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Synthetic approach towards sulfated chondroitin di-, tri- and tetrasaccharides corresponding to the repeating unit

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Abstract

Chondroitin di-, tri- and tetrasaccharides, as well as their 4-, 6-mono- and 4,6-disulfates as their 4-methoxyphenyl glycosides, were systematically synthesized. Target disaccharides having βGalNAc-(1 → 4)-βGlcA sequences were obtained starting from the corresponding pivotal chondroitin disaccharide precursor. A trisaccharide intermediate, which was synthesized by coupling of glucuronate imidate with a known disaccharide acceptor, was transformed into the sulfated and non-sulfated chondroitin trisaccharides. Chondroitin tetrasaccharide and the corresponding 4-disulfate, 6-disulfate as well as 4,6-tetrasulfate were also obtained based on the strategy developed above starting from the reported tetrasaccharide having [$\beta GalN_3$-(1 → 4)-$\beta GlcA_2$] sequence. © 1998 Elsevier Science Ltd.

Keywords: Glycosaminoglycan; Chondroitin sulfate; Oligosaccharide synthesis

1. Introduction

Chondroitin sulfates are sulfated only at the hydroxyl groups, in contrast to heparin and heparan sulfate that bear *N*-sulfo groups. Especially the 4-*O*- and 6-*O*-positions of the GalNAc residue, and in some cases, the 2-*O*-position of GlcA residue are found sulfated. Several types of chondroitin sulfate monomers (A, C, D, E and K) having sulfate(s) at the various positions are well known. Those sulfation patterns give rise to biologically important roles

Abbreviations: TBDMS = *tert*-butyldimethylsilyl; TBDPS = *tert*-butyldiphenylsilyl; TBAF = tetra-*n*-butylammonium fluoride; DIPEA = diisopropylethylamine; CAN = cerium IV ammonium nitrate; DMAP = 4-dimethylaminopyridine

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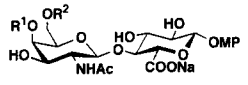
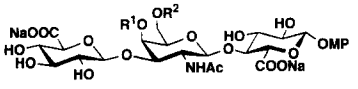
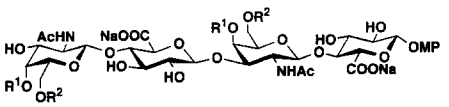
deeply related to the position and the number of sulfate groups. For example, chondroitin 6-sulfate immunologically participates as an inhibitor of human Clq [1]. As found for heparan sulfate [2], chondroitin 6-sulfate could also be a potent ligand for NKR-P1 protein which activates NK cells and induces cytotoxity of tumour cells [2]. Furthermore, oversulfated chondroitin acts in the blood coagulation system. The anticoagulant activity of rabbit thrombomodulin is due to the accumulated sulfates at both of the 4,6-positions of the GalNAc residue at the non-reducing end of the saccharide chain [3]. Additionally, a disulfated GalNAc unit at the non-reducing end might induce the chain termination during biosynthesis [4]. Chondroitin and its 6-sulfate exist in human colon carcinoma in increased levels [5]. A new type of branched glycosaminoglycan, fucosylated chondroitin sulfate, from an echinoderm that had anticoagulant activity was reported recently [6]. In order to be able to study the biological mechanisms in detail, the availability of synthesized chondroitin oligosaccharides displaying various sulfation patterns was investigated.

The synthesis of the chondroitin repeating disaccharide having GlcAGalNAc(4S) sequence as the methyl glycoside was first reported by Sinaÿ's group in 1989 [7]. The following year, chondroitin disaccharide methyl glycosides which have both GlcA-GalNAc and the reverse sequences with sulfate group at 4- or 6-position of the GalNAc residues were synthesized by Jacquinet [8]. His group also reported the synthesis of the chondroitin 4-sulfate trisaccharide as the methyl glycoside in 1995 via conversion of the stereochemistry at the 4-position of the internal GlcNAc moiety of the hyaluronic acid [9]. In the same year, Tamura et al. [10] reported the syntheses of chondroitin 4-sulfate di- and tetrasaccharide as 4-methoxyphenyl glucosides by the use of a disaccharide intermediate suitably designed for chain elongation aimed towards higher chondroitin type polysaccharides. The common disaccharide (**13**) has 4-methoxyphenyl and levulinoyl groups at the reducing and non-reducing end, respectively. These protecting groups were independently removable; therefore, **13** was readily converted into the corresponding glycosyl donor and acceptor. Additionally, we used 4-methoxybenzoyl ester as one of the protecting groups of which the methyl group is suitable as a marker in NMR spectrum.

In the present paper, we describe a systematic approach towards chondroitin di-, tri- and tetrasaccharides having 4-, 6-mono and 4,6-disulfate groups, along with their non-sulfates, as 4-methoxyphenyl glycosides (**1**–**12**) (Scheme 1). The enclosed ^{1}H and ^{13}C NMR data, as well as FABMS data, proved to be of high importance for the determination of the sulfation pattern and structure elucidation.

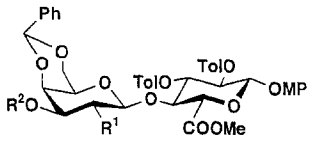
2. Results and discussion

Starting from methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-D-glucopyranosid)uronate (**13**) [10], chondroitin disaccharides (**1**–**4**) were synthesized as follows. The azido derivative of **13** was converted to the corresponding acetamide with thioacetic acid (AcSH) in pyridine [11] in 96% yield. The levulinoyl group of **14** was quantitatively removed using hydrazine acetate [12] to give **15**. The target non-sulfated disaccharide **1** was obtained by the hydrogenolytic re-

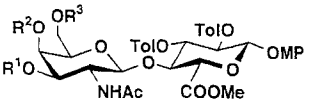
	R^1= H R^2= H	R^1= SO_3Na R^2= H	R^1= H R^2= SO_3Na	R^1= SO_3Na R^2= SO_3Na
	1	2	3	4
	5	6	7	8
	9	10	11	12

Scheme 1. Target oligosaccharides.

moval of the benzylidene acetal of **15** and subsequent saponification. ^{1}H and ^{13}C NMR data of the target compounds (**1–12**) are given in Tables 1–6.



	R^1	R^2
13	N_3	Lev
14	NHAc	Lev
15	NHAc	H
16	N_3	H
17	NHAc	Ac
18	N_3	Piv
19	NHAc	Piv



	R^1	R^2	R^3
20	Ac	H	H
21	Piv	H	H
22	Piv	SO_3Na	SO_3Na
23	Piv	Ac	H
24	Piv	Piv	H
25	Piv	H	H

Reviewing our planned synthetic route towards regioselective sulfation, we were concerned about the

Table 1
^{1}H NMR data[a] for compounds **1–4**

Proton	1	2	3[b]	4
GlcA H-1	4.99	5.00	5.04	5.01
H-2	3.60	3.59	3.62	3.61
H-3	3.70	3.70	3.73	3.73
H-4	3.84	3.82	3.84	3.83
H-5	3.84	3.80	3.96	3.83
GalNAc H-1′	4.49	4.56	4.52	4.59
H-2′	3.89	3.87	3.91	3.91
H-3′	3.70	3.88	3.74	3.88
H-4′	3.92	4.68	3.99	4.72
H-5′	3.70	3.82	3.97	4.12
H-6′	3.76, 3.83	3.82	4.23	4.25, 4.32
NAc	2.04	2.05	2.03	2.04
OMe	3.79	3.80	3.80	3.80

[a]Measured at 400 MHz referenced by *t*-BuOH in deuterium oxide as $\delta = 1.23$ ppm.
[b]At 600 MHz.

Table 2
^{1}H NMR data[a] for compounds **5–8**

Proton	5	6[b]	7	8
GlcA H-1	4.99	4.99	5.00	5.02
H-2	3.60	3.59	3.61	3.62
H-3	3.69	3.70	3.71	3.73
H-4	3.83	3.83	3.81–3.86	3.84
H-5	3.83	3.82	3.81–3.86	3.88
GalNAc H-1′	4.53	4.58	4.56	4.62
H-2′	4.01	4.06	4.03	4.08
H-3′	3.80	4.04	3.99	4.08
H-4′	4.17	4.82	4.23	4.84
H-5′	3.76	3.77–3.82	3.81–3.86	4.13
H-6′	3.76–3.83	3.77–3.82	4.23	4.23, 4.30
GlcA H-1″	4.48	4.45	4.49	4.47
H-2″	3.30	3.33	3.31	3.34
H-3″	3.45	3.45	3.46	3.46
H-4″	3.48	3.51	3.46	3.53
H-5″	3.67	3.64	3.67	3.68
NAc	2.02	2.03	2.02	2.03
OMe	3.80	3.80	3.80	3.80

[a]Measured at 400 MHz referenced by *t*-BuOH in deuterium oxide as $\delta = 1.23$ ppm.
[b]At 600 MHz.

Table 3
^{1}H NMR data[a] for compounds **9–12**

Proton	9	10	11	12
GlcA H-1	4.99	5.00	5.09	5.08
H-2	3.59	3.59	3.63	3.63
H-3	3.69	3.79–3.85	3.77	3.77
H-4	3.77	3.86	3.89	3.88
H-5	3.83	3.82	4.12	4.08
GalNAc H-1′	4.52	4.58	4.58	4.64
H-2′	4.00	4.04	4.03	4.06
H-3′	3.79	4.02	3.87	4.08
H-4′	4.11	4.74	4.16	4.79
H-5′	3.70–3.76	[b]	3.97	4.12
H-6′	3.68–3.80	3.79–3.85	4.21	4.21–4.32
GlcA H-1″	4.48	4.45	4.57	4.43
H-2″	3.34	3.36	3.38	3.40
H-3″	3.56	3.57	3.64	3.64
H-4″	3.73	3.77	3.77	3.82
H-5″	3.84	3.65	3.95	3.91
GalNAc H-1‴	4.45	4.53	4.50	4.59
H-2‴	3.87	3.88	3.89	3.91
H-3‴	3.67	3.84	3.73	3.91
H-4‴	3.90	4.67	3.98	4.71
H-5‴	3.70–3.76	[b]	3.97	4.12
H-6‴	3.68–3.80	3.79–3.85	4.21	4.21–4.32
NAc	2.01	2.03	1.99	2.01
	2.03	2.04	2.02	2.02
OMe	3.80	3.80	3.80	3.80

[a]Measured at 400 MHz referenced by *t*-BuOH in deuterium oxide as $\delta = 1.23$ ppm.
[b]3.70 or 3.79–3.85.

Table 4
^{13}C NMR data[a] for compounds **1–4**

Carbon	1	2	3[b]	4
GlcA C-1	102.33	102.27	102.70[c]	102.69
C-2	73.64	73.56	73.85	73.82[d]
C-3	74.79	74.74	75.33	75.48
C-4	80.52	80.88	82.22	83.01
C-5	77.66	77.65	76.93	78.13
GalNAc C-1′	102.10	102.06	103.22[c]	103.19
C-2′	53.37	53.61	53.68	53.92
C-3′	72.11	71.02	72.17	71.33
C-4′	68.86	76.78	68.88	76.93
C-5′	76.30	75.56	74.24	73.91[d]
C-6′	62.14	62.09	68.63	69.23
$NCOCH_3$	23.52	23.55	23.96	24.02
OMe	56.84	56.87	57.30	57.30
C=O	175.06	174.98	176.49	176.56
	176.12	176.12		

[a]Measured at 100 MHz referenced by *t*-BuOH in deuterium oxide as $\delta = 31.1$ ppm.
[b]At 150 MHz.
[c,d]May be exchanged.

possible reduction of the levulinoylitic ketone moieties during benzylidene acetal cleavage. To avoid this undesired reaction, we decided to interchange the levulinoyl group with acetyl. However, as reported in the previous paper [10], some portion of the acetyl group at *O*-3 of the non-reducing end GalNac in the

Table 5
^{13}C NMR data[a] for compounds **5–8**

Carbon	5	6[b]	7	8
GlcA C-1	102.82	102.79	102.7	102.68
C-2	74.06	74.01	74.0	74.01
C-3	75.28	75.28	75.2	75.51
C-4	81.12	81.52	82.7	83.19
C-5	78.16	78.16	81.4	77.75
GalNAc C-1′	102.43	102.43	103.0	103.02
C-2′	52.56	53.18	52.3	52.98
C-3′	81.81	76.51	74.2	76.33
C-4′	69.21	78.04	69.0	77.58
C-5′	76.53	76.20	78.1	73.86
C-6′	62.64	62.64	69.0	69.32
GlcA C-1″	105.67	104.95	105.5	104.93
C-2″	74.24	74.08	74.3	73.95
C-3″	76.83	76.61	76.7	76.58
C-4″	73.34	73.37	73.2	73.27
C-5″	77.65	77.83	77.5	77.58
$NCOCH_3$	24.02	24.06	24.0	24.07
OMe	57.30	57.30	57.3	57.30
C=O	175.49	175.45	N.D.	N.D.
	176.54	176.51		
	177.49	177.46		

[a]Measured at 100 MHz referenced by *t*-BuOH in deuterium oxide as $\delta = 31.1$ ppm.
[b]At 150 MHz.

Table 6
^{13}C NMR data[a] for compounds **9–12**

Carbon	9	10	11	12
GlcA C-1	102.83	102.71	102.66	102.64
C-2	74.06	74.01	[b]	73.52
C-3	77.85	76.15[i]	75.48	75.31
C-4	81.06[d]	81.83	83.03	81.33
C-5	78.16[e]	81.43	76.99	75.48
GalNAc C-1′	102.48	102.36	103.01	103.09
C-2′	52.53	53.07	52.89	52.41
C-3′	76.50	76.94	[b]	72.02
C-4′	69.28[f]	78.14	77.47	[c]
C-5′	75.28[g]	71.61[i]	[b]	74.11[k]
C-6′	62.56[h]	62.51[j]	69.31	[c]
GlcA C-1″	105.85	105.23	105.29	105.87
C-2″	74.03	73.78	73.44	73.67
C-3″	75.18	75.03	75.26	75.23
C-4″	81.86[d]	76.02	83.31	82.24[l]
C-5″	81.11[e]	78.14	77.29	82.19[l]
GalNAc C-1‴	102.42	102.71	103.40	103.30
C-2‴	53.86	54.10	53.92	53.69
C-3‴	72.63	71.27	71.27	71.26
C-4‴	69.33[f]	77.25	76.84	[c]
C-5‴	76.76[g]	75.28[i]	[b]	74.24[k]
C-6‴	62.63	62.61[j]	69.09	[c]
$NCOCH_3$	24.01	23.99	24.01	23.89
Ome	57.30	57.30	57.30	57.28
C=0	175.47	N.D.	176.52	176.41
	176.23			
	176.56			
	176.59			

[a]Measured at 100 MHz referenced by *t*-BuOH in deuterium oxide as $\delta = 31.1$ ppm.
[b]73.75, 73.80, 73.91.
[c]68.63, 68.81, 68.90.
[d–l]may be exchanged.

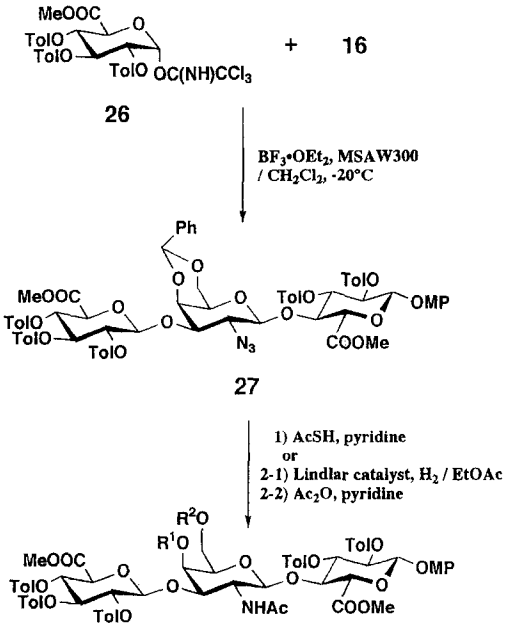
chondroitin tetrasaccharide **37** migrated to the 4-position during the reductive conversion. Therefore, we finally adopted the less migratable pivaloyl ester as protecting group at *O*-3 and examined the synthetic route for the disaccharide moiety. In this paper, both results using the acetyl and pivaloyl esters are described.

