Carbohydrate Research, 172 (1988) 37–64
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF METHYL GLYCOSIDE DERIVATIVES OF TRI- AND PENTA-SACCHARIDES RELATED TO THE ANTITHROMBIN III-BINDING SEQUENCE OF HEPARIN, EMPLOYING CELLOBIOSE AS A KEY STARTING-MATERIAL*

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(Received January 15th, 1987; accepted for publication in revised form, June 18th, 1987)

ABSTRACT

Two key synthons for the title pentasaccharide derivative, methyl *O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside and *O*-(methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-β-D-glucopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide, were prepared from a common starting material, cellobiose. They were coupled to give a tetrasaccharide derivative that underwent *O*-dechloroacetylation to the corresponding glycosyl acceptor. Its condensation with the known 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide afforded a 77% yield of suitably protected pentasaccharide, methyl *O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside. Sequential deprotection and sulfation gave the decasodium salt of methyl *O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-3,6-di-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6-*O*-sulfo-β-D-glucopyranoside (**3**). In a similar way, the trisaccharide derivative, the hexasodium salt of methyl *O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-3,6-di-*O*-sulfo-α-D-glucopyranoside (**4**) was synthesized from methyl *O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranoside. The pentasaccharide **3** binds strongly to antithrombin III with an association constant almost equivalent to that of high-affinity heparin, but the trisaccharide **4** appears not to bind.

* Synthetic Studies on Mucopolysaccharides, Part V. For Part IV, see ref. 1.
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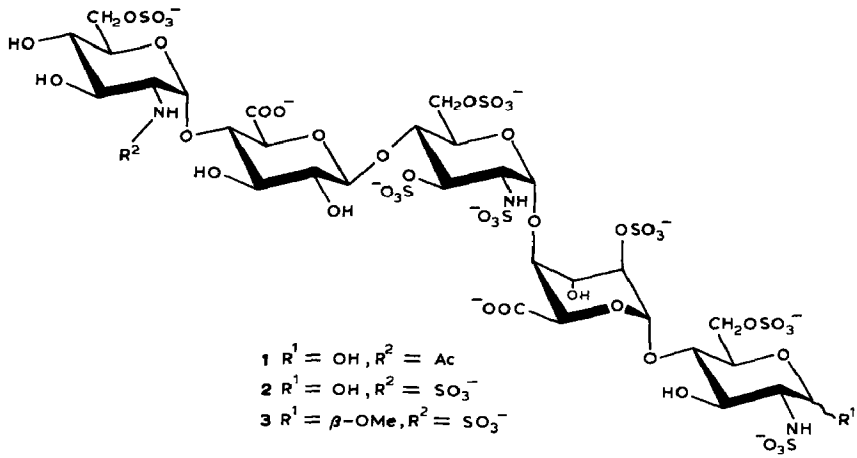

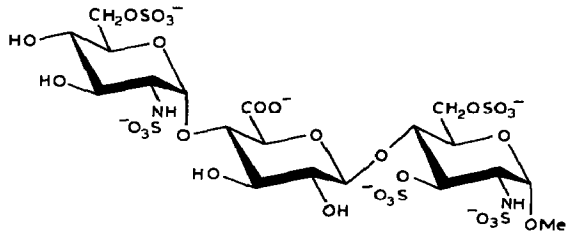

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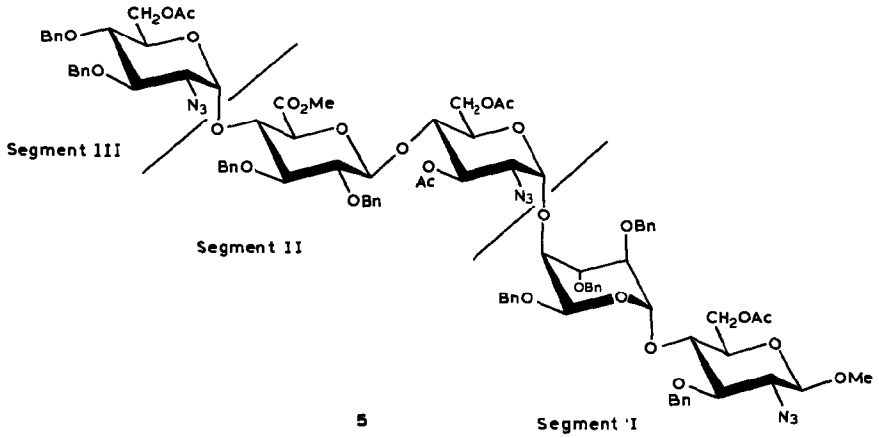
INTRODUCTION

Since the structure of complex pentasaccharide **1** was presented by Lindahl and coworkers[2] in 1982 as containing the minimum sugar sequence in heparin required for its specific binding to antithrombin III (AT III), the synthesis of **1** or its *N*-sulfo analog[3] **2** has been keenly pursued. The anticoagulant activity of heparin is mediated through the activation of AT III by its binding to heparin[3]. Thus, the synthesis of **1** or **2** was expected to clarify partly the many-sided physiological activity of heparin on a molecular basis. In the past two years, three groups, in France[4], Holland[5], and Japan[1], have published on the synthesis of **2**. The French first reported the synthesis of **2**, and found an association constant, with AT III, having the same order of magnitude as that of high-affinity heparin[4,6].

The characteristic feature of our synthesis[1] that distinguished it from the others[4,5] lay in the fact that a couple of disaccharide synthons for the construction of **2** were prepared from cellobiose, utilizing its internal glycosidic linkage. Several fundamental methodologies[7–9] developed for the specific modification of such disaccharidic starting-materials were of great help for the preparation of those synthons. Our preparation of **2**, however, suffered from a low yield in the final stage of

liberation of hemiacetal; this was probably because the interaction between the hemiacetal and free amino groups resulted in unidentified byproducts. Consequently, we were prompted to prepare **3**, a β-methyl glycoside derivative of **2****, whose preparation avoided the wasteful final synthetic stage in the synthesis of **2**. We expected a binding activity like that of **2**, as the presence of a free hemiacetal group was not considered essential for the activity. Save for the preparation of Segment **I** as the methyl glycoside, the main synthetic route is identical with that employed before[1] for **2**. We now describe the synthesis, and association constants with AT III, of **3** and **4**.



RESULTS AND DISCUSSION

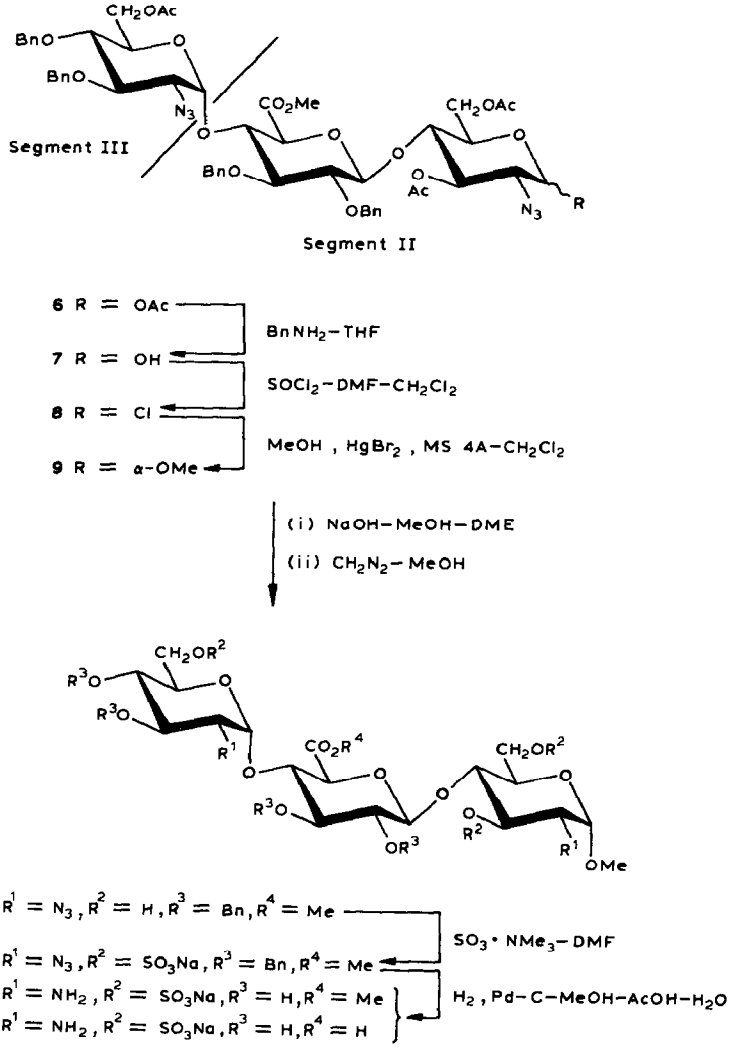
General synthetic strategy. — Our construction of the sugar chains of **5** and **9** employs a disaccharide as the building block. That is, **5** is made up of Segments **I, II,** and **III**, and the two disaccharide synthons **39** and **42** that correspond to **I** and **II** are both derived from cellobiose. We had already succeeded in the transformation[9] of cellobiose into **40**, which is a precursor of **42**, and had reported the preparation[9] of **6**, a precursor of the trisaccharide **9**, by coupling of Segment **II** (derived from **40**) with Segment **III**. Transformation of cellobiose derivative[8] **14** into **39** entails the most novel and challenging stages in the total synthesis of **3**, involving two difficult problems, as follows: (*i*) achievement of efficient discrimination between the 2′- and the 3′-hydroxyl groups of **14** for the eventual selective sulfation at *O*-2 of the L-idosyluronic residue in **3**, and (*ii*) successful configurational inversion at C-5′ (D-*gluco* → L-*ido*). The first was achieved by selective benzylation of the 3′-hydroxyl group. For the second, our plan involved hydroboration[7] of **32**, the key intermediate (which

** The synthesis of the α anomer of **3** was reported by Sinaÿ *et al.* at the XIIIth International Carbohydrate Symposium in 1986; see ref. 10.

is a 5′-eno derivative prepared from **14**), in order to construct the L-idopyranosyluronic portion.

Deprotection and sulfation of **5** and **9** were to be performed in a stepwise manner, according to the previously described French methodology[4]. Prior to the synthesis of **3**, the trisaccharide **9** was converted into **4** as a model for the sulfation reactions, by *O*-deacetylation and *O*-sulfation that gave **11**, and subsequent *N*-sulfation. Likewise, the pentasaccharide derivative **5** was successfully transformed into **3**, *via* **48**.

Synthesis of the trisaccharide **4**. — The fully protected trisaccharide **9** was



Scheme 1

prepared from the glycosyl acetate **6** in 55% overall yield, as illustrated in Scheme 1.

Subsequent deprotection and sulfation (**9** → **10** → **11**) were carried out according to the procedure already reported[4a]. Catalytic hydrogenation of **11** unexpectedly afforded two products (53 and 27% yields, respectively), after purification on a column of Avicel. ^{1}H-N.m.r.- and mass-spectral data revealed that the major product was **12**, and that the minor product was **13**, resulting from hydrolysis of the methyl carboxylate in **12**. Compounds **12** and **13** were each subjected to *N*-sulfation, to give **4** in 44 and 52% yields, respectively. The high-resolution f.a.b.-mass spectrum of **4** was successfully recorded.

Synthesis of the key intermediate **32**. — The sulfation of *O*-2′ of the L-idosyluronic part of **3** requires the regioselective benzylation of the 3′-hydroxyl group of **14**, and various reaction conditions were tested. When silver(I) oxide or benzyl triflate[11] was employed as the reagent, the product was a mixture of 2′,3′-dibenzyl ether[8] **15**, the desired 3′-benzyl ether **16**, and 2′-benzyl ether **17** (see Entries 1 and 2 in Table I). The structures of the two isomers were elucidated on the basis of the ^{1}H-n.m.r. spectra of their acetate derivatives **18** and **19**, prepared from **16** and **17**, respectively (see Experimental section). The desired mono-benzylation was achieved *via* the stannylene intermediate **21**. Thus, treatment of **14** with dibutyltin oxide in toluene and alkylation of the product with benzyl bromide in the presence of tetrabutylammonium iodide[12] for 5 h at 100° gave **16** with high selectivity, in 97% yield (Entry 3). The reason for this unexpectedly high regioselectivity is not yet well understood, but it might be attributable to the combined effects of the stannylene and disiloxane groups in **21**.

After benzoylation of the 2′-hydroxyl group of **16**, the silyl group of the resulting benzoate **20** was removed in aqueous media[8] with fluoride anion, giving the diol **22** in high yield.

