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SYNTHESIS OF DISACCHARIDE FRAGMENTS OF DERMATAN SULFATE

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ABSTRACT

Condensation of crystalline methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside with methyl (2,3,4-tri-*O*-acetyl-α-L-idopyranosyl bromide)-uronate in dichloromethane, in the presence of silver triflate and molecular sieve, provided 54% of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-L-idopyranosyluronate)-β-D-galactopyranoside. The use of methyl (2,3,4-tri-*O*-acetyl-α-L-idopyranosyl trichloroacetimidate)uronate as glycosyl donor, in the presence of trimethylsilyl triflate, improved the yield to 68%. Regioselective opening of the benzylidene group with sodium cyanoborohydride followed successively by *O*-sulfation with the sulfur trioxide–trimethylamine complex, saponification, catalytic hydrogenolysis and selective *N*-acetylation gave the disodium salt of methyl 2-acetamido-2-deoxy-3-*O*-(α-L-idopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside. Condensation of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate in dichloromethane, in the presence of silver triflate and molecular sieve, gave methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-β-D-galactopyranoside in 85% yield. The sequence already described then gave the disodium salt of methyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside.

INTRODUCTION

Dermatan sulfate is a hybrid polymer built from two types of disaccharidic units: *N*-acetylchondrosine (Fig. 1, type A) and *N*-acetyldermosine (Fig. 1, type B). Both units are sulfated mainly at position 4 of the 2-acetamido-2-deoxy-D-galactose residue[1].

As part of a programme on the chemical synthesis of dermatan sulfate fragments, we now report on the synthesis of two disaccharides: methyl 2-

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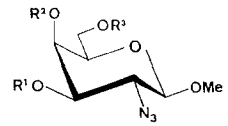
Fig. 1. Repeating units: **A**, *N*-acetylchondrosine; **B**, *N*-acetyldermosine.

acetamido-2-deoxy-3-*O*-(α-L-idopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside disodium salt (**26**) and methyl 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-4-*O*-sulfo-β-D-galactopyranoside disodium salt (**27**). Methyl glycosides with the appropriate configuration corresponding to that in dermatan sulfate have been selected as targets since they facilitate the synthesis work and n.m.r. study without interfering with the potential biological properties. Compound **26** was previously prepared through a more circuitous route[2].

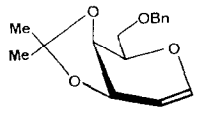
RESULTS AND DISCUSSION

The strategy that was investigated initially involved the expected regioselective glycosylation of the more reactive equatorial hydroxyl group of the known[2] methyl 2-azido-6-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**2**), followed by sulfation of the axial hydroxyl group. A novel route to the key diol **2** was first developed. 1,5-Anhydro-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-D-*lyxo*-hex-1-enitol[3] (**6**) was submitted to azidonitration[4] in acetonitrile. The major isolated product (36%) was crystalline 2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl nitrate (**7**). Also isolated in pure form after column chromatography were the corresponding crystalline β-nitrate **8** (19%) and the two by-products **9** (5%) and **10** (14%) resulting from the reaction of acetonitrile on the anomeric centre[4]. The α-nitrate **7** was treated with sodium methoxide in methanol for 4 h at room temperature to give, selectively, methyl 2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranoside (**11**) in 68% yield. Hydrolytic removal of the isopropylidene group with aqueous 80% acetic acid at 100° for 15 min gave the diol **2** (92%), identical with the authentic compound[2].

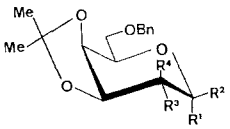
Condensation of **2** with freshly prepared methyl (2,3,4-tri-*O*-acetyl-α-L-idopyranosyl bromide)uronate[5] (**13**) in dichloromethane in the presence of activated 4 Å powdered molecular sieve and silver triflate gave the expected disaccharide **22** in poor yield (16%). A mixture of the two monoacetates **4** [ref. 2] and **5** was also isolated in 25% yield. 1,2-Orthoacetates are intermediates in silver triflate-promoted Koenigs–Knorr synthesis of disaccharide-glycosides[6]. The presence of a vicinal hydroxyl group appeared to complicate the rearrangement of **18** into **22** in the acidic medium and monoacetates were produced. The orthoester **18** was obtained (66%) when the bromide **13** was condensed with **2** in dichloromethane in the presence of activated 4 Å powdered molecular sieve, silver triflate, and 2,4,6-trimethylpyridine[6]



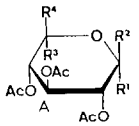
1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = H$, $R^3 = Bn$
3 $R^1 = H$, R^2, $R^3 = PhCH$<
4 $R^1 = H$, $R^2 = Ac$, $R^3 = Bn$
5 $R^1 = Ac$, $R^2 = H$, $R^3 = Bn$



6



7 $R^1 = ONO_2$, $R^2 = R^4 = H$, $R^3 = N_3$
8 $R^1 = R^4 = H$, $R^2 = ONO_2$, $R^3 = N_3$
9 $R^1 = R^4 = H$, $R^2 = NHAc$, $R^3 = N_3$
10 $R^1 = NHAc$, $R^2 = R^3 = H$, $R^4 = N_3$
11 $R^1 = R^4 = H$, $R^2 = OMe$, $R^3 = N_3$

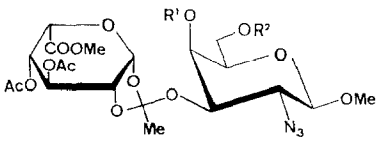


12 $R^1 = R^4 = H$, $R^2 = OAc$, $R^3 = COOMe$
13 $R^1 = R^4 = H$, $R^2 = Br$, $R^3 = COOMe$
14 R^1, $R^2 = H$, OH, $R^3 = COOMe$, $R^4 = H$
15 $R^1 = R^4 = H$, $R^2 = OCNHCCl_3$, $R^3 = COOMe$
16 $R^1 = OCNHCCl_3$, $R^2 = R^4 = H$, $R^3 = COOMe$
17 $R^1 = Br$, $R^2 = R^3 = H$, $R^4 = COOMe$

