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SYNTHESIS OF DERMATAN SULFATE FRAGMENTS: A CHEMICAL SYNTHESIS OF METHYL 2-ACETAMIDO-2-DEOXY-3-*O*-(α-L-IDOPYRANOSYLURONIC ACID)-4-*O*-SULFO-β-D-GALACTOPYRANOSIDE DISODIUM SALT AND ITS NON-SULFATED ANALOGUE

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ABSTRACT

Methyl 2-azido-2-deoxy-β-D-galactopyranoside was subjected in sequence to isopropylidenation, benzylation, acid hydrolysis, and selective acetylation of HO-4, to give amorphous methyl 4-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-β-D-galactopyranoside. Condensation with 2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-α-L-idopyranosyl chloride (easily prepared in three steps from known 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-L-idopyranose) in 1,2-dichloroethane, in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieves, provided, after *O*-dechloroacetylation, 58% of amorphous methyl 4-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-L-idopyranosyl)-β-D-galactopyranoside. Reduction with sodium borohydride, followed successively by *N*-acetylation, oxidation in the presence of chromium trioxide in acetone–sulfuric acid, *O*-sulfation of the sodium salt with the sulfur trioxide–trimethylamine complex, and catalytic hydrogenolysis, then gave the disodium salt of methyl 2-acetamido-2-deoxy-3-*O*-(α-L-idopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside. The β-linked disaccharide was also isolated (30%) and converted, through the same sequence, into methyl 2-acetamido-2-deoxy-3-*O*-(β-L-idopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside. The non-sulfated analogue of the title disaccharide was obtained on catalytic hydrogenolysis of methyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(α-L-idopyranosyluronic acid)-β-D-galactopyranoside. The following compounds were also synthesized: methyl 2-acetamido-2-deoxy-3-*O*-methyl-4-*O*-sulfo-β-D-galactopyranoside, methyl 2-acetamido-2-deoxy-3-*O*-methyl-6-*O*-sulfo-β-D-galactopyranoside, and methyl 2-acetamido-2-deoxy-3-*O*-methyl-4,6-di-*O*-sulfo-β-D-galactopyranoside. The conformation of the L-idopyranosiduronate residue of the synthetic disaccharides is discussed.

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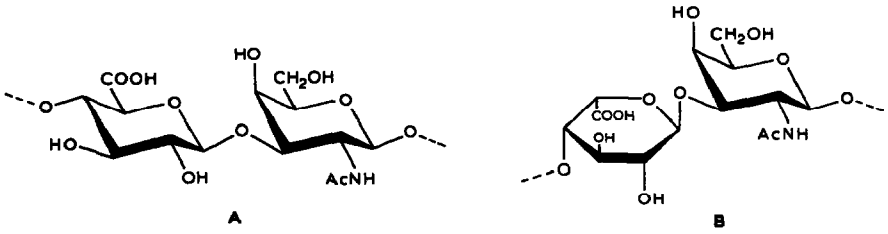


Fig. 1. Repeating units: **A**, *N*-acetylchondrosine; **B**, *N*-acetyldermosine.

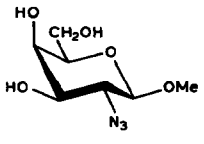
INTRODUCTION

Dermatan sulfate is a sulfated glycosaminoglycuronan which was first isolated[1] from pig skin. Studies of dermatan sulfates from different origins[2] showed them to be hybrid polymers built from two types of disaccharide units, *N*-acetylchondrosine (Fig. 1, type A) and *N*-acetyldermosine (Fig. 1, type B).

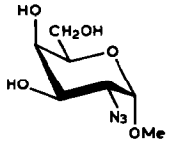
The type A unit is sulfated at either position 4 or 6 of the 2-acetamido-2-deoxy-D-galactose residue, whereas the type B unit is mainly sulfated at position 4 of this residue. Occasionally, iduronic acid is sulfated on position 2, the adjacent 2-acetamido-2-deoxy-D-galactose residue being either 4-sulfated or non-sulfated. To a large extent, this microheterogeneity complicates chemical or enzymic studies. High-resolution n.m.r. spectroscopy has proved useful in the determination of the structure of heparin[4] and the availability of synthetic fragments with defined structure[5] has played a critical role. As part of a similar program on the chemical synthesis of dermatan sulfate fragments, we now report on the synthesis of the title disaccharide and its desulfated analogue. Methyl glycosides with the appropriate configuration corresponding to that in the natural product have been used rather than reducing disaccharides, since they facilitate the synthetic work and the subsequent n.m.r. study without interfering with the potential biological properties.

RESULTS AND DISCUSSION

Azidonitration of 3,4,6-tri-*O*-acetyl-D-galactal gave[6] a crystalline product which was mainly 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl nitrate, treatment of which with sodium methoxide gave, after column chromatography, 60% of crystalline methyl 2-azido-2-deoxy-β-D-galactopyranoside (**1**). Compound **1** was obtained by Paulsen and Paal[7], but the physical properties reported {syrup, $[\alpha]_D^{24}$ −72° (methanol)} are at variance with our data {m.p. 114–115°, $[\alpha]_D$ +11° (methanol)}. The β configuration was apparent from the ^{1}H-n.m.r. signal for H-1 (δ 4.38, $J_{1,2}$ 8.3 Hz). Crystalline methyl 2-azido-2-deoxy-α-D-galactopyranoside (**2**) was also isolated (9%) from this reaction and the α configuration was indicated both by the $J_{1,2}$ value (3.8 Hz) for the H-1 doublet and by the $[\alpha]_D$ value of +189° (methanol).



1



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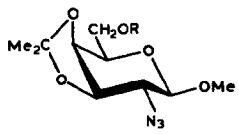
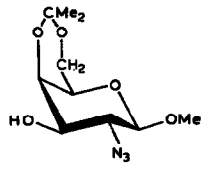
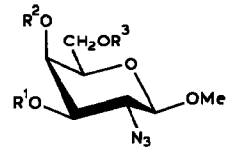


3 R = H
4 R = Ac

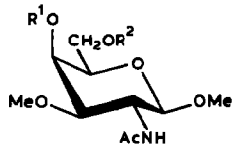


5

Treatment of **1** at room temperature in acetone, in the presence of toluene-*p*-sulfonic acid monohydrate, gave the crystalline thermodynamic 3,4-*O*-isopropylidene derivative **3** as the major product (61%). The amorphous 4,6-*O*-isopropylidene derivative **5** was also isolated (27%). Treatment of **3** with acetic anhydride in pyridine gave an excellent yield of the crystalline acetate **4** and the location of the acetyl group at position 6 was indicated by the chemical shift (δ 4.32) of the n.m.r. signal for H-6,6′. The kinetic isopropylidenation of **1** in *N,N*-dimethylformamide with 2-methoxypropene in the presence of toluene-*p*-sulfonic acid monohydrate gave a good yield of amorphous methyl 2-azido-4,6-*O*-isopropylidene-β-D-galacto-pyranoside (**5**), which has also been prepared[7] using 2,2-dimethoxypropane. The $[\alpha]_D^{24}$ value of $-8°$ (*c* 1, methanol) reported is at variance with our value, $[\alpha]_D$ $+8°$ (*c* 1, methanol). Compound **5**, which was identical with the side-product previously prepared under thermodynamic conditions, was converted into crystalline **6** (87%) in a straightforward manner by methylation and then treatment with aqueous 90% trifluoroacetic acid. Catalytic hydrogenolysis (Pd/C) of **6** gave the amine which was then *N*-acetylated to give 90% of crystalline **9**. Selective *O*-sulfation of HO-6 of **9** was achieved with the sulfur trioxide–trimethylamine complex in *N,N*-dimethylformamide (6 h, 50°), and the trimethylammonium salt was purified by chromatography on Sephadex LH-20, silica gel, and Sephadex SP-C25 (Na^+), to afford 86% of the crystalline sulfate **10** as the sodium salt. Total *O*-sulfation was achieved by using an excess of sulfating agent (18 h, 50°) to give, after purification on Sephadex LH-20 and SP-C25 (Na^+), 85% of the crystalline disulfate **11** as the disodium salt.



6 R^1 = Me, R^2 = R^3 = H
7 R^1 = R^2 = H, R^3 = Bn
8 R^1 = H, R^2 = Ac, R^3 = Bn



9 R^1 = R^2 = H
10 R^1 = H, R^2 = SO_3Na
11 R^1 = R^2 = SO_3Na
12 R^1 = H, R^2 = Bn
13 R^1 = SO_3Na, R^2 = Bn
14 R^1 = SO_3Na, R^2 = H

In order to synthesize the 4-*O*-sulfo derivative, **3** was benzylated and the isopropylidene group was then removed by treatment with aqueous 90% trifluoroacetic acid to give 87% of crystalline **7**. Selective methylation of HO-3 of **7** was readily achieved through the tin procedure[8]. The azido group was then converted into an acetamido group using sodium borohydride–nickel dichloride hexahydrate[9], followed by treatment with acetic anhydride, to give **12** which was *O*-sulfated with sulfur trioxide–trimethylamine to give 86% of **13**, isolated as the crystalline sodium salt after purification on Sephadex LH-20 and SP-C25 (Na^+). Catalytic hydrogenolysis (Pd/C) of **13** cleaved the benzyl ether to give the crystalline sulfate **14** isolated as the sodium salt.

The structures of the three sulfated monosaccharides **10**, **11**, and **14** were confirmed by ^{13}C- and ^1H-n.m.r. studies. Sulfation of a hydroxyl group causes[10–13] a down-field shift (6–10 p.p.m.) of the signal of the carbon atom to which it is attached, whereas the signals of adjacent carbon atoms are shifted upfield by 0.6–2.5 p.p.m. The results in Tables I and II are in agreement with this generalization. Thus, the sulfate group caused a down-field displacement of 7.93 p.p.m. of the signal of C-4 of the 4-monosulfate **14** and of 7.80 p.p.m. for the 4,6-disulfate **11**. Similarly, sulfation of HO-6 caused a down-field shift of 6.11 p.p.m. of the signal of C-6 in the 6-sulfate **10** and of 6.94 p.p.m. in the 4,6-disulfate **11**. ^1H-N.m.r. spectroscopy has also been used[14] to locate the sulfate groups in carbohydrate sulfates, and the relevant data for **9–11** and **14** (300 MHz) are summarized in Table III. A solution of the non-sulfated parent compound **9** in D_2O exhibited signals at δ 4.21 for H-4 and at 3.78–3.84 for H-6a,6b. Sulfation of HO-4 (axial) caused a down-field displacement of 0.72 p.p.m. of the signal of H-4 in 4-sulfate **14** and of 0.75 p.p.m. in the 4,6-disulfate **11**. Similarly, sulfation of HO-6 caused a down-field shift of the signal of H-6a,6b of ~0.5 p.p.m. These data are in agreement with previous observations[14] and constitute a firm basis for the further structural assignment of the target synthetic disaccharide (a preliminary communication of this part of the work has been presented[15]).

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS[a] (22.6 MHz) FOR SODIUM SUGAR SULFATES AND THE PARENT NON-SULFATED SUGAR

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*$NHCOCH_3$*
Non-sulfated **9**	102.27	51.23	80.28	63.84	75.21	61.30	56.62 57.21	22.44
4-Sulfate **14**	102.64	51.55	79.18	71.77	74.56	61.37	57.34 57.60	22.44
6-Sulfate **10**	102.84	51.23	80.15	63.64	72.87	67.41	56.75 57.40	22.44
4,6-Disulfate **11**	102.64	51.49	79.11	71.64	72.55	68.26	57.47 57.66	22.44

[a]In p.p.m. for solutions in D_2O at 25°, relative to the chemical shift of acetone (30.50 p.p.m.)

TABLE II

^{13}C-N.M.R. SHIFT DIFFERENCES[a] IN WATER BETWEEN SODIUM SUGAR SULFATES AND THE PARENT SUGAR

	Shift differences (p.p.m.)					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
4-Sulfate **14**	−0.37	−0.32	+1.10	−7.93	+0.65	−0.07
6-Sulfate **10**	−0.57	0.00	+0.15	+0.20	+2.34	−6.11
4,6-Disulfate **11**	−0.37	−0.26	+1.17	−7.80	+2.66	−6.94

[a]Positive values are upfield with respect to tetramethylsilane.

