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Note

Synthesis of 2,5-anhydro-(β-D-glucopyranosyluronate)- and (α-L-idopyranosyluronate)-D-mannitol hexa-*O*-sulfonate hepta sodium salt ☆

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Dedicated to the memory of Christian Pedersen who passed away September 14, 2003

Abstract—Glycosidation of 2,5-anhydro-1,6-di-*O*-benzoyl-D-mannitol with methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl-1-*O*-trichloroacetimidate)uronate in the presence of trimethylsilyl triflate afforded the corresponding 3-*O*-β-glycoside, which after deprotection was converted into its hexa-*O*-sulfate with $DMF{\cdot}SO_3$ to give after treatment with sodium acetate and subsequent saponification of the methyl ester with sodium hydroxide the hepta sodium salt of 2,5-anhydro-3-*O*-(β-D-glucopyranosyl uronate)-D-mannitol hexa-*O*-sulfate. Glycosidation of the same acceptor with the α-thiophenylglycoside of methyl 2,4-di-*O*-acetyl-3-*O*-benzyl-L-idopyranosyl uronate in the presence of NIS/TfOH afforded the corresponding 3-*O*-α-glycoside in very low yield, therefore the α-thiophenylglycoside of 2-*O*-acetyl-2,4-*O*-benzylidene-3-*O*-benzyl-L-idopyranose was used as donor. The terminal hydroxymethyl group of the obtained disaccharide was subsequently oxidised with NaOCl/TEMPO and the obtained iduronic acid derivative was converted into the hepta sodium salt of 2,5-anhydro-3-*O*-(-α-L-idopyranosyluronate)-D-mannitol hexa-*O*-sulfonate with $DMF{\cdot}SO_3$ and subsequent treatment with sodium acetate.

Keywords: D-Glucuronic acid derivatives; L-Iduronic acid derivatives; Supersulfated disaccharides

Heparin is a member of the large family of glycosaminoglycans, which are polyanionic, complex carbohydrate macromolecules. It was discovered in 1916 and became well known as an antithrombotic agent, which has been used clinically since 1935.[1] However, this macromolecule possess other very important and significant biological activities too, among others antiinflammatory, antiallergic and even antiasthmatic properties.[2–5] Despite the fact that heparin is constructed from a repeating disaccharide building block containing an uronic acid attached to glucosamine at O-4, it is a very complex molecule, as the uronic acid residue can be either β-D-glucuronic- or α-L-iduronic acid both of which can be sulfated at O-2. Furthermore the α-D-glucosamine unit may either be N-acetylated or N-sulfated, both are 6-sulfated and may also be 3-sulfated. It was presumed that the aforementioned different biological activities can be attributed to different regions of the macromolecules, but their microheterogeneity made the exact structure elucidation very difficult. It took decades until the researchers were able to prove,[6,7] that a pentasaccharide unit **1** is responsible for the antithrombotic effect (Fig. 1). As far as the whole molecule of heparin is concerned, it should be mentioned that the ratio of L-iduronic to D-glucuronic acid varies between 9:1 and 7:3, depending of the source of isolation.[8] The antiinflammatory, antiallergic and antiasthmatic activity is however independent from the anticoagulant activity of native heparin, and is displayed by low, and even ultraslow molecular weight heparin fragments, obtained

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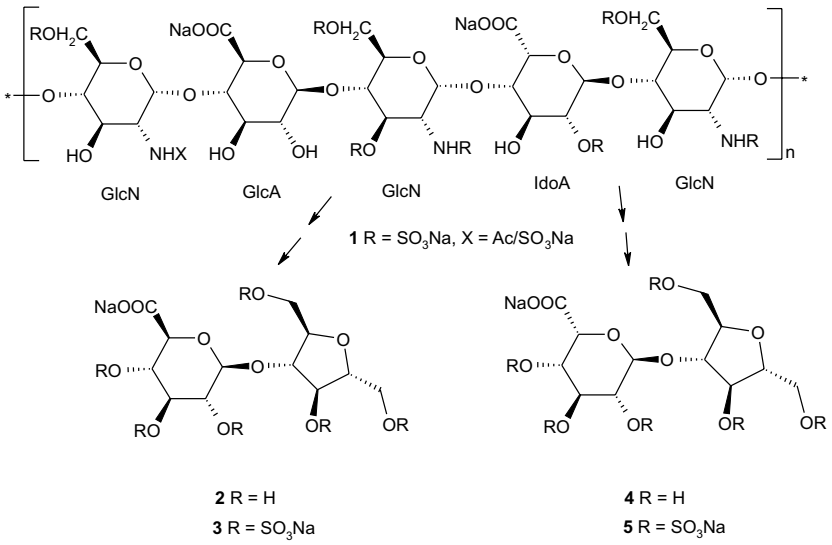


Figure 1.

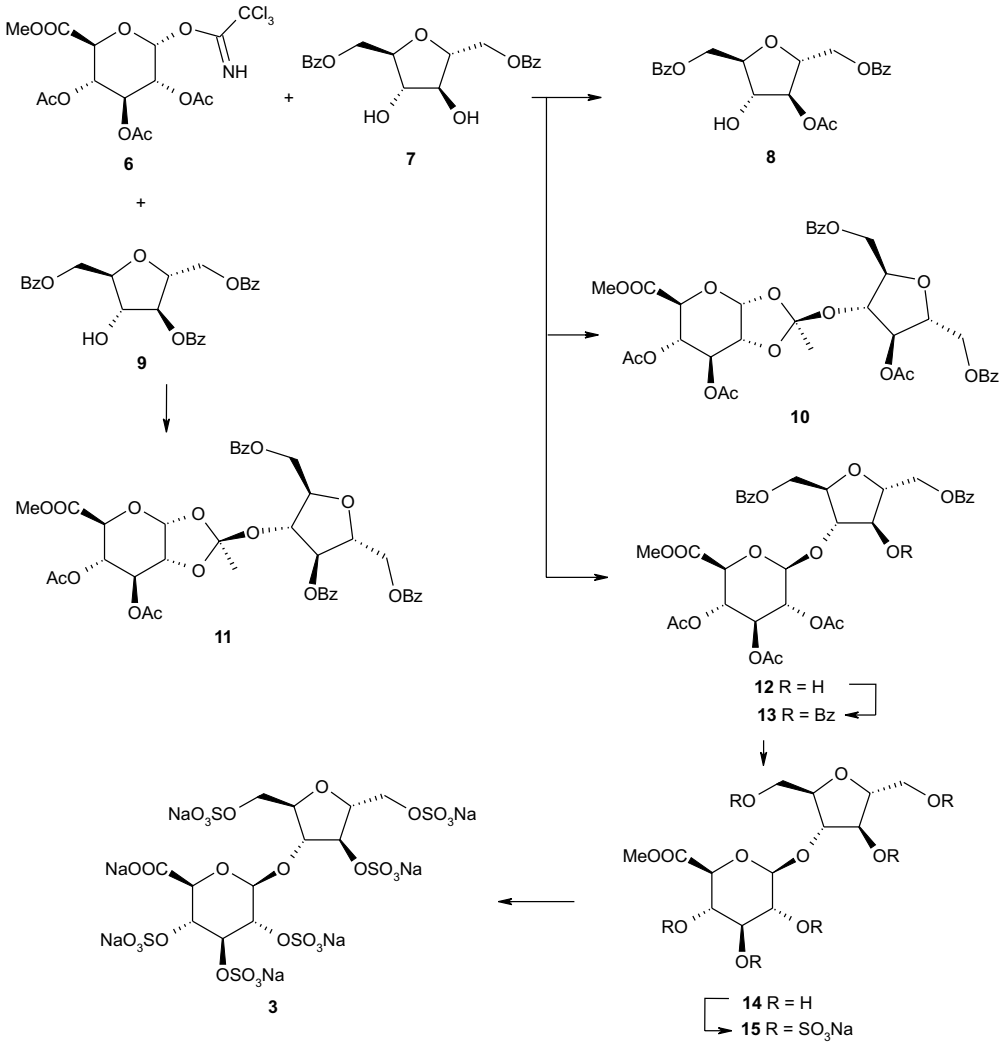
by degradation of the original GAGs.[9,10] Recently it was claimed by Ahmed and Smith,[11] that hypersulfated disaccharides, obtained by degradation of heparin with HNO_2 subsequent reduction by $NaBH_4$ followed by O-sulfation possess pronounced antiasthmatic activity. It is well known,[12] that the primary degradation product obtained by depolymerisation of GAGs by HNO_2 when treated with $NaBH_4$ results in a mixture, containing the two disaccharides **2** and **4**. Accordingly, the structure of the main components obtained after sulfation corresponds to the hepta sodium salt of 2,5-anhydro-3-*O*-(β-D-glucopyranosyluronate)- and (α-L-idopyranosyluronate)-D-mannitol hexa-*O*-sulfate **3** and **5**, respectively (Fig. 1). As their separation represents a very difficult problem, it was decided to synthesise both of them for comparing their biological activity.

