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SYNTHESIS, FROM CELLOBIOSE, OF A TRISACCHARIDE CLOSELY RELATED TO THE GlcNAc→GlcA→GlcN SEGMENT OF THE ANTITHROMBIN-BINDING SEQUENCE OF HEPARIN†

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ABSTRACT

O-(2-Deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-1,6-anhydro-2-deoxy-2-sulfamido-6-*O*-sulfo-β-D-glucopyranose pentasodium salt (**14**) was synthesized as a heparin-related oligosaccharide. The glycosyl acceptor (derived from cellobiose) and a glycosyl donor, 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide, were coupled in the presence of mercuric bromide and molecular sieves 4A to afford a 69% yield of fully protected trisaccharide, namely, *O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-3-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**10**), which was converted into the partially sulfated trisaccharide **14**. Compound **10** also underwent acetolysis to afford the glycosyl acetate, for further elongation of the glycosyl chain.

INTRODUCTION

The mucopolysaccharide heparin shows blood-anticoagulant activity by binding to the protease inhibitor antithrombin III, thereby accelerating inactivation of the serine proteases involved in the blood coagulation[2]. In 1982, Lindahl and his collaborators[3] proposed that the pentasaccharide **1** is the antithrombin III-binding sequence of heparin. This oligosaccharide is attracting much attention from the viewpoint of its antithrombotic activity[4].

In the course of our studies toward the total synthesis of **1**, we have developed several new reactions involving cellobiose[1,5,6], with the expectation that the disaccharide might be utilizable for preparation of the building blocks of **1**. We now describe the synthesis, employing cellobiose as one of the starting materials, of **14**, which can be approximately regarded as a close analog of the 1,6-anhydro derivative of the A–B–C unit in **1**. In addition, the fully protected intermediate **10** under-

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went acetolysis to afford **15**, which might be an important synthon for the preparation of a pentasaccharide closely resembling* **1**.

RESULTS AND DISCUSSION

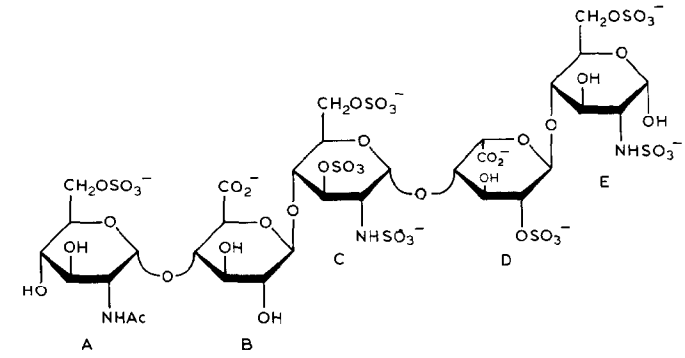
We succeeded in partial protection of the 2′- and 3′-hydroxyl groups of 1,6-anhydro-4′,6′-*O*-benzylidenecellobiose *via* regioselective silylation at O-2 and O-3 with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[1]. The 2′,3′-di-*O*-benzyl derivative (**2**) obtained by this procedure was the main starting material for the synthesis of **14**.

Introduction of an azido group at C-2 of **2** was the first functional manipulation used. Employing the Hicks–Fraser-Reid procedure[7], **2** was treated with 1-tosylimidazole in chloroform–hexane in the presence of sodium methoxide, to give the expected epoxide **4** in good yield, along with some recovery of **2**. The D-*manno* configuration of the monosaccharide moiety carrying the epoxy group was confirmed on the basis of its ^{1}H-n.m.r. spectrum. Opening the epoxy ring of **4** with azide anion was not easy; employment of ethanol–water, ethylene glycol–water, or ethylene glycol monomethyl ether–water as the solvent led to no reaction. However, the reaction proceeded when **4** was treated with sodium azide in *N,N*-dimethylformamide (DMF)–water in the presence of ammonium chloride, during 2 days at 100°, and the resulting alcohol, without isolation, was acetylated, giving **3** in 64% overall yield from **4**. Removal of the benzylidene group in **3** was achieved by treatment with cupric chloride dihydrate[8] in oxolane–ethanol, to afford diol **5** in high yield.

