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Synthesis of hyaluronic-acid-related oligosaccharides and analogues, as their 4-methoxyphenyl glycosides, having *N*-acetyl-β-D-glucosamine at the reducing end

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Abstract

To contribute to the possibilities to study the ability of oligosaccharide fragments of hyaluronic acid to induce angiogenesis, several hyaluronic-acid-related oligosaccharides and their 6-*O*-sulfated analogues were synthesised as their 4-methoxyphenyl glycosides having 2-acetamido-2-deoxy-D-glucopyranose at the reducing end. In all syntheses described, the D-glucopyranosyluronic acid residue was obtained by oxidation at C-6 of a corresponding D-glucopyranosyl residue after construction of the oligosaccharide backbone, using pyridinium dichromate and acetic anhydride.

Keywords: Oxidation; Hyaluronic acid oligosaccharides; Hyaluronic acid sulfated analogues

1. Introduction

Hyaluronic acid (HA) is a linear extracellular polysaccharide [1], consisting of disaccharide repeating units of 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid, namely, [→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→]$_n$. It is a major component of several soft connective tissues and has also been found in certain bacterial strains [2]. HA is biosynthesised at the inner side of plasma membranes by a membrane-bound HA synthetase, and is then extruded to the cell surface [3].

HA obtained from different tissue sources exhibits considerable variation in size, and biological activity has been shown to be critically dependent

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on molecular mass in a number of experimental systems [4]. Native high-molecular-mass HA is anti-angiogenic [5], whereas HA degradation products, containing 3–10 disaccharide units, stimulate endothelial cell proliferation and migration [6], and induce angiogenesis in the chick chorioallantoic membrane (CAM) assay [6,7]. However, biological studies performed so far always used a mixture of oligosaccharides obtained by digestion of HA from biological sources. Therefore, it is not known which oligosaccharides are responsible for the observed stimulating effects on angiogenesis. To circumvent the drawbacks connected to the use of oligosaccharides isolated from biological sources, a synthetic program was initiated in our laboratory to obtain a wide range of medium-sized, well-defined oligosaccharide elements of HA.

As a result of this research program, several oligosaccharide fragments constituted of even or odd numbers of monosaccharides with either 2-acetamido-2-deoxy-D-glucose or D-glucuronic acid at the reducing end have been synthesised. In our synthetic strategy, the D-glucuronic acid residue was obtained by oxidation of the primary hydroxyl function of a corresponding D-glucose residue. Using the above mentioned approach, so far β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc, β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc, β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc [8,9], β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A and β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A [10,11] have been synthesised as their 4-methoxyphenyl glycosides. Using a similar approach, the disaccharide β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc as its methyl glycoside was synthesised [12]. Recently, the expeditious and stereocontrolled syntheses of HA di-, tri- [13], tetra-, hexa-, and octasaccharides [14] fragments having a 1-*O*-methyl β-D-glucuronic acid residue at the reducing end, by direct coupling of D-glucuronic acid derivatives with suitably protected 2-deoxy-2-trichloroacetamido-D-glucose residues [15] were described.

In this paper we demonstrate, by synthesis of several HA oligosaccharide 4-methoxyphenyl glycosides (Table 1, compounds **1**–**3**), that oxidation of D-glucose into D-glucuronic acid of the larger oligosaccharide fragments in the final stage of the synthetic route is possible, using pyridinium dichromate and acetic anhydride [16,17]. Since our synthetic approach offers also the possibility to introduce other groups instead of the normal carboxylic acid function, two sulfated analogues of HA fragments are synthesised (Table 1, compounds **4** and **5**) because it is known that other sulfated oligosaccharides [18] decrease the angiogenesis in CAM experiments. Therefore, it is interesting to study the behaviour of these sulfated analogues of HA oligosaccharides in the above mentioned biological assays.

2. Results and discussion

To establish a convenient and systematic synthesis of the target oligosaccharides, several key intermediates (Scheme 1; **6**–**9**) were synthesised as described previously by Slaghek et al. [8–11]. Starting from these intermediates, the backbone of larger oligosaccharides can be synthesised. The unique properties of the protecting group of the primary hydroxyl function of the glucose residues, the levulinoyl group, allows its selective removal. The obtained hydroxyl functions can be oxidised, and after deprotection the target structures will be obtained.

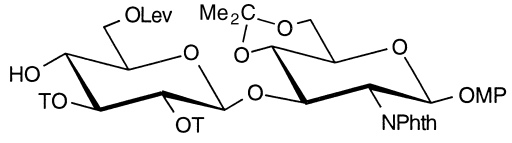
For the synthesis of pentasaccharide **1**, having a GlcNAc residue at the reducing end, disaccharide acceptor **6** was coupled with donor **7** in dichloromethane in the presence of boron trifluoride ethyl etherate (0.3 equiv based on **6**) which gave trisaccharide derivative **10** in 69% yield (Scheme 2). Removal of the allyloxycarbonyl group, applying tetrakis(triphenylphosphine)palladium and morpholine [19,20], afforded **11** in 89% yield. Disaccharide donor **13** was prepared by oxidative removal [21] of the 4-methoxyphenyl group from **8**

Table 1
Synthesised oligosaccharide fragments of hyaluronic acid and analogues (MP = 4-methoxyphenyl)

β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→O)MP (**1**)
β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→O)MP (**2**)
β-$\Delta^{4,5}$Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→O)MP (**3**)
β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*6*S*-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*6*S*-(1→3)-β-D-Glc*p*NAc-(1→O)MP (**4**)
β-D-Glc*p*6*S*-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*6*S*-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*6*S*-(1→3)-β-D-Glc*p*NAc-(1→O)MP (**5**)

with ammonium cerium(IV) nitrate (→ **12**), followed by imidoylation [22] (trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene in dichloromethane), in an overall yield of 67%.

Condensation of **11** with **13** in dichloromethane, using trimethylsilyl triflate (0.05 equiv based on **11**), gave the pentasaccharide derivative **14** in 81% yield (Scheme 3). The allyloxycarbonyl group was split off using tetrakis(triphenylphosphine)palladium and morpholine to yield the pentasaccharide derivative **15** (89%). Acidic removal of the isopropylidene functions of **15**, followed by conventional acetylation of the hydroxyl functions, provided **16** (overall yield 90%), which was delevulinoylated



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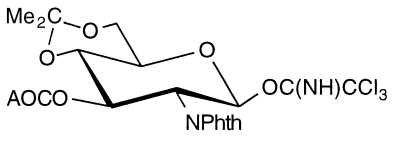

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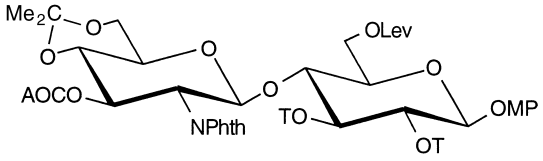

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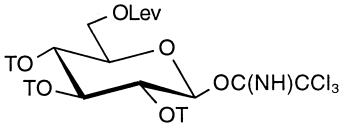

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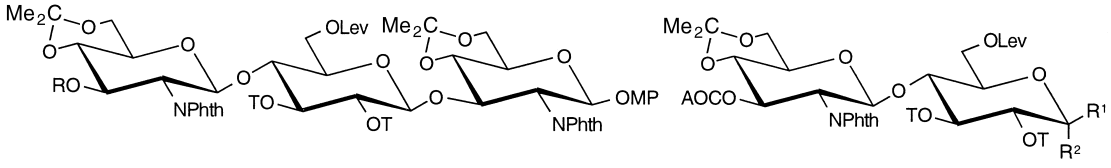
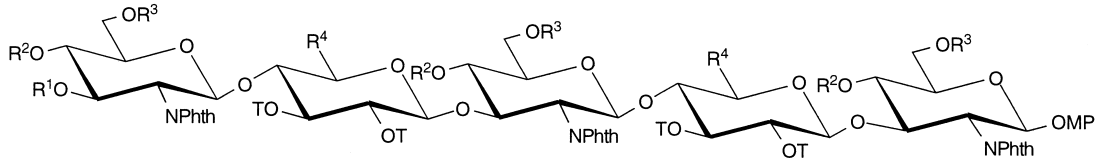
Scheme 1. Key intermediates as synthesised by Slaghek et al. [8–11]



	R
10	AOC
11	H

	R^1	R^2
12	H,OH	
13	H	$OC(NH)CCl_3$

Scheme 2. Synthetic intermediates towards pentasaccharide derivative **14**



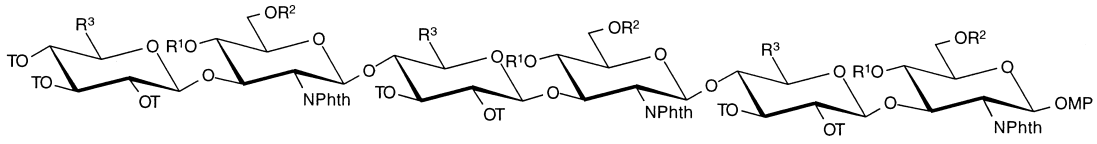
	R^1	R^2	R^3	R^4
14	AOC	CMe_2		CH_2OLev
15	H	CMe_2		CH_2OLev
16	Ac	Ac	Ac	CH_2OLev
17	Ac	Ac	Ac	CH_2OH
18	Ac	Ac	Ac	COOH

