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SYNTHESIS OF HEPARIN FRAGMENTS. A CHEMICAL SYNTHESIS OF THE PENTASACCHARIDE *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-D-GLUCOPYRANOSE DECASODIUM SALT, A HEPARIN FRAGMENT HAVING HIGH AFFINITY FOR ANTITHROMBIN III

MAURICE PETITOU, PHILIPPE DUCHAUSSOY, ISIDORE LEDERMAN, JEAN CHOAY,
Institut Choay, 46, Avenue Théophile Gautier, 75782 Paris (France)

PIERRE SINAŸ, JEAN-CLAUDE JACQUINET,
Laboratoire de Biochimie Structurale, U.A. 499, U.E.R. de Sciences Fondamentales et Appliquées, 45046 Orléans (France)

AND GIANGIACOMO TORRI
G. Ronzoni Institute for Chemical and Biochemical Research, Via G. Colombo 81, 20133 Milan (Italy)
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ABSTRACT

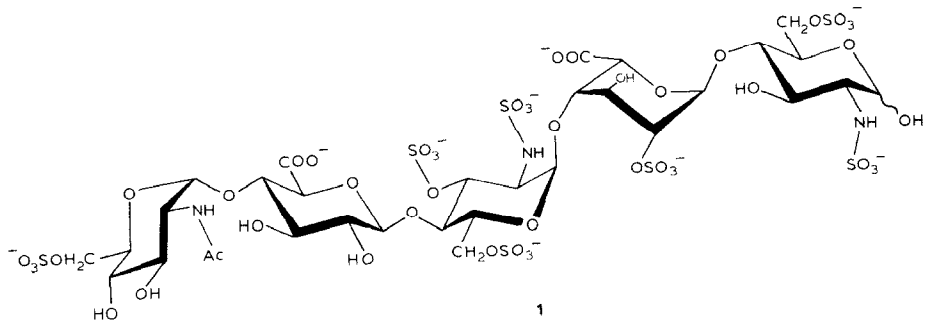
Known allyl 4,6-*O*-benzylidene-α-D-glucopyranoside was first converted into methyl (prop-1-enyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-glucopyranosid)-uronate. Acid hydrolysis, followed by treatment with (bromomethylene)dimethylammonium bromide, gave methyl (2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-glucopyranosyl bromide)uronate. Condensation of this bromide with 3-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose gave 3-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-β-D-glucopyranosyluronate)-β-D-glucopyranose. Acetolysis, followed by treatment with titanium tetrabromide, then gave 3,6-di-*O*-acetyl-2-azido-2-deoxy-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl bromide. Condensation of this bromide with benzyl 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 provided benzyl *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. *O*-Dechloroacetylation followed by condensation with 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide provided benzyl *O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-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 in 70% yield. *O*-Deacetylation followed by re-esterification, *O*-sulfation, saponification, catalytic hydrogenolysis, and *N*-sulfation gave the decasodium salt of *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-D-glucopyranose. This synthetic pentasaccharide binds to antithrombin III with an association constant similar to that of high-affinity heparin and elicits a potent anti-factor Xa activity in plasma.

INTRODUCTION

Heparin is a sulfated glucosaminoglycuronan with a well-known anticoagulant activity[1], and the active molecules have a high affinity for antithrombin III (AT III), thereby enhancing the effects of this inhibitor on procoagulant proteases. The structure of the antithrombin III-binding domain in heparin has been investigated[2] and the hypothesis formulated[3] that the minimum sequence that binds to AT III was contained in the pentasaccharide **1**.

However, no pentasaccharide *per se* has been obtained up to now from natural sources in quantities sufficient for precise investigation. We now report the total synthesis[4] of the title pentasaccharide **30**, the first synthetic heparin fragment to have a high affinity for AT III and thus display biological activity[5]. The tri-*N*-sulfated structural variant **30** was chosen as the synthesis target since replacement of the *N*-acetyl group of fragment D in **1** by a sulfamido group occurs in beef-lung heparin[2c]. The homogeneous sulfation of the three amino functions in **30**, in contrast with **1**, clearly simplifies the synthesis strategy.



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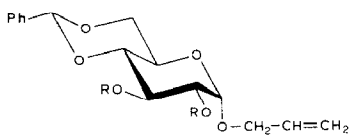
RESULTS AND DISCUSSION

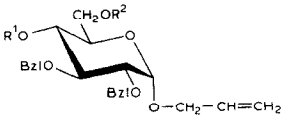
We recently reported[6] the chemical synthesis of the heptasodium salt of the trisaccharide *O*-(2-deoxy-2-sulfamido-3,6-di-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-*O*-sulfo-D-glucopyranose, a fragment of the minimal antithrombin III-binding region in heparin. The general strategy of this synthesis was based on benzyl ethers as permanent blocking-groups, and the alcohol **21** was selected[6] as a progenitor of the regular disaccharide moiety of the heparin fragment. For clarity, the synthetic route to pentasaccharide **30** was as follows: **14** + **16** → **20** $\xrightarrow{21}$ **23** $\xrightarrow{24}$ **25** → **30** in which the known[6] alcohol **21** and the disaccharide glycosyl bromide **20** were the two key building-blocks of the protected target molecule **25**. The synthesis of **20** was undertaken first.

Easily available[7] allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**2**) was first converted into crystalline allyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-trityl-α-D-glucopyranoside (**5**) by benzylation (→**3**), followed by acid hydrolysis (→**4**), selective tritylation, and acetylation. Detritylation of **5** at room temperature with aqueous 80% acetic acid, followed by oxidation of the primary hydroxyl group of the product with chromium trioxide in acetone–sulfuric acid at room temperature (→**6**) and esterification with diazomethane, gave the crystalline derivative **8**. Isomerisation of the allyl group of **8** into the prop-1-enyl group with tris(triphenylphosphine)-rhodium(I) chloride[8] provided 88% of the crystalline glycoside **9**, *O*-deacetylation of which with sodium methoxide gave ~75% of the expected alcohol **10**, but some elimination occurred and yielded 20% of **12**. In order to avoid this side reaction, **6** was saponified and then esterified with etheral diazomethane to afford **7**. Isomerisation of **7** with the rhodium catalyst then gave **10**. Problems with the reproducibility of this isomerisation, especially for large-scale reactions and the cost of the rhodium catalyst, prompted the development of a third route to **10**. The potassium salt of the crude acid **6** was treated with potassium *tert*-butoxide in methyl sulfoxide, and the product which was esterified with diazomethane gave a reproducible and excellent yield of **10**.