The levulinoyl group of **13** was quantitatively removed with hydrazine acetate to give the known methyl (2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-D-glucopyranosid)uronate (**16**) [10,13]. Simultaneous conversion of the azido and alcohol groups to acetamide and acetate, respectively, by the action of AcSH in pyridine yielded **17** (75%). Compound **17** was also made available by conventional acetylation in 88% yield from **15**. On the other

hand, **16** was pivaloylated with pivaloyl chloride and pyridine in the presence of 4,4-dimethylaminopyridine (DMAP) to afford **18** (81%). The azido group of **18** was hydrogenolyzed in the presence of Lindlar catalyst, and the resultant amine was acetylated to give **19** in high yield (96%). The benzylidene acetal of **17** and **19** could be regioselectively reduced to the corresponding 6-*O*-benzyl ethers with sodium cyanoborohydride in THF by the addition of HCl in Et_2O in 87% and quantitative yields, respectively. None of the regioisomers was observed. Then, alcohol **20** so obtained was sulfated with the sulfur trioxide–trimethylamine complex at 50–60°C. After gel-permeation chromatography, the sulfate was exposed to saponification. The desired chondroitin disaccharide 4-sulfate **2** was obtained following the removal of the benzyl ether by catalytic hydrogenation (88%, three steps). The corresponding pivaloate **21** was also converted to sulfate **22** in the same manner as above. This sulfate **22** was saponified, and hydrogenolysis that followed afforded **2** in 88% yield in two steps.

Chondroitin disaccharide 6-sulfate **3** was synthesized as follows. Initially, the regioselective sulfation of diol **25**, which was obtained by the quantitative removal of the benzylidene acetal of **19**, was investigated. However, no sulfate was obtained by the use of sulfur trioxide–trimethylamine complex in *N*,*N*-dimethylformamide at room temperature, followed by 50–60°C within a limited period (25 min). After the removal of the acyl groups, ^{1}H NMR spectroscopy showed that mainly the desired 6-sulfate **3** was obtained (65% yield). Along with the expected regioisomers, the 4-sulfate **2** and the 4,6-disulfate **4** were obtained in 11 and 7% yields, respectively, together with 17% of unsulfated **1**. As the products were difficult to separate, another strategy using stepwise conversion was developed. Thus, the hydroxyl group of **21** was acetylated, and the product was exposed to hydrogenation in the presence of Pd–C to give the 6-OH derivative **23** without migration of the acetate from the 4-position. Alcohol **23** was sulfated and saponified in the usual manner to afford the desired 6-sulfate **3** in 87% yield (three steps). Target 6-sulfate **3** could also be obtained via dipivaloate **24**. The chemical shifts found in the ^{1}H NMR spectrum of the disaccharides (**2** and **3**) were in good agreement with those of the corresponding methyl glycosides, especially on the GalNAc residues already reported by Jacquinet [8].

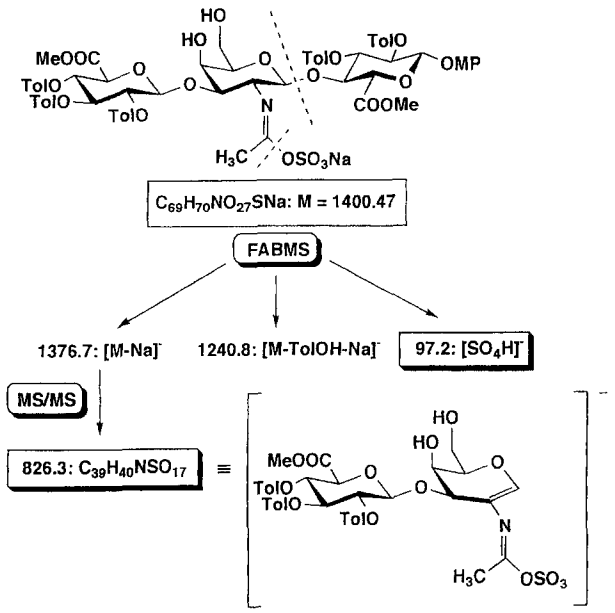
The synthesis of the 4,6-disulfate **4** was first examined by the stepwise sulfation via the 4-sulfate. According to this approach, the benzyl ether of **22** was hydogenolytically removed, and the resultant alcohol was further sulfated to afford **4** (71%, based on **22**). When diol **25** was exposed to the sulfation conditions overnight, the 4,6-disulfate was also obtained. Subsequent saponification quantitatively gave the target compound **4**.



	R^1	R^2
28	PhCH<	
29	H	H
30	H	Bn
31	Bn	H
32	SO_3Na	Bn
33	Ac	H

Scheme 2. Trisaccharide synthesis.

The trisaccharide sequence: GlcAGalNAcGlcA has been reported by Coutant and Jacquinet [9] via the stereochemical conversion of the 4-position of the GlcNAc residue. We obtained the chondroitin trisaccharide by coupling of methyl glucuronate trichloroacetimidate **26** [14] and acceptor **16** (Scheme 2). By the action of borontrifluoride ether complex ($BF_3 \cdot OEt_2$), the desired trisaccharide **27** was obtained in 39% yield. The azido group of **27** was converted into the corresponding acetamide with AcSH (78%). A better yield (93%) for **28** was obtained when the conversion was performed by hydrogenolysis in the presence of Lindlar catalyst, followed by conventional acetylation. Subsequently, the benzylidene acetal was removed hydrolytically to give **29** in 83% yield. Sulfate-free chondroitin trisac-



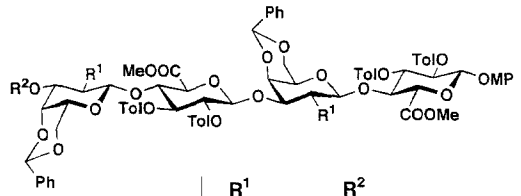
Scheme 3.

charide **5** was obtained via saponification in the same manner as described (quantitatively).

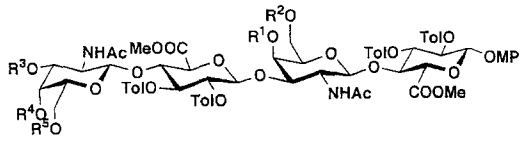
The reductive benzylation of **28** gave the desired 6-benzylated compound **30** in 69% yield. In contrast to the results found for the disaccharide, the regioisomer **31**, with the benzyl ether at *O*-4, was also obtained in 12% yield. The hydroxyl group of **30** was sulfated to yield 4-sulfate **32** in 92% yield. Subsequent saponification of **32** (61%), followed by hydrogenation in the presence of palladium hydroxide, gave 4-sulfate **6** in 82% yield. The chemical shifts found in both the ^{1}H and ^{13}C NMR spectra were in accordance with reported data [9].

Thereafter, the regioselective sulfation of the primary alcohol was examined for the trisaccharide 4,6-diol **29**. The sulfation was performed in the same manner as above but proceeded slowly for one day to give only one spot on TLC. After purification using gel-permeation and ion-exchange resin chromatography, FABMS confirmed the presence of a monosulfated product. As shown in Scheme 3, the MS/MS fragment at 826.3 from 1376.7 $[M - Na]^-$ indicates the presence of sulfate on the GlcAGalN moiety, and the signal at 97.2 is typical for the presence of an *O*-sulfur trioxide fragment, not of an *N*-sulfur trioxide. Surprisingly, it was not transformed to the desired **7** or **8**, but was converted to the non-sulfated **5** after deprotection by applying the usual saponification conditions. Taking the stability of the *O*-sulfate into consideration, we are suggesting a formation of the imine-like $[-N{=}C(OSO_3^-)CH_3]$ product and not formation of the 6-sulfate during the sulfation reaction. The postulated structure of the sulfate is indicated in Scheme 3. Therefore, we explored the same synthetic strategy for 6-sulfate **7** as for the disaccharide case. In order to make the 6-OH derivative **33** available, acetylation at *O*-4 and subsequent removal of benzyl group at *O*-6 were carried out quantitatively. No migrated product was detected during this sequence. Alcohol **33** was sulfated at *O*-6, and the resultant sulfate trisaccharide was saponificated as above to give 6-sulfate **7** in 67% yield (three steps).

The 4,6-disulfate could not be obtained from the 4,6-diol directly, but it was available via the 4-sulfate as examined in the case of the 4,6-disulfated disaccharide. The benzyl ether moiety of **32** was removed in the presence of palladium-on-charcoal under hydrogen in a yield of 71%, followed by sulfation as described. The prolonged reaction time required (6 days) might be due to the electronegativity of the neighboring sulfate. After saponification, the desired disulfate **8** (71% yield) was obtained.



	R^1	R^2
34	N_3	Lev
35	NHAc	Lev
36	N_3	H
37	N_3	Piv
38	NHAc	Ac
39	NHAc	Piv



	R^1	R^2	R^3	R^4	R^5
40	H	Bn	Ac	H	Bn
41	H	Bn	H	Ac	Bn
42	Bn	H	Ac	H	Bn
43	H	Bn	Piv	H	Bn
44	Bn	H	Piv	H	Bn
45	H	H	Piv	H	H
46	Ac	H	Piv	Ac	H

The final targets chondroitin tetrasaccharides (**9**–**12**) exhibit both internal and external GalNAc residues with or without sulfate groups. The synthetic strate-

gies for both types of residue have been evaluated on the di- and trisaccharide case. Tetrasaccharides (**9–12**) were synthesized from the known intermediate **34** [13]. The diazide **34** was converted to diacetamide **35** by the action of AcSH in pyridine in a moderate 43% yield. As found in the case of the trisaccharide, Lindlar catalyst proved to be effective to give the desired diacetamide **35** in higher yield (60%) after conventional acetylation. The latter method seems to be more expedient for the longer oligosaccharides. Treatment of **35** under saponification conditions (88%), followed by hydrogenation (68%), gave non-sulfate **9**.

As reported previously [10], an acetyl group was first used for the protection of *O*-3 on the non-reducing end GalNAc. The transformation from levulinoate **35** into acetate **38** was performed by the chemoselective removal and the acetylation in 64% yield (two steps). The corresponding pivaloate was also examined as an alternative intermediate. Thus, levulinoate **34** was chemoselectively converted to pivaloate **37** via **36** in quantitative yield. This pivaloate gave rise to the corresponding acetamide **39** applying the hydrogenolytic pathway (87%). Reductive ring opening was first executed for **38** in the same manner as described. The desired compound **40** having a 6-OBn group on both GalNAcs was obtained in 51% yield, together with 19% of acetyl-migrated product **41** and a trace amount of regioisomer **42** with the benzyl group at *O*-4 of the inner GalNAc residue. These yields and ratios were not easily reproducible, presumably due to the concentration of volatile HCl in Et_2O. On the other hand, pivaloate **39** afforded the desired secondary diol **43** in 65% yield, but also gave the 4-OBn regioisomer **44** on the internal GalNAc in 15% yield. No pivaloyl-migrated product was observed during reaction analysis. In order to make chondroitin 4-sulfate tetrasaccharide **10** available, the obtained diols (**40** and **43**) were sulfated in 92 and 98% yield, respectively. Both sulfates could be transformed into **10** via saponification and subsequent hydrogenation in the presence of palladium hydroxide in 70% yield.

For the chondroitin 6-sulfate tetrasaccharide **11** synthesis, we tried a regioselective sulfation of tetraol **45**, which was furnished by the removal of two benzylidene acetals of **39** under acidic conditions (78%). Even though the sulfation proceeded slowly, a single product was obtained after two days, which was exposed to basic conditions. The final product proved to be the tetrasulfate **12**. The yields were 78 and 89% for sulfation and deprotection, respectively. In contrast to the results obtained during trisaccharide sulfation, no other product was observed. Thus, the 6-sulfate **11** could be obtained by stepwise synthetic strategy as follows. The acetylation of **43** and the deblocking of the two benzyl groups were carried out in 92 and 99% yield, respectively, to yield **46**. Conventional sulfation overnight and subsequent saponification afforded the title compound **11** in 82% yield (three steps).

In summary, a series of chondroitin 4-, 6- and 4,6-di-sulfates, as well as their non-sulfated analogs, were systematically synthesized for the di-, tri- and tetrasaccharides of which GlcA residues locate at the reducing end as 4-methoxyphenyl glycosides.

3. Experimental

Optical rotations were measured at 22 ± 3°C with a JASCO DIP 310 polarimeter in the solution of specified solvents, and ^1H NMR spectra were measured with JEOL EX 270 MHz spectrometer. Some key compounds were measured with JEOL 400 and 600 MHz spectrometers as indicated. Chemical shifts were expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$ and CD_3OD. For solutions in deuterium oxide, *tert*-butanol served as the reference 1.23 ppm. The FABMS for the synthetic compounds were measured with a triple-stage quadrupole mass spectrometer (Finnigan MAT TSQ 700) equipped with an FAB ion source. Samples were typically dissolved in water, and triethanolamine was used as a matrix in the negative-ion mode. The FAB spectra were recorded under the following conditions: primary beam, xenon; accelerating voltage of the primary ion, 8 kV; collision gas, argon; collision energy, 30 eV; collision gas pressure, 0.2 Pa. Silica gel chromatography, analytical TLC, preparative TLC (PTLC) and high-performance TLC (HPTLC) were done on glass plates coated with Silica Gel F_{254} (E. Merck). Column chromatography was carried out on columns of Silica Gel 60 (E. Merck). Gels for size-exclusion chromatography (Sephadex LH-20, Biobeads S-X1 and S-X2) were purchased from Pharmacia and Bio-Rad, respectively. Molecular sieves were purchased from GL Science, and activated at 180°C under vacuum prior to use. Melting points were determined with a Yanaco mp apparatus and a Büchi mp apparatus model 510 and are uncorrected. All reactions in organic solvents were performed under a nitrogen or argon atmo-

sphere, and organic solvents were distilled according to standard procedures prior to use.

Methyl (2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-levulinoyl-β-D-galactopyranosyl)-2→4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**14**).—To a solution of methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-D-glucopyranosid)uronate (**13**) [10] (73.3 mg, 79.3 μmol) in pyridine (0.5 mL) was added 1.0 mL of AcSH with stirring. An additional 1.0 mL of AcSH was added after 42 h, and the mixture was stirred for 29 h. The reaction mixture was concentrated, and the residue was subjected to silica gel column chromatography (2:1–1:1 toluene–EtOAc, then 10:1 EtOAc–MeOH) to give 71.3 mg of **14** in 96% yield; R_f 0.26 (5:5:1 toluene–EtOAc–Et_3N), $[\alpha]_D$ +51.8° (*c* 1.46, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.88–7.53 (m, 4 H, aromatic H), 7.36–7.28 (m, 5 H, aromatic H), 7.17–7.08 (m, 4 H, aromatic H), 6.93–6.87 (m, 2 H, aromatic H), 6.77–6.74 (m, 2 H, aromatic H), 5.80 (t, 1 H, $J_{2,3} = J_{3,4} = 8.91$ Hz, H-3), 5.55 (d, 1 H, $J_{2,NH}$ 9.56 Hz, NH′), 5.53 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2), 5.28 (s, 1 H, PhC*H*), 5.22 (d, 1 H, H-1), 5.15 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.30 Hz, H-3′), 4.94 (d, 1 H, $J_{1,2}$ 8.24 Hz, H-1′), 4.54 (t, 1 H, $J_{4,5}$ 8.91 Hz, H-4), 4.29 (d, 1 H, H-5), 4.03 (d, 1 H, H-4′), 3.91 (ddd, 1 H, H-2′), 3.78, 3.73 (2 s, 6 H, COOMe, *Me*OPh), 3.76 (d, 1 H, H-6′a), 3.56 (d, 1 H, J_{gem} 10.89 Hz, H-6′b), 2.96 (s, 1 H, H-5′), 2.71–2.48 (m, 4 H, 2 CH_2), 2.34, 2.30 (2 s, 6 H, 2 *Me*Ph), 2.04 (s, 3 H, MeCO), 1.94 (s, 3 H, NAc). Anal. Calcd for $C_{50}H_{53}NO_{17} \cdot 0.5H_2O$: C, 63.27; H, 5.75; N, 1.48. Found: C, 63.25; H, 5.77; N, 1.46.