Scheme 3. Synthetic intermediates towards pentasaccharide **1**

using hydrazinium acetate [23,24] to afford **17** (74%). Slaghek et al. [8–11] described good results for the oxidation of primary hydroxyl to carboxyl functions using a Swern oxidation with oxalyl chloride and methyl sulfoxide [25] followed by oxidation with $NaClO_2$ [26]. However, using this method in the present case gave unsatisfactory results for the oxidation of **17**. Application of pyridinium dichromate [27] in combination with molecular sieves [28] generated the desired product **18** (61%), but the extremely long reaction time (72 h) made this procedure less attractive. Previously, Garegg et al. [16] described that addition of acetic anhydride accelerated a pyridinium chlorochromate-mediated oxidation reaction, whereas Corey et al. [17] published that addition of acetic anhydride and *tert*-butanol to a pyridinium dichromate-mediated oxidation reaction afforded the *tert*-butyl ester of the glucuronic acid residues. We found that oxidation of diol **17** using pyridinium dichromate and acetic anhydride without the addition of *tert*-butanol in dichloromethane gave, after a reaction period of 3 h, **18** in 70% yield. In order to demonstrate the presence of two D-glucuronic acid residues in **18**, a small amount of the product was esterified with diazomethane in ether and analysed by ^{1}H NMR spectroscopy. The detection of two singlets at 3.499 and 3.442 ppm ($COOCH_3$) and two doublets at 3.706 and 3.543 ppm (H-5′ and H-5‴) showed unambiguously the presence of two glucuronic acid residues. Dephthaloylation/deacylation of **18** using methylamine [29] in ethanol (10 days), and re-*N*-acetylation with acetic anhydride in methanol at 0 °C, afforded the target compound **1** (64%).

The starting compound for the synthesis of the target structure **2** was the pentasaccharide derivative **15** (Scheme 3). Acceptor **15** was condensed with **9** (Scheme 1) in dichloromethane in the presence of trimethylsilyl triflate (0.05 equiv based on **15**) to afford the hexasaccharide derivative **19** in 62% yield (Scheme 4). Removal of the isopropylidene functions under acidic conditions, followed by conventional acetylation of the hydroxyl functions gave **20** in an overall yield of 84% based on **19**. Delevulinoylation using hydrazinium acetate provided triol **21** (75%), and subsequent oxidation of the primary hydroxyl functions, as described for the preparation of **18**, yielded **22** (58%), as verified by ^{1}H NMR analysis of the methyl-esterified derivative. Dephthaloylation/deacylation of compound **22** using methylamine in ethanol (10 days), followed by re-*N*-acetylation, gave a complex mixture. Treatment of this mixture with sodium methoxide in methanol (pH 11), Dowex-50 (H^+), and acetic anhydride—methanol resulted in the formation of the elimination product **3**, containing a terminal unsaturated uronic acid residue which could be isolated in 40% yield. Although being an undesired side product, **3** could be of interest to test in biological assays since it reflects the nonreducing terminus of enzymatic degradation products of native hyaluronic acid when incubated with testicular hyaluronidase. To avoid this side reaction, the deprotection strategy was adapted and **22** was heated with ethylenediamine in 1-butanol at 90 °C [30], followed by re-*N*,*O*-acetylation using acetic anhydride and pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine. Finally, de-*O*-acetylation using aq 2 M sodium hydroxide in tetrahydrofuran at 0 °C [31] and subsequent neutralisation yielded the desired compound **2** (73%).

For the preparation of **4** and **5**, representing the sulfated hyaluronic acid analogues of **1** and **2**, the pentasaccharide derivative **17** (Scheme 3) and hexasaccharide derivative **21** (Scheme 4) were each



	R^1	R^2	R^3
19	CMe_2		CH_2OLev
20	Ac	Ac	CH_2OLev
21	Ac	Ac	CH_2OH
22	Ac	Ac	COOH

Scheme 4. Synthetic intermediates towards hexasaccharides **2** and **3**

treated with the sulfur trioxide-trimethylamine complex (5 equiv/OH-group) to afford the pentasaccharide disulfate analogue **23** (88%) and the hexasaccharide trisulfate analogue **24** (78%), respectively (Scheme 5). Deblocking of both compounds, using the procedure as described for compound **2** including conversion into the sodiated form, rendered the sulfated pentasaccharide **4** and hexasaccharide **5** in yields of 68 and 88%, respectively.

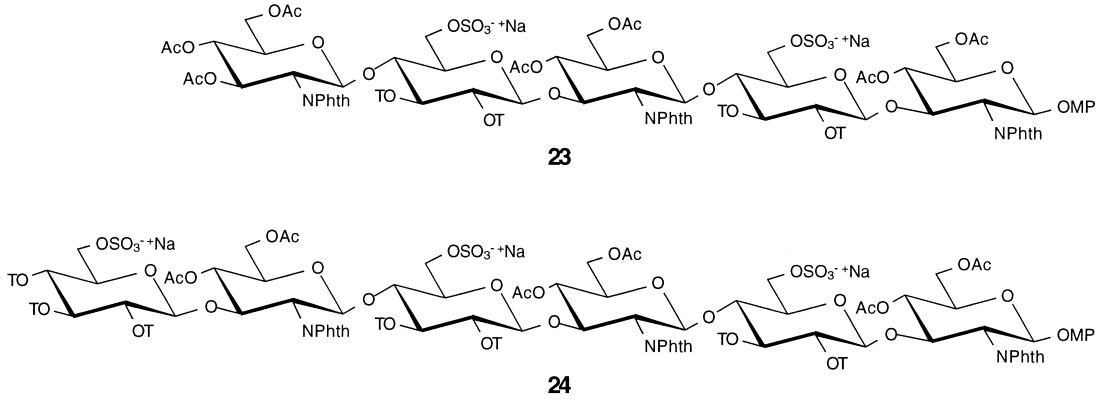
The ^{1}H NMR spectra of **1**–**5** are in full agreement with the expected structures, and in accordance with those reported for the smaller synthetic oligosaccharide 4-methoxyphenyl glycosides [8–11]. The synthesised 4-methoxyphenyl glycosides **1**–**5** will be tested in biological assays for their ability to influence the angiogenesis.

3. Experimental

General methods.—Reactions were monitored by TLC on Kieselgel 60 F_{254} (E. Merck); compounds were visualised by charring with aq 50% H_2SO_4, after examination under UV light. In the work-up procedures of reaction mixtures, organic solutions were washed with appropriate amounts of the indicated aq solutions, then dried ($MgSO_4$), and concentrated under reduced pressure at 20–40 °C (water-bath). Column chromatography was performed on Kieselgel 60 F_{254} (E. Merck, 70–230 mesh).

Optical rotations were measured at 20 °C for solutions in $CHCl_3$ with a Perkin–Elmer 241 polarimeter, using a 10 cm 1 mL cell. ^{1}H NMR spectra were recorded with Bruker AC 300, Bruker AMX 500 or Bruker AMX 600 spectrometers; the values of δ_H are given in ppm relative to the signal for internal Me_4Si (δ 0) for solutions in $CDCl_3$, or by reference to acetone (δ 2.225) for solutions in D_2O. ^{13}C (APT, 75 MHz) NMR spectra were recorded at 27 °C with a Bruker AC 300 or a Varian Gemini-300 instrument; indicated ppm values for δ_C are relative to the signal of $CDCl_3$ (δ 76.9) for solutions in $CDCl_3$. Two-dimensional double-quantum filtered ^{1}H–^{1}H correlation spectra (2D DQF ^{1}H–^{1}H COSY) were recorded using a Bruker AMX 500 apparatus (500 MHz) at 27 °C. Fast-atom-bombardment mass spectrometry (FABMS) was performed on a JEOL JMS SX/SX 102A four-sector mass spectrometer, operated at 10 kV accelerating voltage, equipped with a JEOL MS-FAB 10 D FAB gun, operated at 10 mA emission current, producing a beam of 6 keV Xe atoms. Elemental analyses were carried out by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany).

*4-Methoxyphenyl (3-*O*-allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranoside* (**10**).—To a solution of 4-methoxyphenyl (6-*O*-levulinoyl-2,3-di-*O*-*p*-toluoyl-*β*-D-glucopyranosyl)-(1→3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-D-glucopyranoside [9] (**6**; 0.87 g, 0.91 mmol) and 3-*O*-allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-D-glucopyranosyl trichloroacetimidate [8] (**7**; 0.95 g, 1.64 mmol) in dry CH_2Cl_2 (10 mL), containing 3 Å molecular sieves (0.8 g), was added $BF_3{\cdot}OEt_2$ (34 μL). After stirring for 30 min, TLC (95:5 CH_2Cl_2–acetone) showed the disappearance of **7** and the formation of **10** (R_f 0.21). Then the mixture was neutralised with Et_3N, diluted with CH_2Cl_2 (150 mL), filtered through Celite, and