The second functional manipulation was oxidation of the 5′-(hydroxymethyl) group to the corresponding uronate. Prior to such oxidation, the protection of the 4′-hydroxyl group with a selectively removable group was necessary for the subsequent glycosidation. Compound **5** was tritylated in the usual way, and the ether treated with monochloroacetic anhydride, to give the 4′-*O*-(chloroacetyl)-6′-*O*-trityl derivative (**6**) in 79% overall yield. A series of reactions for the conversion of the 6′-*O*-trityl compound into the corresponding uronate was performed continuously, without characterization of the intermediates as described in our previous paper[6]. After *O*-detritylation with perchloric acid, the resulting alcohol was subjected to Jones oxidation to the uronic acid, which was esterified with diazomethane to afford **7** in 33% overall yield from **6**. The monochloroacetyl group was selectively removed from **7** by treatment with thiourea[9], giving **8** in 71% yield.

The next step was the glycosidation of **8** with **9**, which was prepared from D-glucose according to the Paulsen–Stenzel procedure[10]. When **8** was treated with **9** in dichloromethane, in the presence[11] of mercuric bromide and molecular sieves 4A, the condensation proceeded readily, to afford trisaccharide **10** in 69% yield. Its

*The only difference from **1** is the presence of the sulfated amino group in the nonreducing end, instead of the acetylated one in **1**.



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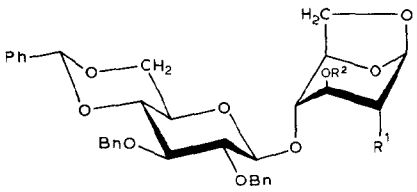
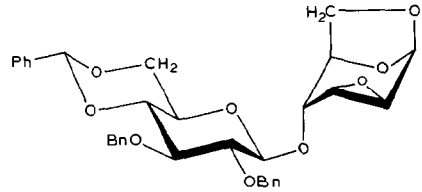


2 R^1 = OH, R^2 = H
3 R^1 = N_3, R^2 = Ac



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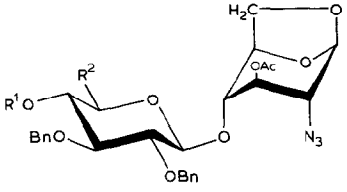
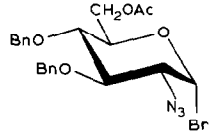


5 R^1 = H, R^2 = CH_2OH
6 R^1 = $COCH_2Cl$, R^2 = CH_2OTr
7 R^1 = $COCH_2Cl$, R^2 = CO_2Me
8 R^1 = H, R^2 = CO_2Me



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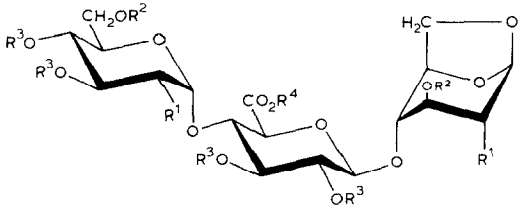
^{1}H-n.m.r. spectrum exhibited the signals due to H-1″ and H-2″ at δ 5.53 as a doublet with $J_{1'',2''}$ 3.90 Hz, and δ 3.26 as a doublet of doublets with J 3.90 and $J_{2'',3''}$ 10.3 Hz, respectively. These data revealed that the newly produced glycosidic bond was α-oriented.

