

140. Synthesis of an *N*-Acetylated Heparin Pentasaccharide and its Anticoagulant Activity in Comparison with the Heparin Pentasaccharide with High Anti-Factor-Xa Activity

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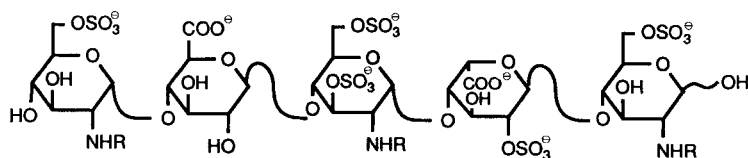
(7. VII. 89)

The synthesis of tri-*N*-acetylated heparin pentasaccharide **2** is described. It was assembled from five suitably blocked monosaccharide units (**3–7**). Glucuronic-acid building block **4** was prepared from glucose by direct Jones oxidation of the 6-*O*-trityl derivative **18**. The resulting acid **16** was esterified to **17** in large amounts using methyl chloroformate/base. Trimethylsilyl bromide proved to be an excellent reagent for the hydrolysis of a prop-1-enyl glycoside (**19** → **21**). The pentasaccharide **29** was obtained by a [2 + 2] + 1 synthesis, the glycosylation reactions furnished good to very good yields. The identity of protected oligosaccharides was confirmed by ¹H-NMR spectroscopy. Sequential deblocking of the pentasaccharide, *O*-sulfation, and *N*-acetylation gave **2** which was shown to exhibit *ca.* 600 times lower anticoagulant activity than pentasaccharide **1**.

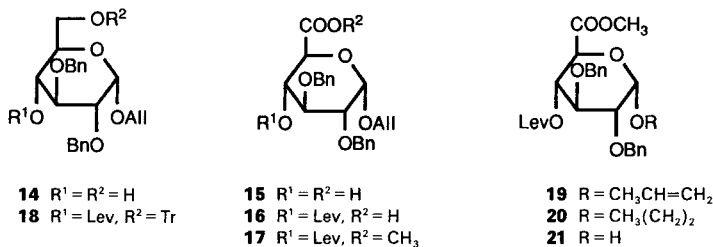
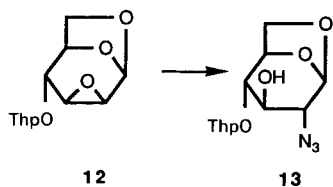
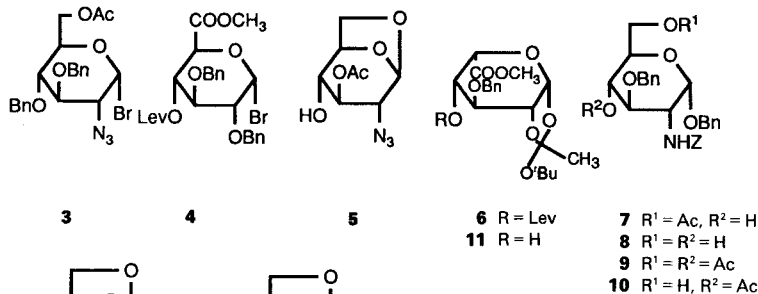
Introduction. – Heparin is a sulfated glucosaminoglycuronan used in the clinic for over 50 years because of its anticoagulant properties mediated by interaction with antithrombin III (AT III). Degradation of heparin followed by investigation of heparin fragments led to the hypothesis that a specific heparin sequence represents the binding-site for AT III [1] [2]. Since then, three groups [3] have reported syntheses of the heparin pentasaccharide **1**, the fragment with high affinity for AT III. Several analogues have been prepared to study structure-activity relations [4]. In addition to its anticoagulant properties, heparin exerts a number of biological effects, including antilipemic [5], antiangiogenic [6], and antiproliferative activities [7]. Therefore, we turned our attention to non-anticoagulant heparin fractions. A de-*N*-sulfated, *N*-acetylated heparin was described that lacked its anticoagulant activity, but retained the anticomplementary activity and the ability to inhibit growth of smooth muscle cells [8]. We now report on the synthesis of the tri-*N*-acetylated heparin pentasaccharide **2**.

Results and Discussion. – For the assembly of the heparin pentasaccharide we adopted the approach of Sinaý *et al.* [3a] starting with five suitably blocked monosaccharide units, namely **3** [9], **4** [4f], **5** [10], **6**, and **7** [11]. For the selective acetylation of **8** [12], we employed equimolar amounts of Ac₂O instead of *N*-acetylimidazole [11], furnishing monoacetate **7** in 83% yield, along with diacetate **9** ([13], 6%), 4-*O*-acetate **10** (6%), and starting material **8** (3%). Iduronic ester **11** [11] was prepared from glucose or, in a shorter synthesis, from 6,3-glucuronolactone *via* alkylating lactone opening [14]. For temporary protection of the 4-OH group, we preferred a levulinic ester (= 4-oxopentanoate) which usually is cleaved in excellent yield. Thus, treatment of **11** with levulinic anhydride in pyridine gave **6**. Azido sugar **5** was prepared as described [10] but using LiN₃ (*cf.* [3b])

Scheme 1



- 1** R = SO₃⁻
2 R = Ac
30 R = H



Bn = benzyl, Lev = levulinoyl, Z = benzyloxycarbonyl, Thp = tetrahydropyranyl, Tr = trityl, AlI = allyl

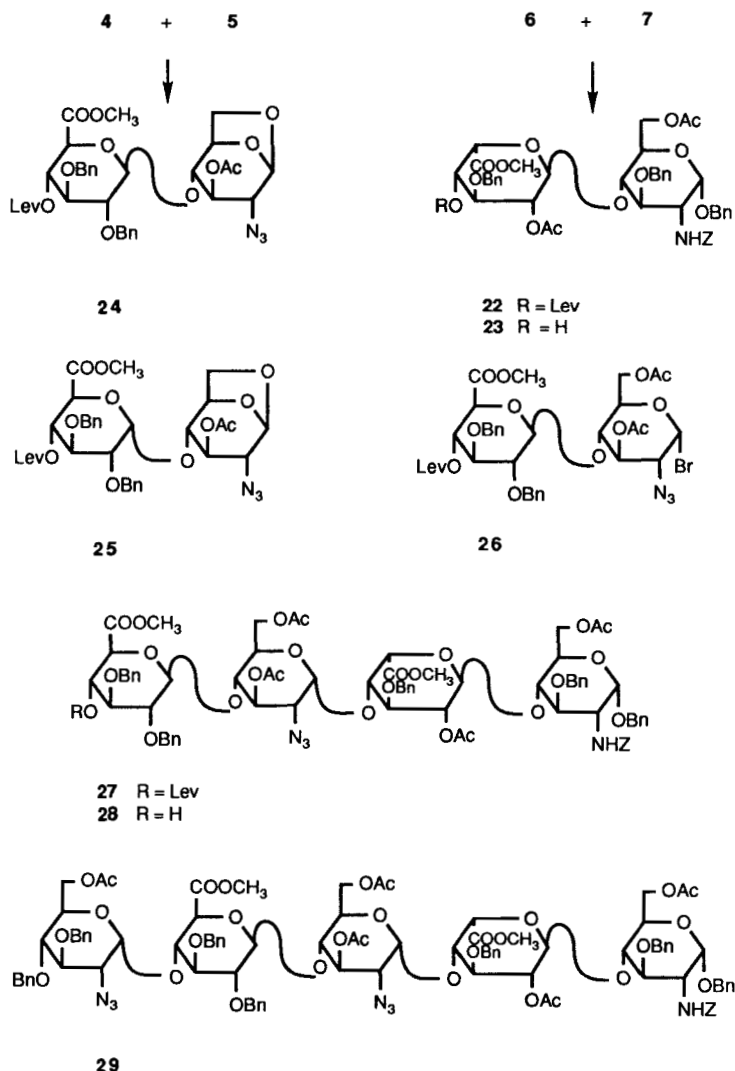
instead of NaN₃ [9] in the opening of the 2,3-anhydro moiety of **12** to furnish **13** in improved yield (95%).

