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Synthesis of hyaluronic acid related di- and tetra-saccharides having a glucuronic acid at the reducing end

Ted M. Slaghek[a,#], Teija K. Hyppönen[a], Tomoya Ogawa[b], Johannis P. Kamerling[a], and Johannes F. G. Vliegenthart[a,*]

[a] Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht, Netherlands
[b] The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-01 Japan

[#] Present Address: Agricultural Research Institute (ATO-DLO), P.O. Box 17, NL-6700 AA Wageningen, Netherlands

Abstract: The synthesis is reported of 4-methoxyphenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-β-D-glucopyranosyluronic acid (**1**) and 4-methoxyphenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-β-D-glucopyranosyluronic acid (**5**), which represent structural elements of hyaluronic acid. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**3**) was condensed with 4-methoxyphenyl 6-*O*-levulinoyl-2,3-di-*O*-*p*-toluoyl-β-D-glucopyranoside (**4**) in dichloromethane, using boron trifluoride etherate as a promoter, yielding 4-methoxyphenyl *O*-(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-glucopyranoside (**2**). Subsequent delevulinoylation, oxidation, complete deprotection, and *N*-acetylation gave **1**. Coupling of 3-*O*-allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**9**) with **4**, followed by de-allyloxycarbonylation of the obtained disaccharide derivative gave 4-methoxyphenyl *O*-(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 (**8**). Demethoxyphenylation and subsequent imidation of **2** afforded *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-6-*O*-levulinoyl-2,3-di-*O*-*p*-toluoyl-α/β-D-glucopyranosyl trichloroacetimidate (**7**). Condensation of **7** with **8** in dichloromethane, with trimethylsilyl trifluoromethanesulfonate as a promoter, gave tetrasaccharide derivative **15**. Subsequent de-isopropylidenation, *O*-acetylation, delevulinoylation, oxidation, complete deprotection, and *N*-acetylation yielded **5**.

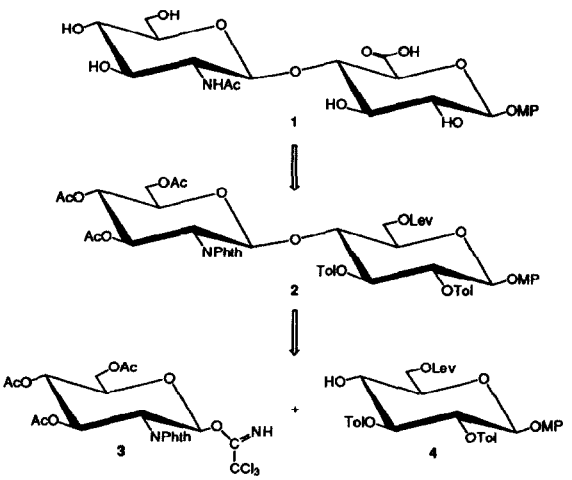
INTRODUCTION

Hyaluronic acid (HA), a unique carbohydrate polymer belonging to the class of glycosaminoglycans, is the major component of several soft connective tissues and has also been found in certain bacterial strains.[1] It is a linear, negatively charged polysaccharide[2] consisting of a repeating disaccharide unit, namely, [→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→]$_n$. The biopolymer is biosynthesised at the innerside of plasma membranes,[3] and it plays an important role in structural functions,[4] cell-cell recognition,[5] cell migration,[6] and angiogenesis.[7] As a medical aid, HA is used in opthalmic surgery[8] and woundhealing,[9] and to combat joint diseases.[10] Endo-

thelial cell growth is inhibited by HA, but stimulated by enzymically generated fragments of HA (3-10 disaccharide units).[1,7] Isolation of the various oligosaccharides is laborious and sofar mainly mixtures of even numbered oligosaccharides were used in biological testing.[7,11] The stimulating effect of HA oligosaccharides on angiogenesis initiated a research program on the synthesis of a wider range of medium-sized oligosaccharide elements constituted of even or odd numbers of monosaccharides with 2-acetamido-2-deoxy-D-glucose or D-glucuronic acid at the reducing end. Using these synthetic oligosaccharides it is aimed to study the biological activity of HA oligosaccharides in more detail. In earlier studies we have reported on the organic synthesis of the 4-methoxyphenyl (MP) glycosides of 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, and 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.[12] This report describes the stereoselective synthesis of a di- and a tetra-saccharide fragment having the MP glycoside of β-D-glucuronic acid at the reducing end, namely, β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-OMP 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-OMP. A short communication with respect to the synthesis of the tetrasaccharide has appeared,[13] and recently the synthesis of the methyl glycoside analogue of the disaccharide has been published by others.[14]

