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SYNTHESIS OF HEPARIN FRAGMENTS: A METHYL α-PENTAOSIDE WITH HIGH AFFINITY FOR ANTITHROMBIN III*

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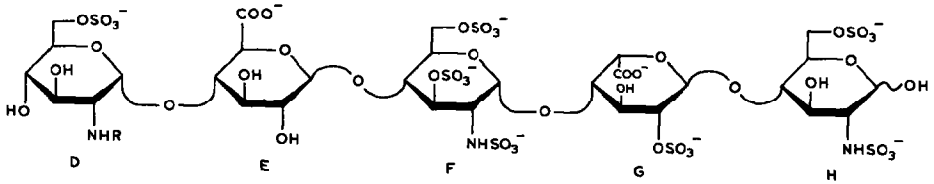
ABSTRACT

The synthesis is described of the methyl α-glycoside of the pentasaccharide which represents the sequence in heparin responsible for binding and activation of the anticoagulant protein Antithrombin III. It was obtained in a yield much better than that of the previously synthesised pentasaccharide and exhibited the same biological properties.

INTRODUCTION

The pentasaccharide sequence **1** is required in heparin for binding to the plasma protein Antithrombin III (AT III), thus eliciting the well known anticoagulant properties of the polysaccharide[1,2]. We have synthesised[3] the pentasaccharide **1** (R = SO_3^-) which binds to AT III with an affinity the same as that of high-affinity heparin species[4] and induces a high anti-factor-Xa activity in plasma and antithrombotic activity *in vivo* in animal models[5].

A late step in the synthesis of **1** (R = SO_3^-) involved hydrogenolysis of the benzyl and benzyloxycarbonyl groups and azide reduction of the pentasaccharide



1 R = Ac or SO_3^-

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intermediate **15** to give **16**. This reaction generally required several days and also gave polymeric products whose size was estimated by g.p.c. on Sephadex G-50 to correspond to deca- and pentadeca-saccharides (Fig 1). These products probably originated from an intermolecular reaction of the terminal reducing unit and an amino group.

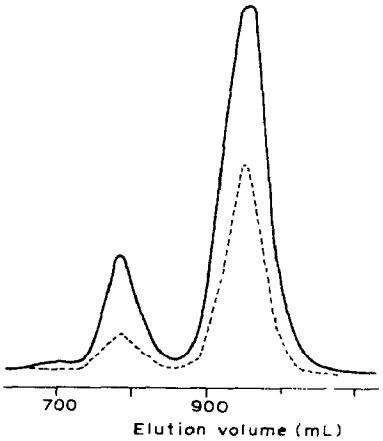


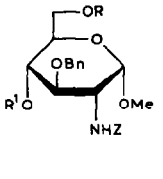
Fig. 1. Chromatography on a column G-50 (300 × 2.5 cm) of Sephadex G-50 of the crude product obtained after hydrogenolysis and *N*-sulfation of **16**; ———, uronic acids; - - - -, optical rotation. The column was calibrated with heparin oligosaccharides of known molecular weight. The peak at 950 mL is a pentasaccharide, that at ~800 mL corresponds to a decasaccharide, and that at 700 mL corresponds to a pentadecasaccharide. ^{1}H-N.m.r. analysis of these compounds indicated them to be complex mixtures (3 decasaccharides and 9 pentadecasaccharides can be formed theoretically).

In order to avoid this side reaction, the methyl α-glycoside (**21**) of **1** (R = SO_3^-) was prepared in the hope that the extra methyl group would not affect the biological properties and might be beneficial by fixing the reducing terminal in the α-pyranoid form as in heparin.

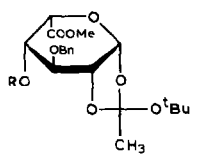
RESULTS AND DISCUSSION

The general strategy developed[3] for the synthesis of **1** (R = SO_3^-) was applied for the preparation of **21**. Thus, two disaccharide blocks (**7** and **9**) were prepared and coupled, and the monosaccharide **14** was then added.

