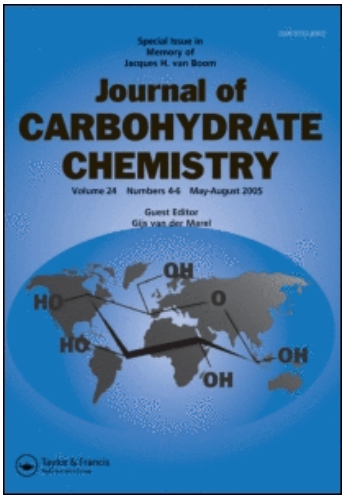

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Synthesis of a Pentasaccharide Corresponding to the Antithrombin III Binding Fragment of Heparin

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SYNTHESIS OF A PENTASACCHARIDE CORRESPONDING TO THE ANTITHROMBIN III BINDING FRAGMENT OF HEPARIN.

C.A.A. van Boeckel, T. Beetz, J.N. Vos, A.J.M. de Jong, S.F. van Aelst, R.H. van den Bosch, J.M.R. Mertens and F.A. van der Vlugt.

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ABSTRACT

The synthesis of a protected pentasaccharide 27b corresponding to the antithrombin III binding region of heparin is presented. This pentasaccharide was prepared from two disaccharides (12c and 23) and a monosaccharide (1). The glucuronic acid containing disaccharide 12c was prepared from easily available monomers 6 and 7. Oxidation to the uronic acid was performed in the disaccharide stage. L-Idose derivative 16, prepared via a new route, was coupled with 1,6-anhydro derivative 17, oxidized and transformed into disaccharide 23. Coupling of 12c and 23 to tetrasaccharide 24a has been investigated. Better yields were obtained without collidine, the reason for which is explained. Coupling of 24b and 1 afforded the pentasaccharide 27b, protected with acetyl at the positions to be sulphated, benzyl at the other hydroxyl functions and azide at the 2-position of the glucosamine residues. Conversion of 27b into the sulphated pentasaccharide Ib can be performed according to published procedures.

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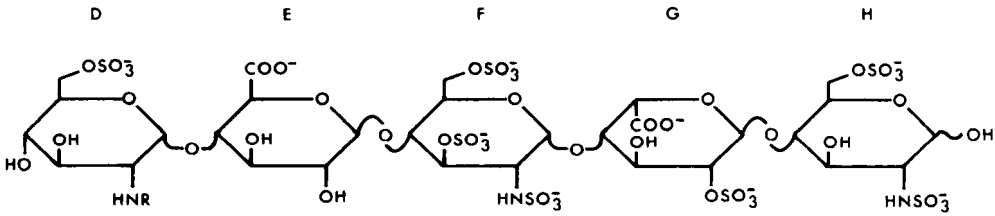
INTRODUCTION

Heparin, which is an important drug in anticoagulant therapy, consists of a mixture of sulphated glycosaminoglycans. About 35% of this mixture binds with antithrombin III (AT III), thereby accelerating deactivation of serine proteases in the coagulation cascade. It has been shown that a unique pentasaccharide fragment of heparin is involved in the AT III binding. The structure of this pentasaccharide has been elucidated and found to be Ia (Fig.1).[1,2,3] A minor variant of this structure has recently been synthesized by Sinaÿ and Petitou et al. (i.e. Ib).[4,5] This structural difference does not affect the binding with AT III. Also Lindahl et al.[6] showed that the N-acetyl group at unit D is not essential for interaction with AT III.

In this communication we wish to present an alternative synthesis of pentasaccharide Ib.

RESULTS AND DISCUSSION

The synthetic approach of Sinaÿ and Petitou[4,5] has been based on coupling of protected uronic acids and glucosamine monomers 1-5 to give fully protected pentasaccharide 27a (see upper part of Fig.2).



I a) R= $-COCH_3$

b) R= $-SO_3^-$

Fig. 1

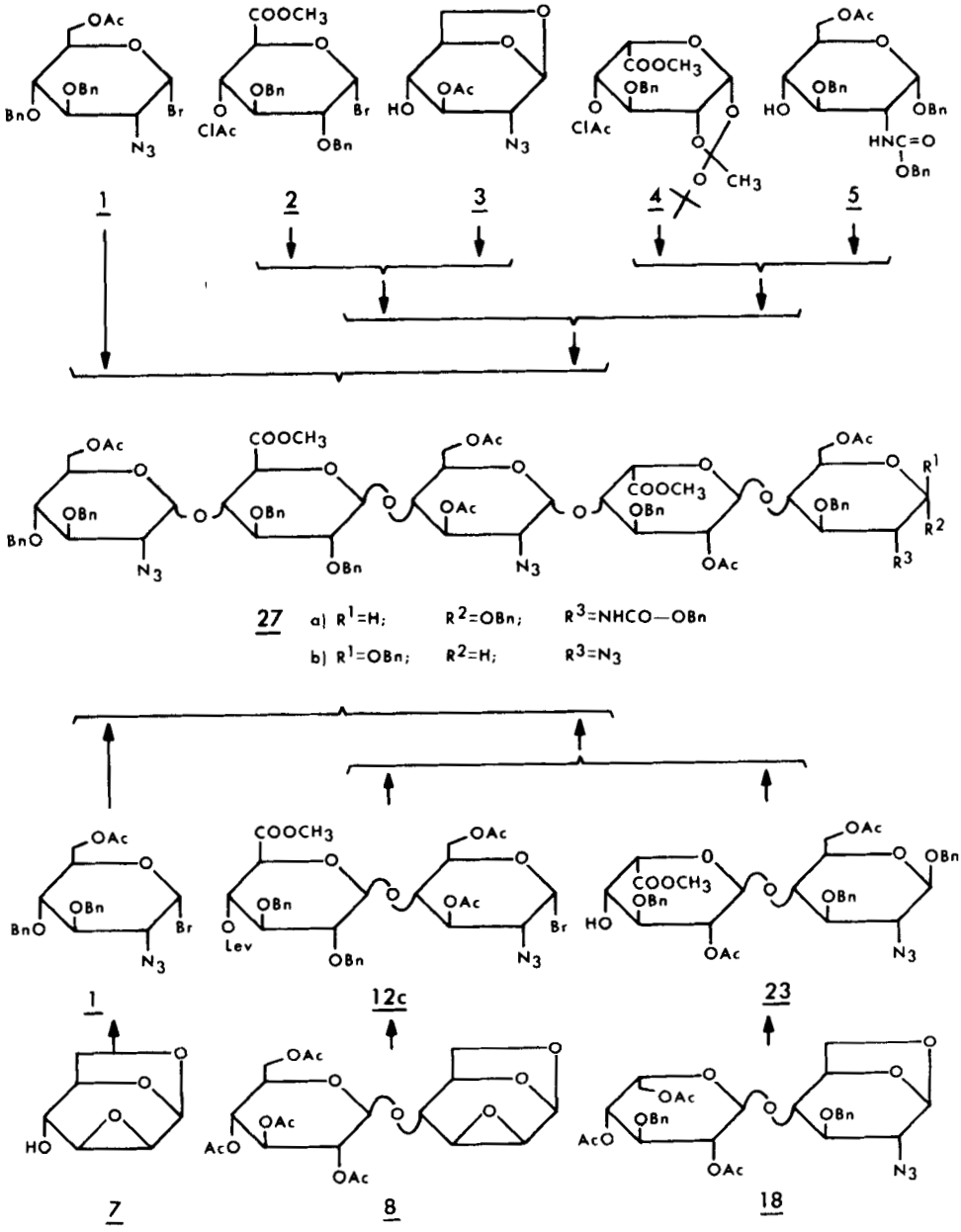


Fig. 2

In this strategy they selected acetyl protective groups for the sulphated hydroxyl functions and benzyl groups for the free hydroxyl functions. The ß-glycosidic linkage between 2 and 3 was introduced by taking advantage of the insoluble promoter silver carbonate.[7] The α-L-iduronic linkage was formed via acid catalyzed coupling [7] of orthoester 4 with 5. The introduction of the two α-glucosamine linkages required non participating azide[8] functions at units 1 and 3 and reactive silver triflate,[8] because non-reactive 4-hydroxyl functions had to be coupled. Protected heparin fragment 27a was converted into Ib by deprotection and sulphation procedures. However, a drawback of this synthesis is the laborious preparation of 2 and 4 followed by coupling reactions with low efficiency (50 and 40% yield respectively).