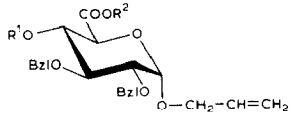
Treatment of **10** with chloroacetyl chloride in pyridine gave **11** which, with mercuric chloride and yellow mercuric oxide in acetone[9], gave 81% of the crystalline hemiacetal **13**. Mild bromination of **13** with (bromomethylene)dimethylammonium bromide[10] gave 90% of the bromide **14**. The alcohol **16** was prepared from 1,6-anhydro-2-azido-2-deoxy-4-*O*-(tetrahydropyran-2-yl)-β-D-glucopyranose[11] (**15**) by acetylation followed by mild acid hydrolysis. Condensation of **14** with 3 mol of **16** in dichloromethane at room temperature in the presence of silver carbonate was slow and, after 6 days, the crystalline disaccharide derivative **17** (70% based on bromide **14**) was obtained. ^{1}H-N.m.r. data for **17** (δ 4.74, d, $J_{1',2'}$ 7.5 Hz, H-1′) indicated that the new glycosidic bond was β. A small amount (6.4%) of the crystalline α-anomer **18** was also isolated (δ 5.10, d, $J_{1',2'}$ 3.5 Hz, H-1′). Treatment of **17** with acetic anhydride–trifluoroacetic acid opened the



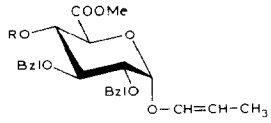
2 R = H
3 R = Bzl



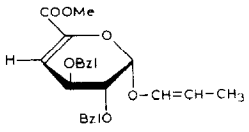
4 $R^1 = R^2 = H$
5 R^1 = Ac, R^2 = Tr



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



9 R = Ac
10 R = H
11 R = $ClCH_2CO$



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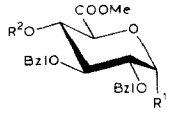
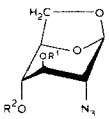
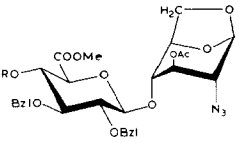


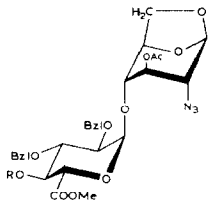
13 R^1 = OH, R^2 = $ClCH_2CO$
14 R^1 = Br, R^2 = $ClCH_2CO$



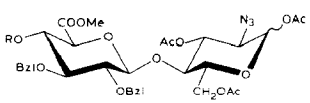
15 R^1 = H, R^2 = tetrahydropyran-2-yl
16 R^1 = Ac, R^2 = H



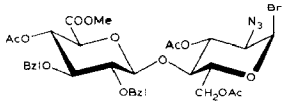
17 R = $ClCH_2CO$



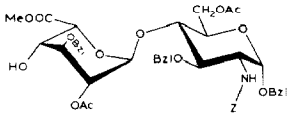
18 R = $ClCH_2CO$



19 R = $ClCH_2CO$



20 R = $ClCH_2CO$



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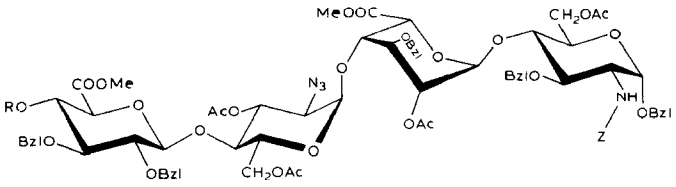
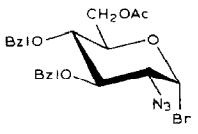


22 R = $ClCH_2CO$
23 R = H



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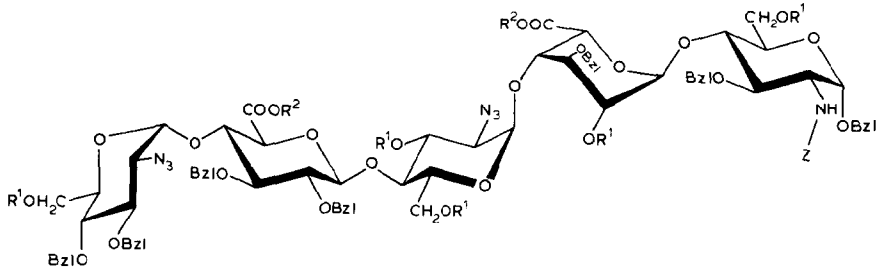
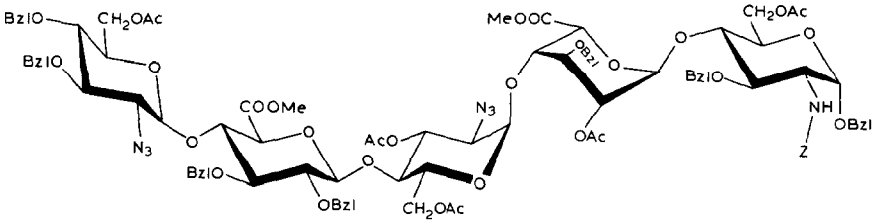


25 R^1 = Ac, R^2 = Me
26 R^1 = H, R^2 = Me
27 R^1 = SO_3Na, R^2 = Me
28 R^1 = SO_3Na, R^2 = Na



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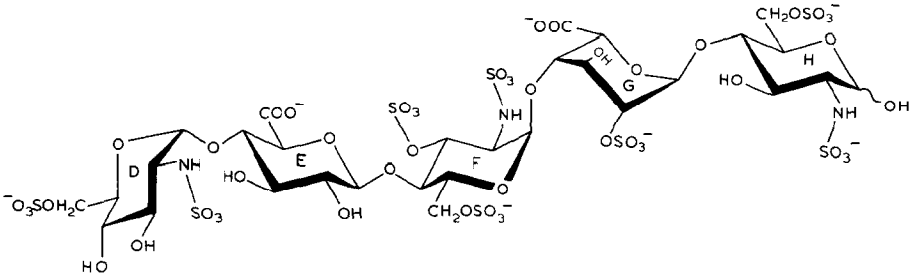

30

anhydro ring and gave an α,β-mixture of the acetates **19**, which, with titanium tetrabromide, gave 60% of the key bromide **20**. The ^{1}H-n.m.r. spectrum of **20** accorded with the structure assigned.