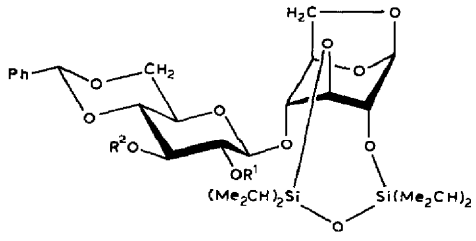
Chemical modifications of the 1,6-anhydroglucose moiety of **22** were perfor-

TABLE I

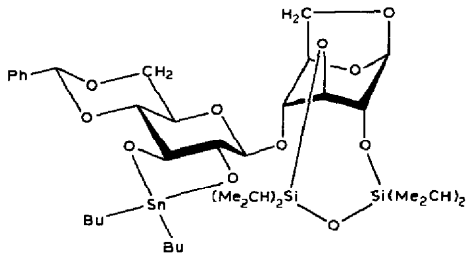
RESULTS OF THE SELECTIVE BENZYLATION OF **14**

Entry	*Reagents*	*Solvents*	*Temp. (°C)*	*Products*[a]	*Yields (%)*	*Ratios* (**16**:**17**)
1	Ag_2O, BnBr, Bu_4NI	THF[b]	r.t.[c]	2′,3′-di-*O*-Bn[d]	10.5	
				2′-*O*-Bn	24.5	1:12.2
				3′-*O*-Bn	2.0	
2	BnOTf[e] 2,4,6-collidine	CH_2Cl_2	−60	2′,3′-di-*O*-Bn	1.4	
				2′-*O*-Bn	4.7	4.2:1
				3′-*O*-Bn	19.6	
3	Bu_2SnO, BnBr, Bu_4NI	Toluene	100	2′-*O*-Bn	trace	>99:1
				3′-*O*-Bn	97.0	

[a]These structures were elucidated on the basis of the ^{1}H-n.m.r. spectra of their acetate derivatives. [b]Oxolane. [c]Room temperature. [d]Bn, benzyl derivative. [e]Benzyl triflate.



14 $R^1 = R^2 = H$
15 $R^1 = R^2 = Bn$
16 $R^1 = H, R^2 = Bn$
17 $R^1 = Bn, R^2 = H$
18 $R^1 = Ac, R^2 = Bn$
19 $R^1 = Bn, R^2 = Ac$
20 $R^1 = Bz, R^2 = Bn$



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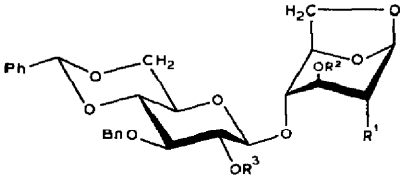
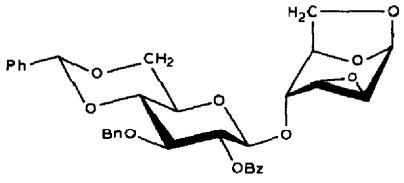


22 $R^1 = OH, R^2 = H, R^3 = Bz$
23 $R^1 = OH, R^2 = H, R^3 = Bn$
24 $R^1 = N_3, R^2 = Ac, R^3 = Bn$
25 $R^1 = OTs, R^2 = H, R^3 = Bz$
26 $R^1 = N_3, R^2 = H, R^3 = Bz$
27 $R^1 = N_3, R^2 = Bn, R^3 = Bz$



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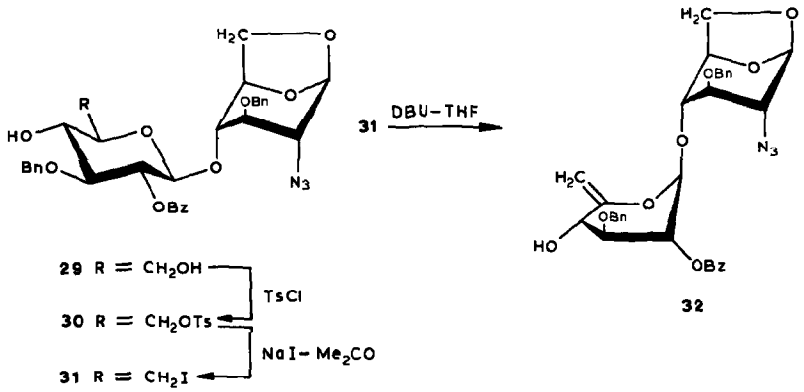
med similarly to the preparation[9] of **24** from **23**. Tosylation of the 2-hydroxyl group of **22**, followed by treatment with sodium hydride, gave epoxide **28**, and the epoxy ring was opened with azide anion to give azidoalcohol **26**. Benzylation of the 3-hydroxyl group of **26** (carrying the base-labile benzoyl group) was difficult, because that hydroxyl group resisted benzylation under mild conditions (Ag_2O–BnBr or benzyl triflate). However, when sodium hydride was added to a mixture of **26**, benzyl bromide, and tetrabutylammonium iodide in oxolane[8,13], the desired benzylation proceeded satisfactorily to give **27** in 91% yield. Removal[14] of the benzylidene group of **27** afforded the diol **29**.

Finally, the substrate, 5′-exo-alkene derivative **32**, was prepared from **29** as depicted in Scheme 2. During the synthetic course, selective tosylation of the 6′-hydroxyl group of **29** was conducted either by the method involving stannylation with bis(tributyltin) oxide[15] or by using the stoichiometric amount of *p*-toluenesulfonyl chloride. Both gave the 6′-*O*-tosyl derivative **30** in high yield.

Construction of **39** (*segment* I) *and* **42** (*segment* II). — The exocyclic-alkene-



Scheme 2

containing β-D-glucopyranosyl moiety in **32** was shown to have a modified $^1C_4$ conformation[7,16] on the basis of the $J_{1',2'}$ and $J_{2',3'}$ values (2.69 and 5.37 Hz, respectively) in its ^{1}H-n.m.r. spectrum. The axially oriented β-glycosidic oxygen atom bearing the bulky aglycon sterically interferes with the desired approach from the β-side of solvated borane[7]. However, hydroboration of **32** with the naked borane prepared from tetrabutylammonium borohydride and methyl iodide[17] gave a 19% yield of the desired L-idopyranosyl derivative **33**, together with a 39% yield of D-glucopyranosyl derivative **29**, that could be recycled for the preparation of **33**. Compound **33** was shown to be the α-L-idopyranosyl-containing disaccharide on the basis of its ^{1}H-n.m.r. spectrum, respectively having the H-1′ and H-2′ resonances at δ 5.22 as a singlet and δ 5.32 as a broad triplet with J 1.22 Hz. This spectrum was in marked contrast to that of **29**, which showed the H-1′ and H-2′ resonances at δ 4.68 as a doublet with $J_{1',2'}$ 8.06 Hz, and δ 5.26 as a doublet of doublets with $J_{1',2'}$ 8.06 and $J_{2',3'}$ 9.28 Hz, respectively.

Tritylation of the diol **33**, and chloroacetylation[18] of the ether, giving **34**, followed by oxidation[4a] of **34**, afforded the uronate **35** (see Scheme 3). Acetolysis[19] of the 1,6-anhydro ring of **35** and bromination[19] of the product gave bromide **37**, which was condensed with methanol in the presence of silver trifluoromethanesulfonate and 2,4,6-collidine[20], giving only the β anomer **38**. *O*-Dechloroacetylation[21] of **38** provided the glycosyl acceptor **39**, Segment **I**.

For the preparation of Segment **II**, compound **40**, already prepared[9] from cellobiose, was converted into **42** according to the method reported by French group[4c] (see Scheme 4).

Synthesis of the pentasaccharide **3**. — Coupling of **39** (Segment **I**) and **42** (Segment **II**) in the presence of silver trifluoromethanesulfonate and 2,4,6-collidine[20] gave tetrasaccharide **43** as a single product, which was *O*-dechloroacetylated, to afford the glycosyl acceptor **44** (Segment **I–II**) in 36% overall yield. Condensation of **44** with the known bromide[19] **45** (Segment **III**) under the same conditions as for the synthesis of **43** gave pentasaccharide **5** in 77% yield. The ^{1}H-n.m.r.

33 $R^1 = H, R^2 = CH_2OH$

34 $R^1 = ClCH_2CO, R^2 = CH_2OTr$

35 $R^1 = ClCH_2CO, R^2 = CO_2Me$

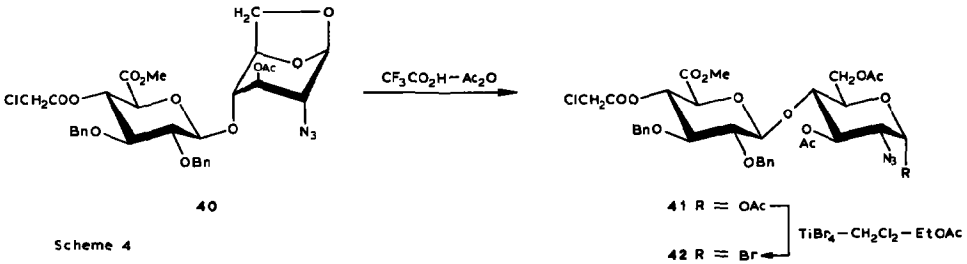
36 $R^1 = OAc, R^2 = ClCH_2CO$

37 $R^1 = \alpha\text{-Br}, R^2 = ClCH_2CO$

38 $R^1 = \beta\text{-OMe}, R^2 = ClCH_2CO$

39 $R^1 = \beta\text{-OMe}, R^2 = H$

Scheme 3.

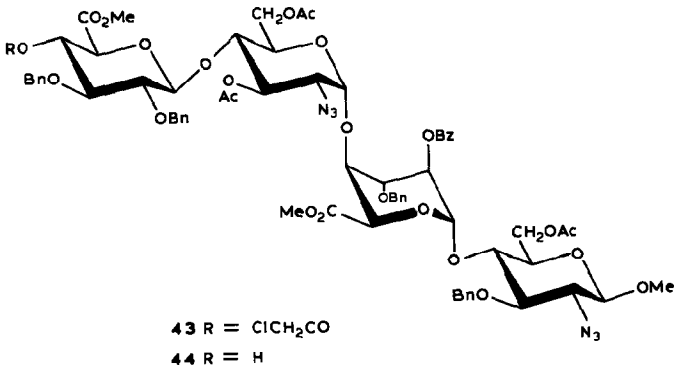


40

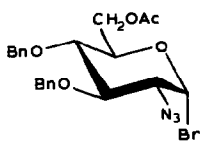
41 R = OAc
42 R = Br

Scheme 4

spectrum of **5** exhibited a signal, due to H-1‴″, at δ 5.50 as a doublet with $J_{1''',2'''}$ 3.66 Hz, showing that the new glycosidic bond had the α configuration. The α anomer **5** was accompanied by a 15% yield of β anomer **46**, which, in its ^{1}H-n.m.r. spectrum, gave the signal due to H-1‴″ at δ 4.34 as a doublet with $J_{1''',2'''}$ 8.06 Hz.



43 R = $ClCH_2CO$
44 R = H



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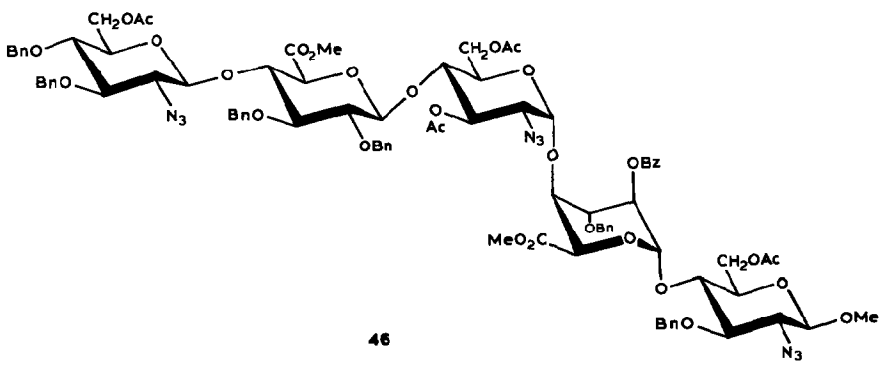

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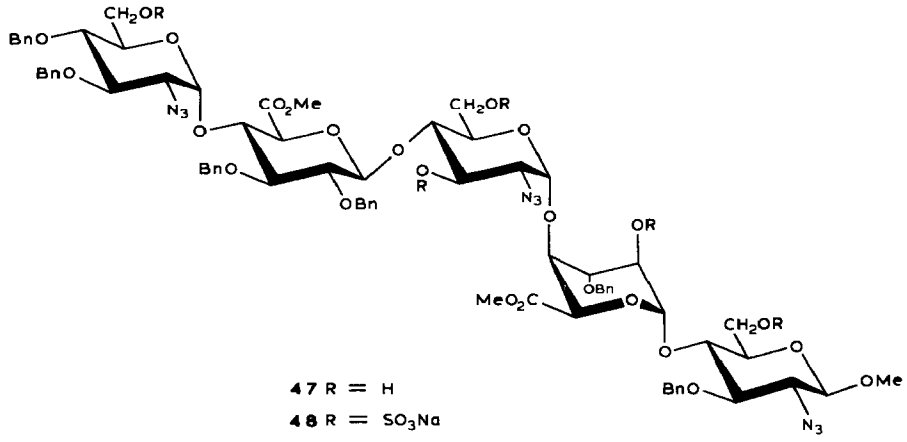

Conversion of the pentasaccharide derivative **5** into **3** was accomplished similarly to the preparation of trisaccharide **4**. Removal of the acyl groups of **5** was followed by *O*-sulfation, giving **48**, which afforded satisfactory analytical results. Hydrogenation of **48** in neutral media proceeded satisfactorily, in contrast to the corresponding step in the former preparation[1] of **2**. Some of the resulting amino groups resisted *N*-sulfation in basic media; however, employment of a large proportion of the reagent (sulfur trioxide–trimethylamine complex) and a long reaction-time (ten days) resulted in complete *N*-sulfation. Alkaline hydrolysis of the methyl alduronate and chromatographic purification[4c] of the product gave **3**, the structure of which was elucidated from the well-defined ^{1}H- (see Fig. 1) and ^{13}C-n.m.r. spectra, and the satisfactory results of elemental analyses.

