by saponification with an excess (5–6 mol equiv for each ester function) of aqueous 3 M sodium hydroxide in methanol–water, and purification, afforded the target molecules **27** and **1–3**, as their sodium salts, in 80–83% overall yields.



	R = $COCCl_3$	R = Ac
n = 0	**8**	**23**
n = 1	**20**	**24**
n = 2	**1**	**25**
n = 3	**22**	**26**



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The ^{1}H NMR spectra of **1–3** and **27** are in full agreement with the expected structures, and in accord with those reported both for synthetic disaccharide methyl glycosides [16,31] and for a tetrasaccharide fragment [32] isolated from HA by digestion with bovine testicular hyaluronidase [33]. The ^{13}C NMR spectra (Table 1) are also in accord with the structures. Signals for GlcNAc C-3 (δ_C ~ 82.5 ppm) and GlcA C-4 (δ_C ~ 80 ppm), in glycosylated units, are shifted downfield ($\Delta\delta$ ~ 9 ppm) compared with those of non-substituted structures, and allow easy assignments and location of the substitutions.

In conclusion, expeditious, stereocontrolled, and high-yielding syntheses of hyaluronic acid tetra-, hexa-, and octa-saccharide methyl glycosides are reported. These compounds, readily obtained in ~ 100 mg amounts, are currently being evaluated in biological and conformational studies.

3. Experimental

General methods.—Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–25 °C with a

Table 1
^{13}C NMR data [a] (proposed assignments) for synthetic oligosaccharides (Na salts)

Residue in product	No.	C-1	C-2	C-3	C-4	C-5	C-6	NAc	OMe
β-GlcNAc-(1 → 4)	**27**	101.05	55.55	73.75	69.90	76.05	60.73	22.55	–
β-GlcA-(1 → OMe)		103.56	72.84	74.05	**80.20** [b]	76.31	175.00	–	57.51
β-GlcNAc-(1 → 4)	**1**	100.97	55.55	73.75	69.87	75.52	60.70	22.59	–
β-GlcA-(1 → 3)		103.21	72.56	73.97	**80.04**	75.95	175.02	–	–
β-GlcNAc-(1 → 4)		100.86	54.45	**82.56**	68.56	75.85	60.70	22.59	–
β-GlcA-(1 → OMe)		103.55	72.79	74.17	**80.26**	76.05	175.02	–	57.50
β-GlcNAc-(1 → 4)	**2**	100.71	55.57	73.76	69.92	75.55	60.74	22.69	–
β-GlcA-(1 → 3)		103.33	72.67	73.76	**80.00**	76.08	175.10	–	–
β-GlcNAc-(1 → 4)		100.75	54.48	**82.73**	68.65	75.55	60.74	22.62	–
β-GlcA-(1 → 3)		103.33	72.67	74.07	**80.14**	76.50	175.10	–	–
β-GlcNAc-(1 → 4)		100.86	54.48	**82.78**	68.65	75.56	60.73	22.62	–
β-GlcA-(1 → OMe)		103.57	72.90	74.07	**80.30**	76.66	175.02	–	57.47
β-GlcNAc-(1 → 4)	**3**	100.73	55.59	73.78	69.92	75.55	60.74	22.67	–
β-GlcA-(1 → 3)		103.34	72.68	73.78	**80.00**	76.43	175.08	–	–
β-GlcNAc-(1 → 4)		100.73	54.50	**82.68**	68.65	75.55	60.74	22.67	–
β-GlcA-(1 → 3)		103.34	72.68	73.78	**80.15**	76.43	175.08	–	–
β-GlcNAc-(1 → 4)		100.88	54.50	**82.72**	68.65	75.57	60.75	22.68	–
β-GlcA-(1 → 3)		103.58	72.89	74.08	**80.16**	76.77	175.12	–	–
β-GlcNAc-(1 → 4)		100.88	54.50	**82.78**	68.65	75.57	60.75	22.68	–
β-GlcA-(1 → OMe)		103.58	72.89	74.08	**80.30**	76.77	175.72	–	57.47

[a] In D_2O at 27 °C quoted in ppm from external Me_4Si, measured from internal acetone (δ_C 30.50 ppm).
[b] Values in bold type reflect the location of the substitutions by a sugar residue.

Perkin–Elmer Model 141 polarimeter. The ^1H (300 MHz) and ^{13}C (75.4 MHz) NMR spectra were recorded at 27 °C, unless otherwise stated, with a Bruker AM-300WB spectrometer. Chemical shifts (δ) are given from the signal of internal Me_4Si, unless otherwise stated. In oligosaccharide structures, the amino sugar (GlcN) and uronic acid (GlcA) units are numbered starting from the reducing end. The purity of the products was determined by TLC on Silica Gel F_{254} (E. Merck), with detection by charring with H_2SO_4. Flash-column chromatography was performed on silica gel (Merck, 40–63 μm). Elemental analyses were performed by the Service Central de Microanalyse du Centre National de la Recherche Scientifique (Vernaison, France).

4,6-O-Benzylidene-2-deoxy-2-trichloroacetamido-D-glucopyranose (**13**).—A mixture of 2-deoxy-2-trichloroacetamido-D-glucopyranose (**12**) [21] (10 g, 30.8 mmol), benzaldehyde (80 mL), and freshly fused $ZnCl_2$ (10 g) was stirred for 16 h at room temperature, then poured under vigorous stirring into ice-cold 1:1 Et_2O–heptane (500 mL). The resulting solid was filtered off, washed with cold Et_2O, and recrystallized from EtOH to give **13** (9.5 g, 74%); mp 189–190 °C; $[\alpha]_D$ −12° (*c* 1, equil., MeOH); ^1H NMR [$(CD_3)_2SO$]: δ 8.74 (d, 0.5 H, *J* 9.0 Hz, NHβ), 8.46 (d, 0.5 H, *J* 8.0 Hz, NHα), 7.40 (m, 5 H, Ph), 6.99 (d, 0.5 H, *J* 4.8 Hz, HO-1α), 6.97 (d, 0.5 H, *J* 6.0 Hz, HO-1β), 5.62, 5.61 (2 s, 1 H, Ph*CH*α,β), 5.36 (d, 0.5 H, *J* 6.5.Hz, HO-3β), 5.21 (d, 0.5 H, *J* 6.0 Hz, HO-3α), 5.12 (t, 0.5 H, $J_{1,2}$ 4.8 Hz, H-1α), and 4.81 (dd, 0.5 H, $J_{1,2}$