It is of advantage to perform these difficult coupling reactions in an earlier stage with simple building blocks. As a consequence such a strategy demands oxidation and other modifications to be performed at the stage of the disaccharides.[9] Our strategy, based upon this approach, is depicted in the lower part of Fig.2. Thus, the simple disaccharides 8 and 18 were easily obtained and smoothly converted into building blocks 12c and 23, respectively. Moreover, an additional advantage appeared to be the occurrence of many crystalline intermediates and the considerable reduction of silica column chromatography, which enabled us to prepare (on lab-scale) disaccharide building blocks in large quantities (see Experimental Part).

In our approach the three glucosamine units were derived from 1,6-anhydro derivatives 7, 8 and 18, which on acetolysis afford the corresponding 6-O-acetyl protected compounds.Because the 6-O-functions have to

be sulphated, it is most convenient to select also acetyl protection for the remaining hydroxyl functions to be sulphated. Coupling of saccharides 1, 12c and 23 gives pentasaccharide 27b, which shows resemblance with 27a, obtained by Sinaÿ and Petitou.[4,5]

Preparation of ß-D-glucuronic acid (1-4) -D-glucosamine disaccharide 12c

The synthesis of disaccharide 12c is illustrated in Fig.3. The ß-glycosidic linkage of 8 was introduced between the easily available monomers 6[10] and 7[11] under modified Koenigs-Knorr conditions.[12] The crystalline disaccharide 8 was converted into 9 (yield 70%) in three steps:

(a) KOtBu

(b) 2,2-dimethoxypropane/pTSOH/DMF.[13]

(c) BnBr/NaH/THF/Bu_4NI.[14]

The epoxide 9 was opened by lithium azide treatment to give 10a, which was acetylated and treated with aqueous acetic acid to afford 10b. Selective oxidation[15] of 10b with Pt/O_2 in water (pH 8-9) at elevated temperature failed, because of acetyl hydrolysis. However, selective oxidation of the 6'-hydroxyl function was possible when the 3-O-acetyl group of 10b was replaced by a 3-O-methoxymethyl (MOM) function. In this case, after methylation, the yield of 11a was 55 %. On large scale however, it proved to be more convenient to use a non-selective oxidation procedure. Thus, 10b was selectively protected at its 6'-hydroxyl group by 4,4'-dimethoxytritylchloride[16] treatment in pyridine. Crude 10c was levulinoylated[17] with levulinic acid

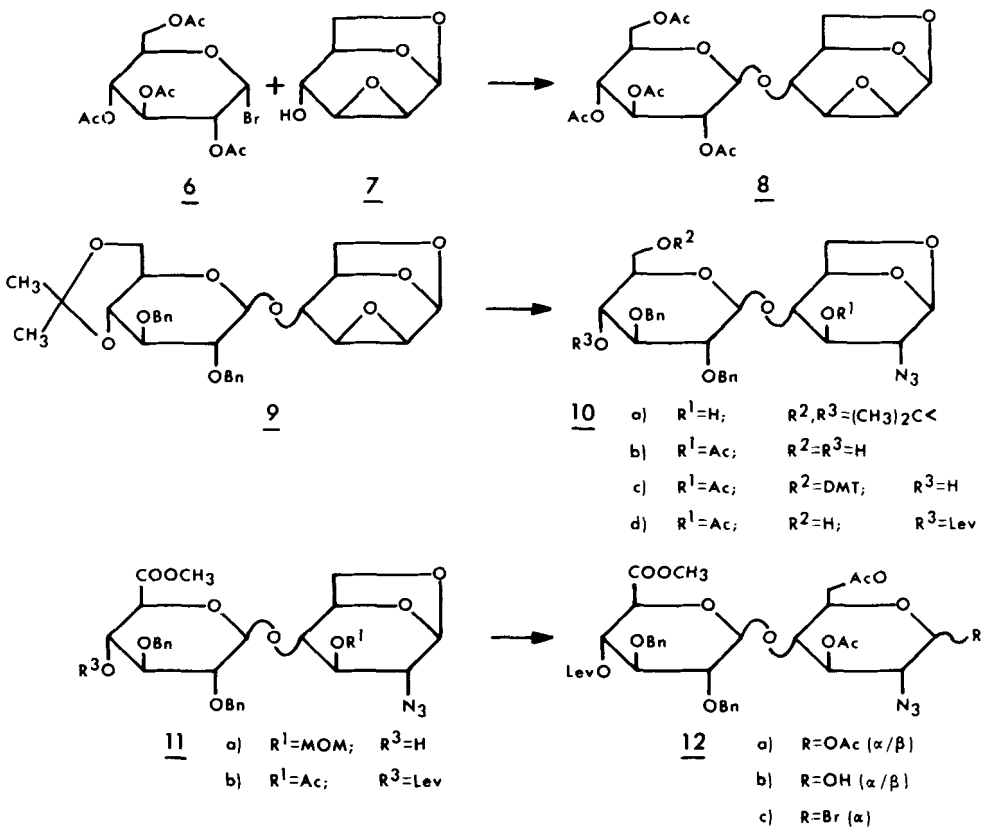


Fig. 3

anhydride in pyridine, in the presence of 4-N,N-dimethylaminopyridine (DMAP). Cleavage of the 4,4'-dimethoxytrityl group in aqueous acetic acid gave, after purification by column chromatography, pure 10d in 71% overall yield from 10b.

Compound 10d was oxidized with chromium(VI) oxide at low temperature[18] and the carboxylic acid function obtained was methylated with diazomethane or by the method described by Rao et al[19] to give 11b. Compound 11b was then converted into the required building block

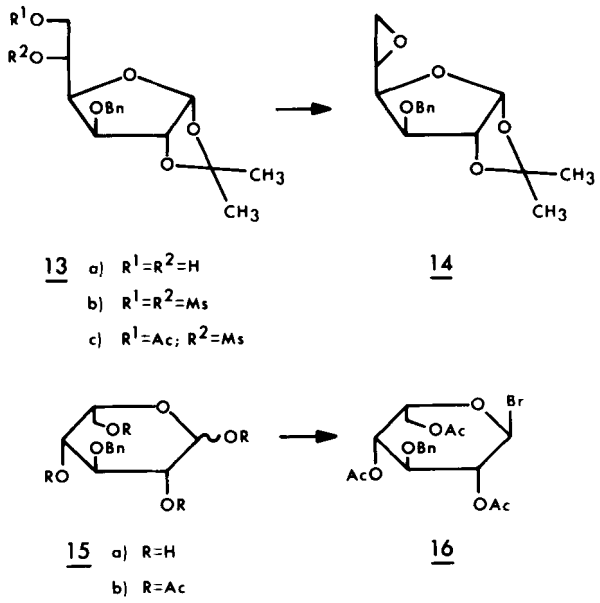


Fig. 4

12c (yield 92%) by a three step procedure:
(a) Ac_2O/TFA (12a).[7b]
(b) piperidine/THF (12b).[20]
(c) oxalyl bromide/$CHCl_3$/DMF (12c).[21]