Condensation of **20** with the known[5] alcohol **21** in dichloromethane at −20°, in the presence of freshly prepared silver triflate[12] and 2,4,6-trimethylpyridine, gave 55% of the amorphous tetrasaccharide derivative **22**. ^{1}H-N.m.r. data for **22** (δ 5.29,

d, $J_{1,2}$ 3.5 Hz, H-1 of fragment F) demonstrated that the new glycosidic bond was α. The corresponding β-anomer was not detected. Selective *O*-dechloroacetylation of **22** gave the alcohol **23**, which was condensed with the known bromide[13] **24** in dichloromethane at −20°, in the presence of silver triflate and 2,4,6-trimethylpyridine, to give 70% of the amorphous pentasaccharide derivative **25**. The 300-MHz ^{1}H-n.m.r. data for **25** (δ 5.50, d, $J_{1,2}$ 3.6 Hz, H-1 of fragment D) demonstrated that the new glycosidic bond was α. The β-anomer **29** was also isolated (14%; δ 3.17, d, $J_{1,2}$ 7.9 Hz, H-1 of fragment D). The $[\alpha]_D$ values (chloroform) (+65° and +46°, respectively) of **25** and **29** confirmed the assigned configurations.

Conversion of the pentasaccharide derivative **25** into **30** was accomplished essentially along lines previously reported[6]. *O*-Deacetylation of **25** at room temperature with sodium hydroxide in chloroform–methanol–water, followed by esterification with diazomethane, gave the amorphous derivative **26** (73% after column chromatography) which had no ^{1}H-n.m.r. signal for acetyl. *O*-Sulfation of **26** was achieved with the sulfur trioxide–trimethylamine complex in *N*,*N*-dimethylformamide, and the trimethylammonium salt of the product was purified by chromatography on Sephadex LH-20 and then on silica gel. Final purification on Sephadex SP-25 (Na^+) afforded 90% of the amorphous pentasulfate **27** sodium salt. Compound **27** was saponified, and the product was catalytically hydrogenated in methanol–water in the presence of Pd/C and then *N*-sulfated in aqueous solution of pH 9.5 at room temperature with the sulfur trioxide–trimethylamine complex. The resulting pentasaccharide fractions were separated on Sephadex G-50 from deca- and pentadeca-saccharide fractions, probably resulting from reaction of the reducing fragment H with free amino groups. Final purification of the pentasaccharide on an anion-exchange resin gave **30**. A 270-MHz ^{1}H-n.m.r. spectrum of **30** has been reported[5], as have one- and two-dimensional 500-MHz ^{1}H-n.m.r. spectra[14]. The ^{13}C-n.m.r. spectrum of **30** (Fig. 1) accords with the structure assigned. Sulfation of the primary hydroxyl groups of the three 2-amino-2-deoxyglucose residues is indicated by the chemical shifts (68–70 p.p.m.) of the signals for C-6. Sulfation at position 3 of 2-amino-2-deoxyglucose residue F is demonstrated by a slightly higher-field shift (59.4 p.p.m.) of the signal for C-2 compared with that (60.71 p.p.m.) of C-2 in units D and H.

The synthetic tri-[6] (F–G–H) and tetra-saccharide[15] (E–F–G–H; this letter code was proposed by Choay *et al.*[21]) do not bind to AT III, whereas **30** binds strongly; the association constant of $7.10^6 M^{-1}$ has the same order of magnitude as that for high-affinity heparin. Furthermore, in the presence of AT III, **30** is markedly inhibitory with respect to factor Xa but does not activate AT III in the thrombin inhibition process. On the assumption[2h,3a] that unit H is important for high biological activity, then **30** (and **1**) probably corresponds to the minimum sequence required in heparin species for strong binding to AT III. The synthesis of **30** reflects the current possibilities for the synthesis of substantial amounts of complex oligosaccharides, which augur well for future developments of the understanding of the biological role of oligosaccharides.

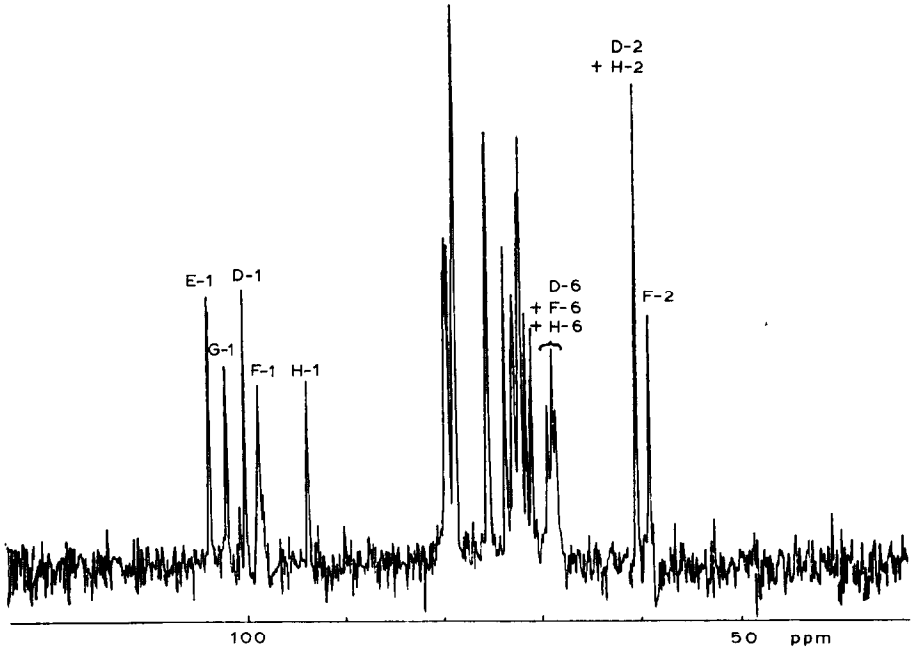

Fig. 1. ^{13}C-N.m.r. spectrum of the synthetic pentasaccharide **30** (D–E–F–G–H).

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–23° with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Perkin–Elmer R-32 or a Bruker CXP 300 instrument for solutions in $CDCl_3$ (internal Me_4Si) unless otherwise stated. ^{13}C-N.m.r. spectra were recorded with a Varian FT 20 instrument. 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 mm) which was used without pre-treatment. Elemental analyses were done at the Service d'Analyse de l'Institut Choay (Mr. M. Zuber).

*Allyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-*D*-glucopyranoside* (**3**). — A solution of allyl 4,6-*O*-benzylidene-α-D-glucopyranoside[7] (**2**, 45 g) in dry *N,N*-dimethylformamide (500 mL) was stirred for 30 min at room temperature in the presence of sodium hydride (28 g of a 50% suspension in oil) and, after cooling to 0°, freshly distilled benzyl bromide (52 mL) was added dropwise. After completion of the reaction (t.l.c.; ether–hexane, 1:1), the excess of benzyl bromide was destroyed by the slow addition of methanol (150 mL) and stirring for 1 h. The mixture was diluted with chloroform, washed with water, dried (Na_2SO_4), and concentrated. The residue was crystallised from ether–hexane to give **3** (50 g, 71%), m.p. 83–84°,

$[\alpha]_D$ −4° (*c* 1.4, chloroform). ^{1}H-N.m.r. data: δ 7.30 (m, 15 H, 3 Ph), 5.90 (m, 1 H, -OCH_2CH=CH_2), 5.51 (s, 1 H, C*H*Ph), 5.20 (m, 2 H, -OCH_2CH=CH_2), 4.80 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.55 (dd, 1 H, $J_{2,3}$ 9 Hz, H-2).

Anal. Calc. for $C_{30}H_{32}O_6$: C, 73.74; H, 6.60. Found: C, 73.53; H, 6.44.

*Allyl 2,3-di-*O*-benzyl-α-*D*-glucopyranoside* (**4**). — A solution of **3** (56 g) and toluene-*p*-sulfonic acid (17 g) in water (450 mL) and methanol (1 L) was heated for 2 h at 80°, then cooled, and concentrated. A solution of the residue in chloroform (1 L) was washed with water until neutral, dried (Na_2SO_4), and concentrated. The amorphous residue (46 g, 100%) of **4**, which was used directly in the next reaction, had $[\alpha]_D$ +43° (*c* 0.96, chloroform). ^{1}H-N.m.r. data: δ 5.80 (m, 1 H, -OCH_2CH=CH_2), 5.20 (m, 2 H, -OCH_2CH=CH_2), 4.80 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.45 (dd, 1 H, $J_{2,3}$ 9 Hz, H-2), 2.90 and 2.45 (2 bs, 2 H, OH).

Anal. Calc. for $C_{23}H_{28}O_6$: C, 68.90; H, 7.04. Found: C, 68.93; H, 7.04.

*Allyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-trityl-α-*D*-glucopyranoside* (**5**). — A mixture of **4** (60 g) and freshly purified chlorotriphenylmethane (50 g) in dry pyridine (500 mL) was heated for 2 h at 80° and then cooled to 0°. Acetic anhydride (140 mL) was added, and the mixture was heated at 80° until **4** had disappeared (t.l.c.; ether–hexane, 1:2). The mixture was concentrated and a solution of the residue in chloroform (1 L) was washed with aqueous 10% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. The crude **5** was used in the next reaction. Crystallisation from ethyl acetate–hexane gave material having m.p. 107–108°, $[\alpha]_D$ +28° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.40–7.10 (m, 25 H, 5 Ph), 4.86 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.60 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 1.60 (s, 3 H, Ac).

Anal. Calc. for $C_{44}H_{44}O_7$: C, 77.16; H, 6.47. Found: C, 77.04; H, 6.49.

*Methyl (allyl 2,3-di-*O*-benzyl-α-*D*-glucopyranosid)uronate* (**7**). — A solution of crude **5** (45 g) in the minimum amount of dichloromethane was stirred at room temperature with aqueous 80% acetic acid (1 L) until t.l.c. (toluene–acetone, 5:1) demonstrated the disappearance of **5**. Water (600 mL) was then added with stirring, the solid was removed, and the filtrate was concentrated. A solution of the crude residue (24 g) in acetone (300 mL) was cooled to −5° and a solution of chromium trioxide (8 g) in 3.5M sulfuric acid (70 mL) was added dropwise with stirring. The mixture was allowed to attain room temperature, and, after 5 h, poured into ice-cold water (1 L) and extracted with chloroform. The extract was washed with water and concentrated, and a solution of the crude **6** (25 g) in methanol (320 mL) was treated with 6M sodium hydroxide (30 mL) at room temperature. After 3 h, the methanol was evaporated, water (500 mL) was added, and the solution was washed with ether and, after acidification with hydrochloric acid, extracted with chloroform. The extract was washed with water and concentrated, and the residue (19 g) was esterified with etheral diazomethane. The product was eluted from a column of silica gel (800 g) with chloroform–ethyl acetate (20:1) to give amorphous **7** (15.8 g, 68% from **4**), $[\alpha]_D$ +35° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.30 (m, 10 H, 2 Ph), 5.90 (m, 1 H, -OCH_2CH=CH_2), 4.88 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 2.95 (s, 1 H, OH).

Anal. Calc. for $C_{24}H_{28}O_7$: C, 67.30; H, 6.58. Found: C, 66.97; H, 6.58.

Methyl (allyl 4-O-acetyl-2,3-di-O-benzyl-α-D-glucopyranosid)uronate (**8**). — Treatment of crude allyl 4-*O*-acetyl-2,3-di-*O*-benzyl-α-D-glucopyranosiduronic acid (**6**) with etheral diazomethane gave a quantitative yield of **8**, m.p. 87–88° (from ethanol), $[\alpha]_D$ +12° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 7.25 (m, 10 H, 2 Ph), 5.89 (m, 1 H, $-OCH_2CH{=}CH_2$), 5.30 (m, 2 H, $-OCH_2CH{=}CH_2$), 5.05 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.85 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.19 (d, 1 H, H-5), 3.90 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 3.66 (s, 3 H, COOMe), 3.59 (dd, 1 H, H-2), 1.88 (s, 3 H, Ac).

Anal. Calc. for $C_{26}H_{30}O_8$: C, 66.36; H, 6.42. Found: C, 66.24; H, 6.30.