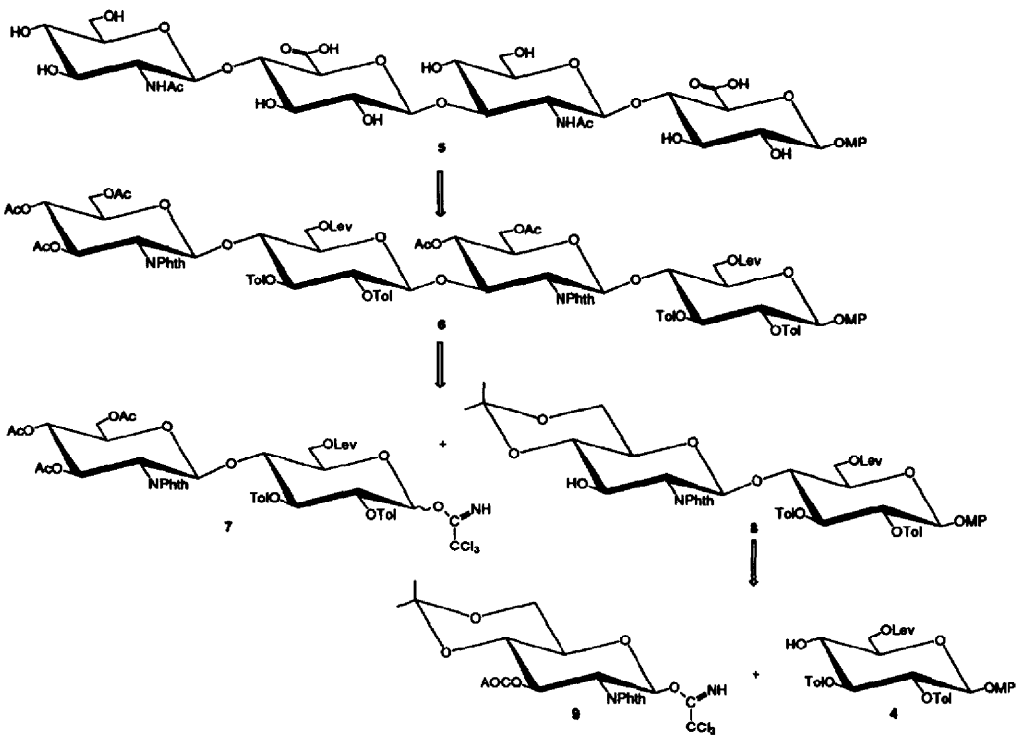
RESULTS AND DISCUSSION

For the synthesis of the two oligosaccharides **1** and **5** a series of monosaccharide coupling synthons were designed, namely **3**,[15] **4**,[12] and **9**,[12] which in principle also allow the extension to higher oligosaccharides (Schemes 1 and 2). 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**3**) and 3-*O*-allyloxycarbonyl-2-deoxy-4,6-di-*O*-isopropylidene-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**9**) are the precursors for the 2-acetamido-2-deoxy-D-glucose element in non-reducing terminal and internal position, respectively, whereas 4-methoxyphenyl 6-*O*-levulinoyl-2,3-di-*O*-*p*-toluoyl-β-D-glucopyranoside (**4**) is the precursor for the D-glucuronic acid element in glycosidic and internal position.



Scheme 1. Retrosynthetic analysis of disaccharide structure **1**. Lev = levulinoyl; MP = 4-methoxyphenyl; Tol = *p*-toluoyl; Phth = phthaloyl; Ac = acetyl.

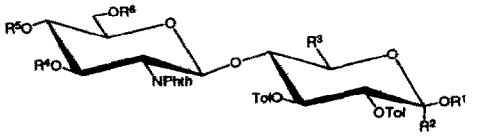
Condensation of **3** with **4** (Scheme 1) in dichloromethane at room temperature, using boron trifluoride etherate as a promoter, gave disaccharide **2** (81%), which was delevulinoylated using hydrazine acetate[16,17] in 1:2 toluene-ethanol to afford **10** (86%). The oxidation of the primary hydroxyl group of the glucose unit in **10** was conducted in two steps, namely first a Swern oxidation with oxalyl chloride and methylsulfoxide[18] followed by oxidation with $NaClO_2$,[19] and it gave **11** in 68% yield (Scheme 3). Then, **11** was treated with methylamine[20] in ethanol (de-acylation), followed by selective *N*-acetylation using acetic anhydride in methanol to give **1** (93%).



Scheme 2. Retrosynthetic analysis of tetrasaccharide structure **5**. AOC = allyloxycarbonyl.

Following the retrosynthetic analysis as presented in Scheme 2 for the synthesis of **5**, glycosyl donor **7** and glycosyl acceptor **8** were prepared. Demethoxyphenylation[21] of **2** with ammonium cerium(IV) nitrate (→ **12**, 96%) followed by imidation with trichloroacetonitrile[15] in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene gave **7** (93%). Condensation of **9** with **4** in dichloromethane at room temperature, using boron trifluoride etherate as a promoter, gave disaccharide derivative **13** (90%). De-allyloxycarbonylation of **13** with tetrakis(triphenylphosphine) palladium in tetrahydrofuran and morpholine[22,23] yielded disaccharide derivative **8** (94%).

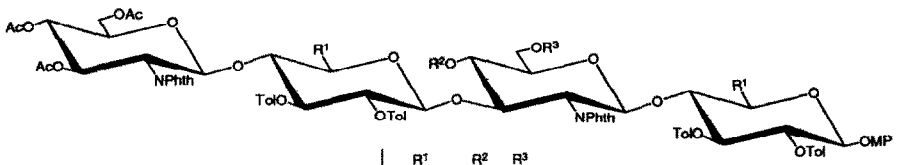
Condensation of **7** with **8** in dichloromethane at 0°C, using trimethylsilyl trifluoromethanesulfonate as a promoter afforded tetrasaccharide derivative **14**. However, **14** could not be purified on silica; therefore the mixture containing **14** was treated with aqueous trifluoroacetic acid in dichloromethane (de-isopropylidenation) to



	R¹	R²	R³	R⁴	R⁵	R⁶
10	MP	H	CH_2OH	Ac	Ac	Ac
11	MP	H	COOH	Ac	Ac	Ac
12	H, OH		CH_2OLev	Ac	Ac	Ac
13	MP	H	CH_2OLev	AOC	CMe_2	

Scheme 3.

give **15** in 77% overall yield. After conventional *O*-acetylation (→**6**, 99%) and delevulinoylation using hydrazine acetate in 1:2 toluene-ethanol (→**16**, 87%), a Swern oxidation with oxalyl chloride and methylsulfoxide followed by oxidation with $NaClO_2$ afforded **17** (76%) (Scheme 4). Finally, **17** was de-acylated with methylamine in ethanol followed by selective *N*-acetylation using acetic anhydride in methanol. Because the ^1H NMR spectrum showed the presence of one remaining *O*-acetyl group, an additional treatment with sodium methoxide in methanol was carried out to yield **5** (61%).



	R¹	R²	R³
14	CH_2OLev	CMe_2	
15	CH_2OLev	H	H
16	CH_2OH	Ac	Ac
17	COOH	Ac	Ac

Scheme 4.

The synthesised MP glycosides **1** and **5** will be tested in biological systems.

