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SYNTHESIS OF HEPARIN FRAGMENTS. A CHEMICAL SYNTHESIS 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 HEPTASODIUM SALT*

JEAN-CLAUDE JACQUINET[†], MAURICE PETITOU[‡], PHILIPPE DUCHAUSSOY[‡], ISIDORE LEDERMAN[‡], JEAN CHOAY[‡], GIANGIACOMO TORRI[§], AND PIERRE SINAY[†]**

[†]*Laboratoire de Biochimie Structurale, E.R.A. 739, U.E.R. de Sciences Fondamentales et Appliquées, 45046 Orléans (France);* [‡]*Institut Choay, 46 Avenue Théophile Gautier, 75782 Paris (France); and* [§]*G. Ronzoni Institute for Chemical and Biochemical Research, Via G. Colombo 81, 20133 Milan (Italy)*

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ABSTRACT

Known 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose was first converted into methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-idofuranuronate. Acid hydrolysis, followed by acetylation and treatment with titanium tetrabromide, gave methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl bromide)uronate, which was immediately transformed into methyl 4-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate 1,2-(*tert*-butyl orthoacetate). A two-step replacement of the 4-*O*-acetyl by a 4-*O*-chloroacetyl group gave the key derivative, crystalline methyl 3-*O*-benzyl-4-*O*-chloroacetyl-β-L-idopyranuronate 1,2-(*tert*-butyl orthoacetate). Condensation of this orthoester with an excess of crystalline benzyl 6-*O*-acetyl-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside in chlorobenzene in the presence of 2,6-dimethylpyridinium perchlorate gave crystalline benzyl 6-*O*-acetyl-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-4-*O*-(methyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-chloroacetyl-α-L-idopyranosyluronate)-α-D-glucopyranoside in 40% yield. *O*-Demonochloroacetylation, followed by condensation with known 3,6-di-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide in dichloromethane in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieve provided benzyl *O*-(3,6-di-*O*-acetyl-2-azido-4-*O*-benzyl-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-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside in 88% yield. *O*-Deacetylation with sodium hydroxide, followed successively by *O*-sulfation in *N,N*-dimethylformamide in the presence of sulfur trioxide–trimethylamine complex, catalytic hydrogenolysis, and *N*-sulfation

*Dedicated to Professor Raymond U. Lemieux.
**To whom enquiries should be sent.

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in water with the same sulfating agent, gave the heptasodium salt of *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 trisaccharide, which is a fragment of the minimal antithrombin III-binding region in heparin, neither binds to antithrombin III nor induces anti-Xa activity.

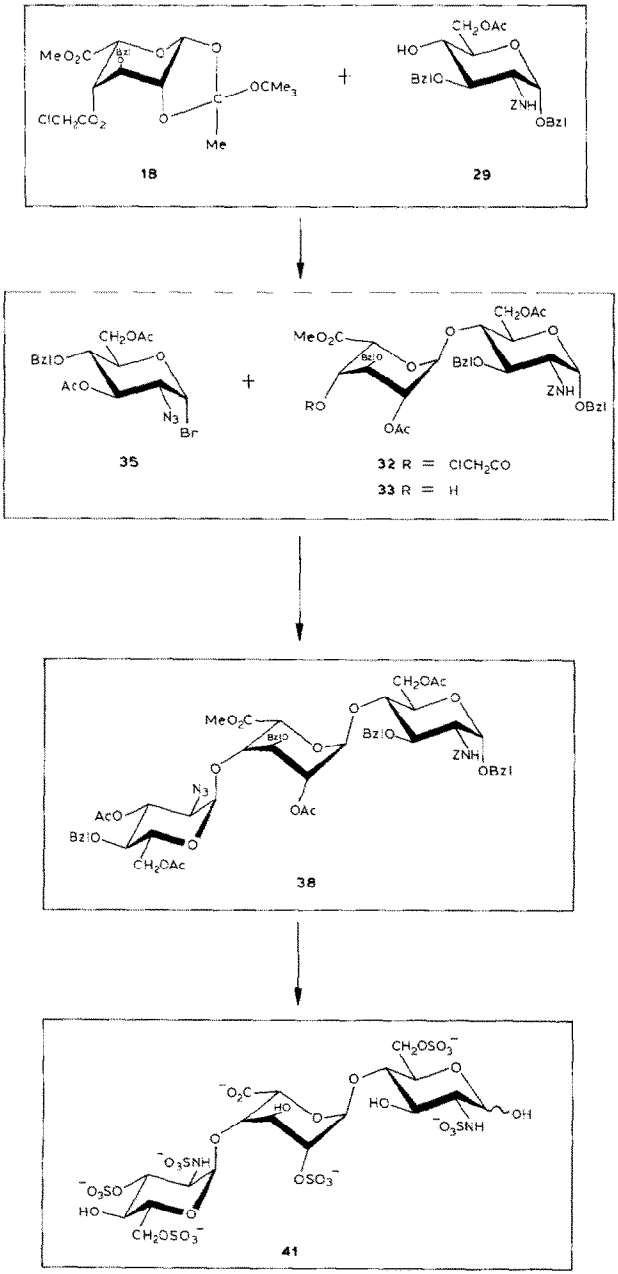
INTRODUCTION

Heparin is a sulfated glucosaminoglycuronan having a well known effect on blood coagulation[1]. There is now a general agreement that this effect is mediated by antithrombin III; about one-third of the polysaccharide preparations have high affinity for antithrombin III, thereby dramatically enhancing the interaction of the inhibitor. The structure of the antithrombin III-binding region in heparin has recently been extensively investigated[2,3], and structure **1** was suggested[4]. In order to test this hypothesis, we started a synthetic program that recently culminated in the total synthesis[5] of pentasaccharide **1**, which binds to antithrombin III and induces specific anti-Xa activity[6]. As a part of this program, we report the total synthesis of the title trisaccharide **41**, which is a fragment of the minimal antithrombin III-binding region of heparin.

RESULTS AND DISCUSSION

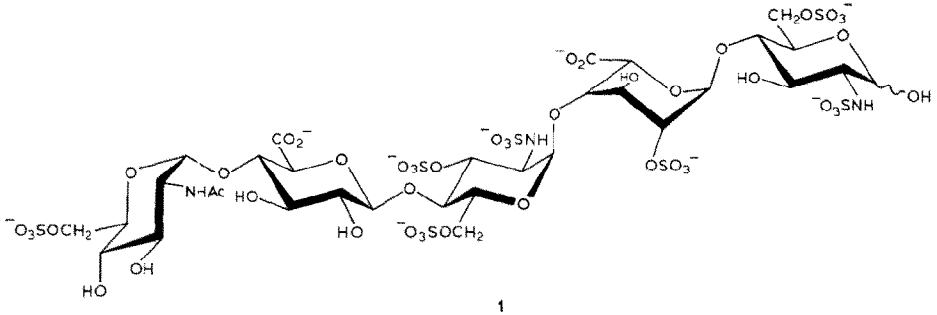
The general strategy of this synthesis is based on benzyl ethers as permanent blocking groups. Although, in the past, problems have been encountered during the catalytic hydrogenolysis of benzyl ethers in the presence of sulfate groups[7], a preliminary study of monosaccharide models[3] demonstrated that such a reaction was indeed possible. For clarity, the synthetic route to trisaccharide **41** is presented in Scheme 1, in which the orthoester **18** is a pivotal derivative of L-iduronic acid, and the synthesis of this key compound was first undertaken.

Easily available 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[8] (**2**) was first converted into amorphous methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranuronate (**4**) in a straightforward manner involving firstly the routine conversion into 5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-6-*O*-trityl-α-D-glucofuranose (**3**). Oxidation of the primary hydroxyl group was achieved directly on the crude trityl derivative **3** (chromium trioxide in acetone–sulfuric acid at room temperature) to give, after *O*-deacetylation and esterification of the free acid with diazomethane, the derivative **4** in 51% yield from the starting material **2**. Czuk *et al.*[9] have reported the inversion in high yield from the D-gluco to the L-ido configuration starting from 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone. We selected this approach, which had been recommended for compounds having an inherent tendency towards elimination during nucleophilic displacements. Treatment of the secondary alcohol **4** with trifluoromethanesulfonic anhydride according to Flechtner[10] gave a triflate which was treated with sodium trifluoroacetate in *N,N*-



Scheme 1 Z = CO_2Bzl

dimethylformamide[9]. Although Czuk *et al.*[9] had observed an inversion within 30 min at room temperature, in the case of a glucofuranurono-6,3-lactone, an overnight heating at 80° was necessary in the present case. *O*-Detrifluoroacetylation in methanol at room temperature proceeded smoothly to give methyl 3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-idofuranuronate (**5**) in 56% yield from **4**. Removal of the isopropylidene group with aqueous 90% trifluoroacetic acid at room temperature for 15 min gave a quantitative yield of crystalline methyl 3-*O*-benzyl-L-idopyranuronate (**6**). The ^{1}H-n.m.r. spectrum for its solution in ($^2H_4$)methanol demonstrated that, in that solvent, the $^1C_4$(L) conformation had been favored ($J_{2,3} = J_{3,4}$ 3 Hz, and $J_{4,5}$ 1.5 Hz), both α and β anomers being present (two singlets for the methyl ester). When **6** was treated with acetone in acidic medium, the furanose derivative **5** was obtained. The sequence **2**→**6**, which was performed several times on a large scale in our laboratories, provided an attractive preparation of 3-*O*-benzyl-L-idopyranuronic acid derivatives, in about 30% yield, from the easily available starting material **2**. The *ido* configuration of **5** was evident from the ^{1}H-n.m.r. parameters of **6**.



