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Syntheses of chondroitin 4- and 6-sulfate pentasaccharide derivatives having a methyl β-D-glucopyranosiduronic acid at the reducing end

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Abstract

The syntheses are reported of β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→OMe), O-sulfonated at C-4 or C-6 of the aminosugar moieties, which represent structural elements of chondroitin 4- and 6-sulfate proteoglycans. Starting from a synthetic disaccharide glycosyl acceptor, the stepwise or blockwise construction of the sugar backbone with appropriate synthons led to a pentasaccharide tetraol, which was used as a common intermediate. Selective 6-O-sulfonation of this tetraol, followed by saponification, gave the 6-sulfate derivative, whereas selective 6-O-benzoylation, followed by O-sulfonation and saponification, afforded the 4-sulfate derivative as their sodium salts.

Keywords: Pentasaccharide; Chondroitin 4- and 6-sulfate; Glycosaminoglycans

1. Introduction

Chondroitin sulfates occur in many tissues as side chains of proteoglycans. They are found at the cell surface or intracellularly in secretory granules, as well as in various body fluids [1,2]. They are linear copolymers built essentially from dimeric units composed of D-glucuronic acid (GlcA) and 2-acetamido-2-deoxy-D-galactose (GalNAc). In the major variants, the 4- and 6-*O*-positions of the GalNAc residues are found sulfonated, but several types having one or more sulfate groups at various positions are also known. Their biological roles are highly diversified, ranging from simple mechanical support functions to more intricate effects such as cell recognition [3], development of osteoarthritis [4], inhibition of human C1q factor [5], AT-III-mediated anticoagulant activity [6], but many other, still poorly understood, effects have been reported. Determination of the precise structure of the sequences involved in such events is highly complicated by the microheterogeneity of the polymers. Chemical or enzymatic controlled degradation affords complex mixtures of products from which pure fragments could be isolated with great difficulty and in low yields. Thus, chemical synthesis of molecules of definite size and structure remains one of the most efficient ways to answer these questions.

Several syntheses of chondroitin 4- and 6-sulfate fragments have been reported in the

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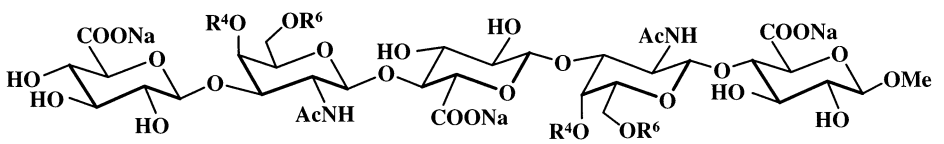
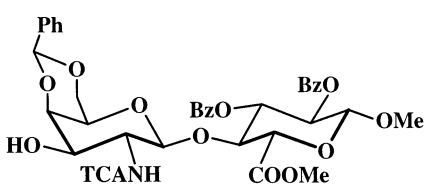
last decade. The basic reducing disaccharides [7], their methyl [8,9] or 4-methoxyphenyl [10] glycosides have been prepared, as well as structures of higher molecular size such as trisaccharide [10–12] or tetrasaccharide [10] derivatives. We now report for the first time the syntheses of chondroitin 4- and 6-sulfate pentasaccharide derivatives **1** and **2** having a methyl β-D-glucopyranosiduronic acid at the reducing end.

2. Results and discussion

For the syntheses of the target pentasaccharides **1** and **2**, the tetraol **15**, which may be obtained from a glycosyl donor (**5**) and a tetrasaccharide glycosyl acceptor (**12**), was designed as a common intermediate. This latter may, in turn, be prepared from a disaccharide glycosyl acceptor (**3**) by stepwise addition of appropriate monosaccharide glycosyl donors



	R^4	R^6
1	SO_3Na	H
2	H	SO_3Na



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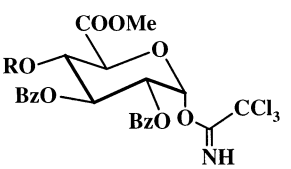
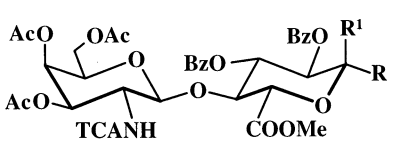
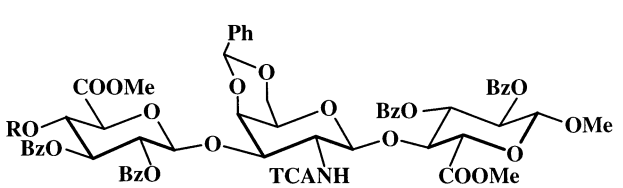


	R
4	MCA
5	Bz



	R	R^1
6	OMP	H
7	H	$OC(NH)CCl_3$



	R
8	MCA
9	H

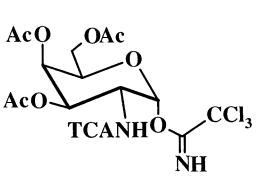


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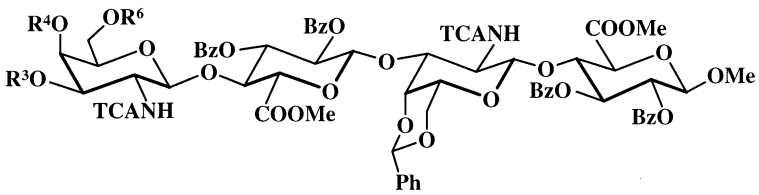
Fig. 1. Structures **1**–**10**.