EXPERIMENTAL

General methods.—The ^1H (300 and 500 MHz) and ^{13}C (APT [attached proton test], 50 and 75 MHz) NMR spectra were recorded at 25°C with Bruker AC 300, AM 500, or WP-200 spectrometers in $CDCl_3$ unless stated otherwise. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si ($CDCl_3$) or internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (D_2O; indirectly to internal acetone, δ 2.225) for ^1H, and to the signal for internal Me_4Si ($CDCl_3$; indirectly to $CDCl_3$, δ 76.9) or external Me_4Si (D_2O; indirectly to internal acetone, δ 31.55) for ^{13}C.

Reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by examination under UV light and by charring with aq 50% sulfuric acid. Column chromatography was performed on Kiesel-

gel 60 (Merck, 70-230 mesh). Optical rotations were measured for solutions in CH_2Cl_2, unless stated otherwise, at 20°C with a Perkin-Elmer 241 polarimeter, using a 10-cm 1-mL cell. Solvents were evaporated under reduced pressure at 40°C (bath). All solvents were distilled from the appropriate drying agents.

4-Methoxyphenyl O-*(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*-glucopyranoside* (**2**).—To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**3**; 320 mg, 0.55 mmol) and 4-methoxyphenyl 6-*O*-levulinoyl-2,3-di-*O*-*p*-toluoyl-β-D-glucopyranoside (**4**; 228 mg, 0.37 mmol) in CH_2Cl_2 (3.6 mL) containing 4A molecular sieves (0.37 g) was added a solution of M BF_3·Et_2O in CH_2Cl_2 (204 μL). After stirring the mixture for 1 h at room temperature, TLC (1:1 toluene-acetone) showed the disappearance of **4** and the formation of **2** (R_F 0.62). Then the mixture was neutralised with triethylamine, diluted with EtOAc (100 mL), and washed with aq 5% NaCl (30 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (1:1 toluene-acetone) of the residue yielded **2**, isolated as a syrup (310 mg, 81%), $[\alpha]_D$ +57 (*c* 1). ^{1}H-NMR data: δ 1.793, 1.918, and 1.965 (3 s, each 3 H, 3 Ac), 2.185 (s, 3 H, $COCH_2CH_2COCH_3$), 2.355 and 2.389 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 2.36-2.50 and 2.63-2.65 (2 m, each 2 H, $COCH_2CH_2COCH_3$), 3.706 (s, 3 H, $C_6H_4OCH_3$), 4.248 (dd, 1 H, $J_{2',1'}$ 8.4, $J_{2',3'}$ 10.6 Hz, H-2'), 5.027 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 5.460 (dd, 1 H, $J_{2,3}$ 8.8 Hz, H-2), 5.514 (d, 1 H, H-1'), 6.704 and 6.823 (2 d, each 2 H, $C_6H_4OCH_3$), 7.154, 7.195, 7.801, and 7.901 (4 d, each 2 H, 2 $COC_6H_4CH_3$).

Anal. Calc. for $C_{54}H_{55}NO_{20}$: C, 62.48; H, 5.34. Found: C, 62.10; H, 5.28.

4-Methoxyphenyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→4)-2,3-di-*O*-*p*-toluoyl-β-*D*-glucopyranoside* (**10**).—To a solution of **2** (500 mg, 0.48 mmol) in EtOH (69 mL) and toluene (34 mL) was added NH_2NH_2·HOAc (222 mg). The mixture was stirred for 40 min, when TLC (1:1 toluene-EtOAc) showed the conversion of **2** into **10** (R_F 0.55). Then the mixture was concentrated, and column chromatography (1:1 toluene-EtOAc) of the residue yielded **10**, isolated as a syrup (390 mg, 86%), $[\alpha]_D$ +86 (*c* 1). NMR data: ^{1}H, δ 1.799, 1.909, and 1.980 (3 s, each 3 H, 3 Ac), 2.349 and 2.393 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.704 (s, 3 H, $C_6H_4OCH_3$), 4.243 (dd, 1 H, $J_{2',1'}$ 8.4, $J_{2',3'}$ 10.7 Hz, H-2'), 5.069 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.437 (dd, 1 H, $J_{2,3}$ 9.3 Hz, H-2), 5.627 (d, 1 H, H-1'), 6.701 and 6.805 (2 d, each 2 H, $C_6H_4OCH_3$), 7.145, 7.213, 7.795, and 7.909 (4 d, each 2 H, 2 $COC_6H_4CH_3$); ^{13}C, δ 20.2 (2 C) and 20.3 (3 $COCH_3$), 21.3 ($COC_6H_4CH_3$), 54.7 and 55.2 ($C_6H_4OCH_3$, C-2'), 60.2 and 60.9 (C-6,6'), 97.8 (C-1'), 100.2 (C-1), 114.2 (2 C), 118.3 (2 C), 150.7, and 155.3 ($C_6H_4OCH_3$), 164.7 and 164.9 (2 $COC_6H_4CH_3$), 169.0, 169.8, and 170.2 (3 $COCH_3$).

Anal. Calc. for $C_{49}H_{49}NO_{18}$: C, 62.61; H, 5.26. Found: C, 62.04; H, 5.38.

4-Methoxyphenyl O-*(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* (**11**).—To a cold (-78°C) 2 M solution of oxalyl chloride in CH_2Cl_2 (0.52 mL) was added Me_2SO (157 μL). After stirring for 10 min, a solution of **10** (100 mg, 0.11 mmol) in CH_2Cl_2 (2.0 mL) was added, and the mixture was stirred for 1 h at -78°C, whereby within 30 min a precipitate was observed. Diisopropylethylamine (0.77 mL) was added, and after 10 min the mixture was diluted with EtOAc (35 mL), washed with M HCl (10 mL) and aq saturated NaCl (10 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated. To a solution of the residue in *t*-BuOH (4.36 mL), 2-methylbutene (1.65