TABLE III

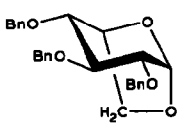
^{1}H-N.M.R. PARAMATERS[a] (300 MHz) FOR SODIUM SUGAR SULFATES AND THE PARENT NON-SULFATED SUGAR

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a,6b*
Non-sulfated **9**	4.37 (8.8)	3.88 (10.0)	3.41 (3.5)	4.21 (0.8)	3.66	3.78–3.84
4-Sulfate **14**	4.44 (8.8)	3.88 (11.0)	3.55 (3.2)	4.93 (0.8)	3.83	3.80
6-Sulfate **10**	4.41 (8.9)	3.92 (11.1)	3.45 (3.3)	4.27 (0.8)	3.91	4.24
4,6-Disulfate **11**	4.47 (8.8)	3.90 (11.2)	3.57 (3.2)	4.96 (0.8)	4.04	4.24–4.35

[a]Solvent, D_2O; temperature, 25°; chemical shifts in p.p.m. from internal TSP; coupling constants in Hz (in brackets).

As in the synthesis of heparin fragments[16–18], the general strategy of the synthesis of the title disaccharides is based on benzyl ethers as permanent blocking-groups.

Benzylation of the readily available[19] 1,6-anhydro-β-L-idopyranose gave the known[20] crystalline 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-L-idopyranose (**15**). Acetolysis of **15** with acetic anhydride–trifluoroacetic acid gave 91% of the known[20] syrupy **16**. In large-scale experiments, crude **16** was *O*-deacetylated with methanolic sodium methoxide to provide 79% of crystalline 2,3,4-tri-*O*-benzyl-L-idopyranose (**17**), the ^{1}H-n.m.r. spectrum ($CDCl_3$) of which indicated it to be a 1:1 α,β-mixture. The coupling constants of the β anomer were small ($J_{1,2}$ 2.4, $J_{2,3} = J_{3,4}$ = 3.7 Hz) and there was a long-range coupling, $J_{2,4}$ 1.2 Hz. These data are in agreement with an almost exclusive $^{1}C_4$ conformation for this derivative.



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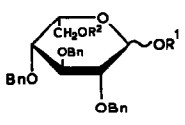
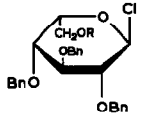
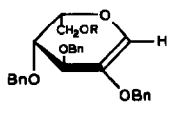


16 $R^1 = R^2 = Ac$
17 $R^1 = R^2 = H$
18 $R^1 = Bz, R^2 = H$
19 $R^1 = R^2 = COCH_2Cl$



20 R = $COCH_2Cl$

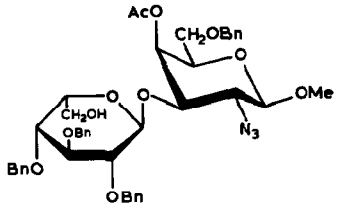


21 R = $COCH_2Cl$

The data obtained for the α anomer ($J_{1,2}$ 3.2, $J_{2,3}$ 5.7, $J_{3,4}$ 5.0, $J_{4,5}$ 3.8 Hz) indicate a significant departure from the $^1C_4$ conformation in solution. Similar constants have been observed for aqueous solutions of methyl α- and β-D-idopyranosiduronic acids[21]. For the α isomer, the $^3J_{H,H}$ values were interpreted utilizing Altona's equation[22] and the molecular geometries obtained from force-field calculations[23]. An equilibrium between $^1C_4$ (58%) and $^4C_1$ (42%) was found[24] at 40°. By analogy, it is proposed tentatively that the J values for 2,3,4-tri-*O*-benzyl-α-L-idopyranose in $CDCl_3$ are best fitted by an ~3:2 equilibrium of the $^1C_4$ and $^4C_1$ forms.

An attempt was made to convert **17** into an L-idopyranosyluronic acid derivative with potential for glycosylation reactions. Conventional tritylation and benzoylation of **17** followed by detritylation in chloroform with perchloric acid[25] gave 51% of crystalline 1-*O*-benzoyl-2,3,4-tri-*O*-benzyl-α-L-idopyranose (**18**). The rather large coupling constants ($J_{1,2}$ 4.0, $J_{2,3} = J_{3,4} = 5.3$, $J_{4,5}$ 4.0 Hz) for this pure α-derivative again suggest an equilibrium of the two chair forms in $CDCl_3$. Attempts to oxidize the alcohol function with chromium trioxide–sulfuric acid in acetone gave a complex mixture of products which was not investigated further.

Compound **17** was monochloroacetylated with chloroacetyl chloride at −20° for 5 min in dichloromethane–pyridine, to give 84% of amorphous **19** as an ~5:2 α,β-mixture (^{1}H-n.m.r. data). Treatment of **19** with a saturated solution of dry hydrogen chloride in dichloromethane for 3 h at 0° gave 90% of the unstable chloride **20**, which was condensed immediately with methyl 4-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**8**, easily obtained by regioselective acidic opening[26] of a methyl orthoester prepared from **7** by treatment with trimethyl orthoacetate and toluene-*p*-sulfonic acid monohydrate in dry toluene) in 1,2-dichloroethane, in the presence of freshly prepared silver triflate[27], 2,4,6-trimethylpyridine, and activated powdered molecular sieve (4 Å). Column chromatography of the mixture of products gave, first, the glycal **21** (16% from **20**). The next fraction was *O*-dechloroacetylated with hydrazine dithiocarbonate[28] to give the α-linked disaccharide **22** (58% from **8**). The last fraction was also *O*-dechloroacetylated to give the β-linked disaccharide **23** (30% from **8**). The α configuration of the newly synthesized linkage of **22** was deduced from the $[\alpha]_D$ value of −21° (chloroform), (*cf.* +42° for **23**). Oxidation of HO-6 of **22** with chromium trioxide in acetone–sulfuric acid at 0° gave, after *O*-deacetylation, the crystalline acid **24** (63% from **22**). Esterification of **24** with diazomethane gave 90% of crystalline **25**. The free acid **24** was converted into the sodium salt **26**, which was then *O*-sulfated. The resulting trimethylammonium salt was purified on Sephadex LH-20 and SP-C25 (Na^+) to afford 87% of the amorphous disaccharide **27** as the disodium salt. Comparison of the 300-MHz ^{1}H-n.m.r. spectra (CD_3OD) of **27** and **26** showed the expected down-field shift[14] (0.69 p.p.m.) of the signal for H-4 but, on the addition of one drop of a solution of trifluoroacetic acid in $CDCl_3$, the signal for H-5′ was immediately shifted down-field by 0.30 p.p.m. This shift reflects the formation of the carboxylic acid[29].



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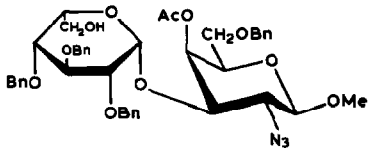

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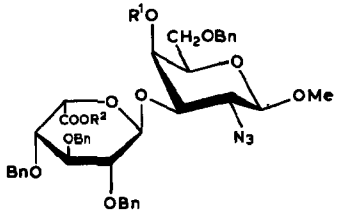
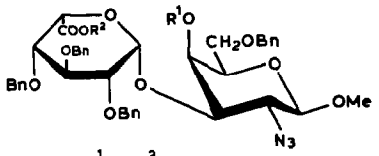


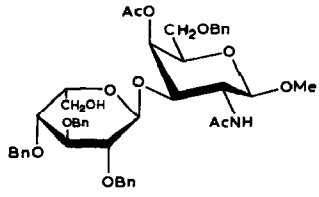
24 $R^1 = R^2 = H$
25 $R^1 = H, R^2 = Me$
26 $R^1 = H, R^2 = Na$
27 $R^1 = SO_3Na, R^2 = Na$



28 $R^1 = R^2 = H$
29 $R^1 = H, R^2 = Na$
30 $R^1 = SO_3Na, R^2 = Na$

The last steps of the synthesis, namely, catalytic hydrogenolysis (Pd/C) of **27** followed by selective *N*-acetylation, were not clean reactions and the title disaccharide was difficult to purify. Equally unsatisfactory were attempts to convert **24** or **26** into the *N*-acetylated disaccharide derivative **32**. Although the sodium salt **26** was converted into **32** in the presence of Pd/C and ammonium formate[30] in methanol–*N*,*N*-dimethylformamide, followed by treatment with acetic anhydride in methanol, the crystalline product **32** was contaminated (~60%) by a compound which appeared to be a tetrasaccharide derivative, the structure of which was not investigated further. Although fractionation of these two products was achieved after esterification with diazomethane to give pure **33**, it was difficult because of the low solubilities of the products. Reduction of **25** with sodium borohydride in the presence of nickel chloride hexahydrate and boric acid[31] followed by selective *N*-acetylation gave crude **33**, but complete purification was difficult for solubility reasons. It was therefore concluded that the azido group should be converted into the acetamido group at an earlier stage. Nevertheless, the syntheses of the azido derivatives **24–27** provided data relevant to the discussion on the conformation of α-L-idopyranosiduronate derivatives. The glycosylation reaction was conducted with the azido derivative **8** rather than the corresponding acetamido compound since β-glycosides of 2-acetamido-2-deoxy-D-galactose are somewhat insoluble in chlorinated solvents at low temperature.

The azido derivative **22** was reduced with sodium borohydride in ethanol in the presence of nickel dichloride hexahydrate and boric acid, and the product was *N*-acetylated immediately to give 81% of the crystalline disaccharide **31**. Oxidation



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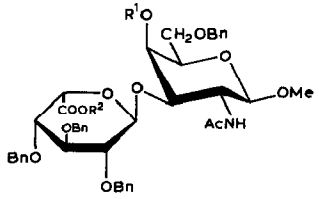
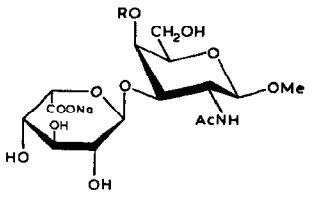


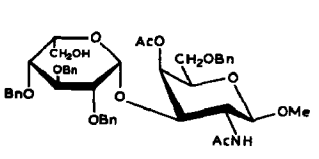
32 $R^1 = R^2 = H$
33 $R^1 = H, R^2 = Me$
34 $R^1 = SO_3Na, R^2 = Na$



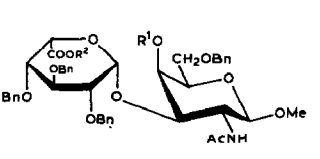
35 $R = SO_3Na$
36 $R = H$

of HO-6 of **31** was easily achieved as described above, to give 67% of the crystalline acid **32** which, on esterification with diazomethane, gave **33**. The sodium salt of **32** was *O*-sulfated with the sulfur trioxide–trimethylamine complex and the resulting trimethylammonium salt was purified by chromatography on Sephadex LH-20, silica gel, and Sephadex SP-C25 (Na^+) to afford 81% of the amorphous disaccharide **34** as the disodium salt. Comparison of the 300-MHz ^{1}H-n.m.r. spectra of **34** and **32** showed the expected down-field shift[14] (0.63 p.p.m.) of the signal of H-4 of **34**. Catalytic hydrogenolysis (Pd/C) of **34** gave the amorphous title disaccharide **35** as the disodium salt (86% after purification on Sephadex G-10). The non-sulfated analogue **36** was obtained (86%) on catalytic hydrogenolysis (Pd/C) of the sodium salt of **32**. Comparison of the 300-MHz ^{1}H-n.m.r. spectra of **35** and **36** showed a down-field displacement of the signal for H-4 in **35** of 0.66 p.p.m., a value close to that (0.72 p.p.m.) observed for the model compounds **9** and **14**.