8.0 Hz, H-1β). Anal. Calcd for $C_{15}H_{16}Cl_3NO_6$: C, 43.66; H, 3.91; N, 3.99. Found: C, 43.69; H, 4.03; N, 3.36.

4,6-O-Benzylidene-1,3-di-O-chloroacetyl-2-deoxy-2-trichloroacetamido-α,β-D-glucopyranose (**14**).—Chloroacetic anhydride (1.026 g, 6 mmol) was added at 0 °C to a solution of **13** (825 mg, 2 mmol) in CH_2Cl_2 (8 mL) and pyridine (1.5 mL), and the mixture was stirred for 30 min at this temperature. Ice-cold water (1 mL) was then added, and the mixture was diluted with CH_2Cl_2 (30 mL), washed successively with water, satd aq $NaHCO_3$, and water, dried ($MgSO_4$), and concentrated. The residue was crystallized from EtOAc–Et_2O to give **14** (1.05 g, 93%); mp 188–190 °C; ^{1}H NMR ($CDCl_3$): δ 7.40 (m, 5 H, Ph), 7.09 (d, 0.3 H, *J* 10.0 Hz, NHβ), 6.92 (d, 0.7 H, *J* 8.5 Hz, NHα), 6.33 (d, 0.7 H, $J_{1,2}$ 4.0 Hz, H-1α), 5.91 (d, 0.3 H, $J_{1,2}$ 8.5 Hz, H-1β), 5.58, 5.52 (2 s, 1 H, PhC*H*α,β), 5.55, 5.53 (2 dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.0 Hz, H-3α,β), 4.48 (m, 0.7 H, H-2α), and 4.13, 4.09 (2 ABq, 2 H, $COCH_2Cl$α,β). Anal. Calcd for $C_{19}H_{18}Cl_5NO_8$: C, 40.35; H, 3.21; N, 2.47. Found: C, 40.50, H, 3.21; N, 2.63.

4,6-O-Benzylidene-2-deoxy-2-trichloroacetamido-3-O-trichloroacetimidoyl-α-D-glucopyranosyl trichloroacetimidate (**15**) *and 4,6-O-benzylidene-3-O-chloroacetyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl trichloroacetimidate* (**10**).—A mixture of **14** (1.5 g, 2.65 mmol) and silica gel (Merck, 40–63 μm, 1.0 g) in anhyd MeOH (80 mL) was vigorously stirred for 30 min at room temperature. The silica gel was filtered off and washed with MeOH (2 × 10 mL), the filtrate was concentrated, and the residue dried in vacuo at 40 °C.

A solution of the residue, trichloroacetonitrile (2.65 mL, 26.5 mmol), and DBU (80 μL, 0.53 mmol) in CH_2Cl_2 (10 mL) was stirred for 45 min at room temperature, then concentrated. The residue was eluted from a column (120 g) of silica gel with 3:1 heptane–EtOAc containing 0.2% of Et_3N to give, first, the 1,3-di-*O*-imidoylated derivative **15** (186 mg, 10%); $[\alpha]_D$ +46° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.80, 8.60 (2 s, 2 H, C=NH), 7.96 (d, 1 H, *J* 7.0 Hz, NH), 7.35 (m, 5 H, Ph), 6.60 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.82 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, H-3), 5.68 (s, 1 H, PhC*H*), 4.54 (m, 1 H, H-2), 4.42 (dd, 1 H, $J_{5,6eq}$ 4.5, $J_{6ax,6eq}$ 11.0 Hz, H-6eq), 4.10 (m, 2 H, H-5,6ax), and 3.88 (t, 1 H, $J_{4,5}$ 9.0 Hz, H-4). Anal. Calcd for $C_{19}H_{16}Cl_9N_3O_6$: C, 32.53; H, 2.30; N, 5.99. Found: C, 32.64; H, 2.46; N, 6.05.

Next eluted was **10** (1.38 g, 82%); mp 133–134 °C (from Et_2O–heptane); $[\alpha]_D$ +44° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.83 (s, 1 H, C=NH), 7.42 (m, 5 H, Ph), 7.01 (d, 1 H, *J* 9.0 Hz, NH), 6.47 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.60 (dd, 1 H, $J_{2,3}$ 10 0, $J_{3,4}$ 9.0 Hz, H-3), 5.59 (s, 1 H, PhC*H*), 4.54 (m, 1 H, H-2), 4.38 (dd, 1 H, $J_{5,6eq}$ 4.5, $J_{6ax,6eq}$ 10.5 Hz, H-6eq), 4.11 (m, 1 H, $J_{5,6ax}$ 10.5 Hz, H-5), 4.10 (ABq, 2 H, $COCH_2Cl$), 3.93 (t, 1 H, H-4), and 3.85 (t, 1 H, H-6ax). Anal. Calcd for $C_{19}H_{17}Cl_7N_2O_7$: C, 36.02; H, 2.70; N, 4.42. Found: C, 36.17; H, 2.77; N, 4.36.

Methyl (4-methoxyphenyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**17**).—A mixture of methyl 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronate (**16**) [28] (6.0 g, 15.9 mmol), 4-methoxyphenol (6.92 g, 56 mmol), and *p*-toluenesulfonic acid (monohydrate, 150 mg) was stirred for 30 min at 110 °C under water-pump vacuum, then cooled. The mixture was diluted with CH_2Cl_2 (100 mL), washed with aq 2 M NaOH and with water, dried ($MgSO_4$), and concentrated. The residue was crystallized from Et_2O, and recrystallized from EtOAc–heptane to give **17** (5 g, 71%); mp 136–137 °C; $[\alpha]_D$ −33° (*c* 1,

$CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.90 (m, 4 H, aromatic H), 5.32 (m, 2 H, H-3,4), 5.24 (dd, 1 H, $J_{1,2}$ 7.0, $J_{2,3}$ 9.0 Hz, H-2), 5.01 (d, 1 H, H-1), 4.12 (d, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.78, 3.75 (2 s, 6 H, OMe,COOMe), and 2.08, 2.05, 2.04 (3 s, 9 H, Ac). Anal. Calcd for $C_{20}H_{24}O_{11}$: C, 54.54; H, 5.49. Found: C, 54.44; H, 5.49.