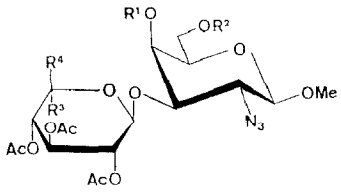
(30 min at −20°). The presence of an orthoacetate group was unambiguously demonstrated by ^1H- and ^{13}C-n.m.r. spectroscopy[7] (see Experimental), and the position of glycosylation was established by addition of trichloroacetyl isocyanate to the n.m.r. sample[8], resulting in the expected downfield shift of the signal for H-4 (1.57 p.p.m.). Attempts to rearrange[6] this orthoester into **22** with tin tetrachloride were disappointing and another strategy was developed.

Treatment of methyl 2-azido-2-deoxy-β-D-galactopyranoside[2,9] (**1**) with α,α-dimethoxytoluene and camphorsulfonic acid in nitromethane gave crystalline methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (**3**, 68%). However, the physical properties of **3** {m.p. 170–171°, $[\alpha]_D$ −0.3° (methanol)} were at variance with those reported[9] {syrup, $[\alpha]_D^{26}$ −14° (methanol)}, and the elemental analysis and n.m.r. data were erroneous.

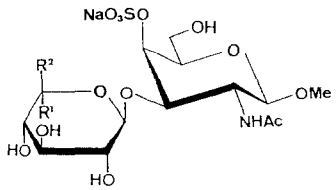
Condensation of **3** with freshly prepared **13**[5] in dichloromethane for 30 min at −20°, in the presence of activated 4 Å powdered molecular sieve, silver triflate, and 2,4,6-trimethylpyridine, gave only the crystalline orthoester derivative **19** (82%). In the absence of 2,4,6-trimethylpyridine, the expected disaccharide derivative **20** was isolated (54%). The ^1H-n.m.r. spectrum (400 MHz, $CDCl_3$) of **20** showed $J_{1',3'}$ and $J_{2',4'}$ values of 1.0 and ~0.4 Hz, respectively, which accord with a $^1C_4$ conformation for the acetylated L-iduronic acid residue and indicate H-1′ to be equatorial (α anomer). In order to improve the yield of the glycosylation reaction, the bromide **13** was converted into the crude hemiacetal **14** by treatment with silver carbonate in moist acetone (the preparation of **14** from the fully acetylated sugar **12** by mild treatment[10] with sodium methoxide failed). Compound **14** was then



18 R^1 = H, R^2 = Bn
19 R^1, R^2 = PhCH<



20 R^1, R^2 = PhCH<, R^3 = COOMe, R^4 = H
21 R^1, R^2 = PhCH<, R^3 = H, R^4 = COOMe
22 R^1 = R^4 = H, R^2 = Bn, R^3 = COOMe
23 R^1 = R^3 = H, R^2 = Bn, R^4 = COOMe
24 R^1 = SO_3Na, R^2 = Bn, R^3 = COOMe, R^4 = H
25 R^1 = SO_3Na, R^2 = Bn, R^3 = H, R^4 = COOMe



26 R^1 = COONa, R^2 = H
27 R^1 = H, R^2 = COONa

reacted directly at 0° with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane. The α-trichloroacetimidate **15** was isolated (62%), after column chromatography, as the major isomer. Reaction of **3** with 1.3 equiv. of **15** for 15 min at −20° in dichloromethane in the presence of trimethylsilyl triflate (1.3 equiv.) gave the disaccharide derivative **20** (68%). Compound **20** was hydrogenolyzed[11] to give **22** (70%). The regioselectivity of this reaction was indicated by the $J_{4,OH}$ value of 3.0 Hz for **22**. The J values ($J_{1',2'}$ 2.8, $J_{2',3'}$ 5.0, $J_{3',4'}$ 4.3, $J_{4',5'}$ 3.0 Hz) observed for the α-L-idopyranosyluronate unit (after addition of trichloroacetyl isocyanate) indicate a significant departure from the $^1C_4$ conformation in chloroform solution. *O*-Sulfation of **22** with the sulfur trioxide–trimethylamine complex in *N,N*-dimethylformamide (36 h at 60°) gave the sulfated disaccharide derivative **24** (94%). Sulfation of HO-4 of **22** (→**24**) caused a downfield shift of the signal for H-4, in accord with previous observations[12]. Compound **24** was saponified with sodium hydroxide, and catalytic hydrogenolysis (Pd/C) of the product followed by selective *N*-acetylation gave the known[2] disaccharide-glycoside **26** as the disodium salt (43% from **24** after purification by h.p.l.c. on a Mono-Q column).

Condensation of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate[13] with **3** in dichloromethane, in the presence of silver triflate and activated 4 Å powdered molecular sieve, gave the crystalline disaccharide derivative **21** (85%). That the new glycosidic linkage was β was apparent from the n.m.r. signal for H-1′ ($J_{1',2'}$ 7.6 Hz). Compound **21** was hydrogenolyzed regioselectively[11] to give the crystalline disaccharide derivative **23** (78%), the ^{1}H-n.m.r. spectrum (400 MHz, $CDCl_3$) of which contained a long-range coupling ($^4J_{OH,5}$ 1.3 Hz). Such ^4J couplings are observed whenever a zigzag arrangement is possible, which

requires the O–H and C–H bonds in the above fragment[14] to be axial. This requirement is met in **23**. Compound **23** was converted into the disaccharide-glycoside **27** (60% overall yield) as previously described. Compound **27** is also the repeating unit of chondroitin 4-sulfate[15].