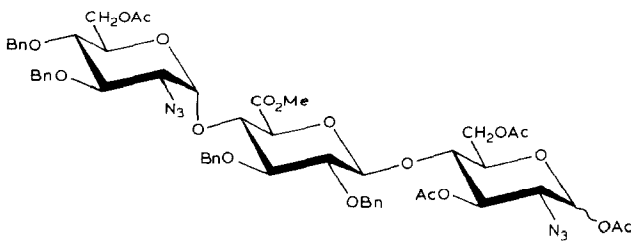
Sulfations (*O* and *N*) were performed in two steps. Basic hydrolysis of the *O*-acetyl groups in **10** was accompanied by concomitant hydrolysis of the methyl

carboxylate. The re-esterification of the resulting carboxylic acid with diazomethane in methanol–ether gave **11** in 69% overall yield from **10**. Sulfation of the free hydroxyl groups in **11** was achieved by treatment with the sulfur trioxide–trimethylamine complex in DMF[12]. After separation of the reaction mixture with Dowex 50W-X8 (Na^+) resin, the 3,6″-disulfate (**12**) was obtained in 74% yield. Simultaneous reduction of the azido groups and hydrogenolysis of the *O*-benzyl groups in **12** was achieved by catalytic hydrogenation in the presence of 10% Pd/C in 3:1:1 (v/v) methanol–acetic acid–water, and subsequent purification in an Avicell column, to give **13** in 51% yield. Selective *N*-sulfation of **13** was performed by treatment with a large excess of sulfur trioxide–trimethylamine complex in aqueous sodium hydroxide[12] at pH 9.5, and subsequent hydrolysis of the methyl carboxylate with sodium hydroxide at pH 12, to give **14** in 61% yield. Its structure was elucidated on the basis of the ^{1}H-n.m.r. spectrum, mass spectra (s.i.-m.s. and f.a.b.- m.s.), and elemental analysis. Compound **14** showed a very faint anti-(Factor Xa) activity[13].



10 $R^1 = N_3, R^2 = Ac, R^3 = Bn, R^4 = Me$
11 $R^1 = N_3, R^2 = H, R^3 = Bn, R^4 = Me$
12 $R^1 = N_3, R^2 = SO_3Na, R^3 = Bn, R^4 = Me$
13 $R^1 = NH_2, R^2 = SO_3Na, R^3 = H, R^4 = Me$
14 $R^1 = NHSO_3Na, R^2 = SO_3Na, R^3 = H, R^4 = Na$

The fully protected trisaccharide **10** may also be regarded as a key compound for further elongation of the glycosyl chain. It was found that **10** underwent acetolysis with 6% trifluoroacetic acid–acetic anhydride[10], to give the glycosyl acetate **15** in 60% yield. Compound **15**, or the corresponding glycosyl halide derived from it, should be able to play an important role in the elongation reaction as a new glycosyl donor.



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EXPERIMENTAL

General methods. — Melting points were determined with a Yamato micro melting-point apparatus, and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241MC polarimeter. Chromatography was performed on columns of Silica Gel Merck (70–230 mesh; E. Merck, Darmstadt, Germany). Thin-layer chromatography was conducted on precoated plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany) of Silica Gel $60F_{254}$. Preparative thin-layer chromatography was performed with precoated plates (layer thickness, 2 mm; E. Merck, Darmstadt, Germany) of Silica Gel $60F_{254}$. I.r. spectra of compounds as Nujol mulls were recorded with a Shimadzu IR-27 spectrophotometer. ^{1}H-N.m.r. spectra were recorded with a JEOL JNM-FX 400 or a JNM-GX 400 spectrometer, using tetramethylsilane as the internal standard, for solutions in chloroform-*d*. Secondary ion (s.i.) mass spectra were recorded with a Hitachi H80 spectrometer at an ionizing voltage of 3 kV (primary ion) and 8–9 kV (secondary ion); each sample was applied as a glycerol matrix. The fast-atom bombardment (f.a.b.) mass spectra were recorded with a JEOL DX300 spectrometer in the negative-ion mode; each sample was applied as a glycerol matrix and bombarded with xenon atoms having a kinetic energy equivalent to 3 keV. Solutions were evaporated under diminished pressure; and solvent extracts were dried with magnesium sulfate.