Preparation of α-L-iduronic acid (1-4)-D-glucosamine disaccharide 23

The synthesis of disaccharide 23 is illustrated in Fig. 4 and 5. For the synthesis of 23 we required an L-idopyranosyl building block (i.e. 16).
Because L-idose derivatives are not commercially available we devised a synthetic route to 16 from D-glucose. Thus, 3-O-benzyl-1,2-O-isopropylidene-α-D-glucofuranose 13a[22] was mesylated[23] to give 13b. The primary mesylate group of 13b was selectively substituted to 13c by potassium acetate in the presence

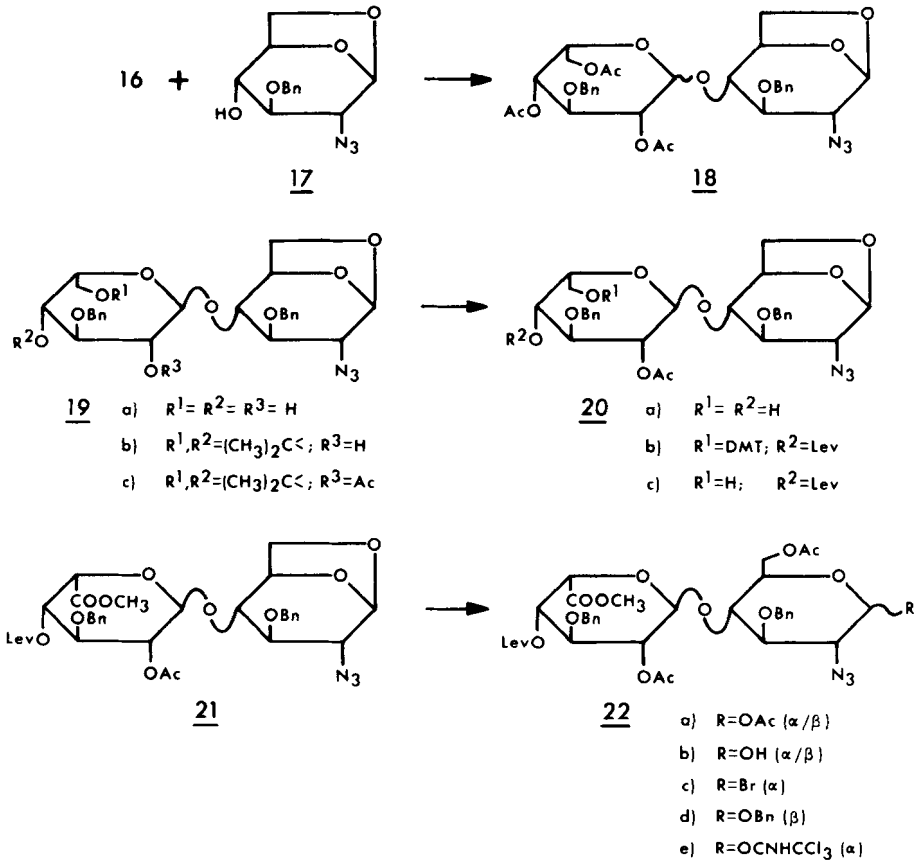


Fig. 5

of crown-ether. Potassium t-butoxide treatment of 13c afforded 14,[22] which possesses the L-idose configuration.

The epoxide opening of 14 was performed with acid to give a diol with retention of configuration. Under these conditions the isopropylidene group was removed simultaneously to yield 15a. Acetylation of 15a gives an α/β mixture of 15b, which was purified by column chromatography. This mixture was transformed into the bromide 16 by the action of titanium tetrabromide.[24] Glycon 16 was coupled with aglycon 17[25] under modified

Koenigs-Knorr conditions to give disaccharide 18 as an α/β mixture in a ratio of 7/1. The α/β mixture of 18 was deacylated to give, after purification by column chromatography, pure α-anomer 19a. Compound 19a was converted into 20a (87% yield) in three steps:

(a) 2,2-dimethoxypropane/ pTSOH/ DMF (19b).[13]

(b) Ac_2O/pyridine (19c).

(c) AcOH/water (20a).

Compound 20a was protected and oxidized as described for 10d and 11b to give L-iduronic acid containing disaccharide 21 (yield 49%) in five steps:

(a) 4,4'-dimethoxytritylchloride/THF/pyridine.

(b) levulinic acid anhydride/pyridine/DMAP (20b).

(c) AcOH/water (20c).

(d) CrO_3/acetone/H_2SO_4.

(e) CH_2N_2/CH_2Cl_2 (21).

The structure and purity of 21 was confirmed by ^1H and ^{13}C-NMR. The α-L-idose configuration was unambiguously proved by 2D-COSY ^1H-NMR and by measuring C-H coupling constants (see Experimental Part).

Compound 21 was converted into bromide derivative 22c (yield 93%) by procedures as described for 12b and 12c:

(a) Ac_2O/TFA (22a).

(b) piperidine/THF (22b).

(c) oxalyl bromide/$CHCl_3$/DMF (22c).

Bromide derivative 22c was coupled with benzyl alcohol at -20°C in the presence of silver silicate[26] to give, after column chromatography, β-benzyl derivative 22d in 60% yield. Apart from bromide derivative 22c we also prepared α-imidate[27] derivative 22e (Cl_3CCN, NaH), which could also be converted into 22d (55% yield). Selective removal of the levulinoyl protective group with NH_2NH_2/HOAc in pyridine at 0°C for 10 min. gave building block 23 in 90% yield.

Synthesis of tetrasaccharide 24a and pentasaccharide 27b

Coupling of 12c with 23 has to be performed with silver triflate[8] as promoter, because aglycon and glycon are both of low reactivity. The reaction was performed in the presence of molecular sieves at -30°C under nitrogen to give crude tetrasaccharide 24a, which was treated with NH_2NH_2/HOAc to give 24b in 52% overall yield (see Fig.6). The analogous tetrasaccharide synthesis of Sinaÿ and Petitou was accomplished in 30% yield. It should be stressed, however, that they used molecular sieves as well as 2,4,6-collidine as acid scavenger. We obtained also a lower coupling yield under the latter conditions and found considerable amounts of hydrolyzed glycon (i.e. 12b).

