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Preparation of spacer-containing di-, tri-, and tetrasaccharide fragments of the circulating anodic antigen of *Schistosoma mansoni* for diagnostic purposes

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Abstract

The chemical synthesis of β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→O)$CH_2CH=CH_2$, β-D-Gal*p*NAc-(1→6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc-(1→O)$CH_2CH=CH_2$, and β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc-(1→O)$CH_2CH=CH_2$ is described. These oligosaccharides represent fragments of the circulating anodic antigen, secreted by the parasite *Schistosoma mansoni* in the circulatory system of the host. The applied synthesis strategy includes the preparation of a non-oxidised backbone oligosaccharide, with a levulinoyl group at O-6 of the β-D-glucose residue. After the selective removal of the levulinoyl group, the obtained hydroxyl functions were converted into carboxyl groups, using pyridinium dichromate and acetic anhydride in dichloromethane, to afford the desired glucuronic-acid-containing oligosaccharides. Subsequently, the allyl glycosides have been elongated with cysteamine to give the corresponding amine-spacer-containing oligosaccharides.

Keywords: Oxidation; *Schistosoma mansoni*; Circulating anodic antigen

1. Introduction

Human schistosomiasis is one of the major parasitic diseases in the world [1], affecting 200 million people predominantly in third world countries. Schistosomiasis is caused by the presence of the blood-fluke *Schistosoma* (Trematode) [2] in the blood-vessels of the human host (final host). Because for cure of the infection chemotherapy [3–5] is available, early diagnosis is important. At present, diagnosis relies mainly on the parasitological

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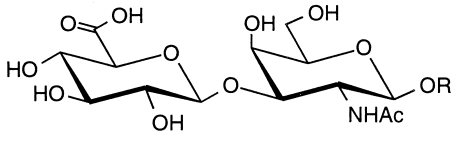
examination of urine and faeces for the presence of *Schistosoma* eggs. In addition, for diagnosis, serological methods are increasingly used, aiming at detection of specific antibodies or of specific antigens. Diagnosis based on the presence of *Schistosoma* antigens in the circulatory system or the urine of the host is increasingly used [6–9]. The gut of the parasite is an important source of these antigens since many gut-associated antigens are excreted into the circulation of the host following digestion of food (e.g., blood cells, proteins) by the parasite. One of the major gut-associated antigens is the circulating anodic antigen (CAA). CAA is a glycoprotein in which the major threonine-linked carbohydrate part consists of disaccharide repeating units, namely $\{\rightarrow 6)$-[β-D-Glc*p*A-$(1\rightarrow 3)$]-β-D-Gal*p*-NAc-$(1\rightarrow\}_n$, probably connected to the protein via a, yet unknown, core saccharide with GlcNAc at the reducing end [10]. As CAA is an important circulating antigen and the glycan part strongly immunogenic, it would be an interesting target antigen for antibody assays. However, this approach is limited by the availability of CAA from biological sources in sufficient amounts. In order to replace isolated CAA in diagnostic methods, a synthetic program for the preparation of a wide range of medium-sized oligosaccharide fragments of CAA was initiated, to determine the optimal epitope of CAA that can act as an immunologic determinant.

In this report, the stereoselective synthesis of the CAA fragments **1**, **3**, and **5** and their cysteamine elongated derivatives **2**, **4**, and **6** is described (Fig. 1). A preliminary report on the synthesis of **5** has appeared [11].

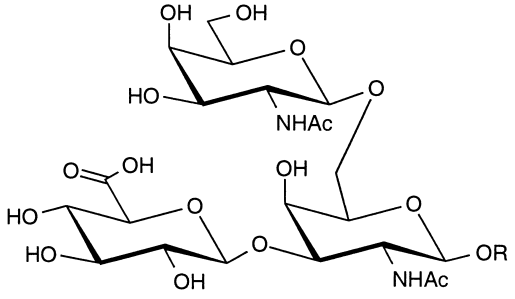
The compounds **2**, **4**, and **6** are suitable for conjugation [12–14] with protein carriers, to be tested in immunological assays with the aim to develop a new diagnostic method for schistosomiasis.