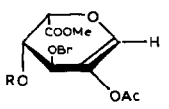
Selective 6-acetylation of the known[6] methyl 3-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (**2**) by *N*-acetylimidazole in dichloroethane gave only 59% of **3**, due to the formation of some 4,6-diacetate. When the ortho ester **4** (ref. 6) was used in glycosylation reactions under the usual conditions (dichloroethane–pyridinium perchlorate)[7], 59% of methyl 5-*O*-acetyl-2,6-anhydro-4-*O*-benzyl-3-*O*-chloroacetyl-D-*xylo*-hex-5-enonate (**5**) was isolated. In an attempt to reduce this side reaction, a 2.5 molar excess of **3** was treated with **4**; this gave 40% of the disaccharide derivative **6**, and crystalline unreacted **3** was recovered



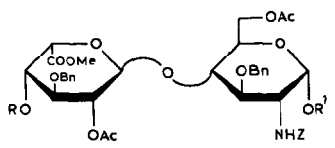
2 R = R' = H
3 R = Ac, R' = H



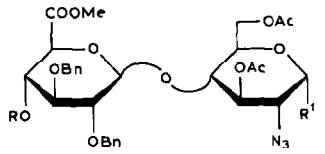
4 R = ClCH$_2$CO



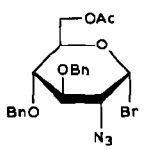
5 R = ClCH$_2$CO



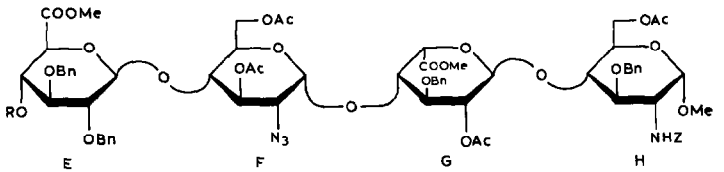
6 R = ClCH$_2$CO, R' = Me
7 R = H, R' = Me
8 R = ClCH$_2$CO, R' = Bn



9 R = ClCH$_2$CO, R' = Br
10 R = levulinyl, R' = Br



14



E F G H

11 R = ClCH$_2$CO
12 R = H
13 R = levulinyl

easily. When the benzyl glycoside analogue[6] of **6** was re-prepared[8] using this procedure, the yield was improved from 22.5% to 45%.

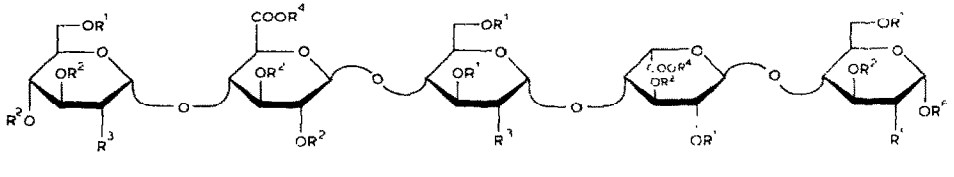
Dechloroacetylation of **6**, using hydrazinedithiocarbonate[9], gave 85% of the crystalline disaccharide derivative **7**.

The glycosyl bromide **9** (ref. 3) and **7** were coupled at -20°, using silver triflate as catalyst and 2,4,6-trimethylpyridine as acid acceptor. Since excess of **7** survived these reaction conditions, a limiting amount of **9** was used because it decomposes during the reaction. Thus, it was possible to isolate the tetrasaccharide derivative **11** in yields of 41% from **9** and 67% from **7**. ^{1}H-N.m.r. spectroscopy indicated exclusive formation of the α-anomer.

The chloroacetyl group of **11** was then removed as described above, to give **12** (84% after chromatography). In an alternative procedure[8], **12** was prepared by the reaction of **7** and **10** (ref. 10) followed by selective removal of the levulinyl group. The overall yield was comparable with that obtained using the chloroacetate, and only the α-glycoside could be detected.

Glycosylation[3] of **12** with **14** (ref. 11) gave 70% of the pentasaccharide derivative **17** together with 10% of another compound which seemed to correspond (n.m.r. data) to the β-anomer.

Saponification of **17** followed by g.p.c. on Sephadex LH-20 gave 72% of **18**, which was *O*-sulfated, without protection of carboxyl groups, using trimethylamine –sulphur trioxide in *N,N*-dimethylformamide, to give 84% of **19**. Conductimetric analysis clearly demonstrated the presence of sulfate and carboxyl groups in **19**. Catalytic hydrogenolysis of **19** cleaved the benzyl ethers and exposed the amino