For the synthesis of building block **4** [4f], we started with allyl 4,6-*O*-benzylidene- α -D-glucopyranoside which is favourably obtained by *Fischer* glycosylation using trifluoromethanesulfonic acid [15] followed by benzylidenation. Benzylation and acetal cleavage gives intermediate **14** [10] which can be converted to glucuronic acid **15** by selective tritylation, acetylation, selective detritylation, oxidation, and deacetylation [10]. In our scheme, we omitted the detritylation step and directly oxidized the trityl compound. To

avoid large-scale esterification of the uronic acid with diazomethane, alternative approaches were investigated. Esterification of **15** using DMF dimethyl acetal [16] gave only small amounts of the corresponding methyl ester. Therefore, the 4-OH group of **15** was first protected by levulinoylation to give **16**. With the carboxyl group now being the only unprotected function, esterification of **16** proceeded smoothly to **17**, without intermediate purification, using methyl chloroformate in the presence of Et₃N and 4-(dimethylamino)pyridine [18]. We then found that the levulinoyl group is compatible with the oxidation step allowing omission of the intermediate protection/deprotection of the 4-OH function. Thus, diol **14** was selectively tritylated at the primary OH group and converted to the 4-*O*-levulinoyl derivative **18** in 93% yield. Direct Jones oxidation of the trityl compound **18** furnished glucuronic acid **16** which was transformed into the uronic ester **17** without intermediate purification, as described above. Isomerization of the allyl double bond in **17** was performed with Wilkinson's catalyst and diazobicyclooctane as base to give the prop-1-enyl derivative **19** [4f] in good yield (82%) along with some propyl glycoside **20**. Separation from by-products was more effective after removal of the prop-1-enyl group so that at this stage no purification was carried out. Cleavage of the prop-1-enyl group is usually accomplished with mercuric salts [18] which in our hands were difficult to remove quantitatively. We found that trimethylsilyl bromide in CH₂Cl₂ in the presence of molecular sieves is an excellent reagent for the hydrolysis of the prop-1-enyl glycoside. No glycosyl bromide was detected in this reaction. Thus, hemiacetal **21** was obtained from **19** in 75% yield. Bromination with Vilsmeier salt [19], preferentially prepared by the *in situ* method with catalytic amounts of DMF [20], furnished building block **4** [4f].

For the synthesis of disaccharide **22**, orthoester **6** was coupled with OH component **7** according to the method of Kochetkov *et al.* [21]. Removal of the levulinic ester afforded the known [11] disaccharide glycosyl acceptor **23** in 98% yield. The second disaccharide unit **24** [3b] was obtained in good yield (71%) from bromide **4** and OH component **5** employing silver zeolite [22] as insoluble catalyst; the α -D-linked isomer **25** was isolated as a by-product (11%). Opening of the 1,6-anhydro ring of **24** with Ac₂O/CF₃COOH followed by bromination produced glycosyl donor **26** as described [3b]. Coupling of disaccharide **26** with glycosyl acceptor **23** was performed with Ag₂CO₃/AgClO₄ 10:1 [23], a catalyst system which in many instances is more effective than silver triflate/base systems, and the results (61% of **27**) compare well with those obtained upon use of silver triflate alone in the synthesis of the benzyl β -D-tetrasaccharide analogue [3b]. As a by-product in this glycosylation, only the hydrolysis product of the disaccharide bromide **26** was obtained, but no tetrasaccharide β -D-linked at the newly formed glycosidic bond was found. The levulinic ester in **27** could again be removed in excellent yield to give known tetrasaccharide **28** [10]. The pentasaccharide synthesis from **28** and bromide **3** [9] proceeded as described [10] to give **29** (81%) along with some undesired β -D glycoside (16%). In this case, silver triflate was the catalyst of choice being superior to Ag₂CO₃/AgClO₄ with respect to yield and α -D/ β -D selectivity. The identity of the blocked oligosaccharides was proven by a complete analysis of the ¹H-NMR spectra using the COSY technique in uncertain cases. These data led to the revision of some tentative assignments in the literature [3b] [10]. Deblocking and *O*-sulfation of the pentasaccharide was carried out as described [10] to give **30**. This pentasaccharide was treated with Ac₂O in H₂O yielding the desired tri-*N*-acetylated heparin pentasaccharide **2**.

Scheme 2



This new heparin pentasaccharide was investigated with respect to its anticoagulant properties using amidolytic assays for thrombin and factor Xa as well as clotting assays. The chromogenic-substrate assays demonstrate the direct inhibition of thrombin (factor IIa) or Xa. In the anti-Xa clotting assay (modified *Denson* and *Bonnar* assay [24]), bovine factor Xa is inactivated with heparin-containing plasma. Residual factor Xa leads to clotting of factor-X-deficient substrate plasma upon recalcification. The aPTT (activated partial thromboplastin time) is a plasma recalcification time in which addition of a platelet substitute eliminates variable platelet procoagulant activity. Since heparin complexed to antithrombin III inhibits several clotting factors, the test has been advocated to

reflect the overall effect of heparin on blood coagulation [25]. Due to the non-parallelism of the dose/response curves with standard heparin in these tests, the effects of the pentasaccharides are characterized by IC_{50} values [$\mu\text{g/ml}$] indicating the concentration of the compound leading to a clotting time of twice the control.

Inspection of the *Table* shows that the effect of the tri-*N*-acetylated pentasaccharide **2** is much less pronounced by a factor of *ca.* 600 if compared to the tri-*N*-sulfated pentasaccharide **1**. Both compounds do not act on thrombin (no inhibition at 1000 $\mu\text{g/ml}$);

Table. *Biological Data of Pentasaccharides*

	IC_{50} [$\mu\text{g/ml}$]			
	Clotting assays		Amidolytic assays	
	aPTT	Xa	IIa	Xa
2	> 1000	66	> 1000	970
1	525	0.115	> 1000	1.55

moreover, the tri-*N*-acetylated pentasaccharide has practically no effect on the overall blood coagulation as shown by the aPTT value. The latter finding is in keeping with literature reports indicating that *N*-desulfated *N*-acetylated heparin has no effect on coagulation as assessed by whole-blood clotting tests [26] [27].