*Methyl (4-methoxyphenyl 2,3-di-*O*-benzoyl-β-*D*-glucopyranosid)uronate* (**11**).—A solution of **17** (2.64 g, 6 mmol) in dry MeOH (50 mL) was treated with methanolic NaOMe (1.0 M, 1 mL) for 1 h at room temperature. The mixture was neutralized with Amberlite IR-120 (H^+) resin, filtered, concentrated, and dried in vacuo at 40 °C.

A mixture of the residue and dibutyltin oxide (1.57 g, 6.3 mmol) was heated for 20 h in refluxing benzene (80 mL) with azeotropic removal of water. Solvent (50 mL) was then slowly distilled off at atmospheric pressure, and the mixture was cooled, then diluted with anhyd THF (20 mL). To this solution were added successively benzoyl chloride (1.45 mL, 12.6 mmol) and Et_3N (1.26 mL, 9 mmol), and the mixture was stirred for 1 h at room temperature, then concentrated. The residue was eluted from a column (150 g) of silica gel with 5:1 toluene–EtOAc to give, first, a mixture of the 2,4- and 3,4-di-*O*-benzoylated isomers (564 mg, 18%). Next eluted was **11** (1.98 g, 63%); mp 138–139 °C (from EtOAc–heptane); $[\alpha]_D$ +57° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–6.90 (m, 14 H, aromatic H), 5.67 (dd, 1 H, $J_{2,1}$ 7.0, $J_{2,3}$ 9.5 Hz, H-2), 5.62 (t, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 5.19 (d, 1 H, H-1), 4.31 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{4,OH}$ 3.5 Hz, H-4), 4.18 (d, 1 H, H-5), 3.86, 3.75 (2 s, 6 H, OMe,COOMe), and 3.36 (d_1, 1 H, OH). Anal. Calcd for $C_{28}H_{26}O_{10}$: C, 64.36; H, 5.01. Found: C, 64.29; H, 4.90.

Methyl [4-methoxyphenyl O*-(4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-trichloroacetamido-β-*D*-glucopyranosyl)-(1 → 4)-2,3-di-*O*-benzoyl-β-*D*-glucopyranosid]uronate* (**9**).—A mixture of **10** (0.51 g) [**11** (0.35 g, 0.66 mmol), and powdered in 4 Å molecular sieves (0.5 g)] in anhyd CH_2Cl_2 (8 mL) was stirred for 30 min at room temperature under dry Ar. Trimethylsilyl triflate in toluene (1.0 M, 0.12 mL) was added, and the mixture was stirred for 30 min at room temperature. Et_3N (0.1 ml) was added, and the mixture was diluted with CH_2Cl_2 (20 mL), filtered, and concentrated. The residue was eluted from a column (40 g) of silica gel with 12:1 toluene–EtOAc to give **9** (590 mg, 89%); mp 254–256 °C (from EtOAc–heptane); $[\alpha]_D$ −34° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–6.85 (m, 19 H, aromatic H), 6.91 (d, 1 H, *J* 8.5 Hz, NH), 5.74 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.58 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 5.30 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN H-3), 5.23 (d, 1 H, GlcA H-1), 5.19 (s, 1 H, PhC*H*), 5.05 (d, 1 H, $J_{1,2}$ 8.5 Hz, GlcN H-1), 4.40 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 4.25 (d, 1 H, GlcA H-5), 4.05 (m, 1 H, GlcN H-2), 4.02 (s, 2 H, COCH_2Cl), 3.76, 3.75 (2 s, 6 H, OMe,COOMe), 3.54 (dd, 1 H, $J_{5,6eq}$ 5.0, $J_{6ax,6eq}$ 10.5 Hz, GlcN H-6eq), 3.50 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcN H-4), 3.38 (m, 1 H, $J_{5,6ax}$ 10.5 Hz, GlcN H-5), and 2.55 (t, 1 H, GlcN H-6ax). Anal. Calcd for $C_{45}H_{41}Cl_4NO_{16}$: C, 54.40; H, 4.16; N, 1.41. Found: C, 54.47; H, 4.22; N, 1.32.

*Methyl [*O*-(4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-trichloroacetamido-β-*D*-glucopyranosyl)-(1 → 4)-2,3-di-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate]uronate* (**7**).—A mixture of **9** (1.0 g, 1.01 mmol) and ceric ammonium nitrate (4.43 g, 8.1 mmol) in 1:1.5:1 toluene–MeCN–H_2O (35 mL) was vigorously stirred for 45 min at room temperature, then extracted with EtOAc (3 × 30 mL). The organic extracts were washed with satd aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The residue

was eluted from a column (40 g) of silica gel with 19:1 CH_2Cl_2–acetone to give the corresponding free hemiacetal (753 mg, 84%).

A mixture of the above isolated hemiacetal, trichloroacetonitrile (0.85 mL, 8.5 mmol), and DBU (25 μL, 0.17 mmol) in CH_2Cl_2 (8 mL) was stirred for 30 min at room temperature, then concentrated. The residue was eluted from a column (50 g) of silica gel with 6:1 toluene–EtOAc containing 0.1% of Et_3N to give **7** (816 mg, 78% from **9**); mp 144–145 °C (from Et_2O–heptane); $[\alpha]_D$ +13° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.70 (s, 1 H, C=NH), 8.0–7.30 (m, 15 H, Ph), 6.97 (d, 1 H, *J* 9.0 Hz, NH), 6.72 (d, 1 H, $J_{1,2}$ 3.5 Hz, GlcA H-1), 6.00 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, GlcA H-3), 5.47 (dd, 1 H, GlcA H-2), 5.32 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, GlcN H-3), 5.19 (s, 1 H, PhC*H*), 5.02 (d, 1 H, $J_{1,2}$ 8.5 Hz, GlcN H-1), 4.53 (d, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-5), 4.33 (t, 1 H, GlcA H-4), 4.03 (s, 2 H, COC*H*$_2$Cl), 4.01 (m, 1 H, GlcN H-2), 3.86 (s, 3 H, COOMe), 3.58 (dd, 1 H, $J_{5,6eq}$ 5.0, $J_{6ax,6eq}$ 10.5 Hz, GlcN H-6eq), 3.51 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcN H-4), 3.38 (m, 1 H, GlcN H-5), and 2.63 (t, 1 H, $J_{5,6ax}$ 10.5 Hz, GlcN H-6ax). Anal. Calcd for $C_{40}H_{35}Cl_7N_2O_{15}$: C, 46.56; H, 3.42; N, 2.71. Found: C, 46.43; H, 3.31; N, 2.62.