AT III-binding activities of **3** *and* **4** *by fluorescence measurement.* — It is known that the interaction of heparin fragments with AT III induces change in the protein fluorescence[22]. On excitation at 280 nm, AT III produces a fluorescence emission spectrum, with a maximum at 330 nm, that is typical of tryptophan or tyrosine, or both. The fluorescence intensity is increased by addition of heparin fragments, and binding parameters are obtained from the increase[22]. This method is

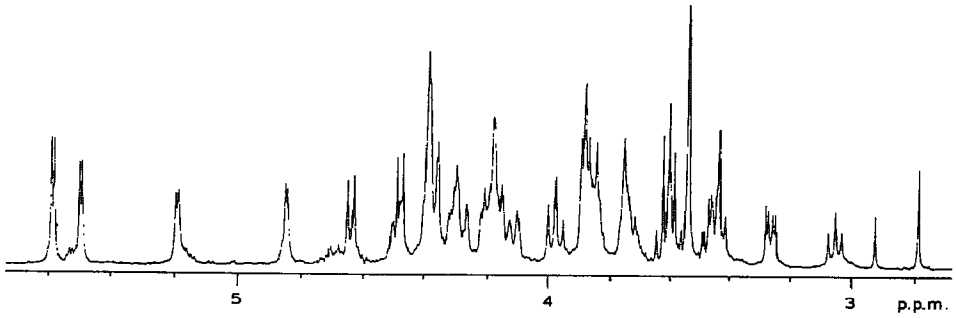

Fig. 1. ^{1}H-N.m.r. spectrum of the synthetic pentasaccharide **3** in D_2O.

known to require a very small amount of the sample. We found the affinity between AT III and the synthetic pure pentasaccharide **3** to be $6 \times 10^6\ \text{M}^{-1}$, similar to the K_a value recently reported[6] for **2**. The fluorescence remained unchanged on addition of the trisaccharide **4** to AT III, suggesting that binding may not take place.

Preparation of the methyl β-glycoside **3** of the pentasaccharide **2** was achieved by employing the silylated cellobiose derivative **14** as the starting-material. The absence of a free hemiacetal group in **3** apparently made no difference to the affinity for AT III; the association constant between **3** and AT III was almost equivalent to that of **2** and AT III, and of the same order of magnitude as that of high-affinity heparin[6]. The trisaccharide **4** did not exhibit any ligand-induced fluorescence change in AT III.

The present work demonstrates the versatile usefulness of 1,6-anhydro-β-cellobiose for the preparation of "disaccharide synthons" for the synthesis of a complex oligosaccharide.

EXPERIMENTAL

General methods. — Melting points were determined with a Yamato micro melting-point apparatus, and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 241MC polarimeter. Solutions were evaporated under diminished pressure; and solvent extracts were dried with magnesium sulfate unless otherwise specified. Chromatography was performed on columns of Silica Gel Merck (70–230 mesh; E. Merck, Darmstadt, Germany). Thin-layer chromatography was conducted with precoated plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany) of Silica Gel $60F_{254}$. Preparative thin-layer chromatography was performed with precoated plates (layer thickness, 2 mm; E. Merck, Darmstadt, Germany) of Silica Gel $60F_{254}$. I.r. spectra of compounds as Nujol mulls were recorded with a Shimadzu IR-27 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 400 MHz with a JEOL JNM-GX 400 spectrometer, using tetramethylsilane as the internal standard, for solutions in chloroform-*d* at 21°, unless otherwise specified. ^{13}C-N.m.r. spectra were recorded at 100 MHz with a JEOL JNM-GX 400 spectrometer, using 1,4-dioxane as the internal standard (67.40 p.p.m.), for solutions in deuterium oxide. Secondary-ion (s.i.) mass spectra were recorded with a Hitachi H-80 spectrometer at an ionizing voltage of 3 kV (primary ion) and 8–9 kV (secondary ion); each sample was applied in a glycerol matrix. Field desorption (f.d.) mass spectra were recorded with the same spectrometer. Fast-atom-bombardment (f.a.b.) mass spectra were recorded with a JEOL DX303 spectrometer in the negative-ion mode; the sample was applied in a triethanolamine matrix and bombarded with xenon atoms having a kinetic energy equivalent to 3 kV.

O-(6-O-Acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(methyl 2,3-di-O-benzyl-β-D-glucopyranosyluronate)-(1→4)-3,6-di-O-acetyl-2-azido-2-deoxy-D-glucopyranose (**7**). — A mixture of **6** (ref. 9; 347 mg, 0.31 mmol) and benzylamine[23] (101 mg, 0.94 mmol) in oxolane (10 mL) was stirred for 10 h at

room temperature. The mixture was poured into ice–water and extracted with chloroform. The extracts were successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 9:2 (v/v) toluene–ethyl acetate as the eluant, to give syrupy **7** (299 mg, 90%); $[\alpha]_D^{25}$ +5.3° (*c* 0.66, chloroform; equilibrium); ν_{max} 3450, 2100, and 1745 cm^{-1}; δ_H: 3.76 (s, 3 H, CO_2CH_3) and 5.51 (d, 1 H, *J* 3.66 Hz, H-1″).

Anal. Calc. for $C_{53}H_{60}N_6O_{18}$: C, 59.54; H, 5.66; N, 7.86. Found: C, 59.32; H, 5.65; N, 7.38.

Glycosidation of **7**. — To a cooled solution of **7** (27 mg, 25 μmol) in dichloromethane (1 mL) were successively added thionyl chloride (42 μL) and *N,N*-dimethylformamide[24] (1 drop) at 0–5°, and the mixture was stirred for 3 h at room temperature, poured into ice–water, and extracted with ethyl acetate. The extracts were combined and successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residue (**8**) was employed for the next step without purification. A solution of **8** in dichloromethane (1 mL) was added to a mixture of methanol (7 mg, 0.25 mmol), molecular sieves 4A (154 mg), and mercuric bromide (6 mg) in dichloromethane (1 mL) under an argon atmosphere, and the mixture was stirred for 2 days at room temperature, diluted with dichloromethane, and filtered. The filtrate was successively washed with aqueous silver nitrate, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The residue was chromatographed on silica gel, with 16:1 (v/v) benzene–ethyl acetate as the eluant, to give syrupy **9**, methyl *O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranoside (17 mg, 62%); $[\alpha]_D^{25}$ +7.2° (*c* 0.97, chloroform); ν_{max} 2100 and 1740 cm^{-1}; δ_H: 2.03 (s, 3 H, Ac), 2.66 (s, 3 H, Ac), 2.70 (s, 3 H, Ac), 3.25 (dd, 1 H, *J* 3.41 and 10.74 Hz, H-2), 3.27 (dd, 1 H, *J* 3.66 and 10.50 Hz, H-2″), 3.43 (s, 3 H, OMe), 3.44 (dd, 1 H, *J* 7.81 and 8.54 Hz, H-2′), 3.49–3.51 (2 H, H-4″,5″), 3.69 (dd, 1 H, *J* 8.79 and 9.28 Hz, H-4), 3.71 (dd, 1 H, *J* 8.54 and 9.03 Hz, H-3′), 3.75 (s, 3 H, CO_2CH_3), 3.84 (dd, 1 H, *J* 6.35 and 10.50 Hz, H-3″), 3.85 (d, 1 H, *J* 9.28 Hz, H-5′), 4.05 (t, 1 H, *J* 9.28 Hz, H-4′), 4.32 (d, 1 H, *J* 7.81 Hz, H-1′), 4.79 (d, 1 H, *J* 3.41 Hz, H-1), 5.43 (dd, 1 H, *J* 9.28 and 10.74 Hz, H-3), and 5.51 (d, 1 H, *J* 3.66 Hz, H-1″); *m/z* (f.d.-m.s.) 1082 [(M^+), calc. for $C_{54}H_{62}N_6O_{18}$: 1082.11].

O-Deacetylation of **9**. — Aqueous sodium hydroxide (3.2 mL, M solution) was added to a cooled solution of **9** (88 mg, 0.08 mmol) in a mixture of methanol (4 mL) and 1,2-dimethoxyethane (4 mL) at 0–5°; the mixture was stirred for 5 h at room temperature, treated with Dowex 50W-X8 (H^+) ion-exchange resin, and filtered. The filtrate was evaporated to dryness below 25°; the residue was dissolved in methanol, re-esterified with ethereal diazomethane, and the solution evaporated. The product was purified by preparative t.l.c. with 9:1 (v/v) benzene–ethyl acetate (developed three times), to give syrupy **10**, methyl *O*-(2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyl-

uronate)-(1→4)-2-azido-2-deoxy-α-D-glucopyranoside (70 mg; 90%); $[\alpha]_D^{25}$ +6.6° (*c* 0.50, chloroform); ν_{max} 3490, 2080, and 1750 cm^{-1}; δ_H: 3.19 (dd, 1 H, *J* 3.76 and 10.25 Hz, H-2), 3.24 (dd, 1 H, *J* 3.91 and 10.26 Hz, H-2″), 3.42 (s, 3 H, OMe), 3.52 (dd, 1 H, *J* 7.56 and 8.79 Hz, H-2′), 3.78 (s, 3 H, CO_2CH_3), 4.06 (d, 1 H, *J* 9.52 Hz, H-5′), 4.57 (d, 1 H, *J* 7.56 Hz, H-1′), 4.74 (d, 1 H, *J* 3.67 Hz, H-1), and 5.50 (d, 1 H, *J* 3.91 Hz, H-1″); *m/z* (f.d.-m.s.): 957 [(M^+), calc. for $C_{48}H_{56}N_6O_{15}$: 957.00].

Methyl 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)-2-azido-2-deoxy-3,6-di-O-sulfo-α-D-glucopyranoside, trisodium salt (**11**). — Sulfur trioxide–trimethylamine complex (230 mg, 1.65 mmol) was added to a cooled solution of **10** (105 mg, 0.11 mmol) in *N,N*-dimethylformamide (5 mL) at 0°, and the mixture was stirred for 36 h at 50°. After being cooled, the mixture was diluted with methanol (1 mL) and chromatographed on a column of Sephadex LH-20 (120 mL), with 1:1 (v/v) chloroform–methanol as the eluant. The product was purified by preparative t.l.c. with 2:1 (v/v) chloroform–methanol and then by use of a column of Dowex 50W-X8 (Na^+) resin with methanol as the eluant, to give sticky **11** (101 mg, 73%); $[\alpha]_D^{18}$ +3.6° (c 0.67, methanol); ν_{max} 2100, 1745, 1250, and 1060 cm^{-1}; $\delta_H(CD_3OD)$: 3.24 (dd, 1 H, *J* 3.42 and 10.26 Hz, H-2), 3.36 (dd, 1 H, *J* 3.42 and 10.49 Hz, H-2″), 3.38 (s, 3 H, OMe), 3.64 (dd, 1 H, *J* 7.81 and 8.30 Hz, H-2′), 3.80 (s, 3 H, CO_2CH_3), 4.69 (dd, 1 H, *J* 8.79 and 10.26 Hz, H-3), 4.80 (d, 1 H, *J* 3.42 Hz, H-1), 4.93 (d, 1 H, *J* 7.81 Hz, H-1′), and 5.53 (d, 1 H, *J* 3.42 Hz, H-1″); *m/z* (f.d.-m.s.): 1284 [$(M + Na - 2H)^+$, calc. for $C_{48}H_{51}N_6Na_4O_{24}S_3$: 1284.09].