(**4**, then **10**), or blockwise addition of a disaccharide glycosyl donor (**7**) (Fig. 1).

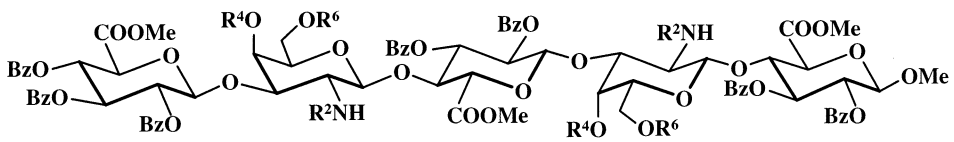
Preparation of the target glycosyl acceptor **12** was achieved as follows. In the first route, methyl 2,3-di-*O*-benzoyl-4-*O*-chloroacetyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (**4**) [13] (1.2 equivalents) was reacted with methyl (4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate (**3**) [12] in toluene at room temperature, in the presence of trimethylsilyl triflate, to give the crystalline trisaccharide derivative **8** in 84% yield. When the same reaction was performed in dichloromethane (details not presented in Section 3), a major product was isolated, the ^{1}H NMR spectrum of which indicated it to be an orthoester derivative [δ 5.95 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1^{III}), 4.62 (m, 1 H, $J_{2,3}$ 4.0, $J_{2,4}$ 1.0 Hz, H-2^{III})]. This derivative could not be rearranged cleanly into **8** by treatment with trimethylsilyl triflate in toluene or dichloromethane. Selective removal of the monochloroacetyl group in **8** with thiourea afforded the crystalline acceptor **9** in 87% yield. Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-*O*-trichloroacetimidoyl-α-D-galactopyranose (**10**) [12,14] (1.4 equivalents) with **9** in dichloromethane, as described above for the preparation of **8**, gave the crystalline tetrasaccharide derivative **11** in 78% yield. In the second route, the crystalline disaccharide imidate **7** was prepared in 74% yield from methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate (**6**) [12] by oxidative removal of the 4-methoxyphenyl group with ceric ammonium nitrate, followed by imidoylation with trichloroacetonitrile and 1,8-diazabicyclo[4,5,0]undec-7-ene (DBU). Coupling of imidate **7** (1.2 equivalents) with alcohol **3** in dichloromethane, as described above for the preparation of **8**, afforded crystalline **11** in 50% yield. Both routes, achieved with moderate excess (1.2–1.4 equivalents) of the corresponding glycosyl donors, compare well (57 and 50% yield, respectively), and no formation of α-linked species was observed. Transformation of **11** into a suitable glycosyl acceptor was then studied. Attempted selective removal of the acetyl groups in **11** with guanidine [15] led to important degradation, whereas the use of a catalytic amount of sodium methoxide at various temperatures and in various solvents led to extensive β-elimination reaction at the GlcA moieties. This problem was overcome by the use of acid-catalysed methanolysis [16]. Thus, careful treatment of **11** with methanolic hydrogen chloride in dichloromethane, followed by benzylidenation of the crude intermediate with benzaldehyde and trifluoroacetic acid, afforded the crystalline tetrasaccharide acceptor **12** in 60% overall yield. Under these conditions, the benzylidene group on the first amino sugar residue was partially removed, but easily reintroduced in the benzylidenation reaction (Fig. 2).

Condensation of methyl 2,3,4-tri-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (**5**) [11] (2 equivalents) with alcohol **12** in toluene, as described above for the preparation of **8**, afforded the crystalline pentasaccharide derivative **13** in 55% yield, and no orthoester formation was observed. This result contrasts with those reported [12] for a similar coupling reaction of **5** with the disaccharide acceptor **3** (85%), and the use of a larger excess of **5** (3–4 equivalents) did not significantly increase the yield. The *N*-trichloroacetyl group in **13** was transformed into *N*-acetyl by treatment [17] with tributylstannane and azobisisobutyronitrile (AIBN) in benzene at 80 °C to afford the crystalline diacetamide **14** in 79% yield. Treatment of **14** with aqueous acetic acid at 100 °C gave the crystalline tetraol **15**, the common intermediate, in 79% yield.