O-(2,3-Di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6:2,3-dianhydro-β-D-mannopyranose (**4**). — A mixture of **2** (ref. 1) (4.5 g, 7.6 mmol), 1-tosylimidazole[7] (3.8 g, 16.7 mmol), sodium methoxide (1.1 g, 20.4 mmol) in chloroform (60 mL, alcohol-free), and hexane (60 mL) was boiled under reflux for 5.5 h. The mixture was cooled, diluted with chloroform, washed with water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 20:1 (v/v) toluene–ethyl acetate as the eluant, to give **4** (2.89 g, 91% based on the **2** consumed); m.p. 185.0–186.5° (from chloroform–ether), $[\alpha]_D^{25}$ −37° (*c* 0.46, chloroform); δ_H: 3.38 (m, 1 H, H-3), 3.43 (ddd, 1 H, *J* 4.15, 4.89, and 9.30 Hz, H-5′), 3.49 (dd, 1 H, *J* 2.93 and 3.17 Hz, H-2), 3.54 (dd, 1 H, *J* 7.57 and 9.30 Hz, H-2′), 3.68–3.82 (m, 5 H, H-6a,6b,3′,4′,6′a), 3.92 (s, 1 H, H-4), 4.35 (dd, 1 H, *J* 5.13 and 10.5 Hz, H-6′b), 4.45 (m, 1 H, H-5), 4.67 (d, 1 H, *J* 7.57 Hz, H-1′), 4.82–4.92 (m, 4 H, benzyl groups), 5.58 (s, 1 H, benzylidene), and 5.74 (s, 1 H, H-1).

Anal. Calc. for $C_{33}H_{34}O_9$: C, 68.98; H, 5.96. Found: C, 68.95; H, 5.94.

O-(2,3-Di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**3**). — A mixture of **4** (1.2 g, 2.1 mmol), sodium azide (900 mg, 14 mmol), and ammonium chloride (1.5 g, 28 mmol) in *N,N*-dimethylformamide (26 mL) and water (4 mL) was heated for 37 h at 100°. The mixture was cooled, poured into ice–water, and extracted with ethyl acetate. The extracts were combined, washed with water, dried, and evaporated. Acetic anhydride (2 mL) was added to a cooled solution of the residue in dichloromethane (10 mL) and pyridine (4 mL) at 0–5°, and the mixture was stirred

for 3 h at room temperature, evaporated, and the residual syrup chromatographed on silica gel with 20:1 (v/v) toluene–ethyl acetate as the eluant, to give **3** (884 mg, 64%) as an amorphous powder; $[\alpha]_D^{22}$ −9° (*c* 0.44, chloroform); ν_{max} 2140 and 1730 cm^{-1}; δ_H: 2.11 (s, 3 H, -OAc), 3.22 (s, 1 H, H-2), 3.41–3.48 (m, 1 H, H-5'), 4.67 (d, 1 H, *J* 7.57 Hz, H-1'), 5.28 (m, 1 H, H-3), 5.49 (s, 1 H, H-1), and 5.58 (s, 1 H, benzylidene).

Anal. Calc. for $C_{35}H_{37}N_3O_{10}$: C, 63.72; H, 5.65; N, 6.37. Found: C, 63.85; H, 5.65; N, 5.75.

O-*(2,3-Di-O-benzyl-β-D-glucopyranosyl)-(1→4)-3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose* (**5**). — A mixture of **3** (773 mg, 1.17 mmol) and cupric chloride dihydrate[8] (1.40 g, 8.2 mmol) in oxolane (10 mL) and ethanol (16 mL) was refluxed for 1 h. The mixture was cooled, made neutral by addition of saturated sodium hydrogencarbonate, and filtered. The filtrate was extracted with chloroform, and the extracts were dried (without being washed with water), and evaporated. The residual syrup was chromatographed on silica gel, with 100:1 (v/v) chloroform–methanol as the eluant, to give **5** (672 mg, 94%); m.p. 84–86° (from ethanol), $[\alpha]_D^{22}$ +18° (*c* 0.57, chloroform); ν_{max} 3500 cm^{-1}.