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Experimental Part

General. See [28]. Chromatography (CC), if not otherwise specified, refers to separations on silica gel 60 (*Merck*, 0.063–0.200 mm); MPLC = medium-pressure liquid chromatography, gel permeation chromatography fractions were monitored with an *LKB 2238 Uvicord S II* at 206 nm, signals were recorded with an *LKB 2210* recorder. ^{13}C -NMR: *Bruker AM 400* (100.6 MHz) with *Aspect 3000* computer, chemical shifts in ppm relative to 3-(trimethylsilyl)(D₄)propionate as internal standard.

Biological Methods. A. Anticoagulant Activities of the Pentasaccharides. For the aPTT [25], 100 μl of citrated human plasma containing various concentrations of the pentasaccharide was mixed with 100 μl of 'activated *Thrombofax*' (*Ortho Diagnostics*, Raritan, N.J., USA) at 37° for 8 min. Then, 100 μl of prewarmed 25 mM CaCl_2 was added and the clotting time registered using a semiautomatic fibrometer coagulation timer (*Becton Dickinson AG*, Basel, CH).

For the anti-Xa clotting assay [24], 25 μl of citrated plasma containing various concentrations of pentasaccharide were mixed with 75 μl of factor Xa (*Diagnostic Reagents*, Thame, Oxon, GB) diluted 1:100 in buffer (0.63% trisodium citrate, 41 mM imidazole, 82 mM NaCl and 0.1% bovine serum albumin, pH 7.3). After prewarming for 2 min at 37°, 200 μl of a 1:1 mixture of factor-X-deficient plasma and platelet substitute (both from *Diagnostic Reagents*) were added and incubated for 20 s. Clotting time was determined in the fibrometer upon adding 100 μl of prewarmed 50 mM CaCl_2 .

Heparins of low molecular weight show nonparallel dose response curves as compared with the international standard for heparin [29]. Therefore, the activities of the oligosaccharides are not expressed in heparin-like IU/mg, but characterized by the IC_{50} ($\mu\text{g/ml}$), indicating the concentration of the compound leading to a clotting time of twice the control.

B. Inhibition of Thrombin or of Factor Xa Determined in Chromogenic-Substrate Assays. Direct inhibition by the pentasaccharide of thrombin and of factor Xa has been measured using chromogenic substrates. The compounds dissolved in plasma with a surplus of antithrombin III were incubated with the enzyme. Residual amidolytic activity for the chromogenic substrates H-D-Phe-Pip-Arg-NH·pNA (*S*-2238; *Kabi Diagnostica*, Möln-dal, S) and Bz-CO-Ile-Glu-Gly-Arg-NH·pNA (*S*-2222) was determined according to *Teien et al.* [30] in a *Cobas Bio* centrifugal automatic spectrophotometer (*Roche Diagnostica*, Basel, CH). The buffer used consisted of 50 mM *Tris*, 180 mM NaCl, 7.5 mM EDTA-Na₂, 1% polyethyleneglycol 6000 and 0.02% *Tween 80*, pH 8.4. The sample consisted of 50 µl of buffer, 30 µl of antithrombin III (1 U/ml, *Kabi Diagnostica*) and of 20 µl of plasma containing various concentrations of the oligosaccharides.

To measure the inhibition of thrombin, the *Cobas Bio* pipetted into the test cuvette 30 µl of the sample and 20 µl of H₂O and mixed it with 180 µl of thrombin (1 U/ml in buffer, *Thrombin Reagent*, *Roche Diagnostica*). After incubating at 37° for 240 s, 60 µl of *S*-2238 (0.75 mM in H₂O) plus 20 µl of H₂O were added as starting reagent. The release of pNA (*para*-nitroaniline) was followed at 405 nm in 10-s intervals for 60 s in comparison to a H₂O blank.

The inhibition of factor Xa was measured likewise using bovine factor Xa (2.8 nkat/ml) and *S*-2222 (2 mM in H₂O), respectively.

Again, the inhibitory potency of the pentasaccharides was expressed as the *IC*₅₀ (µg/ml), i.e. the concentration reducing the amidolytic activity of thrombin or factor Xa by 50% as compared to the plasma control sample.

(*Methyl 3-O-benzyl-4-O-levulinoyl-β-L-idopyranuronate*) 1,2-(*tert*-Butyl orthoacetate) (**6**). Levulinic anhydride (5.0 g, 23.4 mmol) was added to an ice-cold soln. of crude **11** [11] (prepared from 4.52 g (10.6 mmol) of methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate in three steps without intermediate purification according to [11]) and 4-(dimethylamino)pyridine (0.12 g, 0.98 mmol) in pyridine (25 ml). After 17 h at 0°, the mixture was poured onto ice/sat. NaHCO₃ soln. and extracted with Et₂O. The combined org. soln. was washed with H₂O, ice-cold 10% KHSO₄ soln. (3×), brine, and sat. NaHCO₃ soln., dried (Na₂SO₄), and evaporated. The resulting brownish syrup was chromatographed on silica gel (Et₂O/hexane 1:2 → 1:1, containing 0.5% (*v/v*) of Et₃N) to give a yellowish syrup of **6** (1.1 g, 21%; two other runs each starting from 6.70 g of methyl 1,2,4-tri-*O*-acetyl-3-*O*-benzyl-β-L-idopyranuronate gave pure **6** in overall yields of 31 and 33%, resp.) containing 0.2 equiv. of Et₃N. $[\alpha]_D^{20} = -18.4$ (*c* = 1.0, CHCl₃). IR (film): 1767s, 1743s, 1720s, 1214s, 1155s, 1120s, 749m, 699m. ¹H-NMR (400 MHz, CDCl₃): 7.36–7.22 (*m*, 5 arom. H); 5.44 (*d*, *J*(1,2) = 2.4, H–C(1)); 5.19 (*dd* ≈ *t*, H–C(4)); 4.81, 4.67 (2 *d*, *J* = 12.0, PhCH₂); 4.52 (*d*, *J*(4,5) = 3.0, H–C(5)); 4.083–4.077 (*m*, H–C(2), H–C(3)); 3.79 (*s*, CH₃O); 2.76–2.71, 2.57–2.53 (2*m*, (CH₂)₂); 2.17 (*s*, CH₃CO); 1.78 (*s*, CH₃COO); 1.29 (*s*, (CH₃)₃C). EI-MS: 420 (1, *M*⁺ – (CH₃)₃COH), 99 (100, Lev⁺), 91 (78, Bn⁺). Anal. calc. for C₂₅H₃₄O₁₀ + 0.2 C₆H₁₅N (494.54 + 20.24 = 514.78): C 61.13, H 7.25, N 0.54; found: C 61.17, H 7.18, N 0.61.

Benzyl 6-O-Acetyl-3-O-benzyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside (**7**). To a soln. of **8** [12] (177.8 g, 360 mmol) in CH₂Cl₂ (2.0 l) and pyridine (350 ml) was added dropwise a soln. of Ac₂O (34 ml, 360 mmol) in CH₂Cl₂ (1.5 l) and stirred for 4.5 h at r.t. The soln. was concentrated and successively extracted with 6*N* HCl, 0.5*M* NaHCO₃ soln., and H₂O, dried (MgSO₄), and evaporated. The residue was crystallized from AcOEt/hexane to furnish pure material **8** (5.8 g, 3%, after one recrystallization). Further crystallization gave only impure product so that the mother liquor was chromatographed on silica gel (AcOEt/hexane 3:5 → AcOEt), furnishing successively pure diacetate **9** (11.7 g, 5.6%), **7** (161.2 g, 83.6%), and monoacetate **10** (10.8 g, 5.6%).