Preparation of the target molecules **1** and **2** was then achieved as follows. Treatment of **15** with the sulfur trioxide–trimethylamine complex (3.5 equivalents for each hydroxyl group) in *N*,*N*-dimethylformamide at 50 °C for 3 h, followed by ion-exchange chromatography, afforded the crystalline sodium salt **16** in 87% yield. Since comparison of the ^{1}H NMR spectra of **16** and **15** (see Table 2) did not clearly give evidence of the 6-O-sulfonation, a fraction of **16** (details not presented in Section 3) was O-acetylated (acetic anhydride in pyridine). The ^{1}H NMR spectrum of this derivative showed, inter alia, two telling signals at δ 5.27



	R3	R4	R6
11	Ac	Ac	Ac
12	H	PhCH	



	R2	R4	R6
13	TCA	PhCH	
14	Ac	PhCH	
15	Ac	H	H
16	Ac	H	SO_3Na
17	Ac	H	Bz
18	Ac	SO_3Na	Bz

Fig. 2. Structures **11**–**18**.

and 5.20 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4^{II,IV}), and two signals at δ 1.80 and 1.78 (2 s, 3 H each, Ac), characteristic of a 4-O-acetylated-D-*galacto* derivative, thus ensuring that O-sulfonation occurred exclusively at O-6. Treatment of the tetraol **15** with benzoyl cyanide (2 molar equivalents) in pyridine at room temperature afforded the 6-O-benzoylated derivative **17** in 77% yield. Comparison of the ^{1}H NMR spectra (see Table 2) of **17** (chloroform-*d*) and **15** (methanol-d_4) showed no significant downfield shift of the signals for H-4^{II,IV}, thus excluding any O-benzoylation at C-4. Sulfation of **17**, as described for the preparation of **16**, was somewhat troublesome, and required a large excess of reagent (15 equivalents for each hydroxyl group) and prolonged reaction time to go to completion, but the crystalline sodium salt **18** was nevertheless isolated in 88% yield. Comparison of the ^{1}H NMR spectra of **18** and **17** showed (Table 2) the expected [9] downfield shifts ($\sim$1 ppm) of the signals for H-4^{II,IV} in **18**. Saponification of the ester groups was then achieved by treatment of **16** and **18** with lithium hydroperoxide [18] in aqueous tetrahydrofuran (THF) at -5 °C, followed by methanolic sodium hydroxide, to afford the target molecules **2** and **1** in 80 and 78% yields, respectively. The ^{1}H (Table 2) and ^{13}C NMR spectra of **1** and **2** are in full agreement with the expected structures, and in accord with those reported for synthetic di-, tri-, and tetrasaccharide derivatives [9–11] and for polymeric chondroitin 4- and 6-sulfates [19].

In conclusion, stereocontrolled syntheses of chondroitin 4- and 6-sulfate pentasaccharide derivatives **1** and **2** are reported. These molecules are currently being evaluated in conformational studies and in biological assays.

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 Perkin–Elmer 241 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C with a Bruker DPX-250 spectrometer operating at 250 and 63 MHz, respectively, with Me_4Si as internal standard, unless otherwise stated. Assignments were based on homo- and heteronuclear correlations using the supplier's software. Mass spectra were obtained on a Perkin–Elmer SCIEX API 300 spectrometer operating in the ion-spray (IS) mode. Flash-column chromatography was performed on Silica Gel (E. Merck, 40–63 μm). Elemental analyses were performed by the Service Central de Microanalyse du CNRS (Vernaison, France).

*Methyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-β-*D*-galactopyranosyl)-(1→4)-2,3-di-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-*D*-glucopyranuronate* (**7**).—A mixture of methyl (3,4,6 - tri - *O* - acetyl - 2 - deoxy - 2 - trichloroacetamido - β - D - galactopyranosyl) - (1→4) - (4-methoxyphenyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate (**6**) [12] (0.3 g, 0.31 mmol) and ceric ammonium nitrate (1.8 g, 3.3 mmol) in 1:1.5:1 toluene–MeCN–water (15 mL) was vigorously stirred for 45 min at room temperature (rt). The mixture was then diluted with EtOAc (50 mL), washed with water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column (25 g) of silica gel with 1:1 EtOAc–petroleum ether to give the corresponding free hemiacetal (0.23 g, 86%).

A mixture of the above isolated hemiacetal, CCl_3CN (0.32 mL, 0.32 mmol), and DBU (8 μL, 50 μmol) in anhyd CH_2Cl_2 (4 mL) was stirred for 1 h at rt, then concentrated. The residue was eluted from a column (20 g) of silica gel with 1:1 EtOAc–petroleum ether containing 0.5% of Et_3N to give **7** (232 mg, 74% from **6**); mp 129–130 °C (from EtOAc–petroleum ether); $[\alpha]_D$ +32° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): carbohydrate ring protons (see Table 1); 8.65 (s, 1 H, C=N*H*), 8.0–7.20 (m, 10 H, Ph), 3.82, (s, 3 H, COOCH_3), 2.10, 2.0, 1.95 (3 s, 9 H, Ac). Anal. Calcd for $C_{37}H_{36}Cl_6N_2O_{17}$: C, 44.73; H, 3.65; N, 2.82. Found: C, 44.64; H, 3.85; N, 2.85.