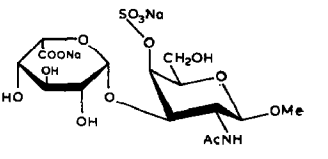
In a similar manner, the β-linked azido disaccharide **23** was converted into the amorphous disaccharide derivative **37** (81%) and then, after oxidation of HO-6, into the amorphous acid **38** (63%). The sodium salt of **38** was *O*-sulfated with the sulfur trioxide–trimethylamine complex and the resulting trimethylammonium salt was purified by chromatography on Sephadex LH-20, silica gel, and Sephadex SP-C25 (Na^+) to afford 83% of the amorphous disaccharide derivative **39** as the disodium salt. The significant down-field displacement (0.80 p.p.m.) of the signal for H-4 in the 300-MHz ^{1}H-n.m.r. spectrum of **39**, in comparison with that in **38**, reflects the 4-sulfation. Catalytic hydrogenolysis (Pd/C) of **39** gave the disaccharide **40** as the disodium salt (87% after purification on Sephadex G-10). The chemical shift (δ 4.92) of the signal for H-4 is close to that (δ 4.87) observed for the model compound **14** and accords with 4-*O*-sulfation. The derivatives **28–30** were also synthesized for comparison with **24, 26**, and **27**.



37



38 $R^1 = R^2 = H$
39 $R^1 = SO_3Na, R^2 = Na$



40

TABLE IV

OPTICAL ROTATIONS AND $^3J_{H,H}$ COUPLING CONSTANTS FOR THE L-IDURONIC MOIETY OF α-LINKED DISACCHARIDES

Compound	$[\alpha]_D^{20}$ [a] *(degrees)*	$J_{1,2}$ [b]	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
22	−21 (*A*)	4.0 (*A*)	7.0	5.1	3.6
24	−20 (*C*)	4.2 (*C*)	5.1	5.0	4.0
25	−27 (*A*)	5.5 (*A*)	5.8	5.4	4.8
26	n.d.	3.6 (*B*)	6.2	4.3	3.4
27	−26 (*B*)	4.0 (*B*)	4.2	4.0	3.6
31	−5 (*A*)	5.0 (*A*)	7.5	5.5	4.3
32	−20 (*C*)	4.6 (*C*)	5.6	4.5	4.2
33	n.d.	5.0 (*C*)	5.2	4.8	4.4
34	−21 (*B*)	4.2 (*B*)	6.8	4.4	3.8
35	−38 (*D*)	5.0 (*D*)	7.8	6.5	4.3
36	−38 (*D*)	4.6 (*D*)	7.5	5.8	3.9

[a]Optical rotations in *A*, chloroform; *B*, methanol; *C*, *N*,*N*-dimethylformamide; *D*, water. [b]^{1}H-N.m.r. (300 MHz) spectra in *A*, chloroform-*d*; *B*, methanol-d_4; *C*, chloroform-*d*–methanol-d_4 (1:1, v/v); *D*, D_2O (internal TSP).

TABLE V

OPTICAL ROTATIONS AND $^3J_{H,H}$ COUPLING CONSTANTS FOR THE L-IDURONIC MOIETY OF β-LINKED DISACCHARIDES

Compound	$[\alpha]_D^{20}$ [a] *(degrees)*	$J_{1,2}$ [b]	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{2,4}$
23	+42 (*A*)	2.2 (*A*)	5.3	5.3	3.3	—
28	+32 (*A*)	1.8 (*B*)	n.d.	3.0	2.4	n.d.
29	n.d.	1.8 (*B*)	3.0	2.8	2.4	0.8
30	+39 (*B*)	1.8 (*B*)	2.8	2.4	2.2	0.8
37	+51 (*A*)	1.8 (*A*)	2.8	2.2	2.0	1.0
38	+28 (*A*)	1.6 (*C*)	3.2	2.8	2.2	1.0
39	+28 (*B*)	1.5 (*B*)	3.2	2.6	2.0	0.8
40	+27 (*D*)	1.5 (*D*)	3.2	3.0	1.6	1.0

[a,b]See footnotes to Table IV.

A comparison of the $[\alpha]_D$ values (Tables IV and V) of the α- and β-linked disaccharides **23**–**40** confirms the assigned anomeric configurations. The data in Tables IV and V also show that ^{1}H-n.m.r. parameters should be used with caution to deduce the anomeric configuration of L-idopyranosiduronates. With the exception of **23**, the coupling constants of the seven β-linked disaccharides **28**–**30** and **37**–**40** are all small (Table V) and there was a long-range coupling ($J_{2,4}$ ~1.0 Hz). These data indicate that the L-iduronate residue in these compounds adopts the $^1C_4$ conformation almost exclusively. In such a conformation, a $J_{1,2}$ value of 1.50–1.80 Hz is indicative of a β anomer. The *J* values for the various α-linked disaccharides are rather large and indicate a significant departure from the $^1C_4$ con-

formation in solution and a substantial contribution[23] of possibly $^4C_1$ or ^2S_o forms. The main difference between the two sets of J values is in the value of $J_{3,4}$ which, in the former compounds, remains small, even when $J_{2,3}$ is quite large[23]. As both $J_{2,3}$ and $J_{3,4}$ are rather large in the eleven derivatives (Table IV), the J values are best-fitted by an equilibrium of the $^1C_4$ and $^4C_1$ forms. The observed J values ($J_{1,2}$ 5.0, $J_{2,3}$ 7.8, $J_{3,4}$ 6.5, $J_{4,5}$ 4.3 Hz) for methyl 2-acetamido-2-deoxy-3-*O*-(α-L-idopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside disodium salt are similar to those reported for an α-L-idopyranosiduronate residue located at the non-reducing terminus of two tetrasaccharides isolated from heparan sulfate, where the population[32] of the $^4C_1$ form was ~65%. Thus, a larger value for $J_{1,2}$ of an α-L-idopyranosiduronate compared to the β form must be expected frequently because of the propensity of the α-linked derivatives to adopt the $^4C_1$ form. Where an α anomer adopts almost exclusively a $^1C_4$ form, $J_{1,2}$ is small and the signal for H-1 generally appears as a broad singlet as a consequence of a long-range $J_{1,3}$ coupling of ~1 Hz.

The ^{13}C-n.m.r. data for **35**, **36**, and **40** are summarized in Tables VI and VII and accord with those reported for model compounds. 4-Sulfation caused a downfield displacement of 7.60 p.p.m. in the signal for C-4 in the disaccharide **35** and of 6.17 p.p.m. in the disaccharide **40**.

To the best of our knowledge, this work represents the first synthesis of disaccharide fragments of dermatan sulfate.

TABLE VI

^{13}C-N.M.R. CHEMICAL SHIFTS[a] (22.6 MHz) OF THE 2-AMINO-2-DEOXY-D-GALACTOSE MOIETY FOR SODIUM DISACCHARIDE SULFATES AND THE PARENT NON-SULFATED DISACCHARIDE

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Non-sulfated **36**	102.44	51.42	79.37	68.39	75.34	61.37
Disaccharide **35**	102.18	52.07	76.64	75.99	74.82	61.30
Disaccharide **40**	103.09	50.90	74.56	74.56	73.85	61.37
Dermatan sulfate[33]	102.60	53.40	81.50	76.70	75.80	62.50

[a]In p.p.m. for solutions in D_2O at 25°.

TABLE VII

^{13}C-N.M.R. SHIFT DIFFERENCES[a] FOR THE 2-AMINO-2-DEOXY-D-GALACTOSE MOIETY BETWEEN SODIUM DISACCHARIDE SULFATES AND THE PARENT NON-SULFATED DISACCHARIDE

	Shift differences (p.p.m.)					
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Disaccharide **35**	+0.26	−0.65	+2.73	−7.60	+0.52	+0.07
Disaccharide **40**	−0.65	+0.52	+4.81	−6.17	+1.49	0.00

[a]Positive values are upfield with respect to tetramethylsilane.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–24° with a Perkin–Elmer Model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with Perkin–Elmer R-32 (90 MHz) and AM-300 (300 MHz) instruments. ^{13}C-N.m.r. spectra were recorded with Bruker WH-90 (22.63 MHz) and AM-300 (75 MHz) instruments. The purity of products was determined by t.l.c. on Silica Gel 60 F_{154} (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 63–200 μm) which was used without pretreatment. Elemental analyses were performed by the Service Central de Micro-Analyse du Centre National de la Recherche Scientifique (Vernaison, France).

Methyl 2-azido-2-deoxy-β- (**1**) *and -α-*D*-galactopyranoside* (**2**). — A suspension of crude 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl nitrate (3.76 g) in anhydrous methanol (15 mL) was treated with methanolic M sodium methoxide (15 mL) for 1 h at room temperature. The clear solution was then neutralized with 80% acetic acid and concentrated, a solution of the residue in acetone (25 mL) was filtered through a bed (2 × 5 cm) of silica gel and concentrated, and the residue was crystallized from ethyl acetate–hexane to give **1** (1.32 g, 60%), m.p. 114–115°, $[\alpha]_D$ +11° (*c* 1, methanol); lit.[7] amorphous, $[\alpha]_D$ −72° (*c* 1, methanol). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 4.38 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1), 3.92 (dd, 1 H, $J_{3,4}$ 3.50, $J_{4,5}$ 0.60 Hz, H-4), 3.61 (s, 3 H, OMe), 3.48 (dd, 1 H, $J_{1,2}$ 8.30, $J_{2,3}$ 10.20 Hz, H-2).

Anal. Calc. for $C_7H_{13}N_3O_5$: C, 38.36; H, 5.98; N, 19.17. Found: C, 38.25; H, 6.03; N, 19.12.

Column chromatography of the product in the mother liquors of **1** on silica gel (25 g), using ethyl acetate–methanol (11:1), gave **2** (124 mg, 9%), m.p. 132–133° (from ethyl acetate–hexane), $[\alpha]_D$ +189° (*c* 1, methanol). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 4.97 (d, 1 H, $J_{1,2}$ 3.80 Hz, H-1), 3.99 (dd, 1 H, $J_{3,4}$ 3.50, $J_{4,5}$ 0.60 Hz, H-4), 3.74 (dd, $J_{1,2}$ 3.80, $J_{2,3}$ 10.20 Hz, H-2), 3.42 (s, 3 H, OMe).

Anal. Calc. for $C_7H_{13}N_3O_5$: C, 38.36; H, 5.98; N, 19.17. Found: C, 38.55; H, 5.97; N, 19.23.

*Methyl 2-azido-2-deoxy-3,4-*O*-isopropylidene-β-*D*-galactopyranoside* (**3**). — A mixture of **1** (635 mg), freshly distilled acetone (20 mL), and toluene-*p*-sulfonic acid monohydrate (40 mg) was stirred for 5 h at room temperature. Triethylamine (0.5 mL) was then added, the mixture was concentrated, and the residue was eluted from a column of silica gel (40 g) with dichloromethane–ethyl acetate (4:1, containing 0.1% of triethylamine) to give, first, amorphous **5** (202 mg, 27%). Further elution gave **3** (458 mg, 61%), m.p. 84–85° (from ether–hexane), $[\alpha]_D$ +43° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 4.10 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.53 (s, 3 H, OMe), 3.32 (t, 1 H, $J_{1,2} = J_{2,3}$ = 8 Hz, H-2), 2.30 (s, 1 H, OH), 1.49 and 1.31 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{10}H_{17}N_3O_5$: C, 46.33; H, 6.61; N, 16.20. Found: C, 46.33; H, 6.52; N, 16.53.

Methyl 6-O-acetyl-2-azido-2-deoxy-3,4-O-isopropylidene-β-D-galactopyranoside (**4**). — Compound **3** (50 mg) was acetylated (pyridine–acetic anhydride) to give **4** (58 mg, 97%), m.p. 86–87° (from hexane–ether), $[\alpha]_D$ +50° (*c* 1, chloroform), ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 4.32 (m, 2 H, H-6a,6b), 4.07 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.52 (s, 3 H, OMe), 3.32 (t, 1 H, $J_{1,2} = J_{2,3} = 8$ Hz, H-2), 2.05 (s, 3 H, Ac), 1.50 and 1.31 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{12}H_{19}N_3O_6$: C, 47.83; H, 6.35; N, 13.95. Found: C, 47.70; H, 6.40; N, 13.97.