Methyl (prop-1-enyl 4-O-acetyl-2,3-di-O-benzyl-α-D-glucopyranosid)uronate (**9**). — A solution of **8** (8 g) in ethanol (240 mL), benzene (102 mL), and water (34 mL) was heated under reflux, and tris(triphenylphosphine)rhodium(I) chloride (1.1 g) and then 1,4-diazabicyclo[2.2.2]octane (340 mg) were added. After 4 h under reflux, the solution was cooled, filtered, and concentrated. The residue was eluted from a column of silica gel (500 g) with chloroform–ethyl acetate (50:1) to give **9** (7.03 g, 88%), m.p. 88–91° (from ethanol), $[\alpha]_D$ +10° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.25 (m, 10 H, 2 Ph), 6.00 (dd, 1 H, prop-1-enyl), 5.02 (t, 1 H, $J_{3,4}$ 9.5 Hz, H-4), 4.96 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.13 (d, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 3.97 (t, 1 H, $J_{2,3}$ 9.5 Hz, H-3), 3.62 (s, 3 H, COOMe), 3.59 (dd, 1 H, H-2), 1.87 (s, 3 H, Ac).

Anal. Calc. for $C_{26}H_{30}O_8$: C, 66.36; H, 6.42. Found: C, 66.47; H, 6.45.

Methyl (prop-1-enyl 2,3-di-O-benzyl-α-D-glucopyranosid)uronate (**10**). — (*a*) *From* **6**. Crude **6** (see above) (21.7 g) was dissolved in methanol and absorbed on to a column (2 × 60 cm) of Dowex 1-X4 (HO^-) resin (20–50 mesh, 200 mL) equilibrated with methanol–water (98:2). The pure acid was eluted with methanol–acetic acid–water (45:45:10) and then transformed into its potassium salt by passage through a column (2 × 30 cm) of Dowex 50-X4 (K^+) resin (20–50 mesh, 100 mL) using methanol–water (9:1). The solution was concentrated and the residue (13.7 g) was dried for 12 h under vacuum at 50°. A solution in dry methyl sulfoxide (25 mL) was stirred for 1 h under argon in the presence of potassium *tert*-butoxide (10 g), then cooled, diluted with ice–water, acidified with 4M hydrochloric acid (30 mL), and extracted with ether (3 × 100 mL). The combined extracts were treated with etheral diazomethane, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (200 g) with dichloromethane–ethyl acetate (20:1) to give amorphous **10** (11.6 g, 97%), $[\alpha]_D$ +32.5° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.25 (m, 10 H, 2 Ph), 6.00 (dd, 1 H, prop-1-enyl), 4.95 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.68 (s, 3 H, COOMe), 3.00 (1 H, OH).

Anal. Calc. for $C_{24}H_{28}O_7$: C, 67.27; H, 6.58. Found: C, 67.37; H, 6.59.

(*b*) *From* **7**. A solution of **7** (160 mg) in ethanol (5.6 mL), benzene (2.4 mL), and water (0.8 mL), containing 1,4-diazabicyclo[2.2.2]octane (8 mg) and tris(triphenylphosphine)rhodium(I) chloride (24 mg), was heated under reflux for 4 h and then cooled. The mixture was diluted with dichloromethane (100 mL), washed with

ice-cold 0.1M hydrochloric acid and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (10 g) with chloroform-ethyl acetate (10:1) to give **10** (145 mg, 90%), identical with the product obtained in (*a*).

(*c*) *From* **9**. To a solution of **9** (6 g) in methanol (100 mL) was added methanolic 2M sodium methoxide (8 mL). After storage for 2 h at room temperature, the solution was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated. The residue was eluted from a column of silica gel (300 g) with chloroform–ethyl acetate (40:1) to give, first, syrupy methyl (prop-1-enyl 2,3-di-*O*-benzyl-β-L-*threo*-hex-4-enopyranosid)uronate (**12**; 0.83 g, 20.2%). ^{1}H-N.m.r. data: δ 6.13 (dd, 1 H, $J_{3,4}$ 2.5 Hz, H-4), 5.13 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.60 (dd, 1 H, $J_{2,3}$ 7 Hz, H-3), 3.80 (dd, 1 H, H-2), 3.70 (s, 3 H, COOMe).

Further elution gave **10** (4.18 g, 76.5%), identical with the product obtained in (*a*).

*Methyl (prop-1-enyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-*D*-glucopyranosid)uronate* (**11**). — 1.25M Chloroacetyl chloride in anhydrous dichloromethane (8 mL) was added dropwise at 0° to a solution of **9** (2.8 g) in pyridine (30 mL). After 30 min, the mixture was concentrated, and a solution of the residue in chloroform was washed with aqueous 10% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (150 g) with hexane–ethyl acetate (3:1) to give **11** (2.67 g, 80%), $[\alpha]_D$ +2° (*c* 1.5, chloroform). ^{1}H-N.m.r. data: δ 7.38 (m, 10 H, 2 Ph), 5.07 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.85 (d, 2 H, $ClCH_2CO$), 3.74 (dd, 1 H, H-2), 3.74 (s, 3 H, COOMe).

Anal. Calc. for $C_{26}H_{30}ClO_8$: C, 61.71; H, 5.97. Found: C, 61.44; H, 5.93.

*Methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-*D*-glucopyranuronate* (**13**). — A solution of mercuric chloride (3.9 g) in acetone (27 mL) was added dropwise at room temperature to a stirred mixture of **11** (2.67 g), yellow mercuric oxide (3.09 g), and acetone (67 mL). After 5 min, the mixture was filtered and concentrated, and a solution of the residue in chloroform was washed with saturated aqueous potassium iodide and water, dried (Na_2SO_4), and concentrated. The residue was crystallised from hexane–ethyl acetate to give **13** (2 g, 81%), m.p. 105–106°, $[\alpha]_D$ −7° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.37 (m, 10 H, 2 Ph), 5.36 (d, $J_{1,2}$ 3.5 Hz, H-1), 4.10 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 8.5 Hz, H-3), 3.85 (d, 2 H, $ClCH_2CO$), 3.72 (s, 3 H, COOMe).

Anal. Calc. for $C_{23}H_{25}ClO_8$: C, 59.41; H, 5.42. Found: C, 59.11; H, 5.35.