Initially, we attempted to circumvent hydrolysis of bromide 12c by extensive drying of all reagents and solvents and by performing the reaction under dry nitrogen atmosphere in a glove-box. However, the hydrolyzed glycon was still formed in the same quantity. In our opinion, the formation of 12b is due to collidine substitution at the sulphur of triflate intermediate 25 (see Fig.7), resulting into formation of 26a, which rapidly rearranges to 26b.[28] This side reaction is favoured because of the relative stability of 25 and the low reactivity of the 4'-hydroxyl group of 23. Fortunately we did not observe formation of 12b in the reaction without collidine, thus affirming the disadvantageous effect of the nucleophilic base.[29] On the other hand less effectively scavenging of formed trifluorosulphonic acid led to some debenzylation.[29]

The synthesis of fully protected pentasaccharide 27b (Fig.8) was realized by coupling of excess of 1 with 24b in the presence of silver triflate, 2,4,6-collidine

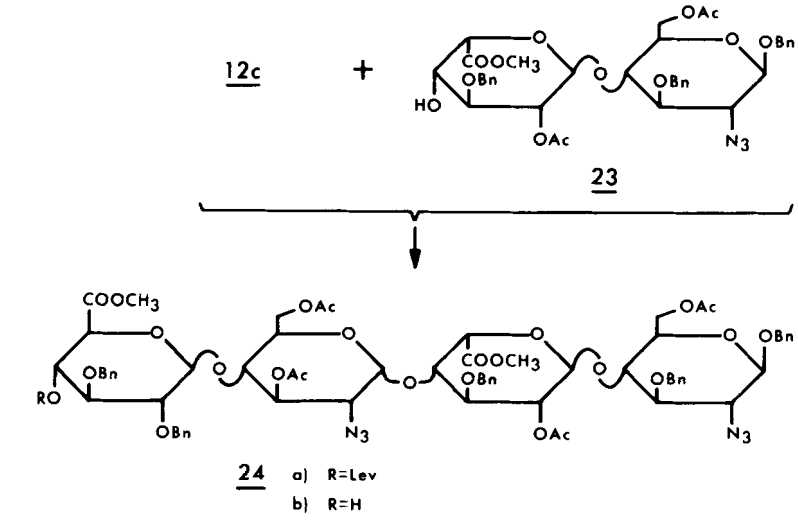


Fig. 6

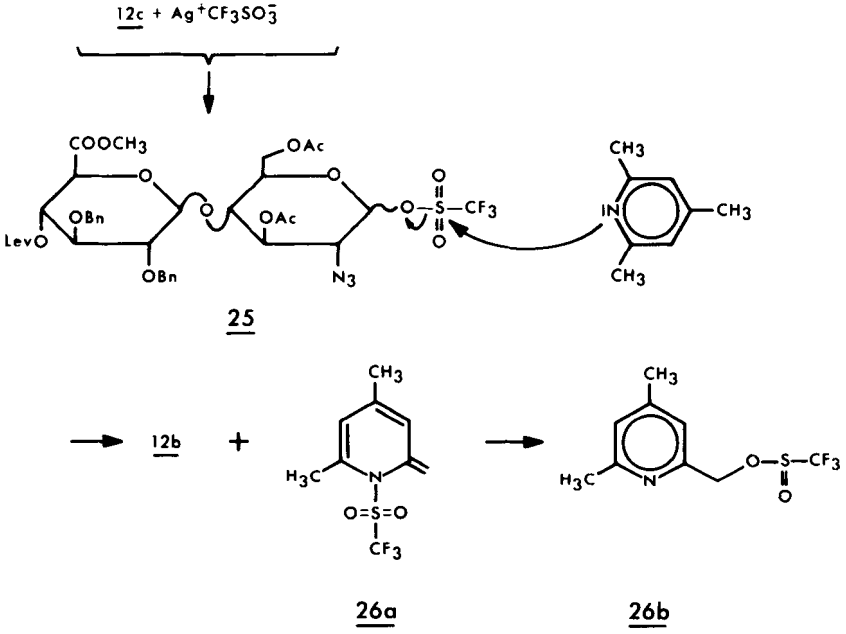


Fig. 7

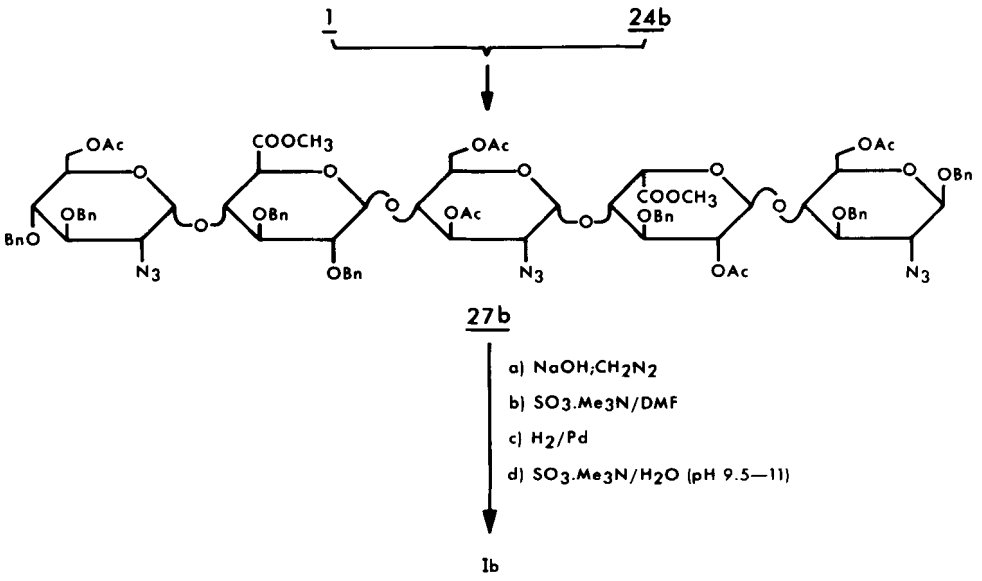


Fig. 8

and molecular sieves to give pentasaccharide 27b in 96% yield, after purification by Sephadex LH-20 chromatography. The structure of compound 27b was confirmed by ^{1}H-NMR and ^{13}C-NMR spectroscopy as well as ^{1}H-^{1}H and ^{13}C-^{1}H scalar COSY NMR spectroscopy. Conversion of 27b into Ib was performed according to the procedures (see Fig.8) of Sinaÿ and Petitou.[4,5]

In conclusion, the synthesis presented here is an improvement of the published one, because the overall yield is higher (0.22% against 0.053%) and fewer chromatographic purifications are necessary.

EXPERIMENTAL

General Procedures. Triethylamine, tetrahydrofuran, acetonitrile and pyridine were dried by heating

with CaH_2 under reflux and then distilled; DMF was stirred with CaH_2 at r.t. and distilled at reduced pressure. Methanol was heated with magnesium and then distilled. Dichloromethane, chloroform, 1,2-dichloroethane and toluene were distilled from P_2O_5; nitromethane was dried with $CaCl_2$. Acetonitrile, pyridine, 1,2-dichloroethane and nitromethane were stored over molecular sieves 4A, methanol over molecular sieves 3A, toluene over sodium-wire and dichloromethane over alumina. Melting points are corrected, optical rotations were recorded at ambient temperature with a Perkin-Elmer 241 polarimeter. TLC analysis was performed on Merck-Fertigplatten (Kieselgel 60 F 254, 5 x 10 cm). Compounds were visualized by spraying with sulphuric acid/ethanol (1/9, v/v). ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Bruker WM-360 spectrometer, equipped with an ASPECT 3000 computer; chemical shifts are given in ppm (δ) relative to TMS as internal reference.