Methyl 2-azido-2-deoxy-4,6-O-isopropylidene-β-D-galactopyranoside (**5**). — A mixture of **1** (265 mg), *N,N*-dimethylformamide (8 mL), 2-methoxypropene (0.5 mL), and toluene-*p*-sulfonic acid monohydrate (3 mg) was stirred for 5 h at room temperature. Triethylamine (0.5 mL) was then added, the mixture was concentrated, and the residue was eluted from a column of silica gel (20 g) with dichloromethane–ethyl acetate (5:1, containing 0.5% of triethylamine) to give amorphous **5** (266 mg, 85%), $[\alpha]_D$ +8° (*c* 1, methanol); lit.[7] syrup, $[\alpha]_D$ −8° (*c* 1, methanol). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 3.53 (s, 3 H, OMe), 2.75 (bs, 1 H, OH), 1.45 (s, 6 H, CMe_2).

Anal. Calc. for $C_{10}H_{17}N_3O_5$: C, 46.33; H, 6.61; N, 16.20. Found: C, 46.14; H, 6.67; N, 16.06.

Methyl 2-azido-2-deoxy-3-O-methyl-β-D-galactopyranoside (**6**). — A mixture of **5** (520 mg), *N,N*-dimethylformamide (10 mL), barium oxide (1.35 g), barium hydroxide octahydrate (0.35 g), and methyl iodide (0.65 mL) was stirred for 2 h at room temperature. Methanol (2 mL) was then added, and the mixture was stirred for 30 min and concentrated. A solution of the residue in dichloromethane (50 mL) was washed with cold aqueous 60% acetic acid, aqueous 5% sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. A solution of the residue in aqueous 90% trifluoroacetic acid (20 mL) was stirred for 15 min at room temperature and then concentrated. Water (3 × 10 mL) was evaporated from the residue which then crystallized from ethyl acetate–hexane to give **6** (406 mg, 87%), m.p. 109–110°, $[\alpha]_D$ +4° (*c* 1, methanol). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 4.14 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.57 (s, 3 H, OMe), 3.49 (s, 3 H, OMe), 3.08 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3), 2.90 (2 H, 2 OH).

Anal. Calc. for $C_8H_{15}N_3O_5$: C, 41.20; H, 6.48; N, 18.02. Found: C, 41.36; H, 6.25; N, 18.17.

Methyl 2-azido-6-O-benzyl-2-deoxy-β-D-galactopyranoside (**7**). — Sodium hydride (92 mg) was added to a stirred solution of **4** (520 mg) in *N,N*-dimethylformamide (8 mL). After 30 min, benzyl bromide (0.36 mL) was added dropwise, and the mixture was stirred for 1 h. Excess of benzyl bromide was then destroyed by the dropwise addition of methanol (1 mL) and stirring for 30 min. The mixture was diluted with dichloromethane (50 mL), washed twice with water, dried (Na_2SO_4), and concentrated. A solution of the residue in aqueous 90% trifluoroacetic acid (10 mL) was stirred for 10 min at room temperature. After co-concentration of this solution with water (3 × 10 mL) and then with toluene, the

residue was eluted from a column of silica gel (30 g) with ethyl acetate–hexane (1:1) to give **7** (540 mg, 87%), m.p. 69–70° (from ether–hexane), $[\alpha]_D$ +5° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 7.27 (m, 5 H, Ph), 4.53 (s, 2 H, OCH_2Ph), 4.11 (d, 1 H, $J_{1,2}$ 7 Hz, H-1), 3.89 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 1 Hz, H-4), 3.51 (s, 3 H, OMe), 3.18 (2 H, 2 OH).

Anal. Calc. for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.19; N, 13.58. Found: C, 54.24; H, 5.90; N, 13.54.

*Methyl 4-O-acetyl-2-azido-6-O-benzyl-2-deoxy-β-*D*-galactopyranoside* (**8**). — A mixture of **7** (450 mg), dry toluene (12 mL), trimethyl orthoacetate (1.5 mL), and toluene-*p*-sulfonic acid monohydrate (3 mg) was stirred for 1 h at room temperature. Triethylamine (0.5 mL) was then added, and the mixture was stirred for 5 min, diluted with toluene (50 mL), washed twice with water, dried (Na_2SO_4), and concentrated. A solution of the residue in aqueous 80% acetic acid (10 mL) was stirred for 10 min at room temperature and then concentrated. Water (3 × 5 mL) and then toluene were evaporated from the residue to give amorphous **8** (458 mg, 95%), $[\alpha]_D$ −45° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.25 (m, 5 H, Ph), 5.33 (dd, 1 H, $J_{3,4}$ 3.41, $J_{4,5}$ 1.0 Hz, H-4), 4.21 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.75 (m, 1 H, $J_{4,5}$ 1.0, $J_{5,6a}$ 6.0, $J_{5,6b}$ 6.5 Hz, H-5), 3.65 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.4 Hz, H-3), 3.59 (s, 3 H, OMe), 3.58 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.53 (dd, 1 H, $J_{5,6b}$ 6.5, $J_{6a,6b}$ 10.2 Hz, H-6b), 3.49 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.2 Hz, H-2), 2.50 (bs, 1 H, OH), 2.08 (s, 3 H, Ac).

Anal. Calc. for $C_{16}H_{21}N_3O_6$: C, 54.69; H, 6.02; N, 11.96. Found: C, 54.41; H, 5.79; N, 11.96.

*Methyl 2-acetamido-2-deoxy-3-O-methyl-β-*D*-galactopyranoside* (**9**). — A solution of **6** (320 mg) in methanol (10 mL) was hydrogenated in the presence of 10% Pd/C (200 mg) for 24 h, then filtered, and concentrated. Acetic anhydride (0.5 mL) was added to a solution of the residue in water (10 mL). After 1 h, the mixture was concentrated and the residue was washed through a bed (1 cm) of silica gel with methanol. Concentration of the filtrate gave a solid which crystallized from ethyl acetate–methanol to give **9** (308 mg, 90%), m.p. 247–249°, $[\alpha]_D$ +10° (*c* 1, water). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 4.37 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.21 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 0.8 Hz, H-4), 3.88 (dd, 1 H, $J_{1,2}$ 8.8, $J_{2,3}$ 10.0 Hz, H-2), 3.50 (s, 3 H, OMe), 3.41 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.5 Hz, H-3), 3.38 (s, 3 H, OMe), 2.03 (s, 3 H, Ac). For the ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{10}H_{19}NO_6$: C, 48.18; H, 7.68; N, 5.62. Found: C, 48.02; H, 7.57; N, 5.56.

*Methyl 2-acetamido-2-deoxy-3-O-methyl-6-O-sulfo-β-*D*-galactopyranoside, sodium salt* (**10**). — A mixture of **9** (125 mg), *N*,*N*-dimethylformamide (3 mL), and the sulfur trioxide–trimethylamine complex (84 mg) was stirred for 4 h at 50°. More sulfating agent (15 mg) was then added, the mixture was stirred for 2 h at 50° and then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (3 × 38 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (1:1). Elution with the same solvent gave a product that was eluted from

a column of silica gel (10 g) with dichloromethane–methanol (2:1) to give a pure fraction that was dissolved in methanol (2 mL) and eluted from a column (1.5 × 12 cm) of Sephadex SP-C25 (Na^+) with methanol–water (9:1) to afford **10** (151 mg, 86%), m.p. 184–186° (dec.; from ethanol–water), $[\alpha]_D$ +5.5° (*c* 1, water). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 4.41 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.27 (dd, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ 0.8 Hz, H-4), 4.26 (m, 2 H, H-6a,6b), 3.92 (dd, 1 H, $J_{1,2}$ 8.9, $J_{2,3}$ 11.1 Hz, H-2), 3.52 (s, 3 H, OMe), 3.45 (dd, 1 H, $J_{2,3}$ 11.1, $J_{3,4}$ 3.3 Hz, H-3), 3.41 (s, 3 H, OMe), 2.03 (s, 3 H, Ac). For the ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{10}H_{18}NNaO_9S \cdot 0.5\ H_2O$: C, 33.33; H, 5.31; N, 3.88. Found: C, 33.30; H, 5.40; N, 3.76.

Methyl 2-acetamido-2-deoxy-3-O-methyl-4,6-di-O-sulfo-β-D-galactopyranoside, disodium salt (**11**). — A mixture of **9** (70 mg), *N,N*-dimethylformamide (2.5 mL), and the sulfur trioxide–trimethylamine complex (200 mg) was stirred for 16 h at 50°. More sulfating agent (70 mg) was added, the mixture was stirred for 8 h at 50° and then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (3 × 38 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (1:1). Elution with the same solvent gave a residue that was eluted from a column (2 × 60 cm) of Sephadex SP-C25 (Na^+) with water to afford **11** (106 mg, 85%), m.p. 180–182° (dec.; from methanol–water), $[\alpha]_D$ +20° (*c* 1, water). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 4.96 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 0.8 Hz, H-4), 4.47 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.30 (m, 2 H, H-6a,6b), 3.90 (dd, 1 H, $J_{1,2}$ 8.8, $J_{2,3}$ 11.2 Hz, H-2), 3.57 (dd, 1 H, $J_{2,3}$ 11.2, $J_{3,4}$ 3.2 Hz, H-3), 3.54 and 3.40 (2 s, each 3 H, 2 OMe), 2.03 (s, 3 H, Ac). For the ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{10}H_{17}NNa_2O_{12}S_2 \cdot 0.5\ H_2O$: C, 25.97; H, 3.92; N, 3.02. Found: C, 25.85; H, 4.04; N, 2.91.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-methyl-β-D-galactopyranoside (**12**). — A mixture of **6** (712 mg), dry methanol (25 mL), and dibutyltin oxide (747 mg) was heated under reflux for 2 h, then cooled, and concentrated to dryness. To a solution of the residue in *N,N*-dimethylformamide (12 mL) was added methyl iodide (1 mL), and the mixture was stirred at 45° for 8 h. After cooling, the mixture was concentrated, diluted with dichloromethane (50 mL), washed with aqueous 5% sodium thiosulfate and then water, dried (Na_2SO_4), and concentrated. The residue was eluted from a short column of silica gel (20 g) with hexane–ethyl acetate (3:2) to give a colorless syrup which was dissolved in ethanolic 4% nickel dichloride hexahydrate (100 mL). A solution of sodium borohydride in ethanol (10 mg/mL) was then added dropwise until the green solution turned to persistent black. Acetic anhydride (15 mL) was added, the mixture was stirred for 2 h and then concentrated, the residue was extracted with pyridine (3 × 10 mL), and the combined extracts were concentrated. A solution of the residue in methanol (10 mL) was added to a column (2 × 10 cm) of silica gel and eluted with methanol to give **12** (492 mg, 63%), m.p. 156–157° (from ethyl acetate–methanol), $[\alpha]_D$ +4° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.40 (m, 5 H, Ph), 4.30 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.07 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8 Hz, H-4), 3.95 (dd, 1 H, $J_{1,2}$ 8.1,

$J_{2,3}$ 10.2 Hz, H-2), 3.45 and 3.38 (2 s, each 3 H, 2 OMe), 3.30 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.0 Hz, H-3), 1.95 (s, 3 H, Ac).

Anal. Calc. for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.42; N, 4.13. Found: C, 59.98; H, 7.58; N, 3.87.

*Methyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-methyl-4-*O*-sulfo-β-*D*-galacto-pyranoside, sodium salt* (**13**). — A mixture of **12** (288 mg), *N,N*-dimethylformamide (3 mL), and the sulfur trioxide–trimethylamine complex (278 mg) was stirred at 50° for 4 h and then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (3 × 38 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (1:1). Elution with the same solvent gave a product, a solution of which in methanol (2 mL) was added to a column (1.5 × 10 cm) of Sephadex SP-C25 (Na^+) and eluted with methanol–water (9:1) to give **13** (330 mg, 88%), m.p. 156–157° (from methanol), $[\alpha]_D$ +18° (*c* 1, water). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.32 (m, 5 H, Ph), 4.87 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.8 Hz, H-4), 4.35 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 3.45 and 3.43 (2 s, each 3 H, 2 OMe), 3.40 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.0 Hz, H-3), 1.95 (s, 3 H, Ac).