mL), and water (2.7 mL) were added NaH_2PO_4 (271 mg) and $NaClO_2$ (271 mg). After stirring overnight, TLC (10:9:1 CH_2Cl_2-EtOAc-HOAc) showed the conversion of **10** into **11** (R_F 0.65). Then the mixture was concentrated, and a solution of the residue in water was washed with hexane, acidified with M HCl, and extracted with EtOAc (3 x 20 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (3:2 CH_2Cl_2-EtOAc followed by 10:9:1 CH_2Cl_2-EtOAc-HOAc) of the residue yielded **11**, isolated as a syrup (69 mg, 68%), $[\alpha]_D$ +4 (*c* 1). ^{1}H-NMR data: δ 1.790, 1.900, and 1.917 (3 s, each 3 H, 3 Ac), 2.357 (s, 6 H, 2 $COC_6H_4CH_3$), 3.690 (s, 3 H, $C_6H_4OCH_3$), 4.045 (d, 1 H, $J_{5,4}$ 9.2 Hz, H-5), 4.252 (dd, 1 H, $J_{2',1'}$ 8.4, $J_{2',3'}$ 10.7 Hz, H-2'), 5.122 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 5.546 (d, 1 H, H-1'), 5.595 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 6.691 and 6.840 (2 d, each 2 H, $C_6H_4OCH_3$), 7.159, 7.165, 7.815, and 7.898 (4 d, each 2 H, 2 $COC_6H_4CH_3$). A small amount of **11** was esterified with diazomethane in ether, and analysed by ^{1}H NMR: δ 1.800, 1.920, and 1.950 (3 s, each 3 H, 3 Ac), 2.356 and 2.381 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.493 (s, 3 H, $COOCH_3$), 3.707 (s, 3 H, $C_6H_4OCH_3$), 4.031 (d, 1 H, $J_{5,4}$ 9.2 Hz, H-5), 4.205 (dd, 1 H, $J_{2',1'}$ 8.4, $J_{2',3'}$ 10.7 Hz, H-2'), 5.095 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1), 5.464 (d, 1 H, H-1'), 5.492 (dd, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 6.700 and 6.814 (2 d, each 2 H, $C_6H_4OCH_3$), 7.161, 7.189, 7.803, and 7.900 (4 d, each 2 H, 2 $COC_6H_4CH_3$).

4-Methoxyphenyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→4)-β-*D-*glucopyranosyluronic acid* (**1**).—A solution of **11** (69 mg, 70 μmol) in ethanolic 30% methylamine (20 mL) was stirred for 3 days, when TLC (4:2:2:1 *n*-BuOH-EtOH-H_2O-HOAc) showed the conversion of **11** into an intermediate amino compound (R_F 0.70). The mixture was concentrated, and a solution of the residue in MeOH (15.6 mL) and acetic anhydride (219 μL) was stirred for 2 h at 0°C, then concentrated, and 1:1 toluene-MeOH (3 x 15 mL) was evaporated from the residue. Compound **1** was purified by FPLC on Q-Sepharose using a concentration gradient of 0 to 150 mM NaCl. After desalting on Bio-Gel P-2 and lyophilisation, **1** was obtained as an amorphous, white powder (33 mg, 93%), $[\alpha]_D$ -40 (*c* 1, water). NMR data (D_2O): ^{1}H, δ 2.068 (s, 3 H, $NHCOCH_3$), 3.612 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 3.728 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2'), 3.802 (s, 3 H, $C_6H_4OCH_3$), 4.576 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1'), 4.991 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 6.964 and 7.095 (2 d, each 2 H, $C_6H_4OCH_3$); ^{13}C, δ 23.7 ($NHCOCH_3$), 56.7 and 57.1 ($C_6H_4OCH_3$, C-2'), 61.9 (C-6'), 102.1 and 102.6 (C-1,1'), 116.4 (2 C), 119.5 (2 C), 152.2, and 156.0 ($C_6H_4OCH_3$), 175.2 and 176.2 ($NHCOCH_3$, *C*OOH); FAB-MS *m/z* 504 $[M+H]^+$.

O-*(3,4,6-Tri-*O-*acetyl-2-deoxy-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 **2** (3.00 g, 2.89 mmol) in toluene (121 mL) and acetonitrile (170 mL) was added water (121 mL) and ammonium cerium(IV) nitrate (16.0 g). After stirring for 75 min, TLC (6:1 CH_2Cl_2-acetone) showed a complete conversion of **2** into **12** (R_F 0.30). Then, the mixture was diluted with EtOAc (300 mL), washed with aq saturated $NaHCO_3$ (2 x 100 mL) and water (2 x 100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2-acetone) of the residue yielded **12**, isolated as a syrup (2.8 g, 96%), $[\alpha]_D$ +93 (*c* 1) (α:β 2.7:1). ^{13}C-NMR data: δ 20.4, 20.5, and 20.7 (3 $COCH_3$), 21.6 ($COC_6H_4CH_3$), 27.7, 29.9, and 38.0 ($COCH_2CH_2COCH_3$), 54.9 (C-2'), 61.3 and 62.1 (C-6,6'), 90.1 (C-1α), 95.4 (C-1β), 97.7 (C-1'), 165.0 and 166.0 (2 $COC_6H_4CH_3$), 169.3, 170.2, and 170.6 (3 $COCH_3$), 172.0 ($COCH_2CH_2COCH_3$), 206.8 ($COCH_2CH_2COCH_3$).

Anal. Calc. for $C_{47}H_{49}NO_{19}$: C, 60.57; H, 5.30. Found: C, 60.06; H, 5.25.

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

toluoyl-α/β-D-glucopyranosyl trichloroacetimidate (7).—To a solution of **12** (2.6 g, 2.8 mmol) in CH_2Cl_2 (8.0 mL) and trichloroacetonitrile (3.0 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (101 μL). After stirring overnight, TLC (9:1 CH_2Cl_2-acetone) showed a complete conversion of **12** into **7** (R_F 0.47), and the mixture was purified by column chromatography (9:1 CH_2Cl_2-acetone) to yield **7**, isolated as a syrup (2.8 g, 93%), $[\alpha]_D$ +80 (*c* 1) (α:β 3:2). NMR data: ^1H, δ 1.789, 1.912, and 1.978 (3 s, each 3 H, 3 Ac), 2.209 (s, 3 H, $COCH_2CH_2COCH_3$), 2.327 and 2.379 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 2.27-2.52 and 2.67-2.72 (2 m, each 2 H, $COCH_2CH_2COCH_3$), 5.585 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 6.600 (d, 0.6 H, $J_{1,2}$ 3.7 Hz, H-1α), 6.631 (d, 0.4 H, $J_{1,2}$ 8.8 Hz, H-1β); ^{13}C, δ 20.3, 20.5, and 20.7 (3 $COCH_3$), 21.7 ($COC_6H_4CH_3$), 27.6, 29.9, and 38.0 ($COCH_2CH_2COCH_3$), 54.9 (C-2'), 61.1 and 61.5 (C-6,6'), 93.0 (C-1α), 93.6 (C-1β), 98.3 (C-1'), 160.7 ($NHCCl_3$), 164.8 and 165.6 (2 $COC_6H_4CH_3$), 169.2, 170.1, and 170.5 (3 $COCH_3$), 171.8 ($COCH_2CH_2COCH_3$), 206.1 ($COCH_2CH_2COCH_3$).