1,6:2,3-Dianhydro-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-mannopyranose. (8) 1,6:2,3-Dianhydro-β-D-mannopyranose 7 (109 g, 757 mmol) was dissolved in dry acetonitrile (1500 ml). To this solution was dropwise added 2,3,4,6-tetra-O-acetyl--α-D-glucopyranosyl bromide 6 (411 g, 1000 mmol) in dry acetonitrile (800 ml). Simultaneously mercury (II) bromide (152 g, 420 mmol) and mercury (II) cyanide (106 g, 420 mmol) were added in portions. The reaction mixture was stirred under a stream of nitrogen for 36 h at -30°C and was then poured into dichloromethane (1500 ml), washed with saturated $NaHCO_3$ solution (1500 ml), KBr solution (3x1000 ml), filtered, dried ($MgSO_4$) and evaporated in vacuo to give a residue (535 g), which was purified by short column chromatography (silica

gel, 5 kg; dichloromethane/acetone 97/3, v/v). Crystallization of the appropriate fraction from ether/acetone (2/1, v/v) afforded pure β-disaccharide 8 (223 g, 62%).
Rf = 0.66 (dichloromethane/acetone; 9/1, v/v)
m.p. 179°C
$[\alpha]_D^{20}$ = + 6.53 (c = 0.75, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.71 (H-1, d, J = 2.9 Hz);4.77 (H-1', d, J= 7.9 Hz)

1,6:2,3-Dianhydro-4-O-(2,3-di-O-benzyl-4,6-O-isopropylidene-β-D-glucopyranosyl)-β-D-mannopyranose. (9)

Compound 8 (208 g, 438 mmol) and a catalytic amount of potassium t-butoxide (208 mg) were dissolved in dry methanol (2000 ml) and stirred at 30-35°C. After 1.5 h TLC revealed complete deacetylation. The solution was neutralized with Dowex 50 WX4 (H form), filtered over hyflo and evaporated to dryness. After coevaporation with toluene 133 g (99%) of deacetylated product was obtained, which was dissolved in DMF (1000 ml). To this solution was added 2,2-dimethoxypropane (266 ml, 2165 mmol) and p-toluenesulphonic acid (370 mg), and the reaction mixture was stirred under nitrogen for 3 days at r.t.. An additional 200 mg and 100 mg of p-toluenesulphonic acid was added after one and two days respectively. After completion of the reaction, 50 ml of water was added and the mixture stirred for 0.5 h. The reaction mixture was made slightly basic with saturated $NaHCO_3$ solution and evaporated to dryness. The residue was dissolved in dichloromethane and washed with brine until neutral, the organic layer was dried ($MgSO_4$) and evaporated to give the isopropylidene compound (131 g, 87%).
Rf = 0.68 (dichloromethane/methanol; 8/2, v/v)
The crude product (131 g, 379 mmol) was dissolved in dry THF (1300 ml) and to this solution was added

benzylbromide (135 ml, 1136 mmol) and tetrabutylammonium iodide (41.5 g).[14] The mixture was stirred in the dark under nitrogen at 50° and sodium hydride (31.6 g, 57.5% dispersion in oil, 757 mmol) was added in 3 h. The mixture was stirred another 20 h at 50°C, cooled in ice after which methanol (40 ml) was carefully added to destroy the excess of sodium hydride. The reaction mixture was evaporated to a small volume, diluted with dichloromethane and poured into water (500 ml). The organic layer was washed with water until neutral, dried ($MgSO_4$) and evaporated to dryness. The crude product was purified by silica gel chromatography to give pure 9 (162 g, 81%).

Rf = 0.58 (dichloromethane/acetone; 93/7, v/v)

m.p. 135

$[\alpha]_D^{20}$ = -21 (c = 0.65, $CHCl_3$)

^{1}H-NMR ($CDCl_3$): 5.72 (H-1, d, 3.0 Hz); 4.60 (H-1', d, 7.9 Hz); 4.43 (H-5, c); 3.92 (H-6'a, dd, J = 10.2, 5.3 Hz); 3.88 (H-4, br); 3.78 (H-6'b, t, 10.2 Hz); 3.72 (H-3', t, J = 9.2 Hz); 3.58 (H-4', t, J = 9.2 Hz); 3.47 (H-2', dd, J = 9.2, 7.9 Hz); 3.35 (H-3, c); 3.24 (H-5', ddd, J = 10.2, 9.2, 5.3 Hz); 1.50, 1.45 (s, 2 x CH_3, isopropylidene).

3-O-Acetyl-1,6-anhydro-2-azido-2-deoxy-4-O-(2,3-di-O-benzyl-ß-D-glucopyranosyl)-ß-D-glucopyranose.(10b)

Compound 9 (132 g, 250 mmol), lithium azide (36.2 g, 740 mmol), 2,4,6-triisopropylbenzenesulphonic acid (78.7 g, 277 mmol) and 2,6-lutidine (32.1 ml, 277 mmol) were dissolved in N,N-dimethylformamide (DMF, 400 ml). This mixture was stirred under nitrogen for 20 h at 100°C; DMF was removed by evaporation in vacuo, the residue dissolved in ethyl acetate (500 ml) and washed with brine (4 x 200 ml). The organic layers were dried ($MgSO_4$), evaporated and the residue purified by

filtration over a thin layer of silica gel to afford pure 10a (156 g, 95%) as an oil, which crystallized in the refrigerator.
Rf = 0.36 (dichloromethane/acetone; 9/1, v/v)
m.p. 99°C
$[\alpha]_D^{20}$ = -29.2 (c = 0.7, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.38 (H-1, s); 4.52 (H-1', d, J = 7.9 Hz); 1.43, 1.51 (2 x CH_3)
Derivative 10a was dissolved in a mixture of pyridine (600 ml) and acetic anhydride (200 ml) and stirred for 16 h at r.t.. After evaporation of the solvents and coevaporation with toluene (3 x 500 ml) in vacuo, the acetylated derivative was isolated in quantitative yield (171 g, 100%).
Rf = 0.69 (dichloromethane/acetone; 9/1, v/v)
^{1}H-NMR ($CDCl_3$): 5.48 (H-1, s); 4.62 (H-1', d, J = 7.9 Hz); 5.25 (H-3, q, J = 1 Hz)
This compound (171 g) was dissolved in a mixture of acetic acid (500 ml) and water (210 ml) and stirred for 16 h at r.t.. Toluene (1000 ml) was added and the solvents were evaporated in vacuo to give compound 10b in quantitative yield (160 g, 100%).
Rf = 0.10 (dichloromethane/acetone; 9/1, v/v)
m.p. 98°C
$[\alpha]_D^{20}$ = + 9.6 (c = 0.8, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.58 (H-1, s); 5.39 (H-3, q, J = 1 Hz)

3-O-Acetyl-1,6-anhydro-2-azido-4-O-(2,3-di-O-benzyl-4-O-levulinoyl-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranose. (10d) A solution of 4,4'-dimethoxytriphenylmethylchloride (131 g, 387 mmol) in THF (600 ml) was added in 2 h to a solution of compound 10b (160 g, 280 mmol) in dry pyridine (1600 ml) at a temperature of 5°C. The reaction mixture was stirred overnight at r.t. and poured into a solution of $NaHCO_3$ (2500 g) in water

(25000 ml). The aqueous solution was extracted with dichloromethane and the combined organic layers were washed with water, dried ($MgSO_4$) and evaporated to dryness to give crude 10c, which was dissolved in dry pyridine (1300 ml). To this solution levulinoyl acid anhydride in THF (0.5 M, 900 ml) was added in 1 h at 0°C. The reaction mixture was stirred overnight at r.t., after which water (600 ml) was added, stirred for 15 min and evaporated to dryness. The crude reaction product was dissolved in acetic acid (3200 ml), and water (1200 ml) was slowly added. The mixture was put in the refrigerator overnight, after which time dichloromethane (5000 ml) was added. The mixture was washed with icewater, cold aq. $NaHCO_3$ solution until basic and cold brine until neutral. The aqueous layers were washed with dichloromethane and the combined organic layers dried ($MgSO_4$) and evaporated to dryness. The crude product was purified by silica gel chromatography to give pure 10d (133 g, 71%).
Rf = 0.44 (dichloromethane/acetone; 95/5, v/v)
$[\alpha]_D^{20}$ = + 28 (c = 1.3, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.58 (H-1, br); 5.36 (H-3, br); 4.89 (H-4, t, J = 9.8 Hz); 2.16, 2.12 (s, 2 x CH_3, levulinoyl, acetyl)