Anal. Calc. for $C_{17}H_{24}NNaO_9S \cdot 0.7\ H_2O$: C, 44.97; H, 5.64; N, 3.08. Found: C, 44.95; H, 5.78; N, 3.07.

*Methyl 2-acetamido-2-deoxy-3-*O*-methyl-4-*O*-sulfo-β-*D*-galactopyranoside, sodium salt* (**14**). — A solution of **13** (200 mg) in methanol–water (9:1, 10 mL) was hydrogenated in the presence of 10% Pd/C (150 mg) for 16 h, then filtered, and concentrated. The residue was eluted from a column (2 × 60 cm) of Sephadex SP-C25 (Na^+) with water, and the product was crystallized from methanol–water to give **14** (137 mg, 86%), m.p. 186–188° (dec.), $[\alpha]_D$ +14° (*c* 1, water). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 4.93 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ 0.7 Hz, H-4), 4.44 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 3.88 (dd, 1 H, $J_{1,2}$ 8.8, $J_{2,3}$ 11.0 Hz, H-2), 3.55 (dd, 1 H, $J_{2,3}$ 11.0, $J_{3,4}$ 3.2 Hz, H-3), 3.52 and 3.43 (2 s, each 3 H, 2 OMe), 2.03 (s, 3 H, Ac). For the ^{13}C-n.m.r. data, see Table I.

Anal. Calc. for $C_{10}H_{18}NNaO_9S$: C, 34.19; H, 5.16; N, 3.99; Na, 6.54. Found: C, 33.86; H, 5.20; N, 3.84; Na, 6.66.

*1,6-di-*O*-Acetyl-2,3,4-tri-*O*-benzyl-*L*-idopyranose* (**16**). — A mixture of **15** (405 mg), acetic anhydride (10 mL), and trifluoroacetic acid (2 mL) was stirred for 4 h at room temperature and then concentrated, and xylene was twice evaporated from the residue which was eluted from a column (15 g) of silica gel with hexane–ethyl acetate (5:2) to give **16** as a colorless syrup (455 mg, 91%), $[\alpha]_D$ +3° (*c* 1, chloroform); lit.[20] $[\alpha]_D$ −4° (*c* 1.9, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 7.25 (m, 15 H, 3 Ph), 6.12 (d, *J* 3.5 Hz, H-1α), 6.10 (d, *J* 2.0 Hz, H-1β), 2.08 and 1.98 (2 s, each 3 H, 2 Ac).

*2,3,4-Tri-*O*-benzyl-*L*-idopyranose* (**17**). — Compound **15** (1 g) was acetolyzed as described above. The crude residue was *O*-deacetylated (methanolic sodium methoxide) overnight at 4° to give **17** (822 mg, 79% from **15**), m.p. 81–82° (from ether–hexane), $[\alpha]_D$ +11° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.30 (m, 15 H, 3 Ph), 5.18 (d, $J_{1,2}$ 3.20 Hz, H-1α), 4.96 (d, $J_{1,2}$ 2.40 Hz, H-1β), 3.77

(dd, $J_{2,3}$ 5.70, $J_{3,4}$ 5.0 Hz, H-3α), 3.75 (t, $J_{2,3} = J_{3,4} = 3.70$ Hz, H-3β), 3.55 (dd, $J_{3,4}$ 5.0, $J_{4,5}$ 3.80 Hz, H-4α), 3.45 (dd, $J_{1,2}$ 3.20, $J_{2,3}$ 5.70 Hz, H-2α), 3.43 (m, $J_{1,2}$ 2.40, $J_{2,3}$ 3.70, $J_{2,4}$ 1.20 Hz, H-2β), 3.33 (m, $J_{3,4}$ 3.70, $J_{4,5}$ 2.50, $J_{2,4}$ 1.20 Hz, H-4β).

Anal. Calc. for $C_{27}H_{30}O_6$: C, 71.98; H, 6.71. Found: C, 72.04; H, 6.83.

1-O-Benzoyl-2,3,4-tri-O-benzyl-α-L-idopyranose (**18**). — A mixture of **17** (228 mg), pyridine (10 mL), and freshly purified chlorotriphenylmethane (155 mg) was stirred at 80° for 8 h and then cooled to 0°. Benzoyl chloride (0.2 mL) was added, and the mixture was stirred for 2 h at room temperature, poured into ice–water (100 mL), stirred for 2 h, and extracted with dichloromethane (3 × 20 mL). The combined extracts were washed with aqueous 10% potassium hydrogensulfate, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. A solution of the residue in chloroform (20 mL) was stirred for 5 min at room temperature with aqueous 60% perchloric acid (0.2 mL). Saturated aqueous sodium hydrogencarbonate (10 mL) was added, and the mixture was vigorously stirred for 10 min, washed twice with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column (20 g) of silica gel with hexane–ethyl acetate (2:1) and crystallized from the same mixture of solvents to give **18** (142 mg, 51% from **17**), m.p. 105–106°, $[\alpha]_D$ −25° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 8.0–7.10 (m, 20 H, 4 Ph), 6.30 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.28 (m, 1 H, $J_{4,5} = J_{5,6a} = 4.0$, $J_{5,6b}$ 8.40 Hz, H-5), 4.01 (dd, 1 H, $J_{5,6b}$ 8.40, $J_{6a,6b}$ 12.40 Hz, H-6b), 3.86 (t, 1 H, $J_{2,3} = J_{3,4} = 5.30$ Hz, H-3), 3.73 (dd, 1 H, $J_{1,2}$ 4.0, $J_{2,3}$ 5.30 Hz, H-2), 3.70 (dd, 1 H, $J_{3,4}$ 5.30, $J_{4,5}$ 4.0 Hz, H-4), 3.69 (dd, 1 H, $J_{5,6a}$ 4.0, $J_{6a,6b}$ 12.40 Hz, H-6a), 2.23 (dd, 1 H, *J* 3.10 and 9.80 Hz, OH).

Anal. Calc. for $C_{34}H_{34}O_7$: C, 73.63; H, 6.18. Found: C, 73.71; H, 6.28.

2,3,4-Tri-O-benzyl-1,6-di-O-chloroacetyl-L-idopyranose (**19**). — A solution of chloroacetyl chloride (0.60 mL) in anhydrous dichloromethane (2 mL) was added dropwise, at −20° during 5 min, to a solution of **17** (740 mg) in pyridine (1 mL) and dichloromethane (8 mL). After 45 min, crushed ice (5 g) was added, and the mixture was stirred at 0° for 1 h, diluted with dichloromethane (50 mL), washed with aqueous 10% potassium hydrogen sulfate, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column (30 g) of silica gel with hexane–ethyl acetate (7:2) to give **19** as a colorless syrup (854 mg, 84%), $[\alpha]_D$ +5° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.30 (m 15 H, 3 Ph), 6.18 (d, $J_{1,2}$ 3.30 Hz, H-1), 6.16 (d, $J_{1,2}$ 2.80 Hz, H-1), 3.95 (2 ABq, 4 H, 2 $ClCH_2CO$).

Anal. Calc. for $C_{31}H_{32}Cl_2O_8$: C, 61.70; H, 5.34, Found: C, 61.54; H, 5.43.

2,3,4-Tri-O-benzyl-6-O-chloroacetyl-α-L-idopyranosyl chloride (**20**). — A saturated solution of dry hydrogen chloride in dichloromethane (10 mL) was added to **19** (250 mg). The mixture was stirred at 0° for 3 h with the exclusion of moisture, then diluted with dichloromethane (30 mL), washed with cold water, cold aqueous 5% sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a short column of silica gel (1 × 3 cm) with dichloromethane to give **20** (205 mg, 90%) as an unstable, colorless syrup, $[\alpha]_D$

−41° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 7.30 (m, 15 H, 3 Ph), 6.10 (bs, 1 H, H-1), 3.93 (s, 2 H, $COCH_2Cl$).

Methyl 4-O-acetyl-2-azido-6-O-benzyl-2-deoxy-3-O-(2,3,4-tri-O-benzyl-α-L-idopyranosyl)-β-D-galactopyranoside (**22**). — A mixture of **8** (285 mg), freshly prepared **20** (680 mg), activated powdered molecular sieve (4 Å) (500 mg), and dry 1,2-dichloroethane (10 mL) was stirred for 15 min at room temperature under dry argon and then cooled to −20°. 2,4,6-Trimethylpyridine (0.26 mL) and freshly prepared silver triflate (435 mg) were added, and the mixture was stirred for 1 h at −20° and then allowed to attain room temperature overnight. The mixture was diluted with dichloromethane (50 mL), filtered through a bed of Celite, washed with water, cold 0.1M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (100 g) with hexane–ethyl acetate (5:2) gave, first, 1,5-anhydro-2,3,4-tri-*O*-benzyl-6-*O*-chloroacetyl-L-*xylo*-hex-1-enitol (**21**) (102 mg, 16% from **20**), m.p. 76–77° (from ether–hexane), $[\alpha]_D$ −59° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.30 (m, 15 H, 3 Ph), 6.40 (s, 1 H, H-1), 4.41 (dd, 1 H, $J_{5,6a}$ 5.50, $J_{6a,6b}$ 11.30 Hz, H-6a), 4.22 (dd, 1 H, $J_{5,6b}$ 7.10, $J_{6a,6b}$ 11.30 Hz, H-6b), 3.97 (d, 1 H, $J_{3,4}$ 2.40 Hz, H-3), 3.94 (ABq, 2 H, $COCH_2Cl$), 3.56 (dd, 1 H, $J_{3,4}$ 2.40, $J_{4,5}$ 1.60 Hz, H-4).

Anal. Calc. for $C_{29}H_{29}ClO_6$: C, 68.43; H, 5.74. Found: C, 68.63; H, 5.81.

Further elution gave a fraction (501 mg), to a solution of which in 2,6-dimethylpyridine (7.2 mL) and acetic acid (2.4 mL) was added dropwise a freshly prepared solution of hydrazine dithiocarbonate (8 mL). The mixture was stirred for 20 min at room temperature, then poured into ice-water (100 mL), and extracted with dichloromethane (5 × 10 mL), and the combined extracts were washed with cold 0.1M hydrochloric acid and then water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (40 g) with dichloromethane–ethyl acetate (6:1) to give syrupy **22** (368 mg, 58%), $[\alpha]_D$ −21° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.30 (m, 20 H, 4 Ph), 5.43 (dd, 1 H, $J_{3,4}$ 3.30, $J_{4,5}$ 1.0 Hz, H-4), 5.10 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1′), 4.23 (d, 1 H, $J_{1,2}$ 7.50 Hz, H-1), 4.04 (m, 1 H, $J_{4',5'}$ 3.60, $J_{5',6'a}$ 6.60, $J_{5',6'b}$ 4.40 Hz, H-5′), 3.86 (dd, 1 H, $J_{5',6'a}$ 6.60, $J_{6'a,6'b}$ 12.40 Hz, H-6′a), 3.76 (dd, 1 H, $J_{5',6'b}$ 4.40, $J_{6'a,6'b}$ 12.40 Hz, H-6′b), 3.75 (m, 1 H, $J_{4,5}$ 1.0, $J_{5,6a}$ and $J_{5,6b}$ 6.0 Hz, H-5), 3.74 (dd, 1 H, $J_{2',3'}$ 7.0, $J_{3',4'}$ 5.10 Hz, H-3′), 3.65 (dd, 1 H, $J_{2,3}$ 10.40, $J_{3,4}$ 3.30 Hz, H-3), 3.62 (dd, 1 H, $J_{3',4'}$ 5.10, $J_{4',5'}$ 3.60 Hz, H-4′), 3.58 (s, 3 H, OMe), 3.54 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 9.70 Hz, H-6a), 3.53 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 7.0 Hz, H-2′), 3.45 (dd, 1 H, $J_{5,6b}$ 6.0, $J_{6a,6b}$ 9.70 Hz, H-6b), 2.64 (t, 1 H, *J* 6.5 Hz, OH), 1.91 (s, 3 H, Ac).