9: Colourless crystals. M.p. 132–133° ([13]: m.p. 130–132°). $[\alpha]_D^{20} = +88.8$ (*c* = 0.5, CHCl₃; [14]: $[\alpha]_D^{23} = +87$ (*c* = 0.673, CHCl₃)). ¹H-NMR (270 MHz, CDCl₃): 7.39–7.17 (*m*, 15 arom. H); 5.15 (*dd* ≈ *t*, *J*(3,4) = 10, H–C(4)); 5.11, 5.03 (2 *d*, *J* = 12.0, PhCH₂ of Z); 4.97 (*d*, *J*(1,2) = 3.5, H–C(1)); 4.90 (*d*, *J* = 10.0, NH–C(2)); 4.69, 4.50 (2 *d*, *J* = 11.6, PhCH₂); 4.58 (*s*, CH₂O); 4.20 (*dd*, *J*(6a,6b) = 12.4, H_a–C(6)); 4.12 (*ddd*, H–C(2)); 4.02 (*dd* ≈ *br. d*, H_b–C(6)); 3.90 (*ddd*, *J*(5,6_a) = 5.0, *J*(5,6_b) = 2.3, H–C(5)); 3.73 (*dd* ≈ *t*, *J*(2,3) = 9.5, H–C(3)); 2.10 (*s*, AcO–C(6)); 1.96 (*s*, AcO–C(4)).

7: Colourless crystals. M.p. 113° ([11]: m.p. 114–115°). $[\alpha]_D^{20} = +84.7$ (*c* = 1, CHCl₃), $[\alpha]_D^{20} = +124$ (*c* = 0.5, dioxane; [11]: $[\alpha]_D^{23} = +88$ (*c* = 1, CHCl₃)).

10: Colourless crystals. M.p. 150–151°. $[\alpha]_D^{20} = +93.6$ (*c* = 0.5, CHCl₃). ¹H-NMR (270 MHz, CDCl₃): 7.39–7.19 (*m*, 15 arom. H); 5.12, 5.04 (2 *d*, *J* = 12.0, PhCH₂ of Z); 5.06 (*dd* ≈ *t*, H–C(4)); 4.99 (*d*, *J*(1,2) = 3.0, H–C(1)); 4.90 (*d*, *J* = 10.0, NH–C(2)); 4.68, 4.50 (2 *d*, *J* = 11.8, PhCH₂); 4.61 (*s*, CH₂O); 4.09 (*ddd*, *J*(2,3) = 10, H–C(2)); 3.78 (*dd* ≈ *t*, *J*(3,4) = 10, H–C(3)); 2.48 (*br. t*, OH–C(6)); 2.00 (*s*, AcO–C(4)). EI-MS: 400 (1, *M*⁺ – COOBn), 91 (100, Bn⁺). Anal. calc. for C₃₀H₃₃NO₈ (535.59): C 67.28, H 6.21, N 2.62; found: C 66.70, H 6.10, N 2.63.

Allyl 2,3-Di-O-benzyl-4-O-levulinoyl-6-O-trityl-α-D-glucopyranoside (**18**). A soln. of allyl 2,3-di-*O*-benzyl-α-D-glucopyranoside [10] (**14**; 399.6 g, 997.8 mmol) in pyridine (950 ml) was reacted with trityl chloride

(= chlorotriphenylmethane; 317.5 g, 1.14 mol) at 80° for 1.5 h. More trityl chloride was added (16 g, 57 mmol), and heating was continued for 2 h. To this crude trityl derivative, 4-(dimethylamino)pyridine (23.2 g, 19 mmol) and a soln. of levulinic anhydride (329 g, 1.54 mol) in pyridine (100 ml) were added at 3°. The mixture was stirred at r.t. for 19 h, poured into ice/dil. NaHCO₃ soln., and extracted with CH₂Cl₂. Successively washing with dil. NaHCO₃ soln., 0.5N HCl, and H₂O followed by drying (MgSO₄) and CC (Et₂O/hexane 1:1) gave **18** (692.2 g, 93%) as a syrup. $[\alpha]_D^{20} = +21.5$ ($c = 0.2$, CHCl₃). ¹H-NMR (270 MHz, CDCl₃): 7.48–7.19 (*m*, 25 arom. H); 6.02 (*dddd*), 5.38, 5.27 (2 *dddd* $\approx$ *dq*), and 4.30, 4.13 (2 *dddd* $\approx$ *ddt*, allyl); 4.92 (*dd*, $J(4,5) = 11.0$, H–C(4)); 4.89 (*d*, H–C(1)); 4.85, 4.63 (2 *d*, $J = 11.8$, PhCH₂); 4.78, 4.65 (2 *d*, $J = 12.0$, PhCH₂); 3.90 (*dd* $\approx$ *t*, $J(3,4) = 9.5$, H–C(4)); 3.88 (*ddd*, $J(5,6_a) = 5.7$, $J(5,6_b) = 2.9$, H–C(5)); 3.62 (*dd*, $J(1,2) = 3.8$, $J(2,3) = 9.8$, H–C(2)); 3.10 (*dd*, $J(6_a,6_b) = 10.0$, H_a–C(6)); 3.04 (*dd*, H_b–C(6)); 2.50–2.15 (*m*, (CH₂)₂); 2.08 (*s*, CH₃CO). EI-MS and CI-MS: no *M*⁺ or high-mass fragment detectable. Anal. calc. for C₄₇H₄₈O₈ (740.89): C 76.19, H 6.53; found: C 76.27, H 6.52.

Methyl (Allyl 2,3-di-O-benzyl-4-O-levulinoyl-α-D-glucopyranosid)uronate (17). *A*: Diol **14** (355 g, 886 mmol) was selectively tritylated and acetylated essentially as described [10]. To a stirred soln. of the crude product in acetone at 3° (10.7 l) was added dropwise *Jones* reagent (3.0 l, prepared by dissolving CrO₃ (802 g) in conc. H₂SO₄ (690 ml) and diluting with H₂O to a volume of 3 l) during 2 h keeping the temp. < 10°. The mixture was then stirred at r.t. for 20 h (green precipitate). The acetone soln. was decanted and concentrated to a thin syrup. The precipitate was dissolved in ice/H₂O/CH₂Cl and, together with the syrup from the acetone soln., extracted with CH₂Cl₂. The org. phase was washed twice with H₂O and dried (Na₂SO₄). The crude product was deacetylated to **15** with NaOH/MeOH as described [10]. To the ice-cold soln. of crude **15** (280 g) in pyridine (730 ml), 4-(dimethylamino)pyridine (7.74 g, 63 mmol) and levulinic anhydride (261 g, 1.22 mol) were added. After stirring for 2 h at 0°, the mixture was poured into ice/H₂O and extracted with CH₂Cl₂. The combined org. soln. was dried (Na₂SO₄) and evaporated: crude **16** as a syrup. To a stirred soln. of crude **16** in CH₂Cl₂ (3.6 l) were added Et₃N (136 ml, 981 mmol), methyl chloroformate (72.8 ml, 944 mmol), and 4-(dimethylamino)pyridine (11.5 g, 94.2 mmol) keeping the temp. between 5 and 10°. After 45 min at that temp., the mixture was poured into ice/H₂O and extracted with CH₂Cl₂. The combined org. soln. was dried (Na₂SO₄), evaporated, and subjected to CC (Et₂O/hexane 1:1 → 2:1) to give **17** (31 g) along with impure fractions that were rechromatographed. All fractions of **17** were pooled and crystallized from Et₂O/hexane: pure **17** (92.75 g, 20% with respect to **14**, over 6 steps).