Scheme 5. Synthetic intermediates towards the sulfated hyaluronic acid analogues **4** and **5**

washed with aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue gave **10**, isolated as a white foam (0.86 g, 69%); $[\alpha]_D$ +38° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.725, 7.302, 7.134, and 6.977 (4 d, each 2 H, 2 $COC_6H_4CH_3$), 6.68–6.60 (m, 4 H, $C_6H_4OCH_3$), 5.61–5.51 (m, 1 H, $COOCH_2CH{=}CH_2$), 5.537 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.367 (dd, 1 H, $J_{2'',3''}$ 10.2, $J_{3'',4''}$ 9.3 Hz, H-3″), 5.337 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1″), 5.307 (dd, 1 H, $J_{2',3'}$ 9.6, $J_{3',4'}$ 10.0 Hz, H-3′), 5.07–4.93 (m, 2 H, $COOCH_2CH{=}CH_2$), 5.035 (dd, 1 H, H-2′), 4.811 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.398 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 4.153 (dd, 1 H, H-2″), 3.657 (s, 3 H, $C_6H_4OCH_3$), 2.352 and 2.336 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 2.249 (s, 3 H, $COCH_2CH_2COCH_3$), 1.485, 1.336, 1.221, and 1.174 (4 s, each 3 H, 2 $C(CH_3)_2$); ^{13}C, δ 171.7 ($COCH_2CH_2COCH_3$), 164.8 and 164.5 (2 $COC_6H_4CH_3$), 154.0 ($COOCH_2CH{=}CH_2$), 131.1 ($COOCH_2CH{=}CH_2$), 118.2 ($COOCH_2CH{=}CH_2$), 99.3 (2 $C(CH_3)_2$), 99.8, 98.3, and 97.7 (C-1,1′,1″), 55.3 (2 C) and 55.0 (C-2,2″ and $C_6H_4OCH_3$), 37.8, 29.7, and 27.5 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 28.9, 28.5, 18.9, and 18.4 (2 $C(CH_3)_2$). Anal. Calcd for $C_{72}H_{75}N_2O_{25}$: C, 63.24; H, 5.46. Found: C, 63.18; H, 5.49.

*4-Methoxyphenyl (2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranoside* (**11**).—To a solution of **10** (0.86 g, 0.63 mmol) in THF (12 mL) and morpholine (0.47 mL) was added tetrakis(triphenylphosphine)palladium (142 mg). The mixture was stirred and boiled under reflux until the de-allyloxycarbonylation was complete (**11**; R_f 0.45, TLC (9:1 CH_2Cl_2–acetone)). Then the mixture was diluted with EtOAc (100 mL) and washed with aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue gave **11**, isolated as a syrup (0.72 g, 89%); $[\alpha]_D$ +45.5° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.715, 7.294, 7.117, and 6.971 (4 d, each 2 H, 2 $COC_6H_4CH_3$), 6.68–6.60 (m, 4 H, $C_6H_4OCH_3$), 5.536 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.228 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1″), 5.032 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.5 Hz, H-2′), 4.813 (d, 1 H, H-1′), 4.394 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 4.033 (dd, 1 H, $J_{2'',3''}$ 10.4 Hz, H-2″), 3.644 (s, 3 H, $C_6H_4OCH_3$), 2.344 and 2.325 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 2.236 (s, 3 H, $COCH_2CH_2COCH_3$), 1.484, 1.332, 1.234, and 1.198 (4 s, each 3 H, 2 $C(CH_3)_2$); ^{13}C, δ 171.7 ($COCH_2CH_2COCH_3$), 164.8 and 164.5 (2 $COC_6H_4CH_3$), 99.4 and 99.3 (2 $C(CH_3)_2$), 99.8, 98.5, and 97.7 (C-1,1′,1″), 61.7, 61.6, and 60.8 (C-6,6′,6″), 56.8, 55.4, and 55.1 (C-2,2″ and $C_6H_4OCH_3$), 37.9, 29.8, and 27.5 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 29.0, 28.6, 18.9, and 18.5 (2 $C(CH_3)_2$). Anal. Calcd for $C_{68}H_{70}N_2O_{23}$: C, 63.64; H, 5.50. Found: C, 63.49; H, 5.59.

*(3-*O*-Allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*α*/*β*-*D*-glucopyranose* (**12**).—To a solution of 4-methoxyphenyl (3-*O*-allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-6-*O*-levulinoyl-2,3-di-*O*-*p*-toluoyl-*β*-D-glucopyranoside [10] (**8**; 1.29 g, 1.24 mmol) in toluene (50 mL) and acetonitrile (75 mL) was added water and ammonium cerium(IV) nitrate (6.8 g, 12.4 mmol). After stirring for 2.5 h, TLC (6:1 CH_2Cl_2–acetone) showed the disappearance of **8** (R_f 0.83) and the formation of **12** (R_f 0.58). The mixture was diluted with EtOAc (300 mL), washed with aq 10% $NaHCO_3$ (2×) and aq 5% NaCl (2×), dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue gave **12**, isolated as a yellow syrup (0.85 g, 73%); $[\alpha]_D$ +50° (*c* 1); ^{13}C NMR ($CDCl_3$): δ 171.8 ($COCH_2CH_2COCH_3$), 165.7 and 164.9 (2 $COC_6H_4CH_3$), 154.1 ($COOCH_2CH{=}CH_2$), 131.0 ($COOCH_2CH{=}CH_2$), 118.2 ($COOCH_2CH{=}CH_2$), 99.3 [$C(CH_3)_2$], 98.4 (C-1′), 95.4 (C-1β), 89.7 (C-1α), 68.2 ($COOCH_2CH{=}CH_2$), 61.8 and 60.8 (C-6,6′), 55.3 (C-2′), 37.7, 29.6, and 27.4 ($COCH_2CH_2COCH_3$), 21.3 ($COC_6H_4CH_3$), 28.5 and 18.4 [$C(CH_3)_2$]. Anal. Calcd for $C_{48}H_{51}NO_{18}$: C, 61.99; H, 5.53. Found: C, 61.67; H, 5.64.

*(3-*O*-Allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*α*-*D*-glucopyranosyl trichloroacetimidate* (**13**).—To a solution of **12** (0.85 g, 0.79 mmol) in dry CH_2Cl_2 (12 mL) and trichloroacetonitrile (1.0 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (30 μL). After stirring overnight, TLC (9:1 CH_2Cl_2–acetone) showed a complete conversion of **12** into **13** (R_f 0.89), and the mixture was purified by column chromatography (93:7 CH_2Cl_2–acetone) to yield **13**, isolated as a colorless syrup (0.87 g, 91%); $[\alpha]_D$ +23° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.919, 7.817, 7.236, and 7.118 (4 d, each 2 H, 2 $COC_6H_4CH_3$), 6.585 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.64–5.51 (m, 1 H, $COOCH_2CH{=}CH_2$),

5.496 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 5.342 (dd, 1 H, $J_{2,3}$ 10.2 Hz, H-2), 5.338 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 5.09–4.95 (m, 2 H, $COOCH_2CH{=}CH_2$), 4.254 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2′), 2.386 and 2.326 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 2.211 (s, 3 H, $COCH_2CH_2COCH_3$), 1.253 (s, 6 H, $C(CH_3)_2$). Anal. Calcd for $C_{50}H_{51}N_2O_{18}Cl_3$: C, 55.90; H, 4.78. Found: C, 55.73; H, 4.71.

*4-Methoxyphenyl (3-*O*-allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-(2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranoside* (**14**).—To a solution of **13** (0.58 g, 0.54 mmol) and **11** (0.23 g, 0.18 mmol) in dry CH_2Cl_2 (7.5 mL), containing 3 Å molecular sieves (0.5 g), was added Me_3SiOTf (17 μL). After stirring for 10 min, TLC (9:1 CH_2Cl_2–acetone) showed the disappearance of **11**, and the formation of **14** (R_f 0.44). Then Et_3N was added, and the mixture was diluted with EtOAc (150 mL), filtered through Celite, and washed with aq 5% NaCl. The organic layer was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue gave **14**, isolated as a white foam (0.32 g, 81%); $[\alpha]_D$ +78° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.687, 7.665, 7.265, 7.220, 7.114, 7.089, 6.959, and 6.952 (8 d, each 2 H, 4 $COC_6H_4CH_3$), 6.66–6.59 (m, 4 H, $C_6H_4OCH_3$), 5.63–5.49 (m, 1 H, $COOCH_2CH{=}CH_2$), 5.07–4.92 (m, 2 H, $COOCH_2CH{=}CH_2$), 5.502, 5.258, and 5.018 (3 d, each 1 H, $J_{1,2/1'',2''/1'''',2''''}$ 8.4, 8.4, and 8.3 Hz, H-1,1″,1‴″), 4.962 and 4.917 (2 dd, each 1 H, $J_{1',2'/1''',2'''}$ 7.9 and 8.0 Hz, $J_{2',3'/2''',3'''}$ 9.8 and 9.6 Hz, H-2′,2‴), 4.719 and 4.658 (2 d, each 1 H, H-1′,1‴), 4.496, 4.354, and 4.024 (3 dd, each 1 H, $J_{2,3/2'',3''/2'''',3''''}$ 10.4, 10.4, and 10.3 Hz, H-2,2″,2‴″), 3.637 (s, 3 H, $C_6H_4OCH_3$), 2.356, 2.329, 2.321, and 2.307 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 2.236 and 2.207 (2 s, each 3 H, 2 $COCH_2CH_2COCH_3$), 1.420, 1.263, 1.204, 1.153, 1.144, and 1.134 (6 s, each 3 H, 3 $C(CH_3)_2$); ^{13}C, δ 171.7 and 171.5 (2 $COCH_2CH_2COCH_3$), 164.8, 164.7, 164.5, and 164.4 (4 $COC_6H_4CH_3$), 154.1 ($COOCH_2CH{=}CH_2$), 130.9 ($COOCH_2CH{=}CH_2$), 99.7, 99.6, 98.3 (2 C), and 97.8 (C-1,1′,1″,1‴1‴″), 99.4 (2 C) and 98.9 ($3C(CH_3)_2$), 61.8, 61.5 (2 C), and 60.8 (2 C) (C-6,6′,6″,6‴,6‴″), 55.4 (2 C), 55.3, and 55.2 (C-2,2″,2‴″ and $C_6H_4OCH_3$), 37.9, 29.7, and 27.5 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 28.9, 28.8, 28.6, 18.9, 18.7, and 18.4 (3 $C(CH_3)_2$). FABMS (positive-ion mode; $C_{116}H_{119}N_3O_{40}$): *m*/*z* 2216 $[M+Na]^+$.