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi Model 510 capillary apparatus and are uncorrected. Optical rotations were measured at 20 ±2° with a Perkin–Elmer Model 241 polarimeter. Elemental analyses were performed at the University Pierre et Marie Curie (Paris VI). ^{1}H-N.m.r. spectra were recorded with Cameca 250 and Bruker AM-400 spectrometers. The values of δ ($CDCl_3$ or CD_3OD) are expressed in p.p.m. downfield from the signal for internal Me_4Si. Values of δ (D_2O) are expressed in p.p.m. downward from Me_4Si, by reference to internal Me_2CO (2.225). ^{13}C-N.m.r. spectra (100.57 MHz) were recorded for solutions in $CDCl_3$, adopting δ 77.0 for the central line of $CDCl_3$. Assignments were aided by the J-MOD technique[16,17]. Reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck) with detection by charring with sulfuric acid. Flash column chromatography[18] was performed on Silica Gel 60 (230–400 mesh, Merck). The ion-exchange chromatography (i.e.c.) column (Mono-Q) was purchased from Pharmacia AB.

*Methyl 2-azido-4,6-O-benzylidene-2-deoxy-β-*D*-galactopyranoside* (**3**). — A mixture of **1** (1.10 g, 5 mmol), (±)-10-camphorsulfonic acid (50 mg), α,α-dimethoxytoluene (8 mL, 53 mmol), and anhydrous nitromethane (10 mL) was stirred for 1.5 h at 20°, then treated with triethylamine (0.5 mL), and concentrated. Water (2 × 10 mL) and then toluene (2 × 30 mL) were evaporated from the residue. Column chromatography of the crude product with 1:1 ethyl acetate–hexane gave **3** (1.05 g, 68%), m.p. 170–171° (from ethyl acetate–hexane), $[\alpha]_D$ −10° (*c* 1.1, chloroform), $[\alpha]_D$ −0.3° (*c* 0.8, methanol); lit.[9] syrup, $[\alpha]_D^{26}$ −14° (*c* 0.1, methanol). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.61–7.53 and 7.48–7.41 (2 m, 5 H, Ph), 5.62 (s, 1 H, PhC*H*), 4.40 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.26–4.21 (m, 2 H, H-1,4), 4.12 (dd, 1 H, $J_{5,6b}$ 1.7 Hz, H-6b), 3.69–3.56 (m, 2 H, H-2,3), 3.63 (s, 3 H, MeO), 3.48 (m, 1 H, H-5), 2.60 (m, 1 H, OH); (250 MHz, $CDCl_3$ + CCl_3CONCO): δ 8.74 (s, 1 H, NH), 7.61–7.53 and 7.48–7.41 (2 m, 5 H, Ph), 5.60 (s, 1 H, PhC*H*), 4.83 (dd, 1 H, $J_{2,3}$ 11.0, $J_{3,4}$ 3.7 Hz, H-3), 4.49 (dd, 1 H, $J_{4,5}$ ~0.8 Hz, H-4), 4.41 (dd, 1 H, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.39 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.13 (dd, 1 H, $J_{5,6b}$ 1.6 Hz, H-6b), 3.94 (dd, 1 H, H-2), 3.68 (s, 3 H, MeO), 3.57 (ddd, 1 H, H-5).

Anal. Calc. for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.57; N, 13.67. Found: C, 54.80; H, 5.56; N, 13.77.

Azidonitration of **6**. — A solution of **6** (1.38 g, 5 mmol) in anhydrous acetonitrile (25 mL) was added dropwise to a stirred, cooled (−20°) mixture of sodium azide (0.49 g, 7.5 mmol) and cerium(IV) ammonium nitrate (8.22 g, 15 mmol). The

suspension was stirred vigorously for 5 h at −20°, then diluted with ice-cold ether (200 mL), washed with cold water until neutral, dried ($MgSO_4$), and concentrated. The residue was eluted from a short column (3 × 6 cm) of silica gel with dichloromethane (150 mL) then ethyl acetate (100 mL), both containing 0.5% of triethylamine, to give fractions *A* (1.15 g) and *B* (0.57 g). Column chromatography of fraction *A* (from 2:1 to 4:1 dichloromethane–carbon tetrachloride) gave, first, 2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl nitrate (**7**; 0.68 g, 36%), m.p. 60–61° (from hexane), $[\alpha]_D$ +109° (*c* 1, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.46–7.32 (m, 5 H, Ph), 6.26 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.65 and 4.55 (2 d, 2 H, *J* 12.2 Hz, PhCH_2), 4.39 (dd, 1 H, $J_{2,3}$ 7.8, $J_{3,4}$ 5.5 Hz, H-3), 4.39 (ddd, 1 H, $J_{4,5}$ 2.5, $J_{5,6a}$ 6.0, $J_{5,6b}$ 6.8 Hz, H-5), 4.31 (dd, 1 H, H-4), 3.84 (dd, 1 H, H-2), 3.79 (dd, 1 H, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.72 (dd, 1 H, H-6b), 1.54 and 1.38 (2 s, 6 H, 2 Me).

Anal. Calc. for $C_{16}H_{20}N_4O_7$: C, 50.52; H, 5.30; N, 14.73. Found: C, 51.02; H, 5.47; N, 14.50.

Eluted second was 2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranosyl nitrate (**8**; 0.36 g, 19%), m.p. 56–58°, $[\alpha]_D$ +29° (*c* 1, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.46–7.32 (m, 5 H, Ph), 5.53 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.65 and 4.55 (2 d, 2 H, *J* 12.0 Hz, PhCH_2), 4.24 (dd, 1 H, $J_{3,4}$ 5.5, $J_{4,5}$ 2.2 Hz, H-4), 4.15 (dd, 1 H, $J_{2,3}$ 7.2 Hz, H-3), 4.13 (m, 1 H, H-5), 3.85–3.73 (m, 2 H, 2 H-6), 3.96 (dd, 1 H, H-2), 1.56 and 1.37 (2 s, 6 H, 2 Me).