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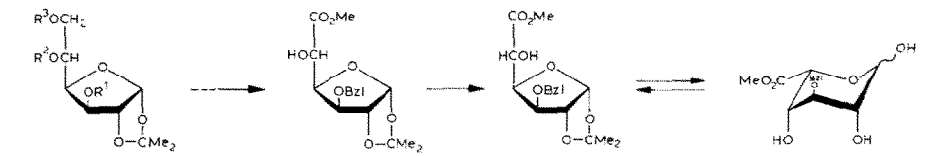
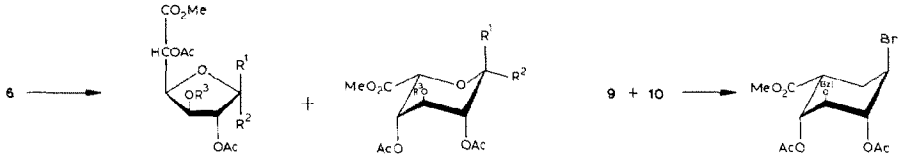


2 R^1 = Bzl, R^2 = R^3 = H
3 R^1 = Bzl, R^2 = Ac, R^3 = H

4 5 6

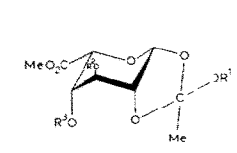
Acetylation of **6** (acetic anhydride and pyridine at room temperature) gave an almost quantitative yield of a mixture of the four isomeric peracetates **7**, **8**, **9**, and **10**. They were carefully separated and unambiguously characterized. The major compound was the β-pyranose acetate **10**, isolated in crystalline form in 63% yield. The anomeric configuration of **9** and **10** was unambiguously deduced from the optical rotation values (respectively −43 and +9° for a solution in chloroform). The ^{1}H-n.m.r. spectra for a solution in (^{2}H)chloroform demonstrated that, in that solvent, the $^1C_4$(L) conformation had been adopted ($J_{2,3} = J_{3,4}$ 3 Hz). In the

case of **9**, the signal of the anomeric proton appeared as a broad singlet at δ 6.23, and examination of the signal for H-3 at δ 3.88 revealed a long-range coupling of about 1 Hz between H-1 and -3, which was ascribed to a "W" arrangement of bonds. The corresponding H-1 signal of **10** appeared as a doublet ($J_{1,2}$ 1.5 Hz) at higher field (δ 6.08). Practically, the mixture of the furanose acetates **7** and **8** (28% yield) could be easily separated on a silica gel column from the mixture of the pyranose acetates **9** and **10** which, upon treatment with titanium tetrabromide for 24 h at room temperature in (10:1, v/v) dichloromethane–ethyl acetate, gave a very high yield (96%) of the syrupy bromide **11**. The configuration was deduced from the optical rotation value ($[\alpha]_D$ −48° in chloroform) and the ^{1}H-n.m.r. spectrum for a solution in (^{2}H)chloroform exhibited a broad H-1 singlet at δ 6.41. Compound **11** was immediately used for the preparation of the appropriate orthoester. The mixture of **7** and **8** could be *O*-deacetylated to give back the crystalline hemiacetal **6**, so that the conversion **6**→**11** may be considered as a high-yielding, overall process.

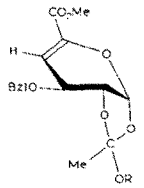


In order to study the conversion of bromide **11** into the orthoester **18** and its glycosylating properties, a model study of a methyl orthoacetate was firstly investigated. Treatment of **11** with methanol in the presence of 2,4,6-trimethylpyridine[11] gave the syrupy orthoester **12** in 73% yield as the pure *exo*-isomer[12]. The critical *O*-deacetylation of this base-sensitive molecule was best achieved at −20° in methanol in the presence of potassium carbonate. Under these conditions, the syrupy alcohol **13** was isolated, after silica gel column chromatography, in 63% yield, and separated from a minimal proportion (7%) of the elimination product **19**. Trichloroacetylation of **13** was achieved at −20° in dichloromethane, in the presence of trichloroacetic anhydride, to give the crystalline trichloroacetate **14** in 90% yield. After this demonstration that an acyl exchange at O-4 was indeed possible under carefully controlled experimental conditions (an essential step for the synthesis of the target trisaccharide), the glycosylating properties of the model methyl orthoacetate **12** were next investigated. For this purpose, methyl 6-*O*-benzoyl-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (**27**) was synthesized, as follows. Known methyl 4,6-*O*-benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside[13] (**24**) was benzylated with benzyl bromide in *N*,*N*-dimethylformamide, in the presence of barium oxide and barium hydroxide octahydrate, to give the crystalline 3-*O*-benzyl derivative **25** (91%). Removal of the benzylidene group with aqueous 60% acetic acid provided, in 89%

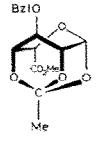
yield, the crystalline diol **26** which was then selectively *O*-benzoylated with benzoyl cyanide in dichloromethane–pyridine[14] to give a 90% yield of the crystalline, secondary alcohol **27**. The ^{1}H-n.m.r. spectrum of this compound exhibited a signal due to H-4 at δ 3.80, which demonstrated that the OH-4 group had not been benzoylated. Glycosylation of **27** with the previously prepared methyl orthoacetate **12** according to Kochetkov *et al.*[15] auspiciously gave the crystalline disaccharide **30** in 50% yield, the unreacted starting material **27** being recovered in 38% yield. The anomeric configuration of the interglycoside linkage of **30** was suggested by the value of the optical rotation, **22** and **23** serving as standards for the calculation. Furthermore, the ^{1}H-n.m.r. spectrum of a solution of **30** in (^{2}H)chloroform exhibited a signal due to H-1′ at δ 5.06 as a broad singlet. In view of these results the pivotal *tert*-butyl orthoacetate **18** could be synthesized with confidence as follows.



12 R^1 = Me, R^2 = Bzl, R^3 = Ac
13 R^1 = Me, R^2 = Bzl, R^3 = H
14 R^1 = Me, R^2 = Bzl, R^3 = CCl_3CO
15 R^1 = CMe_3, R^2 = Bzl, R^3 = Ac
16 R^1 = CMe_3, R^2 = Bzl, R^3 = H
17 R^1 = CMe_3, R^2 = Bzl, R^3 = CCl_3CO
18 R^1 = CMe_3, R^2 = Bzl, R^3 = $ClCH_2CO$



19 R = Me
20 R = CMe_3



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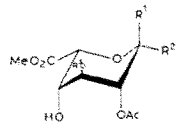
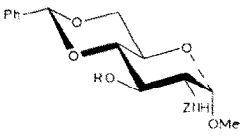
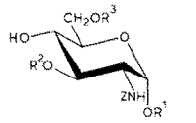


22 R = OBzl, R^2 = H, R^3 = Bzl
23 R^1 = H, R^2 = OBzl, R^3 = Bzl

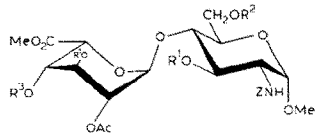


24 R = H
25 R = Bzl

Z = CO_2Bzl



26 R^1 = Me, R^2 = Bzl, R^3 = H
27 R^1 = Me, R^2 = Bzl, R^3 = Bz
28 R = R^2 = Bzl, R^3 = H
29 R^1 = R^2 = Bzl, R^3 = Ac



30 R^1 = Bzl, R^2 = Bz, R^3 = Ac
31 R^1 = Bzl, R^2 = Bz, R^3 = $ClCH_2CO$
32 R^1 = Bzl, R^2 = Ac, R^3 = $ClCH_2CO$
33 R^1 = Bzl, R^2 = Ac, R^3 = H

Bromide **11** was transformed into the syrupy *tert*-butyl orthoacetate **15** in 73% yield. The aforementioned, mild *O*-deacetylation was applied to **15** to give **16** in 62% yield, after fast-flow silica gel-column chromatography to remove a small proportion (7%) of the elimination product **20**; a slow-flow chromatography resulted into the formation of various proportions of the tricyclic orthoester **21**, unambiguously characterized by ^{1}H-n.m.r. spectroscopy. Attempts at crystallizing the alcohol **16** indeed gave a pure crystalline substance but in low yield (~30%), owing to concomitant, extensive formation of the orthoester **21**. For this reason, purified **16** was not crystallized, but immediately trichloroacetylated with trichloroacetic anhydride in dichloromethane–pyridine for 30 min at −20°, to give

the crystalline trichloroacetate **17** in 91% yield. As initial glycosylation experiments with **17** gave rather disappointing results, the more stable monochloroacetate **18** was selected as the key derivative. In order to optimize the yield of its formation, the crude product from the mild *O*-deacetylation of **15** was not purified, but instead directly monochloroacetylated with monochloroacetyl chloride at −20° for 30 min in dichloromethane–pyridine. The pure key compound **18** was then isolated in crystalline form, in 67% yield from **15**, after silica gel-column chromatography, a small proportion (7%) of **20** being separated and found identical with the by-product previously isolated. Thus, the key intermediate (**18**) for the synthesis of the complex trisaccharide **38** was synthesized in a few steps from the L-iduronic acid derivative **6** in ~30% yield.