1. Synthesis of the glucuronic acid derivative 3

For the coupling reaction, 1,6-di-*O*-benzoate **7**[13,14] was used as acceptor and methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[15] as the donor molecule in the presence of mercury cyanide as promoter in acetonitrile as solvent at −5 °C. No reaction took place according to TLC, therefore the temperature was raised to 20 °C and later to 50 °C, but only decomposition of the acetobromo derivative took place and the acceptor molecule remained unchanged.

Next, the more active imidate **6**[16] was used as donor 1,6-di-*O*-benzoate **7** as acceptor and trimethylsilyl trifluoromethanesulfonate as promoter. The reaction was carried out in CH_2Cl_2 at −40 °C and was finished in ~5 min. From the resulting complex mixture, the 3-*O*-acetate of the acceptor anhydride (**8**)—an *ortho*-ester type glycoside containing the 3-*O*-acetate group at the aglycon unit (**10**)—as well as the expected glycoside **12** could be separated by column chromatography (Scheme 1). The latter compound was obtained as a syrup in a relatively low yield (36%) and according to NMR its purity was ~60%. For further purification, it was converted into its crystalline tribenzoate **13**. For avoiding the migration of the 2-*O*-acetyl group of the donor to the acceptor, the same glycosylation reaction was repeated using tribenzoate **9** as acceptor—which was obtained as a by-product[14] in the benzoylation reaction of 2,5-anhydro-D-mannitol.[13,17] Despite the fact, that in this reaction crude imidate was applied, the yield of the obtained pseudotrisaccharide (counted on the imidate content) was excellent (92%), but the isolated material did not crystallise and was not identical with **13**. Detailed investigation of the isolated product by NMR revealed the fact, that instead of **13** the *ortho*-ester **11** was formed. This could not be rearranged into the former on treatment with TfOH or $BF_3{\cdot}Et_2O$ in CH_2Cl_2 as only decomposition took place (Scheme 1).

For the obtention of the target molecule **3**, the tribenzoate **13** was submitted to Zemplén deacylation, which was a very slow process, as the isolated 3-*O*-benzoyl group is very resistant towards transesterification.[18] The obtained hexahydroxy compound **14** was converted with $DMF{\cdot}SO_3$ into its hexa-*O*-sulfate, which after treatment with sodium acetate afforded the hexa-*O*-sulfate hexa sodium salt **15**. Finally the terminal methyl ester was saponified with NaOH to give the corresponding hepta-sodium salt **3**, which was submitted to biological testing. It should be mentioned, that according to NMR the glucuronide part of this molecule adopts a rather distorted boat conformation, due to the electrostatic repulsion of the adjacent *O*-sulfate groups in position 2′,

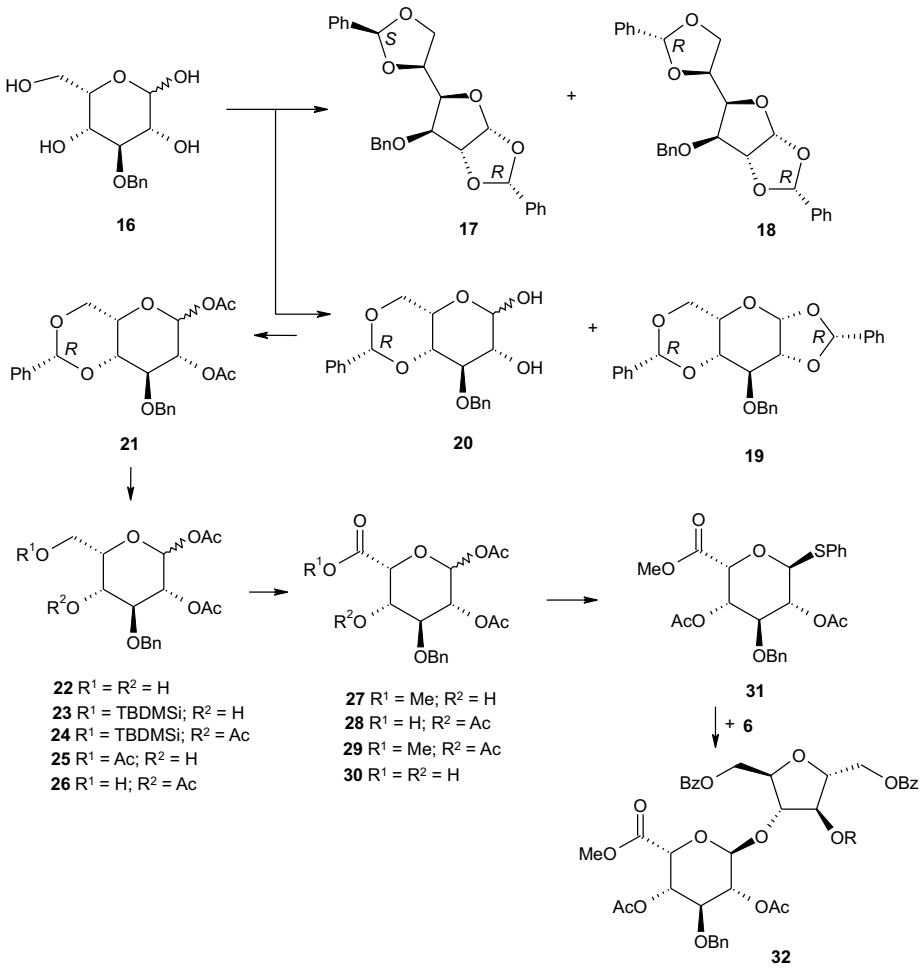


Scheme 1.