Anal. Calc. for $C_{43}H_{49}N_3O_{11}$: C, 65.89; H, 6.30; N, 5.36. Found: C, 66.12; H, 6.44; N, 5.21.

Further elution gave a fraction (285 mg) that was treated as described for the preceding fraction. The product was eluted from a column of silica gel (30 g) with dichloromethane–ethyl acetate (4:1) to give amorphous **23** (190 mg, 30%), $[\alpha]_D$ +42° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.30 (m, 20 H, 4 Ph),

5.52 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.50 Hz, H-4), 4.96 (d, 1 H, $J_{1',2'}$ 2.20 Hz, H-1'), 4.18 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.83 (t, 1 H, $J_{2',3'}$ and $J_{3',4'}$ 5.30 Hz, H-3'), 3.75 (dd, 1 H, $J_{2,3}$ 10.60, $J_{3,4}$ 3.20 Hz, H-3), 3.57 (s, 3 H, OMe), 3.55 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.60 Hz, H-2), 3.49 (dd, 1 H, $J_{1',2'}$ 2.20, $J_{2',3'}$ 5.30 Hz, H-2'), 3.40 (dd, 1 H, $J_{3',4'}$ 5.30, $J_{4',5'}$ 3.30 Hz, H-4'), 2.47 (1 H, OH), 1.96 (s, 3 H, Ac).

Anal. Calc. for $C_{43}H_{49}N_3O_{11}$: C, 65.89; H, 6.30; N, 5.36. Found: C, 66.07; H, 6.41; N, 5.26.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-idopyranosyluronic acid)-β-*D*-galactopyranoside* (**24**). — A portion (0.5 mL) of a solution of chromium trioxide (1.34 g) in concentrated sulfuric acid (1.15 mL) and water (2.85 mL) was added dropwise at 0° to a stirred solution of **22** (300 mg) in acetone (6 mL). After 30 min at 0°, 2-propanol was added dropwise to destroy the excess of oxidant, and the blue mixture was concentrated. A solution of the residue in chloroform (20 mL) was filtered through a bed of Celite and concentrated. To a solution of the residue in 1,2-dimethoxyethane (5 mL) and methanol (1 mL) was added 3M sodium hydroxide (1 mL); the mixture was stirred for 3 h at room temperature and then cooled to 0°. M Hydrochloric acid (12 mL) was added, the mixture was extracted with chloroform (5 × 15 mL), and the combined extracts were washed with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (25 g) with dichloromethane–methanol (10:1) and crystallized from methanol–dichloromethane–ether to give **24** (183 mg, 63%), m.p. 206–208°, $[\alpha]_D$ −20° (*c* 0.5, *N,N*-dimethylformamide). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$–CD_3OD, 1:1): δ 7.30 (m, 20 H, 4 Ph), 5.31 (d, 1 H, $J_{1',2'}$ 4.20 Hz, H-1'), 4.77 (d, 1 H, $J_{4',5'}$ 4.0 Hz, H-5'), 4.23 (d, 1 H, $J_{1,2}$ 7.30 Hz, H-1), 4.05 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.80 Hz, H-4), 3.96 (dd, 1 H, $J_{3',4'}$ 5.0, $J_{4',5'}$ 4.0 Hz, H-4'), 3.78 (dd, 1 H, $J_{2',3'}$ 5.10, $J_{3',4'}$ 5.0 Hz, H-3'), 3.64 (dd, 1 H, $J_{1,2}$ 7.30, $J_{2,3}$ 10.0 Hz, H-2), 3.59 (dd, 1 H, $J_{1',2'}$ 4.20, $J_{2',3'}$ 5.10 Hz, H-2'), 3.57 (s, 3 H, OMe).

Anal. Calc. for $C_{41}H_{45}N_3O_{11}$: C, 65.15; H, 6.00; N, 5.56. Found: C, 64.93; H, 5.94; N, 5.43.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-*L*-idopyranosyluronate)-β-*D*-galactopyranoside* (**25**). — A suspension of **24** (38 mg) in methanol (5 mL) was treated at room temperature with an excess of ethereal diazomethane. After 20 min, acetic acid (0.5 mL) was added to destroy the excess of reagent, and the mixture was concentrated and applied to a short column (2 g) of silica gel. Elution with dichloromethane–ethyl acetate (10:1) gave **25** (35 mg, 90%), m.p. 158–159° (from methanol–dichloromethane), $[\alpha]_D$ −27° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.30 (m, 20 H, 4 Ph), 5.37 (d, 1 H, $J_{1',2'}$ 5.50 Hz, H-1'), 4.72 (d, 1 H, $J_{4',5'}$ 4.80 Hz, H-5'), 4.20 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.80 Hz, H-4), 4.18 (d, 1 H, $J_{1,2}$ 7.80 Hz, H-1), 3.92 (dd, 1 H, $J_{3',4'}$ 5.40, $J_{4',5'}$ 4.80 Hz, H-4'), 3.83 (dd, 1 H, $J_{2',3'}$ 5.80, $J_{3',4'}$ 5.40 Hz, H-3'), 3.65 (s, 3 H, COOMe), 3.57 (s, 3 H, OMe).

Anal. Calc. for $C_{42}H_{47}N_3O_{11}\cdot H_2O$: C, 64.02; H, 6.27; N, 5.33. Found: C, 64.14; H, 6.25; N, 5.34.

Methyl 2-azido-6-O-benzyl-2-deoxy-4-O-sulfo-3-O-(2,3,4-tri-O-benzyl-α-L-idopyranosyluronic acid)-β-D-galactopyranoside, disodium salt (**27**). — M Sodium hydroxide (0.285 mL) was added dropwise to a suspension of **24** (210 mg, 0.28 mmol) in methanol (20 mL), the resulting clear mixture was stirred for 2 h at room temperature and concentrated, and the residue was dried *in vacuo* to give the sodium salt of **26** (218 mg) as a white hygroscopic powder. ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.30 (m, 20 H, 4 Ph), 5.18 (d, 1 H, $J_{1',2'}$ 3.60 Hz, H-1'), 4.57 (d, 1 H, $J_{4',5'}$ 3.40 Hz, H-5'), 4.26 (d, 1 H, $J_{1,2}$ 7.40 Hz, H-1), 4.04 (dd, 1 H, $J_{3',4'}$ 4.30, $J_{4',5'}$ 3.40 Hz, H-4'), 3.94 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.70 Hz, H-4), 3.77 (dd, 1 H, $J_{2',3'}$ 6.20, $J_{3',4'}$ 4.30 Hz, H-3'), 3.55 (s, 3 H, OMe).

A solution of the sodium salt (218 mg) in *N,N*-dimethylformamide (3.5 mL) was stirred for 36 h at 50° with the sulfur trioxide–trimethylamine complex (140 mg). More sulfating agent (70 mg) was then added, the mixture was stirred for 12 h at 50° and then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (32 × 600 mm) of Sephadex LH-20, equilibrated with chloroform–methanol (1:1). Elution with the same solvent gave a product that was eluted slowly from a column (15 × 300 mm) of Sephadex SP-C25 (Na^+) with methanol–water (9:1) to afford **27** (198 mg, 87%), isolated as a colorless glass, $[\alpha]_D$ −26° (*c* 1, methanol). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.30 (m, 20 H, 4 Ph), 5.15 (d, 1 H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.93 (d, 1 H, $J_{4',5'}$ 3.60 Hz, H-5'), 4.63 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ 0.5 Hz, H-4), 4.26 (d, 1 H, $J_{1,2}$ 7.80 Hz, H-1), 4.09 (dd, 1 H, $J_{3',4'}$ 4.0, $J_{4',5'}$ 3.60 Hz, H-4'), 3.76 (dd, 1 H, $J_{2',3'}$ 4.20, $J_{3',4'}$ 4.0 Hz, H-3'), 3.75 (dd, 1 H, $J_{1',2'}$ 4.0, $J_{2',3'}$ 4.20 Hz, H-2'), 3.69 (dd, 1 H, $J_{2,3}$ 10.60, $J_{3,4}$ 3.0 Hz, H-3), 3.60 (dd, 1 H, $J_{1,2}$ 7.80, $J_{2,3}$ 10.60 Hz, H-2), 3.55 (s, 3 H, OMe).

Anal. Calc. for $C_{41}H_{43}N_3Na_2O_{14}S \cdot H_2O$: C, 54.87; H, 5.05; N, 4.68. Found: C, 54.99; H, 5.01; N, 4.62.

Methyl 2-azido-6-O-benzyl-2-deoxy-3-O-(2,3,4-tri-O-benzyl-β-L-idopyranosyluronic acid)-β-D-galactopyranoside (**28**). — The chromic solution (0.5 mL, see the preparation of **24**) was added dropwise at 0° to a stirred solution of **23** (510 mg) in acetone (10 mL). After 30 min at 0°, the mixture was worked-up and treated with 3M sodium hydroxide (3 mL) as described for the preparation of **24**. The residue was eluted from a column (35 g) of silica gel with ethyl acetate–methanol–water (24:2:1) to give pure **28**, isolated as a colorless glass (300 mg, 61%), $[\alpha]_D$ +32° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.30 (m 20 H, 4 Ph), 5.09 (d, 1 H, $J_{1',2'}$ 1.80 Hz, H-1'), 4.38 (d, 1 H, $J_{4',5'}$ 2.40 Hz, H-5'), 4.29 (d, 1 H, $J_{1,2}$ 7.80 Hz, H-1), 4.01 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.70 Hz, H-4), 3.90 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.20 Hz, H-3), 3.85 (dd, 1 H, $J_{3',4'}$ 3.0, $J_{4',5'}$ 2.40 Hz, H-4'), 3.63 (dd, 1 H, $J_{1,2}$ 7.80, $J_{2,3}$ 10.0 Hz, H-2), 3.52 (s, 3 H, OMe).

Anal. Calc. for $C_{41}H_{45}N_3O_{11}$: C, 65.15; H, 6.00; N, 5.56. Found: C, 65.56; H, 6.01; N, 5.56.

Methyl 2-azido-6-O-benzyl-2-deoxy-4-O-sulfo-3-O-(2,3,4-tri-O-benzyl-β-L-idopyranosyluronic acid)-β-D-galactopyranoside, disodium salt (**30**). — M Sodium hydroxide (0.240 mL) was added dropwise to a solution of **28** (180 mg, 0.238 mmol)

in methanol (5 mL). The mixture was stirred for 3 h at room temperature and then concentrated, and the residue was dried *in vacuo* to give **29** as a hygroscopic foam. ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.30 (m, 20 H, 4 Ph), 4.96 (d, 1 H, $J_{1',2'}$ 1.80 Hz, H-1'), 4.33 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.29 (d, 1 H, $J_{4',5'}$ 2.40 Hz, H-5'), 4.01 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.80 Hz, H-4), 3.93 (m, 1 H, $J_{3',4'}$ 2.80, $J_{4',5'}$ 2.40, $J_{2',4'}$ 0.80 Hz, H-4'), 3.83 (dd, 1 H, $J_{2,3}$ 10.60, $J_{3,4}$ 3.20 Hz, H-3), 3.77 (dd, 1 H, $J_{2',3'}$ 3.0, $J_{3',4'}$ 2.80 Hz, H-3'), 3.65 (m, 1 H, $J_{1',2'}$ 1.80, $J_{2',3'}$ 3.0, $J_{2',4'}$ 0.80 Hz, H-2'), 3.61 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.60 Hz, H-2), 3.56 (s, 3 H, OMe).