B: To a stirred soln. of **18** (342.6 g, 462.4 mmol) in acetone (5.6 l) at 5° was added dropwise *Jones* reagent (930 ml) during 50 min, then stirring was continued at r.t. for 19 h. To complete the reaction, more *Jones* reagent (93 ml) was added at 5–10°. After stirring at r.t. for 24 h, the mixture was worked up as described under *A*. The crude **16** was esterified as described above, workup furnished pure **17** (86.12 g, 35.4%; 34% with respect to **14** over 4 steps) as colourless crystals. M.p. 73.5–75°. $[\alpha]_D^{20} = +65.8$ ($c = 0.5$ dioxane). ¹H-NMR (270 MHz, CDCl₃): 7.32–7.26 (*m*, 10 arom. H); 5.92 (*dddd*), 5.33, 5.25 (2 *dddd* $\approx$ *dq*), and 4.19, 4.00 (2 *dddd* $\approx$ *ddt*, allyl); 5.04 (*dd* $\approx$ *t*, H–C(4)); 4.87, 4.60 (2 *d*, $J = 12.0$, PhCH₂); 4.86 (*d*, H–C(1)); 4.77, 4.70 (2 *d*, $J = 11.8$, PhCH₂); 4.23 (*d*, $J(4,5) = 10.2$, H–C(5)); 3.99 (*dd* $\approx$ *t*, $J(3,4) = 9.3$, H–C(3)); 3.70 (*s*, CH₃COO); 3.61 (*dd*, $J(1,2) = 3.5$, $J(2,3) = 9.6$, H–C(2)); 2.70–2.31 (*m*, (CH₂)₂); 2.15 (*s*, CH₃CO). EI-MS: 435 (2, *M*⁺ – Bn), 99 (36, Lev⁺), 91 (100, Bn⁺), 43 (10, CH₃CO). Anal. calc. for C₂₉H₃₄O₉ (526.58): C 66.15, H 6.51; found: C 66.22, H 6.54.

Methyl (Prop-1-enyl 2,3-Di-O-benzyl-4-O-levulinoyl-α-D-glucopyranosid)uronate (19). To a refluxing soln. of **17** (4.6 g, 8.7 mmol) in EtOH/toluene/H₂O 8:3:1 (60 ml) were added diazabicyclooctane (980 mg, 8.7 mmol) and (PPh₃)₃RhCl (200 mg). After 3 h at reflux, more *Wilkinson* catalyst was added (200 mg) and the reaction continued for 3.5 h. The mixture was cooled and filtered over a pad of *Speedex*, the residue was washed with acetone. The combined org. soln. was evaporated, suspended in AcOEt/hexane 1:1, and filtered. The filtrate was evaporated and submitted to CC (AcOEt/hexane 1:1) to give *methyl (propyl di-O-benzyl-4-O-levulinoyl-α-D-glucopyranosid)uronate* (**20**; 230 mg, 5%) followed by **19** (3.76 g, 82%).

19: Colourless crystals. M.p. 65–67.5° ([4f]: m.p. 80–81°). $[\alpha]_D^{20} = +10.0$ ($c = 0.2$, CHCl₃); [4f]: $[\alpha]_D^{20} = +6$ ($c = 1$, CHCl₃). ¹H-NMR (250 MHz, CDCl₃): 6.10, 5.98 (2 *dq*, 1 H, C = CHO, (*Z/E*) = 7:3).

20: Colourless crystals. M.p. 71–71.5°. $[\alpha]_D^{20} = +18.5$ ($c = 0.2$, CHCl₃). ¹H-NMR (270 MHz, CDCl₃): 7.40–7.26 (*m*, 10 arom. H); 5.04 (*dd*, $J(3,4) = 9.2$, $J(4,5) = 10.0$, H–C(4)); 4.88, 4.70 (2 *d*, PhCH₂); 4.79 (*d*, $J(1,2) = 3.6$, H–C(1)); 4.79, 4.62 (2 *d*, PhCH₂); 4.21 (*d*, H–C(5)); 3.98 (*dd* $\approx$ *t*, H–C(3)); 3.61, 3.40 (2 *dt*, $J_{\text{gem}} = 10.0$, $J_{\text{vic}} = 6.9$, CH₂O); 3.60 (*dd*, H–C(2)); 2.75–2.32 (*m*, (CH₂)₂); 1.67 (*ddq* $\approx$ *td*, CH₃CH₂); 0.92 (*t*, $J = 7.4$, CH₃CH₂). EI-MS: 437 (1, *M*⁺ – Bn), 331 (2, 437 – PhCHO), 91 (100, Bn⁺), 99 (30, Lev⁺). Anal. calc. for C₂₉H₃₆O₉ (528.58): C 65.89, H 6.86; found: C 65.60, H 6.88.

Methyl 2,3-Di-O-benzyl-4-O-levulinoyl-α-D-glucopyranuronate (21). To a soln. of crude **19** (from **17**; 46.0 g, 87.4 mmol) in CH₂Cl₂ (250 ml) containing 4-Å molecular sieves (5 g) was added Me₃SiBr (33.2 ml, 255 mmol) at 0°. After stirring for 5 h at 0°, the mixture was filtered. The filtrate was poured into ice/dil. NaHCO₃ soln. and

extracted with CH_2Cl_2 . Org. extracts were washed with H_2O , dried (Na_2SO_4), evaporated, and submitted to CC. Elution with AcOEt /hexane 3:2 furnished **21** (32.1 g, 75%), colourless crystals. M.p. 103–104°, after one crystallization from Et_2O /hexane ([4f]: m.p. 99–101°). $[\alpha]_{\text{D}}^{20} = +6.2$ ($c = 1$, CHCl_3); [4f]: $[\alpha]_{\text{D}} = +8$ ($c = 1$, CHCl_3).