Methyl 3-O-Acetyl-1,6-anhydro-2-azido-2-deoxy-4-O-(2,3-di-O-benzyl-4-O-levulinoyl-β-D-glucopyranosyl-uronate)-β-D-glucopyranoside. (11b) To a solution of compound 10d (15.9 g, 23.7 mmol) in acetone (200 ml) was added at -10°C chromium (VI) oxide (13 g, 130 mmol) dissolved in diluted H_2SO_4 (17 ml, 3.5 M). The reaction mixture was stirred for 24 h at -10°C, when TLC showed nearly complete conversion of 10d into the corresponding carboxylic acid derivative (Rf = 0.55 → 0.18; dichloromethane/methanol; 9/1, v/v). The reaction was

stopped by the addition of methanol (10 ml) and neutralized with potassium acetate, diluted with dichloromethane (300 ml), washed with water (5 x 100 ml), dried ($MgSO_4$) and evaporated in vacuo. Methylation of the derivative was performed on the crude material by adding excess of diazomethane (50 mmol) in dichloromethane. Crude 11b thus obtained, was purified by short column chromatography (200 g silica gel) and the appropriate fractions crystallized to give pure 11b (10.1 g, 61%).

Rf = 0.62 (dichloromethane/acetone; 9/1, v/v)

m.p. 117°C

$[\alpha]_D^{20}$ = - 3.33 (c = 0.7, $CHCl_3$)

^{1}H-NMR ($CDCl_3$): 5.49 (H-1, br); 3.23 (H-2, br); 5.23 (H-3, q, J = 1Hz); 3.64 (H-4, br); 4.58 (H-5, d, J = 5.6 Hz); 4.66 (H-1', c); 3.62-3.68 (H-2';3',c); 5.13 - 5.20 (H-4', c); 3.93 (H-5, d, J = 9.2 Hz); 2.09, 2.15 (s, CH_3,levulinoyl, acetyl); 3.72 (s, $COOCH_3$)

^{13}C-NMR ($CDCl_3$): 100.2 (C-1); 102.8 (C-1')

Methyl 3,6-Di-O-acetyl-2-azido-4-O-(2,3-di-O-benzyl-4-O-levulinoyl-β-D-glucopyranosyluronate)-2-deoxy--α/β-D-glucopyranoside. (12b) Compound 11b (10.1 g, 14.5 mmol) was dissolved in a mixture of acetic anhydride (116 ml), acetic acid (5 ml) and trifluoroacetic acid (16 ml) and stirred for 3 days at 25°C.[7b] The mixture was concentrated to a small volume in vacuo and water (1250 ml) was added. The aqueous mixture was extracted with dichloromethane, the organic layer washed with aq. $NaHCO_3$ solution and water, dried ($MgSO_4$) and evaporated to dryness to give crude 12a, which was dissolved in dry THF (200 ml). To this solution dry piperidine (12 ml) was added.[20] After standing at r.t. for 24 h the anomeric acetyl was completely cleaved. The reaction mixture was evaporated

to dryness, dissoluted in dichloromethane, washed with 5% aq. acetic acid and water. The organic layer was dried ($MgSO_4$) and evaporated to dryness, after which the residue was purifed by silica gel chromatography to give pure 12b (10.1 g, 92%).
Rf = 0.38 (dichloromethane/acetone; 9/1, v/v)

Methyl 3,6-Di-O-acetyl-2-azido-4-O-(2,3-di-O-benzyl-4-O-levulinoyl-β-D-glucopyranosyluronate)-2-deoxy-α-D-glucopyranosylbromide. (12c) Compound 12b (758 mg, 1 mmol) was dried by coevaporation with dry toluene, dissolved in dry chloroform (9 ml) and stirred under nitrogen in a reaction flask sealed with a rubber septum. Dry DMF (1.6 ml) was added via a syringe and the flask was cooled at 5-10°C. Oxalyl bromide[21] (3.1 ml of a M solution in chloroform) was dropwise added and the reaction mixture stirred during 1.5 h. Dry ether (100 ml) was added and the mixture was poured into cold sat. $NaHCO_3$ solution. The organic layer was separated and washed with cold brine, dried ($MgSO_4$) and evaporated to dryness to give a quantitative yield of α-bromide 12c.
Rf = 0.52 (dichloromethane/acetone; 95/5, v/v)
$[\alpha]_D^{20}$ = + 36 (c = 2.2, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 6.34 (H-1, d, J = 3.9 Hz); 5.49 (H-3, dd, J = 9.2 Hz); 5.05 (H-4', dd, J = 9.8 Hz); 3.71 (s, $COOCH_3$)

1,2,4,6-Tetra-O-acetyl-3-O-benzyl-α/β-L-idopyranose. (15b) 3-O-Benzyl-1,2-O-isopropylidene-α-D-glucofuranose (310 g, 1000 mmol) 13a was dissolved in pyridine (1500 ml), to which mesyl chloride (186 ml, 2400 mmol) was added dropwise at 0°C. This mixture was stirred for 16 h at 4°C. The reaction mixture was poured into warm water (50°C; 5000 ml), cooled and the

residue isolated by filtration to give 13b as a solid material which was dried in vacuo (424 g, 91%).
Rf = 0.62 (dichloromethane/methanol; 97/3, v/v)
^{1}H-NMR ($CDCl_3$): 5.88 (H-1,d, J = 3.5 Hz); 5.24 (H-5, ddd, J = 2.3, 5.6, 7.8 Hz); 3.00, 3.08 (s,2x CH_3, mesyl); 1.31, 1.49 (2 x CH_3, isopropyl)
Compound 13b (201 g, 432 mmol) was dissolved in acetonitrile (4000 ml), after which dry potassium acetate (400 g) and 18-crown-6 (12.5 g) were added. The mixture was stirred and boiled under reflux for 24 h, then filtered, concentrated in vacuo, diluted with dichloromethane (500 ml), washed with water (2 x 500 ml) and crystallized from ethanol to give pure 13c (158 g, 85%).
Rf = 0.54 (toluene/ethanol; 7/3, v/v)
m.p. 117°C
$[\alpha]_D^{20}$ = -9.6 (c = 1.8, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.88 (H-1, d, J = 3.5 Hz); 5.25 (H-5, ddd, J = 2.2, 5.8, 7.8 Hz); 3.02 (s, CH_3, mesyl); 2.09 (s, CH_3, acetyl)
Compound 13c (180 g, 418 mmol) was dissolved in dichloromethane (1750 ml) and t-butanol (900 ml). Potassium t-butoxide (94 g) was added and the mixture was stirred for 16 h at 0°C, after which TLC revealed that the reaction was complete (Rf 0.54⟶0.63, toluene/ethanol; 7/3, v/v). After work-up and filtration over silica gel (300 g), 5,6-anhydro-3-0-benzyl-1,2-0--isopropylidene-ß-L-idofuranose 14 was obtained as a yellow oil (113 g, 93%). Compound 14 (113 g) was dissolved in 0.1 M H_2SO_4 (1000 ml) and stirred for 16 h at 60°C. The reaction was stopped by adding of $Ba(OH)_2 . 8H_2O$ (36 g), after which $BaSO_4$ was removed by filtration. Water was evaporated in vacuo and the residue was dried by coevaporation with toluene/ethanol. The residue 15a was dissolved in pyridine (600 ml), cooled

(-15°C) and acetic anhydride (350 ml) was added. This mixture was stirred for 16 h at 0°C, after which water (50 ml) was added. After evaporation of the solvents the crude α/β mixture of 15b was purified by silica gel chromatography to afford α/β-15b (54%; β/α= 1/7). A small portion of α/β-15b was separated by column chromatography to give pure α-15b as well as pure β-15b.