*4-Methoxyphenyl (2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-(2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-*D*-glucopyranoside* (**15**).—To a solution of **14** (0.30 g, 0.14 mmol) in THF (4 mL) and morpholine (91 μL) was added tetrakis(triphenylphosphine)palladium (30 mg). The mixture was stirred and boiled under reflux until the de-allyloxycarbonylation was complete (**15**; R_f 0.17, TLC (85:15 CH_2Cl_2–acetone)). Then the mixture was diluted with EtOAc (100 mL) and washed with aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (85:15 CH_2Cl_2–acetone) of the residue gave **15**, isolated as a glass (0.26 g, 89%); $[\alpha]_D$ +80° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.679, 7.666, 7.264, 7.213, 7.103, 7.093, 6.958, and 6.952 (8 d, each 2 H, 4 $COC_6H_4CH_3$), 6.66–6.59 (m, 4 H, $C_6H_4OCH_3$), 5.282 and 5.272 (2 dd, each 1 H, $J_{2',3'}=J_{2''',3'''}=9.5$ Hz, $J_{3',4'}=J_{3''',4'''}=9.3$ Hz, H-3′,3‴), 5.503, 5.192, and 5.019 (3 d, each 1 H, $J_{1,2/1'',2''/1''''2''''}$ 8.4, 8.2, and 8.3 Hz, H-1,1″,1‴″), 4.963 and 4.913 (2 dd, each 1 H, $J_{1',2'/1''',2'''}$ 7.9 and 8.0 Hz, H-2′,2‴), 4.718 and 4.659 (2 d, each 1 H, H-1′,1‴), 4.493, 4.352, and 4.021 (3 dd, each 1 H, $J_{2,3/2'',3''/2''''3''''}$ 10.4, 10.4, and 10.3 Hz, H-2,2″,2‴″), 3.642 (s, 3 H, $C_6H_4OCH_3$), 2.358, 2.333, 2.319, and 2.311 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 2.234 and 2.209 (2 s, each 3 H, 2 $COCH_2CH_2COCH_3$), 1.419, 1.262, 1.230, 1.183, 1.143, and 1.136 (6 s, each 3 H, 3 $C(CH_3)_2$); ^{13}C, δ 171.7 and 171.6 (2 $COCH_2CH_2COCH_3$), 164.8 and 164.5 ($COC_6H_4CH_3$), 99.4 (2 C) and 99.3 (3 $C(CH_3)_2$), 99.7, 99.6, 98.5, 98.3, and 97.8 (C-1,1′,1″,1‴,1‴″), 61.8, 61.5 (2 C), and 60.9 (2 C) (C-6,6′,6″,6‴,6‴″), 56.8, 55.4, 55.3, and 55.2 (C-2,2″,2‴″ and $C_6H_4OCH_3$), 37.9, 29.7, and 27.6 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 28.9, 28.8, 28.7, 18.9, 18.7, and 18.6 (3 $C(CH_3)_2$). Anal. Calcd for $C_{112}H_{115}N_3O_{38}$: C, 63.72; H, 5.49. Found: C, 63.28; H, 5.36.

*4-Methoxyphenyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-4,6-di-*O*-*

acetyl-2-deoxy-2-phthalimido-β-D-*glucopyranoside* (**16**).—To a solution of **15** (0.23 g, 0.11 mmol) in CH_2Cl_2 (5 mL) and H_2O (12.5 μL) was added CF_3CO_2H (125 μL). The mixture was stirred for 45 min, when TLC (9:1 CH_2Cl_2–MeOH) showed the disappearance of **15** (R_f 0.91) and the formation of a slower moving spot (R_f 0.63). Then the mixture was diluted with EtOAc (100 mL) and washed with aq 10% $NaHCO_3$ and aq 5% NaCl. The aqueous layers were extracted twice with EtOAc. The combined organic layers were dried, filtered, and concentrated. To a solution of the residue in pyridine (5 mL) was added Ac_2O (5 mL) and a catalytic amount of 4-dimethylaminopyridine. After stirring overnight at room temperature, TLC (4:1 CH_2Cl_2–acetone) showed the acetylation to be complete (**16**; R_f 0.68). The mixture was concentrated and co-concentrated with toluene, EtOH, and CH_2Cl_2 (3×20 mL). Column chromatography (85:15 CH_2Cl_2–acetone) of the residue gave **16**, isolated as a glass (0.22 g, 90%); $[\alpha]_D$ +48° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.680, 7.639, 7.349, 7.338, 7.045, 7.017, 6.998, and 6.973 (8 d, each 2 H, 4 $COC_6H_4CH_3$), 6.67–6.59 (m, 4 H, $C_6H_4OCH_3$), 5.596 (dd, 1 H, $J_{3'''',4''''}$ 9.1 Hz, H-3''''), 5.335, 5.272, and 4.849 (3 d, each 1 H, $J_{1,2/1'',2''/1'''',2''''}$ 8.3, 8.2, and 8.4 Hz, H-1,1'',1''''), 4.995 and 4.983 (2 dd, each 1 H, $J_{1',2'/1''',2'''}$ 7.5 and 7.6 Hz, $J_{2',3'} = J_{2''',3'''} = 9.4$ Hz, H-2',2'''), 4.518, 4.391, and 4.004 (3 dd, each 1 H, $J_{2,3/2'',3''/2'''',3''''}$ 10.7, 10.7, and 10.8 Hz, H-2,2'',2''''), 4.322 and 4.252 (2 d, each 1 H, H-1',1'''), 3.655 (s, 3 H, $C_6H_4OCH_3$), 2.383, 2.359, 2.295, and 2.285 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 2.214 and 2.199 (2 s, each 3 H, 2 $COCH_2CH_2COCH_3$), 2.056, 1.896, 1.888, 1.869, 1.859, 1.838, and 1.759 (7 s, each 3 H, 7 Ac); ^{13}C, δ 171.7 and 171.5 (2 $COCH_2CH_2COCH_3$), 170.5, 170.3, 170.2, 169.8, 169.1, 169.0, and 168.9 (7 $COCH_3$), 164.9 and 164.7 (3 C) (4 $COC_6H_4CH_3$), 100.8, 100.6, and 97.5 (3 C) (C-1,1',1'',1''',1''''), 62.4 (2 C), 62.2, 61.8, and 61.4 (C-6,6',6'',6''',6''''), 55.4, 55.2 (2 C), and 54.6 (C-2,2'',2'''' and $C_6H_4OCH_3$), 37.6, 29.7, and 27.5 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 21.5, 20.5 (3 C), 20.4 (2 C), and 20.1 (7 $COCH_3$). Anal. Calcd for $C_{117}H_{117}N_3O_{45}$: C, 61.50; H, 5.16. Found: C, 61.48; H, 5.26.

4-Methoxyphenyl (3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyl)-(1→3)-(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyl)-(1→3)-4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**17**).—To a solution of **16** (200 mg, 88 μmol) in EtOH (12 mL) and toluene (6 mL) was added NH_2NH_2·HOAc (76 mg, 0.88 mmol). The mixture was stirred for 40 min, when TLC (4:1 CH_2Cl_2–acetone) showed the conversion of **16** into **17** (R_f 0.57), then concentrated. Column chromatography (4:1 CH_2Cl_2–acetone) of the residue yielded **17**, isolated as a glass (136 mg, 74%); $[\alpha]_D$ +45° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.716, 7.659, 7.436, 7.420, 7.115, 7.062, 7.027, and 7.006 (8 d, each 2 H, 4 $COC_6H_4CH_3$), 6.67–6.61 (m, 4 H, $C_6H_4OCH_3$), 5.588 (dd, 1 H, $J_{3'''',4''''}$ 9.1 Hz, H-3''''), 5.369 and 5.318 (2 t, each 1 H, $J_{2',3'} = J_{2''',3'''} =$ 8.8 Hz, $J_{3',4'/3''',4'''}$ 8.7 and 8.8 Hz, H-3',3'''), 5.452, 5.362, and 5.024 (3 d, each 1 H, $J_{1,2/1'',2''/1'''',2''''}$ 8.4, 8.5, and 8.4 Hz, H-1,1'',1''''), 4.925 and 4.872 (2 dd, each 1 H, H-2',2'''), 4.545 and 4.488 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 7.2 and 6.9 Hz, H-1',1'''), 3.661 (s, 3 H, $C_6H_4OCH_3$), 2.365, 2.352, 2.336, and 2.311 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 2.053, 1.910, 1.905, 1.891, 1.880, 1.819, and 1.775 (7 s, each 3 H, 7 Ac). FABMS (positive-ion mode; $C_{107}H_{105}N_3O_{41}$): *m*/*z* 2110 $[M+Na]^+$, 2088 $[M+H]^+$.