D E F G H

	R^1	R^2	R^3	R^4	R^5	R^6
15	SO_3Na	Bn	N_3	Me	NHZ	Bn
16	SO_3Na	H	NH_2	Me	NH_2	H
17	Ac	Bn	N_3	Me	NHZ	Me
18	H	Bn	N_3	H	NHZ	Me
19	SO_3Na	Bn	N_3	Na	NHZ	Me
20	SO_3Na	H	NH_2	Na	NH_2	Me
21	SO_3Na	H	$NHSO_3Na$	Na	$NHSO_3Na$	Me

groups to give a quantitative yield of **20**, which was not characterised but *N*-sulfated to give the pentasaccharide-glycoside **21**. Chromatography of the crude product on Sephadex G-50 gave a single peak corresponding to a pentasaccharide and demonstrated that no "polymerisation" had occurred. Pure **21** (63% from **19**), obtained by ion-exchange chromatography, had biological properties[12] identical to those of **1** (ref. 4) (R = SO_3). The 500-MHz ^{1}H-n.m.r. spectrum of **21** was almost superimposable on that of **1** (ref. 13) (R = SO_3), except for the signals arising from unit **H**. The conversion of **19** into **21** was achieved in 63% yield, compared to 35% in the synthesis of **1** (ref. 37 (R = SO_3).

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at ambient temperature with a Perkin-Elmer 241 polarimeter. ^{1}H- and ^{13}C-n.m.r. spectra were recorded with a Bruker AC 100, CPX 300, or WH 500 instrument. T.l.c. was performed on Kieselgel 60 F_{254} (Merck) with detection by charring with sulphuric acid. Column chromatography was performed on silica gel (Merck, 63–200 μm) which was used without pre-treatment. Elemental analyses were done at the "Service d'Analyse" of Institut Choay (Mr. Zuber).

Methyl 6-O-acetyl-3-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (**3**). — A solution of methyl 3-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside[6] (**2**; 8 g, 19.16 mmol) and *N*-acetylimidazole (24 mmol)

in dichloromethane (80 mL) was boiled under reflux overnight, then diluted with dichloromethane (200 mL), washed with M HCl, water, and aqueous 10% $NaHCO_3$, dried, and concentrated. Column chromatography (chloroform–acetone, 20:1) of the residue gave **3** (5.2 g, 59%) which, after crystallisation from ethyl acetate–hexane, had m.p. 124°, $[\alpha]_D^{23}$ +64° (*c*, 1.1 chloroform). ^{1}H-N.m.r. data (100 MHz, $CDCl_3$, internal Me_4Si): δ 7.3 (m, 10 H, 2 Ph), 5.08 (ABq, 2 H, CO_2CH_2Ph), 4.94 (d, 1 H, $J_{2,NH}$ 9.6 Hz, NH), 4.71 (s, 2 H, OCH_2Ph), 4.68 (d, 1 H, $J_{1,2}$ 3,5 Hz, H-1), 3.33 (s, 3 H, OMe), 2.97 (d, 1 H, OH), 2.08 (s, 3 H, Ac).

Anal. Calc. for $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.04. Found: C, 62.61; H, 6.35; N, 2.95.

*Methyl 6-*O*-acetyl-3-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-chloroacetyl-α-*L*-idopyranosyluronate)-α-*D*-glucopyranoside* (**6**). — A solution of **3** (6 g, 13 mmol) in chlorobenzene (40 mL) was heated in an oil bath until 20 mL of solvent had distilled. A solution of 2,6-dimethylpyridinium perchlorate (0.05 mmol) in chlorobenzene (0.5 mL) was then introduced followed dropwise, during 5 min, by a solution of **4** (2.4 g, 5 mmol) in chlorobenzene (24 mL), thus allowing distillation to continue. After 15 min, the mixture was cooled, diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_2), and concentrated. Column chromatography (chloroform–ethyl acetate, 4:1) of the combined products from two experiments yielded **6** (3.6 g, 40% from **4**) and **3** (8.28 g). Crystallisation of **6** from ethyl acetate–hexane gave material with m.p. 135–137°, $[\alpha]_D^{23}$ +12° (*c* 1.2, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.2 (m, 15 H, 3 Ph), 5.10 (m, 2 H, H-2′, 5′), 4.96 (bs, 1 H, H-1′), 4.86 (t, 1 H, H-4′), 4.82 (d, 1 H, NH), 4.64 (d, 1 H, H-1), 4.48 (H-6a), 4.20 (H-6b), 4.02 (H-2), 3.95 ($ClCH_2$), 3.92 (H-4), 3.82 (H-3′), 3.54 (dd, 1 H, H-3), 3.42 (s, 3 H, COOMe), 3.34 (s, 3 H, OMe), 2.02 and 2.10 (2 s, 6 H, 2 Ac).