2. Results and discussion

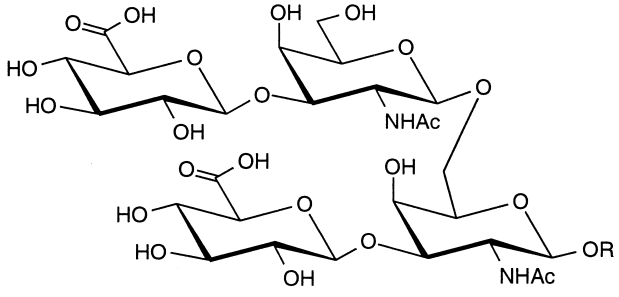
To establish a convenient and systematic route for the synthesis of the target structures **1**–**6**, the disaccharide building block allyl (6-*O*-levulinoyl-2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (**16**) (Scheme 1) was developed which can easily be transformed into an acceptor (**17**) or a donor (**18**), both suitable for the synthesis of the larger oligosaccharides.



1 R = All
2 R = $(CH_2)_3S(CH_2)_2NH_2$

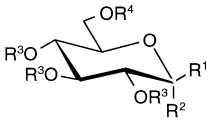


3 R = All
4 R = $(CH_2)_3S(CH_2)_2NH_2$

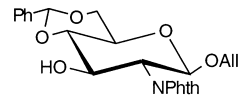


5 R = All
6 R = $(CH_2)_3S(CH_2)_2NH_2$

Fig. 1. Target structures for the synthetic route.



	R^1	R^2	R^3	R^4
7	OMP	H	Ac	Ac
8	OMP	H	T	TBDMS
9	OMP	H	T	Lev
10	H,OH		T	Lev
11	H	$OC(NH)CCl_3$	T	Lev



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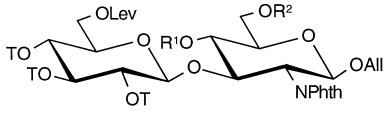
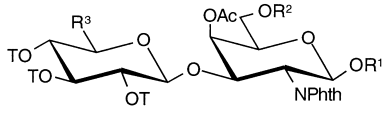


	R^1	R^2
13	CHPh	
14	H	H
15	H	TBDMS



	R^1	R^2	R^3
16	All	TBDMS	CH_2OLev
17	All	H	CH_2OLev
18	$C(NH)CCl_3$	TBDMS	CH_2OLev
19	All	Ac	CH_2OLev
20	All	Ac	CH_2OH
21	All	Ac	COOH

Scheme 1. Synthesis of the mono- and disaccharide building blocks; OMP, $OC_6H_4OCH_3$; TBDMS, $((CH_3)_3C)(CH_3)Si$; Lev, $COCH_2CH_2COCH$; T, $COC_6H_4CH_3$

After the construction of the backbone oligosaccharide, the unique protecting group at the C-6 position of the β-D-glucose residue, the levulinoyl group, can be selectively removed to obtain a hydroxyl function which can be oxidised to prepare the desired β-D-glucuronic acid residue.

The monosaccharide building blocks used for the synthesis of **16** are depicted in Scheme 1. Starting from β-D-glucose pentaacetate, the 4-methoxyphenyl function was introduced at the anomeric center using trimethylsilyl triflate [15] as a catalyst (→**7**, 77%). Subsequent Zemplén deacetylation and selective silylation [16] of the primary hydroxyl function, using *tert*-butyldimethylsilyl chloride in pyridine at 0 °C, followed by toluoylation of the remaining hydroxyl groups with *p*-toluoyl chloride in pyridine, afforded **8** in 63% overall yield. After removal of the *tert*-butyldimethylsilyl ether group, using *p*-toluenesulfonic acid in acetonitrile–water, levulinoylation of the HO-6 function with levulinic acid in the presence of 2-chloro-1-methylpyridinium iodide and 1,4-diazabicyclo[2.2.2]octane in 1,2-dichloroethane [17,18] gave **9** (80%). Slaghek et al. [19] followed for the synthesis of monosaccharide **9** another strategy than reported here. Although the overall yield of the latter synthetic route is slightly higher, our present strategy is less time-consuming and involves less purification steps. Oxidative removal [20] of the 4-methoxyphenyl group at C-1 in a two-phase system using ammonium cerium(IV) nitrate in acetonitrile–toluene–water (→**10**), followed by imidoylation [21] of the anomeric center in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene yielded donor **11** (overall yield 78%). Condensation of allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**) [22,23] with **11** in dichloromethane in the presence of trimethylsilyl triflate (0.05 equiv based on **11**), afforded disaccharide **13** (89%). After removal of the benzylidene function in trifluoroacetic acid–dichloromethane-water (→**14**, 98%), the primary hydroxyl function of the glucosamine unit was selectively protected with a silyl ether group using *tert*-butyldimethylsilyl chloride in pyridine at 0 °C (→**15**, 96%).