3′ and 4′. This is evident from the value of the corresponding coupling constants ($J_{2',3'}$ 3.4, $J_{3',4'}$ 5.3, $J_{4',5'}$ 3.8 Hz) which in the case of the unsubstituted starting material **15** had much higher values ($J_{2',3'}$ 8.2, $J_{3',4'}$ 9.0, $J_{4',5'}$ 9.3 Hz) as expected for the normal chair conformation $^4C_1$.

2. Attempted synthesis of 5 using an L-iduronic acid derivative as donor

In our first attempt we decided to use an L-iduronic acid thioglycoside as donor, which was synthesised by using the method described by Tabeur et al.[19] for the synthesis of a 4-*O*-levulinoyl analogue. Accordingly 3-*O*-benzyl-L-idose **16** was prepared by the method elaborated by van Boeckel et al.[20] and was treated with benzaldehyde in the presence of trifluoroacetic acid.[19] From the resulting mixture the following components could be separated by column chromatography: 1,2-(*R*):5,6-(*S*)-di-*O*-benzylidene-L-idofuranose (**17**) a 1:1 mixture of the aforementioned compound and the corresponding 1,2-(*R*):5,6-(*R*)-di-*O*-benzylidene isomer (**17** + **18**), 1,2-(*R*):4,6-(*R*)-di-*O*-benzylidene-L-idopyranose (**19**) and the needed 4,6-(*R*)-*O*-benzylidene-L-idopyranose (**20**) in yields of 1%, 3%, 7% and 58%, respectively (Scheme 2). The configuration of the benzylidene groups was determined by NMR using NOE measurements. The monobenzylidene derivative **20** gave on acetylation the known[19] 1,2-di-*O*-acetate **21** as an anomeric mixture. The *O*-benzylidene group of the latter was split off without separation of the anomers with aqueous trifluoroacetic acid in CH_2Cl_2 solution and the obtained 4,6-dihydroxy derivative **22** (61%) gave on treatment with *tert*-butyldimethylsilyl chloride in the presence of Et_3N the 6-*O*-silylated derivative **23** containing the α/β-anomers in a ratio of 1:2. When acetic anhydride was added after silylation to the same reaction mixture, no acetylation



Scheme 2.

took place. Finally isolated **23** could be converted into triacetate **24** on treatment with acetyl chloride in pyridine. The two anomers of **24** could be partially separated by column chromatography, but the further reactions were carried out with the anomeric mixture. When the 6-*O*-silyl group was removed from **24** by treating its methanolic solution with aqueous HCl at 0 °C, besides hydrolysis a partial 4-*O* → 6-*O* acetyl migration took place resulting in anomeric mixtures of two isomeric tetraacetates **25** and **26**. They could be separated by column chromatography yielding the needed 6-OH isomer **26** in a yield of 40%. Oxidation of the terminal hydroxymethyl group was carried out with sodium hypochlorite in the presence of TEMPO[21] and the sodium salt of the formed carboxylic acid **28** was treated with methyl iodide affording the methyl ester **29** in an overall yield of 24% counted on **22**. This low overall yield could be increased to 42% by oxidising **22** directly without protecting the secondary 4-OH group. The formed carboxylic acid **30** was converted into its methyl ester **27**, which on acetylation gave **29**. Treatment of triacetate **29** with thiophenol in the presence of $BF_3{\cdot}Et_2O$ afforded the thioglycoside **31** as α-anomer in moderate yield (52%). This was used as donor for the glycosidation of the 1,6-di-*O*-benzoate **7** applying NIS as activator and a catalytic amount of TfOH as promoter and carrying out the reaction at −40 °C in CH_2Cl_2. The formed protected disaccharide **32** was obtained after column chromatography as α-anomer in a very low yield (21%). As the yield could not be increased by changing the reaction conditions, this approach had to be abandoned.

3. Synthesis of 5 using an L-idopyranose thioglycoside derivative as donor

In our further attempts, 3-*O*-benzyl-L-idopyranose-peracetate **33**[20] was converted into its 1-bromide **34**[20], which was coupled with dibenzoate **7** using Helferich's conditions, which according to literature data[20] were successfully applied in the synthesis of the corresponding levoglucosan disaccharide. However in our case no reaction took place at room temperature only a slow

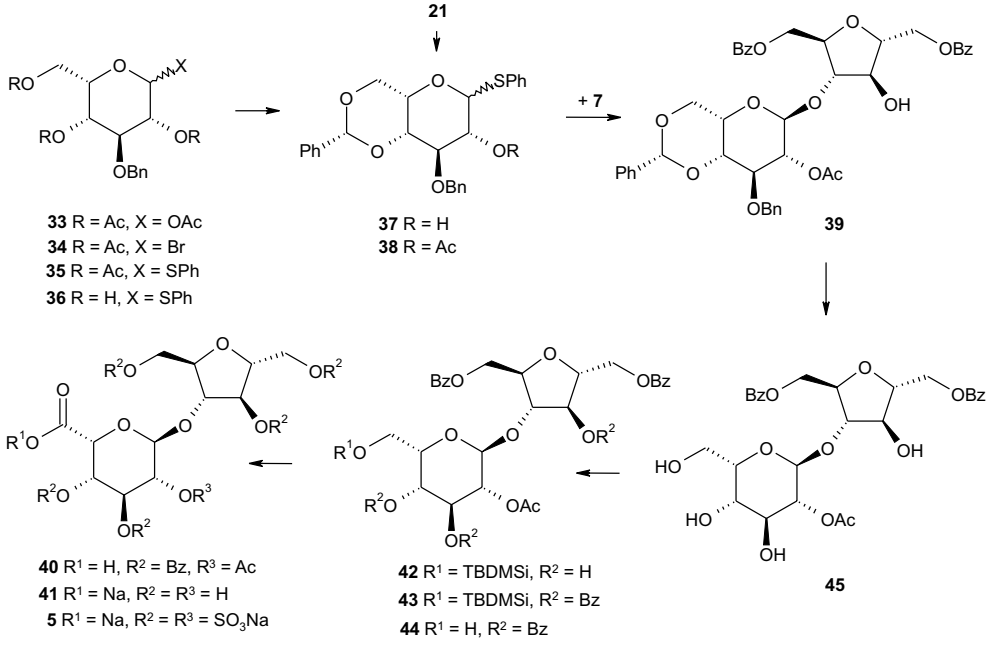
decomposition of **34** was observed. Therefore **33** was treated with thiophenol in the presence of $BF_3{\cdot}Et_2O$[19] affording thioglycoside **35** as an $\alpha/\beta \sim 5:1$ anomeric mixture, which could be separated by column chromatography (Scheme 3). Deacetylation of the major anomer **35**α afforded **36**α, which was converted into the corresponding 4,6-*O*-benzylidene derivative **37**α on treatment with benzaldehyde dimethylacetal. When, in the same reaction sequence the anomeric mixture of **35** was applied as starting material, both anomers **37**α and **37**β were obtained, which could be separated by column chromatography. Acetylation of **37**α afforded the 2-*O*-acetate **38**α, which could be obtained by an alternative approach using benzylidene diacetate **21** as starting material and converting it into **38**α by treatment with thiophenol in the presence of $BF_3{\cdot}Et_2O$. Nevertheless the overall yields of these two approaches counted on the tetraacetate **33** were almost the same (Scheme 3).