4-Methoxyphenyl (3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyluronic acid)-(1→3)-(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyluronic acid)-(1→3)-4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**18**).—To a solution of **17** (55 mg, 26 μmol) in dry CH_2Cl_2 (4 mL) containing Ac_2O (26 μL) was added pyridinium dichromate (39 mg, 104 μmol). After stirring for 1 h, the brown suspension became a brown solution, and after 3 h TLC (10:5:0.5 CH_2Cl_2–acetone–HOAc) showed the conversion of **17** (R_f 0.87) into **18** (R_f 0.13). After addition of EtOAc (25 mL), the suspension was applied to column chromatography (98:2 EtOAc–HOAc) to yield **18**, isolated as a glass (38 mg, 70%); $[\alpha]_D$ +30° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 5.347, 5.231, and 4.881 (3 d, each 1 H, $J_{1,2/1'',2''/1'''',2''''}$ 8.6, 8.5, and 8.3 Hz, H-1,1'',1''''), 4.392 and 4.365 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 8.1 and 7.8 Hz, H-1',1'''), 3.658 (s, 3 H, $C_6H_4OCH_3$), 2.389, 2.367, 2.295, and 2.277 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 2.061, 1.886, 1.862, 1.855, 1.828, 1.797, and 1.762 (7 s, each 3 H, 7 Ac); ^{13}C, δ 170.7 (2 C), 170.4 (2 C), 169.7 (2 C), 169.5, and 169.2 (2 C) (7 $COCH_3$ and 2 COOH), 164.7 and 164.2 ($COC_6H_4CH_3$), 101.5, 100.8, and 97.5 (3 C) (C-1,1',1'',1''',1''''), 62.3 (2 C) and 61.7 (C-

6,6″,6‴), 55.4, 55.2 (2 C), and 54.5 (C-2,2″,2‴ and $C_6H_4OCH_3$), 21.4 ($COC_6H_4CH_3$), 21.5, 20.6 (2 C), 20.5, 20.4 (2 C), and 20.1 (7 $COCH_3$).

A small amount of **18** was esterified with diazomethane in ether, and analysed by ^{1}H NMR: δ 7.689, 7.651, 7.337, 7.328, 7.071, 7.025, 6.992, and 6.967 (8 d, each 2 H, 4 $COC_6H_4CH_3$), 6.625 (bs, 4 H, $C_6H_4OCH_3$), 5.606 (dd, 1 H, $J_{3''',4'''}$ 9.1 Hz, H-3‴), 5.336, 5.279, and 4.801 (3 d, each 1 H, $J_{1,2/1'',2''/1''',2'''}$ 8.5, 8.4, and 8.6 Hz, H-1,1″,1‴), 5.334 and 5.244 (2 dd, each 1 H, $J_{3',4'/3''',4'''}$ 9.0 and 9.2 Hz, H-3′,3‴), 5.024 and 4.969 (2 dd, each 1 H, $J_{1',2'/1''',2'''}$ 7.4 and 7.6 Hz, $J_{2',3'/2''',3'''}$ 9.2 and 9.3 Hz, H-2′,2‴), 4.629, 4.519, and 4.388 (3 dd, each 1 H, $J_{2,3/2'',3''/2''',3'''}$ 10.9, 10.7, and 10.9 Hz, H-2,2″,2‴), 4.363 and 4.326 (2 d, each 1 H, H-1′,1‴), 3.706 and 3.543 (2 d, each 1 H, $J_{4',5'/4''',5'''}$ 9.6 and 9.3 Hz, H-5′,5‴), 3.657 (s, 3 H, $C_6H_4OCH_3$), 3.499 and 3.442 (2 s, each 3 H, 2 $COOCH_3$), 2.391, 2.362, 2.306, and 2.281 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 2.057, 1.890, 1.881, 1.853, 1.837, 1.764, and 1.743 (7 s, each 3 H, 7 Ac). FABMS (positive-ion mode; $C_{109}H_{105}N_3O_{43}$): *m*/*z* 2166 $[M+Na]^+$.

*4-Methoxyphenyl (2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-(*β*-D-glucopyranosyluronic acid)-(1→3)-(2-acetamido-2-deoxy-*β*-D-glucopyranosyl)-(1→4)-(*β*-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-*β*-D-glucopyranoside* (**1**).—A solution of **18** (30 mg, 14 μmol) in ethanolic 33% $MeNH_2$ (7.5 mL) was stirred for 10 days at room temperature, during which the mixture was concentrated repeatedly and new reagent (5×7.5 mL) added. After concentration, the residue was dissolved in dry MeOH (5 mL), Ac_2O (100 μL) was added at 0 °C, and the mixture was stirred for 2 h. Then, TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed the complete disappearance of **18** and the formation of **1** (R_f 0.45), and the solution was concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL). Gel filtration on Sephadex G-10 (water) of the residue yielded **1**, isolated after lyophilisation as a white, amorphous powder (10 mg, 64%); $[\alpha]_D$ +12° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 7.06–6.96 (m, 4 H, $C_6H_4OCH_3$), 5.054 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.564 and 4.533 (2 d, each 1 H, $J_{1'',2''/1''',2'''}$ 8.5 and 8.4 Hz, H-1″,1‴), 4.511 and 4.466 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 7.8 and 7.9 Hz, H-1′,1‴), 4.089 (dd, 1 H, $J_{2,3}$ 10.9 Hz, H-2), 3.851 and 3.704 (2 dd, each 1 H, $J_{2'',3''/2''',3'''}$ 10.7 and 10.9 Hz, H-2″,2‴), 3.387 and 3.347 (2 dd, each 1 H, $J_{2',3'/2''',3'''}$ 9.4 and 9.5 Hz, H-2′,2‴), 3.808 (s, 3 H, $C_6H_4OCH_3$), 2.049, 2.023, and 2.019 (3 s, each 3 H, 3 $NHCOCH_3$). FABMS (negative-ion mode; $C_{43}H_{63}N_3O_{29}$): *m*/*z* 1084 $[M-H]^-$.

*4-Methoxyphenyl (6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-*β*-D-glucopyranosyl)-(1→3)-(2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-D-glucopyranosyl)-(1→3)-(2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-D-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-*β*-D-glucopyranosyl)-(1→3)-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-*β*-D-glucopyranoside* (**19**).—To a solution of **15** (141 mg, 67 μmol) and 6-*O*-levulinoyl-2,3,4-tri-*O*-*p*-toluoyl-*β*-D-glucopyranosyl trichloroacetimidate [8] (**9**; 129 mg, 167 μmol) in dry CH_2Cl_2 (5 mL), containing 3 Å molecular sieves (0.2 g), was added at 0 °C Me_3SiOTf (6.5 μL). After stirring for 45 min, TLC (93:7 CH_2Cl_2–acetone) showed the disappearance of **15** and the formation of **19** (R_f 0.27). Then the mixture was neutralised with Et_3N, diluted with CH_2Cl_2 (150 mL), filtered through Celite, and washed with aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue gave **19**, isolated as a glass (112 mg, 62%); $[\alpha]_D$ +32° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.534, 7.528, 7.490, 7.460, 7.255, 7.246, 7.204, 7.108, 7.093, 7.080, 6.970, 6.960, 6.952, and 6.949 (14 d, each 2 H, 7 $COC_6H_4CH_3$), 6.68–6.58 (m, 4 H, $C_6H_4OCH_3$), 5.502, 5.040, and 5.001 (3 d, each 1 H, $J_{1,2/1'',2''/1''',2'''}$ 8.4, 8.8, and 8.6 Hz, H-1,1″,1‴), 5.095, 4.956, and 4.872 (3 dd, each 1 H, $J_{1',2'/1''',2'''/1'''',2''''}$ 7.8, 7.9, and 8.0 Hz, $J_{2',3'/2''',3'''/2'''',3''''}$ 9.0, 9.5, and 9.6 Hz, H-2′,2‴,2⁗), 4.879, 4.713, and 4.586 (3 d, each 1 H, H-1′,1‴,1⁗), 4.491, 4.366, and 4.352 (3 dd, each 1 H, $J_{2,3/2'',3''/2''',3'''}$ 10.4, 10.4, and 10.2 Hz, H-2,2″,2‴), 3.636 (s, 3 H, $C_6H_4OCH_3$), 2.357, 2.351, 2.326, 2.301, and 2.240 (5 s, 3,3,6,6,3 H, 7 $COC_6H_4CH_3$), 2.219, 2.203, and 2.146 (3 s, each 3 H, 3 $COCH_2CH_2COCH_3$), 1.416, 1.278, 1.258, 1.253, 1.248, and 1.087 (6 s, each 3 H, 3 $C(CH_3)_2$); ^{13}C, δ 172.1 and 171.5 (2 C) (3 $COCH_2CH_2COCH_3$), 165.4, 164.7 (2 C), 164.5 (2 C), and 164.3 (2 C) (7 $COC_6H_4CH_3$), 99.4, 99.1, and 98.9 (3 $C(CH_3)_2$), 99.7 (3 C), 98.3 (2 C), and 97.8 (C-1,1′,1″,1‴,1⁗,1⁗′), 62.5, 61.8, 61.5 (2 C), and 60.8 (2 C) (C-6,6′,6″,6‴,6⁗,6⁗′), 55.4 (C-2,2″,2‴ and $C_6H_4OCH_3$), 37.7, 29.7, and 27.6 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 28.9 (3 C), 18.9, and 18.7 (2 C) (3 $C(CH_3)_2$). FABMS (positive-ion mode; $C_{147}H_{149}N_3O_{48}$): *m*/*z* 2748 $[M+Na]^+$.