Methyl (2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**15**).—Disaccharide **14** (23.4 mg, 25.0 μmol) was dissolved in 2.4 mL of 5:1 EtOH–toluene and hydrazine acetate (39.5 mg, 0.429 mmol, 17 equiv) was added while stirring for 12 min prior to the removal of the solvents in vacuo. The residue was subjected to purification by gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to yield **15** (20.7 mg, 99%); R_f 0.31 (10:1 EtOAc–MeOH); $[\alpha]_D$ +12.3° (*c* 0.473, $CHCl_3$). ^1H NMR (CDCl3): δ 7.88–7.82 (m, 4 H, aromatic H), 7.33–7.23 (m, 5 H, aromatic H), 7.17–7.07 (m, 4 H, aromatic H), 6.94–6.89 (m, 2 H, aromatic H), 6.79–6.75 (m, 2 H, aromatic H), 6.38 (d, 1 H, $J_{2,NH}$ 5.28 Hz, NH′), 5.82 (t, 1 H, $J_{2,3} = J_{3,4} = 8.90$ Hz, H-3), 5.55 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.32 (s, 1 H, PhC*H*), 5.24 (d, 1 H, H-1), 4.63 (d, 1 H, $J_{1,2}$ 7.91 Hz, H-1′), 4.50 (t, 1 H, $J_{4,5}$ 9.24 Hz, H-4), 4.34 (d, 1 H, H-5), 3.91 (d, 1 H, $J_{3,4}$ 2.97 Hz, H-4′), 3.85–3.74 (m, 3 H, H-2′,3′,6′a), 3.81, 3.74 (2 s, 6 H, COOMe, *Me*OPh), 3.56 (d, 1 H, J_{gem} 11.21 Hz, H-6′b), 2.93 (s, 1 H, H-5′), 2.35, 2.29 (2 s, 6 H, 2 *Me*Ph), 2.03 (s, 3 H, NAc). Anal. Calcd for $C_{45}H_{47}NO_{15} \cdot H_2O$: C, 62.85; H, 5.75; N, 1.63. Found: C, 63.06; H, 5.66; N, 1.63.

Sodium (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**1**).—A suspension of **15** (32.8 mg, 39.0 μmol), 2 drops CH_3COOH and a catalytic amount of 10% Pd–C in 3 mL of EtOH was stirred under hydrogen for 5 days. The mixture was filtered over Celite, the volatiles were removed under reduced pressure, and the residue was finally eluted from a column of silica gel with 20:1–5:1 EtOAc–MeOH to give the debenzylidenated product (21.7 mg, 74%). The crude material was dissolved in 3.15 mL of 20:1 THF–water, 1.25 N lithium hydroxide (0.15 mL) was added dropwise at 0°C, and the mixture was stirred for 2 h at the same temperature. The mixture was evaporated under reduced pressure, taken up in 3 mL of MeOH, and 0.2 N NaOMe (0.25 mL) was added. After 3.5 and 5 h, more NaOMe (0.1 mL) and water were added, respectively. The volatiles were removed under reduced pressure, and the residue was applied to gel-permeation chromatography over LH-20 (water). The fractions containing the desired compound were treated with Dowex-50 (H^+) and Dowex-50 (Na^+) and then freeze-dried to give **1** (12.1 mg, 80%). Some minor impurities could be removed by repeated gel-permeation chromatography; R_f 0.15 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ −21.4° (*c* 0.66, H_2O). For ^1H and ^{13}C NMR data, see Tables 1 and 4, respectively. FABMS: *m/z* 524.2 $(M - H)^-$, 502.2 $(M - Na)^-$.

Methyl (2-acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**17**).—(a) By conventional acetylation (Ac_2O, pyridine) **15** (14.0 mg, 16.6 μmol) was converted into its acetate. After the usual work-up, the crude product was purified by PTLC (20:1 EtOAc–MeOH) to give **17** (10.2 mg, 69%); R_f 0.54 (10:1 EtOAc–MeOH); $[\alpha]_D$ +51.6° (*c* 0.68, $CHCl_3$). ^1H NMR ($CDCl_3$); δ 7.88–7.82 (m, 4 H, aromatic H), 7.35–7.28 (m, 5 H, aromatic H), 7.17–7.09 (m, 4 H, aromatic H), 6.93–6.88 (m, 2 H, aromatic H), 6.79–6.75 (m, 2 H, aromatic H), 5.80 (t, 1 H, $J_{2,3} = J_{3,4} = 8.91$ Hz, H-3), 5.53 (dd, 1 H, $J_{1,2}$ 6.93 Hz,

H-2), 5.48 (d, 1 H, $J_{2,NH}$ 8.25 Hz, NH′), 5.29 (s, 1 H, PhC*H*), 5.22 (d, 1 H, H-1), 5.20 (dd, 1 H, $J_{2,3}$ 7.59, $J_{3,4}$ 3.63 Hz, H-3′), 4.94 (d, 1 H, $J_{1,2}$ 8.24 Hz, H-1′), 4.54 (dd, 1 H, $J_{4,5}$ 9.24 Hz, H-4), 4.29 (d, 1 H, H-5), 4.10 (d, 1 H, H-4′), 3.89 (ddd, 1 H, H-2′), 3.81 (dd, 1 H, $J_{5,6a}$ 1.32, J_{gem} 12.53 Hz, H-6′a), 3.79, 3.74 (2 s, 6 H, COOMe, *Me*OPh), 3.57 (dd, 1 H, $J_{5,6b}$ 1.65 Hz, H-6′b), 2.97 (s, 1 H, H-5′), 2.35, 2.31 (2 s, 6 H, 2 *Me*Ph), 2.02, 1.94 (2 s, 6 H, OAc, NAc). Anal. Calcd for $C_{48}H_{51}N_3O_{15}$: C, 63.86; H, 5.60; N, 1.58. Found: C, 63.58; H, 5.67; N, 1.58.

(b) To azide **16** (33.2 mg, 40.2 μmol) in pyridine (0.5 mL) was added 1.0 mL of AcSH while stirring. After 1 and 2 days, 0.5 mL of AcSH was added, respectively, and stirring was continued for 4 h. The reaction mixture was concentrated, and the residue was subjected to silica gel column chromatography (5:1–4:1–2:1–1:1–1:3 toluene–EtOAc) to give 26.5 mg of **17** in 75% yield.

*Methyl (2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**18**).—Pivaloyl chloride (8 μL, 16 μmol) and a catalytic amount of DMAP were added to a solution of **16** [10,13] (25.4 mg, 30.8 μmol) in 1 mL of pyridine at 80°C with stirring. After 1 day, an excess of pivaloyl chloride (0.1 mL) was added, and the mixture was stirred for 1 h. To this solution was added MeOH (0.1 mL), and the resultant mixture was cooled to room temperature and diluted with $CHCl_3$. The organic phase was washed with brine followed by aq $NaHCO_3$, and finally, dried over anhydrous $MgSO_4$. The solution was filtered, the volatiles were removed under diminished pressure, and the residue was subjected to column separation on silica gel (10:1–5:1–2:1–1:1 *n*-hexane–EtOAc) to give **18** (22.6 mg, 81%); R_f 0.64 (3:1 toluene–EtOAc); $[\alpha]_D$ +32.97° (*c* 0.907, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.89–7.83 (m, 4 H, aromatic H), 7.31–7.32 (m, 5 H, aromatic H), 7.17–7.08 (m, 4 H, aromatic H), 6.94–6.89 (m, 2 H, aromatic H), 6.80–6.75 (m, 2 H, aromatic H), 5.79 (t, 1 H, $J_{2,3} = J_{3,4} = 9.24$ Hz, H-3), 5.59 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2), 5.26 (s, 1 H, PhC*H*), 5.22 (d, 1 H, H-1), 4.52 (dd, 1 H, $J_{2,3}$ 10.56, $J_{3,4}$ 3.63 Hz, H-3′), 4.42 (d, 1 H, H-1′), 4.33 (d, 1 H, H-5), 4.15 (d, 1 H, H-4′), 3.82, 3.74 (2 s, 6 H, COOMe, *Me*OPh), 3.82–3.72 (m, 2 H, H-2′,6′a), 3.60 (s, 1 H, H-6′b), 3.12 (s, 1 H, H-5′), 2.34, 2.30 (2 s, 6 H, 2 *Me*Ph), 1.18 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{48}H_{51}N_3O_{15}$: C, 63.35; H, 5.66; N, 4.62. Found: C, 62.62; H, 5.86; N, 4.48.

*Methyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**19**).—To a solution of **18** (33.1 mg, 36.4 μmol) in EtOAc (1 mL) was added Lindlar catalyst (400 mg), and the mixture was stirred under hydrogen for 13 h. The reaction mixture was filtered through Celite and evaporated to dryness. The residue was diluted with pyridine (1 mL) and Ac_2O (1 mL) and stirred for 10 h. Ice was added to the reaction mixture, and stirring was continued overnight. The reaction mixture was diluted with $CHCl_3$, and the organic phase was treated as usual. Separation by gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) gave **19** (32.4 mg) in 96% yield: R_f 0.46 (1:1 toluene–EtOAc); $[\alpha]_D$ +56.9° (*c* 0.253, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.89–7.82 (m, 4 H, aromatic H), 7.30 (br s, 5 H, aromatic H), 7.16–7.10 (m, 4 H, aromatic H), 6.92–6.89 (m, 2 H, aromatic H), 6.77–6.74 (m, 2 H, aromatic H), 5.80 (t, 1 H, $J_{2,3} = J_{3,4} = 8.91$ Hz, H-3), 5.54 (dd, 1 H, $J_{1,2}$ 7.25 Hz, H-2), 5.45 (d, 1 H, $J_{2,NH}$ 8.58 Hz, NH′), 5.30 (s, 1 H, PhC*H*), 5.21 (d, 1 H, H-1), 5.07 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.62 Hz, H-3′), 4.88 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.53 (dd, 1 H, $J_{4,5}$ 9.24 Hz, H-4), 4.28 (d, 1 H, H-5), 4.10 (d, 1 H, H-4′), 3.98 (ddd, 1 H, H-2′), 3.84–3.73 (m, 1 H, H-6′a), 3.78, 3.73 (2 s, 6 H, COOMe, *Me*OPh), 3.57 (d, 1 H, J_{gem} 12.53 Hz, H-6′), 2.95 (s, 1 H, H-5′), 2.35, 2.32 (2 s, 6 H, 2 *Me*Ph), 1.91 (s, 3 H, NAc), 1.13 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{50}H_{55}NO_{16}$: C, 64.82; H, 6.00; N, 1.51. Found: C, 64.82; H, 5.99; N, 1.63.

*Methyl (2-acetamido-3-*O*-acetyl-6-*O*-benzyl-2-deoxy-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)-uronate* (**20**).—To a mixture of **17** (12.6 mg, 14.3 μmol), powdered molecular sieves 3 Å (80 mg), and a small amount of methyl orange in THF (1 mL), was added sodium cyanoborohydride (30 mg, 0.48 mmol, 33 equiv). The mixture was stirred for 5 h, then cooled to 0°C, and HCl–Et_2O was added dropwise until the color changed to pink. Additional sodium cyanoborohydride (54 mg, 0.86 mmol, 59 equiv) and HCl–Et_2O were supplied, then the mixture was allowed to warm up to room temperature. The reaction mixture was neutralized with aq $NaHCO_3$, diluted with $CHCl_3$, and filtered through Celite. After the conventional work-up, the residue was subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to yield **20** (11.0 mg, 87%); R_f 0.55 (10:1 EtOAc–MeOH); $[\alpha]_D$ +52.3° (*c* 0.22, $CHCl_3$). ^{1}H NMR ($CDCl_3$); δ 7.89–7.82 (m, 4 H, aromatic H), 7.37–7.26 (m, 3 H,

aromatic H), 7.21–7.14 (m, 6 H, aromatic H), 6.93–6.89 (m, 2 H, aromatic H), 6.81–6.76 (m, 2 H, aromatic H), 5.70 (t, 1 H, $J_{2,3} = J_{3,4} = 8.90$ Hz, H-3), 5.58 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.54 (d, 1 H, $J_{2,NH}$ 8.91 Hz, NH′), 5.20 (d, 1 H, H-1), 4.99 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 2.97 Hz, H-3′), 4.72 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.42 (dd, 1 H, $J_{4,5}$ 9.24 Hz, H-4), 4.27 (d, 1 H, H-5), 4.21, 4.13 (ABq, 2 H, J 11.87 Hz, Ph*CH*$_2$), 3.97 (ddd, 1 H, H-2′), 3.90 (m, 1 H, H-4′), 3.79, 3.75 (2 s, 6 H, COOMe, *Me*OPh), 3.41 (m, 1 H, H-5′), 2.96–2.91 (m, 2 H, H-6′a,b), 2.36 (s, 6 H, 2 *Me*Ph), 2.25 (d, 1 H, $J_{4,OH}$ 6.27 Hz, OH-4′), 2.06, 1.91 (2 s, 6 H, OAc, NAc). Anal. Calcd for $C_{47}H_{51}NO_{16}$: C, 63.71; H, 5.81; N, 1.58. Found: C, 64.12; H, 5.98; N, 1.51.

Methyl (2-acetamido-6-O-benzyl-2-deoxy-3-O-pivaloyl-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**21**).—To a mixture of **19** (65.9 mg, 71.2 μmol), powdered molecular sieves 3 Å (300 mg), and a small amount of methyl orange in THF (4.5 mL) was added a sodium cyanoborohydride (124 mg, 0.48 mmol, 33 equiv). The mixture was stirred for 2 h, then cooled to 0°C, and HCl–Et_2O was added dropwise until the color changed to pink. Additional sodium cyanoborohydride (120 + 90 mg, total 75 equiv) and HCl–Et_2O were supplied, and the mixture was allowed to warm up to room temperature. The reaction mixture was finally treated as described above to afford **21** (59.1 mg, 90%); R_f 0.57 (1:3 *n*-hexane–EtOAc); $[\alpha]_D$ +33.6° (*c* 0.607, $CHCl_3$). ^{1}H NMR ($CDCl_3$); δ 7.88–7.82 (m, 4 H, aromatic H), 7.37–7.27 (m, 2 H, aromatic H), 7.21–7.13 (m, 7 H, aromatic H), 6.91–6.88 (m, 2 H, aromatic H), 6.78–6.74 (m, 2 H, aromatic H), 5.71 (dd, 1 H, $J_{2,3}$ 9.24, $J_{3,4}$ 8.91 Hz, H-3), 5.58 (dd, 1 H, $J_{1,2}$ 6.92 Hz, H-2), 5.48 (d, 1 H, $J_{2,NH}$ 9.24 Hz, NH′), 5.22 (d, 1 H, H-1), 4.87 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.30 Hz, H-3′), 4.64 (d, 1 H, $J_{1,2}$ 8.24 Hz, H-1′), 4.40 (dd, 1 H, $J_{4,5}$ 9.24 Hz, H-4), 4.29 (d, 1 H, H-5), 4.19, 4.12 (ABq, 2 H, J 11.87 Hz, Ph*CH*$_2$), 4.10 (m, 1 H, H-2′), 3.88 (m, 1 H, H-4′), 3.79, 3.74 (2 s, 6 H, COOMe, *Me*OPh), 3.40 (m, 1 H, H-5′), 2.95–2.89 (m, 2 H, H-6′a,b), 2.34 (s, 6 H, 2 *Me*Ph), 2.11 (m, 1 H, OH-4′), 1.88 (s, 3 H, NAc), 1.16 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{50}H_{57}NO_{16}$: C, 64.70; H, 6.20; N, 1.51. Found: C, 64.43; H, 6.18; N, 1.43.