Anal. Calc. for $C_{49}H_{49}Cl_3N_2O_{19}$: C, 54.68; H, 4.59. Found: C, 54.68; H, 4.50.

4-Methoxyphenyl O-(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 (**13**).—To a solution of 3-*O*-allyloxycarbonyl-2-deoxy-4,6-*O*-isopropylidene-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**9**; 200 mg, 0.35 mmol) and **4** (143 mg, 0.23 mmol) in CH_2Cl_2 (1.8 mL) containing 4A molecular sieves (0.14 g) was added a solution of M BF_3-Et_2O in CH_2Cl_2 (851 μL). After stirring the mixture for 45 min at room temperature, TLC (95:5 CH_2Cl_2-acetone) showed the disappearance of **4** and the formation of **13** (R_F 0.43). Then, triethylamine was added to neutralize and the mixture was diluted with EtOAc (100 mL), washed with aq 5% NaCl, and the organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2-acetone) of the residue yielded **13**, isolated as a syrup (215 mg, 90%), $[\alpha]_D$ +25 (*c* 1). NMR data: ^1H, δ 1.224 and 1.254 [2 s, each 3 H, $C(CH_3)_2$], 2.188 (s, 3 H, $COCH_2CH_2COCH_3$), 2.338 and 2.390 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 2.37-2.50 and 2.63-2.80 (2 m, each 2 H, $COCH_2CH_2COCH_3$), 3.695 (s, 3 H, $C_6H_4OCH_3$), 4.220 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2'), 4.963 and 5.059 (2 m, each 1 H, $COOCH_2CH{=}CH_2$), 5.026 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 5.408 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 5.490 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 6.696 and 6.824 (2 d, each 2 H, $C_6H_4OCH_3$), 7.138, 7.237, 7.810, and 7.921 (4 d, each 2 H, 2 $COC_6H_4CH_3$); ^{13}C, δ 18.4 [$C(CH_3)_2$], 21.4 (2 $COC_6H_4CH_3$), 27.4, 29.6, and 37.7 ($COCH_2CH_2COCH_3$), 55.3 (C-2', $C_6H_4OCH_3$), 60.8 and 61.7 (C-6,6'), 68.2 ($COOCH_2CH{=}CH_2$), 98.4 (C-1'), 99.3 [$C(CH_3)_2$], 100.2 (C-1), 114.2 (2 C), 118.7 (2 C), 150.8, and 155.4 ($C_6H_4OCH_3$), 118.3 ($COOCH_2CH{=}CH_2$), 130.8 ($COOCH_2CH{=}CH_2$), 164.9 ($COC_6H_4CH_3$), 171.6 ($COCH_2CH_2COCH_3$).

Anal. Calc. for $C_{55}H_{57}NO_{19}$: C, 63.75; H, 5.55. Found: C, 63.52; H, 5.64.

4-Methoxyphenyl O-(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 (**8**).—To a solution of **13** (433 mg, 0.42 mmol) in tetrahydrofuran (7.0 mL) and morpholine (300 μL) was added tetrakis(triphenylphosphine) palladium (83.6 mg). The mixture was boiled under reflux for 30 min, when TLC (9:1 CH_2Cl_2-acetone) showed the de-*O*-allyloxycarbonylation to be complete (**8**; R_F 0.29). Then the mixture was diluted with EtOAc (50 mL), washed with aq 5% NaCl (3 x 20 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2-acetone) of the residue yielded **8**, isolated as a syrup (359 mg, 94%), $[\alpha]_D$ +31 (*c* 1). NMR data: ^1H, δ 1.239 and 1.250 [2 s, each 3 H, $C(CH_3)_2$], 2.160 (s, 3 H, $COCH_2CH_2COCH_3$), 2.316 and

2.370 (2 s, each 3 H, 2 $COC_6H_4CH_3$), 3.678 (s, 3 H, $C_6H_4OCH_3$), 5.048 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 5.287 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1'), 5.489 (dd, 1 H, $J_{2,3}$ 9.1 Hz, H-2), 6.688 and 6.822 (2 d, each 2 H, C_6H_4O-CH_3), 7.132, 7.213, 7.802, and 7.901 (4 d, each 2 H, 2 $COC_6H_4CH_3$); ^{13}C, δ 18.6 [$C(CH_3)_2$], 21.3 (CO-$C_6H_4CH_3$), 27.4, 29.5, and 37.7 ($COCH_2CH_2COCH_3$), 55.3 and 56.9 (C-2', $C_6H_4OCH_3$), 60.9 and 61.8 (C-6,6'), 98.7 (C-1'), 98.2 [$C(CH_3)_2$], 100.0 (C-1), 114.3 (2 C), 118.6 (2 C), 150.8, and 155.5 (C_6H_4O-CH_3), 164.8 ($COC_6H_4CH_3$), 171.5 ($COCH_2CH_2COCH_3$).

Anal. Calc. for $C_{51}H_{53}NO_{17}$: C, 64.34; H, 5.61. Found: C, 64.33; H, 5.66.