*4-Methoxyphenyl (6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside* (**20**).—To a solution of **19** (90 mg, 33 μmol) in CH_2Cl_2 (5 mL) and H_2O (5 μL) was added CF_3CO_2H (50 μL). The mixture was stirred for 30 min, when TLC (9:1 CH_2Cl_2–MeOH) showed the disappearance of **19** (R_f 0.93) and the formation of a slower moving spot (R_f 0.70). Then the mixture was diluted with EtOAc (100 mL), and washed with aq 10% $NaHCO_3$ and aq 5% NaCl. The aqueous layers were extracted twice with EtOAc. The combined organic layers were dried, filtered, and concentrated. To a solution of the mixture in pyridine (5 mL) were added Ac_2O (5 mL) and a catalytic amount of 4-dimethylaminopyridine. After stirring overnight at room temperature, TLC (95:5 CH_2Cl_2–MeOH) showed the acetylation to be complete (**20**; R_f 0.37). The mixture was concentrated and co-concentrated with toluene, EtOH, and CH_2Cl_2 (3×20 mL). Column chromatography (95:5 CH_2Cl_2–MeOH) of the residue gave **20**, isolated as a glass (80 mg, 84%); $[\alpha]_D$ +24° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 6.68–6.59 (m, 4 H, $C_6H_4OCH_3$), 5.510 (t, 1 H, $J_{3'''',4'''',5'''',6a''''}$ = $J_{4'''',5'''',6a'''',6b''''}$ = 9.6 Hz, H-4''''), 5.337, 4.885, and 4.843 (3 d, each 1 H, $J_{1,2/1'',2''/1'''',2''''}$ 8.5, 8.5, and 8.4 Hz, H-1,1'',1''''), 5.141, 4.992, and 4.927 (3 dd, each 1 H, $J_{1',2'/1''',2'''/1''''',2'''''}$ 7.8, 7.5, and 7.5 Hz, $J_{2',3'/2''',3'''/2''''',3''''}$ 10.3, 9.1, and 9.2 Hz, H-2',2''',2'''''), 4.486, 4.324, and 4.162 (3 d, each 1 H, H-1',1''',1'''''), 4.391, 4.061, and 3.982 (3 dd, each 1 H, $J_{2,3/2'',3''/2'''',3''''}$ 10.8, 10.7, and 10.8 Hz, H-2,2'',2''''), 3.647 (s, 3 H, $C_6H_4OCH_3$), 2.394, 2.374, 2.352, 2.308, 2.279, and 2.220 (6 s, 3,3,3,3,6,3 H, 7 $COC_6H_4CH_3$), 2.215, 2.192, and 2.136 (3 s, each 3 H, 3 $COCH_2CH_2COCH_3$), 2.053, 1.994, 1.897, 1.872, 1.851, 1.786 (6 s, each 3 H, 6 Ac); ^{13}C, δ 171.9, 171.5, and 171.4 (3 $COCH_2CH_2COCH_3$), 170.5, 170.3 (2 C), 169.0, 168.9, and 168.7 (6 $COCH_3$), 165.4, 164.7 (2 C), 164.6 (2 C), and 164.5 (2 C) (7 $COC_6H_4CH_3$), 100.7 (2 C), 100.5, 97.3 (3 C) (C-1,1',1'',1''',1'''',1'''''), 62.1 (3 C), 62.0, and 61.6 (2 C) (C-6,6',6'',6''',6'''',6'''''), 55.3, 55.2, and 55.1 (2 C) (C-2,2'',2'''' and $C_6H_4OCH_3$), 37.5, 29.5, and 27.4 ($COCH_2CH_2COCH_3$), 21.4 and 21.3 ($COC_6H_4CH_3$), 20.5 (3 C), 20.4, 20.3, and 20.2 (6 $COCH_3$). FABMS (positive-ion mode; $C_{150}H_{149}N_3O_{54}$): *m/z* 2880 $[M+Na]^+$, 2858 $[M+H]^+$.

*4-Methoxyphenyl (2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside* (**21**).—To a solution of **20** (65 mg, 23 μmol) in EtOH (6 mL) and toluene (3 mL) was added NH_2NH_2·HOAc (30 mg, 0.34 mmol). The mixture was stirred for 2 h, when TLC (1:1 CH_2Cl_2–EtOAc) showed the conversion of **20** into **21** (R_f 0.49). Then, the mixture was concentrated, and column chromatography (3:1 CH_2Cl_2–acetone) of the residue yielded **21**, isolated as a glass (44 mg, 75%); $[\alpha]_D$ +37° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.741, 7.671, 7.652, 7.543, 7.445, 7.416, 7.403, 7.125, 7.082, 7.055, 7.029, 7.000, 6.983, and 6.970 (14 d, each 2 H, 7 $COC_6H_4CH_3$), 6.70–6.60 (m, 4 H, $C_6H_4OCH_3$), 5.621 (t, 1 H, $J_{3'''',4'''''}$ = $J_{4'''',5''''}$ = 9.6 Hz, H-4''''), 5.360, 5.045, and 5.012 (3 d, each 1 H, $J_{1,2/1'',2''/1''',2'''}$ 8.5, 8.5, and 8.4 Hz, H-1,1'',1'''), 5.151, 4.834, and 4.723 (3 dd, each 1 H, $J_{1',2'/1''',2'''/1'''',2''''}$ 7.7, 7.3, and 7.1 Hz, $J_{2',3'/2''',3'''/2'''',3''''}$ 9.6, 8.6, and 9.1 Hz, H-2',2''',2''''), 4.588, 4.535, and 4.396 (3 d, each 1 H, H-1',1''',1''''), 4.482, 4.148, and 4.095 (3 dd, each 1 H, $J_{2,3/2'',3''/2''',3'''}$ 11.1, 11.0, and 11.3 Hz, H-2,2'',2'''), 3.656 (s, 3 H, $C_6H_4OCH_3$), 2.379, 2.355, 2.348, 2.325, 2.305, and 2.217 (6 s, 3,3,3,6,3,3 H, 7 $COC_6H_4CH_3$), 2.052, 2.010, 1.920, 1.903, 1.877, and 1.781 (6 s, each 3 H, 6 Ac); ^{13}C, δ 170.5, 170.4, 170.3, 169.5, 169.4, and 169.2 (6 $COCH_3$), 165.9, 165.5, 164.7 (2 C), 164.6, and 164.5 (2 C) (7 $COC_6H_4CH_3$), 99.9 and 99.5 (2 C) (C-1',1''',1''''), 97.9, 97.8, and 97.5 (C-1,1'',1'''), 62.0, 61.4 (2 C), 60.9, and 60.3 (2 C) (C-6,6',6'',6''',6'''',6'''''), 55.4, 55.5, 55.3, and 55.2 (C-2,2'',2''' and $C_6H_4OCH_3$), 21.5, 21.4, and 21.3 ($COC_6H_4CH_3$), 20.6, 20.5, and 20.4 ($COCH_3$). FABMS (positive-ion mode; $C_{135}H_{131}N_3O_{48}$): *m/z* 2584 $[M+Na]^+$, 2562 $[M+H]^+$.

*4-Methoxyphenyl (2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranosyluronic acid)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-p-toluoyl-β-D-glucopyranosyluronic acid)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-(2,3-di-*O*-p-toluoyl-β-D-glucopyranosyluronic acid)-(1→3)-4,6-di-*O*-acetyl-*

2-deoxy-2-phthalimido-β-D-*glucopyranoside* (**22**).—To a solution of **21** (40 mg, 16 μmol) in dry CH_2Cl_2 (2 mL), containing Ac_2O (22 μL), was added pyridinium dichromate (35 mg, 93 μmol). After stirring for 1 h the brown suspension became a brown solution, and after 6 h TLC (5:5:1 CH_2Cl_2–EtOAc–HOAc) showed the conversion of **21** (R_f 0.70) into **22** (R_f 0.19). After the addition of EtOAc (25 mL), the suspension was applied to column chromatography (98:2 EtOAc–HOAc) to yield **22**, isolated as a glass (24 mg, 58%); $[\alpha]_D$ −2° (*c* 1).

A small amount of **22** was esterified with diazomethane in ether, and analysed by ^{1}H NMR: δ 7.692, 7.645, 7.559, 7.407, 7.324, 7.299, 7.115, 7.021, 6.993, and 6.966 (10 d, 2,4,2,2,2,2,2,4,4,4 H, 7 $COC_6H_4CH_3$), 6.624 (bs, 4 H, $C_6H_4OCH_3$), 5.577 (t, 1 H, $J_{3'''',4''''}=J_{4'''',5''''}=9.5$ Hz, H-4''''), 5.335, 4.831, and 4.796 (3 d, each 1 H, $J_{1,2/1'',2''/1''',2'''}$ 8.5, 8.4, and 8.4 Hz, H-1,1'',1''''), 4.513, 4.354, and 4.212 (3 d, each 1 H, $J_{1',2'/1''',2'''/1'''',2''''}$ 7.8, 7.4, and 7.6 Hz, H-1',1''',1'''''), 3.657 (s, 3 H, $C_6H_4OCH_3$), 3.591, 3.427, and 3.418 (3 s, each 3 H, 3 $COOCH_3$), 2.402, 2.383, 2.359, 2.327, 2.286, 2.277, and 2.238 (7 s, each 3 H, 7 $COC_6H_4CH_3$), 2.079, 1.874, 1.850, 1.844, 1.833, and 1.710 (6 s, each 3 H, 6 Ac). FABMS (positive-ion mode; $C_{138}H_{131}N_3O_{51}$): *m*/*z* 2668 $[M+Na]^+$, 2646 $[M+H]^+$.