Anal. Calc. for $C_{42}H_{48}ClNO_{16}$: C, 58.77; H, 5.63; N, 1.63. Found: C, 58.80; H, 5.62; N, 1.44.

*Methyl 6-*O*-acetyl-3-*O*-benzyl-2-benzyloxycarbonylamino-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-α-*L*-idopyranosyluronate)-α-*D*-glucopyranoside* (**7**). — To a solution of **6** (3.52 g, 4.1 mmol) in methanol (60 mL) and dichloromethane (10 mL) was added 2,6-dimethylpyridine (29.2 mL, 250 mmol) followed by acetic acid (9.5 mL, 160 mmol) and hydrazinedithiocarbonate (70 mL of a solution[9] of 0.35 mmol/mL). After 10 min, dichloromethane (500 mL) was added, and the solution was washed with aqueous 10% $KHSO_4$, water, saturated aqueous $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (hexane–ethyl acetate, 1:2) of the residue and crystallisation from ethyl acetate–hexane gave **7** (2.74 g, 85%), m.p. 144–146°, $[\alpha]_D^{23}$ +25° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ 7.2 (m, 15 H, 3 Ph), 5.08 (d, 1 H, $J_{1',2'}$ 0.6 Hz, H-1′), 4.98 (d, 1 H, $J_{4',5'}$ 2.5 Hz, H-5′), 4.92 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2′), 4.93 (d, 1 H, $J_{2,NH}$ 10 Hz, NH), 4.65 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.44 (dd, 1 H, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.6 Hz, H-6a), 4.21 (dd, 1 H, $J_{5,6b}$ 4.1 Hz, H-6b), 4.05 (ddd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.94 (t, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′), 3.90 (t, 1 H, $J_{3,4}$ 9 Hz, H-4), 3.80 (m, 1 H, $J_{4,5}$ 9.6 Hz, H-5), 3.70 (t, 1 H,

H-3'), 3.57 (dd, 1 H, H-3), 3.48 (s, 3 H, COOMe), 3.35 (s, 3 H, OMe), 2.60 (d, 1 H, *J* 11 Hz, OH), 2.10 and 2.0 (2 s, 6 H, Ac).

Anal. Calc. for $C_{40}H_{47}NO_{15}$: C, 61.44; H, 6.05; N, 1.79. Found: C, 61.84; H, 6.09; N, 1.69.

Methyl O-(methyl 2,3-di-O-benzyl-4-O-chloroacetyl-β-D-glucopyranosyluronate)-(1→4)-O-(3,6-di-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(methyl 2-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-(1→4)-6-O-acetyl-3-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (**11**). — A solution of **9** (ref. 3) (1.8 g, 2.25 mmol) and **7** (2.84 g, 3.63 mmol) in dichloroethane (10 mL) was stirred at −20° under argon in the presence of 2,4,6-trimethylpyridine (0.35 mL, 2.6 mmol) and 4Å molecular sieves. Silver triflate (0.56 g, 2.6 mmol) was then introduced and the reaction was allowed to proceed in the dark overnight. Dichloromethane was added (300 mL), and the mixture was filtered, washed with aqueous 10% $KHSO_4$ and water, dried, and concentrated. Column chromatography (hexane–ethyl acetate, 1:1) of the residue yielded **11** (1.38 g, 41% from **9**), isolated as a foam, $[\alpha]_D^{23}$ +49° (*c* 0.95, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ, **E** 4.36 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.05 (dd, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 10 Hz, H-4), 3.70 ($ClCH_2$); **F** 5.0 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **G** 4.65 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **H** 4.65 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.34 (s, 3 H, OMe), 7.20 (m, 25 H, 5 Ph), 2.0, 2.10, and 2.20 (3 s, 12 H, 4 Ac).

Anal. Calc. for $C_{73}H_{83}ClN_4O_{28}$: C, 58.45; H, 5.57; N, 3.73. Found: C, 58.16; H, 5.51; N, 3.74.