Methyl (sodium 2-acetamido-6-O-benzyl-2-deoxy-3-O-pivaloyl-4-O-sulfonato-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**22**).—To a solution of **21** (18.5 mg, 20.0 μmol) in Me_2NCHO (2.5 mL) was added sulfur trioxide–trimethylamine complex (85.7 mg, 0.62 mmol, 31 equiv), and the mixture was stirred for 2 h at 50–60°C. The reaction mixture was cooled to room temperature and subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) and ion-exchange resin over a column of Dowex-50 (Na^+) (8:1 MeOH–water) to give **22** (14.5 mg, 71%); R_f 0.27 (10:1 EtOAc–MeOH); $[\alpha]_D$ +40.9° (*c* 0.58, MeOH). ^{1}H NMR (CD_3OD): δ 7.88–7.81 (m, 4 H, aromatic H), 7.34–7.17 (m, 9 H, aromatic H), 6.91–6.86 (m, 2 H, aromatic H), 6.82–6.77 (m, 2 H, aromatic H), 5.72 (dd, 1 H, $J_{2,3}$ 7.92, $J_{3,4}$ 8.25 Hz, H-3), 5.46 (dd, 1 H, $J_{1,2}$ 8.25 Hz, H-2), 5.41 (d, 1 H, H-1), 4.86 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.30 Hz, H-3′), 4.70 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.69 (d, 1 H, H-4′), 4.45 (dd, 1 H, $J_{4,5}$ 9.24 Hz, H-4), 4.40 (d, 1 H, H-5), 4.28, 4.07 (ABq, 2 H, J 11.22 Hz, PH*CH*$_2$), 3.99 (dd, 1 H, H-2′), 3.82, 3.72 (2 s, 6 H, COOMe, *Me*OPh), 3.51–3.40, 3.04–2.99 (m, 3 H, H-5′,6′a,b), 2.36, 2.34 (2 s, 6 H, 2 *Me*Ph), 1.88 (s, 3 H, NAc), 1.17 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{50}H_{56}NSO_{19}Na \cdot 2H_2O$: C, 56.32; H, 5.68; N, 1.31. Found: C, 56.48; H, 5.55; N, 1.33.

Sodium (sodium 2-acetamido-2-deoxy-4-O-sulfonato-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**2**).—(a) To a solution of **20** (6.6 mg, 7.4 μmol) in Me_2NCHO (1.5 mL) was added sulfur trioxide–trimethylamine complex (29.3 mg, 0.21 mmol, 28 equiv), and the mixture was stirred for 16 h at 50–60°C. The reaction mixture was cooled to room temperature and subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to give crude sulfate (8.4 mg), which was dissolved in THF (1.5 mL) and water (0.1 mL). To this solution was added 1.25 N lithium hydroxide (0.04 mL, 6.9 equiv) while stirring at 0°C. After 40 min, the volatiles were removed under reduced pressure, and the residue was dissolved in 2 mL of MeOH and treated with 0.2 N NaOMe (2.0 mL). After stirring overnight, the reaction mixture was subjected to gel-permeation chromatography (LH-20, water) to give crude 4-sulfate (4.8 mg, 91% in 2 steps), which was immediately dissolved in 2 mL of 3:1 water–MeOH, and stirred overnight under hydrogen in the presence of a catalytic amount of palladium hydroxide. Insoluble material was filtered off, and the filtrate was subjected to gel-permeation chromatography (LH-20, water), Amberlist (CG-50, H^+) and Dowex-50 (Na^+) to give 3.1 mg of **2** in 74% yield after freeze drying; R_f 0.33 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ +8.2° (*c* 0.147, H_2O). For ^{1}H and ^{13}C NMR data, see Tables 1 and 4, respec-

tively. FABMS: *m/z* 604.1 $(M - Na)^-$, 582.2 $(M - 2Na + H)^-$.

(b) Sulfate **22** (9.4 mg, 9.0 μmol) was dissolved in THF (1.5 mL) and water (0.1 mL). To this solution was added 1.25 N lithium hydroxide (0.05 mL, 7 equiv) while stirring at 0°C. After 2 h, the volatiles were removed under reduced pressure, and the residue was dissolved in MeOH (1 mL) and $CHCl_3$ (0.3 mL). To this solution, 0.5 N NaOH (0.6 mL) was added, and stirring was continued overnight before the reaction mixture was diluted with meOH. The solution was concentrated to 0.5 mL under diminished pressure and the residue was eluted from a column of CG-50 (H^+) and Dowex-50 (Na^+) in turn (8:1 MeOH–water). The fraction residue thus obtained was subjected to gel-permeation chromatography (LH-20, 4:2:1 MeOH–water–$CHCl_3$), and the crude product was dissolved in water (2.5 mL) and stirred overnight under hydrogen in the presence of catalytic amount of palladium hydroxide. Insoluble material was filtered off. The solution was exposed to Dowex-50 (Na^+) and purified by gel-permeation chromatography (G-50, water) to give 5.0 mg of **2** (three steps, 88%). Some minor impurities could be removed by additional gel-permeation chromatography (LH-20, water).

*Methyl (2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**23**).—To a solution of **20** (3.4 mg, 3.7 μmol) in pyridine (0.2 mL) was added Ac_2O (0.2 mL) and a catalytic amount of DMAP with stirring for 1.5 h, and then ice was added. After conventional work-up, 2.9 mg of acetylated product was obtained by PTLC (1:1 toluene–EtOAc) purification in 81% yield. The crude product was diluted with EtOAc (1.5 mL) and stirred under hydrogen in the presence of Pd–C for 1 day. The reaction mixture was filtered to give **23** (2.1 mg, 88%); R_f 0.16 (2:3 toluene–EtOAc); $[\alpha]_D$ +26.3° (*c* 0.32, $CHCl_3$). ^1H NMR ($CDCl_3$); δ 7.89–7.84 (m, 4 H, aromatic H), 7.22–7.16 (m, 4 H, aromatic H), 6.94–6.89 (m, 2 H, aromatic H), 6.81–6.76 (m, 2 H, aromatic H), 5.72 (5, 1 H, $J_{2,3} = J_{3,4} =$ 8.58 Hz, H-3), 5.60 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2), 5.36 (d, 1 H, $J_{2,NH}$ 8.91, NH′), 5.22 (d, 1 H, H-1), 5.13 (d, 1 H, $J_{3,4}$ 3.30 Hz, H-4′), 4.97 (dd, 1 H, $J_{2,3}$ 11.22 Hz, H-3′), 4.71 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.45 (dd, 1 H, $J_{4,5}$ 8.91 Hz, H-4), 4.31 (d, 1 H, H-5), 4.14 (m, 1 H, H-2′), 3.79, 3.75 (2 s, 6 H, COOMe, *Me*OPh), 3.49, 3.03–2.97 (m, 3 H, H-5′,6′a,b), 2.38, 2.36 (2 s, 6 H, 2 *Me*Ph), 2.02, 1.83 (2 s, 6 H, OAc, NAc), 1.10 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{45}H_{53}NO_{17} \cdot 0.5H_2O$: C, 60.79; H. 6.13; N, 1.58. Found: C, 60.74; H, 6.15; N, 1.54.

*Methyl (2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**24**).—To a solution of **21** (11.4 mg, 12.3 μmol) in pyridine (0.4 mL) were added pivalic anhydride (0.4 mL) and a catalytic amount of DMAP with stirring at 50–60°C. After 6 days, further pivalic anhydride (0.3 mL) and DMAP were added. Six days later, the reaction mixture was cooled to room temperature, 4 mL of MeOH, was added, and the mixture was diluted with $CHCl_3$ and worked up by the standard procedure. Purification by PTLC (3:2 toluene–EtOAc) afforded the pivaloylated compound (5.3 mg, 43%). A part of this pivalate (3.2 mg, 3.2 μmol) was dissolved in EtOAc (0.7 mL) and hydrogenolized as described above. By applying PTLC (1:1 toluene–EtOAc) purification, **24** was obtained (2.5 mg, 86%); R_f 0.24 (1:1 toluene–EtOAc); $[\alpha]_D$ +24.6°C (*c* 0.167, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.89–7.84 (m, 4 H, aromatic H), 7.31–7.16 (m, 4 H, aromatic H), 6.95–6.87 (m, 2 H, aromatic H), 6.84–6.75 (m, 2 H, aromatic H), 5.71 (dd, 1 H, $J_{2,3}$ 8.57, $J_{3,4}$ 8.91 Hz, H-3), 5.59 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.38 (d, 1 H, $J_{2,NH}$ 9.24, NH′), 5.22 (d, 1 H, H-1), 5.10 (d, 1 H, $J_{3,4}$, 2.96 Hz, H-4′), 5.00 (dd, 1 H, $J_{2,3}$ 11.22 Hz, H-3′), 4.71 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.46 (dd, 1 H, $J_{4,5}$ 8.58 Hz, H-4), 4.30 (d, 1 H, H-5), 4.18 (ddd, 1 H, H-2′), 3.79, 3.75 (2 s, 6 H, COOMe, *Me*OPh), 3.55, 3.06, 2.94 (3 m, 3 H, H-5′,6′a,b), 2.36, 2.36 (2 s, 6 H, 2 *Me*Ph), 1.80 (s, 3 H, NAc), 1.14, 1.09 (2 s, 18 H, *tert*-Bu). Anal. Calcd for $C_{48}H_{59}NO_{17}$: C, 62.52; H, 6.46; N, 1.52. Found: C, 63.10; H, 6.81; N, 1.47.

*Methyl (2-acetamido-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**25**).—Camphorsulfonic acid (3.0 mg) was added to the stirring mixture of **19** (11.3 mg, 12.2 μmol) in CH_2Cl_2 (0.5 mL) and MeOH (0.5 mL). After 5.5 days, the reaction was quenched with aq $NaHCO_3$ and diluted with $CHCl_3$. After aqueous work-up, the crude product was purified by gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to quantitatively give 10.2 mg of **25**; R_f 0.56 (40:1 EtOAc–MeOH); $[\alpha]_D$ +40.2° (*c* 0.687, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.95–7.81 (m, 4 H, aromatic H), 7.23–7.15 (m, 4 H, aromatic H), 6.93–6.90 (m, 2 H, aromatic H), 6.79–6.75 (m, 2 H, aromatic H), 5.75 (t, 1 H, $J_{2,3} = J_{3,4} = 8.58$ Hz, H-3), 5.60 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.54 (d, 1 H, $J_{2,NH}$ 9.24, NH′), 5.27 (d, 1 H, H-1), 4.85 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 2.97 Hz,

H-3′), 4.72 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.45 (dd, 1 H, $J_{4,5}$ 8.91 Hz, H-4), 4.35 (d, 1 H, H-5), 4.17 (ddd, 1 H, H-2′), 3.86 (br s, 1 H, H-4′), 3.77, 3.74 (2 s, 6 H, COOMe, *Me*OPh), 3.38–3.18 (m, 3 H, H-5′,6′a,b), 2.75 (br, 1 H, OH-4′), 2.37, 2.35 (2 s, 6 H, 2 *Me*Ph), 1.98 (br, 1 H, OH-6′), 1.81 (s, 3 H, NAc), 1.16 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{43}H_{51}NO_{16} \cdot 0.5H_2O$: C, 60.97; H, 6.20; N, 1.65. Found: C, 61.17; H, 6.17; N, 1.58.

Sodium (sodium 2-acetamido-2-deoxy-6-O-sulfonato-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**3**).—(a) To a solution of **23** (2.1 mg, 2.4 μmol) in Me_2NCHO (0.5 mL) was added a sulfur trioxide–trimethylamine complex (6.9 mg, 50 μmol, 21 equiv) with stirring at 50–60°C overnight. The reaction mixture was treated as described in the synthesis of **22**. By this process, crude sulfate was obtained, and it was diluted in THF (1 mL) and water (0.12 mL). To this solution, 0.12 mL of 1.25 N lithium hydroxide was added at 0°C. The reaction mixture was stirred for 1 h, and the volatiles were removed under diminished pressure. The residue was dissolved in 1 mL of 4:1 mixture of MeOH–CH_2Cl_2. To this, 0.4 mL of 0.5 N NaOH was added, and the reaction mixture was stirred overnight. Then 0.2 mL of 0.5 N NaOH was added, and the reaction mixture was stirred for additional 1 day. After careful addition of 50% CH_3COOH, the reaction mixture was neutralized and evaporated. The residue was subjected to gel-permeation chromatography (LH-20, water) twice, and treated with Dowex-50 (Na^+) to give **3** (1.3 mg, 87% in three steps); R_f 0.21 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ −44.7° (*c* 0.047, H_2O). For ^1H and ^{13}C NMR data, see Tables 1 and 4, respectively. FABMS: *m/z* 626.1 $(M - H)^-$, 604.1 $(M - Na)^-$.

(b) Pivaloate **24** (1.7 mg, 1.8 μmol) in Me_2NCHO (0.5 mL) was sulfated with a sulfur trioxide–trimethylamine complex (7.0 mg, 50 μmol, 28 equiv) with stirring at 50–60°C overnight. Applying the above described procedure, 0.7 mg of 3 (64% in three steps) was obtained.

(c) To a solution of **25** (3.8 mg, 4.5 μmol) in Me_2NCHO (0.5 mL) was added a sulfur trioxide–trimethylamine complex (17.3 mg, 124 μmol, 28 equiv) with stirring at 50–60°C for 25 min. The reaction mixture was treated as described in the synthesis of **22**. The crude sulfate so obtained was saponified as above to give a mixture (2.8 mg) of **3** (65%), **1** (17%), **2** (11%) and **4** (7%), of which ratio and yields were calculated from ^1H NMR spectra.

Sodium (disodium 2-acetamido-2-deoxy-4,6-di-O-sulfonato-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**4**).—(a) Diol **25** (2.8 mg, 3.3 μmol) was dissolved in Me_2NCHO (0.5 mL), and sulfur trioxide–trimethylamine complex (20.7 mg, 149 μmol, 23 × 2 equiv) was added with stirring at 50–60°C overnight. The elution from gel-permeation chromatography (LH-20, 1:1 $CHCl_3$ MeOH) was collected and evaporated. The residue was exposed to the saponification procedure as above to give **4** quantitatively; R_f 0.06 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha_D$ −14.9° (*c* 0.047, H_2O). For ^1H and ^{13}C NMR data, see Tables 1 and 4, respectively. FABMS: *m/z* 706.2 $(M - Na)^-$, 684.2 $(M - 2Na + H)^-$, 604.2 $(M - SO_3 - 2Na + H)^-$, 582.2 $(M - SO_3 - 3Na + 2H)^-$.

(b) Sulfate **22** (2.6 mg, 2.5 μmol) was dissolved in 1:1 mixture of EtOAc–MeOH (1 mL) and stirred for 4.5 days under hydrogen in the presence of a catalytic amount of palladium hydroxide. Insoluble material was filtered off, the volatiles were removed under reduced pressure, and the residue was subjected to a column of Dowex-50 (Na^+) (8:1 MeOH–water) to give 1.8 mg of debenzylated product, which was sulfated in a similar way as described in the synthesis of **22** to give 2.1 mg of disulfate from a column of Dowex-50 (Na^+) (8:1 MeOH–water). This disulfate was also saponified as above to give 1.2 mg of **4** (71% from **22**).

Methyl (methyl 2,3,4-tri-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1→3)-(2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**27**).—A mixture of **16** (83.4 mg, 0.101 mmol) and powdered molecular sieves AW300 (1.1 g) in toluene (10 mL) and CH_2Cl_2 (1 mL) was stirred at room temperature. After 40 min, the solution was cooled to −20°C, and then $BF_3 \cdot OEt_2$ (7.2 μL, 78.3 μmol) and a solution of methyl(2,3,4-tri-*O*-4-methylbenzoyl-α-D-glucopyranosyl trichloroacetimidate)uronate (**26**) [13,15] (139.3 mg, 0.197 mmol) in toluene (8 mL) were added dropwise. The reaction temperature was raised gradually to room temperature overnight before adding 7 μL of $BF_3 \cdot OEt_2$. After 1 h, the reaction mixture was diluted with EtOAc, quenched with aq $NaHCO_3$, filtered on Celite, and extracted with EtOAc. By the conventional manner, the crude product was roughly fractionized using gel-permeation chromatography (S-X1, toluene) and, finally, purified by silica gel column chromatography (6:1–3:1 toluene–EtOAc) to

give **27** (54.4 mg, 39%); R_f 0.59 (2:1 toluene–EtOAc); $[\alpha]_D$ −8.02° (*c* 1.21, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.86–7.70 (m, 10 H, aromatic H), 7.33–7.27 (m, 5 H, aromatic H), 7.19–7.05 (m, 10 H, aromatic H), 6.94–6.88 (m, 2 H, aromatic H), 6.79–6.74 (m, 2 H, aromatic H), 5.85 (dd, 1 H, $J_{2,3}$ 8.91, $J_{3,4}$ 9.23 Hz, H-3″), 5.75 (t, 1 H, $J_{2,3} = J_{3,4} = 8.91$ Hz, H-3), 5.64 (t, 1 H, H-4″), 5.54 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2), 5.51 (dd, 1 H, $J_{1,2}$ 7.59 Hz, H-2″), 5.34 (s, 1 H, PhC*H*), 5.20 (d, 1 H, H-1), 5.16 (d, 1 H, H-1″), 5.51 (t, 1 H, H-4), 4.39 (d, 1 H, $J_{1,2}$ 7.92 Hz, H-1′), 4.30 (d, 1 H, H-5″), 4.29 (d, 1 H, H-5), 4.17 (d, 1 H, $J_{3,4}$ 3.30 Hz, H-4′), 3.76, 3.74, 3.62 (3 s, 9 H, 2 COOMe, *Me*OPh), 3.71–3.62 (m, 2 H, H-2′, 6′b), 3.53 (d, 1 H, J_{gem} 12.19 Hz, H-6′b), 3.46 (dd, 1 H, $J_{2,3}$ 9.56 Hz, H-3′), 3.09 (s, 1 H, H-5′), 2.36, 2.34, 2.33, 2.30, 2.29 (5 s, 15 H, 5 *Me*Ph). Anal. Calcd for $C_{74}H_{71}N_3O_{23} \cdot H_2O$: C, 64.01; H, 5.31; N, 3.03. Found: C, 64.00; H, 5.15; N, 3.25.