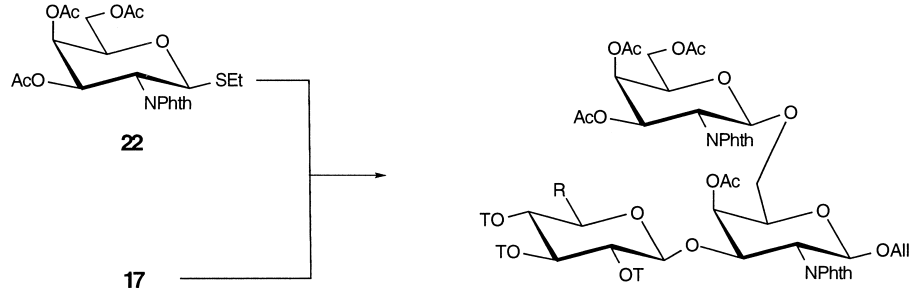
At this stage of the synthetic route, the β-D-glucosamine moiety was transformed into the desired β-D-galactosamine residue by epimerisation

of the hydroxyl function at C-4 [24,25]. To this end, a triflate group was introduced applying trifluoromethanesulfonic anhydride in dichloromethane–pyridine (0 °C), and via a S_N2 displacement using tetrabutylammonium acetate in *N*,*N*-dimethylformamide, the desired 4-*O*-acetyl-protected building block **16** was obtained in 78% yield.

Removal of the silyl ether group under acidic conditions (*p*-toluenesulfonic acid in acetonitrile–water) gave disaccharide derivative **17** (91%). Compound **17** is a suitable acceptor for the synthesis of the larger oligosaccharides (see below). However, compound **17** can also be used as an intermediate in the synthesis of disaccharide target structure **1**. For the last mentioned application, the primary hydroxyl function of the galactosamine unit in **17** was acetylated with acetic anhydride in pyridine (→**19**, 88%). Then the levulinoyl group at HO-6′ was removed using hydrazinium acetate [26,27] (→**20**, 94%), and subsequently the β-D-glucose residue could be converted into the desired β-D-glucuronic acid moiety through oxidation. In the case of the disaccharide derivative the best result was obtained using a Swern oxidation with oxalyl chloride and methyl sulfoxide [28] yielding the aldehyde intermediate, which was further oxidised with $NaClO_2$ [29] to afford disaccharide derivative **21** (83%). Dephthaloylation/deacylation of **21** using methylamine [30] in ethanol (2 days), and subsequent re-*N*-acetylation with acetic anhydride in methanol at 0 °C, afforded target compound **1** (88%).

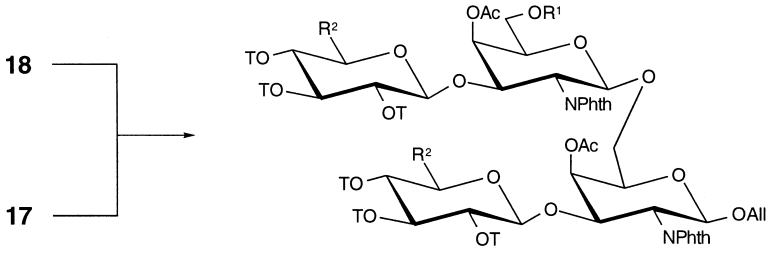
For the synthesis of the target trisaccharide **3**, acceptor disaccharide **17** was coupled with ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**22**) in dichloromethane, using *N*-iodosuccinimide and triflic acid [31] as catalytic system, to afford **23** (80%) (Scheme 2). Removal of the levulinoyl function of the glucose residue (see **19**), gave trisaccharide derivative **24** (91%). Contrary to the oxidation of **20**, no satisfactory results were obtained for the oxidation of **24** via the Swern method. However, the mild oxidation of **24** with pyridinium dichromate in dichloromethane in the presence of pulverised 4 Å molecular sieves [32] resulted in the formation of **25** in 86% yield. Dephthaloylation/deacylation of **25** using methylamine [30] in ethanol (5 days), and subsequent re-*N*-acetylation with acetic anhydride in methanol at 0 °C, afforded the target compound **3** (74%).