Condensation of the thioglycoside **38**α with the aglycon **6** in CH_2Cl_2 at low temperature (−40 °C) using NIS as activator and TfOH as promoter afforded the needed glycoside **39** as α-anomer in excellent yield (81%). The benzylidene and benzyl group of the latter were removed by hydrogenation over Pd/C and the obtained trihydroxy derivative **45** was converted into its 6-*O*-*t*-butyldimethylsilyl derivative, which was not isolated but converted directly into its 4,4,4′-tri-*O*-benzoyl derivative **44**. The terminal silyl group of **44** was removed by treatment with aqueous sulfuric acid in EtOH to yield the 6-OH compound **44** in excellent yield. Partial oxidation of the terminal hydroxymethyl group of **44** was carried out with NaOCl in the presence of TEMPO[21] affording after column chromatography **40** in satisfactory yield (70%) as a ~1:3 mixture of the free carboxylic acid and its sodium salt. This was converted into its polysulfate with $SO_3{\cdot}DMF$ to yield the hepta sodium salt **5** after removing the excess of sulfate ions with $Sr(OAc)_2$ and treatment of the solution with a CHELEX 100 (Na) resin.

4. Experimental

4.1. General methods

Organic solutions were dried over $MgSO_4$ and concentrated under diminished pressure at or below 40 °C. TLC: E. Merck precoated Silica Gel 60 F_{254} plates, with EtOAc (A), EtOAc–hexane mixtures (B, 1:1; C, 1:2; D, 1:3; E, 1:5; F, 2:1; G, 2:3; H, 3:1), EtOAc–EtOH mixture (I, 5:1); detection by spraying the plates with a 0.02 M solution of I_2 and a 0.3 M soln of KI in 10% aqueous H_2SO_4 solution followed by heating at ca. 200 °C. For column chromatography Kieselgel 60 was used. The mp are uncorrected. Optical rotations were determined on 1.0% solutions in $CHCl_3$ at 20 °C unless stated otherwise. The NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 (^{1}H, see Supplementary Material, Tables 1,2,4 and 5) and 125 (^{13}C see Supplementary Material, Tables 3 and 6) MHz, respectively, at ambient temperature. The chemical shifts were referenced to $\delta_{TMS} = 0$ ppm. The solvent is indicated at the ^{1}H NMR spectral data. For structure determination ^{1}H,



Scheme 3.

^{1}H COSY, TOCSY, HMQC, HMBC as well as selective 1D TOCSY and NOESY spectra were recorded.

4.2. 2,5-Anhydro-3-*O*-(β-D-glucopyranosyl uronate)-D-mannitol hexa-*O*-sulfate hepta sodium salt (3)

To a stirred slurry of DMF·SO_3 (48%, 1.7 g, 10 mmol) in DMF (3 mL) a soln of **14** (0.35 g, 1 mmol) in DMF (3 mL) was gradually added at −20 °C at such a rate to keep the temperature below −15 °C. After 15 min, the temperature of the mixture was raised to −5 °C and kept there for 45 min. Thereafter it was cooled to −25 °C and EtOH (0.5 mL) was added at such a rate to keep the temperature below −15 °C. Thereafter the mixture was poured into a stirred and cooled (−5 °C) soln of NaOAc (2 g) in MeOH (20 mL). The resulting precipitate was filtered and washed with MeOH. The solid residue was dissolved in water (5 mL), the pH of the solution was adjusted to 8–9 with M NaOH. Thereafter M NaOH (1 mL) was added to hydrolyse the formed methyl ester **15**. The mixture was kept for 15 h at rt thereafter it was diluted with water (25 mL) and a soln of aq M $Sr(OAc)_2$ was added until no more precipitate ($SrSO_4$) was formed (∼10 mL). The precipitate was filtered off and the filtrate was submitted to a column loaded with CHELEX 100 (Na form) (10 mL) for removing Sr ions. The column was eluted with distilled water and the eluate was concentrated. The residue was filtered with EtOH to yield **3** (0.75 g, 79%); $[\alpha]_D$ +15 (*c* 1, water). Anal. Calcd for $C_{12}H_{13}O_{29}S_6Na_7$: C, 14.79; H, 1.34; Na, 16.51; S, 19.74. Found: C, 14.58; H, 1.72; Na, 16.42; S, 19.55.

4.3. 2,5-Anhydro-3-*O*-(-α-L-idopyranosyluronate)-D-mannitol hexa-*O*-sulfate hepta sodium salt (5)

To a stirred slurry of 48% SO_3·DMF (30 g, 175 mmol) in dry DMF (50 mL) the sodium salt **41** (5.0 g, 14 mmol) was added below −15 °C. Thereafter the temperature was raised to −5 °C and kept there for 45 min. The formed clear soln was kept at 0 °C for 45 min and was then poured into a cooled (0 °C) soln of $NaHCO_3$ (28 g) in water (350 mL). The soln was neutralised with M H_2SO_4 and concentrated. The residue was filtered and washed with MeOH. The resulting crude material was dissolved in water (250 mL) and a soln of aq M $Sr(OAc)_2$ was added until no more precipitate ($SrSO_4$) was formed (∼120 mL). The precipitate was filtered off and the filtrate was submitted to a column loaded with CHELEX 100 (Na form) (20 mL) for removing Sr ions. The column was eluted with distilled water and the eluate was concentrated. The residue was filtered with EtOH to give **5** (12.2 g, 90%) which, according to NMR spectroscopy, contained 0.2 equiv EtOH and 0.75 equiv NaOAc but was free from any other by-product (partially sulfated derivatives); $[\alpha]_D$ −5 (*c* 1, water). Anal. Calcd for $C_{12}H_{13}O_{29}S_6Na_7$: C, 14.79; H, 1.34; Na, 16.51; S, 19.74. Found: C, 14.43; H, 1.48; Na, 16.44; S, 19.52.

4.4. Methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl-1-*O*-trichloroacetimidate)uronate (6)

To a stirred soln of methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranose)uronate[16] (24.7 g, 74 mmol) in 1,2-dichloroethane (120 mL), trichloroacetonitrile (25 mL) and K_2CO_3 (25 g) were added at rt. The mixture was stirred for 3 h, thereafter the solid material was filtered off and the filtrate was concentrated. According to NMR spectroscopy, the imidate content of the residue (34.6 g, 98%) was ∼50%. It could be purified by column chromatography (C → B) and the residue obtained on concentration of the fractions having R_f 0.6 (C) gave after recrystallisation from 2-propanol pure **6** (5.8 g, 16%), mp 114–115 °C, lit.[16] mp 108 °C.