Benzyl 6-O-Acetyl-3-O-benzyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-4-O-(methyl 2-O-acetyl-3-O-benzyl-4-O-levulinoyl- α -L-idopyranosyluronate)- α -D-glucopyranoside (22). From a soln. of **6** (2.8 g, 5.66 mmol) and **7** (12.13 g, 22.65 mmol) in freshly distilled chlorobenzene (95 ml), solvent was distilled off (60 ml). A soln. of 2,6-dimethylpyridinium perchlorate (11.8 mg, 56.6 μmol) in dichloroethane and chlorobenzene (5 ml) was added. The mixture was refluxed for 1.5 h while continuously distilling off the *t*-BuOH formed and keeping the volume constant by addition of chlorobenzene. After cooling, the mixture was diluted with CH_2Cl_2 , extracted with ice/dil. NaHCO_3 soln. and H_2O , dried (Na_2SO_4), and submitted to MPLC. Elution with Et_2O gave **7** (9.42 g, 78%) and colourless crystals of **22** (2.48 g, 46%). M.p. 124.0–124.5°. $[\alpha]_{\text{D}}^{20} = +41.0$ ($c = 0.2$, dioxane). $^1\text{H-NMR}$ (250 MHz, CDCl_3): 7.45–7.12 (*m*, 20 arom. H); 5.11 (*dd* $\approx$ *s*, $J(1',2') = 1.2$, $J(1',3') = 1$, $\text{H-C}(1')$); 5.04 (*dd* $\approx$ *t*, $J(3',4') = 3.0$, $J(4',5') = 2.5$, $\text{H-C}(4')$); 5.00, 4.92 (2 *d*, PhCH_2 of **2**); 4.96 (*d*, $\text{H-C}(1)$); 4.87 (*d*, $\text{H-C}(5')$); 4.86 (*dd*, $\text{H-C}(2')$); 4.83 (*d*, $J = 11.0$, $\text{NH-C}(2)$); 4.74, 4.68 (2 *d*, PhCH_2); 4.68, 4.61, 4.47, 4.45 (4 *d*, $J = 11.5$, 2 PhCH_2); 4.46 (*dd*, $\text{H}_a\text{-C}(6)$); 4.20 (*dd*, $J(5,6b) = 3.8$, $J(6a,6b) = 12.5$, $\text{H}_b\text{-C}(6)$); 4.07 (*ddd*, $J(1,2) = 3.8$, $J(2,3) = 10.0$, $\text{H-C}(2)$); 3.96 (*dd* $\approx$ *t*, $\text{H-C}(4)$); 3.86 (*ddd* $\approx$ *dt*, $J(4,5) = 9.5$, $J(5,6a) = 2.0$, $\text{H-C}(5)$); 3.79 (*ddd* $\approx$ *dd* (*br.*), $J(2',3') = 2.4$, $\text{H-C}(3')$); 3.57 (*dd*, $J(3,4) = 9.2$, $\text{H-C}(3)$); 3.41 (*s*, CH_3OOC); 2.83–2.38 (*m*, $(\text{CH}_2)_2$); 2.16, 2.13, 2.08 (3 *s*, 3 CH_3CO). EI-MS: 848 (3, $M^+ + \text{H} - \text{BnOH}$), 99 (45, Lev^+) 91 (100, Bn^+). Anal. calc. for $\text{C}_{51}\text{H}_{57}\text{NO}_{17}$ (956.01): C 64.01, H 6.00, N 1.47; found: C 63.94, H 6.03, N 1.47.

Benzyl 6-O-Acetyl-3-O-benzyl-2-[(benzyloxycarbonyl)amino]-2-deoxy-4-O-(methyl 2-O-acetyl-3-O-benzyl- α -L-idopyranosyluronate)- α -D-glucopyranoside (23). To a soln. of **22** (5.36 g, 5.6 mmol) in pyridine/ AcOH 4:1 (25 ml) was added hydrazine hydrate (2 ml) at 0°. The resulting white slurry was stirred for 1.5 h at 0° and diluted with acetone (50 ml) to destroy the excess of hydrazine. The mixture was evaporated and the residue taken up in toluene several times and evaporated. MPLC (AcOEt /hexane 1:2) furnished colourless crystals of **23** (4.70 g, 98%). M.p. 153.5–154° ([11]: m.p. 146–147°). $[\alpha]_{\text{D}}^{20} = +52.0$ ($c = 0.3$, CHCl_3); [11]: $[\alpha]_{\text{D}}^{23} = +44$ ($c = 1.0$, CHCl_3).

3-O-Acetyl-1,6-anhydro-2-azido-2-deoxy-4-O-(methyl 2,3-di-O-benzyl-4-O-levulinoyl- β -D-glucopyranosyluronate)- β -D-glucopyranose (24). A soln. of well dried **5** (40.05 g, 147.7 mmol) in dry CH_2Cl_2 (100 ml) was stirred for 2 h in the presence of 4-Å molecular sieves (5 g), *Sikkon* (5 g), and activated silver zeolite [22] (32 g) with exclusion of light. The mixture was cooled to -30° , and a soln. of freshly prepared **4** (24.0 g, 43.7 mmol) in dry CH_2Cl_2 (25 ml) was added during 15 min under a stream of Ar. After stirring for 6 d at r.t. in the dark, the mixture was filtered and the residue washed with CH_2Cl_2 . The combined filtrates were evaporated and subjected to CC (Et_2O /hexane 9:1) to give **5** (31.6 g, 79%). From the disaccharide fractions, **24** (14.4 g, 47%) was obtained by crystallization from AcOEt /hexane. The mother liquor was chromatographed (AcOEt /hexane 1:1) to yield 3-O-acetyl-1,6-anhydro-2-azido-2-deoxy-4-O-(methyl 2,3-di-O-benzyl-4-O-levulinoyl- α -D-glucopyranosyluronate)- β -D-glucopyranose (**25**; 3.4 g, 11%) followed by **24** (7.2 g, 24%; total yield: 71%), colourless crystals, m.p. 123–124° ([3b]: m.p. 117°). $[\alpha]_{\text{D}}^{20} = -10.0$ ($c = 0.6$, CHCl_3), $[\alpha]_{\text{D}}^{20} = -4.5$ ($c = 0.2$, dioxane; [3b]: $[\alpha]_{\text{D}}^{20} = -3.33$ ($c = 0.7$, CHCl_3)).

25: Colourless crystals. M.p. 109.2–109.5°. $[\alpha]_{\text{D}}^{20} = +63.8$ ($c = 0.5$, dioxane). $^1\text{H-NMR}$ (250 MHz, CDCl_3): 7.41–7.26 (*m*, 10 arom. H); 5.60 (*br. s*, $\text{H-C}(1)$); 5.12 (*d*, $J(1',2') = 4.0$, $\text{H-C}(1')$); 5.05 (*dd* $\approx$ *t*, $J(3',4') = 9.5$, $\text{H-C}(4')$); 5.05 (*br. s*, $\text{H-C}(3)$); 4.85, 4.80, 4.72, 4.67 (4 *d*, 2 PhCH_2); 4.68 (*br. d*, $\text{H-C}(5)$); 4.43 (*d*, $J(4',5') = 10.0$, $\text{H-C}(5')$); 4.12 (*dd* $\approx$ *t*, $\text{H-C}(3')$); 4.00 (*br. d*, $J(6a,6b) = 7.5$, $\text{H}_a\text{-C}(6)$); 3.80 (*dd*, $J(5,6b) = 6.0$, $\text{H}_b\text{-C}(6)$); 3.69 (*s*, CH_3OOC); 3.61 (*dd*, $J(2',3') = 9.5$, $\text{H-C}(2')$); 3.48 (*br. s*, $\text{H-C}(4)$); 3.04 (*br. s*, $\text{H-C}(2)$); 2.74–2.27 (*m*, $(\text{CH}_2)_2$); 2.15, 2.12 (2 *s*, 2 CH_3CO). CI-MS: 715 (3, $M + \text{NH}_4^+$), 672 (8, $M^+ + 2 \text{H} - \text{N}_2$). Anal. calc. for $\text{C}_{34}\text{H}_{39}\text{N}_5\text{O}_{13}$ (697.69): C 58.53, H 5.63, N 6.02; found: C 58.40, H 5.67, N 5.96.