Anal. Calc. for $C_{28}H_{33}N_3O_{10}$: C, 58.84; H, 5.82; N, 7.35. Found: C, 58.60; H, 5.83; N, 7.19.

O-*(2,3-Di-O-benzyl-4-O-(chloroacetyl)-6-O-trityl-β-D-glucopyranosyl)-(1→4)-3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose* (**6**). — A mixture of **5** (672 mg, 1.2 mmol) and trityl chloride (492 mg, 1.8 mmol) in pyridine (20 mL) was heated for 4 h at 85°. Chloroacetic anhydride (410 mg, 2.4 mmol) was then added portionwise to the cold mixture at 0–5°; the mixture was stirred for 30 min at room temperature, poured into ice–water, and extracted with ethyl acetate. The extracts were combined, successively washed with cold, dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 5:1 (v/v) hexane–ethyl acetate as the eluant, to give **6** (951 mg, 79%); m.p. 162–163° (from ethyl acetate–hexane), $[\alpha]_D^{21}$ +2° (*c* 0.54, chloroform); δ_H: 1.94 (s, 2 H, -COCH_2Cl) and 5.56 (s, 1 H, H-1).

Anal. Calc. for $C_{49}H_{48}ClN_3O_{11}$: C, 66.02; H, 5.54; Cl, 3.98; N, 4.71. Found: C, 66.14; H, 5.37; Cl, 3.98; N, 4.72.

O-*(Methyl 2,3-di-O-benzyl-4-O-(chloroacetyl)-β-D-glucopyranosyluronate)-(1→4)-3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose* (**7**). — A mixture of **6** (869 mg, 0.98 mmol) and 60% perchloric acid (2 mL) in chloroform (60 mL) was stirred for one min at room temperature, and then diluted with chloroform. The organic layer was successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. Jones's reagent[14] (4M chromium trioxide, 10 mL) was added to a cold solution of the residue in acetone (150 mL) at 0–5°, and the mixture was stirred for 50 min at room temperature. The excess of reagent was decomposed by addition of methanol, the mixture was evaporated, the residue was suspended in ethyl acetate and water, and the aqueous layer

was extracted with ethyl acetate. The organic layers were combined, successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 9:1 (v/v) chloroform–methanol as the eluant, to give the uronic acid, which was esterified with diazomethane in dichloromethane–ether, to afford **7** (214 mg, 33%); m.p. 130–131° (from ethanol), $[\alpha]_D^{22}$ +17° (*c* 0.57, chloroform); δ_H: 2.10 (s, 3 H, -OAc), 3.23 (s, 1 H, H-2), 3.64–3.84 (m, 6 H), 3.72 (s, 3 H, -COOCH_3), 3.95 (d, 1 H, *J* 10.0 Hz, H-5′), 4.01 (d, 1 H, *J* 7.57 Hz, H-6a), 4.58 (d, 1 H, *J* 5.37 Hz, H-5), 4.68 (d, 1 H, *J* 7.57 Hz, H-1′), 5.17 (dd, 1 H, *J* 9.77 and 10.0 Hz, H-4′), 5.27 (m, 1 H, H-3), and 5.50 (s, 1 H, H-1).

Anal. Calc. for $C_{31}H_{34}ClN_3O_{12}$: C, 55.07; H, 5.07; Cl, 5.24; N, 6.22. Found: C, 54.79; H, 5.08; Cl, 5.65; N, 5.77.