Methyl (methyl 2,3,4-tri-O-*4-methylbenzoyl-β*-D-*glucopyranosyluronate)-(1 → 3)-(2-acetamido-4,6*-O-*benzylidene-2-deoxy-β*-D-*galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di*-O-*4-methylbenzoyl-β*-D-*glucopyranosid)uronate* (**28**).—(a) To a solution of **27** (20.6 mg, 15.0 μmol) in pyridine (0.3 mL) was added 0.6 mL of AcSH, and the resulting mixture was stirred for 2 days. The reaction mixture was then evaporated, and the residue was subjected to silica gel column chromatography (*n*-hexane–10:1–5:1–2:1–1:1–1:2 *n*-hexane–EtOAc) to give **28** (16.3 mg, 78%) together with the starting material **27** (4.0 mg, 19%); R_f 0.57 (1:1 toluene–EtOAc); $[\alpha]_D$ +22.3° (*c* 0.815, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.88–7.65 (m, 10 H, aromatic H), 7.36–7.28 (m, 5 H, aromatic H), 7.19–7.04 (m, 10 H, aromatic H), 6.92–6.86 (m, 2 H, aromatic H), 6.78–6.72 (m, 2 H, aromatic H), 5.81 (dd, 1 H, $J_{2,3}$ 9.24, $J_{3,4}$ 9.57 Hz, H-3″), 5.73 (t, 1 H, $J_{2,3} = J_{3,4} = 8.91$ Hz, H-3), 5.61 (d, 1 H, $J_{2,NH}$ 6.93 Hz, NH′), 5.59 (t, 1 H, H-4″), 5.49 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.47 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2″), 5.31 (s, 1 H, PhC*H*), 5.21 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 5.18 (d, 1 H, H-1), 5.10 (d, 1 H, H-1″), 4.69 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.30 Hz, H-3′), 4.52 (t, 1 H, H-4), 4.25 (d, 1 H, H-5″), 4.20 (d, 1 H, H-5), 4.17 (d, 1 H, H-4′), 3.85 (d, 1 H, J_{gem} 11.88 Hz, H-6′a), 3.73, 3.72, 3.58 (3 s, 9 H, 2 COOMe, *Me*OPh), 3.62 (d, 1 H, H-6′b), 3.26 (ddd, 1 H, H-2′), 2.97 (s, 1 H, H-5′), 2.36, 2.34, 2.31, 2.28 (4 s, 15 H, *Me*Ph), 1.64 (s, 3 H, NAc). Anal. Calcd for $C_{76}H_{75}NO_{24}$: C, 65.83; H, 5.46; N, 1.01. Found: C, 66.00; H, 5.56; N, 0.97.

(b) Lindlar catalyst (250 mg) was added to a solution of **27** (54.4 mg, 39.7 μmol) in EtOAc (5 mL), and the reaction mixture was stirred overnight under a hydrogen atmosphere. Insoluble material was removed over Celite and the volatiles were removed in vacuo. The residue was acetylated conventionally, and the resulting mixture was separated by silica gel column chromatography (7:3 toluene–EtOAc) to afford **28** (51.1 mg, 93%).

Methyl (methyl 2,3,4-tri-O-*4-methylbenzoyl-β*-D-*glucopyranosyluronate)-(1 → 3)-(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3di*-O-*4-methylbenzoyl-β*-D-*glucopyranosid)uronate* (**29**).—Camphorsulfonic acid (5.0 mg) was added to a stirred mixture of **28** (16.3 mg, 11.8 μmol) in CH_2Cl_2 (0.5 mL) and MeOH (0.5 mL). After 24 h, the reaction was quenched with Et_3N and evaporated. The crude product was purified by silica gel column chromatography (2:1–1:2 toluene–EtOAc) to give **29** (12.7 mg, 83%); R_f 0.12 (1:1 toluene–EtOAc); $[\alpha]_D$ +30.0° (*c* 0.14, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.91–7.39 (m, 10 H, aromatic H), 7.22–7.06 (m, 10 H, aromatic H), 6.94–6.87 (m, 2 H, aromatic H), 6.79–6.74 (m, 2 H, aromatic H), 5.84 (t, 1 H, $J_{2,3} = J_{3,4} = 9.23$ Hz, H-3″), 5.63 (t, 1 H, $J_{2,3} = J_{3,4} = 8.25$ Hz, H-3), 5.57 (t, 1 H, $J_{4,5}$ 9.57 Hz, H-4″), 5.55 (dd, 1 H, $J_{1,2}$ 6.60 Hz, H-2), 5.48 (dd, 1 H, $J_{1,2}$ 7.59 Hz, H-2″), 5.41 (d, 1 H, $J_{2,NH}$ 6.59 Hz, NH′), 5.19 (d, 1 H, H-1), 5.03 (d, 1 H, $J_{1,2}$ 7.59 Hz, H-1′), 4.91 (d, 1 H, H-1″), 4.61 (dd, 1 H, $J_{2,3}$ 10.56, $J_{3,4}$ 2.97 Hz, H-3′), 4.42 (t, 1 H, H-4), 4.26 (d, 1 H, H-5″), 4.19 (d, 1 H, H-5), 3.95 (d, 1 H, H-4″), 3.73, 3.69, 3.62 (3 s, 9 H, 2 COOMe, *Me*OPh), 3.36–3.24 (m, 3 H, H-5′,6′a,6′b), 3.10 (ddd, 1 H, H-2′), 2.64 (br, 1 H, OH-4′), 2.38, 2.37, 2.36, 2.29 (4 s, 15 H, 5 *Me*Ph), 1.97 (br, 1 H, OH-6′), 1.40 (s, 3 H, NAc). Anal. Calcd for $C_{69}H_{71}NO_{24}$: C, 63.82; H, 5.52; N, 1.08. Found: C, 64.28; H, 5.70; N, 0.99.

Sodium (sodium β-D-*glucopyranosyluronate)-(1 → 3)-(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β*-D-*glucopyranosid)uronate* (**5**).—Diol **29** (4.7 mg, 3.6 μmol) was dissolved in 0.66 mL of 10:1 THF–water and 1.25 N lithium hydroxide (0.06 mL) was added dropwise at 0°C. After 1 h, the mixture was evaporated under reduced pressure, taken up in 0.8 mL of 3:1 MeOH-CH_2Cl_2, and 0.5 N NaOH (0.2 mL) was added. After stirring overnight, the reaction mixture was carefully neutralized with 50% CH_3COOH and the volatiles were removed under reduced pressure. The mixture was applied to gel-permeation chromatography over LH-

20 (water), and the fractions containing the desired compound were treated by Dowex-50 (H^+) and Dowex-50 (Na^+) and then freeze-dried to quantitatively give **5** (2.6 mg). Some minor impurities could be removed by repeated gel-permeation chromatography; $[\alpha]_D$ −45.7° (*c* 0.14, H_2O); R_f 0.18 (3:2:1 BuOH–CH_3COOH–H_2O). For ^1H and ^{13}C NMR data, see Tables 2 and 5, respectively. FABMS: *m/z* 722.2 $(M - H)^-$, 700.1 $(M - Na)^-$.

*Methyl (methyl 2,3,4-tri-*O*-4-methylbenzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**30**) and *methyl (methyl 2,3,4-tri-*O*-4-methylbenzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)-(2-acetamido-4-*O*-benzyl-2-deoxy-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**31**).—To a mixture of **28** (28.1 mg, 20.3 μmol), powdered 3 Å molecular sieves (100 mg), and a small amount of methyl orange in THF (3 mL) was added a sodium cyanoborohydride (95 mg, 1.51 mmol, 74 equiv). After stirring for 80 min, the mixture was cooled to 0°C, and HCl–Et_2O was added dropwise until the color changed to pink. Additional sodium cyanoborohydride (100 + 58 mg, total 198 equiv) and HCl–Et_2O were introduced, and the mixture was allowed to warm up to room temperature. Finally, the reaction mixture was treated as described in the synthesis of **20** to yield **30** (19.4 mg, 69%) and **31** (3.4 mg, 12%).

30: R_f 0.39 (1:1 toluene–EtOAc); $[\alpha]_D$ +21.6° (*c* 0.473, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.86–7.70 (m, 10 H, aromatic H), 7.37–7.06 (m, 15 H, aromatic H), 6.90–6.85 (m, 2 H, aromatic H), 6.77–6.72 (m, 2 H, aromatic H), 5.82 (t, 1 H, $J_{2,3} = J_{3,4} = 9.57$ Hz, H-3″), 5.61–5.55 (m, 3 H, H-2,3,4″), 5.48 (dd, 1 H, $J_{1,2}$ 7.92 Hz, H-2″), 5.37 (d, 1 H, $J_{2,NH}$ 7.60 Hz, NH′), 5.14 (m, 1 H, H-1), 4.98 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.89 (d, 1 H, H-1″), 4.61 (dd, 1 H, $J_{2,3}$ 10.89, $J_{3,4}$ 2.97 Hz, H-3′), 4.39 (m, 1 H, H-4), 4.24 (d, 1 H, $J_{4,5}$ 10.56 Hz, H-5″), 4.22 (s, 2 H, PhCH_2), 4.15 (d, 1 H, $J_{4,5}$ 9.24 Hz, H-5), 4.02 (d, 1 H, H-4′), 3.73, 3.72, 3.55 (3 s, 9 H. 2 COOMe, *Me*OPh), 3.41 (br t, 1 H, H-5′), 3.14–2.97 (m, 3 H, H-2′,6′a,6′b), 2.37, 2.36, 2.35, 2.29 (4 s, 15 H, 5 *Me*Ph), 1.39 (s, 3 H, NAc). Anal. Calcd for $C_{76}H_{77}NO_{24}$: C, 65.74; H, 5.60; N, 1.01. Found: C, 65.39; H, 5.55; N, 0.95.

31: R_f 0.23 (1:1 toluene–EtOAc); $[\alpha]_D$ −8.81° (*c* 0.193, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.87–7.65 (m, 10 H, aromatic H), 7.39–7.05 (m, 15 H, aromatic H), 6.92–6.86 (m, 2 H, aromatic H), 6.78–6.73 (m, 2 H, aromatic H), 5.88 (t, 1 H, $J_{2,3} = J_{3,4} = 9.90$ Hz, H-3″), 5.62–5.54 (m, 3 H, H-2,3,4″), 5.49 (dd, 1 H, $J_{1,2}$ 7.92 Hz, H-2″), 5.29 (d, 1 H, $J_{2,NH}$ 6.60 Hz, NH′), 5.15 (m, 1 H, H-1), 4.92 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.87 (d, 1 H, H-1″), 4.87, 4.57 (ABq, 2 H, *J* 11.88 Hz, PhCH_2), 4.73 (dd, 1 H, $J_{2,3}$ 10.89, $J_{3,4}$ 2.97 Hz, H-3′), 4.36 (m, 1 H, H-4)), 4.26 (d, 1 H, $J_{4,5}$ 9.57 Hz, H-5″), 4.15 (d, 1 H, $J_{4,5}$ 8.91 Hz, H-5), 3.76 (d, 1 H, H-4′), 3.74, 3.70, 3.63 (3 s, 9 H, 2 COOMe, *Me*OPh), 3.24 (br t, 1 H, H-5′), 3.12 (m, 1 H, H-2′), 2.89–2.82 (m. 2 H, H-6′a,6′b), 2.39, 2.37, 2.35, 2.28 (4 s, 15 H, 5 *Me*Ph)$_3$ 1.55 (s, 3 H, NAc). Anal. Calcd for $C_{76}H_{77}NO_{24} \cdot 0.5H_2O$: C, 65.31; H, 5.64; N, 1.00. Found: C, 65.29; H, 5.58; N, 0.98.

*Sodium (sodium β-*D*-glucopyranosyluronate)-(1 → 3)-(sodium 2-acetamido-2-deoxy-4-*O*-sulfonato-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β-*D*-glucopyranosid)uronate* (**6**).—To a solution of **30** (5.5 mg, 4.0 μmol) in Me_2NCHO (0.7 mL) was added a sulfur trioxide–trimethylamine complex (30 mg, 0.22 mmol, 55 equiv), and the reaction mixture was stirred overnight at 50–60°C and treated as described in the synthesis of **22** to give the 4-sulfate **32** (5.4 mg, 92%); R_f 0.39 (5:5:1 EtOAc–MeOH–Et_3N). This compound was used for the next reaction without further purification. Sulfate **32** (5.1 mg, 3.4 μmol) was dissolved in THF (0.5 mL) and water (0.04 mL). To this solution was added 1.25 N lithium hydroxide (0.04 mL, 5 × 3 equiv) while stirring at 0°C. After 1 h, volatiles were removed under reduced pressure. The residue was dissolved in MeOH (0.5 mL) and $CHCl_3$ (0.15 mL), and 0.5 N NaOH (0.5 mL) was added to this solution. After the stirring for 1.5 days, an excess amount of dry ice was added to the reaction mixture, which was then carefully neutralized with 50% CH_3COOH (R_f 0.32, 3:2:1 BuOH–CH_3COOH–H_2O). The solution was eluted from a column of G-50 (2% aq. pyridine), Dowex-50 (H^+) and Dowex-50 (Na^+) (8:1 MeOH–water) to give saponified product (1.9 mg, 61%), which was dissolved in water (1 mL) and stirred for 8.5 h under hydrogen in the presence of catalytic amount of palladium hydroxide. Insoluble material was filtered off. The solution was exposed to Dowex-50 (Na^+), and purificated by gel-permeation chromatography (G-10, water) to give 1.4 mg of **6** (82%); R_f 0.23 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ −41° (*c* 0.093, H_2O). For ^1H and ^{13}C NMR data, see Tables 2 and 5, respectively. FABMS: *m/z* 824.0 $(M - H)^-$, 802.2 $(M - Na)^-$.