Anal. Calc. for $C_{16}H_{20}N_4O_7$: C, 50.52; H, 5.30; N, 14.73. Found: C, 50.71; H, 5.31; N, 14.88.

Column chromatography of fraction *B* (1:1 ethyl acetate–hexane) gave, first, *N*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranosylamine (**9**; 92 mg, 5%), $[\alpha]_D$ +51° (*c* 1.5, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.42–7.32 (m, 5 H, Ph), 6.50 (d, 1 H, $J_{1,NH}$ 9.5 Hz, NH), 5.06 (dd, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.65 and 4.54 (2 d, 2 H, *J* 12.2 Hz, PhCH_2), 4.24–4.15 (m, 2 H, H-3,4), 4.11 (m, 1 H, H-5), 3.78–3.67 (m, 2 H, 2 H-6), 3.44 (dd, 1 H, $J_{2,3}$ 6.8 Hz, H-2), 2.04 (s, 3 H, Ac), 1.56 and 1.36 (2 s, 6 H, 2 Me).

Anal. Calc. for $C_{18}H_{24}N_4O_5$: C, 57.44; H, 6.43; N, 14.88. Found: C, 57.27; H, 6.48; N, 14.75.

Eluted second was *N*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-3,4-*O*-isopropylidene-α-D-talopyranosylamine (**10**; 262 mg, 14%), m.p. 133–135° (from ethyl acetate–hexane), $[\alpha]_D$ +20° (*c* 1, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.42–7.32 (m, 5 H, Ph), 6.24 (d, 1 H, $J_{1,NH}$ 8.6 Hz, NH), 5.84 (dd, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.65 and 4.55 (2 d, 2 H, *J* 12.2 Hz, PhCH_2), 4.47 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 7.0 Hz, H-3), 4.34 (dd, 1 H, $J_{4,5}$ 1.5 Hz, H-4), 4.17 (ddd, 1 H, $J_{5,6a}$ 5.8, $J_{5,6b}$ 6.5 Hz, H-5), 3.92 (dd, 1 H, H-2), 3.70 (dd, 1 H, $J_{6a,6b}$ 9.2 Hz, H-6a), 3.64 (dd, 1 H, H-6b), 2.04 (s, 3 H, Ac), 1.53 and 1.35 (2 s, 6 H, 2 Me).

Anal. Calc. for $C_{18}H_{24}N_4O_5$: C, 57.44; H, 6.43; N, 14.88. Found: C, 57.42; H, 6.42; N, 14.97.

Methyl 2-azido-6-O-benzyl-2-deoxy-3,4-O-isopropylidene-β-D-galactopyrano-

side (**11**). — Sodium methoxide (324 mg, 6 mmol) was added to a stirred solution of **7** (0.76 g, 2 mmol) in anhydrous methanol (6 mL). After 4 h, the solution was neutralized with acetic acid and concentrated, and a solution of the residue in ethyl acetate was filtered through a bed of Celite and concentrated. The crude product was eluted from a column of silica gel with 2:1 hexane–ether (containing 0.5% of triethylamine), to give **11** (0.47 g, 68%), $[\alpha]_D$ +14° (*c* 1.4, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.46–7.34 (m, 5 H, Ph), 4.68 and 4.60 (2 d, 2 H, *J* 12.0 Hz, PhCH_2), 4.15 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.15 (dd, 1 H, $J_{3,4}$ 5.6, $J_{4,5}$ 2.2 Hz, H-4), 3.99–3.93 (m, 1 H, H-5), 3.95 (dd, 1 H, $J_{2,3}$ 7.8 Hz, H-3), 3.86–3.79 (m, 2 H, 2 H-6), 3.60 (s, 3 H, MeO), 3.40 (dd, 1 H, H-2), 1.55 and 1.36 (2 s, 6 H, 2 Me).

Anal. Calc. for $C_{17}H_{23}N_3O_5$: C, 58.44; H, 6.64; N, 12.03. Found: C, 58.71; H, 6.67; N, 12.23.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-β-*D*-galactopyranoside* (**2**). — A solution of **11** (0.70 g, 2 mmol) in aq. 80% acetic acid (15 mL) was stirred for 15 min at 100°, then cooled to room temperature, and concentrated, and toluene (3 × 20 mL) was evaporated from the residue. Column chromatography (1:1 ethyl acetate–hexane) of the crude product gave **2** (0.57 g, 92%), m.p. 70–71° (from ether–hexane), $[\alpha]_D$ +7° (*c* 1, chloroform), identical with the authentic compound[2].

*Methyl (2,3,4-tri-*O*-acetyl-α- and -β-*L*-idopyranosyl trichloroacetimidate)uronate* (**15** *and* **16**). — Silver carbonate (276 mg, 1 mmol) was added to a stirred solution of freshly prepared **13** (0.40 g, 1 mmol) in 25:1 acetone–water (10 mL). After 15 min at room temperature, the suspension was filtered through a bed of Celite and concentrated, and toluene (2 × 20 mL) was evaporated from the residue to leave crude **14**, a solution of which in anhydrous dichloromethane (5 mL) was cooled to 0° and treated with trichloroacetonitrile (1.00 mL, 10 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (150 μL, 1 mmol). The mixture was stirred for 30 min at 0° and 1 h at room temperature, then added to a column of silica gel, and eluted with 1:1 ethyl acetate–hexane (containing 0.5% of triethylamine) to give, first, **15** (0.30 g, 62% from **13**), $[\alpha]_D$ −43° (*c* 1, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 8.87 (s, 1 H, NH), 6.50 (m, 1 H, H-1), 5.25–5.22 (m, 1 H, H-4), 5.20–5.16 (m, 1 H, H-3), 5.05–5.02 (m, 2 H, H-2,5), 3.84 (s, 3 H, MeO), 2.18, 2.16, and 2.12 (3 s, 9 H, 3 Ac).

Anal. Calc. for $C_{15}H_{18}Cl_3NO_{10}$: C, 37.64; H, 3.79; N, 2.93. Found: C, 37.62; H, 3.92; N, 2.85.