Methyl [methyl O-(4,6-O-benzylidene-3-O-chloroacetyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**18**).—(*a*) A mixture of **7** (0.2 g, 0.19 mmol), anhyd MeOH (40 μL, 1 mmol), and powdered 3 Å molecular sieves (0.1 g) in anhyd CH_2Cl_2 (3 mL) was stirred for 1 h at room temperature under dry Ar. Trimethylsilyl triflate in toluene (1.0 M, 30 μL, 30 μmol) was added, and the mixture was stirred for 45 min at room temperature. Et_3N (12 μL) was added, and the mixture was diluted with CH_2Cl_2 (10 mL), filtered, and concentrated. The residue was eluted from a column (15 g) of silica gel with 19:1 CH_2Cl_2–EtOAc to give **18** (137 mg, 80%); mp 144–145 °C (from EtOAc–heptane); $[\alpha]_D$ −30° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 15 H, Ph), 7.08 (d, 1 H, *J* 9.0 Hz, NH), 5.66 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.36 (dd, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-2), 5.32 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, GlcN H-3), 5.17 (s, 1 H, PhC*H*), 4.95 (d, 1 H, $J_{1,2}$ 8.5 Hz, GlcN H-1), 4.67 (d, 1 H, GlcA H-1), 4.22 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 4.13 (d, 1 H, GlcA H-5), 4.05 (m, 1 H, GlcN H-2), 4.04 (s, 2 H, COC*H*$_2$Cl), 3.86 (s, 3 H, COOMe), 3.54 (s, 3 H, OMe), 3.45 (m, 2 H, GlcN H-4,6eq), 3.33 (m, 1 H, $J_{4,5}$ 9.5, $J_{5,6ax}$ 10.5, $J_{5,6eq}$ 5.0 Hz, GlcN H-5), and 2.48 (t, 1 H, $J_{6ax,6eq}$ 10.5 Hz, GlcN H-6ax). Anal. Calcd for $C_{39}H_{37}Cl_4NO_{15}$: C, 51.96; H, 4.14; N, 1.55. Found: C, 52.01; H, 4.16; N, 1.40.

(*b*) A mixture of **10** (1.3 g, 2.05 mmol) and methyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate (**19**) [19] (735 mg, 1.71 mmol) was treated as described for the preparation of **9** to give **18** (1.4 g, 91%); mp 144–145 °C.

Methyl [methyl O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**8**).—A mixture of **18** (0.85 g, 0.94 mmol) and thiourea (215 mg, 2.82 mmol) in pyridine (3 mL) and EtOH (15 mL) was stirred for 20 h at 80 °C, then cooled, and concentrated. The residue was eluted from a column (50 g) of silica gel with 3:1 toluene–EtOAc to give **8** (743 mg, 95%); mp 122–123 °C (from EtOAc–heptane); $[\alpha]_D$ −1° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 15 H, Ph), 7.08 (d, 1 H, *J* 7.5 Hz, NH), 5.66 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.38 (dd, 1 H, $J_{1,2}$ 7.5 Hz, GlcA H-2), 5.18 (s, 1 H,

PhC*H*), 4.99 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN H-1), 4.67 (d, 1 H, GlcA H-1), 4.32 (t, 1 H, $J_{4,5}$ 9.5 Hz, GlcA H-4), 4.16 (d, 1 H, GlcA H-5), 3.98 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 3.5 Hz, GlcN H-3), 3.88 (s, 3 H, COOMe), 3.63 (m, 1 H, GlcN H-2), 3.53 (s, 3 H, OMe), 3.25 (m, 2 H, GlcN H-4,6eq), 2.94 (d, 1 H, OH), and 2.52 (t, 1 H, $J_{5,6ax} = J_{6ax,6eq}$ = 10.0 Hz, GlcN H-6ax). Anal. Calcd for $C_{37}H_{36}Cl_3NO_{14}$: C, 53.86; H, 4.40; N, 1.70. Found: C, 53.76; H, 4.48; N, 1.61.

Methyl [methyl O-*(4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-trichloroacetamido-β-*D*-glucopyranosyl)-(1 → 4)*-O*-(methyl 2,3-di-*O*-benzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)*-O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-*D*-glucopyranosyl)-(1 → 4)-2,3-di-*O*-benzoyl-β-*D*-glucopyranosid]uronate* (**4**).—A mixture of **7** (740 mg, 0.72 mmol) and **8** (455 mg, 0.55 mmol) was treated as described for the preparation of **9**. The residue was eluted from a column (100 g) of silica gel with 4:1 toluene–EtOAc to give **4** (821 mg, 87%); mp 178–179 °C (from EtOAc–heptane); $[\alpha]_D$ −13° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.0–7.30 (m, 30 H, Ph), 6.86 (d, 1 H, *J* 9.0 Hz, GlcN2 [1] NH), 6.75 (d, 1 H, *J* 8.0 Hz, GlcN*1* NH), 5.58, 5.52 (2 t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 HZ, GlcA *1* H-3,GlcA 2 H-3), 5.35, 5.28 (2 dd, 2 H, $J_{1,2}$ 7.0 Hz, GlcA *1* H-2,GlcA 2 H-2), 5.24 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, GlcN2 H-3), 5.18, 5.14 (2 s, 2 H, PhC*H*), 5.07 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN2 H-1), 4.99 (d, 1 H, GlcA 2 H-1), 4.94 (d, 1 H, $J_{1,2}$ 8.5 Hz, GlcN*1* H-1), 4.64 (d, 1 H, GlcA *1* H-1), 4.34, 4.22 (2 t, 2 H, $J_{4,5}$ 9.5 Hz, GlcA *1* H-4,GlcA 2 H-4), 4.31 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN*1* H-3), 4.07, 3.92 (2 d, 2 H, GlcA *1* H-5,GlcA 2 H-5), 3.99 (s, 2 H, COCH_2Cl), 3.98 (m, 1 H, GlcN2 H-2), 3.82, 3.67, 3.51 (3 s, 9 H, COOMe, OMe), 3.49 (m, 1 H, GlcN*1* H-2), and 2.54, 2.50 (2 t, 2 H, $J_{5,6ax} = J_{6ax,6eq}$ = 10.5 Hz, 2 GlcN H-6ax). Anal. Calcd for $C_{75}H_{69}Cl_7N_2O_{18}$: C, 53.16; H, 4.10; N, 1.65. Found. C, 53.10; H, 4.18; N, 1.51.