*Methyl (methyl 2,3,4-tri-*O*-4-methylbenzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)-(2-acetamido-4-*O*-*

acetyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**33**).—Using the conventional procedure, **30** (5.0 mg, 3.6 μmol) was quantitatively converted into its acetate (5.1 mg) (R_f 0.57, 1:1 toluene–EtOAc) with Ac_2O (0.7 mL), pyridine (0.7 mL), and catalytic amount of DMAP. A portion of this acetate (1.0 mg) was hydrogenolized in the presence of Pd–C in EtOAc for 1 day and treated as described in the synthesis of **24** to quantitatively give **33** (1.0 mg); R_f 0.18 (1:1 toluene–EtOAc); $[\alpha]_D$ +28.6° (*c* 0.273, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.86–7.65 (m, 10 H, aromatic H), 7.20–7.05 (m, 10 H, aromatic H), 6.90–6.87 (m, 2 H, aromatic H), 6.76–6.73 (m, 2 H, aromatic H), 5.77 (t, 1 H, $J_{2,3} = J_{3,4} = 9.57$ Hz, H-3″), 5.61–5.54 (m, 3 H, H-2,3,4″), 5.42 (dd, 1 H, $J_{1,2}$ 7.91 Hz, H-2″), 5.35 (d, 1 H, $J_{2,NH}$ 6.60 Hz, NH′), 5.13 (d, 1 H, $J_{1,2}$ 6.93 Hz, H-1), 5.12 (d, 1 H, $J_{3,4}$ 3.96 Hz, H-4′), 5.01 (d, 1 H, $J_{1,2}$ 7.92 Hz, H-1′), 4.80 (d, 1 H, H-1″), 4.74 (dd, 1 H, $J_{2,3}$ 10.89 Hz, H-3′), 4.36 (m, 1 H, H-4), 4.17 (d, 1 H, $J_{4,5}$ 9.89 Hz, H-5″), 4.14 (d, 1 H, $J_{4,5}$ 9.57 Hz, H-5), 3.73, 3.64 (2 s, 9 H, 2 COOMe, *Me*OPh), 3.39 (broad, 1 H, H-5′), 3.05–3.01 (m, 4 H, H-2′,6′a,6′b, OH-6′), 2.37, 2.35, 2.28 (3 s, 15 H, 5 *Me*Ph), 1.96 (s, 3 H, Ac), 1.32 (s, 3 H, NAc). Anal. Calcd for $C_{71}H_{73}NO_{25} \cdot 1.5H_2O$: C, 62.36; H, 5.61; N, 1.02. Found: C, 62.33; H, 5.61; N. 0.91.

Sodium (sodium β-D-glucopyranosyluronate)-(1 → 3)-(sodium 2-acetamido-2-deoxy-6-O-sulfonato-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**7**).—To a solution of **33** (1.0 mg, 0.8 μmol) in Me_2NCHO (0.5 mL) was added sulfur trioxide–trimethylamine complex (4.2 mg, 30.2 μmol, 40 equiv) with stirring at 50–60°C overnight. The reaction mixture was treated as described in the synthesis of **22**. The crude sulfate so obtained was diluted in THF (0.5 mL) and water (0.06 mL). To this solution was added 0.06 mL of 1.25 N lithium hydroxide at 0°C, and the reaction mixture was stirred for 2.5 h before the volatiles were removed under diminished pressure. The residue was dissolved in 0.5 mL of 4:1 MeOH–CH_2Cl_2. To this, 0.2 mL of 0.5 N NaOH was added, and the reaction mixture was stirred overnight. Another 0.2 mL of 0.5 N NaOH was added, and stirring was continued for 1 day. Applying the work-up as described in the synthesis of **3**, we obtained **7** (0.4 mg, 67% in three steps); R_f 0.25 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ −22.2° (*c* 0.027, H_2O). For ^1H and ^{13}C NMR data, see Tables 2 and 5, respectively. FABMS: *m/z* 802.2 $(M - Na)^-$, 780.2 $(M - 2Na + H)^-$.

Sodium (sodium β-D-glucopyranosyluronate)-(1 → 3)-(disodium 2-acetamido-2-deoxy-4,6-di-O-sulfonato-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**8**).—Sulfate **32** (5.4 mg, 3.6 μmol) was diluted with EtOAc (1 mL) and MeOH (1 mL), and the reaction mixture was stirred overnight under a hydrogen atmosphere in the presence of Pd–C. This mixture was filtered to give the 6-OH compound (3.6 mg, 71%); R_f 0.46 (5:5:1 × 2 EtOAc–MeOH–Et_3N). To a solution of this alcohol (3.6 mg, 2.6 μmol) in Me_2NCHO (0.5 mL) was added sulfur trioxide–trimethylamine complex (23.2 mg, 167 μmol, 64 equiv) with stirring at 50–60°C. Additional amounts of sulfur trioxide–trimethylamine complex (26.0 + 20.5 mg) were added after 2 and 4 days, respectively. The reaction mixture was treated as described in the synthesis of **22** to afford the crude sulfate (4.7 mg), which was diluted in THF (1.0 mL) and water (0.12 mL). To this solution was added 0.24 mL of 1.25 N lithium hydroxide at 0°C, and then the reaction mixture was stirred for 1.5 h before the volatiles were removed under diminished pressure. To a MeOH solution (0.8 mL) of the residue was added 0.8 mL of 0.5 N NaOH, and stirring was continued for 2 days. Using the same work-up as described in the synthesis of **3**, the desired **8** (1.7 mg, 71% in three steps) was obtained: R_f 0.03 (3:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ −26.4° (*c* 0.087, H_2O); R_f 0.59 (1:1:1 BuOH–CH_3COOH–H_2O). For ^1H and ^{13}C NMR data, see Tables 2 and 5, respectively, FABMS: *m/z* 904 $(M - Na)^-$, 882 $(M - 2Na + H)^-$, 860 $(M - 3Na + 2H)^-$, 838 $(M - 4Na + 3H)^-$, 802 $(M - SO_3 - 2Na + H)^-$, 780 $(M - SO_3 - 3Na + 2H)^-$, 758 $(M - SO_3 - 4Na + 3H)^-$.

Methyl (2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-levulinoyl-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**35**).—(a) To a solution of **34** [13] (27.8 mg, 17.1 μmol) in pyridine (1.0 mL) was added 1.5 mL of AcSH with stirring at 50°C. After 2 days, an additional 1.0 mL of AcSH was supplied. The mixture was kept at the same temperature for more than 3 days and evaporated. The residue was subjected to silica gel column chromatography (3:1–2:3–1:10 toluene–EtOAc → 40:1 EtOAc–MeOH) and further purified by PTLC (30:1 EtOAc–MeOH) to give **35** (12.1 mg, 43%); R_f 0.57 (1:1 toluene–EtOAc, then 30:1 EtOAc–MeOH); $[\alpha]_D$ +35.7° (*c* 0.76, $CHCl_3$). ^1H NMR ($CDCl_3$): δ

7.98–7.81 (m, 8 H, aromatic H), 7.59–7.56 (m, 2 H, aromatic H), 7.41–7.04 (m, 16 H, aromatic H), 6.91–6.86 (m, 2 H, aromatic H), 6.78–6.73 (m, 2 H, aromatic H), 5.75 (t, 1 H, $J_{2,3} = J_{3,4} = 8.91$ Hz, H-3), 5.64 (s, 1 H, PhCH), 5.57 (dd, 1 H, $J_{2,3}$ 3.30, $J_{3,4}$ 7.91 Hz, H-3″), 5.49 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.48 (d, 1 H, $J_{2,NH}$ 6.93 Hz, NH‴), 5.25 (s, 1 H, PhCH), 5.20 (d, 1 H, $J_{1,2}$ 8.58 Hz, H-1‴), 5.18 (dd, 1 H, $J_{1,2}$ 3.96 Hz, H-2″), 5.17 (d, 1 H, H-1), 5.04 (d, 1 H, H-1″), 4.79 (dd, 1 H, $J_{4,5}$ 10.22 Hz, H-4″), 4.69 (dd, 1 H, $J_{2,3}$ 10.89, $J_{3,4}$ 3.63 Hz, H-3‴), 4.54 (t, 1 H, H-4), 4.47 (m, 1 H, H-4‴), 4.46 (dd, 1 H, $J_{2,3}$ 8.91, $J_{3,4}$ 3.30 Hz, H-3′), 4.31 (d, 1 H, H-5″), 4.27 (d, 1 H, $J_{1,2}$ 9.24 Hz, H-1′), 4.22 (d, 1 H, H-5), 4.17 (d, 1 H, $J_{2,NH}$ 9.23 Hz, NH′), 4.02 (ddd, 1 H, H-2′), 3.91–3.71 (m, 4 H, H-4′, 6 × 3), 3.73, 3.71 (2 s, 9 H, 2 COOMe, *Me*OPh), 3.49 (d, 1 H, J_{gem} 11.55 Hz, H-6 × 1), 3.28 (ddd, 1 H, H-2‴), 3.01 (s, 1 H, H-5′ or H-5‴), 2.73–2.30 (m, 4 H, 2 CH_2), 2.64 (s, 1 H, H-5‴ or H-5′), 2.43, 2.39, 2.35, 2.30 (4 s, 12 H, 4 *Me*Ph), 2.04 (s, 3 H, MeCO), 1.84, 1,77 (2 s, 6 H, 2 NAc). Anal. Calcd for $C_{88}H_{92}N_2O_{30} \cdot 2H_2O$: C, 62.39; H, 5.72; N, 1.65. Found: C, 62.36; H, 5.57; N, 1.66.

(b) To a solution of **34** (34.7 mg, 21.3 μmol) in EtOAc (5 mL) was added Lindlar catalyst (200 mg), and the mixture was stirred overnight under a hydrogen atmosphere. Insoluble material was removed by Celite filtration, and the volatiles were removed in vacuo. The residue was conventionally acetylated to afford **35** (21.1 mg, 60%) after separation of the obtained mixture by silica gel column chromatography (1:1–1:3 toluene–EtOAc → 50:1 EtOAc–MeOH).

Sodium (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(sodium β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**9**).—Acetamide **35** (9.8 mg, 5.9 μmol) was diluted with THF (0.8 mL) and water (0.06 mL). To this solution was added 0.10 mL of 1.25 N lithium hydroxide at 0°C, and the mixture was then stirred for 45 min prior to removal of the volatiles under diminished pressure. The residue was dissolved in 1.2 mL of 3:1 MeOH–CH_2Cl_2 and 0.3 mL of 0.5 N NaOH, and stirring was continued overnight. Using the same work-up as described in the synthesis of 3, the corresponding saponified compound (5.7 mg, 88% in two steps) (R_f 0.47, 3:2:1 BuOH–CH_3COOH–H_2O) was obtained and converted to **9** without further purification. A catalytic amount of palladium hydroxide was added to water (1.8 mL) solution of the product (4.1 mg, 3.7 μmol), and the reaction mixture was stirred under a hydrogen atmosphere for 1.5 days. Insoluble material was filtered off, and the solution was exposed to Dowex-50 (Na^+). After filtration, the volatiles were removed, and the residue was purified by gel-permeation chromatography (LH-20, water) to give 2.3 mg of **9** in 68% yield; $[\alpha]_D$ −40.3° (*c* 0.067, H_2O); R_f 0.13 (3:2:1 BuOH–CH_3COOH–H_2O). For ^1H and ^{13}C NMR data, see Tables 3 and 6, respectively. FABMS: *m/z* 903.4 $(M - Na)^-$, 881.3 $(M - 2Na + H)^-$.

Methyl (2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**36**).—Known tetrasaccharide **34** [10,14] (37.7 mg, 23.2 μmol) was dissolved in 3.6 mL of 5:1 EtOH–toluene, hydrazine acetate (34.2 mg, 0.371 mmol, 16 equiv) was added while stirring for 42 min, and the solvents were then removed in vacuo. The residue was subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to yield **36** (34.3 mg, 97%); R_f 0.46 (1:1 toluene–EtOAc); $[\alpha]_D$ 0° (*c* 0.955, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.86–7.80 (m, 8 H, aromatic H), 7.38–7.27 (m, 10 H, aromatic H), 7.18–7.04 (m, 8 H, aromatic H), 6.92–6.89 (m, 2 H, aromatic H), 6.78–6.74 (m, 2 H, aromatic H), 5.74 (br t, 1 H, *J* 9.16 Hz, H-3), 5.61 (dd, 1 H, $J_{2,3}$ 6.93, $J_{3,4}$ 8.25 Hz, H-3″), 5.57 (dd, 1 H, $J_{1,2}$ 7.26, $J_{2,3}$ 9.23 Hz, H-2), 5.40 (s, 1 H, PhCH), 5.36 (br t, 1 H, *J* 6.28 Hz, H-2″), 5.28 (s, 1 H, PhCH), 5.20 (d, 1 H, H-1), 5.12 (d, 1 H, $J_{1,2}$ 5.94 Hz, H-1″), 4.52 (dd, 1 H, $J_{3,4}$ 8.25, $J_{4,5}$ 10.23 Hz, H-4″), 4.50 (br t, 1 H, *J* 8.90 Hz, H-4), 4.36 (d, 1 H, $J_{1,2}$ 7.92 Hz, H-1′), 4.30 (d, 1 H, H-5″), 4.28 (d, 1 H, $J_{4,5}$ 9.24 Hz, H-5), 4.25 (br d, 1 H, *J* 3.30 Hz, H-4′), 4.06 (d, 1 H, $J_{1,2}$ 7.59 Hz, H-1‴), 3.88 (bd, 1 H, *J* 2.96 Hz, H-4‴), 3.83, 3.74 (2 s, 9 H, 2 COOMe, *Me*OPh), 3.61 (m, 2 H, H-2′,6′a), 3.52 (br d, 1 H, J_{gem} 12.34 Hz, H-6′b), 3.47 (br s, 2 H, H-6‴a,6‴b), 3.41 (dd, 1 H, $J_{2,3}$ 10.22, $J_{3,4}$ 2.97 Hz, H-3′), 3.32 (m, 1 H, H-2‴), 3.28 (m, 1 H, H-3‴), 3.08 (s, 1 H, H-5′), 2.75) (s, 1 H, H-5‴), 2.36, 2.34, 2.31, 2.28 (4 s, 12 H, 4 *Me*Ph). Anal. Calcd for $C_{79}H_{78}N_6O_{26}$: C, 62.11; H, 5.16; N, 5.50. Found: C, 62.04; H, 5.22; N, 5.62.

Methyl (2-azido-4,6-O-benzylidene-2-deoxy-3-O pivaloyl-β-D-galactopyranosyl)-(1 → 4)-(methyl-2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-

4-methylbenzoyl-β-D-glucopyranosid)uronate (**37**).—Pivaloyl chloride (0.04 mL, 0.325 mmol) and a catalytic amount of DMAP were added to a solution of **36** (34.3 mg, 22.5 μmol) in pyridine (1.5 mL) at 80°C with stirring. An excess amount of pivaloyl chloride (0.1 mL) was added after 2 h, and stirring was continued for one more hour. To the solution was added MeOH (0.14 mL), and the mixture was processed as described in the synthesis of **18**. The residue so obtained was subjected to gel-permeation chromatography (S-X8, toluene) to quantitatively give **27** (36.6 mg); R_f 0.61 (2:1 toluene–EtOAc); $[\alpha]_D$ −0.28° (*c* 0.36, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.86–7.78 (m, 8 H, aromatic H), 7.39–7.03 (m, 18 H, aromatic H), 6.94–6.88 (m, 2 H, aromatic H), 6.78–6.74 (m, 2 H, aromatic H), 5.75 (dd, 1 H, $J_{2,3}$ 8.91, $J_{3,4}$ 9.24 Hz, H-3), 5.60 (dd, 1 H, $J_{2,3}$ 6.27, $J_{3,4}$ 7.92 Hz, H-3″), 5.58 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2), 5.42 (s, 1 H, PhC*H*), 5.34 (br t, 1 H, *J* 5.94 Hz, H-2″), 5.23 (s, 1 H, PhC*H*), 5.20 (d, 1 H, H-1), 5.13 (d, 1 H, $J_{1,2}$ 5.61 Hz, H-1″), 4.56 (dd, 1 H, $J_{3,4}$ 7.92, $J_{4,5}$ 10.23 Hz, H-4″), 4.50 (br t, 1 H, *J* 8.90 Hz, H-4), 4.42 (dd, 1 H, $J_{2,3}$ 10.89, $J_{3,4}$ 3.63 Hz, H-3‴), 4.36 (d, 1 H, $J_{1,2}$ 7.92 Hz, H-1′), 4.30 (d, 1 H, H-5″), 4.29 (d, 1 H, $J_{4,5}$ 9.24 Hz, H-5), 4.29 (d, 1 H, $J_{3,4}$ 3.63 Hz, H-4′), 4.08 (d, 1 H, $J_{1,2}$ 7.25 Hz, H-1‴), 4.04 (d, 1 H, H-4‴), 3.86–3.60 (m, 2 H, H-2′,6′ or 6‴ × 1), 3.81 (s, 3 H, COOMe), 3.75, 3.73 (s, 6 H, COOMe, *Me*OPH), 3.67 (dd, 1 H, H-2‴), 3.52 (d, 1 H, J_{gem} 11.88 Hz, H-6′ or H-6‴ × 1), 3.46 (br s, 2 H, H-6 × 2), 3.42 (dd, 1 H, $J_{2,3}$ 10.22 Hz, H-3′), 3.08 (s, 1 H, H-5′ or H-5‴), 2.66 (s, 1 H, H-5‴ or H-5′), 2.36, 2.34, 2.33, 2.27 (4 s, 12 H, 4 *Me*Ph), 1.19 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{84}H_{86}N_6O_{27}$: C, 62.59; H, 5.39; N, 5.22. Found: C, 62.30; H, 5.40; N, 5.07.