Eluted second was **16** (14 mg, 3% from **13**) contaminated by **15**. ^{1}H-N.m.r. selected data (250 MHz, $CDCl_3$): δ 8.76 (s, 1 H, NH), 6.28 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 3.83 (s, 3 H, MeO), 2.19, 2.15, and 2.11 (3 s, 9 H, 3 Ac).

*Methyl 3,4-di-*O*-acetyl-β-*L*-idopyranuronate 1,2-[(methyl 2-azido-6-*O*-benzyl-2-deoxy-β-*D*-galactopyranosid-3-yl) orthoacetate]* (**18**). — A mixture of **2** (155 mg, 0.5 mmol), freshly prepared **13** (260 mg, 0.65 mmol), activated 4 Å powdered molecular sieve (0.20 g), and anhydrous dichloromethane (2 mL) was stirred for 10 min at room temperature and then cooled to −20°. 2,4,6-Trimethylpyridine (100 μL, 0.75 mmol) and silver triflate (192 mg, 0.75 mmol) were added, and the mixture

was stirred for 30 min at −20°, then applied to a column of silica gel, and eluted with 1:1 ethyl acetate–toluene (containing 0.5% of triethylamine) to give **18** (206 mg, 66%), $[\alpha]_D$ −42° (*c* 1, chloroform). N.m.r. data: ^{1}H (250 MHz, $CDCl_3$), δ 7.40–7.32 (m, 5 H, Ph), 5.64 (d, 1 H, $J_{1',2'}$ 2.8 Hz, H-1'), 5.40 (dd, 1 H, $J_{2',3'}$ 1.7, $J_{3',4'}$ 2.7 Hz, H-3'), 5.12 (ddd, 1 H, $J_{4',5'}$ 1.3, $J_{2',4'}$ 0.8 Hz, H-4'), 4.62 (s, 2 H, PhCH_2), 4.48 (d, 1 H, H-5'), 4.15 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.05 (ddd, 1 H, H-2'), 3.99 (ddd, 1 H, $J_{3,4}$ 2.8, $J_{4,5}$ ~0.6, $J_{4,OH}$ 2.2 Hz, H-4), 3.84 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.80 (s, 3 H, MeO), 3.74 (dd, 1 H, $J_{5,6b}$ 5.8 Hz, H-6b), 3.67 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 3.58 (dd, 1 H, H-2), 3.57 (s, 3 H, MeO), 2.56 (d, 1 H, OH), 2.16 and 2.08 (2 s, 6 H, 2 Ac), 1.86 (s, 3 H, Me); ^{1}H (250 MHz, $CDCl_3$ + CCl_3CONCO): δ 5.56 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ ~0.5 Hz, H-4); ^{13}C, δ 169.26, 168.15, and 167.11 (3 C=O), 137.79, 128.40, 127.75, and 127.68 (aromatic), 124.40 (CH_3C), 102.70 (C-1), 96.41 (C-1'), 74.14, 74.08, 73.35, 70.01, 68.31, 66.16, 66.04, and 61.66 (C-2,3,4,5,2',3',4',5'), 73.71 (PhCH_2), 69.30 (C-6), 56.86 (CH_3O), 52.61 ($COOCH_3$), 25.05 (CH_3C), 20.67 and 20.46 (2 CH_3CO).

Anal. Calc. for $C_{27}H_{35}N_3O_{14}$: C, 51.84; H, 5.64; N, 6.72. Found: C, 51.80; H, 5.55; N, 6.63.

*Methyl 3,4-di-O-acetyl-β-*L*-idopyranuronate 1,2-[(methyl 2-azido-4,6-O-benzylidene-2-deoxy-β-*D*-galactopyranosid-3-yl) orthoacetate*] (**19**). — Glycosylation of **3** (153 mg, 0.5 mmol), as for the preparation of **18**, gave, after column chromatography with 2:1 ethyl acetate–hexane (containing 0.5% of triethylamine), **19** (256 mg, 82%), m.p. 118° (softening at 110°) (from ethyl acetate–hexane), $[\alpha]_D$ +8.5° (*c* 1, chloroform). N.m.r. data: ^{1}H (250 MHz, $CDCl_3$): δ 7.61–7.56 and 7.46–7.38 (2 m, 5 H, Ph), 5.63 (s, 1 H, PhC*H*), 5.62 (d, 1 H, $J_{1',2'}$ 2.8 Hz, H-1'), 5.42 (dd, 1 H, $J_{2',3'}$ 1.7, $J_{3',4'}$ 2.6 Hz, H-3'), 5.13 (ddd, 1 H, $J_{4',5'}$ 1.3, $J_{2',4'}$ 0.8 Hz, H-4'), 4.49 (d, 1 H, H-5'), 4.36 (dd, 1 H, $J_{5,6a}$ 1.0, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.26 (dd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ ~0.5 Hz, H-4), 4.21 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.12 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 4.08 (ddd, 1 H, H-2'), 3.81 (s, 3 H, MeO), 3.80 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 3.71 (dd, 1 H, H-3), 3.59 (s, 3 H, MeO), 3.39 (ddd, 1 H, H-5), 2.12 and 2.10 (2 s, 6 H, 2 Ac), 1.86 (s, 3 H, Me); ^{13}C, δ 169.33, 168.29, and 167.11 (3 C=O), 137.66, 128.85, 128.03, and 126.15 (aromatic), 124.33 (CH_3C), 102.59 (C-1), 100.84 (PhCH), 96.29 (C-1'), 74.72, 74.08, 72.63, 70.00, 66.55, 66.22, 66.17, and 60.99 (C-2,3,4,5,2',3',4',5'), 68.96 (C-6), 56.79 (CH_3O), 52.56 ($COOCH_3$), 24.37 (CH_3C), 20.60 and 20.47 (2 CH_3CO).