In order to prepare the tetrasaccharide target structure **5**, disaccharide donor **18** was synthesised from **16** in a two reaction step sequence (Scheme 1). Isomerisation of the double bond of the allyl function at C-1 [33], followed by removal of the 1-propenyl function using *N*-iodosuccinimide in tetrahydrofuran and water [34] afforded the hemiacetal derivative of **16**. Activation of the anomeric center was achieved by imidoylation [21] using trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene in dichloromethane resulting in the disaccharide imidate **18** (overall yield 71%, based on **16**). Condensation of **17** with **18** in dichloromethane in the presence of trimethylsilyl triflate (0.05 eq based on **18**) afforded the tetrasaccharide derivative **26** (73%) (Scheme 3). The silyl ether group could be removed selectively under acidic conditions (*p*-toluenesulfonic acid in acetonitrile–water), and the product was acetylated with acetic anhydride in pyridine to afford **27** (90% over two steps). Note that the desilylated intermediate in the last reaction step sequence can be used as a tetrasaccharide acceptor, suitable for the synthesis of



	R
23	CH_2OLev
24	CH_2OH
25	COOH

Scheme 2. Intermediates towards trisaccharide **3**



	R^1	R^2
26	TBDMS	CH_2OLev
27	Ac	CH_2OLev
28	Ac	CH_2OH
29	Ac	COOH

Scheme 3. Intermediates towards tetrasaccharide **5**

larger oligosaccharide structures. Compound **27** was delevulinoylated (see **19**), to afford diol **28** in 98% yield. Then, oxidation of the two primary hydroxyl functions (see **24**) resulted in the formation of **29** (60%). However, the extremely long reaction time necessary (up to 96 h) to complete the reaction, made this method less attractive. Therefore, pyridinium dichromate in dichloromethane in the presence of acetic anhydride [35] was attempted, since it was claimed that acetic anhydride facilitates the reduction of chromium(VI) from the intermediate ester, thereby accelerating the reaction [36]. Indeed, by using the latter method, the reaction time for the oxidation of tetrasaccharide **28** into **29** was decreased to 4.5 h and the yield slightly increased to 64%. Dephthaloylation/deacylation of **29** using methylamine [30] in ethanol (7 days), and re-*N*-acetylation with acetic anhydride in methanol at 0 °C, afforded the target compound **5** (63%).

To facilitate the conjugation of **1**, **3**, and **5** to bovine serum albumin, their allyl functions were elongated with cysteamine using cysteamine hydrochloride (2 equiv) in water under UV light [37] for initiation of the reaction, to obtain the amine-spacer-containing epitopes **2**, **4**, and **6**. The synthesis of the neoglycoconjugates and subsequent immunological studies are currently under investigation.

3. Experimental

General methods.—Reactions were monitored by TLC on Kieselgel 60 F_{254} (E. Merck); compounds were visualised, after examination under UV light, by charring with aq 50% H_2SO_4. 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. ^1H 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 spectrometer 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 ^1H–^1H correlation spectra (2D DQF ^1H–^1H COSY) of the products **1**–**6** 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 Xenon atoms. Elemental analyses were carried out by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany).

4-Methoxyphenyl 2,3,4,6-tetra-O-*acetyl*-β-D-*glucopyranoside* (**7**).—To a solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (5.0 g, 12.8 mmol),

4-methoxyphenol (2.4 g, 14.7 mmol) in dry CH_2Cl_2 (40 mL), containing 4 Å molecular sieves (5 g), was added at 0 °C Me_3SiOTf (0.25 mL, 1.35 mmol). After stirring for 2.5 h, TLC (2:1 toluene–EtOAc) showed the disappearance of the starting material and the formation of a new spot (R_f 0.66). Then the mixture was neutralised with Et_3N, diluted with CH_2Cl_2 (150 mL), washed with aq 5% $NaHCO_3$ and aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Crystallisation from *i*-PrOH afforded **7**, isolated as white crystals (4.6 g, 77%); $[\alpha]_D$ −21° (*c* 1); mp 102 °C; ^{1}H NMR ($CDCl_3$): δ 6.946 and 6.817 (2 d, each 2 H, $C_6H_4OCH_3$), 4.953 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.288 (dd, 1 H, $J_{5,6b}$ 5.1, $J_{6a,6b}$ 12.1 Hz, H-6b), 4.168 (dd, 1 H, $J_{5,6a}$ 2.2 Hz, H-6a), 3.775 (s, 3 H, $C_6H_4OCH_3$), 2.083, 2.076, 2.041, and 2.032 (4 s, each 3 H, 4 Ac). Anal. Calcd for $C_{21}H_{26}O_{11}$: C, 55.50; H, 5.77. Found: C, 55.48; H, 5.76.