Methyl O-(methyl 2,3-di-O-benzyl-β-D-glucopyranosyluronate)-(1→4)-O-(3,6-di-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(methyl 2-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-(1→4)-6-O-acetyl-3-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (**12**). — Treatment of **11** (1.34 g, 0.89 mmol) as described above for **6** gave **12**, isolated as a foam (1.07 g, 84%) after column chromatography (hexane–ethyl acetate, 2:3); $[\alpha]_D^{23}$ +53° (*c* 1.14, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ **E** 4.32 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.75 (H-4), 3.76 (s, COOMe), 2.58 (s, OH); **F** 5.0 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **G** 5.30 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 3.61 (s, COOMe); **H** 4.65 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.31 (s, 3 H, OMe), 7.20 (m, 25 H, 5 Ph), 2.10 and 2.0 (2 s, 12 H, 4 Ac).

Anal. Calc. for $C_{71}H_{82}N_4O_{27}$: C, 59.90; H, 5.80; N, 3.93. Found: C, 60.01; H, 5.79; N, 3.84.

Methyl 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)-O-(3,6-di-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(methyl 2-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-(1→4)-6-O-acetyl-3-O-benzyl-2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (**17**). — A solution of 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide[11] (**14**; 1.1 g, 2.2 mmol) and **12** (1.2 g, 0.85 mmol) in dichloroethane (30 mL) was stirred at −20° under argon in the presence of 4Å molecular sieves and 2,4,6-trimethylpyridine (0.36 mL, 2.6 mmol). After 30 min, silver triflate (0.64 g, 2.4 mmol) was introduced and the reaction was

allowed to proceed in the dark for 1 h. Dichloromethane (500 mL) was added and, after filtration, the solution was washed with aqueous 10% $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (chloroform–ethyl acetate, 5:1) of the residue gave **17** (1.11 g, 70%), $[\alpha]_D^{23}$ +60° (*c* 0.9, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$): δ, **D** 5.50 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **E** 4.34 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.60 (s, 3 H, COOMe); **F** 5.0 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **G** 5.30 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 3.75 (s, 3 H, COOMe); **H** 4.75 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.32 (s, 3 H, OMe), 7.20 (m, 35 H, 7 Ph), 2.12, 2.10, 2.05, and 2.0 (4 s, 15 H, 5 Ac).

Anal. Calc. for $C_{93}H_{105}N_7O_{32}$: C, 60.94; H, 5.77; N, 5.34. Found: C, 60.88; H, 5.76; N, 5.35.

Methyl O-*(2-azido-3,4-di*-O-*benzyl-2-deoxy-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-*benzyl-β*-D-*glucopyranosyluronic acid)-(1→4)*-O-*(2-azido-2-deoxy-α*-D-*glucopyranosyl)-(1→4)*-O-*(3*-O-*benzyl-α*-L-*idopyranosyluronic acid)-(1→4)-3*-O-*benzyl-2-benzyloxycarbonylamino-2-deoxy-α*-D-*glucopyranoside* (**18**). — To a solution of **17** (1.08 g, 0.59 mmol) in chloroform (25 mL), methanol (88 mL), and water (12.7 mL) was added 5M NaOH (12.3 mL). After 6 h, the mixture was diluted with chloroform (300 mL), water (100 mL) and 6M HCl (20 mL) were added, and the organic phase was washed with water until neutral, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of Sephadex LH-20 with methanol–chloroform (1:1) gave **18**, isolated as a white foam (0.68 g, 72%), $[\alpha]_D^{23}$ +41° (*c* 0.95, chloroform). ^{1}H-N.m.r. data (300 MHz, CD_3OD, internal Me_4Si): δ, **D** 5.55 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **E** 4.72 (d, 1 H, $J_{1,2}$ 8 Hz, H-1); **F** 5.12 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **G** 5.29 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1); **H** 4.70 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.40 (s, 3 H, OMe), 7.20 (m, 35 H, 7 Ph).

Anal. Calc. for $C_{81}H_{91}N_7O_{27}$: C, 61.00; H, 5.75; N. 6.14. Found: C, 60.55; H, 5.77; N. 6.04.