O-*(Methyl 2,3-di*-O-*benzyl-β*-D-*glucopyranosyluronate)-(1→4)-3*-O-*acetyl-1,6-anhydro-2-azido-2-deoxy-β*-D-*glucopyranose* (**8**). — A mixture of **7** (208 mg, 0.31 mmol) and thiourea[9] (27 mg, 0.35 mmol) in ethanol (7 mL) was boiled under reflux for 6 h, cooled, evaporated to half volume, and diluted with dichloromethane. The organic layer was successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The product (**8**) was purified by preparative t.l.c., with 2:1 (v/v) benzene–ethyl acetate, to give amorphous, powdery **8** (131 mg, 71%); $[\alpha]_D^{25}$ +8° (*c* 0.05, chloroform); ν_{max} 3460 cm^{-1}.

Anal. Calc. for $C_{29}H_{33}N_3O_{11} \cdot 0.5\ H_2O$: C, 57.23; H, 5.63; N, 6.90. Found: C, 57.62; H, 5.56; N, 6.25.

O-*(6*-O-*Acetyl-2-azido-3,4-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-(1→4)*-O-*(methyl 2,3-di*-O-*benzyl-β*-D-*glucopyranosyluronate)-(1→4)-3*-O-*acetyl-1,6-anhydro-2-azido-2-deoxy-β*-D-*glucopyranose* (**10**). — A solution of **9** (ref. 10) (205 mg, 0.44 mmol) in dichloromethane (5 mL) was added to a mixture of **8** (131 mg, 0.22 mmol), mercuric bromide (48 mg, 0.13 mmol), and molecular sieves 4A (1.2 g) in dichloromethane (2 mL) under an argon atmosphere, and the mixture was stirred for 3 days at room temperature. The mixture was filtered, and the filtrate was successively washed with aqueous silver nitrate, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The product (**10**) was purified by preparative t.l.c., with 4:1 (v/v) benzene–ethyl acetate, to give amorphous, powdery **10** (91 mg, 69% based on the consumed **8**); $[\alpha]_D^{25}$ +28° (*c* 0.66, chloroform); δ_H: 2.03 (s, 3 H, -OAc), 2.10 (s, 3 H, -OAc), 3.21 (s, 1 H, H-2), 3.26 (dd, 1 H, *J* 3.60 and 10.5 Hz, H-2″), 3.51 (t, 1 H, *J* 8.79 Hz, H-4″), 3.64 (m, 1 H, H-4), 3.75 (s, 3 H, -COOCH_3), 3.88 (dd, 1 H, *J* 8.79 and 10.3 Hz, H-3″), 3.96 (d, 1 H, *J* 6.86 Hz, H-6a), 4.15 (t, 1 H, *J* 8.79 Hz, H-4′), 4.67 (d, 1 H, *J* 7.57 Hz, H-1′), 5.22 (m, 1 H, H-3), 5.47 (s, 1 H, H-1), and 5.53 (d, 1 H, *J* 3.90 Hz, H-1″).

Anal. Calc. for $C_{51}H_{56}N_6O_{16}$: C, 60.71; H, 5.59; N, 8.33. Found: C, 60.93; H, 5.68; N, 7.87.

O-*(2-Azido-3,4-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-(1→4)*-O-*(methyl 2,3-di*-O-*benzyl-β*-D-*glucopyranosyluronate)-(1→4)-1,6-anhydro-2-azido-2-deoxy-β*-D-*glucopyranose* (**11**). — Aqueous sodium hydroxide (10 mL, M solution) was