Benzyl O-(Methyl 2,3-di-O-benzyl-4-O-levulinoyl- β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-O-(3,6-di-O-acetyl-2-azido-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-O-(methyl 2-O-acetyl-3-O-benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-6-O-acetyl-3-O-benzyl-2-[(benzyloxycarbonyl)amino]-2-deoxy- α -D-glucopyranoside (27). A soln. of well dried **23** (1.74 g, 2.03 mmol) in abs. CH_2Cl_2 (30 ml) was stirred together with 4-Å molecular sieves (5 g) and dried $\text{Ag}_2\text{CO}_3/\text{AgClO}_4$ 10:1 (1.1 g) for 2.5 h at r.t. After cooling to 0°, a soln. of well dried **26** (2.506 g, 3.05 mmol) in abs. CH_2Cl_2 (10 ml) was added within 15 min at 0°. The mixture was stirred for 4 d at r.t. and filtered over a pad of *Speedex*, the residue was washed with CH_2Cl_2 . The combined org. soln. was extracted with KHSO_4 soln. and H_2O , evaporated and subjected to MPLC (AcOEt /hexane 3:2): **27** (1.98 g, 61%) as a syrup. $[\alpha]_{\text{D}}^{20} = +60.0$ ($c = 0.2$, CHCl_3). $^1\text{H-NMR}$ (400 MHz, CDCl_3): 7.39–7.19 (*m*, 30 arom. H); 5.37 (*dd*, $J(3'',4'') = 9.0$, $\text{H-C}(3'')$); 5.29 (*d*, $J(1',2') = 3.5$, $\text{H-C}(1')$); 5.03 (*dd* $\approx$ *t*, $\text{H-C}(4'')$); 4.98 (*br. s*, PhCH_2 of **Z**, $\text{H-C}(1'')$); 4.92 (*dd* $\approx$ *t*, $J(2',3') = 5.5$, $\text{H-C}(2')$); 4.88 (*d*, $J(1,2) = 3.2$, $\text{H-C}(1)$); 4.79–4.63 (*m*, PhCH_2 , $\text{NHC}(2)$, $\text{H-C}(5')$); 4.51, 4.46 (2 *d*, PhCH_2); 4.45 (*dd*, $\text{H}_a\text{-C}(6'')$); 4.35 (*dd* $\approx$ *br. d*, $\text{H}_a\text{-C}(6)$); 4.33 (*d*, $J(1'',2'') = 7.9$, $\text{H-C}(1'')$); 4.18 (*dd*,

$J(6a, 6b) \approx J(6a'', 6b'') \approx 12$, $J(5, 6b) \approx J(5'', 6b'') \approx 3.4$, $H_b-C(6)$, $H_b-C(6'')$; 4.06 (*ddd* $\approx$ br. *d*, $H-C(5'')$); 4.04 (*dd* $\approx$ *t*, $J(4'', 5'') = 4.7$, $H-C(4'')$); 4.02 (*ddd*, $H-C(2)$); 3.95 (*dd* $\approx$ *t*, $J(3', 4') \approx 4.7$, $H-C(3')$); 3.92 (*dd* $\approx$ *t*, $H-C(4)$); 3.81 (*d*, $J(4'', 5'') = 10.0$, $H-C(5'')$); 3.80 (*ddd* $\approx$ br. *dd*, $H-C(5)$); 3.69 (*dd* $\approx$ *t*, $J(4'', 5'') = 10$, $H-C(4'')$); 3.69 (*s*, $CH_3OOC(6''')$); 3.62 (*dd* $\approx$ *t*, $J(3''', 4''') = 10.0$, $H-C(3''')$); 3.59 (*dd* $\approx$ *t*, $H-C(3)$); 3.59 (*s*, $CH_3OOC(6')$); 3.43 (*dd*, $J(2'', 3'') = 9.0$, $H-C(2'')$); 3.18 (*dd*, $J(1'', 2'') = 3.3$, $J(2'', 3'') = 10.8$, $H-C(2'')$); 2.72–2.46 (*m*, 3 *H*, Lev); 2.33–2.26 (*m*, 1 *H*, Lev); 2.16, 2.14, 2.09, 2.08, 1.97 (5 *s*, 5 CH_3CO). Anal. calc. for $C_{82}H_{92}N_4O_{29}$ (1597.64): C 61.65, H 5.80, N 3.51; found: C 61.45, H 6.14, N 3.46.

Benzyl O-(Methyl 2,3-di-O-benzyl- β -D-glucopyranosyluronate)-(1 $\rightarrow$ 4)-O-(3,6-di-O-acetyl-2-azido-2-deoxy- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-O-(methyl 2-O-acetyl-3-O-benzyl- α -L-idopyranosyluronate)-(1 $\rightarrow$ 4)-6-O-acetyl-3-O-benzyl-2-[benzyloxycarbonyl]amino]-2-deoxy- α -D-glucopyranoside (28). A soln. of **27** (1.8 g, 1.13 mmol) in pyridine/AcOH 4:1 (25 ml) was reacted with hydrazine hydrate (2.4 ml) for 1 h at 0°. Usual workup and MPLC (AcOEt/hexane 3:2) gave **28** (1.64 g, 97%) as a syrup. $[\alpha]_D^{20} = +62.3$ (*c* = 0.8, $CHCl_3$); $[10]: [\alpha]_D^{20} = +58.5$ (*c* = 0.8, $CHCl_3$). 1H -NMR (400 MHz, $CDCl_3$): 7.39–7.13 (*m*, 30 arom. *H*); 5.36 (*dd*, $J(3', 4') = 9.8$, $H-C(3')$); 5.29 (*d*, $J(1', 2') = 3.5$, $H-C(1')$); 4.98 (br. *d*, $H-C(1'')$); *s*, $PhCH_2$ of *Z*); 4.93 (*dd* $\approx$ *t*, $J(2', 3') = 5.7$, $H-C(2')$); 4.88 (*d*, $H-C(1)$); 4.86, 4.70 (2 *d*, $J = 11.5$, $PhCH_2$); 4.80–4.67 (*m*, 4 *H* of $PhCH_2$); 4.77 (*d*, $NH-C(2)$); 4.77, 4.50 (2 *d*, $J = 11.9$, $PhCH_2$); 4.75 (*d*, $H-C(5'')$); 4.65, 4.46 (2 *d*, $J = 12.0$, $PhCH_2$); 4.44 (*dd*, $J(5'', 6a'') \approx 1.5$, $J(6a'', 6b'') = 12.0$, $H_a-C(6'')$); 4.35 (*dd*, $J(5, 6a) = 3$, $H_a-C(6)$); 4.32 (*d*, $J(1'', 2'') = 7.6$, $H-C(1'')$); 4.19 (2 *dd*, $J(5, 6b) \approx J(5'', 6b'') \approx 3.8$, $J(6a, 6b) \approx J(6a'', 6b'') \approx 12.2$, $H_b-C(6)$, $H_b-C(6'')$); 4.07 (*ddd* $\approx$ br. *d*, $H-C(5'')$); 4.05 (*dd* $\approx$ *t*, $J(4', 5') = 4.7$, $H-C(4')$); 4.03 (*dd*, $J(1, 2) = 3.3$, $H-C(2)$); 3.95 (*dd* $\approx$ *t*, $J(3', 4') = 4.9$, $H-C(3')$); 3.92 (*dd* $\approx$ *t*, $H-C(4)$); 3.80 (*ddd*, $H-C(5)$, $H-C(5'')$); 3.79–3.75 (*m*, $H-C(4'')$, $H-C(5'')$); 3.78 (*s*, $CH_3OOC(6''')$); 3.70 (*dd*, $J(4'', 5'') = 7.2$, $H-C(4'')$); 3.59 (*dd*, $H-C(3)$); *s*, $CH_3OOC(6')$); 3.44 (*dd*, $J(3''', 4''') = 7.8$, $H-C(3''')$); 3.35 (*dd*, $J(2'', 3'') = 9.2$, $H-C(2'')$); 3.19 (*dd*, $J(1'', 2'') = 3.3$, $J(2'', 3'') = 10.8$, $H-C(2'')$); 2.09, 2.08, 2.07, 1.96 (4 *s*, 4 CH_3CO).