*Methyl (2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-acetyl-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-*galactopyranosyl)-(1→4)-(4-methoxyphenyl 2,3-di-O-III δ)*4-methylbenzoyl-β-D-glucopyranosid)uronate* (**38**).—Hydrazine acetate (11.8 mg, 0.128 mmol, 19 equiv) was added to a solution of **35** (11.0 mg, 6.6 μmol) in 1 mL of 4:1 EtOH–toluene while stirring. After 0.5 h, the solution was diluted with $CHCl_3$. The organic phase was washed as usual, and the residue was subjected to conventional acetylation to give **38** (6.8 mg) in 64% yield. For further purification, PTLC (30:1 EtOAc–MeOH) was used; R_f 0.54 (30:1 EtOAc–MeOH); $[\alpha]_D$ +38.0° (*c* 0.213, $CHCl_3$). ^1H NMR data ($CDCl_3$); δ 7.96 (d, 2 H, *J* 8.25 Hz, aromatic H), 7.87–7.78 (m, 6 H, aromatic H), 7.57 (d, 2 H, *J* 6.27 Hz, aromatic H), 7.42–7.13 (m, 16 H, aromatic H), 7.07 (d, 2 H, *J* 7.91 Hz, aromatic H), 6.92–6.86 (m, 2 H, aromatic H), 6.77–6.71 (m, 2 H, aromatic H), 5.75 (br t, 1 H, *J* 8.75 Hz, H-3), 5.62 (s, 1 H, PhC*H*), 5.59 (dd, 1 H, $J_{2,3}$ 3.63, $J_{3,4}$ 7.92 Hz, H-3″), 5.50 (d, 1 H, $J_{2,NH}$ 8.58 Hz, NH‴), 5.49 (dd, 1 H, $J_{1,2}$ 7.26, $J_{2,3}$ 8.58 Hz, H-2), 5.28 (s, 1 H, PhC*H*), 5.24–5.17 (m, 3 H, H-1,2″,1‴), 5.04 (d, 1 H, $J_{1,2}$ 4.29 Hz, H-1″), 4.79 (dd, 1 H, $J_{4,5}$ 10.22 Hz, H-4″), 4.68 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.63 Hz, H-3‴), 4.54 (br t, 1 H, *J* 8.75 Hz, H-4), 4.54 (dd, 1 H, $J_{2,3}$ 11.88, $J_{3,4}$ 3.63 Hz, H-3′), 4.45 (d, 1 H, H-4‴), 4.34 (d, 1 H, $J_{1,2}$ 8.24 Hz, H-1′), 4.31 (d, 1 H, H-5δ), 4.22 (d, 1 H, $J_{2,NH}$ 13.53 Hz, NH′), 4.22 (d, 1 H, $J_{4,5}$ 9.24 Hz, H-5), 4.02 (ddd, 1 H, H-2′), 3.96 (d, 1 H, H-4′), 3.87 (d, 1 H, J_{gem} 12.20 Hz, H-6′ or H-6‴;× 1), 3.77–3.69 (m, 1 H, H-6‴ or H-6′ × 1), 3.75 (m, 1 H, H-6‴ or H-6′δ × 1), 3.72 (s, 9 H, 2 COOMe, *Me*OPh), 3.52 (d, 1 H, J_{gem} 11.22 Hz, H-6′ or H-6‴ × 1), 3.22 (ddd, 1 H, $J_{1,2}$ 7.59 Hz, H-2‴), 3.00 (s, 1 H, H-5‴ or H-5′), 2.65 (s, 1 H, H-5′ or H-5‴), 2.42, 2.38, 2.34, 2.31 (4 s, 12 H, 4 *Me*Ph), 1.98, 1.82, 1,75 (3 s, 9 H, OAc, 2 NAc). Anal. Calcd for $C_{85}H_{88}N_2O_{29}$; C, 63.73; H, 5.55; N, 1.75. Found: C, 63.84; H, 5.72; N, 1.72.

Methyl (2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-pivaloyl-β-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-(1→4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**39**).—To a solution of **37** (13.0 mg, 8.1 μmol) in EtOAc (1.5 mL) was added a catalytic amount of Lindlar catalyst, and resultant residue was stirred overnight under a hydrogen atmosphere. Insoluble material was removed by Celite filtration, and the volatiles were removed in vacuo. The residue was acetylated conventionally. After purification by silica gel column chromatography (3:2–1:5 toluene–EtOAc) **39** (11.6 mg, 87%) was obtained; R_f 0.16 (1:1 toluene–EtOAc); $[\alpha]_D$ +32.8° (*c* 0.50, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 8.00–7.97 (d, 2 H, *J* 8.24 Hz, aromatic H), 7.88–7.82 (m, 6 H, aromatic H), 7.61–7.58 (d, 2 H, *J* 6.60 Hz, aromatic H), 7.41–7.13 (m, 14 H, aromatic H), 7.08–7.05 (d, 2 H, *J* 7.91 Hz, aromatic H), 6.91–6.84 (m, 2 H, aromatic H), 6.78–6.73 (m, 2 H, aromatic H), 5.75 (br t, 1 H, *J* 8.75 Hz, H-3), 5.65 (s, 1 H, PhC*H*), 5.59 (dd, 1 H, $J_{2,3}$ 3.30, $J_{3,4}$ 7.59 Hz, H-3″), 5.52 (d, 1 H, $J_{2,NH}$ 6.93 Hz, NH‴), 5.49 (dd, 1 H, $J_{1,2}$ 8.91, $J_{2,3}$ 8.58 Hz, H-2), 5.29 (s, 1 H, PhC*H*), 5.21–5.17 (m, 3 H, H-1,2″,1‴), 5.04 (d, 1 H, $J_{1,2}$ 3.96 Hz, H-1″), 4.82

(dd, 1 H, $J_{4,5}$ 10.55 Hz, H-4″), 4.69 (dd, 1 H, $J_{2,3}$ 10.89, $J_{3,4}$ 3.63 Hz, H-3‴), 4.54 (br t, 1 H, J 9.15 Hz, H-4), 4.51 (d, 1 H, H-4‴), 4.37 (dd, 1 H, $J_{2,3}$ 10.56, $J_{3,4}$ 3.30 Hz, H-3′), 4.31 (d, 1 H, H-5″), 4.28 (d, 1 H, $J_{1,2}$ 6.27 Hz, H-1′), 4.22 (d, 1 H, $J_{4,5}$ 9.24 Hz, H-5), 4.17–4.01 (m, 2 H, H-2′, NH′), 3.96 (d, 1 H, H-4′), 3.92–3.70 (m, 3 H, H-6′a,6‴ × 2), 3.73, 3.70 (2 s, 9 H, 2 COOMe, *Me*OPh), 3.52 (d, 1 H, J_{gem} 11.55 Hz, H-6′b), 3.30 (m, 1 H, H-2‴), 3.00 (s, 1 H, H-5‴), 2.60 (s, 1 H, H-5′), 2.45, 2.39, 2.35, 2.31 (4 s, 12 H, 4 *Me*Ph), 1.85, 1,71 (2 s, 6 H, 2 NAc), 1.09 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{88}H_{94}N_2O_{29}$: C, 64.29; H, 5.78; N, 1.70 Found: C, 64.30; H. 5.79; N, 1.67.

Methyl (2-acetamido-6-O-benzyl-2-deoxy-3-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-O-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-6-O-benzyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**40**), *methyl (2-acetamido-6-O-benzyl-2-deoxy-4-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-6-O-benzyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate and* (**41**) *methyl (2-acetamido-6-O-benzyl-2-deoxy-3-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-4-O-benzyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate* (**42**).—Sodium cyanoborohydride (34 mg, 0.54 mmol, 63 × 2 equiv), powered 3 Å molecular sieves (60 mg) and small amount of methyl orange were added to a solution of **38** (6.8 mg, 4.3 μmol) in THF (1 mL), and the mixture was stirred for 30 min at room temperature. The mixture was then cooled to 0°C, and HCl–Et_2O was added dropwise until the color changed to pink. The reaction mixture was allowed to warm up to room temperature before additional sodium cyanoborohydride (10 mg) and HCl–Et_2O were added. The reaction mixture was treated as described in the synthesis of **20**, and purified by PTLC (10:1 EtOAc–MeOH) to yield **40** (3.5 mg, 51%), **41** (1.3 mg, 19%) and trace amount of **42**.

40: R_f 0.53 (10:1 EtOAc–MeOH); $[\alpha]_D$ +34.2° (c 1.01, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.85–7.79 (m, 8 H, aromatic H), 7.37–7.11 (m, 18 H, aromatic H), 6.89–6.85 (m, 2 H, aromatic H), 6.77–6.72 (m, 2 H, aromatic H), 5.59–5.51 (m, 4 H, H-2,3,3″, NH‴), 5.30 (dd, 1 H, $J_{1,2}$ 7.26, $J_{2,3}$ 8.91 Hz, H-2″), 5.27 (d, 1 H, $J_{2,NH}$ 6.27 Hz, NH′), 5.13 (m, 1 H, H-1), 4.95 (dd, 1 H, $J_{2,3}$ 10.88, $J_{3,4}$ 2.97 Hz, H-3‴), 4.94 (d, 1 H, $J_{1,2}$ 8.91 Hz, H-1′), 4.79 (d, 1 H, H-1″), 4.70 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1‴), 4.49 (dd, 1 H, $J_{2,3}$ 10.88, $J_{3,4}$ 2.64 Hz, H-3′), 4.41–4.26 (m, 2 H, H-4,4″), 4.23–4.06 (m, 6 H, H-5,5″), 2 PhCH_2), 3.97 (m, 1 H, H-4′), 3.97–3.88 (m, 1 H, H-2‴), 3.88 (d, 1 H, H-4‴), 3.73, 3.72, 3.60 (3 s, 9 H, 2 COOMe PhO*Me*), 3.48–3.29 (m, 2 H, H-5′,5‴), 3.10–2.92 (m, 5 H, H-2′,6′ × 2,6‴ × 2), 2.37, 2.35, 2.34, 2.33 (4 s, 12 H, 4 *Me*Ph), 2.05, 1.88, 1.40 (3 s, 9 H, OAc, 2 NAc). Anal. Calcd for $C_{85}H_{92}N_2O_{29} \cdot H_2O$: C, 62.87; H, 5.85; N, 1.73. Found: C, 62.70; H, 5.80; N, 1.74.

41: R_f 0.39 (10:1 EtOAc–MeOH). ^{1}H NMR data ($CDCl_3$): δ 7.86–7.78 (m, 8 H, aromatic H), 7.36–7.05 (m, 18 H, aromatic H), 6.89–6.84 (m, 2 H, aromatic H), 6.77–6.73 (m, 2 H, aromatic H), 5.58–5.52 (m, 3 H, H-2,3,3″), 5.37 (dd, 1 H, $J_{1,2}$ 7.59, $J_{2,3}$ 9.90 Hz, H-2″), 5.30–5.28 (m, 2 H, NH′, NH‴ or H-4‴), 5.16 (br s, 1 H, H-4‴ or NH‴), 5.13 (m, 1 H, H-1″), 4.58 (dd, 1 H, $J_{2,3}$ 10.56, $J_{3,4}$ 3.30 Hz, H-3′), 4.37 (m, 1 H, H-4″), 4.31 (m, 1 H, H-1‴), 4.25–4.08 (m, 7 H, H-4,5,5″, 2 PhCH_2), 4.00 (br s, 1 H, H-4′), 3.73, 3.73, 3.61 (3 s, 9 H, 2 COOMc, PhO*Me*), 3.69–3.61 (m, 3 H, H-2‴,3‴, OH-3‴), 3.43–3.38 (m, 2 H, H-5′,5‴), 3.09–2.96 (m, 3 H, H-2′,6′ or 6‴ × 2), 2.83 (dd, 1 H, $J_{5,6a}$ 5.94, J_{gem} 9.57 Hz, H-6‴ or H-6′a), 2.71 (dd, 1 H, $J_{5,6b}$ 7.26 Hz, H-6‴ or H-6′b), 2.37, 2.35, 2.34, 2.32 (4 s, 12 H, 4 *Me*Ph), 2.01, 1.91, 1.40 (3 s, 9 H, OAc, 2 NAc).

42: R_f 0.39 (10:1 EtOAc–MeOH); $[\alpha]_D$ −17.5° (c 0.36, $CHCl_3$). ^{1}H NMR data ($CDCl_3$); δ 7.85–7.77 (m, 8 H, aromatic H), 7.34–7.01 (m, 18 H, aromatic H), 6.93–6.87 (m, 2 H, aromatic H), 6.78–6.73 (m, 2 H, aromatic H), 5.61 (t, 1 H, $J_{2,3} = J_{3,4} =$ 8.91 Hz, H-3″), 5.56–5.51 (m, 3 H, H-2,3, NH‴), 5.37 (br t, 1 H, J 8.42 Hz, H-2′), 5.25 (d, 1 H, $J_{2,NH}$ 6.92 Hz, NH′), 5.14 (m, 1 H, H-1), 5.03 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 2.64 Hz, H-3‴), 4.92 (d, 1 H, $J_{1,2}$ 7.92 Hz, H-1′), 4.79 (d, 1 H, $J_{1,2}$ 7.58 Hz, H-1″), 4.78, 4.48 (ABq, 2 H, J_{gem} 12.86 Hz, PhCH_2), 4.71 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1‴), 4.66 (dd, 1 H, $J_{2,3}$ 11.55, $J_{3,4}$ 2.63 Hz, H-3′), 4.35 (m, 1 H, H-4), 4.28 (t, 1 H, $J_{4,5}$ 8.91 Hz, H-4″), 4.20–4.11 (m, 4 H, H-5,5″, PhCH_2), 3.90 (d, 1 H, H-4‴), 3.85 (m, 1 H, H-2‴), 3.77, 3.73, 3.69 (3 s, 9 H, 2 COOMe, PhOMe), 3.63 (br s, 1 H, H-4′), 3.34, 3.22 (2 m, 2 H, H-5′,5‴), 3.08 (m, 1 H, H-2′), 3.01–2.87 (m, 4 H, 6′ × 2, 6‴ × 2), 2.38, 2.36, 2.36, 2.34, 2.32 (4 s, 12 H, 4

*Me*Ph), 2.05, 1.91, 1.56 (3 s, 9 H, OAc, 2 NAc). Anal. Calcd. for $C_{85}H_{92}N_2O_{29} \cdot H_2O$: C, 62.87; H, 5.85; N, 1.73. Found: C, 62.96; H, 6.05; N, 1.75.