Anal. Calc. for $C_{48}H_{53}N_6Na_3O_{24}S_3 \cdot 3H_2O$: C, 43.77; H, 4.51; N, 6.38. Found: C, 43.29; H, 4.42; N, 6.11.

Reduction of the azido groups and O-debenzylation of **11**. — A mixture of **11** (130 mg, 0.10 mmol) and 10% Pd–C (200 mg) in methanol (8 mL), acetic acid (6 mL), and water (6 mL) was shaken under a hydrogen atmosphere for 7 h at room temperature. The mixture was filtered, and the filtrate was evaporated *in vacuo*. The residue, showing two ninhydrin-positive spots in t.l.c. on cellulose, was chromatographed on a column of Avicel, with 8:3:4 (v/v) 1-butanol–acetic acid–water as the eluant, and then subjected to preparative t.l.c. on cellulose with 5:5:4:1 (v/v) pyridine–ethyl acetate–water–acetic acid (developed three times), to give **12**, methyl *O*-(2-amino-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(methyl β-D-glucopyranosyluronate)-(1→4)-2-amino-2-deoxy-3,6-di-*O*-sulfo-α-D-glucopyranoside, trisodium salt (46 mg, 53%) and **13**, methyl *O*-(2-amino-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-2-amino-2-deoxy-3,6-di-*O*-sulfo-α-D-glucopyranoside, trisodium salt (23 mg, 27%).

For **12**: $\delta_H(D_2O)$: 3.36 (s, 3 H, OMe), 3.75 (s, 3 H, CO_2CH_3), 4.52 (d, 1 H, *J* 7.81 Hz, H-1′), 4.83 (d, 1 H, *J* 4.41 Hz, H-1), and 5.38 (d, 1 H, *J* 3.42 Hz, H-1″); *m/z* (s.i.-m.s.): 828 [$(M - Na + H)^+$, calc. for $C_{20}H_{34}N_2Na_2O_{24}S_3$: 828.64].

For **13**: ν_{max} 1610, 1250, and 1050 cm^{-1}; $\delta_H(D_2O)$: 3.44 (s, 3 H, OMe), 4.60 (d, 1 H, *J* 7.81 Hz, H-1′), 5.02 (d, 1 H, *J* 4.41 Hz, H-1), and 5.66 (d, 1 H, *J* 3.42 Hz, H-1″); *m/z* (s.i.-m.s.): 793 [$(M - 2Na + 2H)^+$, calc. for $C_{19}H_{33}N_2NaO_{24}S_3$: 792.63].

N-*Sulfation of* **12** *and* **13**. — *From* **12**. Sulfur trioxide–trimethylamine complex (12 mg, 86 μmol) was added to a solution of **12** (7 mg, 8 μmol) in aqueous sodium hydroxide (2 mL) at pH 9.5, and the mixture was stirred for 5 days at room temperature. The pH of the mixture was maintained at 9.5–10.0 by addition of M sodium hydroxide. During this time, more sulfur trioxide–trimethylamine complex (48 mg, 0.34 mmol) was added, in four portions. Aqueous sodium hydroxide (M solution) was now added, to pH 12, and the mixture was stirred for 6 h at room temperature. After adjustment of the pH to 7.5 by addition of M hydrochloric acid, the mixture was freeze-dried; the residue was eluted from a column of Sephadex G-25 using water, to give a sugar fraction. This was passed through a column of SP-Sephadex C-25 (Na^+), to give amorphous white powdery **4**, methyl *O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-3,6-di-*O*-sulfo-α-D-glucopyranoside, hexasodium salt (5 mg, 44%).

From **13**. Treatment **13** (6 mg, 7 μmol) as described for the synthesis of **4** from **12** gave **4** (4 mg, 52%). The ^{1}H-n.m.r. spectrum was identical with that of **4** prepared from **12**; $[\alpha]_D^{18}$ +3.1° (*c* 0.03, water); δ_H (D_2O, 50°, external Me_4Si): 3.20 (dd, 1 H, *J* 3.42 and 10.01 Hz, H-2″), 3.35 (dd, 1 H, *J* 6.65 and 7.82 Hz, H-2′), 3.37 (s, 3 H, OMe), 3.42 (dd, 1 H, *J* 3.42 and 10.50 Hz, H-2), 3.54 (dd, 1 H, *J* 7.56 and 10.01 Hz, H-3″), 4.38 (dd, 1 H, *J* 7.56 and 10.50 Hz, H-3′), 4.59 (d, 1 H, *J* 7.82 Hz, H-1′), 4.98 (d, 1 H, *J* 3.42 Hz, H-1), and 5.58 (d, 1 H, *J* 3.42 Hz, H-1″); *m/z* (s.i.-m.s.): 1062 [(M^+), calc. for $C_{19}H_{28}N_2Na_6O_{30}S_5$: 1062.66]; (f.a.b.-m.s.): 1038.9070 [M − Na)$^-$, calc. for $C_{19}H_{28}N_2Na_5O_{30}S_5$: 1038.8811].

Destructive elemental analysis was not performed on this compound, because of its limited amount, but the high-field ^{1}H-n.m.r. spectrum showed >97% purity.

O-(3-O-Benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranose (**16**). — A mixture of **14** (ref. 8; 4.58 g, 6.99 mmol) and dibutyltin oxide (2.09 g, 8.39 mmol) in toluene (90 mL) was refluxed for 15 h with azeotropic removal of water, concentrated to half volume, and cooled to 80°. Tetrabutylammonium iodide[12] (2.58 g, 6.99 mmol) and benzyl bromide (2.39 g, 14.0 mmol; 1.7 mL) were added, and the mixture was heated for 5 h at 100°. When t.l.c. in 9:1 (v/v) benzene–ethyl acetate and in 50:47:3 (v/v) chloroform–ethyl acetate–methanol showed only a trace of the starting material **14**, the mixture was cooled, and evaporated *in vacuo*. The residue was, without processing, chromatographed on silica gel, with 50:1 (v/v) toluene–ethyl acetate as the eluant, to give **16** (5.03 g, 97%); m.p. 164.8–165.8° (from ethyl acetate–hexane), $[\alpha]_D^{22}$ −58.8° (*c* 0.15, chloroform); δ_H: 0.92–1.12 (m, 28 H, tetraisopropyl groups), 2.63 (d, 1 H, *J* 2.19 Hz, O*H*-2′), 3.40–3.46 (m, 1 H, H-5′), 3.50 (d, 1 H, H-6a), 3.60–3.76 (8 H, H-2, 3, 4, 6b, 2′ 3′, 4′, 6′a), 4.33 (d, 1 H, *J* 7.33, Hz, H-1′), 4.56 (broad d, 1 H, *J* 4.15 Hz, H-5), 4.82 (d, 1 H, *J* 11.48 Hz, 0.5 OCH_2Ph), 4.95 (d, 1 H, *J* 11.48 Hz, 0.5 OCH_2Ph), 5.29 (s, 1 H, H-1), and 5.57 (s, 1 H, benzylidene).

Anal. Calc. for $C_{38}H_{56}O_{11}Si_2$: C, 61.26; H, 7.58. Found: C, 61.17; H, 7.70.

O-(2-O-Acetyl-3-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-

1,6-anhydro-2,3-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranose (**18**) *and* O-*(3-O-acetyl-2-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranose* (**19**). — A mixture of **14** (ref. 8; 23.0 g, 35.1 mmol), silver (I) oxide (12.2 g, 52.6 mmol), benzyl bromide (9.06 g, 53.0 mmol), and tetrabutylammonium iodide (13.0 g, 35.2 mmol) in oxolane (200 mL) was stirred for 6 days at room temperature, diluted with chloroform, and filtered. The filtrate was evaporated *in vacuo,* and the residue was chromatographed on silica gel, with 40:1 (v/v) toluene–ethyl acetate as the eluant, to give 2′, 3′-dibenzyl ether **15** (ref. 8; 3.08 g, 10.5%), and fractions (containing **16**, **17**, and the starting material **14**) that were combined, acetylated in the usual way with acetic anhydride–pyridine, and evaporated. The residual syrup was chromatographed on silica gel, with 90:1 (v/v) toluene–ethyl acetate as the eluant, to give **18** (558 mg, 2.0%), **19** (6.77 g, 24.5%), and the 2′′,3′-di-*O*-acetyl derivative of **14** (ref. 8; 3.18 g, 12.3%) in that order.

For **18**: syrup, $[\alpha]_D^{21}$ −34.7° (*c* 0.65, chloroform); δ_H: 2.02 (s, 3 H, Ac), 3.71 (t, 1 H, *J* 8.79 Hz, H-3′), 4.54 (d, 1 H, *J* 7.82 Hz, H-1′), 4.99 (dd, 1 H, *J* 7.82 and 8.79 Hz, H-2′), and 5.59 (s, 1 H, H-1).

Anal. Calc. for $C_{40}H_{58}O_{12}Si_2$: C, 61.04; H, 7.43. Found: C, 61.33; H, 7.49.

For **19**: m.p. 156–157° (from ethyl acetate–hexane), $[\alpha]_D^{28}$ −53.8° (*c* 0.60, chloroform); δ_H: 1.96 (s, 3 H, Ac), 3.40 (dd, 1 H, *J* 7.57 and 9.52 Hz, H-2′), 4.61 (d, 1 H, *J* 7.57 Hz, H-1′), 5.29 (t, 1 H, *J* 9.52 Hz, H-3′), and 5.32 (s, 1 H, H-1).

Anal. Calc. for $C_{40}H_{58}O_{12}Si_2$: C, 61.04; H, 7.43. Found: C, 61.24; H, 7.47.

O-*(2-O-Benzoyl-3-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-2,3-O-(tetraisopropyldisiloxane-1,3-diyl)-β-D-glucopyranose* (**20**). — Benzoyl chloride (2.07 g 14.8) mmol) was added dropwise to a cooled solution of **16** (9.18 g, 12.3 mmol) in pyridine (100 mL) at 0–5°, and the mixture was stirred for 5 h at room temperature, poured into ice-water, and extracted with ethyl acetate. The extracts were combined, successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 50:1 (v/v) toluene–ethyl acetate as the eluant, to give **20** (10.3 g, 98%); m.p. 89.5–90.5° (from ethyl acetate–hexane), $[\alpha]_D^{27}$ −19.0° (*c* 0.67, chloroform); δ_H: 0.85–1.35 (m, 28 H, tetraisopropyl groups), 3.40–3.52 (5 H, H-2, 3, 4, 5′, 6′a), 3.69 (t, 1 H, *J* 7.08 Hz, H-6b), 3.80 (t, 1 H *J* 10.50 Hz, H-4′), 3.84–3.88 (2 H, H-6a, 3′), 4.25 (d, 1 H, *J* 4.64 Hz, H-5), 4.37 (dd, 1 H, *J* 4.88 and 10.50 Hz, H-6′b), 4.70 (d, 1 H, *J* 12.0 Hz, 0.5 OCH_2Ph), 4.73 (d, 1 H, *J* 7.81 Hz, H-1′), 4.82 (d, 1 H, *J* 12.0 Hz, 0.5 OCH_2Ph), 5.18 (s, 1 H, H-1), 5.25–5.30 (m, 1 H, H-2′), and 5.62 (s, 1 H, benzylidene).

Anal. Calc. for $C_{45}H_{60}O_{12}Si_2$: C, 63.66; H, 7.12. Found: C, 63.77; H, 7.15.

O-*(2-O-Benzoyl-3-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-β-D-glucopyranose* (**22**). — To a cooled solution of **20** (25.0 g, 29.4 mmol) in acetonitrile (150 mL) and water[8] (5 mL) was added dropwise a M solution of tetrabutylammonium fluoride in oxolane (40 mL) at 0–5°, and the mixture was stirred for 10 h at room temperature, and then evaporated *in vacuo*. The residue was diluted with chloroform and water, and the organic layer was washed with water,

dried, and evaporated. Ether was added to the residual syrup, and the crystalline mass was collected by filtration, to give **22** (15.7 g, 88%), m.p. 212–213° (from chloroform–ether), $[\alpha]_D^{22}$ −2.61° (*c* 0.12, chloroform); δ_H: 2.52 (d, 1 H, *J* 8.79 Hz, O*H*), 3.43 (broad s, 1 H, O*H*), 3.48–3.55 (m, 1 H, H-5′), 3.62 (dd, 1 H, *J* 5.61 and 7.81 Hz, H-6b), 3.75 (s, 1 H, H-4), 3.83–3.95 (5 H, H-2, 3, 3′, 4′, 6′a), 4.06 (d, 1 H, *J* 7.81 Hz, H-6a), 4.37 (d, 1 H, *J* 5.61 Hz, H-5), 4.38 (dd, 1 H, *J* 4.88 and 10.50 Hz, H-6′b), 4.73 (d, 1 H, *J* 7.81 Hz, H-1′), 5.29 (s, 1 H, H-1), 5.31 (dd, 1 H, *J* 7.81 and 8.55 Hz, H-2′), and 5.63 (s, 1 H, benzylidene).