*Methyl (2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-*D*-glucopyranosyl bromide)uronate* (**14**). — A solution of **13** (2 g) in dichloromethane (50 mL) was stirred for 5 h at 0° in the presence of 2,4,6-trimethylpyridine (4.8 mL) and $[Me_2N{=}CHBr]^+Br^-$ (3.48 g), then diluted with dichloromethane (100 mL), washed with ice-cold water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (20 g) with hexane–ethyl acetate (2:1) to give **14** (2.06 g, 90%), $[\alpha]_D$ +82° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.25 (m, 10 H, 2 Ph), 6.36 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.09 (dd, 1 H, $J_{3,4}$ 9, $J_{4,5}$ 10 Hz, H-4), 4.35 (d, 1 H, $J_{4,5}$ 10 Hz, H-5), 3.93 (t, 1 H, $J_{2,3}$ 9 Hz, H-3), 3.70 (d, 2 H, $ClCH_2CO$), 3.63 (s, 3 H, COOMe), 3.53 (dd, 1 H, H-2).

Anal. Calc. for $C_{23}H_{24}BrClO_7$: C, 52.33; H, 4.58. Found: C, 52.18; H, 4.70.

3-O-Acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**16**). — A solution of 1,6-anhydro-2-azido-2-deoxy-4-*O*-(tetrahydropyran-2-yl)-β-D-glucopyranose[12] (**15**, 6.5 g) in pyridine (160 mL) was treated overnight at room temperature with acetic anhydride (80 mL) and then concentrated, and a solution of the residue in chloroform (800 mL) was washed with aqueous 10% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. A solution of the residue (8.6 g) in methanolic 0.25M toluene-*p*-sulfonic acid (165 mL) was stored at room temperature for 4 h, then diluted with chloroform (500 mL), washed with ice-cold water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (200 g) with toluene–acetone (3:1) to give **16** (4.2 g, 77%), $[\alpha]_D$ −6.5° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 5.51 (d, 1 H, H-1), 4.93 (d, 1 H, H-3), 3.50 (d, 1 H, H-2), 3.40 (1 H, OH), 2.20 (s, 3 H, Ac).

Anal. Calc. for $C_8H_{11}N_3O_5$: C, 41.92; H, 4.83; N, 18.33. Found: C, 41.94; H, 4.98; N, 17.93.

3-O-Acetyl-1,6-anhydro-2-azido-2-deoxy-4-O-(methyl 2,3-di-O-benzyl-4-O-chloroacetyl-β-D-glucopyranosyluronate)-β-D-glucopyranose (**17**). — A solution of freshly prepared **14** (8.62 g, 16.33 mmol) and **16** (11 g, 48 mmol) in anhydrous dichloromethane (160 mL) was stirred for 6 days at room temperature under dry argon in the presence of 4 Å activated, powdered molecular sieve (10 g) and silver carbonate (6.6 g). The mixture was then diluted with dichloromethane (150 mL), filtered, washed with water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (400 g) with chloroform–ethyl acetate (6:1) to give **17** (7.717 g, 70% from **14**), m.p. 130–132° (from hexane–ethyl acetate), $[\alpha]_D$ −17° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.40–7.30 (m, 10 H, 2 Ph), 5.54 (bs, 1 H, H-1), 5.32 (bs, 1 H, H-3), 4.74 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 3.80 (AB system, $ClCH_2CO$), 3.77 (s, 3 H, COOMe), 3.29 (bs, 1 H, H-2), 2.14 (s, 3 H, OAc).

Anal. Calc. for $C_{31}H_{34}ClN_3O_{12}$: C, 55.07; H, 5.06; N, 6.21. Found: C, 54.81; H, 5.17; N, 5.92.

Unreacted **16** (7.3 g, 66%) was also recovered from the silica gel column, together with 3-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-glucopyranosyluronate)-β-D-glucopyranose (**18**; 710 mg, 6.4%), m.p. 187–189° (from hexane–ethyl acetate), $[\alpha]_D$ +31° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 7.25 (m, 10 H, 2 Ph), 5.54 (bs, 1 H, H-1), 5.10 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.42 (d, $J_{4',5'}$ 9 Hz, H-5'), 3.66 (s, 3 H, COOMe), 3.05 (bs, 1 H, H-2), 2.09 (s, 3 H, Ac).

Anal. Calc. for $C_{31}H_{34}ClN_3O_{12}$: C, 55.07; H, 5.06; N, 6.21. Found: C, 54.60; H, 4.96; N, 6.21.

1,3,6-Tri-O-acetyl-2-azido-2-deoxy-4-O-(methyl 2,3-di-O-benzyl-4-O-chloroacetyl-β-D-glucopyranosyluronate)-D-glucopyranose (**19**). — A solution of **18** (7 g) in acetic anhydride (100 mL) and trifluoroacetic acid (14 mL) was kept at room temperature for 12 h, and then concentrated. The residue was eluted from a column of silica gel (400 g) with hexane–ethyl acetate (2:1) to give amorphous **19** (6.95 g,

86%). ^{1}H-N.m.r. data: δ 7.35 (m, 10 H, 2 Ph), 6.29 (d, H-1α), 5.57 (d, H-1β), 5.50 (dd, $J_{3,4}$ 8.5 Hz, H-3α), 5.15 (dd, $J_{3,4}$ 8.5 Hz, H-3β), 4.43 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'), 3.75 (s, 3 H, COOMe), 3.60 (dd, $J_{1,2}$ 8.5, $J_{2,3}$ 9 Hz, H-2β), 3.57 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 9 Hz, H-2α), 2.25, 2.20, and 2.09 (3 s, 9 H, 3 Ac).

Anal. Calc. for $C_{35}H_{40}ClN_3O_{15}$: C, 54.00; H, 5.18; N, 5.40. Found: C, 53.66; H, 5.17; N, 5.22.

*3,6-Di-*O*-acetyl-2-azido-2-deoxy-4-*O*-(methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-*β*-*D*-glucopyranosyluronate)-*α*-*D*-glucopyranosyl bromide* (**20**). — A solution of **19** (3 g) in anhydrous dichloromethane–ethyl acetate (10:1, 44 mL) was stirred for 12 h at room temperature in the presence of $TiBr_4$ (2.6 g), then diluted with dichloromethane (150 mL), washed with ice–water, dried (Na_2SO_4), and concentrated. The residue was promptly eluted from a column of silica gel (80 g) with dichloromethane–ethyl acetate (20:1) to give **20** (1.85 g, 60%), $[\alpha]_D$ +63° (*c* 1, dichloromethane). ^{1}H-N.m.r. data: 7.35 (m, 10 H, 2 Ph), 6.40 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.56 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 8.5 Hz, H-3), 5.05 (t, 1 H, $J_{3',4'} = J_{4',5'} = 9$ Hz, H-4'), 4.42 (d, 1 H, $J_{1',2'}$ 7 Hz, H-1'), 3.76 (AB system, 2 H, $ClCH_2CO$), 3.75 (s, 3 H, COOMe), 2.24 and 2.10 (2 s, 6 H, 2 Ac).