Methyl (2-acetamido-6-O-benzyl-2-deoxy-3-O-pivaloyl-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-6-O-benzyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate (**43**) *and methyl (2-acetamido-6-O-benzyl-2-deoxy-3-O-pivaloyl-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-acetamido-4-O-benzyl-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-O-4-methylbenzoyl-β-D-glucopyranosid)uronate* (**44**).— Sodium cyanoborohydride (108 mg, 1.7 mmol, 60 × 2 equiv), powdered 3 Å molecular sieves (120 mg) and a small amount of methyl orange were added to a solution of **39** (23.3 mg, 14.2 μmol) in THF (4 mL), and the mixture was stirred for 90 min at room temperature. The mixture was then cooled to 0°C, and HCl–Et_2O was added dropwise until the color changed to pink. The reaction mixture was allowed to warm up to room temperature before additional sodium cyanoborohydride (114 + 50 mg) and HCl–Et_2O were supplied. The reaction mixture was treated as described in the synthesis of **20**, and purified by the column of silica gel (2:1–1:1–2:3–1:3–1:5 toluene–EtOAc → 30:1 EtOAc–MeOH) to yield **43** (15.1 mg, 65%) and **44** (3.5 mg, 15%). **43**: R_f 0.60 (30:1 EtOAc–MeOH); $[\alpha]_D$ +45.3° (*c* 0.727, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.86–7.81 (m, 8 H, aromatic H), 7.36–7.12 (m, 18 H, aromatic H), 6.90–6.85 (m, 2 H, aromatic H), 6.77–6.73 (m, 2 H, aromatic H), 5.63 (d, 1 H, $J_{2,NH}$ 9.24 Hz, NH‴), 5.59–5.53 (m, 3 H, H-2,3,3″), 5.31 (d, 1 H, $J_{2,NH}$ 7.26 Hz, NH′), 5.30 (dd, 1 H, $J_{1,2}$ 7.26, $J_{2,3}$ 8.25 Hz, H-2′), 5.13 (m, 1 H, H-1), 4.90 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.80 (dd, 1 H, $J_{2,3}$ 10.50, $J_{3,4}$ 3.30 Hz, H-3‴), 4.80 (d, 1 H, H-1″), 4.61 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1‴), 4.42 (dd, 1 H, $J_{2,3}$ 12.87, $J_{3,4}$ 2.97 Hz, H-3′), 4.37 (m, 1 H, H-4), 4.44 (br t, 1 H, *J* 8.91 Hz, H-4″), 4.24–4.05 (m, 7 H, H-5,5″,2‴, 2 PhCH_2), 3.98 (d, 1 H, H-4′), 3.85 (d, 1 H, H-4‴), 3.73, 3.60 (2 s, 9 H, 2 COOMe, PhO*Me*), 3.40, 3.29 (2 m, 2 H, H-5′,5‴), 3.15 (dt, 1 H, H-2′), 3.08–2.87 (m, 4 H, H-6′ × 2,6‴ × 2), 2.37, 2.35, 2.34, 2.33 (4 s, 12 H, 4 *Me*Ph), 1.86, 1.40 (2 s, 6 H, 2 NAc), 1.15 (s, 3 H, *tert*-Bu). Anal. Calcd for $C_{88}H_{98}N_2O_{29}$: C, 64.14; H, 6.01; N, 1.70. Found: C, 63.84; H, 6.01; N, 1.49.

44: R_f 0.30 (30:1 EtOAc–MeOH); $[\alpha]_D$ +16.3° (*c* 0.160, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.86–7.77 (m, 8 H, aromatic H), 7.32–7.10 (m, 18 H, aromatic H), 6.90–6.87 (m, 2 H, aromatic H), 6.77–6.73 (m, 2 H, aromatic H), 5.62 (br t, 1 H, *J* 9.08 Hz, H-3″), 5.56–5.53 (m, 2 H, H-2,3), 5.42 (d, 1 H, $J_{2,NH}$ 9.57 Hz, NH‴), 5.47 (br t, 1 H, *J* 9.06 Hz, H-2″), 5.23 (d, 1 H, $J_{2,NH}$ 6.60 Hz, NH′), 5.14 (m, 1 H, H-1), 4.92 (d, 1 H, $J_{1,2}$ 8.25 Hz, H-1′), 4.90 (dd, 1 H, $J_{2,3}$ 11.22, $J_{3,4}$ 3.30 Hz, H-3‴), 4.78 (d, 1 H, $J_{1,2}$ 7.91 Hz, H-1″), 4.78, 4.48 (ABq, 2 H, J_{gem} 11.87 Hz, PhCH_2), 4.66 (m, 1 H, H-3′), 4.62 (d, 1 H, $J_{1,2}$ 8.58 Hz, H-1‴), 4.34 (m, 1 H, H-4), 4.28 (br t, 1 H, *J* 9.24 Hz, H-4″), 3.88 (m, 1 H, H-4‴), 3.77, 3.73, 3.69 (3 s, 9 H, 2 COOMe, PhOMe), 3.62 (br s, 1 H, H-4′), 3.34, 3.22 (2 m, 2 H, H-5′,5‴), 3.09 (m, 1 H, H-2′), 3.00–2.77 (m, 4 H, 6′ × 2,6‴ × 2), 2.39, 2.39, 2.34, 2.32 (4 s, 12 H, 4 *Me*Ph), 1.88, 1.57 (2 s, 6 H, NAc), 1.16 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{88}H_{98}N_2O_{29}$: C, 64.14; H, 6.01; N, 1.70. Found: C, 63.88; H, 6.26; N, 1.63.

Sodium (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)-(4-methoxyphenyl β-D-glucopyranosid)uronate (**10**).—(a) To a solution of **40** (3.5 mg, 2.2 μmol) in Me_2NCHO (0.7 mL) was added sulfur trioxide–trimethylamine complex (18.9 mg, 0.14 mmol, 31 × 2 equiv.), and the resultant mixture was stirred for 1 day at 50–60°C. The reaction mixture was cooled to room temperature, subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH), and treated on a column of Dowex-50 (N^+) (8:1 MeOH–water) to give crude disulfate (3.6 mg, 92%); R_f 0.44 (3:1 $CHCl_3$–MeOH). This was dissolved in THF (0.6 mL) and water (0.4 mL). To the solution was added 1.25 N lithium hydroxide (30 μL, 10 × 2 equiv) while stirring at 0°C. After 1 h, the volatiles were removed under reduced pressure, the residue was dissolved in MeOH (0.5 mL), and 0.5 N NaOMe (0.5 mL) was added. After stirring overnight, the reaction mixture was eluted from a column of LH-20 (water). The residue so obtained was dissolved in water (3 mL) and stirred for 3.5 days under hydrogen in the presence of a catalytic amount of palladium hydroxide. Insoluble material was filtered off. The solution was exposed to Amberlite CG-50 (H^+), filtered, and evaporated. The residue was diluted in water and exposed to Dowex-50 (NA^+). Gel-permeation chromatography (LH-20, water) gave 1.9 mg of **10** in 70% yield; R_f 0.18

(2:2:1 BuOH–CH_3COOH–H_2O); $[\alpha]_D$ −28.7° (*c* 0.167, H_2O). For ^1H and ^{13}C NMR data, see Tables 3 and 6, respectively. FABMS: *m/z* 1107.3 $(M - Na)^-$, 1085.3 $(M - 2Na + H)^-$, 1063.2 $(M - 3Na + 2H)^-$.

(b) To a solution of **43** (10.5 mg, 6.4 μmol) in Me_2NCHO (1.0 mL) was added sulfur trioxide–trimethylamine complex (59.8 mg, 0.43 mmol, 30 × 2 equiv), and stirring was continued for 1 day at 50–60°C. The reaction mixture was treated as above to give the corresponding disulfate (11.6 mg, 98%); R_f 0.52 (4:1 $CHCl_3$–MeOH), which was dissolved in THF (1.5 mL) and water (0.1 mL). To this solution was added 1.25 N lithium hydroxide (0.1 mL) while stirring at 0°C. After 2 h, the volatiles were removed under reduced pressure, and the residue was dissolved in 30 mL of 3:1 MeOH–CH_2Cl_2, and treated with 0.5 N NaOH (0.5 mL). After stirring for 1.5 days, the reaction mixture was neutralized by careful addition of 50% CH_3COOH and evaporated. The residue was fractionated on a column of LH-20 (water), and the residue was subsequently treated with Dowex-50 (H^+) and Dowex-50 (Na^+). The volatiles were removed, the residue was dissolved in water (5 mL) and stirred overnight under hydrogen in the presence of catalytic amount of palladium hydroxide. Insoluble material was filtered off. The solution was eluted from gel-permeation chromatography (LH-20, water) to yield 5.0 mg of **10** in 70% yield.

*Methyl (2-acetamido-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)-(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**45**).—Camphorsulfonic acid (2.2 mg, 9.5 μmol) was added to a solution of **39** (15.7 mg, 9.6 μmol) in a 1:1 mixture of CH_2Cl_2–MeOH (1.0 mL) while stirring. After 2 days, the reaction was quenched with aq $NaHCO_3$, extracted with $CHCl_3$, and treated as usual. Separation by column of silica gel chromatography (3:2–2:3–1:3–1:10 *n*-hexane–EtOAc → 50:1–20:1 EtOAc–MeOH) afforded **45** (10.9 mg, 78%); R_f 0.50 (10:1 EtOAc–MeOH); $[\alpha]_D$ +28.8° (*c* 0.26, $CHCl_3$). Selected ^1H NMR data ($CDCl_3$): δ 7.88–7.79 (m, 8 H, aromatic H), 7.17–7.09 (m, 8 H, aromatic H), 6.90–6.87 (m, 2 H, aromatic H), 6.75–6.72 (m, 2 H, aromatic H), 6.06, 5.77 (2 br, 2 H, 2 NH), 5.68, 5.62 (2 t, 2 H, $J_{2,3} = J_{3,4} = 8.58$ Hz, H-3,3‴), 5.55 (dd, 1 H, $J_{1,2}$ 6.93 Hz, H-2), 5.30 (br, t, 1 H, *J* 7.92 Hz, H-2″), 5.24 (d, 1 H, H-1), 4.93 (m, 2 H, H-1′,1″ or 1‴), 4.84 (dd, 1 H, $J_{2,3}$ 10.89, $J_{3,4} < 1.0$ Hz, H-3‴), 4.71 (d, 1 H, H-1′,1″ or 1‴), 4.46, 4.41 (2 br t, 2 H, *J* 8.90 and 8.58 Hz, H-44″), 4.23 (br d, 2 H, *J* 8.91 Hz, H-5,5″), 3.80, 3.72, 3.70 (3 s, 9 H, 2 COOMe, *Me*OPh), 2.35, 2.32 (2 s, 12 H, 4 *Me*Ph), 1.81, 1.35 (2 s, 6 H, 2 Ac), 1.14 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{74}H_{86}N_2O_{29} \cdot H_2O$: C, 59.82; H, 5.98; N, 1.89. Found: C, 59.67; H, 6.00; N, 1.71.

*Methyl (2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-pivaloyl-β-*D*-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosyluronate)-(1 → 3)-(2-acetamido-4-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl)(1 → 4)-(4-methoxyphenyl 2,3-di-*O*-4-methylbenzoyl-β-*D*-glucopyranosid)uronate* (**46**).—Diol **43** (15.6 mg) was acetylated in a conventional manner in 92% yield (15.1 mg). A portion of the product (14.6 mg, 8.4 μmol) was diluted in EtOAc (5 mL), and the mixture was stirred in the presence of Pd–C under a hydrogen atmosphere for 2.5 days. Insoluble materials were filtered off, and the volatiles were evaporated to give 13.0 mg of **46** in 99% yield; R_f 0.23 (30:1 EtOAc–MeOH); $[\alpha]_D$ +37.0° (*c* 0.40, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.79–7.71 (m, 8 H, aromatic H), 7.16–7.07 (m, 8 H, aromatic H), 6.83–6.79 (m, 2 H, aromatic H), 6.69–6.66 (m, 2 H, aromatic H), 5.51–5.49 (m, 2 H, H-2,3), 5.46 (t, 1 H, $J_{2,3} = J_{3,4}$ 8.58 Hz, H-3″), 5.36 (d, 1 H, $J_{2,NH}$ 8.91 Hz, NH‴), 5.28 (d, 1 H, *J* 6.93 Hz, NH′), 5.21 (dd, 1 H, $J_{1,2}$ 7.26 Hz, H-2″), 5.13 (d, 1 H, $J_{3,4}$ 3.30 Hz, H-4′), 5.07 (m, 1 H, H-1), 5.02 (d, 1 H, $J_{3,4}$ 3.30 Hz, H-4‴), 4.93 (d, 1 H, $J_{1,2}$ 7.91 Hz, H-1′), 4.86 (dd, 1 H, $J_{2,3}$ 11.22 Hz, H-3‴), 4.69 (d, 1 H, H-1′), 4.62 (dd, 1 H, $J_{2,3}$ 10.88 Hz, H-3′), 4.53 (d, 1 H, $J_{1,2}$ 8.58 Hz, H-1‴), 4.30 (m, 1 H, H-4), 4.24 (br t, 1 H, *J* 8.88 Hz, H-4″), 4.07 (d, 1 H, $J_{4,5}$ 9.24 Hz, H-5), 4.01 (d, 1 H, $J_{4,5}$ 8.90 Hz, H-5″), 3.99 (m, 1 H, H-2‴), 3.71, 3.66 (2 s, 9 H, 2 COOMe, MeOPh), 3.30–3.22 (m, 2 H, H-5′,5‴), 3.05–3.28 (m, 3 H, H-6′,6‴), 2.96 (m, 1 H, H-2′), 2.66–2.50 (m, 1 H, H-6′ or H-6‴), 2.28 (s, 12 H, 4 *Me*Ph), 1.97, 1.96, 1.93, 1.29 (4 s, 12 H, 2 OAc, 2 NAc), 1.01 (s, 9 H, *tert*-Bu). Anal. Calcd for $C_{78}H_{90}N_2O_{31} \cdot H_2O$: C, 59.68; H, 5.92; N, 1.79. Found: C, 59.68; H, 5.81; N, 1.69.

*Sodium (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)-(4-methoxyphenyl β-*D*-glucopyranosid)uronate* (**11**).—To a solution of **46** (3.9 mg, 2.5 μmol) in Me_2NCHO (1.0 mL) was added sulfur trioxide-trimethylamine complex (20.5 mg, 0.147 mmol 29 × 2 equiv) and stirring was continued overnight at 50–60°C. The reaction mixture was

cooled to room temperature and subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH) to give the crude sulfate (R_f 0.16, 4:1 $CHCl_3$–MeOH), which was dissolved in a mixture of THF (1.0 mL) and water (0.12 mL), To this solution was added 1.25 N lithium hydroxide (0.14 mL) while stirring at 0°C. After 2 h, the volatile were removed under reduced pressure, and the residue was dissolved in MeOH (0.8 mL) and treated with 0.5 N NaOH (0.8 mL). After 1.5 days, the reaction mixture was neutralized by careful addition of 50% CH_3COOH and evaporated. The residue was eluted from a column of LH-20 (water) to yield 2.3 mg of **11** in 82% yield; $[\alpha]_D$ −15.0° (*c* 0.10, H_2O); R_f 0.11 (2:2:1 BuOH–CH_3COOH–H_2O). For ^1H and ^{13}C NMR data, see Tables 3 and 6, respectively. FABMS: *m/z* 1107.3 $(M - Na)^-$, 1085.3 $(M - 2Na + H)^-$, 1063.2 $(M - 3Na + 2H)^-$.

*Sodium (disodium 2-acetamido-2-deoxy-4,6-di-O-sulfonato-β-*D*-galactopyranosyl)-(1 → 4)-(sodium β-*D*-glucopyranosyluronate)-(1 → 3)-(disodium 2-acetamido-2-deoxy-4, 6-di-O-sulfonato-β-*D*-galacto-pyranosyl)-(1 → 4)-(4-methoxyphenyl-β-*D*-gluco-pyranosid)uronate* (**12**).—To a solution of **45** (5.0 mg, 3.4 μmol) in Me_2NCHO (0.5 mL) was added sulfur trioxide–trimethylamine complex (17.9 mg, 0.129 mmol), and the mixture was stirred at 50–60°C. After 1 and 4 days, more reagent (24.4 + 21.0 mg, total 0.455 mmol, 33 equiv) were added. The reaction mixture was cooled to room temperature on the fifth day and subjected to gel-permeation chromatography (LH-20, 1:1 $CHCl_3$–MeOH), and the fractions collected were treated with Dowex-50 (NA^+) to give the crude sulfate (R_f 0.46, 15:5:2 $CHCl_3$–MeOH–Et_3N), which was dissolved in a mixture of THF (0.5 mL) and water (0.04 mL). To this was added 1.25 N lithium hydroxide (0.06 mL) while stirring at 0°C. After 2 h, the volatiles were removed under reduced pressure, and the residue was dissolved in MeOH (0.5 mL) and CH_2Cl_2 (0.15 mL). To this, 0.5 N NaOH (0.3 mL) was added, and stirring was continued for 1.5 days. The reaction mixture was neutralized by careful addition of 50% CH_3COOH and the solution was evaporated. The residue so obtained was eluted from a column of LH-20 (water) to yield 4.0 mg of **12** in 89% yield; $[\alpha]_D$ −13.1° (*c* 0.267, H_2O); R_f 0.33 (1:1:1 BuOH–CH_3COOH–H_2O). For ^1H and ^{13}C NMR data, see Tables 3 and 6, respectively. FABMS: *m/z* 1310.9 $(M - Na)^-$, 1289.0 $(M - 2Na + H)^-$, 1267.2 $(M - 3Na + 2H)^-$.

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