*4-Methoxyphenyl 6-*O*-tert-butyldimethylsilyl-2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranoside* (**8**).—To a solution of **7** (4.3 g, 9.25 mmol) in MeOH (40 mL) was added NaOMe (pH 10). The solution was stirred for 8 h, then neutralised by addition of Dowex-50 (H^+). The mixture was filtered and concentrated, after which the residue was dissolved in pyridine (20 mL), and *tert*-butyldimethylsilyl chloride (1.81 g, 12.0 mmol) was added at 0 °C. TLC analysis (R_f 0.52; 9:1 CH_2Cl_2–MeOH) after 4.5 h showed the silylation of the primary hydroxyl function to be complete. The mixture was allowed to warm to room temperature, and *p*-toluoyl chloride (6.5 mL, 55.5 mmol) was added. The mixture was stirred overnight when TLC (R_f 0.87; 97:3 CH_2Cl_2–acetone) showed the reaction to be complete. The mixture was diluted with EtOAc (250 mL), washed with aq 5% $NaHCO_3$ and aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Crystallisation from EtOH gave compound **8**, isolated as light yellow crystals (4.4 g, 63%); $[\alpha]_D$ −38° (*c* 1); mp 86 °C; ^{1}H NMR ($CDCl_3$): δ 7.850, 7.733, 7.298, 7.165, 7.152, and 7.063 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 6.984 and 6.755 (2 d, each 2 H, $C_6H_4OCH_3$), 5.889 (t, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 9.6 Hz, H-3), 5.680 (dd, 1 H, $J_{1,2}$ 7.9 Hz, H-2), 5.501 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 5.212 (d, 1 H, H-1), 3.775 (s, 3 H, $C_6H_4OCH_3$), 2.342, 2.333, and 2.273 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 0.913 (s, 9 H, $Si(CH_3)_2C(CH_3)_3$), 0.055 and 0.026 (2 s, each 3 H, $Si(CH_3)_2C(CH_3)_3$). Anal. Calcd for $C_{43}H_{50}O_{10}Si$: C, 68.41; H, 6.68. Found: C, 68.17; H, 6.79.

*4-Methoxyphenyl 6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranoside* (**9**).—To a solution of **8** (4.4 g, 5.83 mmol) in acetonitrile (45 mL) containing water (5 mL), was added *p*-toluenesulfonic acid (pH 3). After 5 h, TLC (R_f 0.74; 9:1 CH_2Cl_2–EtOAc) showed a complete removal of the silyl function. The mixture was diluted with CH_2Cl_2 (250 mL), washed with aq 5% $NaHCO_3$ and aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. To a solution of the residue in 1,2-dichloroethane (100 mL) was added levulinic acid (1.25 mL, 11.7 mmol) and 2-chloro-1-methylpyridinium iodide (5.96 g, 23.4 mmol). The mixture was stirred for 15 min, then 1,4-diazabicyclo[2.2.2]octane (3.27 g, 29.0 mmol) was added and the stirring was continued for another 20 min, when TLC (R_f 0.86; 6:1 CH_2Cl_2-acetone) revealed the reaction to be complete. The mixture was filtered through Celite, diluted with EtOAc (200 mL), washed with aq 5% NaCl, and the organic phase was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue yielded **9**, isolated as a syrup (3.4 g, 80%); $[\alpha]_D$ +22° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.853, 7.809, 7.740, 7.162, and 7.080 (5 d, 2,2,2,4,2 H, 3 $COC_6H_4CH_3$), 6.961 and 6.779 (2 d, each 2 H, $C_6H_4OCH_3$), 5.704 (dd, 1 H, $J_{1,2}$ 7.7, $J_{2,3}$ 9.5 Hz, H-2), 5.231 (d, 1 H, H-1), 3.744 (s, 3 H, $C_6H_4OCH_3$), 2.676 (m, 4 H, $COCH_2CH_2COCH_3$), 2.348 and 2.289 (2 s, 6,3 H, 3 $COC_6H_4CH_3$), 2.160 (s, 3 H, $COCH_2CH_2COCH_3$); ^{13}C, d 205.8 ($COCH_2CH_2COCH_3$), 171.8 ($COCH_2CH_2COCH_3$), 165.4, 164.9, and 164.8 (3 $COC_6H_4CH_3$), 100.5 (C-1), 63.0 (C-6), 55.2 ($C_6H_4OCH_3$), 37.5, 29.3, and 27.5 ($COCH_2CH_2COCH_3$), 21.2 ($COC_6H_4CH_3$). Anal. Calcd for $C_{42}H_{42}O_{12}$: C, 68.28; H, 5.73. Found: C, 68.35; H, 5.82.