4-Methoxyphenyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→4)-*O*-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-β-*D*-glucopyranosyl)-(1→3)-*O*-(2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→4)-6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-β-*D*-glucopyranoside* (**15**).—To a solution of **7** (761 mg, 0.71 mmol) and **8** (269 mg, 0.28 mmol) in CH_2Cl_2 (2.8 mL) containing 4A molecular sieves (283 mg) was added a solution of M $CF_3SO_3SiMe_3$ in CH_2Cl_2 (66 μL) at 0°C. After stirring the mixture for 60 min at room temperature, TLC (9:1 CH_2Cl_2-acetone) showed the disappearance of **8** and the formation of **14** (R_F 0.50). Then, triethylamine was added to neutralize, and the mixture was diluted with EtOAc (100 mL), washed with aq 5% NaCl (3 x 30 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (5.3 mL), and trifluoroacetic acid (300 μL) and water (37 μL) were added. After stirring the mixture for 60 min, TLC (85:15 CH_2Cl_2-acetone) showed the de-isopropylidenation to be complete (**15**; R_F 0.44). Then, the mixture was washed with aq saturated $NaHCO_3$ (3 x 25 mL) and water (3 x 25 mL), and the organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2-acetone) of the residue yielded **15**, isolated as a syrup (400 mg, 77%), $[\alpha]_D$ +70 (*c* 1). NMR data: ^1H, δ 1.768, 1.889, and 1.923 (3 s, each 3 H, 3 Ac), 2.162 and 2.166 (2 s, each 3 H, 2 $COCH_2CH_2COCH_3$), 2.305, 2.341, and 2.380 (3 s, 3,6,3 H, 4 $COC_6H_4CH_3$), 3.683 (s, 3 H, $C_6H_4OCH_3$), 4.155 and 4.336 (2 dd, each 1 H, $J_{2',1'/2''',1'''}$ 8.2 and 8.3 Hz, $J_{2',3'/2''',3'''}$ 10.7 Hz, H-2',2'''), 4.443 and 4.957 (2 d, each 1 H, $J_{1,2/1'',2''}$ 7.9 and 7.2 Hz, H-1,1''), 5.025 and 5.414 (2 d, each 1 H, H-1',1'''), 5.087 and 5.410 (2 dd, each 1 H, $J_{2,3/2'',3''}$ 8.7 and 9.6 Hz, H-2,2''), 6.667 and 6.779 (2 d, each 2 H, $C_6H_4OCH_3$), 6.865, 7.055, 7.136, 7.181, 7.279, 7.653, 7.773, and 7.836 (8 d, each 2 H, 4 $COC_6H_4CH_3$); ^{13}C, δ 20.2, 20.4, and 20.5 (3 $COCH_3$), 21.5 ($COC_6H_4CH_3$), 27.4, 27.6, 29.2, 29.7, and 37.7 (2 C) ($COCH_2CH_2COCH_3$), 54.8 and 55.5 (2 C) (C-2', 2''', $C_6H_4OCH_3$), 61.2 and 62.0 (C-6,6',6'',6'''), 97.5 (C-1',1'''), 99.9 and 100.8 (C-1,1''), 114.4 (2 C), 118.7 (2 C), 150.9, and 155.5 ($C_6H_4OCH_3$), 164.7 and 165.1 ($COC_6H_4CH_3$), 169.1, 169.8, and 170.3 (3 $COCH_3$), 171.6 ($COCH_2CH_2COCH_3$).

Anal. Calc. for $C_{95}H_{96}N_2O_{35}$: C, 62.49; H, 5.30. Found: C, 62.38; H, 5.42.

4-Methoxyphenyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1→4)-*O*-(6-*O*-levulinoyl-2,3-di-*O*-p-toluoyl-β-*D*-glucopyranosyl)-(1→3)-*O*-(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*-glucopyranoside* (**6**).—To a solution of **15** (313 mg, 0.17 mmol) in pyridine (6.6 mL) and acetic anhydride (6.6 mL) was added 4-dimethylaminopyridine (5 mg). After stirring overnight, TLC (4:1 CH_2Cl_2-acetone) showed the *O*-acetylation to be complete (**6**; R_F 0.75), and the mixture was diluted with EtOAc (100 mL), and washed with aq saturated $NaHCO_3$ (3 x 25 mL) and water (3 x 25 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated, and co-concentrated with toluene, EtOH, and CH_2Cl_2 (each 3 x 25 mL). Column chromatography (4:1 CH_2Cl_2-acetone) of the residue yield-

ed **6**, isolated as a syrup (326 mg, 99%), $[\alpha]_D$ +38 (*c* 1). NMR data: ^{1}H, δ 1.763, 1.861, 1.871, 1.890, and 1.940 (5 s, each 3 H, 5 Ac), 2.159 and 2.226 (2 s, each 3 H, 2 $COCH_2CH_2COCH_3$), 2.294, 2.333, 2.363, and 2.373 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 3.683 (s, 3 H, $C_6H_4OCH_3$), 4.282 and 4.936 (2 d, each 1 H, $J_{1,2/1'',2''}$ 7.6 and 7.4 Hz, H-1,1''), 5.037 and 5.282 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 8.3 and 8.1 Hz, H-1',1'''), 6.670 and 6.769 (2 d, each 2 H, $C_6H_4OCH_3$), 7.001, 7.047, 7.123, 7.132, 7.355, 7.685, 7.751, and 7.807 (8 d, each 2 H, 4 $COC_6H_4CH_3$); ^{13}C, δ 19.6, 20.1, 20.4, 20.5, and 20.6 (5 $COCH_3$), 21.5 ($COC_6H_4CH_3$), 27.4, 27.5, 29.1, 29.7, and 37.7 (2 C) (2 $COCH_2CH_2COCH_3$), 54.5, 55.2, and 55.4 (C-2',2''', C_6H_4O-CH_3), 61.4 and 62.4 (C-6,6',6'',6'''), 97.4 and 97.5 (C-1',1'''), 99.9 and 100.6 (C-1,1''), 114.2 (2 C), 118.6 (2 C), 150.7, and 155.4 ($C_6H_4OCH_3$), 164.7, 164.9 (2 C), and 165.0 (4 $COC_6H_4CH_3$), 168.4, 169.1, 169.9, 170.2, and 170.4 (5 $COCH_3$), 171.5 and 171.7 (2 $COCH_2CH_2COCH_3$).