Methyl [methyl O-*(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamide-β-*D*-glucopyranosyl)-(1 → 4)*-O*-(methyl 2,3-di-*O*-benzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)*-O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-*D*-glucopyranosyl)-(1 → 4)-2,3-di-*O*-benzoyl-β-*D*-glucopyranosid]uronate* (**20**).—Compound **4** (760 mg, 0.45 mmol) was treated as described for the preparation of **8**. The residue was eluted from a column (50 g) of silica gel with 6:1 CH_2Cl_2–EtOAc to give **20** (689 mg, 95%); mp 173–174 °C (from EtOAc–heptane); $[\alpha]_D$ +4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.0–7.30 (m, 30 H, Ph), 7.02 (d, 1 H, *J* 7.0 Hz, GlcN2 NH), 6.77 (d, 1 H, *J* 8.0 Hz, GlcN*1* NH), 5.59, 5.48 (2 t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, 2 GlcA H-3), 5.35, 5.27 (2 dd, 2 H, $J_{1,2}$ 7.0 Hz, 2 GlcA H-2), 5.18, 5.16 (2 s, 2 H, 2 PhC*H*), 5.07, 4.95 (2 d, 2 H, $J_{1,2}$ 8.0 Hz, GlcN*1* H-1,GlcN2 H-1), 5.0, 4.64 (2 s, 2 H, GlcA 2 H-1,GlcA *1* H-1), 4.34, 4.26 (2 t, 2 H, $J_{4,5}$ 9.5 Hz, 2 GlcA H-4), 4.32 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN*1* H-3), 4.07, 3.92 (2 d, 2 H, 2 GlcA H-5), 3.96 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 3.5 Hz, GlcN2 H-3), 3.82, 3.72, 3.50 (3 s, 9 H, COOMe,OMe), 3.60 (m, 1 H, GlcN2 H-2), 2.99 (d, 1 H, OH), and 2.54, 2.43 (2 t, 2 H, $J_{5,6ax} = J_{6ax,6eq}$ = 10.5 Hz, 2 GlcN H-6ax). Anal. Calcd for $C_{73}H_{68}Cl_6N_2O_{27}$: C, 54.19; H, 4.24; N, 1.73. Found: C, 54.09; H, 4.30; N, 1.72.

Methyl [methyl O-*(4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-trichloro-*

[1] In recording NMR data, the amino sugar and uronic acid residues have been numbered starting from the reducing end. Thus GlcN2 refers to the second GlcN residue from the reducing end.

acetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-gluco-pyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glu-copyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**5**).—A mixture of **7** (606 mg, 0.59 mmol) and **20** (634 mg, 0.39 mmol) was treated as described for the preparation of **9**. The residue was eluted from a column (80 g) of silica gel with 6:1 toluene–acetone to give **5** (908 mg, 93%); mp 198–200 °C (from EtOAc–heptane); $[\alpha]_D$ −7° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 45 H, Ph), 6.92 (d, 1 H, *J* 9.0 Hz, GlcN3 NH), 6.77, 6.70 (2 d, 2 H, *J* 8.0 Hz, GlcN*1* NH, GlcN2 NH), 5.60, 5.50, 5.43 (3 t, 3 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 3 GlcA H-3), 5.36, 5.29, 5.27 (3 dd, 3 H, $J_{1,2}$ 7.0 Hz, 3 GlcA H-2), 5.28 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN*3* H-3), 5.19, 5.17, 5.15 (3 s, 3 H, 3 PhC*H*), 5.07 (d, 1 H, $J_{1,2}$ 8.5 Hz, GlcN*3* H-1), 5.06, 4.92 (2 d, 2 H, $J_{1,2}$ 8.0 Hz, GlcN*1* H-1, GlcN2 H-1), 4.97, 4.96, 4.64 (3 d, 3 H, 3 GlcA H-1), 4.38, 4.32, 4.19 (3 t, 3 H, $J_{4,5}$ 9.5 Hz, 3 GlcA H-4), 4.37, 4.35 (2 dd, 2 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN*1* H-3,GlcN2 H-3), 4.07, 3.92, 3.83 (3 d, 3 H, 3 GlcA H-5), 4.03 (s, 2 H, COCH_2Cl), 3.83, 3.67, 3.66, 3.52 (4 s, 12 H, COOMe,OMe), and 2.50 (m, 3 H, 3 GlcN H-6ax). Anal. Calcd for $C_{111}H_{101}Cl_{10}N_3O_{41}$: C, 53.59; H, 4.09; N, 1.70. Found: C, 53.49; H, 4.14; N, 1.60.

Methyl (methyl O-[4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-gluco-pyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyrano-sid]uronate (**21**).—Compound **5** (0.9 g, 0.36 mmol) was treated as described for the preparation of **8**. The residue was eluted from a column (50 g) of silica gel with 6:1 toluene–acetone to give **21** (725 mg, 83%); mp 187–189 °C (from EtOAc–heptane); $[\alpha]_D$ +6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 45 H, Ph), 7.03 (d, 1 H, *J* 7.0 Hz, GlcN*3* NH), 6.72, 6.67 (2 d, 2 H, *J* 8.0 Hz, GlcN*1* NH,GlcN2 NH), 5.58, 5.47, 5.41 (3 t, 3 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 3 GlcA H-3), 5.34, 5.26, 5.25 (3 dd, 3 H, $J_{1,2}$ 7.0 Hz, 3 GlcA H-2), 5.18, 5.16, 5.15 (3 s, 3 H, 3 PhC*H*), 5.06, 5.04, 4.96 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, 3 GlcN H-1), 4.96, 4.94, 4.64 (3 d, 3 H, 3 GlcA H-1), 4.37, 4.31 (2 dd, 2 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN*1* H-3,GlcN2 H-3), 4.36, 4.34, 4.24 (3 t, 3 H, $J_{4,5}$ 9.5 Hz, 3 GlcA H-4), 4.06, 3.90, 3.80 (3 d, 3 H, 3 GlcA H-5), 3.97 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 3.5 Hz, GlcN*3* H-3), 3.82, 3.68, 3.64, 3.49 (4 s, 12 H, COOMe,OMe), 2.98 (d, 1 H, OH), and 2.48 (m, 3 H, 3 GlcN H-6ax). Anal. Calcd for $C_{109}H_{100}Cl_9N_3O_{40}$: C, 54.30; H, 4.18; N, 1.74. Found: C, 54.15; H, 4.27; N, 1.68.