4-Methoxyphenyl (^{4,5}Δ-*glucopyranosyluronic acid*)-(1→3)-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(1→4)-(β-D-*glucopyranosyluronic acid*)-(1→3)-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(1→4)-(β-D-*glucopyranosyluronic acid*)-(1→3)-*2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**3**).—A solution of **22** (12 mg, 4.5 μmol) in ethanolic 33% $MeNH_2$ (7.5 mL) was stirred for 10 days at room temperature, during which the mixture was concentrated and repeatedly new reagent (5×7.5 mL) added. After final concentration, the residue was dissolved in dry MeOH (5 mL) and Ac_2O (100 μL) was added at 0 °C, and the mixture was stirred for 2 h. Then, TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed a complex mixture. The solution was concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL). To a solution of the residue in MeOH (5 mL) was added NaOMe (pH 11), and the mixture was stirred overnight. After concentration a solution of the residue in MeOH (10 mL) was stirred with Dowex-50 (H^+) for 1 h, then the mixture was filtered and concentrated. The residue was dissolved in dry MeOH (5 mL) and Ac_2O (100 μL) was added at 0 °C, and the mixture was stirred for 2 h. Then, TLC (4:2:3:2 1-BuOH–EtOH–H_2O–HOAc) showed the formation of a major compound (R_f 0.23). After concentration, gel filtration on Sephadex G-10 (water) of the residue yielded **3**, isolated after lyophilisation as a white, amorphous powder (3 mg, 40%); $[\alpha]_D$ +108° (*c* 0.25, H_2O); ^{1}H NMR (D_2O): δ 7.11–6.95 (m, 4 H, $C_6H_4OCH_3$), 5.849 (d, 1 H, $J_{3'''',4''''}$ 4.0 Hz, H-4''''), 5.151 (d, 1 H, $J_{1'''',2''''}$ 4.9 Hz, H-1''''), 5.053 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.588 and 4.563 (2 d, each 1 H, $J_{1'',2''/1''',2'''}$ 8.0 and 8.5 Hz, H-1'',1'''), 4.510 and 4.465 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 7.7 and 7.8 Hz, H-1',1'''), 4.136 (t, 1 H, $J_{2'''',3''''}$ 4.3 Hz, H-3''''), 4.089 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 3.845 (dd, 2 H, $J_{2'',3''/2''',3'''}$ 10.2 Hz, H-2'',2'''), 3.750 (dd, 1 H, H-2''''), 3.725 (s, 3 H, $C_6H_4OCH_3$), 3.386 and 3.347 (2 dd, each 1 H, $J_{2',3'/2''',3'''}$ 9.5 and 9.4 Hz, H-2',2'''), 2.064, 2.032, and 2.021 (3 s, each 3 H, 3 $NHCOCH_3$). FABMS (negative-ion mode; $C_{49}H_{69}N_3O_{34}$): *m*/*z* 1264 $[M+Na-H]^-$, 1242 $[M-H]^-$.

4-Methoxyphenyl (β-D-*glucopyranosyluronic acid*)-(1→3)-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(1→4)-(β-D-*glucopyranosyluronic acid*)-(1→3)-(*2-acetamido-2-deoxy*-β-D-*glucopyranosyl*)-(1→4)-(β-D-*glucopyranosyluronic acid*)-(1→3)-*2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**2**).—To a solution of **22** (16 mg, 6 μmol) in 1-BuOH (5 mL) was added ethylenediamine (1 mL). The mixture was stirred overnight at 90 °C under Ar, when TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed the disappearance of **22**. The mixture was concentrated and co-concentrated with toluene (5×10 mL). The residue was dissolved in dry pyridine (5 mL), and Ac_2O (5 mL) and a catalytic amount of 4-dimethylaminopyridine were added. The mixture was stirred overnight at room temperature, then concentrated and co-concentrated with toluene and EtOH (2×20 mL). The yellow, amorphous solid was dissolved in THF (5 mL), and at 0 °C was added aq 2 M NaOH (1 mL) under vigorous stirring. After stirring for 6 h at 0 °C, TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed the conversion into **2** (R_f 0.35), and the solution was neutralised with aq 1 M HCl. After concentration and co-concentration with 1:1 toluene-MeOH (3×10 mL), gel filtration on Sephadex G-10 (water) of the residue yielded **2**, isolated after lyophilisation as a white, amorphous powder (5 mg, 73%); $[\alpha]_D$ +44° (*c* 0.25, H_2O); ^{1}H NMR (D_2O): δ 7.05–6.97 (m, 4 H, $C_6H_4OCH_3$), 5.053 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.563 (d, 2 H, $J_{1'',2''/1''',2'''}$ 8.5 Hz, H-1'',1'''), 4.509 and 4.464 (2 d, 1,2 H, $J_{1',2'/1''',2'''/1'''',2''''}$ 7.7

and 8.1 Hz, H-1′,1‴,1⁗), 4.087 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.842 (dd, 2 H, $J_{2'',3''/2'''',3''''}$ 10.2 Hz, H-2″,2⁗), 3.501, 3.380, and 3.375 (3 dd, each 1 H, $J_{2',3'/2''',3'''/2'''',3''''}$ 9.4, 9.4, and 9.5 Hz, H-2′,2‴,2⁗), 3.806 (s, 3 H, $C_6H_4OCH_3$), 2.030, 2.020, and 2.018 (3 s, each 3 H, 3 $NHCOCH_3$). FABMS (negative-ion mode; $C_{49}H_{71}N_3O_{35}$): *m/z* 1282 $[M+Na-H]^-$, 1260 $[M-H]^-$.

4-Methoxyphenyl (3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(sodium 2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyl 6-sulfate)-(1→3)-(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(sodium 2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyl 6-sulfate)-(1→3)-4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**23**).—To a solution of **17** (50 mg, 24 μmol) in dry DMF (4 mL) was added sulfur trioxide-trimethylamine complex (50 mg, 360 μmol), and the mixture was stirred overnight at 50 °C under Ar. Then, TLC (85:15 CH_2Cl_2–MeOH) showed the disappearance of **17** and the formation of a new spot (R_f 0.57). After the addition of MeOH (1 mL), stirring was continued for 15 min. The mixture was concentrated and a solution of the residue in MeOH (10 mL) was stirred with Dowex-50 (Na^+) for 1 h, then the mixture was filtered and concentrated. Column chromatography (9:1 CH_2Cl_2-MeOH) of the residue afforded **23** as a white, amorphous powder (49 mg, 88%); $[\alpha]_D$ −5° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.666, 7.640, 7.306, 7.301, 7.106, 7.085, 7.036, and 7.019 (8 d, each 2 H, 4 $COC_6H_4CH_3$), 6.67–6.59 (m, 4 H, $C_6H_4OCH_3$), 5.628 (dd, 1 H, $J_{3'''',4''''}$ 9.0 Hz, H-3⁗), 5.599, 5.421, and 5.164 (3 d, each 1 H, $J_{1,2/1'',2''/1'''',2''''}$ 8.2, 8.5, and 8.3 Hz, H-1,1″,1⁗), 4.444 and 4.420 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 6.8 and 7.3 Hz, H-1′,1‴), 3.632 (s, 3 H, $C_6H_4OCH_3$), 2.397, 2.386, and 2.294 (3 s, 3,3,6 H, 4 $COC_6H_4CH_3$), 2.124, 2.103, 2.035, 1.911, 1.852, 1.884, and 1.716 (7 s, each 3 H, 7 Ac); ^{13}C, δ 170.8, 170.7 (2 C), 170.6, 170.4, 169.9, and 169.2 (7 $COCH_3$), 164.9 (2 C), 164.8, and 164.7 (4 $COC_6H_4CH_3$), 100.2, 99.4, 96.9, 95.5, and 95.4 (C-1,1′,1″,1‴,1⁗), 65.4 and 63.8 (C-6′,6‴), 61.7 (2 C) and 61.0 (C-6,6″,6⁗), 55.2, 55.2, 54.7, and 54.2 (C-2,2″,2⁗ and $C_6H_4OCH_3$), 20.7, 20.6, 20.5, and 20.4 (4 $COC_6H_4CH_3$), 20.1, 19.9, 19.7, 19.6 (2 C), 19.5, and 19.3 (7 $COCH_3$).