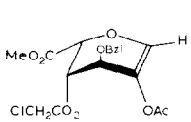
Treatment of **18** with benzyl alcohol in chlorobenzene in the presence of 2,6-dimethylpyridinium perchlorate[15] gave a mixture of glycosides that was fractionated by silica gel column chromatography after *O*-demonochloroacetylation with thiourea[16] in pyridine–ethanol[17]. Syrupy methyl (benzyl 2-*O*-acetyl-3-*O*-benzyl-β (**23**) and -α-L-idopyranosid)uronate (**22**) were obtained in 25 and 50% yield, respectively, from the orthoester **18**. The anomeric configuration of **22** and **23** was unambiguously assigned by the optical rotation values of −65 and +70°, respectively, for solutions in chloroform. In addition, and in full agreement with similar data for compounds **9**–**11**, the ^{1}H-n.m.r. spectrum of the α-glycoside **22** exhibited a signal due to H-1 at δ 5.05 as a broad singlet, whereas the β-glycoside **23** exhibited the corresponding signal at higher field (δ 4.90) as a doublet with $J_{1,2}$ 2 Hz. Both rings adopted a $^1C_4$(L) conformation ($J_{4,5}$ 2 Hz, $J_{2,3}$ and $J_{3,4}$ 3.5 Hz). In this series, the appearance of the signal for H-1 for solutions in (^{2}H)chloroform as a broad singlet at low field thus strongly supports the α configuration at C-1. The benzyl glycosides **22** and **23** proved to be valuable derivatives for the synthesis of heparin fragments.

Condensation of **18** with alcohol **27**, as previously described, gave the crystalline disaccharide **31** in 30% yield, 60% of the unreacted starting material **27** being recovered after silica gel chromatography. No attention was paid, at this stage, on the isolation of the decomposition products from orthoester **18**. The structure of **31** was ascertained after selective *O*-demonochloroacetylation with thiourea in pyridine–methanol[17], followed by acetylation (acetic anhydride in pyridine), to give the disaccharide **30** previously prepared.

On the basis of these model studies, the synthesis of disaccharide **33** was successfully achieved, as follows. Benzyl 3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside[18] (**28**) was selectively acetylated with *N*-acetylimidazole in 1,2-dichloroethane to give crystalline benzyl 6-*O*-acetyl-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (**29**) in 71% yield. The ^{1}H-n.m.r. spectrum of **29** exhibited, at δ 3.95, a signal due to H-4 which demonstrated that OH-4 was not acetylated. Condensation of **18** (1.55 equiv.) with alcohol **29** (1 equiv.) in chlorobenzene in the presence of 2,6-dimethylpyridinium perchlorate gave crystalline disaccharide **32** in 35% yield (based on **29**), and 52%

of unreacted starting **29**. In addition, the syrupy 2-acetoxyglycal **34** was isolated, from the reaction mixture after silica gel chromatography, in 59% yield (based on orthoester **18**). The formation of this glycal is severely competing with the normal glycosylation step, thus lowering the yield of the condensation product when a moderate excess of orthoester **18** was used. Selective *O*-demonochloroacetylation of the protected disaccharide **32** gave **33** in 86% yield. When a four-molar excess of **29** was used in order to increase the yield of the reaction from **18**, disaccharide **33** was obtained, after *O*-demonochloroacetylation, in 40% yield (based on orthoester **18**). The α configuration of the newly synthesized glycoside linkage was deduced from ^{1}H-n.m.r. spectroscopy, as previously demonstrated in this series; the signal for H-1′ appeared as a broad singlet at δ 5.07, the L-iduronic residue adopting a $^1C_4$(L) chair conformation (the signal for H-3′ appeared as a triplet at δ 3.70, with $J_{2',3'} = J_{3',4'}$ 3 Hz).

Condensation of the disaccharide **33** with previously described[19] 3,6-di-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide (**35**) in dichloromethane at −20°, in the presence of freshly prepared silver triflate[20] and



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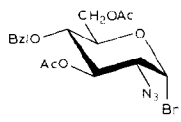

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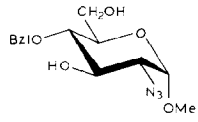

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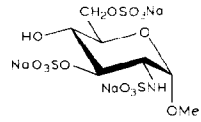

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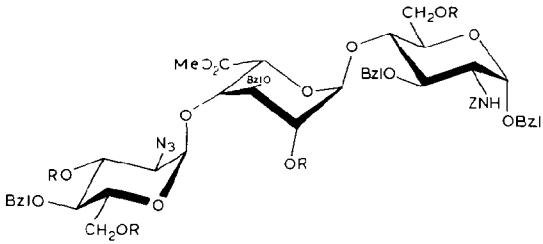
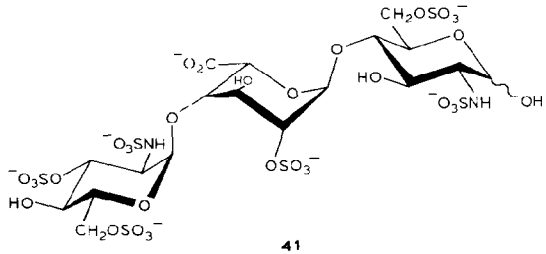


38 R = Ac
39 R = H
40 R = SO_3Na



41

2,4,6-trimethylpyridine, gave the amorphous trisaccharide **38** in 88% yield. The ^{1}H-n.m.r. spectrum of **38** exhibited a signal due to H-1″ at δ 5.26 as a doublet with $J_{1'',2''}$ 3.5 Hz, demonstrating the α configuration of the newly synthesized glycoside bond.

Conversion of the protected trisaccharide **38** into the final compound **41** was accomplished as follows. During previous work on the synthesis of heparin fragments[5], OAc-3 of the 2-amino-2-deoxy-D-glucose residue was found to be rather resistant to the conventional sodium methoxide–methanol treatment. Consequently *O*-deacetylation of **38** was achieved at 0° with M sodium hydroxide in 1,2-dimethoxyethane–methanol. After esterification with diazomethane, the amorphous derivative **39** was isolated in 81% yield after silica gel purification; no ^{1}H-n.m.r. acetyl signal was present in the expected region. *O*-Sulfation of the tetrol **39** was achieved with sulfur trioxide–trimethylamine complex in *N,N*-dimethylformamide. The trimethylammonium salt was purified by chromatography on Sephadex LH-20, and then on silica gel. Final purification on Sephadex SP-25 (Na^+) afforded the amorphous trisaccharide **40**, as a sodium salt, in 87% yield. Comparison of the 300 MHz ^{1}H-n.m.r. spectrum of **40** with that of **39** in the same solvent [($^2H_4$)methanol] showed the expected, downfield displacements of the signals of protons associated with sulfuric esters: H-6 and H-6″, ~0.5 p.p.m.; H-2′, 1.04 p.p.m.; and H-3″, 0.84 p.p.m. Noticeable was also the significant downfield displacement by 0.68 p.p.m. of the signal of H-1′ caused by *O*-sulfation on a vicinal position. These data demonstrated that **40** was indeed a tetra-*O*-sulfate at the expected positions. Catalytic hydrogenolysis in methanol–water in the presence of Pd–C removed all the benzyl groups (no aromatic signal was present in the ^{1}H-n.m.r. spectrum), and converted the azido into an amino group, thus demonstrating that the ionic sulfate group did not interfere with the hydrogenation, as previously stated[7]. *N*-Sulfation was achieved in aqueous solution at pH 9 and room temperature for two days with sulfur trioxide–trimethylamine complex. Purification on Sephadex G-25, then on Sephadex SP-25 (Na^+), gave the expected trisaccharide **41** in 70% yield (based on **40**). The ^{1}H-n.m.r. spectrum of **41** for a solution in deuterium oxide exhibited full signals at δ 4.34 for H-3″, and 4.36 for H-6,6″, and -2′. These chemical shifts are characteristic of corresponding *O*-sulfated positions, as shown earlier by an ^{1}H-n.m.r. study of various synthetic model monosaccharides[3,21]. For example, synthetic compound[21] **36** exhibited signals at δ 3.65 (dd) for H-3, and 3.32 for H-6, whereas sulfated synthetic compound[21] **37** exhibited signals for a solution in deuterium oxide at δ 4.37 for H-3, and 4.32 for H-6. These data demonstrate the uniformity and selectivity of the *O*-sulfation in compound **41**. Except for H-3″, which is typically deshielded, the reported chemical shifts of the pure synthetic compound **41** are in excellent agreement with the correspnding shifts for heparin[22].