added to a cold solution of **10** (300 mg, 0.30 mmol) in methanol (60 mL) at 0–5°; the mixture was stirred for 3 h at room temperature, treated with Dowex 50W-X8 (H^+) resin, the mixture filtered, and the filtrate evaporated. The residue was re-esterified with diazomethane in methanol–ether, and the solvent was evaporated. The product (**11**) was purified by preparative t.l.c., with 10:1 (v/v) benzene–methanol, to give amorphous, powdery **11** (189 mg, 69%); $[\alpha]_D^{22}$ +10° (*c* 0.73, chloroform); ν_{max} 3500 (OH), 2130 (N_3), and 1755 cm^{-1} (CO_2CH_3); δ_H: 3.24 (dd, 1 H, *J* 3.90 and 9.03 Hz, H-2″), 3.26 (d, 1 H, *J* 4.64 Hz, H-2), 3.79 (s, 3 H, -CO_2CH_3), 3.86 (d, 1 H, *J* 4.40 Hz, H-3), 4.00 (d, 1 H, *J* 9.52 Hz, H-5′), 4.60 (d, 1 H, *J* 7.57 Hz, H-1′), 5.39 (s, 1 H, H-1), and 5.46 (d, 1 H, *J* 3.90 Hz, H-1″).

Anal. Calc. for $C_{47}H_{52}N_6O_{14}$: C, 61.03; H, 5.67; N, 9.09. Found: C, 61.05; H, 5.79; N, 8.43.

*O-(2-Azido-3,4-di-O-benzyl-2-deoxy-6-O-sulfo-α-*D*-glucopyranosyl)-(1→4)-O-(methyl 2,3-di-O-benzyl-β-*D*-glucopyranosyluronate)-(1→4)-1,6-anhydro-2-azido-2-deoxy-3-O-sulfo-β-*D*-glucopyranose, disodium salt* (**12**). — Sulfur trioxide–trimethylamine complex (77 mg, 0.55 mmol) was added to a cooled solution of **11** (170 mg, 0.18 mmol) in *N,N*-dimethylformamide[12] (2 mL) at 0°, and the mixture was stirred for 5 h at room temperature. Another portion of the sulfur trioxide–trimethylamine complex (50 mg, 0.37 mmol) was added, and the mixture was stirred for 5 h at 50°, evaporated, and the residue chromatographed on a column of Dowex 50W-X8 (Na^+) resin, with methanol as the eluant, to give a crude **12**. It was re-chromatographed on silica gel, with 9:1 (v/v) chloroform–methanol as the eluant, and further purification was achieved by preparative t.l.c., with 5:2 (v/v) chloroform–methanol, to give amorphous, powdery **12** (154 mg, 74%); ν_{max} 2130 (N_3), 1740 (CO_2CH_3), 1260, and 1030 cm^{-1} (-OSO_3).

*O-(2-Amino-2-deoxy-6-O-sulfo-α-*D*-glucopyranosyl)-(1→4)-O-(methyl β-*D*-glucopyranosyluronate)-(1→4)-2-amino-1,6-anhydro-2-deoxy-3-O-sulfo-β-*D*-glucopyranose, disodium salt* (**13**). — A mixture of **12** (140 mg, 0.12 mmol) and 10% Pd/C (200 mg) in methanol (18 mL), acetic acid (6 mL), and water (6 mL) was shaken under a hydrogen atmosphere for 9 h at room temperature. The mixture was filtered and the filtrate was evaporated. The residual syrup was chromatographed on a column of Avicel, with 8:3:3 (v/v) butanol–acetic acid–water as the eluant, to give amorphous, powdery **13** (45 mg, 51%); ν_{max} 3450 (OH and NH_2), 1745 (CO_2CH_3), and 1050 cm^{-1} (-OSO_3); δ_H (D_2O): 4.71 (d, 1 H, *J* 8.06 Hz, H-1′), 5.60 (d, 1 H, *J* 3.06 Hz, H-1″), and 5.65 (s, 1 H, H-1).