*6-*O*-Levulinoyl-2,3,4-tri-*O*-p-toluoyl-α,β-D-glucopyranose* (**10**).—To a solution of **9** (5.47 g, 7.40 mmol) in 1:1:1 toluene–acetonitrile–water (600 mL) was added ammonium cerium(IV) nitrate (40.7 g, 74.0 mmol). After 30 min, TLC (R_f 0.19; 9:1 CH_2Cl_2–acetone) showed the conversion of **9** into **10** to be complete. The mixture was diluted with EtOAc (500 mL), washed with aq saturated $NaHCO_3$ and water, 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 syrup (4.11 g, 88%); $[\alpha]_D$ +24° (*c* 1) (α:β 2.7:1); ^{1}H NMR ($CDCl_3$): δ 5.733 (d, 0.73 H, $J_{1,2}$ 3.3 Hz, H-1α), 4.664 (d, 0.27 H, $J_{1,2}$ 8.1 Hz, H-1β), 2.67–2.62 (m, 4 H, $COCH_2CH_2$-$COCH_3$),

2.334, 2.325, and 2.266 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.170 (s, 3 H, $COCH_2CH_2COCH_3$). Anal. Calcd for $C_{35}H_{36}O_{11}$: C, 66.45; H, 5.74. Found: C, 66.16; H, 5.82.

6-O-*Levulinoyl-2,3,4-tri*-O-p-*toluoyl*-α-D-*glucopyranosyl trichloroacetimidate* (**11**).—To a solution of **10** (2.45 g, 3.88 mmol) in dry CH_2Cl_2 (12 mL) and trichloroacetonitrile (4.1 mL, 40.8 mmol) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (140 μL). The mixture was stirred overnight and applied to column chromatography (95:5 CH_2Cl_2–acetone) to yield **11**, isolated as a white foam (2.77 g, 89%); $[\alpha]_D$ +38° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 8.631 (s, 1 H, $C(NH)CCl_3$), 7.832, 7.748, 7.168, 7.147, and 7.086 (5 d, 4,2,2,2,2 H, 3 $COC_6H_4CH_3$), 6.961 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.545 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 2.74–2.61 (m, 4 H, $COCH_2CH_2COCH_3$), 2.356, 2.341, and 2.292 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.179 (s, 3 H, $COCH_2CH_2COCH_3$). Anal. Calcd for $C_{37}H_{36}Cl_3NO_{11}$: C, 57.19; H, 4.67. Found: C, 56.76; H, 4.68.