Methyl [methyl O-(4,6-O-benzylidene-3-O-chloroacetyl-2-deoxy-2-trichloro-acetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-gluco-pyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glu-copyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-gluco-pyranosid]uronate (**6**).—A mixture of **7** (160 mg, 0.15 mmol) and **21** (230 mg, 91 μmol) was treated as described for the preparation of **9**. The residue was eluted from a

column (30 g) of silica gel with 6:1 toluene–acetone to give **6** (280 mg, 93%); mp 191–192 °C (from EtOAc–heptane); $[\alpha]_D$ −2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 60 H, Ph), 6.93 (d, 1 H, *J* 8.0 Hz, GlcN*4* NH), 6.71, 6.65, 6.63 (3 d, 3 H, *J* 8.0 Hz, 3 GlcN NH), 5.57, 5.49, 5.39, 5.38 (4 t, 4 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 4 GlcA H-3), 5.37 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0 Hz, GlcN*4* H-3), 5.27, 5.26, 5.23, 5.21 (4 dd, 4 H, $J_{1,2}$ 7.0 Hz, 4 GlcA H-2), 5.17, 5.15, 5.14, 5.13 (4 s, 4 H, 4 PhC*H*), 5.05, 5.03, 5.02 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, 3 GlcN H-1), 4.96 (d, 1 H, $J_{1,2}$ 8.5 Hz, GlcN*4* H-1), 4.95, 4.94, 4.92, 4.64 (4 d, 4 H, 4 GlcA H-1), 4.30 (m, 7 H, 4 GlcA H-4,3 GlcN H-3), 4.06, 4.01, 3.90, 3.80 (4 d, 4 H, $J_{4,5}$ 9.5 Hz, 4 GlcA H-5), 3.99 (s, 2 H, COCH_2Cl), 3.81, 3.80, 3.64, 3.62, 3.50 (5 s, 15 H, COOMe,OMe), 3.92 (m, 1 H, GlcN*4* H-2), and 2.51 (m, 4 H, 4 GlcN H-6ax). Anal. Calcd for $C_{147}H_{133}Cl_{13}N_4O_{54}$: C, 53.82; H, 4.08; N, 1.71. Found: C, 53.62; H, 4.16; N, 1.72.

Methyl [methyl O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**22**).—Compound **6** (160 mg, 49 μmol) was treated as described for the preparation of **8**. The residue was eluted from a column (10 g) of silica gel with 5:1 toluene–acetone to give **22** (134 mg, 86%); mp 190–192 °C (from EtOAc–heptane); $[\alpha]_D$ +8° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 60 H, Ph), 7.02, 6.74, 6.68, 6.66 (4 d, 4 H, *J* 8.0 Hz, 4 GlcN NH), 5.58, 5.47, 5.40, 5.37 (4 t, 4 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 4 GlcA H-3), 5.36, 5.26, 5.25, 5.24 (4 dd, 4 H, $J_{1,2}$ 7.0 Hz, 4 GlcA H-2), 5.17, 5.16, 5.15, 5.13 (4 s, 4 H, 4 PhC*H*), 5.05, 5.04, 5.03, 4.94 (4 d, 4 H, $J_{1,2}$ 8.0 Hz, 4 GlcN H-1), 4.97, 4.95, 4.92, 4.64 (4 d, 4 H, 4 GlcA H-1), 4.30 (m, 7 H, 4 GlcA H-4, 3 GlcN H-3), 4.06, 3.92, 3.83, 3.81 (4 d, 4 H, $J_{4,5}$ 9.5 Hz, 4 GlcA H-5), 3.97 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 3.5 Hz, GlcN*4* H-3), 3.82, 3.69, 3.65, 3.63 3.50 (5 s, 15 H, COOMe,OMe), 2.99 (d, 1 H, OH), and 2.50 (m, 4 H, 4 GlcN H-6ax). Anal. Calcd for $C_{145}H_{132}Cl_{12}N_4O_{53}$: C, 54.35; H, 4.15; N, 1.75. Found: C, 54.20; H, 4.33; N, 1.80.

Methyl [methyl O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**23**).—A solution of **8** (285 mg, 0.34 mmol), tributylstannane (0.42 mL, 1.55 mmol), and AIBN (5 mg) in benzene (10 mL) and *N,N*-dimethylacetamide (3 mL) was stirred for 15 min under a flow of Ar, then heated for 1 h under reflux, cooled, and concentrated. The solid residue was washed with heptane (3 × 3 mL), and crystallized from MeOH to give **23** (202 mg, 81%); mp 250–252°C; $[\alpha]_D$ −5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.0–7.30 (m, 15 H, 3 Ph), 6.62 (d, 1 H, *J* 8.0 Hz, NH), 5.65 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 5.41 (dd, 1 H, $J_{1,2}$ 7.0 Hz, GlcA H-2), 5.20 (s, 1 H, PhC*H*), 4.67 (d, 1 H, GlcA H-1), 4.55 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN H-1), 4.22 (m, 2 H, GlcA H-4,5), 3.92 (s, 3 H, COOMe), 3.82 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 4.0 Hz, GlcN H-3), 3.55 (s, 3 H, OMe), 3.50 (m, 3 H, GlcN H-2,6eq, OH), 3.26 (t, 1 H, $J_{4,5}$ 9.0 Hz, GlcN H-4), 3.16 (m, 1 H, $J_{5,6ax}$ 9.5, $J_{5,6eq}$ 5.0 Hz, GlcN H-5), 2.55 (t, 1 H, $J_{6ax,6eq}$, 9.5 Hz, GlcN H-6ax), and 2.06 (s, 3 H, Ac). Anal. Calcd for $C_{37}H_{39}NO_{14}$: C, 61.58; H, 5.45; N, 1.94. Found: C, 61.68; H, 5.52; N, 1.91.