4.5. 2,5-Anhydro-1,6-di-*O*-benzoyl- (7) and 1,3,6-tri-*O*-benzoyl-D-mannitol (9)

To a stirred soln of 2,5-anhydro-D-mannitol[13,17] (25.5 g, 15.5 mmol) in pyridine (250 mL), a soln of benzoyl chloride (45 mL, 39 mmol) in CH_2Cl_2 (200 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 1.5 h and then kept at 20 °C for 30 min. Thereafter it was poured into water, the separated aq soln was extracted with CH_2Cl_2 and the combined organic solutions gave after usual processing and concentration a residue, which was submitted to column chromatography. As eluent first solvent C was applied for removing the by-products (R_f 0.9 and 0.6) and then A for eluting the product. Concentration of the latter fraction gave crude **7** (33.5 g, 58%), mp 99–101 °C (toluene); R_f 0.5 (F); $[\alpha]_D$ +21 (*c* 1, $CHCl_3$); $[\alpha]_D$ +29 (*c* 1, MeOH), lit.[13] mp 100–102 °C, $[\alpha]_D$ +31 (*c* 1.5, MeOH).

The residue, obtained on concentration of the fractions containing the by-products, afforded a syrup (16 g) from which the tribenzoate **9** (9.9 g, 13.3%) could be obtained after repeated column chromatography with solvent B; R_f 0.5 (C); mp 75–80 °C (ether–hexane); $[\alpha]_D$ +43 (*c* 1, $CHCl_3$). Lit.[14] mp 80–84 °C; $[\alpha]_D$ +54 (*c* 0.1, $CHCl_3$).

4.6. Reaction of trichloroimidate 6 with dibenzoate 7

A soln of **7** (0.74 g, 2 mmol) and **6** (0.96 g, 2 mmol) in CH_2Cl_2 (25 mL) was stirred in the presence of molecular sieve (4 Å) (5 g) for 2 h. Thereafter the mixture was cooled to −40 °C and TMSOTf (0.1 mL) was added. After 5 min the reaction was stopped by adding Et_3N (0.5 mL). After dilution with CH_2Cl_2 (25 mL) it was washed with 5% aq $NaHCO_3$ and water, dried and

concentrated. The residue was submitted to column chromatography (B). Concentration of the fraction having R_f 0.8 afforded 3-*O*-acetyl-1,6-dibenzoyl-2,5-anhydro-D-mannitol (**8**) (0.1 g, 12%). Anal. Calcd for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35. Found: C, 63.67; H, 5.57.

Concentration of the fraction having R_f 0.65 gave methyl 3,4-di-*O*-acetyl-1,2-(*S*)-*O*-[1′-*O*-(3-*O*-acetyl-2,5-anhydro-1,6-di-*O*-benzoyl-D-mannito-4-yl)-ethylidene]-α-D-glucopyranuronate **10** (0.15 g, 11%); $[\alpha]_D$ +12. Anal. Calcd for $C_{35}H_{38}O_{17}$: C, 57.53; H, 5.24. Found: C, 57.45; H, 5.38.

Concentration of the third fraction having R_f 0.5 gave 2,5-anhydro-1,6-di-*O*-benzoyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-D-glucopyranosyl uronate)-D-mannitol (**12**) (0.5 g, 36%), the purity of which was ~60% according to NMR.

4.7. Benzoylation of 12

To a stirred and cooled (0 °C) soln of crude **12** (1.5 g) in pyridine (10 mL), benzoyl chloride (0.5 mL) was added at such a rate to keep the temperature below 20 °C. After 1.5 h at rt the mixture was diluted with CH_2Cl_2 to give after usual processing and concentration of the organic soln 2,5-anhydro-1,3,6-tri-*O*-benzoyl-4-*O*-(methyl 2,3,4-tri-*O*-acetyl-D-glucopyranosyl uronate)-D-mannitol **13** (0.9 g, 52%), mp 140–141 °C (EtOH), R_f 0.6 (B); $[\alpha]_D$ −18. Anal. Calcd for $C_{40}H_{40}O_{17}$: C, 60.60; H, 5.09. Found: C, 60.72; H, 5.12.

4.8. Reaction of trichloroimidate 6 with tribenzoate 9

To a stirred soln of crude imidate **6** (purity ~50%,12 g, 12.5 mmol) and tribenzoate **9** (5.7 g, 12 mmol) in CH_2Cl_2 (100 mL) molecular sieve (4 Å) (20 g) was added. After 30 min the mixture was cooled to −35 °C and TMSOTf (0.5 mL) was added. The temperature was kept at −30 °C for 8 min, thereafter the reaction was quenched with Et_3N (1 mL), diluted with CH_2Cl_2, washed with water, dried and concentrated. The residue was submitted to column chromatography (G) to give after concentration of the fractions having R_f 0.5 a syrup (8 g, 84%) the structure of which according to NMR corresponded to methyl 3,4-di-*O*-acetyl-1,2-(*S*)-*O*-[1′-O-(2,5-anhydro-1,3,6-tri-*O*-benzoyl-D-mannito-4-yl)-ethylidene]-α-D-glucopyranuronate **11**; $[\alpha]_D$ +11. Anal. Calcd for $C_{40}H_{40}O_{17}$: C, 60.60; H, 5.09. Found: C, 60.43; H, 5.18.

4.9. 2,5-Anhydro-3-*O*-(methyl β-D-glucopyranosyl uronate)-D-mannitol (14)

A stirred slurry of **13** (1.2 g, 1.5 mmol) in MeOH (20 mL) and 2 M methanolic NaOMe (0.3 mL) was stirred at 45 °C for 5 h. The cooled soln was neutralised with a cation exchange resin, filtered and concentrated. The residue was dissolved in water, extracted with ether and the aq soln was concentrated to give **14** (0.5 g, 93%) as a syrup. R_f 0.3 (H). Anal. Calcd for $C_{13}H_{22}O_{11}$: C, 44.07, H, 6.26. Found: C, 43.88, H, 6.52.

4.10. Reaction of 3-*O*-benzyl-L-idose (16) with benzaldehyde

To a stirred soln of **16**[20] (3.1 g, 11.5 mmol) in benzaldehyde (10 mL), CF_3COOH (0.5 mL) was added. The mixture was stirred at 20 °C for 3 h, then it was neutralised with Et_3N (1.5 mL), diluted with CH_2Cl_2, washed with water, dried and concentrated. The residue was treated with hexane (2 × 25 mL), the hexane soln was decanted, and the residue (4.3 g) submitted to column chromatography using first E and then B for elution.

Concentration of the first fraction gave 3-*O*-benzyl-1,2-(*R*):5,6-(*S*)-di-*O*-benzylidene-L-idofuranose (**17**) (30 mg, 1%), R_f 0.3 (E). Anal. Calcd for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87. Found: C, 72.55; H, 5.96.

Concentration of the second fraction gave a 1:1 mixture of **17** and the corresponding 3-*O*-benzyl-1,2-(*R*):5,6-(*R*)-di-*O*-benzylidene isomer (**18**), (150 mg, 3%), R_f 0.3 (E). Anal. Calcd for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87. Found: C, 72.47; H, 5.99.

Concentration of the third fraction gave 3-*O*-benzyl-1,2-(*R*):4,6-(*R*)-di-*O*-benzylidene-L-idopyranose (**19**), (360 mg, 7%), R_f 0.2 (E), R_f 0.8 (B). Anal. Calcd for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87. Found: C, 72.47; H, 5.56.