Anal. Calc. for $C_{33}H_{35}O_{11}$: C, 65.23; H, 5.81. Found: C, 65.00; H, 5.61.

O-*(2-*O*-Benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranosyl)-(1→4)-1,6-anhydro-2-*O*-p-tolylsulfonyl-β-*D*-glucopyranose* (**25**). — To a cooled solution of **22** (31.2 g, 51.3 mmol) in alcohol-free chloroform (150 mL) and pyridine (150 mL) was added dropwise a solution of *p*-toluenesulfonyl chloride (16.6 g, 87.3 mmol) in alcohol-free chloroform (70 mL) and pyridine (100 mL) at 0–5°, and the mixture was stirred for 4 days at room temperature, during which time more *p*-toluenesulfonyl chloride (2.95 g, 15.4 mmol) was added portionwise. The mixture was poured into ice–water, and extracted with chloroform. The extracts were combined, successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. Ether was added to the residual syrup, and the crystalline mass was collected by filtration, to give **25** (35.8 g, 91.5%); m.p. 167.0–168.5° (from chloroform–ether), $[\alpha]_D^{22}$ −0.66° (*c* 0.63, chloroform); δ_H: 2.43 (s, 3 H, Ar-C*H*$_3$), 3.00 (d, 1 H, *J* 3.66 Hz, O*H*-3), 3.44–3.52 (3 H, H-4, 6b, 5′), 3.66 (d, 1 H, *J* 7.57 Hz, H-6a), 3.78–3.88 (4 H, H-3, 3′, 4′, 6′a), 4.12 (d, 1 H, *J* 5.27 Hz, H-2), 4.32 (d, 1 H, *J* 5.13 Hz, H-5), 4.36 (dd, 1 H, *J* 4.88 and 10.50 Hz, H-6′b), 4.75 (d, 1 H, *J* 7.81 Hz, H-1′), 5.27 (s, 1 H, H-1), 5.29 (m, 1 H, H-2′), and 5.61 (s, 1 H, benzylidene).

Anal. Calc. for $C_{40}H_{41}O_{13}S$: C, 63.07; H, 5.43; S, 4.21. Found: C, 63.04; H, 5.26; S, 4.21.

O-*(2-*O*-Benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranosyl)-(1→4)-1,6:2,3-dianhydro-β-*D*-mannopyranose* (**28**). — To a cooled solution of **25** (35.5 g, 46.6 mmol) in oxolane (200 mL) and *N*,*N*-dimethylformamide (20 mL) was added portionwise sodium hydride (2.05 g of 60% mineral oil dispersion, 51.3 mmol) at 0–5°, and the mixture was stirred for 5 min at room temperature. When the reaction was complete, the mixture turned gelatinous. The mixture was carefully poured into ice–water and extracted with chloroform. The extracts were washed once with water (if the extracts were washed several times with water, a precipitate, namely, epoxide **28**, formed in the separatory funnel), dried, and evaporated. The solid residue was triturated with ether, and the crystalline mass was collected by filtration, to give **28** (26.0 g, 94.6%); m.p. 74.5–75.0° (from chloroform–ether), $[\alpha]_D^{28}$ +6.47° (*c* 0.54, chloroform); δ_H: 3.29 (dd, 1 H, *J* 0.74 and 3.67 Hz, H-3), 3.41 (ddd, 1 H, *J* 0.73, 3.18, and 3.66 Hz, H-2), 3.51–3.53 (m, 1 H, H-5′), 3.58–3.63 (2 H, H-4,6b), 3.83–3.93 (3 H, H-6a,3′,6′a), 4.24–4.27 (m, 1 H, H-5), 4.39 (dd, 1 H, *J* 4.88 and 10.50 Hz, H-6′b), 4.86 (d, 1 H, *J* 8.06 Hz, H-1′), 5.32–5.37 (m, 1 H, H-2′), 5.61 (d,

1 H, *J* 3.18 Hz, H-1), and 5.62 (s, 1 H, benzylidene).

Anal. Calc. for $C_{33}H_{33}O_{10}$: C, 67.23; H, 5.64. Found: C, 67.18; H, 5.44.

O-(2-O-Benzoyl-3-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (**26**). — A mixture of **28** (1.54 g, 2.60 mmol), sodium azide (1.02 g, 15.7 mmol), and ammonium chloride (1.96 g, 36.6 mmol) in *N,N*-dimethylformamide (15 mL) and water (1.5 mL) was heated for 40 h at 110°, cooled, poured into ice–water, and extracted with ethyl acetate. The extracts were combined, washed with water only (if the extracts were washed with brine, they turned gelatinous in the separatory funnel), dried, and evaporated. The residual syrup was chromatographed on silica gel, with 4:1 (v/v) toluene–ethyl acetate as the eluant, to give **26** (1.45 g, 88.5%); m.p. 174–176° (from ethyl acetate–hexane), $[\alpha]_D^{22}$ −0.77° (*c* 0.52, chloroform); δ_H: 3.20 (d, 1 H, *J* 3.67 Hz, H-2), 3.22 (d, 1 H, *J* 5.86, Hz, O*H*-3), 3.50–3.61 (3 H, H-4,6b,5′), 3.72–3.78 (2 H, H-3,6a), 3.83–3.91 (3 H, H-3′, 4′, 6′a), 4.32 (d, 1 H, *J* 5.13 Hz, H-5), 4.40 (dd, 1 H, *J* 5.13 and 10.50 Hz, H-6′b), 4.78 (d, 1 H, *J* 8.05 Hz, H-1′), 5.27 (s, 1 H, H-1), 5.33-5.37 (m, 1 H, H-2′), and 5.36 (s, 1 H, benzylidene).

Anal. Calc. for $C_{33}H_{34}N_3O_{10}$: C, 62.66; H, 5.42; N, 6.64. Found: C, 62.96; H, 5.23; N, 6.49.

O-(2-O-Benzoyl-3-O-benzyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranose (**27**). — Sodium hydride (1.68 g of 60% mineral oil dispersion, 42.1 mmol) was added portionwise to a cooled mixture of **26** (24.2 g, 38.3 mmol), benzyl bromide (19,6 g, 115 mmol), and tetrabutylammonium iodide (17.0 g, 45.9 mmol) in dry oxolane (400 mL) at 0–5°, and the mixture was stirred for 50 min at room temperature, carefully poured into ice–water, and extracted with ethyl acetate. The extracts were combined, washed with water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 35:1 (v/v) toluene–ethyl acetate as the eluant, to give **27** (25.3 g, 91.4%); m.p. 147.0–148.5° (from ethyl acetate–hexane), $[\alpha]_D^{22}$ +13.4° (*c* 0.52, chloroform); δ_H: 3.25 (s, 1 H, H-2), 3.37–3.42 (m, 1 H, H-5′), 3.63 (dd, 1 H, *J* 5.12 and 7.33 Hz, H-6b), 3.69 (s, 1 H, H-4), 3.73–3.74 (m, 1 H, H-3), 3.78 (t, 1 H, *J* 10.26 Hz, H-6′a), 3.81–3.87 (2 H, H-3′,4′), 3.97 (d, 1 H, *J* 7.32 Hz, H-6a), 4.24 (dd, 1 H, *J* 5.13 and 10.50 Hz, H-6′b), 4.42 (d, 1 H, *J* 5.12 Hz, H-5), 4.59–4.67 (2 H, OCH_2Ph), 4.69 (d, 1 H, *J* 11.96 Hz, 0.5 OCH_2Ph), 4.72 (d, 1 H, *J* 8.06 Hz, H-1′), 4.81 (d, 1 H, *J* 11.96 Hz, 0.5 OCH_2Ph), 5.30–5.34 (m, 1 H, H-2′), 5.34 (s, 1 H, H-1), and 5.58 (s, 1 H, benzylidene).

Anal. Calc. for $C_{40}H_{40}N_3O_{10}$: C, 66.47; H, 5.58; N, 5.81. Found: C, 66.23; H, 5.37; N, 5.67.

O-(2-O-Benzoyl-3-O-benzyl-β-D-glucopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranose (**29**). — A mixture of **27** (845 mg, 1.17 mmol) and cupric chloride dihydrate[14] (1.40 g, 8.18 mmol) in oxolane (5 mL) and ethanol (7 mL) was refluxed for 1.5 h, cooled, made neutral with saturated sodium hydrogencarbonate, and filtered. The filtrate was extracted with chloroform, and the extracts were dried (sodium sulfate) without being washed with water, and

evaporated. The residual syrup was chromatographed on silica gel, with 35:15:1 (v/v) chloroform–ethyl acetate–methanol as the eluant, to give syrupy **29** (636 mg, 85.6%); $[\alpha]_D^{22}$ −7.31° (*c* 0.55, chloroform); δ_H: 2.18 (broad s, 1 H, O*H*), 2.47 (broad s, 1 H, O*H*), 3.28 (s, 1 H, H-2), 3.32 (ddd, 1 H, *J* 4.40, 4.40, and 9.32 Hz, H-5'), 3.63–3.80 (m, 7 H), 3.84 (d, 1 H, *J* 7.32 Hz, H-6a), 4.44 (d, 1 H, *J* 5.13 Hz, H-5), 4.57–4.74 (4 H, 2 OC*H*$_2$Ph), 4.68 (d, 1 H, *J* 8.06 Hz, H-1'), 5.26 (dd, 1 H, *J* 8.06 and 9.28 Hz, H-2'), and 5.35 (s, 1 H, H-1).

Anal. Calc. for $C_{33}H_{36}N_3O_{10}$: C, 62.45; H, 5.67; N, 6.62. Found: C, 62.50; H, 5.58; N, 6.63.

O-*(2-*O*-Benzoyl-3-*O*-benzyl-6-*O*-*p*-tolylsulfonyl-β-*D*-glucopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranose* (**30**). — *Method A.* A mixture of **29** (15.2 g, 23.9 mmol) and bis(tributyltin) oxide[15] (8.57 g, 14.4 mmol) in toluene (250 mL) was refluxed for 3 h with azeotropic removal of water, concentrated to half volume, and cooled to 80°. *p*-Toluenesulfonyl chloride (9.13 g, 47.9 mmol) was added portionwise to the mixture, which was then heated for 13 h at 80°, poured into ice–cold aqueous sodium hydrogencarbonate, and extracted with chloroform. The extracts were combined, washed with water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 8:1 (v/v) chloroform–ethyl acetate as the eluant, to give **30** (9.60 g, 94.6% yield, based on the **29** consumed).

Method B. A solution of *p*-toluenesulfonyl chloride (2.77 g, 14.5 mmol) in alcohol-free chloroform (20 mL) and pyridine (20 mL) was added to a cooled solution of **29** (5.12 g, 8.07 mmol) in alcohol-free chloroform (30 mL) and pyridine (40 mL) at 0–5°, and the mixture was stirred for 40 h at room temperature. During this time, more *p*-toluenesulfonyl chloride (770 mg, 4.0 mmol) was added portionwise to the mixture, which was then poured into ice–water, and extracted with chloroform. The extracts were combined, successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. Ethanol was added to the residual syrup, and the crystalline mass was collected by filtration, to give **30** (5.25 g, 82.5%; m.p. 142.5–143.5° (from ethyl acetate–hexane), $[\alpha]_D^{27}$ +5.73° (*c* 0.19, chloroform); δ_H: 2.39 (s, 1 H, O*H*-4'), 2.42 (s, 3 H, Ar-C*H*$_3$), 3.18 (s, 1 H, H-2), 3.53–3.58 (m, 1 H, H-5'), 3.61–3.68 (3 H, H-6b, 3', 4'), 3.74 (broad s, 1 H, H-4), 3.77 (s, 1 H, H-3), 4.01 (d, 1 H, *J* 7.09 Hz, H-6a), 4.23 (dd, 1 H, *J* 5.69 and 10.98 Hz, H-6'a), 4.30 (dd, 1 H, *J* 1.96 and 10.99 Hz, H-6'b), 4.48 (d, 1 H, *J* 5.13 Hz, H-5), 4.55–4.74 (4 H, 2 OC*H*$_2$Ph), 4.78 (d, 1 H, *J* 8.06 Hz, H-1'), 5.23 (m, 1 H, H-2'), and 5.36 (s, 1 H, H-1).