Methyl [methyl O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**24**).—Compound **20** (300 mg, 0.18 mmol) was treated as described for the preparation of **23** to give **24** (232 mg, 88%); mp 180–182 °C (from MeOH); $[\alpha]_D$ +18° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.0–7.30 (m, 30 H, Ph), 6.65 (d, 1 H, *J* 6.0 Hz, GlcN*2* NH), 5.54, 5.48 (2 t, 2 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, 2 GlcA H-3), 5.42 (d, 1 H, *J* 7.0 Hz, GlcN*1* NH), 5.34, 5.32 (2 dd, 2 H, $J_{1,2}$ 7.0 Hz, 2 GlcA H-2), 5.17, 5.12 (2 s, 2 H, 2 PhC*H*), 5.0 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN*1* H-1), 4.78, 4.61 (2 d, 2 H, 2 GlcA H-1), 4.58 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 8.5 Hz, GlcN*1* H-3), 4.36 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN*2* H-1), 4.27, 4.08 (2 t, 2 H, $J_{4,5}$ 9.5 Hz, 2 GlcA H-4), 4.04, 3.82 (2 d, 2 H, 2 GlcA H-5), 3.77, 3.65, 3.48 (3 s, 9 H, COOMe,OMe), 3.72 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 3.0 Hz, GlcN*2* H-3), 2.88 (m, 1 H, GlcN*1* H-2), 2.58, 2.48 (2 t, 2 H, $J_{5,6ax} = J_{6ax,6eq}$ = 10.0 Hz, 2 GlcN H-6ax), and 1.96, 1.49 (2 s, 6 H, Ac). Anal. Calcd for $C_{73}H_{74}N_2O_{27}$: C, 62.12; H, 5.28; N, 1.98. Found: C, 61.96; H, 5.35; N, 1.94.

Methyl [methyl O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(methyl-2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2,3-di-O-benzoyl-β-D-glucopyranosid]uronate (**25**).—Compound **21** (432 mg, 0.18 mmol) was treated as described for the preparation of **23** to give **25** (344 mg, 91%); mp 181–183 °C (from MeOH); $[\alpha]_D$ +23° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.0–7.30 (m, 45 H, Ph), 6.66 (d, 1 H, *J* 6.0 Hz, GlcN*3* NH), 5.52, 5.47, 5.38 (3 t, 3 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, 3 GlcA H-3), 5.35, 5.33, 5.28 (3 dd, 3 H, $J_{1,2}$ 7.0 Hz, 3 GlcA H-2), 5.34, 5.30 (2-d, 2 H, *J* 8.0 Hz, 2 GlcN NH), 5.12, 5.10, 5.08 (3 s, 3 H, 3 PhC*H*), 4.98, 4.88 (2 d, 2 H, $J_{1,2}$ 8.0 Hz, 2 GlcN H-1), 4.75, 4.74, 4.58 (3 d, 3 H, 3 GlcA H-1), 4.55 (m, 2 H, 2 GlcN H-3), 4.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN*3* H-1), 4.25, 4.17, 4.05 (3 t, 3 H, $J_{4,5}$ 9.5 Hz, 3 GlcA H-4), 4.02, 3.81, 3.70 (3 d, 3 H, 3 GlcA H-5), 3.76, 3.64, 3.54, 3.48 (4 s, 12 H, COOMe,OMe), 3.72 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 2.5 Hz, GlcN*3* H-3), 3.40 (m, 1 H, GlcN*3* H-2), 2.78 (m, 2 H, 2 GlcN H-2), 2.50 (m, 3 H, 3 GlcN H-6ax), and 1.96, 1.43, 1.39 (3 s, 9 H, Ac). Anal. Calcd for $C_{109}H_{109}N_3O_{40}$: C, 62.31; H, 5.23; N, 2.0. Found: C, 62.11; H, 5.28; N, 1.94.

Methyl [methyl O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-O-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-3-di-O-benzoyl-β-D-glucopyranosid]uronate (**26**).—Compound **22** (290 mg, 90 μmol) was treated as described for the preparation of **23** to give **26** (232 mg, 92%); mp 208–210 °C (from MeOH); $[\alpha]_D$ +26° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.0–7.30 (m, 60 H, Ph), 6.66 (d, 1 H, *J* 6.0 Hz, GlcN*4* NH), 5.52, 5.47, 5.38, 5.37 (4 t, 4 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, 4 GlcA H 3), 5.30 (m, 7 H, 4 GlcA H-2,3 GlcN NH), 5.17, 5.10, 5.09, 5.08 (4 s, 4 H, 4 PhC*H*), 4.96, 4.87, 4.84 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, 3 GlcN H-1), 4.74, 4.73, 4.71, 4.58 (4

d, 4 H, $J_{1,2}$ 7.0 Hz, 4 GlcA H-1), 4.53 (m, 3 H, 3 GlcN H-3), 4.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, GlcN*4* H-1), 4.24, 4.16, 4.15, 4.03 (4 t, 4 H, $J_{4,5}$ 9.5 Hz, 4 GlcA H-4), 4.02, 3.80, 3.67, 3.66 (4 d, 4 H, 4 GlcA H-5), 3.72 (m, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.0, $J_{3,OH}$ 3.0 Hz, GlcN*4* H-3), 3.76, 3.64, 3.52, 3.51, 3.47 (5 s, 15 H, COOMe,OMe), 3.42 (m, 1 H, GlcN*4* H-2), 2.50 (m, 4 H, 4 GlcN H-6ax), and 1.95, 1.42, 1.34, 1.28 (4 s, 12 H, Ac). Anal. Calcd for $C_{145}H_{144}N_4O_{53} \cdot H_2O$: C, 62.0; H, 5.24; N, 1.99. Found: C, 61.86; H, 5.33; N, 1.87.

Methyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranosiduronic acid, sodium salt (**27**).—A solution of **23** (148 mg, 0.2 mmol) in HOAc (10 mL) was heated at 100 °C. Water (3 mL) was added dropwise, and the mixture was stirred for 1 h at 100 °C, then cooled, concentrated, evaporated with water (3 × 5 mL), and dried in vacuo at 40 °C. NaOH (3 M, 1 mL) was added at 0°C to a solution of the residue in 3:1 MeOH–H_2O (4 mL), and the mixture was stirred for 16 h at room temperature, then diluted with water (5 mL), deionized with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The resulting solid was washed with EtOAc (3 × 2 mL) to remove benzoic acid, and the residue was eluted from a column (10 g) of silica gel with 10:5:3 EtOAc–MeOH–H_2O, then from a column (1 × 30 cm) of Sephadex SP-C25 (Na^+) with 1:1:1 EtOAc–MeOH–H_2O to give **27** as an amorphous, hygroscopic powder (74 mg, 83%); $[\alpha]_D$ −37° (*c* 1, H_2O); NMR data: ^1H (D_2O, 37 °C, internal H_2O, δ_H 4.754), δ 4.54 (d, 1 H, $J_{1,2}$ 8.2 Hz, GlcN H-1), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, GlcA H-1), 3.92 (dd, 1 H, $J_{5,6a}$ 2.0, $J_{6a,6b}$ 12.5 Hz, GlcN H-6a), 3.74 (m, 3 H, GlcN H-6b,GlcA H-4,5), 3.69 (dd, 1 H, $J_{2,3}$ 10.0 Hz, GlcN H-2), 3.58 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, GlcA H-3), 3.54 (s, 3 H, OMe), 3.48 (m, 3 H, GlcN H-3,4,5), 3.32 (dd, 1 H, GlcA H-2), and 2.04 (s, 3 H, Ac); ^{13}C (D_2O, internal acetone), see Table 1. Anal. Calcd for $C_{15}H_{24}NNaO_{12} \cdot H_2O$: C, 39.92; H, 5.81; N, 3.10. Found: C, 39.82; H, 5.92; N, 3.08.

Methyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(β-D-glucopyranosyluronic acid)-(1 → 3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranosiduronic acid, disodium salt (**1**).—Compound **24** (252 mg, 0.18 mmol) was treated as described for the preparation of **27**. After extraction of the benzoic acid, the residue was eluted from a column (12 g) of silica gel with 4:3:2 EtOAc–MeOH–H_2O, then from a column (1 × 30 cm) of Sephadex SP-C25 (Na^+) with 1:1:1 EtOAc–MeOH–H_2O to give amorphous, hygroscopic **1** (122 mg, 82%); $[\alpha]_D$ −49° (*c* 1, H_2O); NMR data: ^1H (D_2O, 37 °C, internal H_2O), δ 4.57, 4.54 (2 d, 2 H, $J_{1,2}$ 8.2 Hz, 2 GlcN H-1), 4.48, 4.38 (2 d, 2 H, $J_{1,2}$ 7.8 Hz, 2 GlcA H-1), 3.92, 3.91 (2 dd, 2 H, $J_{5,6a}$ 2.0, $J_{6a,6b}$ 12.5 Hz, 2 GlcN H-6a), 3.84, 3.69 (2 dd, 2 H, $J_{2,3}$ 10.0 Hz, 2 GlcN H-2), 3.59, 3.58 (2 t, 2 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 2 GlcA H-3), 3.54 (s, 3 H, OMe), 3.35, 3.33 (2 dd, 2 H, 2 GlcA H-2), and 2.05, 2.02 (2 s, 6 H, Ac); ^{13}C (D_2O, internal acetone), see Table 1. Anal. Calcd for $C_{29}H_{44}N_2O_{23} \cdot 2H_2O$: C, 40.0; H, 5.56; N, 3.22. Found: C, 40.10; H, 5.63; N, 3.11.

Methyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(β-D-glucopyranosyluronic acid)-(1 → 3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-O-(β-D-glucopyranosyluronic acid)-(1 → 3)-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranosiduronic acid, trisodium salt (**2**).—Compound **25** (248 mg, 0.12 mmol) was treated as described for the preparation of **1**. The residue was eluted from a column (1 × 40 cm) of Sephadex SP-C25 (Na^+) with 1:1 MeOH–H_2O to give amorphous, hygroscopic **2** (117 mg, 80%); $[\alpha]_D$ −56° (*c* 1, H_2O);

NMR data: ^{1}H (D_2O, 37 °C, internal H_2O), δ 4.55, 4.54, 4.52 (3 d, 3 H, $J_{1,2}$ 8.0 Hz, 3 GlcN H-1), 4.44, 4.34 (2 d, 3 H, $J_{1,2}$ 7.8 Hz, 3 GlcA H-1), 3.89 (m, 3 H, 3 GlcN H-6a), 3.92, 9.91, 3.68 (3 dd, 3 H, $J_{2,3}$ 10.0 Hz, 3 GlcN H-2), 3.56, 3.55, 3.54 (3 t, 3 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, 3 GlcA H-3), 3.53 (s, 3 H, OMe), 3.32, 3.31, 3.30 (3 dd, 3 H, 3 GlcA H-2), and 2.04, 2.01 (2 s, 9 H, Ac); ^{13}C (D_2O, internal acetone), see Table 1. Anal. Calcd for $C_{43}H_{64}N_3Na_3O_{34} \cdot 3H_2O$: C, 40.03; H, 5.47; N, 3.26. Found: C, 40.01; H, 5.57; N, 3.10.

Methyl O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 4)*-O-*(β-*D*-glucopyranosyluronic acid)-(1 → 3)*-O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 4)*-O-*(β-*D*-glucopyranosyluronic acid)-(1 → 3)*-O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 4)*-O-*(β-*D*-glucopyranosyluronic acid)-(1 → 3)*-O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 4)-β-*D*-glucopyranosiduronic acid, tetrasodium salt* (**3**).—Compound **26** (233 mg, 89 μmol) was treated as described for the preparation of **1**. After removal of the benzoic acid, the residue was dissolved in H_2O (3 mL), and 1.0 M NaOH was added until pH 9 (pH meter). The solution was directly eluted from a column (2.5 × 140 cm) of Sephadex G-10 with H_2O to give amorphous, hygroscopic **3** (111 mg, 81%); $[\alpha]_D$ −61° (*c* 1, H_2O); NMR data: ^{1}H (D_2O, 37 °C, internal H_2O), δ 4.62, 4.61, 4.59, 4.58 (4 d, 4 H, $J_{1,2}$ 8.0 Hz, 4 GlcN H-1), 4.51, 4.50, 4.41 (3 d, 4 H, $J_{1,2}$ 7.8 Hz, 4 GlcA H-1), 3.96 (m, 4 H, 4 GlcN H-6a), 3.88, 3.87, 3.74 (3 dd, 4 H, $J_{2,3}$ 10.0 Hz, 4 GlcN H-2), 3.62 (m, 4 H, 4 GlcA H-3), 3.58 (s, 3 H, OMe), 3.38, 3.37, 3.36 (3 dd, 4 H, $J_{2,3}$ 9.5 Hz, 4 GlcA H-2), and 2.10, 2.08, 2.06 (3 s, 12 H, Ac); ^{13}C (D_2O, internal acetone), see Table 1. Anal. Calcd for $C_{57}H_{84}N_4Na_4O_{45} \cdot 2H_2O$: C, 40.91; H, 5.30; N, 3.35. Found: C, 40.78; H, 5.41; N, 3.21.

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