Antithrombin III-binding experiments by gel filtration[6], and anti-Xa activity measured either by the clotting assay of Yin *et al.*[23] or by the amidolytic assay of Teien and Lie[24] showed that the synthetic trisaccharide **41** neither binds to anti-

thrombin III nor induces anti-Xa activity. These results indicate that, although being a part of the postulated fragment of the heparin to antithrombin III-binding site, the trisaccharide **41** does not contain all the minimal structural requirements to bind antithrombin III and to induce anti-Xa activity. Nevertheless, this work is the first successful approach to synthesize a glycosaminoglycuronan fragment specifically sulfated, thus demonstrating that chemical synthesis may contribute to the study of the structure–activity relationship of glycosaminoglycuronan sulfates.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 22–25° with a Perkin–Elmer model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Perkin–Elmer R-32 instrument for solution in (^{2}H)chloroform unless otherwise stated; the chemical shifts (δ) are given from the signal of internal Me_4Si. The purity of products was determined by t.l.c. on Silica gel 60 F 154 (Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica gel 60 (Merck, 63–200 μm) which was used without pre-treatment. Elemental analyses were done at the Service Central de Micro-Analyse du Centre National de la Recherche Scientifique.

*5-O-Acetyl-3-O-benzyl-1,2-O-isopropylidene-6-O-trityl-α-*D*-glucofuranose* (**3**). — A mixture of 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[8] (**2**; 119 g) and freshly purified chlorotriphenylmethane(128 g) in dry pyridine (500 mL) was heated for 2 h at 80° and then cooled to 0°. Acetic anhydride (360 mL) was added, and the mixture stirred overnight at room temperature and evaporated to dryness. The residue was dissolved in chloroform (1.5 L) and the solution washed successively with 10% aqueous $KHSO_4$, water, 5% aqueous $NaHCO_3$, water, dried (Na_2SO_4), and evaporated to give a crude syrup (303 g) that was used without further purification. A fraction (100 mg) was purified on silica gel (8 g). Elution with 3:1 (v/v) hexane–ethyl acetate gave **3** (72 mg) as a colorless glass, $[\alpha]_D^{23}$ −49° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (m, 20 H, Ph), 5.82 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 3.92 (d, 1 H, $J_{3,4}$ 3 Hz, H-3), 1.86 (s, 3 H, Ac), 1.50 and 1.29 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{37}H_{38}O_7$: C, 74.73; H, 6.44. Found: C, 74.66; H, 6.33.

*Methyl 3-O-benzyl-1,2-O-isopropylidene-α-*D*-glucofuranuronate* (**4**). — Crude **3** (152 g) was dissolved in acetone (1.5 L) and the solution cooled to 0°. A solution of chromium trioxide (192 g) in 3.5M sulfuric acid (250 mL) was added dropwise under stirring, and the mixture allowed to warm up to room temperature. After 3 h, the mixture was poured into ice-cold water (1.5 L) and extracted with chloroform (3 × 500 mL). The combined extracts were washed twice with water, dried (Na_2SO_4), and evaporated. The residue (120 g) was dissolved in methanol (800 mL). A solution of 4M NaOH (120 mL) was added, and the pH of the solution was brought to ~1 with M HCl. After extraction with chloroform (5 × 100 mL), the combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The

residue was esterified with etheral diazomethane, and the product was purified on silica gel (500 g). Elution with 3:2 (v/v) ether–hexane gave amorphous **4** (32 g, 51% from **2**), $[\alpha]_D^{23}$ −27° (*c* 2.5, chloroform); ^{1}H-n.m.r.: δ 7.31 (m, 5 H, Ph), 6.01 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.73 (s, 3 H, CO_2Me), 3.35 (d, 1 H, *J* 9 Hz, OH-5), 1.47 and 1.32 (2 s, 5 H, CMe_2).

Anal. Calc. for $C_{17}H_{22}O_7$: C, 60.34; H, 6.55. Found: C, 60.39; H, 6.54.

*Methyl 3-O-benzyl-1,2-O-isopropylidene-β-*L*-idofuranuronate* (**5**). — A mixture of pyridine (24.5 mL) and dichloromethane (250 mL) was cooled to −20° with exclusion of moisture. A solution of trifluoromethanesulfonic anhydride (23.5 mL) in dichloromethane (450 mL) was then added dropwise. The mixture was allowed to warm up to −10° and a solution of **4** (22.42 g) in dichloromethane (230 mL) was added dropwise under stirring. After 1 h at −10°, the mixture was poured into ice-cold water (1.5 L) containing $NaHCO_3$ (5 g), and stirred for 1 h. The organic layer was washed with 3% HCl, water, dried (Na_2SO_4), and evaporated. A mixture of the crude residue and sodium trifluoroacetate (19.5 g) in dry *N,N*-dimethylformamide (250 mL) was stirred overnight at 80°, cooled, and evaporated to dryness. The residue was dissolved in dichloromethane (200 mL), the organic layer washed twice with water, dried (Na_2SO), and evaporated. The residue was immediately dissolved in methanol (250 mL), and stirred overnight at room temperature. After evaporation to dryness, the mixture was purified on silica gel (200 g). Elution with 2:1 (v/v) ether–hexane gave amorphous **5** (12.55 g, 56% from **4**), $[\alpha]_D^{23}$ −33° (*c* 1.9, chloroform); ^{1}H-n.m.r.: δ 7.31 (m, 5 H, Ph), 5.96 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 3.70 (s, 3 H, CO_2Me), 3.30 (1 H, OH-5), 1.47 and 1.32 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{17}H_{22}O_7$: C, 60.34; H, 6.55. Found: C, 59.99; H, 6.57.

*Methyl 3-O-benzyl-*L*-idopyranuronate* (**6**). — A solution of **5** (13 g) in aqueous 90% trifluoroacetic acid (80 mL) was kept at room temperature for 15 min, evaporated to dryness, and evaporated twice with water (2 × 20 mL) to give a white solid residue (11.20 g, 99%), which was crystallized from ethyl acetate, m.p. 127–129°, $[\alpha]_D^{23}$ +13° (*c* 1, methanol); ^{1}H-n.m.r. (CD_3OD): δ 7.26 (s, 5 H, Ph), 4.92 (broad s, H-1α), 4.48 (d, 1 H, $J_{4,5}$ 1.5 Hz, H-5), 3.94 (m, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 1.5 Hz, H-4), 3.80 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 3 Hz, H-3), 3.70 and 3.60 (2 s, 3 H, CO_2Me, α and β).

Anal. Calc. for $C_{14}H_{18}O_7$: C, 56.37; H, 6.08. Found: C, 56.17; H, 5.85.

Methyl 1,2,4-tri-O-acetyl-3-O-benzyl-α- (**9**) *and -β-*L*-idopyranuronate* (**10**). — Conventional acetylation of **6** (3 g) with acetic anhydride (10 mL) and pyridine (20 mL) for 5 h at room temperature and elution of the residue from a column of silica gel (150 g) with 4:1 (v/v) toluene–ethyl acetate gave a mixture of methyl 1,2,5-tri-*O*-acetyl-3-*O*-benzyl-α- (**7**) and -β-L-idofuranuronate (**8**) (1.194 g, 28%), and then a pure fraction of syrupy **9** (170 mg, 4%), $[\alpha]_D^{23}$ −43° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.32 (s, 5 H, Ph), 6.23 (br. s, 1 H, H-1), 5.24 (m, 1 H, H-4), 4.95 (m, 2 H, H-2,5), 4.72 (s, 2 H, OCH_2Ph), 3.88 (m, 1 H, $J_{2,3}$ and $J_{3,4}$ 3 Hz, H-3), 3.75 (s, 3 H, CO_2Me), 2.06 and 2.04 (2 s, 9 H, Ac).

Anal. Calc. for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70. Found: C, 56.59; H, 5.73.

Further elution gave **10** (2.668 g, 63%), m.p. 112–113° (from ether), $[\alpha]_D^{23}$ +9° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.33 (s, 5 H, Ph), 6.08 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.16 (m, 1 H. $J_{2,4}$ 1, $J_{3,4}$ 3, $J_{4,5}$ 2 Hz, H-4), 5.03 (m, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3, $J_{2,4}$ 1 Hz, H-2), 4.72 (d, 1 H, $J_{4,5}$ 2 Hz, H-5), 4.71 (s, 2 H, OCH_2Ph), 3.96 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 3 Hz, H-3), 3.75 (s, 3 H, CO_2Me), 2.10 and 2.03 (2 s, 9 H, Ac).

Anal. Calc. for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70. Found: C, 56.62; H, 5.55.

*Methyl 1,2,5-tri-*O*-acetyl-3-*O*-benzyl-α-* (**7**) *and -β-*L*-idofuranuronate* (**8**). — A portion (300 mg) of the mixture of **7** and **8** was eluted from a column of silica gel (30 g) with 3:2 (v/v) hexane–ethyl acetate to give syrupy **7** (84 mg), $[\alpha]_D^{23}$ +5° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (s, 5 H, Ph), 6.10 (s, 1 H, H-1), 5.66 (d, 1 H, $J_{4,5}$ 7.5 Hz, H-5), 5.31 (d, 1 H, $J_{2,3}$ 1.5 Hz, H-2), 4.23 (dd, 1 H, $J_{2,3}$ 1.5, $J_{3,4}$ 6 Hz, H-3), 3.60 (s, 3 H, CO_2Me), and 2.10 (s, 9 H, Ac).