Concentration of the last fraction, using solvent B for elution gave 3-*O*-benzyl-4,6-(*R*)-*O*-benzylidene-L-idopyranose (**20**) (2.4 g, 58%), R_f 0.5 (B) which according to NMR contained the α/β-anomers in a ratio of 3:7. According to lit.[19] the two anomers were isolated as a 1:1 mixture in a yield of 49%.

4.11. Conversion of 20 into 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-L-idopyranose (24)

A soln of **20** (13 g, 35.5 mmol) in pyridine (50 mL) and acetic anhydride (30 mL) was kept at rt for 20 h (R_f 0.2 → 0.6, EtOAc–hexane 1:2), to give after usual processing crude 1,2-di-*O*-acetyl-3-*O*-benzyl-4,6-(*R*)-*O*-benzylidene-L-idopyranose **21** (13.9 g, 80%) as syrup containing according to NMR spectroscopy the α,β-anomers in a ratio of 3:7.

To a soln of crude **21** (2.9 g) in CH_2Cl_2 (300 mL) CF_3COOH (10 mL) and water (5 mL) were added at 0 °C. The mixture was kept at this temperature for 4 h (R_f 0.6 → 0.05, C) and was then treated with a cold (0 °C) aq $NaHCO_3$ soln. The organic soln was washed

with water, dried and concentrated to give after column chromatography 1,2-di-*O*-acetyl-3-*O*-benzyl-L-idopyranose **22** (1.3 g, 61%) as syrup R_f 0.3, H) as a mixture of the two anomers. The ^{1}H NMR spectrum of this mixture was identical with that, described in lit.[19]

To a stirred soln of crude **22** (1.3 g, 3.67 mmol) in CH_2Cl_2 (25 mL), Et_3N (0.7 mL), TBDMSi-Cl (0.66 g, 1.2 equiv) and subsequently DMAP (25 mg) were added at rt. After 24 h the reaction (R_f 0.3 → 0.95, H), was not completed, therefore further TBDMSi-Cl (0.1 g) was added. After 24 h at rt acetic anhydride (3 mL) and Et_3N (3 mL) was added but no change could be monitored by TLC (R_f 0.4, D). After 1 h, MeOH (5 mL) was added and after 30 min the mixture was processed the usual way to give after concentration of the organic solution 1,2-di-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-L-idopyranose **23** (1.7 g, ~100%), which according to NMR was a 1:2 mixture of the α,β-anomers). Anal. Calcd for $C_{23}H_{36}O_8Si$: C, 58.95; H, 7.74; Si, 5.99. Found: C, 58.73; H, 7.52; Si, 5.08.

To a stirred soln of an anomeric mixture of crude **23** (1.8 g, 3.85 mmol) in CH_2Cl_2 (20 mL), pyridine (3 mL) and subsequently acetyl chloride (1 mL) were added at 0 °C. The mixture was kept at rt for 3 days, then MeOH (1 mL) was added and after 30 min the mixture was processed the usual way to give after concentration a syrup, which was submitted to column chromatography (D). Concentration of the first fraction afforded 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-α-L-idopyranose **24**α (120 mg, 6.1%), $[\alpha]_D$ −33 (*c* 0.7, $CHCl_3$), R_f 0.5 (D). Anal. Calcd for $C_{25}H_{38}O_9Si$: C, 58.80; H, 7.50; Si, 5.50. Found: C, 58.72; H, 7.57; Si, 5.37.

Concentration of the second fraction gave **24**α + **24**β (550 mg, 28%), while concentration of the third fraction gave pure 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-β-L-idopyranose **24**β (420 mg, 21%), $[\alpha]_D$ +30 (*c* 1, $CHCl_3$), R_f 0.45 (D). Anal. Calcd for $C_{25}H_{38}O_9Si$: C, 58.80; H, 7.50; Si, 5.50. Found: C, 58.68; H, 7.72; Si, 5.39.

4.12. 1,2,6-Tri-*O*-acetyl- (25) and 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-L-idopyranose (26)

To a soln of the anomeric mixture of the 6-*O*-silyl derivative **24**α + **24**β (6.5 g, 12.7 mmol) in acetone (65 mL) 10 N aq sulfuric acid (6 mL) was added at 0 °C. After 2 h the mixture was neutralised with solid $NaHCO_3$ (10 g), filtered, concentrated and the residue submitted to column chromatography using B for elution. Concentration of the first fraction (R_f 0.4) afforded **25** (0.9 g, 18%), which according to NMR was a 1:2 mixture of the α,β-anomers.

Concentration of the second fraction (R_f 0.3) afforded **26** (2.0 g, 40%), which, according to NMR was a 2:3 mixture of the α,β-anomers. Anal. Calcd for $C_{19}H_{24}O_9$: C, 57.57; H, 6.10. Found: C, 57.33; H, 6.18.

4.13. Methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-L-idopyranosyluronate (29)

Method (*a*). To a stirred soln of **26** (1.13 g) and TEMPO (10 mg) in CH_2Cl_2 (15 mL) saturated aqueous $NaHCO_3$ (9 mL), KBr (50 mg) and Bu_4NBr (75 mg) were added. The mixture was cooled to 0 °C and a mixture, containing 0.6 M NaOCl soln (37 mL), satd aq $NaHCO_3$ (5 mL) and satd NaCl soln (10 mL) was added during a period of 45 min. The organic phase was separated and washed with water (2 × 10 mL). The combined aqs solutions were acidified with 5 N HCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic solns were dried over Na_2SO_4 and concentrated to yield crude 6-carboxylic acid **28** (0.76 g), which was dissolved in DMF (20 mL) and stirred in the presence of $KHCO_3$ (1.0 g) and MeI (0.6 mL) at rt for 16 h. The residue of the concentrated mixture was dissolved in CH_2Cl_2, washed with water, dried and concentrated to yield **29** (0.74 g, 61%) as a syrup. According to NMR, this was a mixture containing the α,β-anomers in a ratio of ~1:2. Anal. Calcd for $C_{20}H_{24}O_{10}$: C, 56.60; H, 5.70. Found: C, 56.37; H, 5.88.

Method (*b*). A soln of **27** (0.96 g) in pyridine (7 mL) and Ac_2O (0.5 mL) was kept at rt overnight to give after usual processing **29** (0.75 g, 71%) as a syrup, which was identical with that, obtained according method (*a*).

4.14. Methyl 1,2-di-*O*-acetyl-3-*O*-benzyl-L-idopyranosyluronate (27)

To a stirred soln of **22** (1.42 g) and TEMPO (14 mg) in CH_2Cl_2 (20 mL), satd aq $NaHCO_3$ (12 mL), KBr (66 mg) and Bu_4NBr (100 mg) were added. The mixture was cooled to 0 °C and a mixture, containing a 0.6 M NaOCl soln (50 mL), satd aq $NaHCO_3$ (6.6 mL) and satd NaCl soln (13 mL) was added during a period of 45 min. The organic phase was separated and washed with water (2 × 15 mL). The combined aq solutions were acidified with 5 N HCl and extracted with CH_2Cl_2 (3 × 25 mL). The dried organic soln was concentrated to yield crude 6-carboxylate **30** (1.0 g), which was dissolved in DMF (30 mL) and stirred in the presence of $KHCO_3$ (1.35 g) and MeI (0.8 mL) at rt for 16 h. The residue of the concentrated mixture was dissolved in CH_2Cl_2, washed with water, dried and concentrated to yield **27** (0.96 g, 62%) as a syrup. According to NMR, this was a mixture containing the α,β-anomers in a ratio of ~1:2. Anal. Calcd for $C_{18}H_{22}O_9$: C, 56.54; H, 5.80. Found: C, 56.35; H, 6.00.