α-15b:

Rf = 0.44 (toluene/ethyl acetate; 7/3, v/v)

$[\alpha]_D^{20}$ = +1.5 (c = 1.1, $CHCl_3$)

^{1}H-NMR ($CDCl_3$): 6.08 (H-1, d, J = 1.1 Hz); 4.96 (H-2, dd, J = 1.0, 1.1 Hz); 3.79 (H-3, ddd, J = 1.3, 1.7, 2.1 Hz); 4.94 (H-4, ddd, J = 0.5, 1.0, 1.0 Hz)

^{13}C-NMR ($CDCl_3$): 90.9, 65.6, 76.0, 66.2, 75.7, 61.8 (C1-C6)

β-15b:

Rf = 0.41 (toluene/ethyl acetate; 7/3, v/v)

$[\alpha]_D^{20}$ = -8.9 (c = 1.2, $CHCl_3$)

^{1}H-NMR ($CDCl_3$): 6.09 (H-1, d, J = 1.4 Hz);5.08 (H-2, ddd, J = 1.0, 1.4, 2.9 Hz); 3.89 (H-3, t, J = 3 Hz); 4.88 (H-4, ddd, J = 1.0, 2.2, 3.0 Hz)

^{13}C-NMR ($CDCl_3$): 90.3, 66.5, 73.5, 66.9, 72.0, 62.2 (C1-C6)

1,6-Anhydro-2-azido-3-O-benzyl-4-O-(3-O-benzyl-α-L-idopyranosyl)-2-deoxy-β-D-glucopyranose. (19a)

Compound 15b (39.9 g, 91 mmol; α/β mixture) was dissolved in a mixture of dichloromethane (200 ml) and ethyl acetate (100 ml). A solution of titanium tetrabromide (45 g) in dichloromethane (450 ml) was added and the reaction mixture was stirred under nitrogen for 6 h at room temperature. Toluene (1000 ml) and excess of potassium acetate were added until a colourless solution was obtained. After filtration and

evaporation of the solvents crude bromide 16 was obtained. The bromide 16 was dissolved in acetonitrile (200 ml) and added dropwise to a solution of 17 (35.3 g, 127.4 mmol) in a mixture of dry acetonitrile (400 ml) and nitromethane (20 ml). Mercury (II) cyanide (23.1 g, 91 mmol) was added in portions and the mixture was stirred under a stream of nitrogen for 18 h at room temperature.

The reaction mixture was then poured into dichloromethane (400 ml) and washed with saturated $NaHCO_3$ (500 ml) and KBr (2 x 400 ml, 2 M) solutions. The organic layer was dried ($MgSO_4$) and the solvent evaporated to give the crude reaction mixture (66.6 g). After purification by chromatography on silica gel (2000 g) and elution with hexane/ethyl acetate (4/6, v/v) pure compound 18 was obtained (30.4 g, 51%). Compound 18 (30.4 g, 46.5 mmol) was then dissolved in a mixture of methanol (100 ml) and triethylamine (40 ml) and stirred for 24 h at r.t.. The solvents were evaporated and the crude product was purified over a small layer of silica gel (100 g) to afford pure 19a (19.7 g; 41% overall yield from 15b).

Rf = 0.45 (dichloromethane/methanol; 9/1, v/v)

^{1}H-NMR ($CDCl_3$): 5.13 (H-1', s); 5.52 (H-1, s)

^{13}C-NMR ($CDCl_3$): 101.2 (C-1); 99.3 (C-1')

4-O-(2-O-Acetyl-3-O-benzyl-α-L-idopyranosyl)-1,6--anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranose. (20a) Compound 19a (16.2, 30.6 mmol) in DMF (81 ml) was treated with freshly distilled 2,2-dimethoxypropane (26.8 ml, 218 mmol) and a catalytic amount of p-toluenesulphonic acid in a similar way as described for the synthesis of compound 9.[13] The crude product 19b was acetylated in a mixture of pyridine (89 ml) and

acetic anhydride (29.4 ml) to give 19c as described for 10b. Crude 19c was dissolved in a mixture of acetic acid (60 ml) and water (26 ml) and stirred for 16 h at r.t.. Toluene (120 ml) was added and the solvents were evaporated to give 20a, which was purified by silica gel chromatography (15.2 g, 87%).
Rf 0.11 (dichloromethane/acetone; 95/5, v/v)
$[\alpha]_D^{20}$ = -56 (c = 0.74, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.52 (H-1, s); 5.08 (H-1', s); 5.10 (H-2', c); 3.24 (H-2, br d, J = 3 Hz); 2.11 (s, CH_3, acetyl)

Methyl 4-O-(2-O-Acetyl-3-O-benzyl-4-O-levulinoyl-α-L-idopyranosyluronate)-1,6-anhydro-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranoside. (21) Compound 20a was converted into 21 according to the procedures as described for 10d and 11b (see also text).
Rf = 0.54 (dichloromethane/acetone; 9/1, v/v)
$[\alpha]_D^{20}$ = - 34.1 (c = 0.6, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.49 (H-1, br); 3.23 (H-2, dd); 3.62 (H-3, c); 3.76 (H-4, dd); 4.71 (H-5, dd); 3.74, 4.02 (2 x H-6, 2 x dd); 5.23 (H-1',ddd); 4.98 (H-2', ddd); 3.82 (H-3', ddd); 5.17 (H-4', dddd); 4.84 (H-5', d) 3.72 (s, $COOCH_3$); 2.12, 2.19 (2 x CH_3, levulinoyl, acetyl); $J_{1',2'}$= 1.2, $J_{1',3'}$=1.0, $J_{1',4'}$ =0.5, $J_{2',3'}$=2.5, $J_{2',4'}$=0.9, $J_{3',4'}$=3.2, $J_{4',5'}$=2.3 Hz
^{13}C-NMR ($CDCl_3$): 101.2 (C-1, J_{CH} = 176.2 Hz); 95.9 (C-1', J_{CH} = 169.8 Hz)

Methyl 6-O-Acetyl-4-O-(2-O-acetyl-3-O-benzyl-4-O--levulinoyl-α-L-idopyranosyluronate)-2-azido-3-O-benzyl-2-deoxy-α-D-glucopyranosylbromide. (22c) Compound 21 (1.39 g, 2 mmol) was dissolved in a mixture of acetic anhydride (16 ml), acetic acid (0.7 ml) and

trifluoroacetic acid (2.2 ml) and treated as described for 12a to give 22a. Crude 22a was dissolved in THF (28 ml) and dry piperidine (1.7 ml) was added as for 12b to give 22b, which was converted into the α-bromide 22c (1.5 g, 93%) by treatment with oxalyl bromide according to the procedure as described for 12c.
Rf = 0.81 (hexane/ethylacetate; 4/6, v/v)
^{1}H-NMR ($CDCl_3$): 6.41(H-1, d, J = 4.0 Hz); 3.80, 3.63 (H-2, dd, J = 4.0 Hz); 3.47 (s, $COOCH_3$); 2.18, 2.11, 2.09 (s, 3 x CH_3, levulinoyl, acetyl)