Table 1
^{1}H NMR data [a]: carbohydrate ring protons for compounds **7**–**9**, **11** and **12**

Proton	**7**	**8**	**9**	**11**	**12**
H-1^{I}	6.69 (*3.5*)	4.68 (*7.0*)	4.72 (*7.0*)	4.67 (*7.0*)	4.67 (*7.0*)
H-2^{I}	5.47 (*9.0*)	5.30 (*9.0*)	5.33 (*9.0*)	5.33 (*9.0*)	5.31 (*9.0*)
H-3^{I}	5.98 (*9.0*)	5.61 (*9.0*)	5.46 (*9.0*)	5.52 (*9.0*)	5.55 (*9.0*)
H-4^{I}	4.38 (*9.5*)	4.57 (*9.0*)	4.58 (*9.0*)	4.57 (*9.0*)	4.57 (*9.0*)
H-5^{I}	4.52	4.14	4.10	4.12	4.12
H-1^{II}	4.94 (*8.0*)	5.25 (*8.0*)	5.20 (*8.0*)	5.23 (*8.0*)	5.23 (*8.0*)
H-2^{II}	3.91 (*11.0*)	3.64 (*11.0*)	3.62 (*11.0*)	3.62 (*11.0*)	3.62 (*11.0*)
H-3^{II}	5.14 (*3.5*)	4.59 (*3.5*)	4.57 (*3.5*)	4.53 (*3.5*)	4.51 (*3.5*)
H-4^{II}	5.12 (*1.0*)	4.31 (*1.0*)	4.28 (*1.0*)	4.26 (*1.0*)	4.30 (*1.0*)
H-5^{II}	3.70	3.28	3.53	3.89	3.30
H-6a,b^{II}	3.35	3.85, 3.80	3.95, 3.80	3.95, 3.90	3.90, 3.80
NH^{II}	6.74 (*8.5*)	7.03 (*7.0*)	7.07 (*7.0*)	6.86 (*7.0*)	6.78 (*7.0*)
H-1^{III}		5.14 (*7.0*)	5.12 (*7.0*)	5.09 (*7.0*)	5.11 (*7.0*)
H-2^{III}		5.44 (*9.0*)	5.44 (*9.0*)	5.35 (*9.0*)	5.32 (*9.0*)
H-3^{III}		5.68 (*9.0*)	5.72 (*9.0*)	5.68 (*9.0*)	5.67 (*9.0*)
H-4^{III}		5.48 (*9.5*)	4.10 (*9.5*)	4.35 (*9.5*)	4.48 (*9.5*)
H-5^{III}		4.20	4.15	4.13	4.17
H-1^{IV}				4.78 (*8.0*)	4.59 (*8.0*)
H-2^{IV}				3.65 (*11.0*)	3.65 (*11.0*)
H-3IV				5.04 (*3.5*)	3.92 (*3.5*)
H-4^{IV}				5.10 (*1.0*)	3.95 (*1.0*)
H-5^{IV}				3.72	3.19
H-6a,b^{IV}				3.31, 3.25	3.90, 3.80
NH^{IV}				6.64 (*8.5*)	6.75 (*8.0*)

[a] Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) in $CDCl_3$.

Methyl (methyl 2,3-di-O-benzoyl-4-O-chloroacetyl-β-D-glucopyranosyluronate)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosid)uronate (**8**).—A mixture of methyl (4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosid)uronate (**3**) [12] (0.6 g, 0.73 mmol), methyl 2,3-di-*O*-benzoyl-4-*O*-chloroacetyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (**4**) [13] (0.56 g, 0.87 mmol), and powdered 4 Å molecular sieves (0.5 g) in anhyd toluene (20 mL) was stirred for 1 h at rt under dry Ar. A solution of Me_3SiOTf in toluene (1 M, 0.13 mL) was added, and the mixture was stirred for 1 h at rt. Triethylamine (0.14 mL) was added, and the mixture was filtered, and concentrated. The residue was eluted from a column (100 g) of silica gel with 4:1 toluene–EtOAc containing 0.2% of Et_3N to give **8** (0.8 g, 84%); mp 249–250 °C (from EtOAc–petroleum ether); $[\alpha]_D$ +37° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): carbohydrate ring protons (see Table 1); 8.0–7.20 (m, 25 H, Ph), 5.34 (s, 1 H, PhC*H*), 3.93 (ABq, 2 H, COCH_2Cl), 3.83, 3.75 (2 s, 6 H, COOCH_3), 3.48 (s, 3 H, OCH_3). Anal. Calcd for $C_{60}H_{55}Cl_4NO_{23}$: C, 55.39; H, 4.23; N, 1.07. Found: C, 55.25; H, 4.32; N, 1.12.

Methyl (methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosid)uronate (**9**).—A mixture of **8** (585 mg, 0.45 mmol) and thiourea (0.1 g, 1.35 mmol) in pyridine (5 mL) and abs EtOH (12 mL) was stirred for 2 h at 80 °C, then cooled, diluted with CH_2Cl_2 (60 mL), washed with water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column (40 g) of silica gel with 2:1 toluene–EtOAc to give **9** (0.48 g, 87%); mp 262–264 °C (from EtOAc–petroleum ether); $[\alpha]_D$ +32° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): carbohydrate ring protons (see Table 1); 8.0–7.20 (m, 25 H, Ph), 5.29 (s, 1 H, PhC*H*), 3.80, 3.75 (2 s, 6 H, COOCH_3), 3.48 (s, 3 H, OCH_3), 3.29 (bs, 1 H, *HO*-4^{III}). Anal. Calcd for $C_{58}H_{54}Cl_3NO_{22}$: C, 56.89; H, 4.41; N, 1.14. Found: C, 56.72; H, 4.54; N, 1.29.