Anal. Calc. for $C_{27}H_{33}N_3O_{14}$: C, 52.01; H, 5.33; N, 6.74. Found: C, 52.19; H, 5.45; N, 6.57.

Glycosylation of **2** *with* **13**. — A mixture of **2** (309 mg, 1 mmol), freshly prepared **13** (520 mg, 1.3 mmol), activated 4 Å powdered molecular sieve (0.40 g), and anhydrous dichloromethane (4 mL) was stirred for 10 min at room temperature and then cooled to −20°. Silver triflate (385 mg, 1.5 mmol) was added, the mixture was allowed to attain −5° during 1 h, then triethylamine (0.3 mL) was added, and the suspension was applied to a column of silica gel and eluted with ethyl acetate–hexane (from 2:3 to 2:1) to give, first, an ~1:1 mixture of known[2] **4** and methyl

3-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-β-D-galactopyranoside **5** (88 mg, 25%). ^{1}H-N.m.r. selected data (250 MHz, $CDCl_3$): δ 4.74 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.0 Hz, H-3), 4.26 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.17 (ddd, 1 H, $J_{4,5}$ ~0.6, $J_{4,OH}$ 3.7 Hz, H-4), 2.94 (d, 1 H, OH), 2.19 (s, 3 H, Ac).

Eluted second was **22** (100 mg, 16%), identical with the compound obtained from **20**.

Eluted third was an uncharacterized trisaccharide derivative (75 mg, 8%).

*Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-*L*-idopyranosyluronate)-β-*D*-galactopyranoside* (**20**). — (*a*) A mixture of **3** (307 mg, 1 mmol), freshly prepared **13** (520 mg, 1.3 mmol), activated 4 Å powdered molecular sieve (0.40 g), and anhydrous dichloromethane (4 mL) was stirred for 10 min at room temperature and then cooled to −15°. Silver triflate (385 mg, 1.5 mmol) was added, the mixture was allowed to attain room temperature during 2 h, then triethylamine (0.3 mL) was added, and the suspension was applied to a column of silica gel and eluted with 2:1 ethyl acetate–hexane (containing 0.5% of triethylamine) to give **20** (336 mg, 54%), $[\alpha]_D$ +19° (*c* 1, chloroform). N.m.r. data: ^{1}H (400 MHz, $CDCl_3$), δ 7.47–7.44 and 7.37–7.33 (2 m, 5 H, Ph), 5.44 (s, 1 H, PhC*H*), 5.24 (dd, 1 H, $J_{1',2'}$ 1.4, $J_{1',3'}$ ~1.0 Hz, H-1'), 5.09–5.07 (m, 2 H, H-4',5'), 5.02 (m, 1 H, H-3'), 4.88 (ddd, 1 H, $J_{2',3'}$ 3.0, $J_{2',4'}$ ~0.4 Hz, H-2'), 4.39 (dd, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ ~0.8 Hz, H-4), 4.31 (dd, 1 H, $J_{5,6a}$ 1.3, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.17 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.07 (dd, 1 H, $J_{5,6b}$ 1.7 Hz, H-6b), 3.79 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 3.63 (dd, 1 H, H-3), 3.57 and 3.43 (2 s, 6 H, 2 MeO), 3.40 (ddd, 1 H, H-5), 2.09, 2.05, and 1.99 (3 s, 9 H, 3 Ac); ^{13}C, δ 169.15, 168.98, 168.76, and 168.03 (4 C=O), 137.35, 128.91, 127.98, and 125.82 (aromatic), 102.12 (C-1), 100.58 (Ph*C*H), 99.74 (C-1'), 81.13, 74.01, 66.91, 66.77, 66.35, 66.12, 65.97, and 61.50 (C-2,3,4,5,2',3',4',5'), 68.88 (C-6), 20.57 and 20.36 (3 CH_3CO).

Anal. Calc. for $C_{27}H_{33}N_3O_{14}\cdot 0.5H_2O$: C, 51.26; H, 5.42; N, 6.64. Found: C, 51.45; H, 5.32; N, 6.58.

(*b*) A mixture of **3** (307 mg, 1 mmol), trichloroacetimidate **15** (622 mg, 1.3 mmol), activated 4 Å powdered molecular sieve (0.40 g), and anhydrous dichloromethane (4 mL) was stirred for 10 min at room temperature and then cooled to −20°. Trimethylsilyl triflate (250 μL, 1.3 mmol) was added, and the mixture was stirred for 15 min at −20°, then triethylamine (0.4 mL) was added, and the suspension was applied to a column of silica gel and eluted with 2:1 ethyl acetate–hexane (containing 0.5% of triethylamine) to give **20** (424 mg, 68%), identical with the compound obtained in (*a*).

*Methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-β-*D*-galactopyranoside* (**21**). — A mixture of **3** (307 mg, 1 mmol), freshly prepared **17** (520 mg, 1.3 mmol), activated 4 Å powdered molecular sieve (0.40 g), and anhydrous dichloromethane (4 mL) was stirred for 10 min at room temperature and then cooled to −15°. Silver triflate (385 mg, 1.5 mmol) was added, and the mixture was allowed to attain room temperature during 2 h, then triethylamine (0.3 mL) was added, and the suspension was applied to a column of

silica gel and eluted with 14:1 dichloromethane–acetone (containing 0.5% of triethylamine) to give **21** (530 mg, 85%), m.p. 179–180° (from ethyl acetate–hexane), $[\alpha]_D$ −12° (*c* 1, chloroform). ^{1}H-N.m.r. data (400 MHz, $CDCl_3$): δ 7.55–7.51 and 7.39–7.31 (2 m, 5 H, Ph), 5.55 (s, 1 H, PhC*H*), 5.30–5.23 (m, 2 H, H-3′,4′), 5.11–5.02 (m, 1 H, H-2′), 4.94 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.33 (dd, 1 H, $J_{5,6a}$ 1.2, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.30 (dd, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ ~0.5 Hz, H-4), 4.18 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.08–4.03 (m, 1 H, H-5′), 4.07 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.79 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 3.73 and 3.58 (2 s, 6 H, 2 MeO), 3.53 (dd, 1 H, H-3), 3.39 (ddd, 1 H, H-5), 2.06, 2.03, and 2.02 (3 s, 9 H, 3 Ac).