4.15. Methyl(phenyl-2,4-di-*O*-acetyl-3-*O*-benzyl-1-thio-α-L-idopyranosyl)uronate (31)

To a stirred soln of an anomeric mixture of **29** (2.12 g, 5.0 mmol) and thiophenol (0.7 mL, 6.8 mmol) in CH_2Cl_2 (40 mL), $BF_3{\cdot}Et_2O$ (2 mL) was added at rt. After 2 h the

mixture was poured into 4% aq $NaHCO_3$ solution, washed with water, dried and concentrated. The residue was submitted to column chromatography (C), to yield **31** (1.24 g, 52%), R_f 0.5. Anal. Calcd for $C_{24}H_{26}O_8S$: C, 60.75; H, 5.52; S, 6.76. Found: C, 60.77; H, 5.77; S, 6.66.

4.16. 2,5-Anhydro-1,6-di-*O*-benzoyl-3-*O*-[methyl (2,4-di-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl)uronate]-D-mannitol (32)

A soln of **31** (0.94 g, 2.0 mmol) and **6** (0.74 g, 2.0 mmol) in dry CH_2Cl_2 (25 mL) was stirred in the presence of molecular sieve (5 g) for 30 min. Thereafter the mixture was cooled to −40 °C and NIS (1.35 g, 6.0 mmol) as well as TMSOTf (120 μL) were added. The mixture was stirred at −40 °C for 20 min, then Et_3N (2 mL) was added and the temperature was raised to rt. The mixture was filtered, the filtrate was diluted with CH_2Cl_2 and was washed with aq $Na_2S_2O_3$ and subsequently with a $NaHCO_3$ soln and water. The dried organic soln was concentrated and the residue purified by column chromatography (B). Concentration of the fractions having R_f 0.5 gave **32** (0.3 g, 21%); $[\alpha]_D$ −13 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{38}H_{40}O_{15}$: C, 61.95; H, 5.47. Found: C, 62.17; H, 5.59.

4.17. 2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α-L-idopyranosyl bromide (34)

Under Ar, a soln of $TiBr_4$ (3.1 g, 8.4 mmol) in CH_2Cl_2 (30 mL) was added to a stirred soln of tetraacetate **33** (2.7 g, 6.2 mmol) in CH_2Cl_2 (15 mL) and EtOAc (7.5 mL). The dark brown soln was stirred at rt for 6 h when according to TLC the reaction was completed (R_f 0.3 → 0.2, (C). Thereafter toluene (60 mL) and subsequently sodium acetate (10 g) were added gradually to the mixture until its colour turned yellow. The slurry was filtered, washed with toluene and the filtrate evaporated to yield **34** as a pale yellow syrup (2.8 g, 98%), R_f 0.3 (B). No data are given in lit.[20]

4.18. Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-L-idopyranoside (35)

To a stirred soln of crude **33** (40 g, 91 mmol) in CH_2Cl_2 (500 mL), thiophenol (11 mL, 107 mmol) and $BF_3{\cdot}Et_2O$ (31 mL, 245 mmol) were added at 0 °C. Stirring was continued at rt for 1.5 h, thereafter the mixture was washed with a 5% $NaHCO_3$ soln and water, dried, concentrated and the residue (46 g) was submitted to column chromatography using solvent D for elution.

Concentration of the fraction having R_f 0.5 (C) gave **35**α (28 g, 63%); $[\alpha]_D$ −95 (*c* 1, $CHCl_3$). Lit.[19] $[\alpha]_D$ −91 (*c* 0.56, $CHCl_3$). The ^1H NMR spectrum was identical with that described in lit.[19]

4.19. Phenyl 3-*O*-benzyl-1-thio-α-L-idopyranoside (36α)

To a soln of **35**α (6.4 g) in MeOH (70 mL), 2 M methanolic NaOMe was added and after 2 h at rt the mixture was neutralised with solid CO_2. The residue of the concentrated mixture was purified by column chromatography (B) to yield **36**α (3.4 g, 71.5%), $[\alpha]_D$ −151 (*c* 1, $CHCl_3$), R_f 0.2 (B). Anal. Calcd for $C_{19}H_{22}O_5S$: C, 62.96; H, 6.12; S, 8.85. Found: C, 63.08; H, 6.19; S, 8.72.

4.20. Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-L-idopyranoside (37)

To a soln of **36**α (10 g) and *p*-tolylsulfonic acid (50 mg) in dry DMF (100 mL) benzaldehyde dimethyl acetal (10 mL) was added and the mixture was stirred at 70 °C under diminished pressure (10 kP) for 5 h. The cooled solution was diluted with ether, washed with water, dried over Na_2SO_4 and concentrated. The residue was submitted to column chromatography (E). Concentration of the fraction having R_f 0.55 yielded **37**α (8 g, 64%), $[\alpha]_D$ −145 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{26}H_{26}O_5S$: C, 69.31; H, 5.82; S, 7.12. Found: C, 69.22; H, 5.99; S, 6.89.

When an anomeric mixture of crude **36** was used as starting material, besides **37**α (46%) some of the β-anomer **37**β (1 g, 8%) was isolated too; $[\alpha]_D$ +80 (*c* 1, $CHCl_3$); R_f 0.4. Anal. Calcd for $C_{26}H_{26}O_5S$: C, 69.31; H, 5.82; S, 7.12. Found: C, 69.14; H, 5.70; S, 6.92.

4.21. Phenyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-L-idopyranoside (38α)

(a) A soln of **37**α (3.5 g, 7.8 mmol) in pyridine (10 mL) and Ac_2O (5 mL) was kept overnight at rt to give after usual processing a semisolid residue (3.64 g, 95%), which was recrystallised from 1.5-fold EtOH to give **38**α (2.5 g). The mother liquor was concentrated and the residue purified by column chromatography (E) to give a second crop of **38**α (0.6 g). Combined yield 80%. Mp 120–122 °C; R_f 0.4 (E), $[\alpha]_D$ −123 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{28}H_{28}O_6S$: C, 68.27; H, 5.73; S, 6.51. Found: C, 68.22; H, 5.79; S, 6.60.

When **37**β was used as starting material, the corresponding β-anomer **38**β was obtained as syrup; R_f 0.4 (E), $[\alpha]_D$ +16 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{28}H_{28}O_6S$: C, 68.27; H, 5.73; S, 6.51. Found: C, 68.11; H, 5.95; S, 6.41.

(b) To a stirred soln of diacetate **21** (13.5 g, 30.5 mmol) and thiophenol (4 mL, 36 mmol) in CH_2Cl_2 (240 mL) $BF_3{\cdot}Et_2O$ (10 mL, 79 mmol) was added at −20 °C. The mixture was stirred this temperature for 30 min and subsequently at −10 °C for 45 min. Thereafter it was cooled to −20 °C and Et_3N (17 mL) was added. The mixture was warmed to rt, washed with water, dried and concentrated. The resulting semisolid

residue was recrystallised from 1.5-fold EtOH to give **38**α (5.5 g). The mother liquor was concentrated and the residue purified by column chromatography (E) to give a second crop of **38**α (2 g). Combined yield 50%. Identical with that described above.