Anal. Calc. for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70. Found: C, 56.90; H, 5.81.

Further elution gave a mixture of **7** and **8**, and then pure **8** (75 mg), $[\alpha]_D^{23}$ +88° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.28 (s, 5 H, Ph), 6.41 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 5.35 (m, 2 H, H-5,2), 4.84 (dd, 1 H, $J_{2,3}$ 2.5, $J_{3,4}$ 7.5 Hz, H-3), 4.59 (s, 2 H, OCH_2Ph), 4.48 (t, 1 H, $J_{3,4}$ and $J_{4,5}$ 7.5 Hz, H-4), 3.72 (s, 3 H, CO_2Me), 2.15 and 2.03 (2 s, 9 H, Ac).

Anal. Calc. for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70. Found: C, 56.42; H, 5.87.

*Methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-α-*L*-idopyranosyl bromide)uronate* (**11**). — A solution of **9** and **10** (425 mg) in anhydrous dichloromethane (10 mL) and ethyl acetate (1 mL) was stirred for 24 h at room temperature in the presence of $TiBr_4$ (470 mg). The mixture was diluted with dichloromethane (50 mL), washed with ice-cold water, dried (Na_2SO_4), and evaporated. The syrupy residue (**11**) (435 mg, 96%) was immediately used for the next reaction, $[\alpha]_D^{23}$ −48° (*c* 1, chloroform); ^{1}H-n.m.r : δ 7.30 (m, 5 H, Ph), 6.41 (br. s, 1 H, H-1), 3.75 (s, 3 H, CO_2Me), and 2.04 (s, 6 H, Ac).

*Methyl 4-*O*-acetyl-3-*O*-benzyl-β-*L*-idopyranuronate 1,2-(methyl orthoacetate)* (**12**). — A solution of bromide **11** (435 mg) in anhydrous dichloromethane (10 mL) containing 2,4,6-trimethylpyridine (0.66 mL) and methanol (0.4 mL) was stirred for 20 h at room temperature under a dry atmosphere of argon. The mixture was diluted with dichloromethane (200 mL), washed with aqueous saturated $NaHCO_3$, water, dried (Na_2SO_4), and evaporated. Elution of the residue from a column of silica gel (20 g) with 3:2 (v/v) hexane–ethyl acetate containing triethylamine (0.5%) gave the syrupy orthoacetate **12** (302 mg, 73%), $[\alpha]_D^{23}$ −21°(*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.31 (s, 5 H, Ph), 5.52 (d, 1 H, $J_{1,2}$ 3 Hz, H-1), 5.18 (dd, 1 H, $J_{3,4}$ 3, $J_{4,5}$ 1.5 Hz, H-4), 4.71 (AB system, 2 H, OCH_2Ph), 4.51 (d, 1 H, $J_{4,5}$ 1.5 Hz, H-5). 4.10 (m, 2 H, H-2,3), 3.74 (s, 3 H, CO_2Me), 3.22 (s, 3 H, OMe), 2.00 (s, 3 H, Ac), and 1.71 (s, 3 H, CMe).

Anal. Calc. for $C_{19}H_{24}O_9$: C, 57.57; H, 6.10;. Found: C, 57.72; H, 5.99.

*Methyl 3-*O*-benzyl-β-*L*-idopyranuronate 1,2-(methyl orthoacetate)* (**13**). — A solution of the orthoacetate **12** (470 mg) in anhydrous methanol (15 mL) was stirred for 5 h at −20° in the presence of anhydrous K_2CO_3 (60 mg) under a dry at-

mosphere of argon. The mixture was filtered and the filtrate evaporated. The residue was dissolved in chloroform (50 mL), and the solution washed with aqueous saturated NaCl, with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (30 g). Elution with 3:2 (v/v) hexane–ethyl acetate containing triethylamine (0.5%) gave a syrupy compound (28 mg, 7%) that was identified as methyl 3-*O*-benzyl-β-L-*lyxo*-hex-4-enopyranuronate 1,2-(methyl orthoacetate) (**19**), $[\alpha]_D^{23}$ +98° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.32 (s, 5 H, Ph), 6.28 (dd, 1 H, $J_{3,4}$ 4.5, $J_{2,4}$ 1.5 Hz, H-4), 5.79 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.65 (s, 2 H, OCH_2Ph), 4.55 (m, 1 H, $J_{1,2}$ 3.5, $J_{2,4}$ 1.5, $J_{2,3}$ 2 Hz, H-2), 4.21 (dd, 1 H, $J_{2,3}$ 2, $J_{3,4}$ 4.5 Hz, H-3), 3.84 (s, 3 H, CO_2Me), 3.31 (s, 3 H, OMe), and 1.59 (s, 3 H, CMe); no satisfactory elemental analysis was obtained.

Further elution provided syrupy orthoacetate **13** (261 mg, 62%), $[\alpha]_D^{23}$ −14° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.35 (s, 5 H, Ph), 5.50 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.68 (s, 2 H, OCH_2Ph), 4.49 (d, 1 H, $J_{4,5}$ 1 Hz, H-5), 3.81 (s, 3 H, CO_2Me), 3.30 (s, 3 H, OMe), 2.80 (d, 1 H, *J* 12 Hz, OH), and 1.76 (s, 3 H, CMe).

Anal. Calc. for $C_{17}H_{22}O_8$: C, 57.62; H, 6.26. Found: C, 57.65; H, 6.26.

Methyl 3-O-benzyl-4-O-trichloroacetyl-β-L-idopyranuronate 1,2-(methyl orthoacetate) (**14**). — A solution of trichloroacetic anhydride (0.15 mL) in anhydrous dichloromethane (1 mL) was dropwise added within 5 min at −20° to a solution of **13** (142 mg) in pyridine (3 mL) and dichloromethane (1 mL). After 30 min, the mixture was poured into ice-cold water (50 mL) and extracted with dichloromethane (3 × 15 mL). The organic extract was washed with water, dried (Na_2SO_4), and evaporated. The residue crystallized from ether–hexane to give **14** (180 mg, 90%), m.p. 109–110°, $[\alpha]_D^{23}$ −7° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.36 (s, 5 H, Ph), 5.55 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 5.30 (dd, 1 H, $J_{3,4}$ 2.5, $J_{4,5}$ 1.5 Hz, H-4), 4.78 (AB system, 2 H, OCH_2Ph), 4.60 (d, 1 H, $J_{4,5}$ 1.5 Hz, H-5), 3.77 (s, 3 H, CO_2Me), 3.26 (s, 3 H, OMe), and 1.72 (s, 3 H, CMe).

Anal. Calc. for $C_{19}H_{21}Cl_3O_9$: C, 45.67; H, 4.24. Found: C, 45.59; H, 4.04.

*Methyl 4-O-acetyl-3-O-benzyl-β-L-idopyranuronate 1,2-(*tert*-butyl orthoacetate)* (**15**). — A solution of bromide **11** (2.119 g) in anhydrous dichloromethane (20 mL) containing 2,4,6-trimethylpyridine (2.65 mL) and *tert*-butyl alcohol (3 mL) was stirred for 15 h at room temperature under a dry atmosphere of argon. Elution of the residue from a column of silica gel (120 g) with 2:1 (v/v) hexane–ethyl acetate containing triethylamine (0.5%) gave the syrupy orthoacetate **15** (1.542 g, 73%), $[\alpha]_D^{23}$ −23° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.35 (s, 5 H, Ph), 5.48 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 5.20 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.5 Hz, H-4), 4.76 (AB system, 2 H, OCH_2Ph), 4.55 (d, 1 H, $J_{4,5}$ 1.5 Hz, H-5), 4.10 (m, 2 H, H-2,3), 3.80 (s, 3 H, CO_2Me), 2.06 (s, 3 H, Ac), 1.82 (s, 3 H, CMe), and 1.35 (s, 9 H, CMe_3).

Anal. Calc. for $C_{22}H_{30}O_9$: C, 60.26; H, 6.90. Found: C, 60.59; H, 6.85.

*Methyl 3-O-benzyl-β-L-idopyranuronate 1,2-(*tert*-butyl orthoacetate)* (**16**). — A solution of the orthoacetate **15** (424 mg) in anhydrous methanol (15 mL) was stirred for 5 h at −20° in the presence of anhydrous K_2CO_3 (60 mg) under a dry at-

mosphere of argon. The mixture was filtered and the filtrate evaporated. The residue was dissolved in chloroform (50 mL), and the solution washed with aqueous saturated NaCl, with water, dried (Na_2SO_4), and evaporated. The residue was promptly chromatographed on a column of silica gel (25 g). Elution with 2:1 (v/v) hexane–ethyl acetate containing triethylamine (0.5%) gave a syrupy compound (31 mg, 7%) that was identified as unstable methyl 3-*O*-benzyl-β-L-*lyxo*-hex-4-enopyranuronate 1,2-(*tert*-butyl orthoacetate) (**20**) $[\alpha]_D^{23}$ +103° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.33 (s, 5 H, Ph), 6.27 (dd, 1 H, $J_{3,4}$ 5, $J_{2,4}$ 1 Hz, H-4), 5.67 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 4.63 (s, 2 H, OCH_2Ph), 4.55 (m, 1 H, $J_{1,2}$ 4, $J_{2,3}$ 2, $J_{2,4}$ 1 Hz, H-2), 4.18 (dd, $J_{2,3}$ 2, $J_{3,4}$ 5 Hz, H-3), 3.81 (s, 3 H, CO_2Me), 1.67 (s, 3 H, CMe), and 1.34 (s, 9 H, CMe_3); no satisfactory elemental analysis was obtained.