Benzyl 6-O-Acetyl-2-azido-3-O-benzyl-2-deoxy-4-O-(methyl 2-O-acetyl-3-O-benzyl-4-O-levulinoyl-α-L-idopyranosyluronate)-α-D-glucopyranoside. (22d) To a mixture of silver silicate (1.25 g), powdered molecular sieves (2.5 g) and dry benzylalcohol (1 ml, 9.8 mmol) in dry dichloromethane (25 ml) and dry toluene (6 ml), was added bromide 22c (1.5 g , 1.87 mmol) dissolved in dichloromethane (28 ml) in 1.5 h at -25°C under nitrogen. The mixture was stirred overnight at -25°C, diluted with dichloromethane, filtered over hyflo and the filtrate evaporated to dryness. The crude reaction product was purified by silica gel chromatography to give pure 22d (950 mg, 60%).
Rf = 0.51 (dichloromethane/acetone; 93/7, v/v)
$[\alpha]_D^{20}$ = -39 (c = 0.34, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 5.12 (H-1', br); 5.08 (H-4', t, J = 2.8 Hz), 4.97 (H-5', d, J = 2.8 Hz); 4.84 (H-2', t, J = 1.9 Hz); 4.33 (H-1, d, J = 7.9 Hz); 3.80 (H-3, dd, J = 2.8, 1.9 Hz); 3.46 (s, $COOCH_3$); 3.24 (H-2, t, J = 7.9 Hz); 2.18, 2.13, 2.08 (s, 3 x CH_3, levulinoyl, acetyl)

Benzyl 4-O-(Methyl 2-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-6-O-acetyl-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranoside. (23) Compound 22d (583 mg, 688 mmol) dissolved in dry pyridine (8.4 ml) was cooled

at 0°C, and to this solution was added a mixture of pyridine (4.8 ml), acetic acid (3.2 ml) and hydrazine hydrate (0.4 ml). This mixture was stirred at 0°C for 5 min and then at r.t. for another 10 min. The mixture was diluted with dichloromethane, washed with water, aq. $NaHCO_3$ solution and brine, dried ($MgSO_4$) and evaporated to dryness. The residue was purified by silica gel chromatography to give 23 as a foam (464 mg, 90%).

Rf = 0.45 (dichloromethane/acetone; 95/5, v/v)

$[\alpha]_D^{20}$ = - 31.4 (c = 0.8, $CHCl_3$)

^{1}H-NMR ($CDCl_3$): 4.33 (H-1, d, J = 7.8 Hz): 5.07 (H-1', br); 3.49 (s, $COOCH_3$); 2.08, 2.12 (s, 2 x CH_3, acetyl)

^{13}C-NMR ($CDCl_3$): 100.3 (C-1); 98.0 (C-1')

Benzyl O-(Methyl 2,3-di-O-benzyl-β-D-glucopyranosyluronate)-(1 ⟶ 4)-O-3,6-di-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1 ⟶ 4)-O-(methyl 2-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-(1 ⟶ 4)-6-O-acetyl-2-azido-3-O-benzyl-2-deoxy-β-D-glucopyranoside. (24b) A mixture of glycon 12c (820 mg, 1 mmol), aglycon 23 (464 mg, 0.62 mmol) and activated molecular sieves (4A, 1.2 g) was stirred in 1,2-dichloroethane (12.5 ml) at -30°C under a nitrogen atmosphere. Silver triflate (400 mg) was added and the mixture was stirred for 16 h at -30°C. The reaction mixture was filtered, washed with aq. $NaHCO_3$ (5 ml, 5%) and saturated NaCl solution (5 ml) and dried ($MgSO_4$). After evaporation of the solvent the residue was chromatographed over a small column of silica gel (5 g) to give crude tetrasaccharide 24a (686 mg; 68%) Compound 24a was dissolved in a mixture of pyridine (13 ml) and acetic acid (3 ml). The mixture was cooled (0°C) and hydrazine hydrate (0.4 ml) was added. After 10 min at 0°C, when TLC revealed that the reaction was complete (Rf 0.38 ⟶ 0.35,

dichloromethane/acetone; 95/5, v/v), dichloromethane (50 ml) was added and the solution washed with water (30 ml), aq. $NaHCO_3$ (30 ml, 5%) and brine (30 ml). The organic layer was evaporated and the residue purified on a column of Sephadex-LH20 (2.5 x 90 cm; THF/methanol; 95/5, v/v) to give pure 24b (448 mg, 52%).
Rf = 0.38 (dichloromethane/acetone; 93/7, v/v)
$[\alpha]_D^{20}$ = +10.9 (c = 0.8, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 4.29 (H-1, d, J = 8.1 Hz); 5.29 (H-1', d, J = 3.9 Hz); 5.00 (H-1'', d, J = 3.3 Hz); 4.33 (H-1''', d, J = 7.9 Hz); 5.36 (H-3''', dd, J = 9.1, 10 Hz); 3.59, 3.78 (s, 2 x CH_3, $COOCH_3$);1.99, 2.08 (s, 4 x CH_3, acetyl)
^{13}C-NMR ($CDCl_3$): 100.3 (C-1); 98.1 (C-1'); 97.6 (C-1''); 103.5 (C-1''')

Benzyl O-(6-O-Acetyl-2-azido-3,4-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1 ⟶ 4)-O-(methyl 2,3-di-O-benzyl-β-D-glucopyranosyluronate)-(1 ⟶ 4)-O-3,6-di-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1 ⟶ 4)-O-(methyl 2-O-acetyl-3-O-benzyl-α-L-idopyranosyluronate)-(1⟶4)-6-O-acetyl-2-azido-3-O-benzyl-2-deoxy-β-Dglucopyranoside. (27b)

A mixture of aglycon 24b (395 mg, 0.28 mmol), silver triflate (490 mg, 1.9 mmol), molecular sieves (4A, 1 g) and collidine (0.3 ml) in 1,2-dichloroethane (14 ml) was stirred at -20°C under nitrogen. Glycopyranosylbromide 1 (410 mg, 0.96 mmol), dissolved in 1,2-dichloroethane (2 ml) was added dropwise. The mixture was stirred for 16 h at -20°C and worked-up. The residue was filtered over a small layer of silica gel (1.6 g) and the mixture separated over a column of Sephadex-LH20 (2.5 x 180 cm; THF/methanol; 95/5, v/v) to give pure pentasaccharide 27b (491 mg, 96%).
Rf = 0.44 (toluene/acetone; 8/2, v/v)

$[\alpha]_D^{20}$ = +27.5 (c = 0.5, $CHCl_3$)
^{1}H-NMR ($CDCl_3$): 4.28 (H-1, d, J = 8.1 Hz);5.28 (H-1', d, J = 3.9 Hz); 4.99 (H-1'', d, J = 3.4 Hz); 4.35 (H-1''', d, J = 7.9 Hz); 5.49 (H-1''', d, J = 3.9 Hz); 4.90 (H-2', dd, J = 3.9, 4.1 Hz); 5.35 (H-3'', dd, J = 9.1, 10 Hz); 3.58, 3.74 (s, 2 x CH_3, $COOCH_3$); 2.00, 2.01, 2.08, 2.09 (5 x CH_3, acetyl)
^{13}C-NMR ($CDCl_3$): 100.3 (C-1); 98.1 (C-1'); 97.6 (C-1''); 103.2 (C-1'''); 98.1 (C-1'''')

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