4-Methoxyphenyl (2-acetamido-2-deoxy-β-D-*glucopyranosyl)-(1→4)-(sodium* β-D-*glucopyranosyl 6-sulfate)-(1→3)-(2-acetamido-2-deoxy*-β-D-*glucopyranosyl)-(1→4)-(sodium* β-D-*glucopyranosyl 6-sulfate)-(1→3)-2-acetamido-2-deoxy*-β-D-*glucopyranoside* (**4**).—To a solution of **23** (49 mg, 21 μmol) in 1-BuOH (5 mL) was added ethylenediamine (1 mL). The mixture was stirred overnight at 90 °C under Ar, when TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed the disappearance of **23**. The mixture was concentrated and co-concentrated with toluene (5×10 mL). The residue was dissolved in dry pyridine (5 mL), and Ac_2O (5 mL) and a catalytic amount of 4-dimethylaminopyridine were added. The mixture was stirred overnight at room temperature, then concentrated and co-concentrated with toluene and EtOH (2×20 mL). The yellow, amorphous solid was dissolved in THF (5 mL), and at 0 °C under vigorous stirring, aq 2 M NaOH (1 mL) was added. After stirring for 6 h at 0 °C, TLC analysis (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed the conversion into a new spot with R_f 0.32, then aq 1 M HCl was added to neutralise the solution. The mixture was concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL), and a solution of the residue in MeOH (10 mL) was stirred with Dowex-50 (Na^+) for 1 h, then filtered and concentrated. Gel filtration on Sephadex G-10 (water) of the residue yielded **4**, isolated after lyophilisation as a slightly yellow, amorphous powder (17 mg, 68%); $[\alpha]_D$ −32° (*c* 1, H_2O); ^{1}H NMR (D_2O): δ 7.06–6.97 (m, 4 H, $C_6H_4OCH_3$), 5.073 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.634 and 4.601 (2 d, each 1 H, $J_{1'',2''/1'''',2''''}$ 9.0 and 8.4 Hz, H-1″,1⁗), 4.537 and 4.515 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 7.8 and 8.4 Hz, H-1′,1‴), 4.269 (dd, 2 H, $J_{5',6a'/5''',6a'''}$ 6.0 Hz, $J_{6a',6b'/6a''',6b'''}$ 10.8 Hz, H-6a′,6a‴), 4.112 (dd, 2 H, $J_{5',6b'/5''',6b'''}$ < 1 Hz, H-6b′,6b‴), 4.074 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.810 (s, 3 H, $C_6H_4OCH_3$), 3.364 and 3.327 (2 dd, each 1 H, $J_{2',3'/2''',3'''}$ 9.0 and 8.4 Hz, H-2′,2‴), 2.086, 2.064, and 2.019 (3 s, each 3 H, 3 $NHCOCH_3$). FABMS (positive-ion mode; $C_{43}H_{65}N_3O_{33}S_2Na_2$): *m/z* 1284 $[M+Na]^+$, 1262 $[M+H]^+$.

4-Methoxyphenyl (sodium 2,3,4-tri-O-p-*toluoyl*-β-D-*glucopyranosyl 6-sulfate)-(1→3)-(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(sodium 2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyl 6-sulfate)-(1→3)-(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→4)-(sodium 2,3-di*-O-p-*toluoyl*-β-D-*glucopyranosyl 6-sulfate)-(1→3)-4,6-di*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**24**).—To a solution of **21** (33 mg, 13 μmol) in dry DMF (5 mL) was added sulfur trioxide–trimethylamine complex (37 mg, 268 μmol), and the mixture was stirred overnight at 50 °C under Ar. Then, TLC (4:1 CH_2Cl_2-MeOH) showed

the disappearance of **21** and the formation of **24** (R_f 0.40). After the addition of MeOH (1 mL), stirring was continued for 15 min. The mixture was concentrated and a solution of the residue in MeOH (10 mL) was stirred with Dowex-50 (Na^+) for 1 h, then the mixture was filtered, and concentrated. Column chromatography (85:15 CH_2Cl_2–MeOH) of the residue afforded **24** as a white, amorphous powder (28 mg, 78%); $[\alpha]_D$ +64° (*c* 0.5); NMR ($CDCl_3$): ^{1}H, δ 7.579, 7.531, 7.493, 7.409, 7.311, 7.209, 7.202, 6.993, 6.948, 6.928, 6.889, 6.881, 6.873, and 6.853 (14 d, each 2 H, 7 $COC_6H_4CH_3$), 6.508 (bs, 4 H, $C_6H_4OCH_3$), 5.422 (t, 1 H, $J_{3'''',4'''',5'''',6a'''',6b''''}$ = 9.5 Hz, H-4''''), 5.214, 5.008, and 4.980 (3 d, each 1 H, $J_{1,2/1'',2''/1''',2'''}$ 8.5, 8.2, and 7.9 Hz, H-1,1'',1'''), 4.842, 4.751, and 4.678 (3 dd, each 1 H, $J_{1',2'/1''',2'''/1'''',2''''}$ 7.8, 7.5, and 7.5 Hz, $J_{2',3'/2''',3'''/2'''',3''''}$ 9.5, 9.5, and 9.1 Hz, H-2',2''',2''''), 4.696, 4.183, and 4.105 (3 d, each 1 H, H-1',1''',1''''), 3.543 (s, 3 H, $C_6H_4OCH_3$), 2.288, 2.262, 2.253, 2.198, 2.186, 2.159, and 2.116 (7 s, each 3 H, 7 $COC_6H_4CH_3$), 2.026, 1.985, 1.947, 1.895, 1.796, and 1.756 (6 s, each 3 H, 6 Ac); ^{13}C, δ 171.2 (2 C), 170.9 (2 C), 170.7, and 170.4 (6 $COCH_3$), 165.5, 165.4, 165.0, 164.9 (2 C), and 164.8 (2 C) (7 $COC_6H_4CH_3$), 100.5, 99.7 (2 C), 97.2, 95.7, and 95.4 (C-1,1',1'',1''',1'''',1'''''), 66.5 (2 C), 64.6, and 62.0 (3 C) (C-6,6',6'',6''',6'''',6'''''), 55.3 (C-2,2'',2'''' and $C_6H_4OCH_3$), 21.2, 21.1, and 21.0 ($COC_6H_4CH_3$), 20.4, 20.2, and 20.1 ($COCH_3$).

4-Methoxyphenyl (sodium β-D-*glucopyranosyl 6-sulfate)-(1→3)-(2-acetamido-2-deoxy-*β-D-*glucopyranosyl)-(1→4)-(sodium* β-D-*glucopyranosyl 6-sulfate)-(1→3)-(2-acetamido-2-deoxy-*β-D-*glucopyranosyl)-(1→4)-(sodium* β-D-*glucopyranosyl 6-sulfate)-(1→3)-2-acetamido-2-deoxy-*β-D-*glucopyranoside* (**5**).—To a solution of **24** (25 mg, 8.9 μmol) in 1-BuOH (5 mL) was added ethylenediamine (1 mL). The mixture was stirred overnight at 90 °C under Ar, when TLC (4:2:2:1 1-BuOH–EtOH–H_2O–HOAc) showed the disappearance of **24**. The mixture was concentrated and co-concentrated with toluene (5×10 mL). The residue was dissolved in dry pyridine (5 mL), and Ac_2O (5 mL) and a catalytic amount of 4-dimethylaminopyridine were added. The mixture was stirred overnight at room temperature, then concentrated and co-concentrated with toluene and EtOH (2×20 mL). The yellow, amorphous solid was dissolved in THF (5 mL), and at 0 °C under vigorous stirring, aq 2 M NaOH (1 mL) was added. After stirring for 6 h at 0 °C, TLC (4:2:2:1 1-BuOH–EtOH–H_2O–HOAc) showed the conversion into a new spot with R_f 0.55. The solution was neutralised with aq 1 M HCl, concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL), and a solution of the residue in MeOH (10 mL) was stirred with Dowex-50 (Na^+) for 1 h, then filtered and concentrated. Gel filtration on Sephadex G-10 (water) of the residue yielded **5**, isolated after lyophilisation as a white, amorphous powder (11 mg, 88%); $[\alpha]_D$ −112° (*c* 0.5, H_2O); ^{1}H NMR (D_2O): δ 7.06–6.96 (m, 4 H, $C_6H_4OCH_3$), 5.066 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.638 and 4.632 (2 d, each 1 H, $J_{1'',2''/1'''',2''''}$ 8.4 and 8.5 Hz, H-1'',1''''), 4.532, 4.516, and 4.505 (3 d, each 1 H, $J_{1',2'/1''',2'''/1'''',2''''}$ 8.1, 8.1, and 7.7 Hz, H-1',1''',1'''''), 4.330 and 4.273 (2 dd, 1,2 H, $J_{5',6a'/5''',6a'''/5'''',6a''''}$ 2.2 and 2.9 Hz, $J_{6a',6b'/6a''',6b'''/6a'''',6b''''}$ 11.4 and 12.1 Hz, H-6a',6a''',6a''''), 4.208 and 4.109 (2 dd, 1,2 H, $J_{5',6b'/5''',6b'''/5'''',6b''''}$ 5.2 and 4.1 Hz, H-6b',6b''',6b''''), 4.083 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.809 (s, 3 H, $C_6H_4OCH_3$), 3.384, 3.330, and 3.304 (3 dd, each 1 H, $J_{2',3'/2''',3'''/2'''',3''''}$ 9.1, 9.4, and 8.9 Hz, H-2',2''',2'''''), 2.074, 2.067, and 2.021 (3 s, each 3 H, 3 $NHCOCH_3$). FABMS (positive-ion mode; $C_{49}H_{74}N_3O_{41}S_3Na_3$): *m*/*z* 1526 $[M+H]^+$.

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