Anal. Calc. for $C_{40}H_{42}N_3O_{12}S$: C, 60.91; H, 5.37; N, 5.33; S, 4.06. Found: C, 60.73; H, 5.19; N, 5.20; S, 4.06.

O-*(2-*O*-Benzoyl-3-*O*-benzyl-6-deoxy-6-iodo-β-*D*-glucopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranose* (**31**). — A mixture of **30** (2.43 g, 3.08 mmol) and sodium iodide (1.85 g, 12.3 mmol) in acetone (60 mL) was refluxed for 14 h, cooled, evaporated under diminished pressure, and the residue dissolved in ethyl acetate and water. The organic layer was successively washed with

aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 10:1 (v/v) toluene–ethyl acetate as the eluant, to give **31** (2.20 g, 95.9%); m.p. 144.5–146.0° (from ethyl acetate–hexane), $[\alpha]_D^{28}$ −21.6° (*c* 0.04, chloroform); δ_H: 2.29 (d, 1 H, *J* 2.93 Hz, O*H*-4′), 3.21 (s, 1 H, H-2), 3.25–3.33 (2 H, H-6′a,6′b), 3.54–3.58 (2 H, H-4′,5′), 3.67–3.72 (2 H, H-6b, 3′), 3.80 (s, 1 H, H-4), 3.87 (s, 1 H, H-3), 4.06 (d, 1 H, *J* 8.06 Hz, H-6a), 4.51–4.76 (4 H, 2 OCH_2Ph), 4.59 (d, 1 H, *J* 5.13 Hz, H-5), 4.88 (d, 1 H, *J* 8.05 Hz, H-1′), 5.33 (dd, 1 H, *J* 8.05 and 9.52 Hz, H-2′), and 5.38 (s, 1 H, H-1).

Anal. Calc. for $C_{33}H_{35}IN_3O_9 \cdot 0.5\ H_2O$: C, 52.59; H, 4.81; I, 16.84; N, 5.58. Found: C, 52.50; H, 4.51; I, 16.72; N, 5.52.

O-*(2-*O*-Benzoyl-3-*O*-benzyl-6-deoxy-β-*D*-*xylo*-hex-5-enopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranose* (**32**). — A mixture of **31** (2.20 g, 2.95 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1.35 g, 8.86 mmol) in oxolane (60 mL) was refluxed for 18 h, cooled, and diluted with ethyl acetate. The organic layer was successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated. The residual syrup was chromatographed on silica gel, with 30:1 (v/v) toluene–ethyl acetate containing 1% (v/v) of triethylamine as the eluant, to give syrupy **32** (1.60 g, 88.1%); $[\alpha]_D^{27}$ −4.61° (*c* 0.65, chloroform); δ_H: 2.50 (d, 1 H, *J* 3.66 Hz, O*H*-4′), 3.23 (s, 1 H, H-2), 3.69–3.78 (6 H, H-3, 4, 6b, 3′,6′a, 6′b), 4.09 (d, 1 H, *J* 7.32 Hz, H-6a), 4.61–4.79 (6 H, H-5, 4′, and 2 OCH_2Ph), 5.06 (d, 1 H, *J* 2.69 Hz, H-1′), 5.36 (dd, 1 H, *J* 2.69 and 5.37 Hz, H-2′), and 5.49 (s, 1 H, H-1).

Anal. Calc. for $C_{33}H_{34}N_3O_9 \cdot 1.2\ H_2O$: C, 62.10; H, 5.75; N, 6.58. Found: C, 62.40; H, 5.52; N, 6.17.

O-*(2-*O*-Benzoyl-3-*O*-benzyl-α-*L*-idopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy-β-*D*-glucopyranose* (**33**). — Methyl iodide (967 mg, 6.81 mmol) was added dropwise to a cooled solution of **32** (2.0 g, 3.24 mmol) and tetrabutylammonium borohydride[17] (1.75 g, 6.81 mmol) in dichloromethane (50 mL) under an argon atmosphere during 5 min at 0–5°, and the mixture was stirred for 30 min at room temperature. To the cold mixture were successively added, dropwise, water (1 mL), saturated sodium hydrogencarbonate (1.5 mL), and 30% hydrogen peroxide (1.5 mL), and the mixture was stirred for 50 min at room temperature. After the mixture had turned pale yellow, it was poured into ice–water, and extracted with chloroform. The extracts were combined, dried without being washed with water, and evaporated. The residual syrup was chromatographed on silica gel, with 5:2 (v/v) toluene–ethyl acetate as the eluant, to give the less polar **33** (390 mg, 19.0%), and the more polar **29** (778 mg, 38%).

For **33**: syrup, $[\alpha]_D^{23}$ −20.0° (*c* 0.06, chloroform); δ_H: 1.86 (broad s, 1 H, O*H*-6′), 2.72 (d, 1 H, *J* 9.76 Hz, O*H*-4′), 3.25 (d, 1 H, *J* 2.93 Hz, H-2), 3.63–3.85 (7 H, H-3, 4, 6b, 3′, 4′, 6′a, 6′b), 4.04 (d, 1 H, *J* 7.32 Hz, H-6a), 4.20–4.22 (m, 1 H, H-5′), 5.22 (s, 1 H, H-1′), 5.32 (distorted t, 1 H, *J* 1.22 Hz, H-2′), and 5.53 (s, 1 H, H-1).

Anal. Calc. for $C_{33}H_{36}N_3O_{10}$: C, 62.45; H, 5.72; N, 6.62. Found: C, 62.49; H,

5.64; N, 6.27.

O-*(2-O-Benzoyl-3-O-benzyl-4-O-chloroacetyl-6-O-trityl-α-L-idopyranosyl)-(1→4)-1,6-anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranose* (**34**). — A mixture of **33** (1.47 g, 2.32 mmol) and trityl chloride (904 mg, 3.24 mmol) in pyridine (30 mL) was heated for 12 h at 100°, and cooled. Chloroacetic anhydride[18] (1.98 g, 11.6 mmol) was added portionwise to the mixture at 0–5°; it was then stirred for 2 h at 0–5°, poured into ice-water, and extracted with ethyl acetate. The extracts were combined, successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 36:1 (v/v) toluene–ethyl acetate as the eluant, to give syrupy **34** (2.15 g, 97.4%); $[\alpha]_D^{22}$ −2.33° (*c* 0.65, chloroform); δ_H: 3.10 (dd, 1 H, *J* 6.35 and 9.03 Hz, H-6′a), 3.21 (broad s, 1 H, H-2), 3.38 (dd, 1 H, *J* 6.59 and 9.04 Hz, H-6′b), 3.63 (s, 2 H, $COCH_2Cl$), 3.71 (broad s, 1 H, H-3), 3.75 (distorted dd, 1 H, *J* 5.86 and 7.08 Hz, H-6b), 3.90 (broad s, 1 H, H-3′), 3.92 (broad s, 1 H, H-4), 4.07 (d, 1 H, *J* 7.33 Hz, H-6a), 4.64 (distorted t, 1 H, *J* 6.10 Hz, H-5′), 4.80 (d, 1 H, *J* 5.37 Hz, H-5), 5.11 (broad s, 1 H, H-4′), 5.28 (s, 1 H, H-1′), 5.30 (broad s, 1 H, H-2′), and 5.54 (s, 1 H, H-1).

Anal. Calc. for $C_{54}H_{50}ClN_3O_{11}\cdot 0.5\ H_2O$: C, 67.46; H, 5.35; Cl, 3.69; N, 4.37. Found: C, 67.46; H, 5.34; Cl, 3.75; N, 4.39.

O-*(Methyl 2-O-benzoyl-3-O-benzyl-4-O-chloroacetyl-α-L-idopyranosyluronate)-(1→4)-1,6-anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranose* (**35**). — To a cooled solution of **34** (1.26 g, 1.32 mmol) in acetone (50 mL) was added dropwise a solution of chromium trioxide (2.30 g, 23.0 mmol) in 3.5M sulfuric acid[4a] (3.0 mL) at 0–5°, and the mixture was stirred for 3 h at room temperature, poured into ice-water, and extracted with chloroform. The extracts were combined, washed with water, dried, and evaporated. The residue was esterified with ethereal diazomethane in dichloromethane and the solvents were evaporated. The residual syrup was chromatographed on silica gel, with 15:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **35** (314 mg, 52%); $[\alpha]_D^{22}$ −19.9° (*c* 0.60, chloroform); δ_H: 3.25 (d, 1 H, *J* 2.93 Hz, H-2), 3.66 (m, 1 H, H-3), 3.73 (s, 3 H, CO_2CH_3), 3.76 (dd, 1 H, *J* 5.86 and 7.32 Hz, H-6b), 3.79 (broad s, 1 H, H-4), 3.85 (d, 1 H, *J* 14.90 Hz, 0.5 $ClCH_2CO$), 3.93 (d, 1 H, *J* 14.90 Hz, 0.5 $ClCH_2CO$), 3.97 (broad s, 1 H, H-3′), 4.03 (d, 1 H, *J* 7.33 Hz, H-6a),4.72–4.76 (2 H, H-5, 0.5 OCH_2Ph), 4.92 (d, 1 H, *J* 1.95 Hz, H-5′), 5.26 (broad s, 1 H, H-4′), 5.31 (broad s, 1 H, H-2′), 5.38 (s, 1 H, H-1′), and 5.51 (s, 1 H, H-1).

Anal. Calc. for $C_{40}H_{42}ClN_3O_{15}\cdot 0.5\ H_2O$: C, 57.87; H, 4.99; N, 5.62. Found: C, 57.61; H, 4.86; N, 5.38.

O-*(Methyl 2-O-benzoyl-3-O-benzyl-4-O-chloroacetyl-α-L-idopyranosyluronate)-(1→4)-1,6-di-O-acetyl-2-azido-3-O-benzyl-2-deoxy-D-glucopyranose* (**36**).— A mixture of **35** (261 mg, 0.35 mmol) and trifluoroacetic acid[19] (1.5 mL) in acetic anhydride (10 mL) was stirred for 20 h at room temperature, and evaporated, and the residual syrup was chromatographed on silica gel, with 10:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **36** (274 mg, 94%); α/β = 6:1);

$[\alpha]_D^{22}$ −7.88° (*c* 0.50, chloroform); δ_H (α anomer): 2.09 (s, 3 H, Ac), 2.18 (s, 3 H, Ac), 3.52 (s, 3 H, CO_2CH_3), 3.63 (dd, 1 H, *J* 3.66 and 9.52 Hz, H-2), 3.81 (t, 1 H, *J* 9.52 Hz, H-3), 3.86–4.03 (4 H, H-5, 3', $ClCH_2CO$), 4.06 (t, 1 H, *J* 9.52 Hz, H-4), 4.26–4.38 (2 H, H-6a, 6b), 4.96 (d, 1 H, *J* 3.67 Hz, H-5'), 5.18 (distorted t, 1 H, *J* 3.67 Hz, H-2'), 5.23 (distorted t, 1 H, *J* 4.15 Hz, H-4'), 5.37 (d, 1 H, *J* 3.42 Hz, H-1'), and 6.22 (d, 1 H, *J* 3.66 Hz, H-1).

Anal. Calc. for $C_{40}H_{42}ClN_3O_{15}\cdot 0.5\ H_2O$: C, 56.57; H, 5.10; Cl, 4.17, N, 4.95. Found: C, 56.29; H, 4.98; Cl, 4.89; N, 4.73.

Methyl O-*(methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-chloroacetyl-*α*-*L*-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranoside* (**38**). — A solution of titanium tetrabromide[19] (1.12 g, 3.05 mmol) in ethyl acetate (20 mL) was added to a cooled solution of **36** (855 mg, 1.02 mmol) in dichloromethane (20 mL) at 0–5°, and the mixture was stirred for 10 h at room temperature, poured into ice–water, and extracted with ethyl acetate. The extracts were combined, successively washed with sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup **37** was employed for the next step without purification. A mixture of **37** and molecular sieves 4A[25] (1 g) in dichloromethane (20 mL) was stirred for 10 min at room temperature under an argon atmosphere, and then cooled to −15°. Silver trifluoromethanesulfonate (392 mg, 1.53 mmol), 2,4,6-collidine[20] (185 mg, 1.53 mmol; 0.2 mL), and methanol (4 mL) were added, and the mixture was stirred for 2 h at −10°, diluted with dichloromethane, and filtered. The filtrate was successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 24:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **38** (628 mg, 76%); $[\alpha]_D^{21}$ −15.0° (*c* 0.20, chloroform); δ_H: 2.07 (s, 3 H, Ac), 3.33 (t, 1 H, *J* 9.04 Hz, H-3), 3.41 (dd, 1 H, *J* 7.82 and 9.04 Hz, H-2), 3.47 (s, 3 H, OMe), 3.54 (s, 3 H, CO_2CH_3), 3.42–3.50 (m, 1 H, H-5), 3.86–3.99 (4 H, H-4,3', $ClCH_2CO$), 4.18 (d, 1 H, *J* 7.82 Hz, H-1), 4.29 (dd, 1 H, *J* 3.90 and 12.45 Hz, H-6a), 4.47 (dd, 1 H, *J* 2.44 and 12.45 Hz, H-6b), 4.68–4.82 (4 H, 2 OCH_2Ph), 5.03 (d, 1 H, *J* 2.93 Hz, H-5'), 5.13 (distorted t, 1 H, *J* 2.44 Hz, H-2'), 5.22 (distorted t, 1 H, *J* 3.42 Hz, H-4'), and 5.32 (d, 1 H, *J* 1.56 Hz, H-1').