Anal. Calc. for $C_{33}H_{37}BrClN_3O_{13}$: C, 49.59; H, 4.66; H, 5.25. Found: C, 49.84; H, 4.65; N, 5.23.

Benzyl 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* (**22**). — A solution of freshly prepared **20** (1.85 g) and benzyl 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[6] (**21**, 1.245 g) in anhydrous dichloromethane (25 mL) was stirred for 15 min at room temperature under dry argon in the presence of 4 Å activated, powdered molecular sieve (1 g). The mixture was cooled to −20°, and 2,4,6-trimethylpyridine (0.37 mL) and freshly prepared silver triflate (0.67 g) were added. The mixture was stirred for 12 h in the dark at −20°, then diluted with dichloromethane (150 mL), filtered, washed with aqueous 10% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (100 g) with hexane–ethyl acetate (6:5) to give **22** (1.249 g, 55%) as a colourless glass, $[\alpha]_D$ +57° (*c* 1, chloroform). ^{1}H-N.m.r. data: unit E, δ 5.06 (t, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-5), 4.36 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.68 (s, 3 H, COOMe), 3.46 (dd, 1 H, $J_{2,3}$ 8.5 Hz, H-2); unit F, δ 5.39 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5 Hz, H-3), 5.29 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.19 (dd, 1 H, H-2); unit G, δ 3.59 (s, 3 H, COOMe).

Anal. Calc. for $C_{79}H_{87}ClN_4O_{28}$: C, 58.85; H, 5.69; N, 3.47. Found: C, 58.70; H, 5.66; N, 3.44.

Further elution with ethyl acetate–hexane (2:1) gave **21** (381 mg, 30%).

Benzyl 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 (**23**). — A solution of **22** (765 mg) in pyridine (23 mL) and ethanol (4 mL) was kept for 30 min at 100° in the presence of thiourea (53 mg), then cooled, and concentrated. A solution of the residue in chloroform (250 mL) was washed with aqueous 10% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (40 g) with ethyl acetate–hexane (3:2) to give **23** (598 mg, 82%), $[\alpha]_D$ +58.5° (*c* 0.8, chloroform). ^{1}H-N.m.r. data: unit E, δ 4.32 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 3.78 (s, 3 H, COOMe), 3.44 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9 Hz, H-3), 3.35 (dd, 1 H, H-2), 2.55 (bs, 1 H, OH); unit F, δ 5.29 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 3.19 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2); unit G, δ 3.59 (s, 3 H, COOMe); 7.25 (m, 30 H, 6 Ph), 2.10, 2.08, 2.07, and 1.97 (4 s, 12 H, 4 Ac).

Anal. Calc. for $C_{77}H_{86}N_4O_{27}$: C, 61.67; H, 5.78; N, 3.73. Found: C, 61.13; H, 5.85; N, 3.54.

Benzyl 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 (**25**). — A solution of freshly prepared 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide[14] (**24**, 1.9 g) and **23** (1.246 g) in anhydrous dichloromethane (25 mL) was stirred for 15 min at −20° under dry argon in the presence of 2,4,6-trimethylpyridine (0.67 mL) and 4 Å activated, powdered molecular sieve (1 g). Freshly prepared silver triflate was added, and the mixture was stirred for 1 h in the dark at −20°, diluted with dichloromethane (150 mL), filtered, washed with aqueous 10% $KHSO_4$ and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (80 g) with hexane–ethyl acetate (4:3) to give pentasaccharide fractions which were rechromatographed. Elution with chloroform–ethyl acetate (5:1) gave, first, **25** (1.106 g, 70%), $[\alpha]_D$ +65° (*c* 1, chloroform). ^{1}H-N.m.r. data: unit D, δ 5.50 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.18 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2); unit E, δ 4.35 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 3.42 (dd, 1 H, $J_{2,3}$ 8.7 Hz, H-2), 3.75 (s, 3 H, COOMe); unit F, δ 5.35 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 5.28 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 3.26 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2); unit G, δ 3.57 (s, 3 H, COOMe); 7.30 (m, Ph), 2.09, 2.08, 2.06, 2.03, and 2.00 (5 s, 15 H, 5 Ac).

Anal. Calc. for $C_{99}H_{109}N_7O_{32}$: C, 62.28; H, 5.75; N, 5.13. Found: C, 62.01; H, 5.72; N, 5.40.

Further elution gave the pentasaccharide **29** (219 mg, 14%), $[\alpha]_D$ +46° (*c* 1, chloroform). ^{1}H-N.m.r. data: unit D, δ 3.17 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 10.5 Hz, H-2); unit E, δ 4.33 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.82 (s, 3 H, COOMe), 3.38 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2); unit F, δ 5.34 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5 Hz, H-3), 5.29 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.26 (dd, 1 H, H-2); unit G, δ 3.58 (s, 3 H, COOMe); 7.30 (m, Ph), 2.10, 2.08, 2.04, 1.98, and 1.82 (5 s, 15 H, 5 Ac).

Anal. Calc. for $C_{99}H_{109}N_7O_{32}$: C, 62.28; H, 5.75; N, 5.13. Found: C, 61.66; H, 5.75; N, 5.13.

Benzyl 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)*-O-*(2-azido-2-deoxy-α*-D-*glucopyranosyl)-(1→4)*-O-*(methyl 3*-O-*benzyl-α*-L-*idopyranosyluronate)-(1→4)-3*-O-*benzyl-2-benzyloxycarbonylamino-2-deoxy-α*-D-*glucopyranoside* (**26**). — 5M Sodium hydroxide (13 mL) was added at room temperature to a solution of **25** (1.053 g) in chloroform (26 mL), methanol (94 mL), and water (13 mL). After 6 h, the mixture was diluted with chloroform (200 mL) and water (100 mL), and acidified with 6M HCl (20 mL). The organic layer was washed with water, treated with etheral diazomethane, and concentrated. The residue was eluted from a column of silica gel (50 g) with chloroform–methanol (20:1) to give amorphous **26** (686 mg, 73%), $[\alpha]_D$ +62° (*c* 1, chloroform). ^{1}H-N.m.r. data (CD_3OD): unit D, δ 5.52 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 3.32 (dd, 1 H, $J_{2,3}$ 7.2 Hz, H-2); unit E, δ 4.72 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 3.75 (s, 3 H, COOMe), 3.50 (t, 1 H, $J_{2,3}$ 8.5 Hz, H-2); unit F, δ 5.12 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 3.29 (dd, 1 H, $J_{2,3}$ 9 Hz, H-2); unit G, δ 5.25 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1), 3.18 (s, 3 H, COOMe).