Further elution provided the orthoacetate **16** (271 mg, 62%). Crystallization from ether–hexane provided a poor yield (123 mg, 28%) of **16**, m.p. 68–69°, $[\alpha]_D^{23}$ −19° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.34 (s, 5 H, Ph), 5.41 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.68 (AB system, 2 H, OCH_2Ph), 4.48 (d, 1 H, $J_{4,5}$ 1 Hz, H-5), 3.80 (s, 3 H, CO_2Me), 2.85 (d, 1 H, *J* 12 Hz, OH), 1.82 (s, 3 H, CMe), and 1.33 (s, H, CMe_2).

Anal. Calc. for $C_{20}H_{28}O_8$: C, 60.59; H, 7.12. Found: C, 60.82; H, 7.14.

The mother liquors of the crystallization of **16** were evaporated and the residue was chromatographed on a column of silica gel (10 g). Elution with 5:2 (v/v) hexane–ethyl acetate gave syrupy methyl 3-*O*-benzyl-1,2,4-*O*-ethylidene-β-L-idopyranuronate (**21**) (41 mg, 11%), $[\alpha]_D^{23}$ +21° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (s, 5 H, Ph), 5.83 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 4.37 (dd, 1 H, $J_{1,2}$ 4.5, $J_{2,3}$ 2.5 Hz, H-2), 3.99 (dd, 1 H, $J_{2,3}$ 2.5, $J_{3,4}$ 4.5 Hz, H-3), 3.75 (s, 3 H, CO_2Me), and 1.55 (s, 3 H, CMe).

Anal. Calc. for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.56; H, 5.69.

*Methyl 3-O-benzyl-4-O-trichloroacetyl-β-L-idopyranuronate 1,2-(*tert*-butyl orthoacetate)* (**17**). — A solution of trichloroacetic anhydride (0.185 mL) in anhydrous dichloromethane (2 mL) was dropwise added, at −20° within 5 min, to a solution of **16** (198 mg) in pyridine (5 mL) and dichloromethane (2 mL). After 30 min, the mixture was poured into ice-cold water (50 mL) and extracted with dichloromethane (3 × 15 mL). The organic extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue crystallized from ether–hexane to give **17** (246 mg, 91%), m.p. 133–134°, $[\alpha]_D^{23}$ −12° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.36 (s, 5 H, Ph), 5.46 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 5.29 (dd, 1 H, $J_{3,4}$ 2.5, $J_{4,5}$ 1.5 Hz, H-4), 4.76 (AB system, 2 H, OCH_2Ph), 4.60 (d, 1 H, $J_{4,5}$ 1.5 Hz, H-5), 3.78 (s, 3 H, CO_2Me), 1.77 (s, 3 H, CMe), and 1.30 (s, 9 H, CMe_3).

Anal. Calc. for $C_{22}H_{27}Cl_3O_9$: C, 48.77; H, 5.02. Found: C, 48.65; H, 4.82.

*Methyl 3-O-benzyl-4-O-monochloroacetyl-β-L-idopyranuronate 1,2-(*tert*-butyl orthoacetate)* (**18**). — A solution of orthoacetate **15** (2.20 g) in anhydrous methanol (50 mL) was stirred for 5 h at −20° in the presence of anhydrous K_2CO_3 (200 mg) under a dry atmosphere of argon. The mixture was filtered, and the filtrate immediately diluted with chloroform (250 mL), washed with aqueous saturated NaCl, with water, dried (Na_2SO_4), and evaporated. The residue was not

purified by chromatography, but promptly dissolved in pyridine (20 mL) and dichloromethane (5 mL), and the solution cooled to −20°. A solution of chloroacetyl chloride (1 mL) in dichloromethane (5 mL) was added dropwise. After 30 min, the mixture was poured into ice-cold water (250 mL) and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with aqueous saturated $KHSO_4$, with water, dried (Na_2SO_4), and evaporated. The residue was promptly chromatographed on a column of silica gel (100 g). Elution with 5:2 (v/v) hexane–ethyl acetate containing triethylamine (0.1%) gave the unsaturated derivative **20** (135 mg, 7%), and then orthoacetate **18** (1.592 g, 67% from **15**), m.p. 67–68° (from ether–hexane), $[\alpha]_D^{23}$ +19° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.34 (s, 5 H, Ph), 5.45 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 5.24 (dd, 1 H, $J_{3,4}$ 2.5, $J_{4,5}$ 1.5 Hz, H-4), 4.75 (AB system, 2 H, OCH_2Ph), 4.55 (d, 1 H, $J_{4,5}$ 1.5 Hz, H-5), 4.00 (s, 2 H, $ClCH_2CO$), 3.77 (s, 3 H, CO_2Me), 1.77 (s, 3 H, CMe), and 1.30 (s, 9 H, CMe_3).

Anal. Calc. for $C_{22}H_{29}ClO_9$: C, 55.87; H, 6.23. Found: C, 56.01; H, 6.23.

Methyl [*benzyl 2-*O*-acetyl-3-*O*-benzyl-β-* (**23**) *and α-*L*-idopyranosid*]*uronate* (**22**). — A solution of orthoester **18** (118 mg) and freshly distilled benzyl alcohol (0.15 mL) in chlorobenzene (10 mL) was distilled at atmosphere pressure to remove 8 mL of solvent. After addition of a 0.2M solution of 2,6-dimethylpyridinium perchlorate in 1,2-dichloroethane (2.5 μmol) and chlorobenzene (2 mL), the mixture was distilled for 30 min with dropwise addition of fresh chlorobenzene to maintain a constant volume of about 2 mL. The mixture was cooled, diluted with chloroform (50 mL), washed with aqueous 5% $NaHCO_3$, with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (8 g). Elution with 2:1 (v/v) hexane–ethyl acetate gave a fraction (102 mg, 80%) that was directly dissolved in pyridine (5 mL) and ethanol (1 mL), and heated for 20 min at 100° in the presence of thiourea (25 mg). After being cooled, the mixture was evaporated to dryness, and the residue taken up in water (50 mL) and extracted with chloroform (4 × 10 mL). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (10 g). Elution with 4:3 (v/v) ethyl acetate–hexane gave syrupy **23** (26 mg, 25% from **18**), $[\alpha]_D^{23}$ +70° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (m, 10 H, 2 Ph), 4.90 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.52 (d, 1 H, $J_{4,5}$ 2 Hz, H-5), 3.78 (s, 3 H, CO_2Me), 3.12 (d, 1 H, *J* 10 Hz, OH), and 2.05 (s, 3 H, Ac).

Anal. Calc. for $C_{23}H_{26}O_8$: C, 64.18; H, 6.09. Found: C, 64.04; H, 6.12.

Further elution gave syrupy **22** (54 mg, 50% from **18**), $[\alpha]_D^{23}$ −65° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (m, 10 H, 2 Ph), 5.05 (br. s, 1 H, H-1), 4.85 (d, 1 H, $J_{4,5}$ 2 Hz, H-5), 3.78 (s, 3 H, CO_2Me), 2.80 (d, 1 H, *J* 10 Hz, OH), and 2.06 (s, 3 H, Ac).

Anal. Calc. for $C_{23}H_{26}O_8 \cdot 0.5\ H_2O$: C, 62.86; H, 6.19; Found: C, 62.86; H, 5.96.

*Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-*D*-glucopyranoside* (**25**). — A solution of methyl 4,6-*O*-benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside[13] (**24**; 415 mg) in dry *N,N*-di-

methylformamide (10 mL) was stirred for 5 h in the presence of barium oxide (613 mg), barium hydroxide octahydrate (158 mg), and freshly distilled benzyl bromide (0.15 mL). The excess of benzyl bromide was eliminated by addition of methanol (1 mL) and stirring for 1 h. The mixture was diluted with chloroform (50 mL), washed successively with ice-cold 60% aqueous acetic acid, with water, with aqueous saturated $NaHCO_3$, with water, dried (Na_2SO_4), and evaporated. The residue crystallized from chloroform–ethanol to give **25** (461 mg, 91%), m.p. 202–203°, $[\alpha]_D^{23}$ +46° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (m, 15 H, 3 Ph), 5.52 (s, 1 H, C*H*Ph), 5.06 (AB system, 2 H, CO_2CH_2Ph), 4.72 (AB system, 2 H, OCH_2Ph), 4.67 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), and 3.28 (s, 3 H, OMe).

Anal. Calc. for $C_{29}H_{31}NO_7$: C, 68.89; H, 6.18; N, 2.77. Found: C, 68.95; H, 6.01; N, 2.83.