Anal. Calc. for $C_{39}H_{42}ClN_3O_{14}\cdot H_2O$: C, 56.56; H, 5.32; N, 5.07. Found: C, 56.58; H, 5.11; N, 4.80.

Methyl O-*(methyl 2-*O*-benzoyl-3-*O*-benzyl-*α*-*L*-idopyranosyluronate)-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-*β*-*D*-glucopyranoside* (**39**). — A mixture of **38** (628 mg, 0.77 mmol) and thiourea[21] (88.3 mg, 1.16 mmol) in oxolane (15 mL) and ethanol (15 mL) was refluxed for 10 h, cooled, poured into ice–water, and extracted with chloroform. The extracts were combined, successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 10:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **39** (540 mg, 95%); $[\alpha]_D^{24}$ −16° (*c* 0.39, chloroform); δ_H: 2.06 (s, 3 H, Ac), 2.67 (d, 1 H, *J* 10.74 Hz, O*H*-4'), 3.33 (t, 1 H, *J* 9.77 Hz, H-3), 3.41–3.50 (m, 1 H, H-5), 3.51 (s, 3 H, OMe), 3.55 (s, 3 H, CO_2CH_3),

3.51–3.56 (m, 1 H, H-2), 3.88–3.91 (1 H, H-3'), 3.93 (t, 1 H, *J* 9.77 Hz, H-4), 4.04–4.08 (1 H, H-4'), 4.18 (d, 1 H, *J* 7.82 Hz, H-1), 4.29 (dd, 1 H, *J* 4.16 and 12.46 Hz, H-6a), 4.49 (dd, 1 H, *J* 2.20 and 12.46 Hz, H-6b), 4.66–4.80 (4 H, 2 OC*H*$_2$Ph), 4.98 (d, 1 H, *J* 2.44 Hz, H-5'), 5.13 (broad s, 1 H, H-2'), and 5.26 (broad s, 1 H, H-1').

Anal. Calc. for $C_{37}H_{41}N_3O_{13}\cdot 1.5\ H_2O$: C, 58.26; H, 5.81; N, 5.51. Found: C, 57.97; H, 5.46; N, 5.07.

O-*(Methyl 2,3-di*-O-*benzyl-4*-O-*chloroacetyl-β*-D-*glucopyranosyluronate)-(1→4) - 1,3,6-tri*-O-*acetyl-2-azido-2-deoxy-α*-D-*glucopyranose* (**41**). — A mixture of **40** (ref. 9; 5.30 g, 7.84 mmol) and trifluoroacetic acid (5 mL) in acetic anhydride (60 mL) was stirred for 2 days at room temperature, and evaporated. The residual syrup was chromatographed on silica gel, with 11:1 (v/v) toluene–ethyl acetate as the eluant, to give **41** (4.27 g, 70%); m.p. 138.5–139.5° (from ethyl acetate–hexane, $[\alpha]_D^{23}$ +38.3° (*c* 0.30, chloroform); δ_H: 2.04 (s, 3 H, Ac), 2.22 (s, 6 H, 2 Ac), 3.48 (dd, 1 H, *J* 7.82 and 9.03 Hz, H-2'), 3.54 (dd, 1 H, *J* 3.66 and 10.74 Hz, H-2), 3.65 (dd, 1 H, *J* 9.03 and 10.01 Hz, H-3'), 3.65–3.81 (4 H, H-4,5, ClC*H*$_2$CO), 3.71 (s, 3 H, CO_2CH_3), 3.84 (d, 1 H, *J* 10.01 Hz, H-5'), 4.21 (dd, 1 H, *J* 3.66 and 12.21 Hz, H-6a), 4.33 (d, 1 H, *J* 7.81 Hz, H-1'), 4.36 (dd, 1 H, *J* 1.72 and 12.21 Hz, H-6b), 5.08 (t, 1 H, *J* 10.01 Hz, H-4'), 5.45 (dd, 1 H, *J* 8.79 and 10.74 Hz, H-3), and 6.23 (d, 1 H, *J* 3.66 Hz, H-1).

Anal. Calc. for $C_{35}H_{40}ClN_3O_{15}\cdot 0.5\ H_2O$: C, 53.40; H, 5.25; Cl, 4.50; N, 5.34. Found: C, 53.49; H, 5.16; Cl, 4.41; N, 5.41.

Methyl O-*(methyl 2,3-di*-O-*benzyl-β*-D-*glucopyranosyluronate)-(1→4)*-O-*(3,6-di*-O-*acetyl-2-azido-2-deoxy-α*-D-*glucopyranosyl)-(1→4)*-O-*(methyl 2*-O-*benzoyl-3*-O-*benzyl-α*-L-*idopyranosyluronate)-(1→4)-6*-O-*acetyl-2-azido-3*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**44**). — To a cooled solution of **41** (2.01 g, 2.58 mmol) in dichloromethane (30 mL) was added dropwise a solution of titanium tetrabromide (2.06 g, 5.61 mmol) in ethyl acetate (30 mL) at 0–5°, and the mixture was stirred for 10 h at room temperature, poured into ice–water, and extracted with ethyl acetate. The extracts were combined, successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 20:1 (v/v) dichloromethane–ethyl acetate[4c] as the eluant, to give syrupy **42** (1.40 g, 67.9%); this was employed for the next step without characterization. A mixture of **39** (540 mg, 0.73 mmol), the bromide **42** (1.40 g, 1.75 mmol), and molecular sieves 4A (1.2 g) in 1,2-dichloroethane (30 mL) was stirred for 30 min at room temperature under an argon atmosphere, and then cooled to −15°. To the mixture were added silver trifluoromethanesulfonate (540 mg, 2.1 mmol) and 2,4,6-collidine (276 mg, 2.3 mmol; 0.3 mL), and the mixture was stirred for 24 h at −15°, diluted with dichloromethane, and filtered. The filtrate was successively washed with cold dilute sulfuric acid, aqueous sodium hydrogencarbonate, and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 10:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **43** (517 mg, 49%); δ_H: 1.99 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.16 (s, 3 H, Ac),

3.22 (dd, 1 H, *J* 3.41 and 10.76 Hz, H-2″), 3.49 (s, 3 H, OMe), 3.69 (s, 3 H, CO_2CH_3), 3.72 (s, 3 H, CO_2CH_3), 4.35 (d, 1 H, *J* 7.82 Hz, H-1), 5.06 (t, 1 H, *J* 10.01 Hz, H-4‴), 5.12 (d, 1 H, *J* 3.41 Hz, H-1″), 5.21 (distorted t, 1 H, *J* 6.35 Hz, H-2′), 5.39 (dd, 1 H, *J* 9.28 and 10.75 Hz, H-3″), and 5.61 (d, 1 H, *J* 5.86 Hz, H-1′).

A mixture of **43** (517 mg, 0.35 mmol) and thiourea (78.8 mg, 1.04 mmol) in oxolane (15 mL) and ethanol (15 mL) was refluxed for 15 h, cooled, poured into ice–water, and extracted with chloroform. The extracts were combined, successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 7:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **44** (360 mg, 74%; 36% overall yield from **39**); $[\alpha]_D^{21}$ +14.3° (*c* 0.44, chloroform); δ_H: 1.98 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 3.23 (dd, 1 H, *J* 3.42 and 10.74 Hz, H-2″), 3.48 (s, 3 H, OMe), 3.72 (s, 3 H, CO_2CH_3), 3.78 (s, 3 H, CO_2CH_3), 5.12 (d, 1 H, *J* 3.41 Hz, H-1″), 5.22 (distorted t, 1 H, *J* 6.60 Hz, H-2′), 5.37 (dd, 1 H, *J* 9.28 and 10.74 Hz, H-3″), and 5.62 (d, 1 H, *J* 6.10 Hz, H-1′).

Anal. Calc. for $C_{68}H_{76}N_6O_{25}$: C, 59.30; H, 5.56; N, 6.10. Found: C, 59.19; H, 5.56; N, 5.63.

Methyl O-*(6-*O-*acetyl-2-azido-3,4-di-*O-*benzyl-2-deoxy-*α-D-*glucopyranosyl)-(1→4)-*O-*(methyl 2,3-di-*O-*benzyl-*β-D-*glucopyranosyluronate)-(1→4)-*O-*(3,6-di-*O-*acetyl-2-azido-2-deoxy-*α-D-*glucopyranosyl)-(1→4)-*O-*(methyl 2-*O-*benzoyl-3-*O-*benzyl-*α-L-*idopyranosyluronate)-(1→4)-6-*O-*acetyl-2-azido-3-*O-*benzyl-2-deoxy-*β-D-*glucopyranoside* (**5**). — A mixture of **44** (360 mg, 0.26 mmol), silver trifluoromethanesulfonate (621 mg, 2.42 mmol), molecular sieves 4A (1.5 g), and 2,4,6-collidine (351 mg, 2.89 mmol, 0.8 mL) in 1,2-dichloroethane (10 mL) was stirred for 10 min at room temperature under an argon atmosphere, and then cooled to −15°. To the mixture was added a solution of **45** (ref. 19; 600 mg, 1.22 mmol) in 1,2-dichlorothane (5 mL) and the the mixture was stirred for 40 min at −15°, diluted with dichloromethane, and filtered. The filtrate was successively washed with aqueous sodium hydrogencarbonate and water, dried, and evaporated. The residual syrup was chromatographed on silica gel, with 11:1 (v/v) toluene–ethyl acetate as the eluant, to give amorphous, powdery **5** (357 mg, 77%); $[\alpha]_D^{20}$ +32.2° (*c* 0.13, chloroform); δ_H: 2.01 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 3.22 (dd, 1 H, *J* 3.42 and 10.99 Hz, H-2″), 3.26 (dd, 1 H, *J* 3.66 and 10.50 Hz, H-2‴), 3.48 (s, 3 H, OMe), 3.70 (s, 3 H, CO_2CH_3), 3.74 (s, 3 H, CO_2CH_3), 4.34 (d, 1 H, *J* 7.81 Hz, H-1), 5.12 (d, 1 H, *J* 3.42 Hz, H-1″), 5.21 (distorted t, 1 H, *J* 6.59 Hz, H-2′), 5.36 (t, 1 H, *J* 10.49 Hz, H-3″), 5.50 (d, 1 H, *J* 3.66 Hz, H-1⁗), and 5.61 (d, 1 H, *J* 5.86 Hz, H-1′).

Anal. Calc. for $C_{90}H_{99}N_9O_{30}\cdot H_2O$: C, 59.30; H, 5.69; N, 6.92. Found: C, 59.52; H, 5.56; N, 6.97.

Further elution gave amorphous, powdery pentasaccharide, the β anomer **46** (55 mg, 15%); $[\alpha]_D^{19}$ +16.0° (*c* 0.25, chloroform); δ_H: 1.81 (s, 3 H, Ac), 1.99 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 3.21 (dd, 1 H, *J* 3.42 and 10.75 Hz, H-2″), 3.48 (s, 3 H, OMe), 3.71 (s, 3 H, CO_2CH_3), 3.83 (s, 3 H, CO_2CH_3), 4.34 (d, 2 H, *J*

8.06 Hz, H-1,1‴), 5.10 (d, 1 H, *J* 3.42 Hz, H-1″), 5.21 (distorted t, 1 H, *J* 6.35 Hz, H-2′), 5.34 (dd, 1 H, *J* 9.28 and 10.75 Hz, H-3″), and 5.62 (d, 1 H, *J* 6.14 Hz, H-1′).

Anal. Calc. for $C_{90}H_{99}N_9O_{30}\cdot H_2O$: C, 59.89; H, 5.64; N, 6.98. Found: C, 59.81; H, 5.54; N, 6.73.