Data of Pentasaccharide 29: Colourless foam. $[\alpha]_D^{20} = +72.7$ (*c* = 0.9, $CHCl_3$); $[10]: [\alpha]_D^{20} = +65$ (*c* = 1.0, $CHCl_3$). 1H -NMR (400 MHz, $CDCl_3$): 7.39–7.12 (*m*, 40 arom. *H*); 5.49 (*d*, $J(1''', 2''') = 3.8$, $H-C(1''')$); 5.34 (*dd*, $J(3'', 4'') = 9.1$, $H-C(3'')$); 5.28 (*d*, $J(1', 2') = 3.3$, $H-C(1')$); 4.99, 4.805 (2 *d*, $J = 11.3$, $PhCH_2$); 4.98 (*s*, $PhCH_2$ of *Z*); 4.97 (*d*, $H-C(1'')$); 4.92 (*dd* $\approx$ *t*, $J(2', 3') = 5.5$, $H-C(2')$); 4.88 (*d*, $J(1, 2) = 3.1$, $H-C(1)$); 4.85 (2 *d* $\approx$ *d*, $PhCH_2$); 4.81, 4.54 (2 *d*, $J = 11.1$, $PhCH_2$); 4.795, 4.67 (2 *d*, $J = 11.0$, $PhCH_2$); 4.78 (*d*, $J(2, NH) = 10$, $NH-C(2)$); 4.76, 4.50 (2 *d*, $J = 11.5$, $PhCH_2$); 4.73, 4.69 (2 *d*, $J = 11.8$, $PhCH_2$); 4.73 (*d*, $J(4', 5') = 4.0$, $H-C(5')$); 4.65, 4.45 (2 *d*, $J = 11.6$, $PhCH_2$); 4.43 (*dd*, $J(5'', 6a'') \approx 1.5$, $J(6a'', 6b'') \approx 11$, $H_a-C(6'')$); 4.34 (*dd*, $J(5, 6a) \approx 2$, $J(6a, 6b) \approx 10$, $H_a-C(6)$); 4.33 (*d*, $J(1'', 2'') = 7.9$, $H-C(1'')$); 4.25 (*dd*, $J(5''', 6a''') = 1$, $J(6a''', 6b''') = 12.0$, $H_b-C(6''')$); 4.18 (2 *dd*, $H_b-C(6)$, $H_b-C(6''')$); 4.14 (*dd*, $J(5'', 6b'') \approx 3.5$, $H_b-C(6'')$); 4.04 (*ddd*, $H-C(5'')$); 4.03 (2 *dd* $\approx$ *t*, $J(4', 5') = 4.0$, $H-C(4')$); $J(4'', 5'') = 9.8$, $H-C(4'')$); 4.02 (*ddd*, $H-C(2)$); 3.94 (*dd* $\approx$ *t*, $J(3', 4') = 5.5$, $H-C(3')$); 3.92 (*dd* $\approx$ *t*, $H-C(4)$); 3.84 (*d*, $H-C(5'')$); *dd*, $J(3''', 4''') = 8.6$, $H-C(3''')$); 3.79 (*ddd* $\approx$ *dt*, $J(4, 5) = 8.0$, $J(5, 6b) \approx 2$, $H-C(5)$); 3.74 (*s*, $CH_3OOC(6''')$); 3.69 (*dd* $\approx$ *t*, $J(3'', 4'') = 8.9$, $H-C(3'')$); 3.67 (*dd* $\approx$ *t*, $J(4'', 5'') = 9.9$, $H-C(4'')$); 3.58 (*dd* $\approx$ *t*, $H-C(3)$); 3.57 (*s*, $H_3OOC(6')$); 3.49 (*m* $\approx$ *d*, $H-C(4'')$, $H-C(5'')$); 3.42 (*dd*, $J(2'', 3'') = 9.1$, $H-C(2'')$); 3.26 (*dd*, $J(2''', 3''') = 10.4$, $H-C(2''')$); 3.18 (*dd*, $J(1'', 2'') = 3.5$, $J(2'', 3'') = 10.8$, $H-C(2'')$); 2.09, 2.07, 2.06, 2.02, 1.99 (5 *s*, 5 CH_3CO).

O-(2-Acetamido-2-deoxy-6-O-sulfo- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-O-(β -D-glucopyranosyluronic acid)-(1 $\rightarrow$ 4)-O-(2-acetamido-2-deoxy-3,6-di-O-sulfo- α -D-glucopyranosyl)-(1 $\rightarrow$ 4)-O-(2-O-sulfo- α -L-idopyranosyluronic acid)-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy-6-O-sulfo-D-glucopyranose Heptasodium Salt (2). A soln. of crude **30** [10] (the compd. was not purified after hydrogenation; 92 mg, 65 μ mol) in H_2O (2 ml) and Ac_2O (1 ml) was kept at r.t. for 16 h. The soln. was evaporated and chromatographed twice on *LH-20* using H_2O to separate from by-products of higher molecular weight. The main fraction (42 mg) was chromatographed on *Sephadex G 25 (Fine)* to give pure **2** (25 mg, 25%) as colourless glass. $[\alpha]_D^{20} = +43$ (*c* = 0.2, H_2O). 1H -NMR (400 MHz, D_2O): 2.06, 2.05, 2.04 (3 *s*, 3 *Ac*). ^{13}C -NMR (D_2O): 177.8 ($C=O$); 177.3 (2 $C=O$); 103.9, 102.2 ($C(1')$, $C(1'')$); 99.8, 97.7 ($C(1')$, $C(1'')$); 93.5 ($C(1)$); 69.8, 69.2, 68.8 ($C(6)$, $C(6')$, $C(6'')$); 57.0, 56.4, 54.9 ($C(2)$, $C(2')$, $C(2'')$); 25.3 (*AcN*); 24.8 (2 *AcN*); external dioxane: 69.4. No destructive analysis was carried out on this compd.

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