A solution of **29** (165 mg) in *N,N*-dimethylformamide (3 mL) was stirred for 36 h at 60° in the presence of the sulfur trioxide–trimethylamine complex (140 mg) and then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (32 × 600 mm) of Sephadex LH-20 equilibrated with chloroform–methanol (1:1). Elution with the same solvent gave a product that was eluted slowly from a column (15 × 300 mm) of Sephadex SP-C25 (Na^+) with methanol–water (9:1) to give **30**, isolated as a colorless glass (164 mg, 88%), $[\alpha]_D$ +39° (*c* 1, methanol). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.30 (m, 20 H, 4 Ph), 5.09 (d, 1 H, $J_{1',2'}$ 1.80 Hz, H-1'), 4.85 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.60 Hz, H-4), 4.36 (d, 1 H, $J_{1,2}$ 7.80 Hz, H-1), 4.34 (d, 1 H, $J_{4',5'}$ 2.20 Hz, H-5'), 4.18 (m, 1 H, $J_{1',2'}$ 1.80, $J_{2',3'}$ 2.80, $J_{2',4'}$ 0.80 Hz, H-2'), 3.94 (dd, 1 H, $J_{5,6a}$ 4.40, $J_{6a,6b}$ 10.60, H-6a), 3.87 (dd, 1 H, $J_{2,3}$ 10.60, $J_{3,4}$ 3.20 Hz, H-3), 3.83 (dd, 1 H, $J_{5,6b}$ 7.10, $J_{6a,6b}$ 10.60, H-6b), 3.77 (m, 1 H, $J_{3',4'}$ 2.40, $J_{4',5'}$ 2.20, $J_{2',4'}$ 0.80 Hz, H-4'), 3.68 (dd, 1 H, $J_{2',3'}$ 2.80, $J_{3',4'}$ 2.40 Hz, H-3'), 3.67 (dd, 1 H, $J_{1,2}$ 7.80, $J_{2,3}$ 10.60 Hz, H-2), 3.57 (s, 3 H, OMe).

Anal. Calc. for $C_{41}H_{43}N_3Na_2O_{14}S \cdot 2\,H_2O$: C, 53.76; H, 5.17; N, 4.58. Found: C, 53.75; H, 4.98; N, 4.43.

Methyl 2-acetamido-4-O-acetyl-6-O-benzyl-2-deoxy-3-O-(2,3,4-tri-O-benzyl-α-L-idopyranosyl)-β-D-galactopyranoside (**31**). — Ethanolic 4% nickel dichloride hexahydrate (20 mL) containing 2% of boric acid was added to a solution of **21** (200 mg) in tetrahydrofuran (1.5 mL). A solution of sodium borohydride in ethanol (10 mg/mL) was then added dropwise with stirring until the green solution turned to persistent black. The mixture was concentrated, and a solution of the residue in dichloromethane–methanol (15:1, 3 mL) was applied to a short column (1 × 8 cm) of silica gel. Elution with the same solvent gave a colorless product, the pH of a solution of which in methanol (10 mL) was adjusted to 8 with M sodium hydroxide, and acetic anhydride (2 mL) was added immediately. The mixture was stirred for 15 min and then concentrated, a solution of the residue in ethyl acetate was filtered through a bed of Celite and concentrated, and the residue was crystallized from ethyl acetate–hexane to give **31** (165 mg, 81%), $[\alpha]_D$ −5° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$); δ 7.25 (m, 20 H, 4 Ph), 5.48 (dd, 1 H, $J_{3,4}$ 3.40, $J_{4,5}$ 0.80 Hz, H-4), 4.88 (d, 1 H, $J_{1',2'}$ 5.0 Hz, H-1'), 4.81 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1), 4.58 (dd, 1 H, $J_{2,3}$ 10.80, $J_{3,4}$ 3.40 Hz, H-3), 4.05 (m, 1 H, $J_{4',5'}$ 4.30, $J_{5',6'a}$ 6.70, $J_{5',6'b}$ 4.30 Hz, H-5'), 3.87 (dd, 1 H, $J_{5',6'a}$ 6.70, $J_{6'a,6'b}$ 12.30 Hz, H-6'a), 3.78 (dd, 1 H, $J_{5',6'b}$ 4.30, $J_{6'a,6'b}$ 12.30 Hz, H-6'b), 3.69 (dd, 1 H, $J_{3',4'}$ 5.50, $J_{4',5'}$ 4.30 Hz, H-4'),

3.49 (s, 3 H, OMe), 3.48 (dd, 1 H, $J_{2',3'}$ 7.50, $J_{3',4'}$ 5.50 Hz, H-3'), 3.46 (dd, 1 H, $J_{1',2'}$ 5.0, $J_{2',3'}$ 7.50 Hz, H-2'), 3.37 (dd, 1 H, $J_{1,2}$ 8.30, $J_{2,3}$ 10.80 Hz, H-2), 2.00 (s, 3 H, Ac), 1.84 (s, 3 H, NAc).

Anal. Calc. for $C_{45}H_{53}NO_{12}$: C, 67.57; H, 6.68; N, 1.75. Found: C, 67.30; H, 6.62; N, 1.83.

*Methyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-idopyranosyluronic acid)-β-*D*-galactopyranoside* (**32**). — A portion (1 mL) of the chromic solution (see the preparation of **24**) was added dropwise at 0° to a stirred solution of **31** (240 mg) in acetone (10 mL). After 15 min, 2-propanol was added dropwise to destroy the excess of oxidant, and the blue mixture was concentrated. A solution of the residue in chloroform (20 mL) was filtered through a bed of Celite and concentrated. The residue was immediately dissolved in tetrahydrofuran (5 mL) and methanol (5 mL), the solution was cooled to 0°, and 3M sodium hydroxide (1 mL) was added. The mixture was stirred for 5 h at 0°, cold M hydrochloric acid (10 mL) was then added, the white slurry was extracted with chloroform (10 × 20 mL), and the combined extracts were washed with water, dried (Na_2SO_4), and concentrated to give **32** (155 mg, 67%), m.p. 232–235° (dec.), $[\alpha]_D$ −20° (*c* 0.5, *N,N*-dimethylformamide). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$–CD_3OD, 1:1): δ 7.25 (m, 20 H, 4 Ph), 5.22 (d, 1 H, $J_{1',2'}$ 4.60 Hz, H-1'), 4.78 (d, 1 H, $J_{4',5'}$ 4.20 Hz, H-5'), 4.34 (d, 1 H, $J_{1,2}$ 8.40 Hz, H-1), 4.12 (dd, 1 H, $J_{1,2}$ 8.40, $J_{2,3}$ 10.80 Hz, H-2), 4.07 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.80 Hz, H-4), 3.98 (dd, 1 H, $J_{3',4'}$ 4.50, $J_{4',5'}$ 4.20 Hz, H-4'), 3.86 (dd, 1 H, $J_{2,3}$ 10.80, $J_{3,4}$ 3.20 Hz, H-3), 3.82 (dd, 1 H, $J_{2',3'}$ 5.60, $J_{3',4'}$ 4.50 Hz, H-3'), 3.56 (dd, 1 H, $J_{1',2'}$ 4.60, $J_{2',3'}$ 5.60 Hz, H-2'), 3.47 (s, 3 H, OMe), 1.78 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{49}NO_{12}\cdot H_2O$: C, 65.38; H, 6.50; N, 1.78. Found: C, 65.69; H, 6.30; N, 1.80.

*Methyl 2-acetamido-6-*O*-benzyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-*L*-idopyranuronate)-β-*D*-galactopyranoside* (**33**). — A portion (5 mg) of **32** was esterified with etheral diazomethane as described above to give **33**. ^{1}H-N.m.r. data (300 MHz, $CDCl_3$–CD_3OD, 1:1): δ 7.30 (m, 20 H, 4 Ph), 5.23 (d, 1 H, $J_{1',2'}$ 5.0 Hz, H-1'), 4.78 (d, 1 H, $J_{4',5'}$ 4.40 Hz, H-5'), 4.38 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.10 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.70 Hz, H-4), 4.05 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.70 Hz, H-2), 3.93 (dd, 1 H, $J_{3',4'}$ 4.80, $J_{4',5'}$ 4.40 Hz, H-4'), 3.88 (dd, 1 H, $J_{2,3}$ 10.70, $J_{3,4}$ 3.20, H-3), 3.82 (dd, 1 H, $J_{2',3'}$ 5.20, $J_{3',4'}$ 4.80 Hz, H-3'), 3.66 (s, 3 H, COOMe), 3.55 (dd, 1 H, $J_{1',2'}$ 5.0, $J_{2',3'}$ 5.20 Hz, H-2'), 3.47 (s, 3 H, OMe), 1.87 (s, 3 H, NAc).

*Methyl 2-acetamido-6-*O*-benzyl-2-deoxy-4-*O*-sulfo-3-*O*-(2,3,4-tri-*O*-benzyl-α-*L*-idopyranosyluronic acid)-β-*D*-galactopyranoside, disodium salt* (**34**). — M Sodium hydroxide (0.132 mL) was added dropwise to a suspension of **32** (100 mg, 0.129 mmol) in methanol (8 mL). The resulting clear mixture was stirred for 2 h at room temperature and then concentrated, and the residue was dried *in vacuo* to give an amorphous hygroscopic powder, a solution of which in *N,N*-dimethylformamide (2.5 mL) was stirred for 36 h at 60° in the presence of the sulfur trioxide–trimethylamine complex (140 mg). The mixture was then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (32 × 600 mm) of

Sephadex LH-20 equilibrated in, and eluted with, 1:1 chloroform–methanol. The product was eluted from a column (8 g) of silica gel with ethyl acetate–methanol–water (10:2:1). Pure fractions were slowly eluted from a column (15 × 300 mm) of Sephadex SP-C25 (Na^+) with 9:1 methanol–water, to afford pure **34**, isolated as a colorless glass (94 mg, 81%), $[\alpha]_D$ −21° (*c* 0.5, methanol). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.25 (m, 20 H, 4 Ph), 5.10 (d, 1 H, $J_{1',2'}$ 4.20 Hz, H-1'), 5.07 (d, 1 H, $J_{4',5'}$ 3.80 Hz, H-5'), 4.70 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.80 Hz, H-4), 4.38 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.07 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.20 Hz, H-2), 4.06 (dd, 1 H, $J_{3',4'}$ 4.40, $J_{4',5'}$ 3.80 Hz, H-4'), 3.87 (dd, 1 H, $J_{2,3}$ 10.20, $J_{3,4}$ 3.20 Hz, H-3), 3.76 (dd, 1 H, $J_{2',3'}$ 6.80, $J_{3',4'}$ 4.40 Hz, H-3'), 3.64 (dd, 1 H, $J_{1',2'}$ 4.20, $J_{2',3'}$ 6.80 Hz, H-2'), 3.46 (s, 3 H, OMe), 1.77 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{47}NNa_2O_{15}S \cdot H_2O$: C, 56.51; H, 5.40; N, 1.53. Found: C, 56.55; H, 5.40; N, 1.52.

*Methyl 2-acetamido-2-deoxy-3-*O*-(α-*L*-idopyranosyluronic acid)-4-*O*-sulfo-β-*D*-galactopyranoside, disodium salt* (**35**). — A solution of **34** (85 mg) in 4:1 methanol–water was hydrogenolyzed in the presence of 10% Pd/C (100 mg) for 36 h, then filtered, and concentrated. A solution of the residue in water (1 mL) was added to a column (20 × 900 mm) of Sephadex G-10 and eluted with water to give **35** (44 mg, 86%), isolated by lyophilization as an amorphous powder, $[\alpha]_D$ −38° (*c* 1, water). N.m.r. data: ^{1}H (300 MHz, D_2O, internal TSP), δ 4.83 (d, 1 H, $J_{1',2'}$ 4.60 Hz, H-1'), 4.73 (dd, 1 H, $J_{3,4}$ 2.80, $J_{4,5}$ 0.80 Hz, H-4), 4.64 (d, 1 H, $J_{4',5'}$ 3.90 Hz, H-5'), 4.53 (d, 1 H, $J_{1,2}$ 7.60 Hz, H-1), 4.07 (dd, 1 H, $J_{1,2}$ 7.60, $J_{2,3}$ 11.0 Hz, H-2), 4.05 (dd, 1 H, $J_{2,3}$ 11.0, $J_{3,4}$ 2.80 Hz, H-3), 3.94 (dd, 1 H, $J_{3',4'}$ 5.80, $J_{4',5'}$ 3.90 Hz, H-4'), 3.62 (dd, 1 H, $J_{2',3'}$ 7.50, $J_{3',4'}$ 5.80 Hz, H-3'), 3.54 (s, 3 H, OMe), 3.51 (dd, 1 H, $J_{1',2'}$ 4.60, $J_{2',3'}$ 7.50 Hz, H-2'), 2.05 (s, 3 H, NAc); ^{13}C (22.6 MHz, D_2O, internal acetone), δ 103.35 (C-1'), 102.18 (C-1), 76.64 (C-3), 75.99 (C-4), 74.82 (C-5), 72.68, 71.77 (2 C), 70.66, 61.30 (C-6), 57.34 (OCH_3), 52.07 (C-2), 22.44 ($CO-CH_3$).