Anal. Calc. for $C_{27}H_{33}N_3O_{14}$: C, 52.01; H, 5.33; N, 6.74. Found: C, 52.16; H, 5.38; N, 6.72.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-β-*D*-galactopyranoside* (**23**). — A cold (0°), saturated solution of hydrogen chloride in anhydrous ether was added dropwise to a mixture, kept at 0°, of **21** (312 mg, 0.5 mmol), sodium cyanoborohydride (312 mg, 5 mmol), activated 4 Å powdered molecular sieve (0.50 g), Methyl Orange indicator (~10 mg), and anhydrous tetrahydrofuran (10 mL) until a persistent red colour was obtained. Stirring was continued for 3 h at 0°, then the mixture was diluted with dichloromethane (150 mL) and water, filtered, washed with water and then saturated aqueous sodium hydrogencarbonate, dried ($MgSO_4$), and concentrated. The residue was eluted from a column of silica gel with 1:1 ethyl acetate–hexane to give **23** (244 mg, 78%), m.p. 123–124° (from ethyl acetate–hexane), $[\alpha]_D$ −28° (*c* 1.2, chloroform). ^{1}H-N.m.r. data (400 MHz, $CDCl_3$): δ 7.37–7.28 (m, 5 H, Ph), 5.28 (dd, 1 H, $J_{2',3'}$ 9.0, $J_{3',4'}$ 9.4 Hz, H-3′), 5.22 (dd, 1 H, $J_{4',5'}$ 9.4 Hz, H-4′), 5.07 (dd, 1 H, $J_{1',2'}$ 7.8 Hz, H-2′), 4.84 (d, 1 H, H-1′), 4.59 and 4.55 (2 d, 2 H, *J* 11.8 Hz, PhCH_2), 4.14 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.06 (ddd, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ ~0.7, $J_{4,OH}$ 2.8 Hz, H-4), 4.04 (d, 1 H, H-5′), 3.80 (dd, 1 H, $J_{5,6a}$ 6.0, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.73 (dd, 1 H, $J_{5,6b}$ 6.0 Hz, H-6b), 3.71 and 3.56 (2 s, 6 H, 2 MeO), 3.61 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 3.58 (m, 1 H, $J_{5,OH}$ 1.3 Hz, H-5), 3.42 (dd, 1 H, H-3), 2.63 (dd, 1 H, OH), 2.08 and 2.03 (2 s, 9 H, 3 Ac).

Anal. Calc. for $C_{27}H_{35}N_3O_{14}$: C, 51.84; H, 5.64; N, 6.72. Found: C, 52.11; H, 5.57; N, 6.76.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-*L*-idopyranosyluronate)-β-*D*-galactopyranoside* (**22**). — Treatment of **20** (312 mg, 0.5 mmol), as for the preparation of **23**, gave, after similar work-up and purification, **22** (219 mg, 70%), $[\alpha]_D$ −57° (*c* 1, chloroform). ^{1}H-N.m.r. data (250 MHz, $CDCl_3$): δ 7.44–7.34 (m, 5 H, Ph), 5.43 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 5.24–5.19 (m, 2 H, H-3′,4′), 5.08 (m, 1 H, H-5′), 4.97 (m, 1 H, H-2′), 4.67–4.56 (m, 2 H, PhCH_2), 4.17 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.15 (ddd, 1 H, $J_{3,4} = J_{4,OH}$ = 3.0, $J_{4,5}$ ~0.5 Hz, H-4), 3.82 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 10.3 Hz, H-6a), 3.78 (s, 3 H, MeO), 3.75 (dd, 1 H, $J_{5,6b}$ 5.2 Hz, H-6b), 3.67 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 3.59 (s, 3 H, MeO), 3.58 (ddd, 1 H, H-5), 3.53 (dd, 1 H, H-3), 2.80 (d, 1 H, OH), 2.12 and 2.09 (2 s, 9 H, 3 Ac); (250 MHz, $CDCl_3$ + CCl_3CONCO): δ 5.44 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ ~0.6 Hz,

H-4), 5.38 (d, 1 H, $J_{1',2'}$ 2.8 Hz, H-1'), 5.24 (dd, 1 H, $J_{3',4'}$ 4.3, $J_{4',5'}$ 3.0 Hz, H-4'), 5.14 (dd, 1 H, $J_{2',3'}$ 5.0 Hz, H-3'), 5.03 (d, 1 H, H-5'), 4.94 (dd, 1 H, H-2').

Anal. Calc. for $C_{27}H_{35}N_3O_{14}$: C, 51.84; H, 5.64; N, 6.72. Found: C, 52.00; H, 5.82; N, 6.65.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-*D*-glucopyranosyluronate)-4-*O*-sulfo-β-*D*-galactopyranoside, sodium salt* (**25**). — A mixture of **23** (125 mg, 0.2 mmol), sulfur trioxide–trimethylamine complex (139 mg, 1 mmol), and anhydrous *N,N*-dimethylformamide (2 mL) was stirred for 36 h at 60°, and then cooled to room temperature. Methanol (1 mL) was added, and the mixture was eluted from a column (20 × 600 mm) of Sephadex LH-20 with 1:1 chloroform–methanol to give a product that was eluted from a short column (10 × 40 mm) of Dowex 50-X8-200 (Na^+ form) with 4:1 methanol–water to afford **25** (127 mg, 87%), $[\alpha]_D$ −7.8° (*c* 0.7, methanol). ^{1}H-N.m.r. data (400 MHz, CD_3OD): δ 7.37–7.22 (m, 5 H, Ph), 5.33 (dd, 1 H, $J_{2',3'}$ 9.0, $J_{3',4'}$ 9.5 Hz, H-3'), 5.21 (dd, 1 H, $J_{4',5'}$ 9.8 Hz, H-4'), 5.11 (dd, 1 H, $J_{1',2'}$ 7.8 Hz, H-2'), 5.03 (d, 1 H, H-1'), 4.81 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ ~0.5 Hz, H-4), 4.58 and 4.54 (2 d, 2 H, *J* 11.8 Hz, PhCH_2), 4.26 (d, 1 H, H-5'), 4.24 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.89 (dd, 1 H, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 10.5 Hz, H-6a), 3.79 (dd, 1 H, $J_{5,6b}$ 7.2 Hz, H-6b), 3.73 (ddd, 1 H, H-5), 3.70 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-3), 3.68 and 3.53 (2 s, 6 H, 2 MeO), 3.58 (dd, 1 H, H-2), 2.03, 2.00, and 1.97 (3 s, 9 H, 3 Ac).