Methyl (3,4,6-tri-O-acetyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosid)uronate (**11**).—(a) A mixture of **9** (0.3 g, 0.245 mmol), 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetamido-1-*O*-trichloroacetimidoyl-α-D-galactopyranose (**10**) [12,14] (204 mg, 0.34 mmol) in anhyd CH_2Cl_2 (8 mL) was treated as described for the preparation of **8**. The residue was eluted from a column (40 g) of silica gel with 2:1 toluene–EtOAc containing 0.2% of Et_3N to give **11** (320 mg, 78%); mp 151–153 °C (from EtOAc–petroleum ether); $[\alpha]_D$ +3° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): carbohydrate ring protons (see Table 1); 8.0–7.20 (m, 25 H, Ph), 5.31 (s, 1 H, PhC*H*), 3.82, 3.78 (2 s, 6 H, COOCH_3), 3.48 (s, 3 H, OCH_3), 1.92, 1.90, 1.88 (3 s, 9 H, Ac). Anal. Calcd for $C_{72}H_{70}Cl_6N_2O_{30}$: C, 52.17; H, 4.22; N, 1.69. Found: C, 51.92; H, 4.37; N, 1.71.

(b) A mixture of **3** (100 mg, 0.12 mmol) and **7** (144 mg, 0.14 mmol) was treated as described in (a). The residue was eluted from a column (25 g) of silica gel with 2:1 toluene–EtOAc containing 0.2% of Et_3N, and crystallized from EtOAc–petroleum ether to give **11** (100 mg, 50%); mp 151–153 °C.

Methyl (4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosyluronate)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β-D-glucopyranosid)uronate (**12**).—A solution of AcCl (0.2 mL) in anhyd MeOH (5 mL) was added to a solution of **11** (0.39 g, 0.23 mmol) in anhyd CH_2Cl_2 (5 mL), and the mixture was stirred for 24 h at rt, then deionized with Dowex 1X2-400 (OH^-) resin, filtered, concentrated, and dried. A mixture of the residue, benzaldehyde (6 mL), and CF_3COOH (0.1 mL) was stirred for 1.5 h at rt, then cooled to 0 °C. Triethylamine (0.5 mL) was added, and the mixture was diluted with CH_2Cl_2 (40 mL), washed with water, dried ($MgSO_4$), and concentrated. The residue was eluted from a column (20 g) of silica gel with 1:1→3:1

EtOAc–petroleum ether containing 0.5% of Et_3N, and crystallized from EtOAc–petroleum ether to give **12** (228 mg, 60%); mp 172–174 °C; $[\alpha]_D$ −6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): carbohydrate ring protons (see Table 1); 8.0–7.20 (m, 30 H, Ph), 5.37, 5.23 (2 s, 2 H, PhC*H*), 3.82, 3.81 (2 s, 6 H, COOCH_3), 3.49 (s, 3 H, OCH_3), 2.95 (bs, 1 H, *H*O-3^{IV}). Anal. Calcd for $C_{73}H_{70}Cl_6N_2O_{27}$: C, 54.07; H, 4.32; N, 1.73. Found: C, 54.05; H, 4.29; N, 1.78.

Methyl (methyl 2,3,4-tri-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(4,6*-O-*benzylidene-2-deoxy-2-trichloroacetamido-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(4,6*-O-*benzylidene-2-deoxy-2-trichloroacetamido-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosid)uronate* (**13**).—A mixture of **12** (308 mg, 0.19 mmol) and methyl 2,3,4-tri-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (**5**) [11] (255 mg, 0.38 mmol) in anhyd toluene (9 mL) was treated as described for the preparation of **8**. The residue was eluted from a column (40 g) of silica gel with 4:1 toluene–EtOAc containing 0.2% of Et_3N to give **13** (222 mg, 55%); mp 172–174 °C (from EtOAc–petroleum ether); $[\alpha]_D$ +9.5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): carbohydrate ring protons (see Table 2); 8.0–7.20 (m, 45 H, Ph), 5.34, 5.29 (2 s, 2 H, PhC*H*), 3.81, 3.77, 3.62 (3 s, 9 H, COOCH_3), 3.48 (s, 3 H, OCH_3). Anal. Calcd for $C_{101}H_{90}Cl_6N_2O_{36}$: C, 57.21; H, 4.28; N, 1.32. Found: C, 57.02; H, 4.44; N, 1.40.

Methyl (methyl 2,3,4-tri-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(2-acetamido-4,6*-O-*benzylidene-2-deoxy-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(2-acetamido-4,6*-O-*benzylidene-2-deoxy-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosid)uronate* (**14**).—A mixture of **13** (360 mg, 0.17 mmol), Bu_3SnH (0.46 mL, 1.7 mmol), and AIBN (20 mg) in dry benzene (10 mL) was stirred for 30 min at rt under dry Ar, then heated at 80 °C for 2 h, cooled, and concentrated. The residue was stirred for 1 h at 0 °C with petroleum ether (30 mL), and the precipitate was filtered off, washed with petroleum ether, and eluted from a column (30 g) of silica gel with 1:1 EtOAc–CH_2Cl_2 to give **14** (257 mg, 79%); mp 240–242 °C (from EtOH–petroleum ether); $[\alpha]_D$ +10° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): carbohydrate ring protons (see Table 2); 8.0–7.20 (m, 45 H, Ph), 5.28, 5.27 (2 s, 2 H, PhC*H*), 3.76, 3.67, 3.58 (3 s, 9 H, COOCH_3), 3.47 (s, 3 H, OCH_3), 1.57, 1.54 (2 s, 6 H, NAc); ISMS: *m*/*z* 1915, $[M+H]^+$. Anal. Calcd for $C_{101}H_{96}N_2O_{36}$: C, 63.38; H, 5.06; N, 1.46. Found: C, 63.08; H, 5.20; N, 1.44.