Methyl O-*(2-azido-3,4-di*-O-*benzyl-2-deoxy-6*-O-*sulfo-α*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-*benzyl-β*-D-*glucopyranosyluronic acid)-(1→4)*-O-*(2-azido-2-deoxy-3,6-di*-O-*sulfo-α*-D-*glucopyranosyl)-(1→4)*-O-*(3*-O-*benzyl-2*-O-*sulfo-α*-L-*idopyranosyluronic acid)-(1→4)-3*-O-*benzyl-2-benzyloxycarbonylamino-2-deoxy-6*-O-*sulfo-α*-D-*glucopyranoside heptasodium salt* (**19**). — A solution of **18** (0.6 g, 0.376 mmol) and trimethylamine–sulphur trioxide complex (0.65 g, 4.7 mmol) in *N,N*-dimethylformamide (6 mL) was kept overnight at 50°, and then cooled. Methanol (3 mL) and chloroform (3 mL) were added and the solution was layered on to the top of a column of Sephadex LH-20. Elution with methanol–chloroform (1:1), followed by conversion into the sodium salt by passage through a column of Dowex 50 (Na^+) resin (20 mL), yielded **19** (0.684 g, 85%), isolated as a white powder, $[\alpha]_D^{20}$ +29° (*c* 1, methanol). ^{1}H-N.m.r. data (300 MHz, CD_3OD): δ, **D** 5.53 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1); **F** 5.20 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); **G** 5.42 (bs, 1 H, H-1); **H** 4.62 (d, $J_{1,2}$ 3.6 Hz, H-1), 3.40 (s, 3 H, OMe), 7.20 (m, 35 H, 7 Ph).

Anal. Calc. for $C_{81}H_{84}N_7Na_7O_{42}S_5$: C, 45.27; H, 3.94; N, 4.56. Found: C, 44.86; H, 4.53; N, 4.78.

Methyl O-*(2-deoxy-2-sulfamido-6*-O-*sulfo-α*-D-*glucopyranosyl)-(1→4)*-O-

(β-D-glucopyranosyluronic acid)-(1→4)-O-(2-deoxy-2-sulfamido-3,6-di-O-sulfo-α-D-glucopyranosyl)-(1→4)-O-(2-O-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-O-sulfo-α-D-glucopyranoside decasodium salt (**21**). — A solution of **19** (330 mg, 0.153 mmol) in methanol–water (25 mL, 9:1) was hydrogenated in the presence of 5% Pd/C (165 mg). After 5 days, the spent catalyst was replaced by fresh catalyst. After a further 5 days, u.v. spectroscopy indicated reaction to be complete. The solution was filtered and concentrated to give **20**, which was immediately dissolved in water (10 mL), and the pH was adjusted to 9.5 and maintained thereat throughout the *N*-sulfation step.

Pyridine–sulphur trioxide complex (240 mg, 1.5 mmol) was introduced in 3 portions during 1 h. After 2 h, the mixture was neutralised with HCl, layered on to the top of a column (300 × 2.5 cm) of Sephadex G-50, and eluted with 0.2M NaCl. A single peak emerged; the appropriate fractions were immediately loaded on to a column (1.6 × 9 cm) of Dowex 1-X2 (Na^+) resin and eluted with a sodium chloride (0.5 → 2M) gradient (500 mL, total volume). The fractions containing **21** were combined and desalted on a Sephadex G-25 column to give **21**, obtained as a white powder after lyophilisation (166 mg, 63% from **19**); $[\alpha]_D^{23}$ +48° (*c* 0.61, water). ^{1}H-N.m.r. data (D_2O, internal TSP), ^{1}H (500 MHz): δ, **D** 5.60 (H-1), 3.25 (H-2), 3.61 (H-3), 3.57 (H-4), 3.87 (H-5), 4.36 (H-6), 4.15 (H-6′); **E** 4.62 (H-1), 3.41 (H-2), 3.82 (H-3,4,5); **F** 5.52 (H-1), 3.44 (H-2), 4.34 (H-3), 3.96 (H-4), 4.12 (H-5), 4.48 (H-6), 4.26 (H-6′); **G** 5.18 (H-1), 4.29 (H-2), 4.16 (H-3), 4.17 (H-4), 4.79 (H-5); **H** 5.02 (H-1), 3.27 (H-2), 3.64 (H-3), 3.76 (H-4), 3.95 (H-5), 4.40 (H-6), 4.33 (H-6′). ^{13}C (25 MHz): δ, **D** 100.9 (C-1), 69.5 (C-6), 60.6 (C-2); **E** 103.8 (C-1); **F** 99.1 (C-1), 68.7 (C-6), 59.4 (C-2); **G** 102.2 (C-1); **H** 100.4 (C-1), 69.0 (C-6), 60.4 (C-2), 58.0 (OMe).

Anal. Calc. for $C_{31}H_{43}N_3Na_{10}O_{49}S_8 \cdot 5H_2O$: C, 20.47; H, 2.94; N, 2.31. Found: C, 20.72; H, 3.42; N, 2.34.

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