Anal. Calc. for $C_{27}H_{34}N_3NaO_{17}S \cdot 0.5H_2O$: C, 44.03, H, 4.79; N, 5.70. Found: C, 44.17; H, 4.83; N, 5.67.

*Methyl 2-azido-6-*O*-benzyl-2-deoxy-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-α-*L*-idopyranosyluronate)-4-*O*-sulfo-β-*D*-galactopyranoside, sodium salt* (**24**). — Treatment of **22** (125 mg, 0.2 mmol), as for the preparation of **25**, gave, after similar work-up and purification, **24** (137 mg, 94%), $[\alpha]_D$ −30° (*c* 0.8, methanol). ^{1}H-N.m.r. data (400 MHz, CD_3OD): δ 7.37–7.22 (m, 5 H, Ph), 5.61 (d, 1 H, $J_{1',2'}$ 2.8 Hz, H-1'), 5.26 (dd, 1 H, $J_{2',3'}$ 4.0 Hz, H-2'), 5.12 (d, 1 H, $J_{4',5'}$ 3.0 Hz, H-5'), 5.08 (dd, 1 H, $J_{3',4'}$ 5.8 Hz, H-4'), 4.98 (dd, 1 H, H-3'), 4.67 (dd, 1 H, $J_{3,4}$ 3.0, $J_{4,5}$ ~0.5 Hz, H-4), 4.58 and 4.53 (2 d, 2 H, *J* 12.0 Hz, PhCH_2), 4.24 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.88 (dd, 1 H, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 9.7 Hz, H-6a), 3.82–3.74 (m, 2 H, H-5,6b), 3.71 (s, 3 H, MeO), 3.68 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.53 (s, 3 H, MeO), 2.12, 2.06, and 2.01 (3 s, 9 H, 3 Ac).

Anal. Calc. for $C_{27}H_{34}N_3NaO_{17}S$: C, 44.57; H, 4.71; N, 5.77. Found: C, 44.55; H, 4.91; N, 5.71.

*Methyl 2-acetamido-2-deoxy-3-*O*-(β-*D*-glucopyranosyluronic acid)-4-*O*-sulfo-β-*D*-galactopyranoside, disodium salt* (**27**). — A solution of **25** (145 mg, 0.2 mmol) in 9:1 methanol–water (10 mL) was treated with 6M sodium hydroxide (1 mL) for 3 h at room temperature, then applied to a short column (10 × 40 mm) of Dowex 50-X8-200 (H^+ form), and eluted with 9:1 methanol–water to give a product that was neutralized with saturated aqueous sodium hydrogencarbonate, then concentrated. A solution of the residue in 9:1 *tert*-butyl alcohol–water (10 mL) was stirred with 10% Pd/C (150 mg) under hydrogen for 36 h, then filtered, and partially con-

centrated. The solution was treated with acetic anhydride (100 μL) and aqueous saturated sodium hydrogencarbonate (0.5 mL), with the operation being repeated three times, then concentrated. The residue was added to a column (20 × 800 mm) of Sephadex G-10 and eluted with water to give crude **27** which was further purified by i.e.c.–h.p.l.c. on a Mono-Q column (10 × 100 mm), using a linear sodium chloride gradient (from 0.2 to 0.6M). After desalting and lyophilization, **27** (75 mg, 69%) was obtained as a white powder, $[\alpha]_D$ −33° (*c* 0.5, water). ^{1}H-N.m.r. data (400 MHz, D_2O): δ 4.79 (dd, 1 H, $J_{3,4}$ 2.5, $J_{4,5}$ ~0.6 Hz, H-4), 4.48 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.47 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.08 (dd, 1 H, $J_{2,3}$ 11.0 Hz, H-3), 4.04 (dd, 1 H, H-2), 3.85–3.78 (m, 3 H, H-5,6a,6b), 3.66 (d, 1 H, $J_{4',5'}$ 9.8 Hz, H-5′), 3.54 (dd, 1 H, $J_{3',4'}$ 8.8 Hz, H-4′), 3.52 (s, 3 H, MeO), 3.46 (dd, 1 H, $J_{2',3'}$ 9.2 Hz, H-3′), 3.36 (dd, 1 H, H-2′), 2.02 (s, 3 H, Ac).

Anal. Calc. for $C_{15}H_{23}NNa_2O_{15}S \cdot 2.5H_2O$: C, 31.04; H, 4.86; N, 2.41. Found: C, 30.99; H, 5.04; N, 2.46.

*Methyl 2-acetamido-2-deoxy-3-*O*-(α-*L*-idopyranosyluronic acid)-4-*O*-sulfo-β-*D*-galactopyranoside, disodium salt* (**26**). — Treatment of **24** (145 mg, 0.2 mmol), as for the preparation of **27**, gave, after similar work-up and purification, **26** (46 mg, 43%) as a white powder, $[\alpha]_D$ −30° (*c* 0.4, water), identical with the authentic compound[2].

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