Methyl (methyl 2,3,4-tri-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosid)uronate* (**15**).—A suspension of **14** (192 mg, 0.1 mmol) in AcOH (8 mL) was stirred at 100 °C. Water (4 mL) was added dropwise, and the mixture was stirred for 30 min at 100 °C, then cooled, concentrated, and evaporated with water (3 × 10 mL). The residue was eluted from a column (15 g) of silica gel with 20:1 EtOAc–EtOH to give **15** (138 mg, 79%); mp 197–198 °C (from $CHCl_3$–petroleum ether); $[\alpha]_D$ −3° (*c* 1, MeOH); ^1H NMR (CD_3OD): carbohydrate ring protons (see Table 2); 8.10–7.20 (m, 35 H, Ph), 3.77, 3.68, 3.58 (3 s, 9 H, COOCH_3), 3.42 (s, 3 H, OCH_3), 1.35, 1.34 (2 s, 6 H, NAc); ISMS: *m*/*z* 1741, $[M+H]^+$. Anal. Calcd for $C_{87}H_{90}N_2O_{36}$: C, 60.0; H, 5.21; N, 1.61. Found: C, 59.80; H, 5.32; N, 1.52.

Methyl (methyl 2,3,4-tri-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-6*-O-*sulfonato-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di-O-benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-6*-O-*sulfonato-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosid)uronate* (**16**).—A mixture of **15** (261 mg, 0.15 mmol) and sulfur trioxide–trimethylamine complex (132 mg, 1 mmol) in anhyd DMF (8 mL) was stirred for 3 h at 50 °C under dry Ar, then cooled. Methanol (0.5 mL) was added, and the mixture was concentrated. The residue was eluted from a column (30 g) of silica gel with 6:1 CH_2Cl_2–MeOH, then from a column (1.5 × 25 cm) of Sephadex SP C-25 (Na^+) with 9:5:1 CH_2Cl_2–

Table 2
^{1}H NMR data [a]: carbohydrate ring protons for pentasaccharide derivatives **13**–**18**, **1**, and **2**

Protons		**13**	**14**	**15** [b]	**16** [b]	**17**	**18** [b]	**1** [c]	**2** [c]
GlcA	H-1	5.18, 5.05, 4.67	5.10, 4.90, 4.63	5.20, 488, 4.78	5.17, 4.90, 4.78	4.93, 4.78, 4.60	5.29, 5.25, 5.24	4.35, 4.34, 4.27	4.37, 4.36, 4.25
	H-2	5.49, 5.31, 5.29	5.47, 5.30, 5.28	5.51, 5.25, 5.20	5.49, 5.25, 5.18	5.39, 5.36, 5.35	5.66, 5.62, 5.35	3.25, 3.23, 3.22	3.23, 3.21, 3.18
	H-3	5.77, 5.65, 5.64	5.83, 5.67, 5.64	6.00, 5.58, 5.55	5.95, 5.60, 5.59	5.87, 5.61, 5.53	6.13, 5.83, 5.75	3.55, 3.39, 3.37	3.50, 3.35, 3.34
	H-4	5.55, 4.55, 4.52	5.59, 4.46, 4.39	5.61, 4.35, 4.34	5.60, 4.32, 4.31	5.50, 4.27, 4.25	5.66, 4.39, 4.37	3.57, 3.54, 3.45	3.56, 3.55, 3.43
	H-5	4.27, 4.12, 4.11	4.27, 4.10, 4.08	4.59, 4.18, 4.16	4.59, 4.20, 4.15	4.27, 4.05, 4.01	4.69, 4.35, 4.33	3.70–3.65	3.63–3.58
GalN	H-1	5.21, 5.12	5.11, 4.97	5.18, 5.02	5.01, 4.91	4.90, 4.88	5.30, 5.22	4.47, 4.46	4.42, 4.41
	H-2	3.68, 3.65	3.31, 3.17	3.24, 3.15	3.64, 3.62	3.05, 3.03	3.64, 3.62	3.92, 3.91	3.89, 3.86
	H-3	4.54, 4.48	4.62, 4.52	4.57, 4.47	4.56, 4.43	4.67, 4.58	4.58, 4.57	3.97, 3.96	3.85, 3.84
	H-4	4.32, 4.20	4.14, 4.10	4.09, 3.95	4.11, 4.01	4.01, 3.86	5.10, 5.01	4.72, 4.64	4.08, 4.05
	H-5	3.25, 3.10	2.92, 2.87	3.15, 3.10	3.50, 3.45	3.51, 3.49	3.58, 3.57	3.75, 3.68	3.73, 3.69
	H-6a,b	3.95–3.85	3.80–3.70	3.80–3.70	3.85–3.75	3.98, 3.70–3.60	4.10–3.90	3.75–3.65	4.12–4.08
	NH	6.92, 6.81	5.36, 5.28			5.45, 5.33			

[a] Chemical shifts (δ, ppm) in $CDCl_3$, unless otherwise stated.
[b] CD_3OD.
[c] D_2O.