Methyl 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)-2-azido-3*-O-*benzyl-2-deoxy-6*-O-*sulfo*-β-D-*glucopyranoside, pentasodium salt* (**48**). — To a cooled solution of **5** (430 mg, 0.24 mmol) in chloroform (10 mL), methanol (30 mL), and water (5 mL) was added 5N sodium hydroxide [4c] (5 mL, 25 mmol) at 0–5°, and the the mixture was stirred for 4 h at room temperature, made neutral by addition of Dowex 50W-X8 (K^+) resin, the suspension filtered, and the filtrate evaporated *in vacuo*. The residue was re-esterified with ethereal diazomethane in methanol and the solution evaporated. The residual syrup was chromatographed on silica gel, with 140:60:3 (v/v) chloroform–ethyl acetate–methanol as the eluant, to give sticky **47** (210 mg, 58%). This compound showed no acyl peak in its ^{1}H-n.m.r. spectrum; δ_H (CD_3OD): 3.27 (s, 3 H), 3.54 (s, 3 H), 3.74 (s, 3 H), 4.27 (d, 1 H, *J* 7.57 Hz, H-1), 5.13 (d, 1 H, *J* 3.66 Hz, H-1″), 5.25 (d, 1 H, *J* 4.41 Hz, H-1″), and 5.52 (d, 1 H, *J* 3.67 Hz, H-1‴).

Sulfur trioxide–trimethylamine complex (243 mg, 1.74 mmol) was added to a solution of **47** (210 mg, 0.14 mmol) in *N,N*-dimethylformamide (3.5 mL), and the mixture was stirred for 20 h at 50–55°, and cooled; methanol (1 mL) was added, the mixture was applied to a column (1.5 × 75 cm) of Sephadex LH-20 equilibrated with 1:1 (v/v) chloroform–methanol, and eluted with the same solvent. The product was chromatographed on silica gel, with 4:1 (v/v) chloroform–methanol as the eluant, to give a pure fraction. This was passed through a column (1.5 × 30 cm) of SP-Sephadex C-25 (Na^+) equilibrated with 9:1 (v/v) methanol–water, and using the same solvents, to give sticky **48** (230 mg, 80%); $[\alpha]_D^{25}$ +11° (*c* 0.21, methanol); δ_H (CD_3OD): 3.31 (s, 3 H), 3.36 (dd, 1 H, *J* 3.42 and 10.26 Hz, H-2‴), 3.43 (dd, 1 H, *J* 3.90 and 10.25 Hz, H-2″), 3.54 (s, 3 H), 3.81 (s, 3 H), 5.18 (d, 1 H, *J* 3.90 Hz, H-1″), 5.37 (broad s, 1 H, H-1′), and 5.51 (d, 1 H, *J* 3.42 Hz, H-1‴).

Anal. Calc. for $C_{77}H_{84}N_9Na_5O_{40}S_5\cdot 6H_2O$: C, 42.84; H, 4.48; N, 5.84; S, 7.43. Found: C, 43.20; H, 4.57; N, 5.55; S, 7.38.

Methyl O-*(2-deoxy-2-sulfamido-6*-O-*sulfo*-α-D-*glucopyranosyl)-(1→4)*-O-*(*β-D-*glucopyranosyluronic acid)-(1→4)*-O-*(2-deoxy-2-sulfamido-3,6-di*-O-*sulfo*-α-D-*glucopyranosyl)-(1→4)*-O-*(2*-O-*sulfo*-α-L-*idopyranosyluronic acid)-(1→4)-2-deoxy-2-sulfamido-6*-O-*sulfo*-β-D-*glucopyranoside, decasodium salt* (**3**). — A mixture of **48** (187 mg, 0.09 mmol) and 10% Pd–C (140 mg) in methanol (20 mL) and water (5 mL) was stirred under a hydrogen atmosphere for 3 days at room temperature, filtered, and the filtrate evaporated *in vacuo*. The residue was re-hydrogenated, in the presence of fresh catalyst (150 mg), for 3 days at room temperature, filtered, and the filtrate evaporated. The residue was hydrogenated once more over fresh catalyst in water (20 mL) for 3 days at room temperature. The suspension was filtered by

using a millipore filter (0.22 μm; MILLEX-GS, Millipore Corporation), and the filtrate was evaporated *in vacuo*. The pH of a solution of the residue (74 mg, 53 μmol) in water (15 mL) was adjusted to 9.5 by addition of 0.5M sodium hydroxide. Sulfur trioxide-trimethylamine complex (55 mg, 0.39 mmol) was added, and the mixture was stirred for 10 days at room temperature, the pH being maintained at 9.5–10.0 by appropriate addition of 0.5M sodium hydroxide. During this time, more sulfur trioxide-trimethylamine complex (672 mg, 4.83 mmol) was added to the mixture in seven portions. Aqueous sodium hydroxide (3 M solution) was added to pH 12, and the mixture was stirred for 6 h at room temperature. After neutralization to pH 7.5 by addition of M hydrochloric acid, the mixture was evaporated *in vacuo*. The residue was applied to a column (1.4 × 96 cm) of Sephadex G-25 equilibrated with 0.2M sodium chloride and eluted with the same solvent[4c]. The sugar fraction was eluted from a column (1.4 × 13 cm) of Dowex AG1-X2 ion-exchange resin equilibrated with 0.5M sodium chloride by using a gradient of sodium chloride[4c] (0.5 → 3M). The pure fraction was desalted on a column (1.4 × 55 cm) of Sephadex G-25, using water, to give a pentasaccharide fraction which was eluted from a column (1.2 × 25 cm) of SP-Sephadex C-25 (Na^+) with water. The pentasaccharide fractions were combined and freeze-dried, to give amorphous, white, powdery **3** (25 mg, 16%); $[\alpha]_D^{20}$ +23° (*c* 0.07, water); δ_H (D_2O, 20 or 50°, external Me_4Si): 3.02 (dd, 1 H, *J* 8.30 and 9.28 Hz, H-2), 3.26 (dd, 1 H, *J* 3.91 and 10.01 Hz, H-2''''), 3.44 (broad t, 1 H, *J* 9.01 Hz, H-2'''), 3.47 (dd, 1 H, *J* 3.42 and 10.50 Hz, H-2''), 3.54 (s, 3 H, OMe), 3.58 (t, 1 H, *J* 9.28 Hz, H-4''''), 3.63 (dd, 1 H, *J* 9.28 and 10.01 Hz, H-3''''), 3.97 (t, 1 H, *J* 9.28 Hz, H-4''), 4.11 (broad d, 1 H, *J* 9.77 Hz, H-5''), 4.18–4.22 (H-2'), 4.36–4.41 (H-3''), 4.45 (d, 1 H, *J* 8.30 Hz, H-1), 4.51 (d, 1 H, *J* 7.82 Hz, H-1'''), 4.85 (d, 1 H, *J* 3.17 Hz, H-5'), 5.19 (d, 1 H, *J* 3.90 Hz, H-1'), 5.50 (d, 1 H, *J* 3.42 Hz, H-1''), and 5.59 (d, 1 H, *J* 3.91 Hz, H-1''''); δ_C (D_2O): 58.00 (OMe), 57.48,

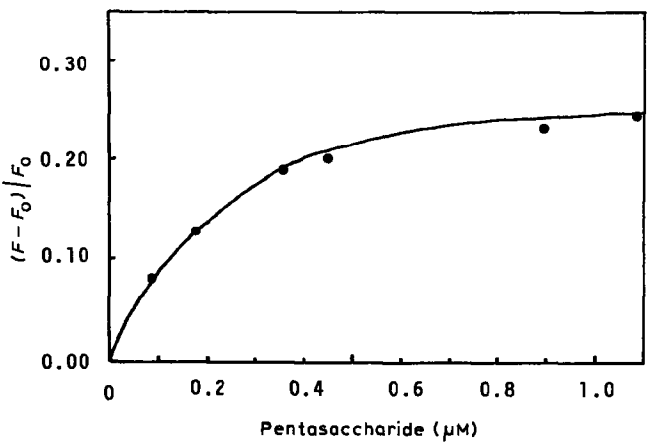

Fig. 2. Changes in fluorescence difference intensities of antithrombin III (AT III) at various concentrations of pentasaccharide **3**, where F_0 and F are, respectively, the fluorescence intensities of AT III alone, and of AT III in the presence of **3**.

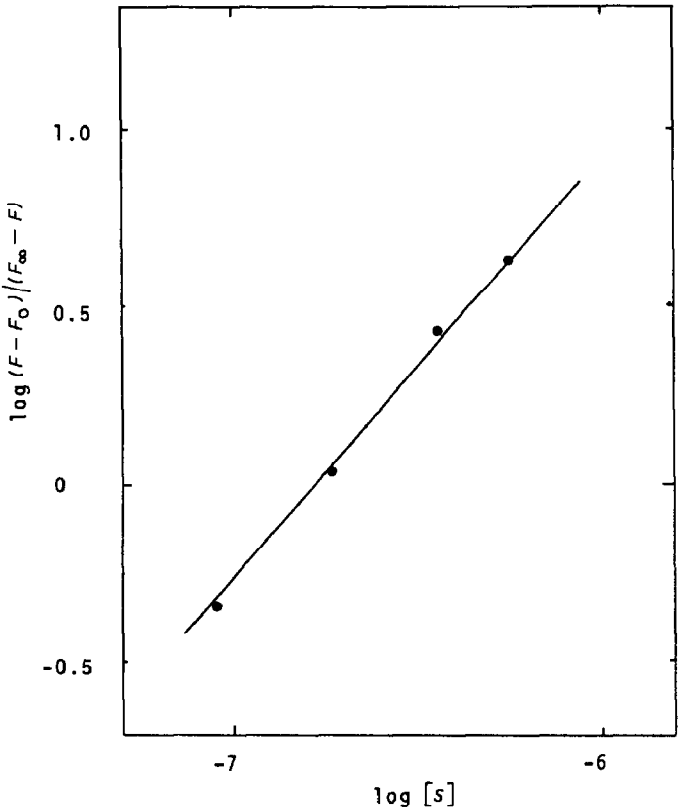

Fig. 3. Plot of log $(F - F_0)/(F_\infty - F)$ *versus* log [*S*].

58.76, and 60.80 (C-2, 2″, 2‴″), 66.77, 67.08, and 67.47 (C-6, 6″, 6‴″), 97.28, 98.64, 100.21, 102.04, and 103.51 (C-1, 1′, 1″, 1‴, 1‴″).

Anal. Calc. for $C_{31}H_{43}N_3Na_{10}O_{49}S_8 \cdot 13\ H_2O$: C, 18.98; H, 3.54; N, 2.14. Found: C, 18.70; H, 3.30; N, 2.43.

Fluorescence measurement. — Fluorescence measurements were made with a Hitachi type 650-60 fluorescence spectrophotometer equipped with a recorder. Correction of emission spectra with Rhodamine B, and measurements of fluorescence difference spectra, were performed with a microcomputer attached to the fluorescence spectrophotometer. A synthetic oliogosaccharide at a concentration of 200μM was added by a syringe to 2.2 mL of human antithrombin III (AT III; commercially available from Boehringer-Mannheim Yamanouchi Corporation) at a concentration of 100nM in 0.01 M 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), pH 7.5, and 0.15M sodium chloride[6c] at 20°. The wavelengths of excitation and emission were 280 and 330 nm, respectively.

The intensity difference $(F - F_0)$ in different spectra depends on the pentasaccharide (**3**) concentration, and plots of $(F - F_0)/F_0$ against the concentration of **3** were hyperbolic, as shown in Fig. 2, where F_0 and F are, respectively, the intensity

of AT III alone and of AT III in the presence of **3**. A plot according to the equation[26] $\log (F - F_0)/(F_\infty - F) = \log [S] + \log K_a$ was linear, with a slope of ~ 1, as shown in Fig. 3, where K_a is the association constant of the AT III–pentasaccharide **3** complex, $[S]$ is the concentration of **3**, and F_∞ is the maximum intensity when all of the AT III is in the form of a complex with **3**. The K_a value of **3** was calculated to be 6.0×10^6 M^{-1}.

ACKNOWLEDGMENTS

We express our gratitude to Profs. N. Seno and I. Matsumoto and Dr. H. Ogawa of Ochanomizu University for their great help in measuring the association constants. We are grateful to Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the ^{1}H- and ^{13}C-n.m.r. spectra, and Mr. Y. Esumi for measuring the s.i. and f.d. mass spectra. We thank Dr. S. Matsunaga of the Univ. of Tokyo and Mrs. S. Shimada of Snow Brand Milk Products Co., Ltd., for measuring the f.a.b.-mass spectra. We also thank Dr. H. Homma and his collaborators for the elemental analyses.

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