4.22. 3-*O*-(2-*O*-Acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-L-idopyranosyl)-2,5-anhydro-1,6-di-*O*-benzoyl-D-mannitol (39)

A soln of thioglycoside **38**α (6.2 g, 12.6 mmol) and 2,5-anhydro-1,6-di-*O*-benzoyl-D-mannitol **7** (5.6 g, 15 mmol) in dry CH_2Cl_2 (180 mL) was stirred in the presence of molecular sieve (18 g) for 30 min. Thereafter the mixture was cooled to −40 °C and NIS (5.6 g, 25 mmol) as well as TfOH (0.35 mL) were added. The mixture was stirred at −40 °C for 20 min, then Et_3N (1 mL) was added and the temperature was raised to rt. The mixture was filtered, the filtrate was diluted with CH_2Cl_2 and was washed with aq $Na_2S_2O_3$ and subsequently with $NaHCO_3$ solution and water. The dried organic solution was concentrated and the residue purified by column chromatography (D → C). Concentration of the fractions having R_f 0.3 (C) gave **39** (7.7 g, 81%) as syrup, $[\alpha]_D$ −28 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{42}H_{42}O_{13}$: C, 66.84; H, 5.61. Found: C, 66.61; H, 5.77.

4.23. 2,5-Anhydro-1,4,6-tri-*O*-benzoyl-3-*O*-(2-*O*-acetyl-3,4-di-*O*-benzoyl-α-L-idopyranosyluronic acid)-D-mannitol (40)

To a soln of **44** (13.85 g, 15.65 mmol) in CH_2Cl_2 (100 mL), TEMPO (50 mg), KBr (260 mg), Bu_4NBr (400 mg) and 4% aq $NaHCO_3$ (100 mL). Thereafter a mixture of 0.6 M aq NaOCl (180 mL), 4% aq $NaHCO_3$ (25 mL) and brine (50 mL) was added gradually at 0 °C during 45 min. The mixture was separated, the aq soln was extracted with CH_2Cl_2, the combined organic solutions were washed water, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography, using first 2:1 EtOAC–hexane for eluting the faster running by-products and then 5:1 EtOAc–EtOH for eluting the main product (R_f 0.85). Concentration of this fraction gave **40** (10.65 g, 74.7%) as solid foam, which was a ∼1:3 mixture of the free carboxylic acid and its sodium salt, $[\alpha]_D$ −40 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{49}H_{42}O_{17}$: C, 65.19; H, 4.69. Found: C, 65.08; H, 4.79.

4.24. Sodium 2,5-anhydro-3-*O*-(-α-L-idopyranosyluronate)-D-mannitol (41)

To a soln of **40** (2.25 g, 2.5 mmol) in dry MeOH (50 mL), 2 M NaOMe (1.5 mL, 3 mmol) was added. The mixture was kept at rt for 30 h, treated with a cation exchange resin (H^+), filtered and concentrated. The residue was dissolved in water (10 mL) and extracted with $CHCl_3$ for removing methyl benzoate. The pH of the aq soln was adjusted to 8 with M NaOH to give after freeze-drying the sodium salt **41** (0.7 g, 82.5%), which was filtered with EtOH. According to NMR investigations this adopts in Me_2SO soln predominantly the $^4C_1$ conformation, $[\alpha]_D$ 0 (*c* 1, water). When TFA was added to the NMR soln, the liberated free acid was present in the $^1C_4$ conformation. The free acid showed a negative optical rotation of $[\alpha]_D$ −12 (*c* 1, water). Anal. Calcd for $C_{12}H_{19}NaO_{11}$: C, 39.79; H, 5.29; Na, 6.35. Found: C, 39.99; H, 5.38; Na, 6.30.

4.25. 3-*O*-(2-*O*-Acetyl-3,4-di-*O*-benzoyl-6-*O*-*t*-butyltrimethylsilyl-α-L-idopyranosyl)-2,5-anhydro-1,2,6-tri-*O*-benzoyl-D-mannitol (43)

To a stirred soln of **45** (11.4 g, 20.14 mmol) in pyridine (70 mL) *tert*-butyldimethylsilyl chloride (3.65 g, 24 mmol) was added at 0 °C. Stirring was continued at 20 °C for 2 h, then further *tert*-butyldimethylsilyl chloride (1 g, 6.6 mmol) was added and the mixture was kept overnight at rt. According to TLC, the starting material was completely converted into **42** (R_f 0.2 → 0.7, A). After cooling the mixture to 0 °C, benzoyl chloride (8.75 mL, 75.5 mmol) was gradually added (10 min) and the temperature was raised to rt. After 2 h, the mixture was poured onto ice, extracted with CH_2Cl_2 to give after usual processing and concentration a residue, which was purified by column chromatography (C). The fractions having R_f 0.7 afforded on concentration **43** (20 g, ∼100%) as syrup, $[\alpha]_D$ −55 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{55}H_{58}O_{16}Si$: C, 65.85; H, 5.83; Si, 2.80. Found: C, 65.61; H, 5.97; Si, 2.62.

4.26. 3-*O*-(2-*O*-Acetyl-3,4-di-*O*-benzoyl-α-L-idopyranosyl)-2,5-anhydro-1,4,6-tri-*O*-benzoyl-D-mannitol (44)

To a stirred soln of **43** (20 g, 20.36 mmol) in MeOH (300 mL) and EtOH (300 mL), M H_2SO_4 (40 mL) was added at rt. After 30 min, further M H_2SO_4 (20 mL) was added and after 5 h when according to TLC the reaction was completed (R_f 0.7 → 0.4, C) solid $NaHCO_3$ was added gradually until a pH of 5 was obtained. The mixture was filtered and concentrated. The residue was dissolved in CH_2Cl_2, washed with 5% $NaHCO_3$ and water, dried and concentrated. The residue was purified by column chromatography (C), to give **44** (17 g, 97%) as syrup, $[\alpha]_D$ −61 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{49}H_{44}O_{16}$: C, 66.21; H, 4.99. Found: C, 66.14; H, 5.09.

4.27. 3-*O*-(2-*O*-Acetyl-α-L-idopyranosyl)-2,5-anhydro-1,6-di-*O*-benzoyl-D-mannitol (45)

A soln of **39** (4.7 g, 6.2 mmol) in MeOH (150 mL) water (15 mL) and AcOH (0.5 mL) was saturated at rt with hydrogen using Pd–C (10%, 0.5 g) as catalyst. After 6 h,

when according to TLC the reaction was completed (R_f $0.9 \rightarrow 0.3$, A) the filtered soln was concentrated. Toluene was added to and evaporated from the residue which, after recrystallisation from EtOAc–hexane, gave **45** (3.44 g, 95%), mp 142–143 °C, R_f 0.3; $[\alpha]_D$ −13 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{28}H_{32}O_{13}$: C, 58.33; H, 5.59. Found: C, 58.29; H, 5.63.

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