MeOH–water to give **16** (254 mg, 87%); mp 184–186 °C (dec., from $CHCl_3$–petroleum ether); $[\alpha]_D$ −5° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): carbohydrate ring protons (see Table 2); 8.10–7.20 (m, 35 H, Ph), 3.79, 3.77, 3.64 (3 S, 9 H, $COOCH_3$), 3.44 (s, 3 H, OCH_3), 1.42, 1.34 (2 s, 6 H, NAc). Anal. Calcd for $C_{87}H_{86}N_2Na_2O_{42}S_2{\cdot}2H_2O$: C, 52.89; H, 4.59; N, 1.42. Found: C, 52.69; H, 4.71; N, 1.29.

O-Acetylated derivative; ^{1}H NMR (CD_3OD): δ 8.0–7.30 (m, 35 H, Ph), 5.89, 5.61, 5.48 (3 t, 3 H, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-$3^{I,III,V}$), 5.58 (dd, 1 H, $J_{4,5}$ 9.5 Hz, H-4^{V}), 5.34, 5.22, 5.13 (3 dd, 3 H, $J_{1,2}$ 7.0 Hz, H-$2^{I,III,V}$), 5.27, 5.20 (2 dd, 2 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-$4^{II,IV}$), 5.13, 4.96, 4.78 (3 d, 3 H, H-$1^{I,III,V}$), 4.97, 4.88 (2 d, 2 H, $J_{1,2}$ 8.0 Hz, H-$1^{II,IV}$), 4.56, 4.51 (2 dd, 2 H, $J_{2,3}$ 11.0 Hz, H-$3^{II,IV}$), 4.47, 4.17, 4.12 (3 d, 3 H, H-$5^{I,III,V}$), 4.32, 4.23 (2 dd, 2 H, H-$4^{I,III}$), 3.85–3.75 (m, 4 H, H-6a,$b^{II,IV}$), 3.79, 3.76, 3.64 (3 s, 9 H, $COOCH_3$), 3.65–3.55 (m, 2 H, H-$2^{II,IV}$), 3.50–3.45 (m, 2 H, H-$5^{II,IV}$), 1.80, 1.78 (2 s, 6 H, OAc), 1.42, 1.36 (2 s, 6 H, NAc).

Methyl (methyl 2,3,4-tri-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(2-acetamido-6*-O-*benzoyl-2-deoxy-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(2-acetamido-6*-O-*benzoyl-2-deoxy-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosid)uronate* (**17**).—A mixture of **15** (244 mg, 0.14 mmol) and benzoyl cyanide (74 mg, 0.57 mmol) in anhyd pyridine (8 mL) was stirred overnight at rt. Methanol (0.75 mL) was added, and the mixture was concentrated, and evaporated with toluene (3 × 10 mL). The residue was eluted from a column (25 g) of silica gel with 30:1 CH_2Cl_2–MeOH to give **17** (210 mg, 77%); mp 188–190 °C (from EtOAc–petroleum ether); $[\alpha]_D$ +19° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): carbohydrate ring protons (see Table 2); 8.20–7.20 (m, 45 H, Ph), 3.78, 3.57, 3.54 (3 s, 9 H, $COOCH_3$), 3.48 (s, 3 H, OCH_3), 1.37, 1.35 (2 s, 6 H, NAc); ISMS: *m/z* 1947, $[M+H]^+$. Anal. Calcd for $C_{101}H_{96}N_2O_{38}$: C, 62.34; H, 4.97; N, 1.44. Found: C, 62.14; H, 4.95; N, 1.55.

Methyl (methyl 2,3,4-tri-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-6*-O-*benzoyl-2-deoxy-4*-O-*sulfonato-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-6*-O-*benzoyl-2-deoxy-4*-O-*sulfonato-β*-D-*galactopyranosyl)-(1→4)-(methyl 2,3-di*-O-*benzoyl-β*-D-*glucopyranosid)uronate* (**18**).—A mixture of **17** (125 mg, 64 μmol) and sulfur trioxide–trimethylamine complex (170 mg, 1.28 mmol) in anhyd DMF was stirred for 48 h at 50 °C under dry Ar. More reagent (85 mg, 0.64 mmol) was then added, and the mixture was stirred for further 48 h at 50 °C, then cooled. Methanol (1 mL) was added, and the mixture was stirred for 1 h at rt, then concentrated. The residue was eluted twice from a column (25 g) of silica gel with 10:1 CH_2Cl_2–MeOH, then from a column (1.5 × 25 cm) of Sephadex SP C-25 (Na^+) with 9:5:1 CH_2Cl_2–MeOH–water to give **18** (120 mg, 88%); 202–204 °C (dec., from $CHCl_3$–petroleum ether); $[\alpha]_D$ +9° (*c* 1, MeOH); ^{1}H NMR (CD_3OD): carbohydrate ring protons (see Table 2); 8.20–7.20 (m, 45 H, Ph), 3.87, 3.68, 3.58 (3 s, 9 H, $COOCH_3$), 3.42 (s, 3 H, OCH_3), 1.35, 1.32 (2 s, 6 H, NAc). Anal. Calcd for $C_{101}H_{94}N_2Na_2O_{44}S_2{\cdot}H_2O$: C, 55.96; H, 4.46; N, 1.29. Found: C, 55.71; H, 4.62; N, 1.18.