Anal. Calc. for $C_{99}H_{100}N_2O_{37}$: C, 62.26; H, 5.28. Found: C, 62.15; H, 5.29.

4-Methoxyphenyl O-*(3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-p-*toluoyl-β*-D-*glucopyranosyl)-(1→3)*-O-*(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)-2,3-di*-O-p-*toluoyl-β*-D-*glucopyranoside* (**16**).—To a solution of **6** (337 mg, 0.18 mmol) in EtOH (25.2 mL) and toluene (12.5 mL) was added NH_2NH_2·HOAc (162 mg). After stirring for 30 min, TLC (4:1 CH_2Cl_2-acetone) showed the reaction to be complete (**16**; R_F 0.63), and the mixture was concentrated. Column chromatography (9:1 CH_2Cl_2-acetone) of the residue yielded **16**, isolated as a syrup (262 mg, 87%), $[\alpha]_D$ +59 (*c* 1). NMR data: ^{1}H, δ 1.779, 1.836, 1.882, 1.913, and 1.946 (5 s, each 3 H, 5 Ac), 2.337 and 2.362 (2 s, each 6 H, 4 $COC_6H_4CH_3$), 3.690 (s, 3 H, $C_6H_4OCH_3$), 4.504 and 4.990 (2 d, each 1 H, $J_{1,2/1'',2''}$ 7.2 and 7.7 Hz, H-1,1''), 5.149 and 5.458 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 8.4 Hz, H-1',1'''), 6.678 and 6.762 (2 d, each 2 H, $C_6H_4OCH_3$), 7.033, 7.118, 7.124, 7.142, 7.444, 7.721, 7.755, and 7.816 (8 d, each 2 H, 4 $COC_6H_4CH_3$); ^{13}C, δ 20.0-20.2 ($COCH_3$), 20.4 and 21.4 (3 C) (4 $COC_6H_4CH_3$), 54.5, 55.2, and 55.3 (C-2',2''', C_6H_4O-CH_3), 60.2, 60.4, 61.0, and 61.4 (C-6,6',6'',6'''), 97.7 (C-1',1'''), 99.3 and 100.1 (C-1,1''), 114.3 (2 C), 118.3 (2 C), 150.7, and 155.4 ($C_6H_4OCH_3$), 164.6 and 164.9 ($COC_6H_4CH_3$), 169.0, 169.2 (2 C), 169.8, and 170.2 (5 $COCH_3$).

Anal. Calc. for $C_{89}H_{88}N_2O_{33}$: C, 62.38; H, 5.18. Found: C, 62.46; H, 5.28.

4-Methoxyphenyl O-*(3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)*-O-*(2,3-di*-O-p-*toluoyl-β*-D-*glucopyranosyluronic acid)-(1→3)*-O-*(4,6-di*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)-2,3-di*-O-p-*toluoyl-β*-D-*glucopyranosyluronic acid* (**17**).—To a cold (-78°C) 2 M solution of oxalyl chloride in CH_2Cl_2 (645 μL) was added Me_2SO (195 μL), and the mixture was stirred for 10 min. Then a solution of **16** (113 mg, 65.9 μmol) in CH_2Cl_2 (1.1 mL) was added, and the mixture was stirred for 5 h, whereby within 15 min a precipitate was observed. Diisopropylethylamine (0.96 mL) was added, and after 10 min the mixture was diluted with EtOAc (50 mL), and washed with M HCl (2 x 15 mL) and aq saturated NaCl (2 x 15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. To a solution of the residue in *t*-BuOH (5.4 mL), 2-methylbutene (2.0 mL), and water (3.4 mL) were added NaH_2PO_4 (336 mg) and $NaClO_2$ (336 mg). The mixture was stirred overnight, when TLC (5:5:1 CH_2Cl_2-EtOAc-HOAc) showed a complete conversion of **16** into **17** (R_F 0.83). Then, the mixture was concentrated, and a solution of the residue in water was washed with hexane, acidified with M HCl, and extracted with EtOAc (3 x 30 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (3:2 CH_2Cl_2-EtOAc followed by 5:5:1 CH_2Cl_2-

EtOAc-HOAc) of the residue yielded **17**, isolated as a syrup (88 mg, 76%), $[\alpha]_D$ +6 (*c* 1). NMR data: ^{1}H, δ 1.757, 1.824, 1.842, and 1.881 (4 s, 3,3,3,6 H, 5 Ac), 2.298, 2.333, 2.344, and 2.382 (4 s, each 3 H, 4 $COC_6H_4CH_3$), 3.689 (s, 3 H, $C_6H_4OCH_3$), 3.783 and 3.859 (2 d, each 1 H, $J_{5,4/5'',4''}$ 9.5 and 9.0 Hz, C-5,5''), 4.438 and 5.057 (2 d, each 1 H, $J_{1,2/1'',2''}$ 7.5 and 7.7 Hz, H-1,1''), 5.370 and 5.567 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 8.3 and 8.4 Hz, H-1',1'''), 6.677 and 6.798 (2 d, each 2 H, $C_6H_4OCH_3$), 7.006, 7.066, 7.114, 7.147, 7.369, 7.706, 7.782, and 7.832 (8 d, each 2 H, 4 $COC_6H_4CH_3$); ^{13}C, δ 20.2, 20.3 (2 C), and 20.5 (2 C) (5 $COCH_3$), 21.5 ($COC_6H_4CH_3$), 54.4, 55.2, and 55.5 (C-2',2''', $C_6H_4OCH_3$), 61.6 and 62.0 (C-6,6', 6'',6'''), 97.3 and 97.7 (C-1',1'''), 100.4 and 100.7 (C-1,1''), 114.4 (2 C), 118.5 (2 C), 150.6, and 155.5 ($C_6H_4OCH_3$), 163.4 (*C*OOH), 164.8, 164.9, 165.0, and 165.1 (4 $COC_6H_4CH_3$), 169.7, 170.0, 170.1, 170.8, and 171.1 (5 $COCH_3$). A small amount of **17** was esterified with diazomethane in ether, and analysed with ^{1}H NMR: δ 1.767, 1.884, 1.894, and 1.908 (4 s, 6,3,3,3 H, 5 Ac), 2.308, 2.340, and 2.385 (3 s, 3,6,3 H, 4 $COC_6H_4CH_3$), 3.386 and 3.577 (2 s, each 3 H, 2 $COOCH_3$), 3.686 (s, 3 H, $C_6H_4OCH_3$), 3.722 and 3.794 (2 d, each 1 H, $J_{5,4/5'',4''}$ 9.7 and 8.9 Hz, C-5,5''), 4.355 and 5.002 (2 d, each 1 H, $J_{1,2/1'',2''}$ 7.6 and 7.0 Hz, H-1,1''), 4.971 and 5.285 (2 d, each 1 H, $J_{1',2'/1''',2'''}$ 8.4 and 8.3 Hz, H-1',1'''), 6.672 and 6.764 (2 d, each 2 H, $C_6H_4OCH_3$), 6.999, 7.074, 7.122, 7.131, 7.353, 7.693, 7.761, and 7.828 (8 d, each 2 H, 4 $COC_6H_4CH_3$).