*Methyl 3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-*D*-glucopyranoside* (**26**). — Compound **25** (300 mg) was heated for 0.5 h at 100° under stirring with 60% aqueous acetic acid (10 mL). After being cooled, the mixture was evaporated and the residue crystallized from 2-propanol to give **26** (220 mg, 89%), m.p. 151–152°, $[\alpha]_D^{23}$ +94° (*c* 1, methanol).

Anal. Calc. for $C_{22}H_{27}NO_7$: C, 63.30; H, 6.52; N, 3.35. Found: C, 63.32; H, 6.43; N, 3.34.

*Methyl 6-O-benzoyl-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-*D*-glucopyranoside* (**27**). — A solution of diol **26** (835 mg) in pyridine (5 mL) and dichloromethane (12 mL) was stirred for 5 h at room temperature in the presence of benzoyl cyanide (400 mg). The excess of reagent was eliminated by addition of methanol (5 mL). The mixture was evaporated and the residue crystallized from ethyl acetate–hexane to give **27** (935 mg, 90%), m.p. 154–155°, $[\alpha]_D^{23}$ +74° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.97–7.25 (m, 15 H, 3 Ph), 5.05 (AB system, 2 H, CO_2CH_2Ph), 4.96 (d, 1 H, *J* 9.5 Hz, NH), 4.70 (s, 2 H, OCH_2Ph), 3.30 (s, 3 H, OMe), and 3.08 (d, 1 H, OH).

Anal. Calc. for $C_{29}H_{31}NO_8$: C, 66.78; H, 5.99; N, 2.68. Found: C, 67.05; H, 5.83; N, 2.61.

*Benzyl 6-O-acetyl-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-*D*-glucopyranoside* (**29**). — A solution of imidazole (340 mg) in dichloromethane (4 mL) was acetylated, under stirring for 15 min at 0°, by dropwise addition of a solution of acetyl chloride (0.18 mL) in dichloromethane (1 mL). Imidazole hydrochloride was filtered off, and the solution of *N*-acetylimidazole was added to a solution of benzyl 3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside[18] (**28**; 987 mg) in 1,2-dichloroethane (15 mL). The mixture was boiled under reflux during 30 h, cooled, diluted with chloroform (50 mL), washed successively with ice-cold aqueous 0.1M HCl, water, aqueous saturated $NaHCO_3$, and water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (50 g). Elution with 15:1 (v/v) dichloromethane–acetone gave **29** (759 mg, 71%). m.p. 114–115° (from ethyl acetate–hexane), $[\alpha]_D^{23}$ +88° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.28 (s, 15 H, 3 Ph), 5.05 (AB system, 2 H, CO_2CH_2Ph),

5.04 (d, 1 H, *J* 8.5 Hz, NH), 4.89 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.70 (s, 2 H, OCH_2Ph), 4.56 (AB system, 2 H, OCH_2Ph), 2.93 (d, 1 H, *J* 3 Hz, OH), and 2.07 (s, 3 H, Ac).

Anal. Calc. for $C_{30}H_{33}NO_8$: C, 67.28; H, 6.21; N, 2.61. Found: C, 67.46; H, 5.97; N, 2.76.

Methyl 6-O-benzoyl-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-4-O-(methyl 2,4-di-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-α-D-glucopyranoside (**30**). — A solution of orthoester **12** (80 mg) and **27** (52 mg) in chlorobenzene (8 mL) was distilled at atmosphere pressure to remove 6 mL of chlorobenzene. After dropwise addition of a 0.2M solution of 2,6-dimethylpyridinium perchlorate in 1,2-dichloroethane (2 μmol) and chlorobenzene (2 mL), the mixture was slowly distilled for 1 h with addition of fresh chlorobenzene to maintain a constant volume of ~2 mL. The mixture was cooled, diluted with chloroform (50 mL), washed with aqueous saturated $NaHCO_3$, with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (15 g). Elution with 4:3 (v/v) hexane–ethyl acetate gave the starting material **27** (20 mg, 38%), and then a fraction that crystallized and was recrystallized from ethyl acetate–hexane to give the disaccharide **30** (44 mg, 50%), m.p. 120–121°, $[\alpha]_D^{23}$ +17° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 8.10–7.10 (m, 20 H, 4 Ph), 5.06 (br. s, 1 H, H-1′), 3.41 (s, 3 H, CO_2Me), 3.34 (s, 3 H, OMe), 1.97 and 1.92 (2 s, 6 H, Ac).

Anal. Calc. for $C_{47}H_{51}NO_{16}$: C, 63.72; H, 5.80. N, 1.58. Found: C, 63.51; H, 6.05; N, 1.69.

Methyl 6-O-benzoyl-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-4-O-(methyl 2-O-acetyl-3-O-benzyl-4-O-chloroacetyl-α-L-idopyranosyluronate)-α-D-glucopyranoside (**31**). — Compound **27** (66 mg) was glycosylated by orthoester **18** (120 mg) as described for the preparation of disaccharide **30**. The residue was chromatographed on a column of silica gel (15 g). Elution with 7:4 (v/v) hexane–ethyl acetate gave starting material **27** (40 mg, 60%), and then a fraction that crystallized from ether–hexane to give **31** (36 mg, 30%), m.p. 143–144°, $[\alpha]_D^{23}$ +9.5° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 8.10–7.10 (m, 10 H, 4 Ph), 5.07 (br. s, 1 H, H-1′), 3.87 (s, 2 H, ClCH_2CO_2), 3.40 (s, 3 H, CO_2Me), 3.34 (s, 3 H, OMe), and 1.97 (s, 3 H, Ac).

Anal. Calc. for $C_{45}H_{50}ClNO_{16}$: C, 61.34; H, 5.48; N, 1.52. Found: C, 61.59; H, 5.53; N, 1.51.

O-Dechloroacetylation of **31** (thiourea in pyridine–ethanol) and acetylation (acetic anhydride–pyridine) gave disaccharide **30** (m.p. 120–121°, from ether–hexane).

Benzyl 6-O-acetyl-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-4-O-(methyl 2-O-acetyl-3-O-benzyl-4-O-chloroacetyl-α-L-idopyranosyluronate)-α-D-glucopyranoside (**32**). — A solution of orthoester **18** (735 mg) and **29** (535 mg) in chlorobenzene (15 mL) was processed for 1.5 h as described for the preparation of disaccharide **30**. The residue was chromatographed on a column of silica gel (80 g). Elution with 4:3 (v/v) hexane–ethyl acetate gave a syrupy compound (369 mg, 59% from **18**), identified as methyl 2-*O*-acetyl-1,5-anhydro-3-*O*-benzyl-4-*O*-chloro-

acetyl-*xylo*-hex-1-enitoluronate (**34**), $[\alpha]_D^{23}$ −29° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.30 (s, 5 H, Ph), 6.69 (s, 1 H, H-1), 5.42 (dd, 1 H, $J_{3,4}$ 4, $J_{4,5}$ 1.5 Hz, H-4), 4.70 (AB system, 2 H, OCH_2Ph), 4.02 (s, 2 H, ClCH_2CO$_2$), 3.79 (s, 3 H, CO_2Me), and 1.92 (s, 3 H, Ac).

Anal. Calc. for $C_{18}H_{19}ClO_8$: C, 54.21; H, 4.80. Found: C, 54.26; H, 4.82.

The starting material **29** was next eluted (175 mg, 52%), followed by disaccharide **32** (327 mg, 35% from **29**), m.p. 146–147° (from ether–hexane), $[\alpha]_D^{23}$ +35° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.25 (m, 20 H, 4 Ph), 3.93 (s, 2 H, ClCH_2CO$_2$), 3.42 (s, 3 H, CO_2Me), 2.11 and 2.02 (2 s, 6 H, Ac).

Anal. Calc. for $C_{48}H_{52}ClNO_{16}$: C, 61.70; H, 5.61; N, 1.50. Found: C, 61.87; H, 5.90; N, 1.43.

*Benzyl 6-O-acetyl-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-4-O-(methyl 2-O-acetyl-3-O-benzyl-α-*L*-idopyranosyluronate)-α-*D*-glucopyranoside* (**33**). — *(a) From* **32**. A solution of disaccharide **32** (56 mg) in pyridine (2.5 mL) and ethanol (0.5 mL) was heated for 30 min at 100° in the presence of thiourea (7 mg). After being cooled, the mixture was evaporated. The residue was taken up in chloroform (20 mL), the organic solution washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (2 g). Elution with 2:1 (v/v) ethyl acetate–hexane gave **33** which crystallized from ether–hexane (44 mg, 86%), m.p. 146–147°, $[\alpha]_D^{23}$ +44° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.25 (m, 20 H, 4 Ph), 5.07 (br. s, 1 H, H-1′), 4.88 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.70 (t, 1 H, $J_{2',3'} = J_{3',4'}$ 3 Hz, H-3′), 3.46 (s, 3 H, CO_2Me), 2.61 (d, 1 H, *J* 10 Hz, OH), 2.10 and 2.06 (2s, 6 H, Ac).

Anal. Calc. for $C_{46}H_{51}NO_{15}$: C, 64.40; H, 5.99; N, 1.63. Found: C, 64.42; H, 5.88; N, 1.68.