Anal. Calc. for $C_{15}H_{23}NNa_2O_{15}S$: C, 33.65; N, 2.61. Found: C, 33.13; N, 2.55.

*Methyl 2-acetamido-2-deoxy-3-*O*-(α-*L*-idopyranosyluronic acid)-β-*D*-galacto-pyranoside, sodium salt* (**36**). — A mixture of **32** (50 mg, 0.065 mmol), methanol (5 mL), and M sodium hydroxide (0.066 mL) was stirred for 2 h at room temperature and then concentrated. A solution of the residue in 4:1 methanol–water (5 mL) was stirred with 10% Pd/C (50 mg) under hydrogen for 24 h, then filtered, and concentrated. A solution of the residue in water (1 mL) was added to a column (20 × 900 mm) of Sephadex G-10 and eluted with water to afford **36** (24 mg, 86%), isolated by lyophilization as a white foam, $[\alpha]_D$ −38° (*c* 1, water). N.m.r. data: ^{1}H (300 MHz, D_2O, internal TSP), δ 4.82 (d, 1 H, $J_{1',2'}$ 5.0 Hz, H-1'), 4.46 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1), 4.45 (d, 1 H, $J_{4',5'}$ 4.30 Hz, H-5'), 4.07 (dd, 1 H, $J_{3,4}$ 3.60, $J_{4,5}$ 0.80 Hz, H-4), 4.05 (dd, 1 H, $J_{1,2}$ 8.30, $J_{2,3}$ 10.80 Hz, H-2), 3.88 (dd, 1 H, $J_{3',4'}$ 6.50, $J_{4',5'}$ 4.30 Hz, H-4'), 3.83 (dd, 1 H, $J_{2,3}$ 10.80, $J_{3,4}$ 3.60 Hz, H-3), 3.64 (dd, 1 H, $J_{2',3'}$ 7.80, $J_{3',4'}$ 6.50 Hz, H-3'), 3.53 (s, 3 H, OMe), 3.47 (dd, 1 H, $J_{1',2'}$ 5.0, $J_{2',3'}$ 7.80 Hz,

H-2'), 2.04 (s, 3 H, NAc); ^{13}C (22.6 MHz, D_2O, internal acetone): δ 103.42 (C-1'), 102.44 (C-1), 79.37 (C-3), 75.34 (C-5), 72.81, 71.57 (2 C), 68.39 (C-4), 61.67 (C-6), 57.21 (OCH_3), 51.42 (C-2), 22.44 (CO–CH_3).

Anal. Calc. for $C_{15}H_{24}NNaO_{12}\cdot H_2O$: C, 39.91; H, 5.80; N, 3.10. Found: C, 39.83; H, 5.84; N, 2.96.

Methyl 2-acetamido-4-O-acetyl-6-O-benzyl-2-deoxy-3-O-(2,3,4-tri-O-benzyl-β-L-idopyranosyl)-β-D-galactopyranoside (**37**). — Compound **23** (120 mg) was treated as described for **31**. The product was eluted from a column of silica gel (8 g) with ethyl acetate to give amorphous **37** (100 mg, 81%), $[\alpha]_D$ +51° (*c* 1, chloroform). ^1H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.25 (m, 20 H, 4 Ph), 5.52 (dd, 1 H, $J_{3,4}$ 3.40, $J_{4,5}$ 0.80 Hz, H-4), 5.04 (d, 1 H, $J_{1,2}$ 8.30 Hz, H-1), 4.88 (dd, 1 H, $J_{2,3}$ 10.80, $J_{3,4}$ 3.40 Hz, H-3), 4.77 (d, 1 H, $J_{1',2'}$ 1.80 Hz, H-1'), 3.94 (dd, 1 H, $J_{5',6'a}$ 8.60, $J_{6'a,6'b}$ 12.30 Hz, H-6'a), 3.79 (m, 1 H, $J_{4',5'}$ 2.0, $J_{5',6'a}$ 8.60, $J_{5',6'b}$ 2.30 Hz, H-5'), 3.62 (dd, 1 H, $J_{2',3'}$ 2.80, $J_{3',4'}$ 2.20 Hz, H-3'), 3.52 (s, 3 H, OMe), 3.35 (dd, 1 H, $J_{5',6'b}$ 2.30, $J_{6'b,6'a}$ 12.30 Hz, H-6'b), 3.14 (m, 1 H, $J_{3',4'}$ 2.20, $J_{4',5'}$ 2.0, $J_{2',4'}$ 1.0 Hz, H-4'), 3.07 (dd, 1 H, $J_{1,2}$ 8.30, $J_{2,3}$ 10.80 Hz, H-2), 2.06 (s, 3 H, Ac), 2.01 (s, 3 H, NAc).

Anal. Calc. for $C_{45}H_{53}NO_{12}$: C, 67.57; H, 6.79; N, 1.75. Found: C, 67.27; H, 6.79; N, 1.79.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-3-O-(2,3,4-tri-O-benzyl-β-L-idopyranosyluronic acid)-β-D-galactopyranoside (**38**). — The chromic solution (see preparation of **24**, 1 mL) was added dropwise at 0° to a stirred solution of **37** (150 mg) in acetone (3.5 mL). After 20 min at 0°, the mixture was worked-up as described for the preparation of **24**. To a solution of the residue in 1:1 tetrahydrofuran–methanol (5 mL) at 0° was added 3M sodium hydroxide (1 mL), and the mixture was stirred for 3 h. Cold M hydrochloric acid was added, the mixture was extracted with chloroform (5 × 10 mL), and the combined extracts were washed with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (12 g) with ethyl acetate–methanol–water (15:2:1) to give **38**, isolated as a colorless glass (91 mg, 63%), $[\alpha]_D$ +28° (*c* 1, chloroform). ^1H-N.m.r. data (300 MHz, $CDCl_3$–CD_3OD, 1:1): δ 7.30 (m, 20 H, 4 Ph), 4.93 (d, 1 H, $J_{1',2'}$ 1.60 Hz, H-1'), 4.34 (d, 1 H, $J_{4',5'}$ 2.20 Hz, H-5'), 4.28 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.22 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 10.20 Hz, H-2), 4.02 (dd, 1 H, $J_{3,4}$ 3.50, $J_{4,5}$ 0.80 Hz, H-4), 3.95 (dd, 1 H, $J_{2,3}$ 10.20, $J_{3,4}$ 3.50 Hz, H-3), 3.93 (m, 1 H, $J_{3',4'}$ 2.80, $J_{4',5'}$ 2.20, $J_{2',4'}$ 1.0 Hz, H-4'), 3.58 (m, 1 H, $J_{1',2'}$ 1.60, $J_{2',3'}$ 3.20, $J_{2',4'}$ 1.0 Hz, H-2'), 3.48 (s, 3 H, OMe), 1.95 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{49}NO_{12}\cdot H_2O$: C, 65.38; H, 6.50; N, 1.78. Found: C, 65.72; H, 6.25; N, 1.67.

Methyl 2-acetamido-6-O-benzyl-2-deoxy-4-O-sulfo-3-O-(2,3,4-tri-O-benzyl-β-L-idopyranosyluronic acid)-β-D-galactopyranoside, disodium salt (**39**). — M Sodium hydroxide (0.21 mL) was added dropwise to a suspension of **38** (159 mg, 0.206 mmol) in methanol (5 mL). The clear solution was stirred for 2 h at room temperature and then concentrated, and the residue was dried *in vacuo*. A solution

of the sodium salt in *N,N*-dimethylformamide (4 mL) was stirred for 36 h at 60° in the presence of the sulfur trioxide–trimethylamine complex (210 mg). The mixture was then cooled, methanol (1 mL) was added, and the mixture was chromatographed on a column (32 × 600 mm) of Sephadex LH-20 equilibrated in, and eluted with, 1:1 chloroform–methanol. The product was eluted from a column of silica gel (15 g) with ethyl acetate–methanol–water (9:2:1) to give a pure fraction which was eluted slowly from a column (15 × 300 mm) of Sephadex SP-C25 (Na^+) with 9:1 methanol–water to give **39**, isolated as a colorless glass (153 mg, 83%), $[\alpha]_D$ +28° (*c* 1, methanol). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ 7.30 (m, 20 H, 4 Ph), 5.07 (d, 1 H, $J_{1',2'}$ 1.50 Hz, H-1'), 4.87 (d, 1 H, $J_{4',5'}$ 2.0 Hz, H-5'), 4.82 (dd, 1 H, $J_{3,4}$ 3.80, $J_{4,5}$ 0.80 Hz, H-4), 4.32 (d, 1 H, $J_{1,2}$ 7.40 Hz, H-1), 4.03 (m, 1 H, $J_{3',4'}$ 2.60, $J_{4',5'}$ 2.0, $J_{2',4'}$ 0.80 Hz, H-4'), 3.95 (dd, 1 H, $J_{2,3}$ 10.50, $J_{3,4}$ 3.20 Hz, H-3), 3.82 (dd, 1 H, $J_{2',3'}$ 3.20, $J_{3',4'}$ 2.60 Hz, H-3'), 3.58 (m, 1 H, $J_{1',2'}$ 1.50, $J_{2',3'}$ 3.20, $J_{2',4'}$ 0.80 Hz, H-2'), 3.46 (s, 3 H, OMe), 1.97 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{47}NNa_2O_{15}S \cdot H_2O$: C, 56.51; H, 5.40; N, 1.53. Found: C, 56.42; H, 5.53; N, 1.48.

*Methyl 2-acetamido-2-deoxy-3-O-(β-*L*-idopyranosyluronic acid)-4-O-sulfo-β-*D*-galactopyranoside, disodium salt* (**40**). — A solution of **39** (98 mg) in 5:1 methanol–water (6 mL) was hydrogenolyzed in the presence of 10% Pd/C (100 mg) for 2 days, then filtered, and concentrated. A solution of the residue in water (1.5 mL) was added to a column (20 × 900 mm) of Sephadex G-10 and eluted with water to give **34** (51 mg, 87%), isolated by lyophilization as an amorphous powder, $[\alpha]_D$ +27° (*c* 1, water). N.m.r. data: ^{1}H (300 MHz, D_2O, internal TSP), δ 5.01 (d, 1 H, $J_{1',2'}$ 1.50 Hz, H-1'), 4.92 (dd, 1 H, $J_{3,4}$ 3.20, $J_{4,5}$ 0.60 Hz, H-4), 4.45 (d, 1 H, $J_{1,2}$ 8.50 Hz, H-1), 4.33 (d, 1 H, $J_{4',5'}$ 1.60 Hz, H-5'), 4.30 (dd, 1 H, $J_{2,3}$ 11.0, $J_{3,4}$ 3.20 Hz, H-3), 4.08 (dd, 1 H, $J_{2',3'}$ 3.20, $J_{3',4'}$ 3.0 Hz, H-3'), 4.03 (dd, 1 H, $J_{1,2}$ 8.50, $J_{2,3}$ 11.0 Hz, H-2), 3.93 (m, 1 H, $J_{3',4'}$ 3.0, $J_{4',5'}$ 1.60, $J_{2',4'}$ 1.0 Hz, H-4'), 3.53 (s, 3 H, OMe), 2.07 (s, 3 H, NAc); ^{13}C (22.6 MHz, D_2O, internal acetone), δ 103.09 (C-1), 95.88 (C-1'), 74.56 (C-3,4), 73.85 (C-5), 72.48, 70.27, 69.75, 68.71, 61.37 (C-6), 57.40 (OCH_3), 50.90 (C-2), 22.63 (CO–CH_3).

Anal. Calc. for $C_{15}H_{23}NNa_2O_{15}S$: C, 33.65; N, 2.61. Found: C, 33.47; N, 2.50.

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