4-Methoxyphenyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→4)*-O-*(β-*D-*glucopyranosyluronic acid)-(1→3)*-O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→4)-β-*D-*glucopyranosyluronic acid* (**5**).—A solution of **17** (50 mg, 29 μmol) in ethanolic 33% methylamine (40 mL) was stirred for 3 days, and after concentration the residue was taken up in MeOH (6.4 mL) and acetic anhydride (179.6 μL). The solution was stirred for 2 h at 0°C, when TLC (4:2:2:1 *n*-BuOH-EtOH-H_2O-HOAc) showed the formation of **5** (R_F 0.55). The mixture was concentrated, and 1:1 MeOH-toluene (3 x 30 mL) was evaporated from the residue. Then the residue was dissolved in MeOH, and NaOMe was added until pH 10. After stirring overnight, 5 drops of water were added and the stirring was continued for 2 h at room temperature. Then Dowex 50 (H^+) was added to neutralize, and the mixture was filtered and concentrated. Gelfiltration of the residue on Sephadex G-10 (water) followed by Bio-Gel P-2 (water), yielded **5**, isolated after lyophilisation as a white, amorphous powder (16 mg, 61%), $[\alpha]_D$ -45 (*c* 1, water). ^{1}H-NMR data (D_2O): δ 2.026 and 2.046 (2 s, each 3 H, $NHCOCH_3$), 3.810 (s, 3 H, $C_6H_4OCH_3$), 4.491, 4.541, and 4.591 (3 d, each 1 H, $J_{1',2'/1'',2''/1''',2'''}$ 7.8, 8.2, and 8.4 Hz, H-1',1'', 1'''), 5.015 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 6.977 and 7.102 (2 d, each 2 H, $C_6H_4OCH_3$); FAB-MS *m/z* 883 $[M+H]^+$.

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REFERENCES

1. Laurent, T. C.; Fraser, J. R. E. *FASEB J.* **1992**, *6*, 2397-2404.
2. Meyer, K. *Fed. Proc., Fed. Am. Chem. Soc. Exp. Biol.* **1958**, *17*, 1075-1077.
3. Prehm, P. *Biochem. J.* **1984**, *220*, 597-600.
4. Aruffo, A.; Stamenkovic, I.; Melnick, M.; Underhill, C. B.; Seed, B. *Cell* **1990**, *61*, 1303-1313.
5. Toole, B. P. In *Cell Biology of Extracellular Matrix*, Hay, E. D. Ed.; Plenum Press, New York, USA, **1991**, pp. 305-341.
6. Ellis, I.; Grey, A. M.; Schor, A. M.; Schor, S. L. *J. Cell Sci.* **1992**, *102*, 447-456.
7. West, D. C.; Kumar, S. In *The Biology of Hyaluronan*, Evered, D.; Whelan, J. Eds.; Ciba Foundation Symposium 143, Wiley, Chichester, UK, **1989**, pp. 187-207.
8. Balazs, E. A.; Denlinger, J. L. In *The Biology of Hyaluronan*, Evered, D.; Whelan, J. Eds.; Ciba Foundation Symposium 143, Wiley, Chichester, UK, **1989**, pp. 265-280.
9. King, S. R.; Hickerson, W. L.; Proctor, K. G.; Newsome, A. M. *Surgery* **1991**, *109*, 76-84.
10. Engström-Laurent, A. In *The Biology of Hyaluronan*, Evered, D.; Whelan, J. Eds.; Ciba Foundation Symposium 143, Wiley, Chichester, UK, **1989**, pp. 233-247.
11. West, D. C.; Hampson, I. N.; Arnold, F.; Kumar, S. *Science* **1985**, *228*, 1324-1326.
12. Slaghek, T. M.; Nakahara, Y.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1994**, *255*, 61-85.
13. Slaghek, T. M.; Hyppönen, T.K.; Ogawa, T.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron Lett.* **1993**, *34*, 7939-7942.
14. Carter, M. B.; Petillo, P. A.; Anderson, L.; Lerner, L. E. *Carbohydr. Res.* **1994**, *258*, 299-306.
15. Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, 1343-1357.
16. Van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, 4875-4878.
17. Jeker, N.; Tamm, C. *Helv. Chim. Acta* **1988**, *71*, 1895-1903.
18. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651-1660.
19. Lindgren, B. O.; Nilsson, T. *Acta Chem. Scand.* **1973**, *27*, 888-890.
20. Motawia, M. S.; Wengel, J.; Abdel-Megid, A. E.-S.; Pedersen, E. B. *Synthesis* **1989**, 384-387.
21. Fukuyama, T.; Laird, A. A.; Hotchkiss, L. M. *Tetrahedron Lett.* **1985**, *26*, 6291-6292.
22. Kunz, H.; Waldmann, H. *Angew. Chem.* **1984**, *96*, 49-50.
23. Hayakawa, Y.; Kato, H.; Uchiyama, M.; Kajino, H.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2400-2402.

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