Anal. Calc. for $C_{89}H_{99}N_7O_{27}$: C, 62.92; H, 5.87; N, 5.77. Found: C, 62.56; H, 5.92; N, 5.70.

Benzyl 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)*-O-*(2-azido-2-deoxy-3,6-di*-O-*sulfo-α*-D-*glucopyranosyl)-(1→4)*-O-*(methyl 3*-O-*benzyl-2*-O-*sulfo-α*-L-*idopyranosyluronate)-(1→4)-3*-O-*benzyl-2-benzyloxycarbonylamino-2-deoxy-6*-O-*sulfo-α*-D-*glucopyranoside pentasodium salt* (**27**). — A solution of **26** (364 mg) in *N,N*-dimethylformamide (3 mL) was stirred for 12 h at 50° in the presence of sulfur trioxide–trimethylamine complex (375 mg). The mixture was then cooled, methanol (1 mL) was added, and the mixture was eluted from a column (2 × 75 cm) of Sephadex LH-20 equilibrated with chloroform–methanol (1:1), using the same solvent. The product was eluted from a column of silica gel (30 g) with ethyl acetate–pyridine–acetic acid–water (8:5:1:3) to yield a pure fraction, a solution of which in methanol (1 mL) was eluted from a column of Sephadex SP-25 (Na^+ form) with methanol–water (9:1) to afford **27** (430 mg, 90%), m.p. 227–228° (dec.), $[\alpha]_D$ +40° (*c* 0.95, methanol). ^{1}H-N.m.r. data (CD_3OD): unit D, δ 5.52 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1); unit E, δ 3.80 (s, 3 H, COOMe); unit F, δ 5.20 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1); unit G, δ 5.37 (bs, 1 H, H-1), 2.90 (s, 3 H, COOMe).

This derivative was used immediately for the next reaction and was not submitted for elemental analysis.

Benzyl 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* (**28**). — A solution of **27** (409 mg) in methanol–water (2:1, 8 mL) was saponified with 2.5M NaOH (2.05 mL) for 3 h at room temperature. The mixture was diluted with aqueous methanol and eluted

successively from columns of Dowex 50W (H^+, 20–50 mesh) and (Na^+, 20–50 mesh) resins with the same solvent. The product **28** (372 mg, 90%), which was directly hydrogenated, had $[\alpha]_D$ +37° (*c* 2, methanol). ^{1}H-N.m.r. data (CD_3OD): unit D, δ 5.53 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1); unit E, δ 4.98 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 3.83 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.3 Hz, H-3), 3.62 (t, 1 H, H-2); unit F, δ 5.32 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1); unit G, δ 5.41 (bs, 1 H, H-1).

Anal. Calc. for $C_{87}H_{88}N_7Na_7O_{42}S_5 \cdot 7\ H_2O$: C, 44.44; H, 4.57; N, 4.17. Found: C, 44.44; H, 4.32; N, 4.32.

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-*D*-glucopyranose decasodium salt* (**30**). — A solution of **28** (560 mg) in methanol–water (4:1, 50 mL) was hydrogenated in the presence of 5% Pd/C (280 mg) for 4 days, then filtered, and concentrated. A solution of the residue in methanol–water (3:2, 40 mL) was hydrogenated again for 4 days using fresh catalyst. The suspension was filtered and concentrated, and a solution of the residue (330 mg, 93%) in water (10 mL) was adjusted to pH 9.5 with 0.5M NaOH. Sulfur trioxide–trimethylamine complex (240 mg) was added, the pH being maintained at 9.5 by automatic addition of 0.5M NaOH. The mixture was filtered and then eluted from a column (2.5 × 300 cm) of Sephadex G-50 (fine) with 0.2M sodium chloride, to give pentasaccharide fractions which were eluted from a column (1.6 × 12 cm) of Dowex AG 1-X2 resin (200–400 mesh) equilibrated with 0.5M sodium chloride, using a gradient of sodium chloride (0.5→3M). The pentasaccharide fractions were combined and desalted on a column (1.6 × 50 cm) of Sephadex G-25. The pure fractions were combined and lyophilised to give **30** as an amorphous, white powder (153 mg, 36%), $[\alpha]_D$ +42° (*c* 1, water). N.m.r. data: ^{1}H, (300 MHz, D_2O, 35°, internal TSP): unit D, δ 5.63 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.25 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2); unit E, δ 4.63 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.42 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.0 Hz, H-2); unit F, δ 5.52 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.37 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9 Hz, H-3), 3.97 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.44 (dd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 10.6 Hz, H-2); unit G, δ 5.21 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.31 (dd, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 7 Hz, H-2), 4.17 (dd, 1 H, $J_{2,3}$ 8.2, $J_{3,4}$ 3.6 Hz, H-3), 4.15 (dd, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ 2.7 Hz, H-4), 4.78 (d, 1 H, $J_{4,5}$ 2.7 Hz, H-5); unit H, δ 5.44 (d, $J_{1,2}$ 3.5 Hz, H-1α), 4.71 (dd, $J_{1,2}$ 8 Hz, H-1β), 3.26 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2α), 3.06 (dd, $J_{1,2}$ 8.2, $J_{2,3}$ 10.5 Hz, H-2β); ^{13}C (20 MHz, F.t.): unit D, δ 100.31 (C-1), 60.71 (C-2), 69.04 (C-6); unit E, δ 103.75 (C-1); unit F, δ 98.92 (C-1), 59.40 (C-2), 68.72 (C-6); unit G, δ 102.21 (C-1); unit H, δ 93.91 (C-1), 60.71 (C-2), 69.57 (C-6).

No destructive elemental analysis has been performed on this precious substance, but the high-field ^{1}H-n.m.r. spectrum demonstrated >97% purity.

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