Sodium (sodium β-D-*glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-4*-O-*sulfonato-β*-D-*galactopyranosyl)-(1→4)-(sodium β*-D-*glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-4*-O-*sulfonato-β*-D-*galactopyranosyl)-(1→4)-(methyl β*-D-*glucopyranosid)uronate* (**1**).—A solution of **18** (120 mg, 56 μmol) in 2:1 THF–water (4 mL) was treated at −5 °C with 30% H_2O_2 (0.45 mL) and LiOH (1 M, 0.84 mL), and the mixture was stirred for 2 h at this temperature and 16 h at rt, then cooled to 0 °C. Methanol (4 mL) and NaOH (4 M, 0.62 mL) were added, and the mixture was stirred for 8 h at rt, then treated with Amberlite IR-120 (H^+) resin to pH 3 (pH meter control), filtered, and concentrated. The residue was stirred for 1 h at 0 °C with abs EtOH (10 mL), and the precipitate was filtered off, washed with cold abs EtOH, and eluted from a column (12 g) of silica gel with 12:5:3 → 5:3:2 EtOAc–MeOH–water, then taken up in water (5 mL). The pH of the solution was brought to 6.5 (pH meter control) with diluted NaOH, and the solution was

filtered, and freeze-dried to give **1** as an amorphous hygroscopic powder (56 mg, 80%); $[\alpha]_D$ −46° (*c* 1, H_2O); ^1H NMR (D_2O, internal H_2O): carbohydrate ring protons (see Table 2); 3.43 (s, 3 H, OCH_3), 1.94, 1.92 (2 s, 6 H, NAc); ^{13}C (D_2O, internal acetone): δ 176.12, 175.24, 175.16, 174.69, 174.46 (C=O), 103.88, 103.72, 103.48, 103.27 (C-1^{II-V}), 101.97 (C-1^{I}), 80.55, 80.27, 80.07 (C-$4^{I,III}$, C-$3^{II,IV}$), 76.79, 76.67, 76.51, 75.58, 75.27, 74.79, 74.14, 73.71, 72.77, 72.41, 72.01 (C-$2,3^{I,III,V}$, C-4^{V}, C-$3,4,5^{II,IV}$), 61.24, 61.05 (C-$6^{II,IV}$), 57.48 (O*C*H_3), 51.80, 51.62 (C-$2^{II,IV}$), 22.74, 22.69 (CO*C*H_3). Anal. Calcd for $C_{35}H_{49}N_2Na_5O_{35}S_2 \cdot 3H_2O$: C, 32.56; H, 4.29; N, 2.17. Found: C, 32.34; H, 4.41; N, 2.07.

*Sodium (sodium β-*D*-glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-6-*O*-sulfonato-β-*D*-galactopyranosyl)-(1→4)-(sodium β-*D*-glucopyranosyluronate)-(1→3)-(sodium 2-acetamido-2-deoxy-6-*O*-sulfonato-β-*D*-galactopyranosyl)-(1→4)-(methyl β-*D*-glucopyranosid)uronate* (**2**).—A solution of **16** (130 mg, 67 μmol) in 2:1 THF–water (8 mL) was treated as described for the preparation of **1**. The residue was freeze-dried to give **2** as an amorphous hygroscopic powder (65 mg, 78%); $[\alpha]_D$ −24° (*c* 1, H_2O); ^1H NMR (D_2O, internal H_2O): carbohydrate ring protons (see Table 2); 3.42 (s, 3 H, OCH_3), 1.89, 1.88 (2 s, 6 H, NAc); ^{13}C (D_2O, internal acetone): δ 176.23, 176.10, 175.81, 175.12, 174.24 (C=O), 103.81, 103.56, 103.36, 103.12 (C-1^{II-V}), 101.75 (C-1^{I}), 81.75, 81.55, 81.04, 80.35 (C-$3^{II,IV}$, C-$4^{I,III}$), 75.86, 75.56, 75.37, 74.56, 74.28, 73.37, 73.08, 71.97, 71.86, 71.77, 71.60 (C-$2,3^{I,III,V}$, C-4^{V}, C$3,4,5^{II,IV}$), 67.78, 67.62 (C-$6^{II,IV}$), 57.56 (O*C*H_3), 51.69, 51.42 (C-$2^{II,IV}$), 22.77, 22.52 (CO*C*H_3). Anal. Calcd for $C_{35}H_{49}N_2Na_5O_{35}S_2 \cdot 2H_2O$: C, 33.03; H, 4.20; N, 1.30. Found: C, 32.80; H, 4.33; N, 1.17.

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