*O-(2-Deoxy-2-sulfamido-6-O-sulfo-α-*D*-glucopyranosyl)-(1→4)-O-(β-*D*-glucopyranosyluronic acid)-(1→4)-1,6-anhydro-2-deoxy-2-sulfamido-3-O-sulfo-β-*D*-glucopyranose, pentasodium salt* (**14**). — Sulfur trioxide–trimethylamine complex (54 mg, 0.39 mmol) was added to a cooled solution of **13** (28 mg, 0.04 mmol) in aqueous sodium hydroxide (3 mL) at[12] pH 9.5 and 0–5°, and the mixture was stirred for 96 h at room temperature. During this time, more sulfur trioxide–trimethylamine complex (81 mg, 0.57 mmol) was added to the mixture in three portions. Aqueous sodium hydroxide (M solution) was added to the mixture, to pH

12, and the mixture was stirred for 6 h at room temperature. After neutralization to pH 7.5 by addition of dilute hydrochloric acid (M solution), the mixture was freeze-dried. The residue was chromatographed on a gel-permeation column (Sephadex G-25), with water as the eluant, to give crude **14**. This was passed through a column of Dowex 50W-X8 (Na^+) resin, with methanol as the eluant, to give amorphous, powdery **14** (23 mg, 61%); $[\alpha]_D^{22}$ +9° (*c* 0.50, water); δ_H (D_2O): 3.20 (dd, 1 H, *J* 3.66 and 9.88 Hz, H-2″), 3.41 (dd, 1 H, *J* 7.81 and 8.79 Hz, H-2′), 3.43 (s, 1 H, H-2), 3.51–3.80 (m, 5 H), 3.53 (dd, 1 H, *J* 2.81 and 9.88 Hz, H-3″), 3.78 (dd, 1 H, *J* 5.37 and 8.05 Hz, H-6b), 4.09–4.17 (m, 3 H), 4.30 (dd, 1 H, *J* 2.19 and 8.79 Hz, H-4′), 4.57 (s, 1 H, H-3), 4.63 (d, 1 H, *J* 7.81 Hz, H-1′), 4.74 (d, 1 H, *J* 5.37 Hz, H-5), 5.58 (s, 1 H, H-1), and 5.60 (d, 1 H, *J* 3.66 Hz, H-1″); *m/z* (s.i.-m.s.) 928 [$(M)^+$, calc. for $C_{18}H_{25}N_2Na_5O_{26}S_4$: 928.25], (f.a.b.-m.s.) 905 [$(M-Na)^-$, calc. for $C_{18}H_{25}N_2Na_4O_{26}S_4$: 905.59].

Anal. Calc. for $C_{18}H_{25}N_2Na_5O_{26}S_4 \cdot H_2O$: C, 22.41; H, 3.03; N, 2.90. Found: C, 522.23; H, 3.03; N, 2.90.

O-*(6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-α-*D*-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-β-*D*-glucopyranosyluronate)-(1→4)-1,3,6-tri-*O*-acetyl-2-azido-2-deoxy-*D*-glucopyranose* (**15**). — A mixture of **10** (47 mg, 0.05 mmol) and trifluoroacetic acid[10] (0.3 mL) in acetic anhydride (5 mL) was stirred for 10 h at room temperature. The mixture was evaporated, and the residue was purified by preparative t.l.c., with 6:1 (v/v) benzene–ethyl acetate, to give amorphous powdery **15** (31 mg, 60%) as the anomeric mixture ($\alpha:\beta$ = 6:1) at C-1; $[\alpha]_D^{20}$ +49° (*c* 0.46, chloroform); δ_H (α anomer): 2.03 (s, 3 H, -OAc), 2.07 (s, 3 H, -OAc), 2.10 (s, 3 H, -OAc), 2.20 (s, 3 H, -OAc), 3.28 (dd, 1 H, *J* 3.66 and 10.3 Hz, H-2″), 3.76 (s, 3 H, $-CO_2CH_3$), 5.41 (dd, 1 H, *J* 8.54 and 10.7 Hz, H-3), 5.50 (d, 1 H, *J* 3.67 Hz, H-1″), and 6.22 (d, 1 H, *J* 3.66 Hz, H-1).

Anal. Calc. for $C_{55}H_{62}N_6O_{19} \cdot H_2O$: C, 58.51; H, 5.71; N, 7.44. Found: C, 58.44; H, 5.66; N, 6.99.

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