(b) From **18**. A solution of orthoester **18** (473 mg, 1 mmol) and **29** (2.140 g, 4 mmol) in chlorobenzene (10 mL) was processed as described for the preparation of **30**. The residue was directly *O*-dechloroacetylated (thiourea in pyridine–ethanol) and chromatographed on a column of silica gel (200 g). Elution with 2:1 (v/v) ethyl acetate–hexane gave **29** (1.82 g), and then a fraction that crystallized in ether–hexane to give **33** (343 mg, 40% from orthoacetate **18**), m.p. 146–147°.

*Benzyl O-(3,6-di-O-acetyl-2-azido-4-O-benzyl-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-(benzyloxycarbonyl)amino-2-deoxy-α-*D*-glucopyranoside* (**38**). — A solution of freshly prepared 3,6-di-*O*-acetyl-2-azido-4-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide[19] (**35**; 110 mg) and **33** (112 mg) in anhydrous dichloromethane (2 mL) was stirred for 15 min at room temperature under a dry atmosphere of argon in the presence of 4A activated, powdered molecular sieve (100 mg). After having cooled the mixture to −20°, 2,4,6-trimethylpyridine (70 μL) and freshly prepared silver triflate[20] (78 mg) were added. The mixture was stirred for 2 h in the dark at −20°, and then diluted with dichloromethane (50 mL) and filtered, and the filtrate washed with aqueous 10% $KHSO_4$, water, aqueous saturated $NaHCO_3$, water, dried (Na_2SO_4), and evaporated. The residue was chro-

matographed on a column of silica gel (18 g). Elution with 4:3 (v/v) hexane–ethyl acetate gave trisaccharide **38** (139 mg, 88%) as a colorless glass, $[\alpha]_D^{23}$ +83° (*c* 1, chloroform); ^{1}H-n.m.r.: δ 7.25 (m, 25 H, Ph), 5.44 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 9 Hz, H-3''), 5.26 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 3.59 (s, 3 H, CO_2Me), 3.06 (dd, 1 H, $J_{1'',2''}$ 3.5, $J_{2'',3''}$ 10.5 Hz, H-2''), 2.12, 2.08, 2.01, and 1.97 (4 s, 12 H, Ac).

Anal. Calc. for $C_{63}H_{70}N_4O_{21}$: C, 62.06; H, 5.79; N, 4.59. Found: C, 61.62; H, 5.81; N, 4.68.

Benzyl O-(2-azido-4-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(methyl 3-O-benzyl-α-L-idopyranosyluronate)-(1→4)-3-O-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (**39**). — A M NaOH solution (2 mL) was dropwise added, at 0° under stirring, to a solution of trisaccharide **38** (122 mg) in 1,2-dimethoxyethane (6 mL) and methanol (2 mL). After 6 h at 0°, the mixture was acidified with M HCl, diluted with ice-cold water and extracted with chloroform (5 × 10 mL). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was dissolved in methanol (2 mL) and esterified with etheral diazomethane for 30 min. Excess of diazomethane was eliminated with acetic acid, and the mixture evaporated to dryness. The residue was chromatographed on a column of silica gel (10 g). Elution with 16:1 (v/v) dichloromethane–methanol gave trisaccharide **39** (85 mg, 81%) as a colorless glass, $[\alpha]_D^{23}$ +77° (*c* 1, chloroform); ^{1}H-n.m.r.: (300 MHz F.t. n.m.r., Bruker CXP; CD_3OD): δ 5.13 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.03 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.74 (br. s, 1 H, H-1'), 4.65 (d, 1 H, $J_{4',5'}$ 3.8 Hz, H-5'), 3.93 (t, 1 H, $J_{3',4'}$ 5.1 Hz, H-4'), 3.84 (t, 1 H, $J_{2',3'}$ 5.1 Hz, H-3'), 3.76 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 9 Hz, H-3''), 3.72 (t, 1 H, $J_{2,3} = J_{3,4}$ 9 Hz, H-3), 3.54 (dd, H-2), 3.53 (d, H-2'), 3.22 (s, 3 H, CO_2Me), and 3.17 (dd, H-2''); the signals from H-4,5,6,4''5''6'' (8 H) were not resolved (δ 3.75–3.65); no acetyl signal was detected in the expected region.

Anal. Calc. for $C_{55}H_{62}N_4O_{17}$: C, 62.84; H, 5.94. Found: C, 62.56; H, 5.92.

Benzyl O-(2-azido-4-O-benzyl-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-(benzyloxycarbonyl)amino-2-deoxy-6-O-sulfo-α-D-glucopyranoside tetrasodium salt (**40**). — A solution of trisaccharide **39** (95 mg) in *N,N*-dimethylformamide (2.5 mL) was stirred for 20 h at 50° in the presence of sulfur trioxide–trimethylamine complex (140 mg). The mixture was cooled, methanol (1 mL) added, and the mixture chromatographed on a column (2.8 × 35 cm) of Sephadex LH-20 equilibrated in 1:1 (v/v) chloroform–methanol. Elution with the same solvent gave a residue that was chromatographed on a column of silica gel (10 g). Elution with 8:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water provided a pure fraction that was dissolved in methanol (1 mL) and chromatographed on a column (1 × 25 cm) of Sephadex SP-25 (Na^+). Elution with 9:1 (v/v) methanol–water afforded pure trisaccharide **40** (115 mg, 87%) as a colorless glass, $[\alpha]_D^{23}$ +54° (*c* 1, methanol); ^{1}H-n.m.r.: (300 MHz F.t. n.m.r., Bruker CXP; CD_3OD): δ 5.42 (br. s, 1 H, H-1'), 5.16 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 5.02 (d, 1 H, $J_{4',5'}$ 1.5 Hz, H-5'), 4.81 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.60 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 9 Hz, H-3''), 4.57 (d,

1 H, $J_{2',3'}$ 2 Hz, H-2'), 4.02 (dd, 1 H, $J_{3',4'}$ 3 Hz, H-4'), 3.88 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 3.37 (dd, 1 H, H-2''), and 3.28 (s, 3 H, CO_2Me); the signals from H-6,3',6'' were not resolved (δ 4.25).

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 heptasodium salt (**41**). — A solution of **40** (50 mg) in 1:1 (v/v) methanol–water (5 mL) was hydrogenated in the presence of 10% Pd–C (50 mg) for 36 h. The suspension was filtered, and the filtrate evaporated to give a colorless glass (30 mg, 94%). This compound was homogeneous on t.l.c. in 4:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water (R_F 0.5), and gave a positive reaction with the ninhydrin spray; ^{1}H-n.m.r. (D_2O): δ 5.89 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.84 (d, $J_{1,2}$ 3.5 Hz, H-1), 5.71 (br. s, 1 H, H-1'), 4.31 (s, 3 H, CO_2Me), 3.98 (dd, 1 H, $J_{1'',2''}$ 3.5, $J_{2'',3''}$ 10 Hz, H-2''); no aromatic signal was detected in the range δ 8.0–7.0.

The free trisaccharide (30 mg) was dissolved in water (2 mL), and the pH of the solution was adjusted to 9 with M NaOH. Sulfur trioxide–trimethylamine complex (35 mg) was added and the pH was maintained at 9 by subsequent addition of M NaOH. After 24 h, more sulfur trioxide–trimethylamine complex (35 mg) was added. After 2 days, the pH was adjusted to 12 with M NaOH, and the mixture was stirred for 2 h. After neutralization with M HCl and concentration *in vacuo* to ~2 mL, the mixture was chromatographed on a column of Sephadex G-25 (fine, 2 × 40 cm). Elution with water afforded a product (unreactive with the ninhydrin spray) that was dissolved in purified water (1 mL) and chromatographed on a column (1 × 15 cm) of Sephadex SP-25 (Na^+). Elution with water was followed polarimetrically. The fractions having an optical activity were pooled and lyophilized to give trisaccharide **41** (27 mg, 70%) as a colorless glass, $[\alpha]_D^{23}$ +35° (*c* 1, water); ^{1}H-n.m.r. [300 MHz F.t. Bruker CXP; D_2O; chemical shifts from signal of internal sodium 3-(trimethylsilyl)-propionate (TSP)]: δ 5.53 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1''), 5.44 (d, $J_{1,2}$ 3.4 Hz, H-1α), 5.21 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.83 (d, 1 H, $J_{4',5'}$ 2.5 Hz, H-5'), 4.71 (d, $J_{1,2}$ 7.5 Hz, H-1β), 4.34 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 9 Hz, H-3''), 3.72 (t, 1 H, $J_{4'',5''}$ 9 Hz, H-4''), 3.41 (dd, H-2''), 3.26 (dd, $J_{2,3}$ 10.5 Hz, H-2α), and 3.06 (dd, $J_{2,3}$ 10 Hz, H-2β); the signals from H-6,2',6'' were not resolved (δ 4.36); ^{13}C-n.m.r. (25.1 MHz, F.t. Varian; D_2O; chemical shifts from signal of external Me_4Si): δ 102.30 (C-1'), 99.19 (C-1''), 93.88 (C-1), 81.61 (C-3''), 60.72 (C-2), and 59.12 (C-2'').

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