Allyl (6-O-*levulinoyl-2,3,4-tri*-O-p-*toluoyl*-β-D-*glucopyranosyl)-(1→3)-4,6*-O-*benzylidene-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**13**).—To a solution of **11** (0.77 g, 0.99 mmol) and allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranoside [24,25] (**12**; 0.29 g, 0.66 mmol) in CH_2Cl_2 (8 mL), containing 4 Å molecular sieves (0.8 g), was added at 0 °C Me_3SiOTf (6.3 μL, 33 μmol). After stirring for 10 min, TLC (9:1 CH_2Cl_2–acetone) showed the disappearance of **12** and the formation of a new product (R_f 0.77). The mixture was neutralised with Et_3N, diluted with CH_2Cl_2 (150 mL), washed with aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue gave **13**, isolated as a glass (0.62 g, 89%); $[\alpha]_D$ +31° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.662, 7.532, 7.249, 7.110, 6.969, and 6.941 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.674 (s, 1 H, CHC_6H_5), 5.63–5.51 (m, 1 H, $OCH_2CH=CH_2$), 5.230 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.6 Hz, H-2′), 5.166 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.07–4.93 (m, 2 H, $OCH_2CH=CH_2$), 4.916 (d, 1 H, H-1′), 4.788 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 2.72–2.50 (m, 4 H, $COCH_2CH_2COCH_3$), 2.350, 2.323, and 2.234 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.191 (s, 3 H, $COCH_2CH_2COCH_3$); ^{13}C, δ 206.2 ($COCH_2CH_2COCH_3$), 172.1 ($COCH_2CH_2COCH_3$), 165.5, 164.8, and 164.3 (3 $COC_6H_4CH_3$), 133.1 ($OCH_2CH=CH_2$), 117.4 ($OCH_2CH=CH_2$), 101.6, 100.2, and 97.5 (CHC_6H_5 and C-1,1′), 55.0 (C-2), 37.8, 29.7, and 27.8 ($COCH_2CH_2COCH_3$), 21.5 (2 C) and 21.3 (3 $COC_6H_4CH_3$).

Allyl (6-O-*levulinoyl-2,3,4-tri*-O-p-*toluoyl*-β-D-*glucopyranosyl)-(1→3)-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**14**).—To a solution of **13** (0.69 g, 0.66 mmol) in CH_2Cl_2 (20 mL) and water (63 μL) was added CF_3CO_2H (0.54 mL). The mixture was stirred for 30 min, when TLC (4:1 CH_2Cl_2–acetone) showed the formation of a slower moving spot (R_f 0.45). The mixture was diluted with CH_2Cl_2 (150 mL), washed with aq 10% $NaHCO_3$ and aq 10% NaCl, and the aqueous phases were twice extracted with CH_2Cl_2 (50 mL). The combined organic layers were dried, filtered, and concentrated. Column chromatography (4:1 CH_2Cl_2–acetone) of the residue gave **14**, isolated as a glass (0.62 g, 98%); $[\alpha]_D$ +4° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.742, 7.521, 7.323, 7.137, 6.964, and 6.823 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.60–5.48 (m, 1 H, $OCH_2CH=CH_2$), 5.390 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.9 Hz, H-2′), 4.997 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.03–4.89 (m, 2 H, $OCH_2CH=CH_2$), 4.816 (d, 1 H, H-1′), 4.629 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 2.82–2.61 (m, 4 H, $COCH_2CH_2COCH_3$), 2.353, 2.313, and 2.224 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.215 (s, 3 H, $COCH_2CH_2COCH_3$); ^{13}C, δ 204.8 ($COCH_2CH_2COCH_3$), 172.1 ($COCH_2CH_2COCH_3$), 165.4, 165.0, and 164.2 (3 $COC_6H_4CH_3$), 130.7 ($OCH_2CH=CH_2$), 117.1 ($OCH_2CH=CH_2$), 101.2 and 97.3 (C-1,1′), 62.8 and 62.6 (C-6,6′), 54.6 (C-2), 37.7, 29.6, and 27.6 ($COCH_2CH_2COCH_3$), 21.5 (2 C) and 21.3 (3 $COC_6H_4CH_3$). Anal. Calcd for $C_{52}H_{53}NO_{17}$: C, 64.79; H, 5.54. Found: C, 64.79; H, 5.49.

Allyl (6-O-*levulinoyl-2,3,4-tri*-O-p-*toluoyl*-β-D-*glucopyranosyl)-(1→3)-6*-O-tert-*butyldimethylsilyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**15**).—To a solution of **14** (0.57 g, 0.59 mmol) in pyridine (18 mL) was added *tert*-butyldimethylsilyl chloride (0.27 g, 1.77 mmol). The mixture was stirred for 6 h at 0 °C when TLC (8:1 CH_2Cl_2–acetone) showed a complete conversion of **14** into **15** (R_f 0.84). The mixture was diluted with EtOAc (150 mL), washed with aq 10% $NaHCO_3$ and aq 10% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue yielded **15**, isolated as a glass (0.60 g, 96%); $[\alpha]_D$ +5° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.741, 7.520, 7.327, 7.135, 6.961, and 6.823 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.61–5.48 (m, 1 H, $OCH_2CH=CH_2$), 5.395 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.9 Hz, H-2′), 4.940 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.01–4.89 (m, 2 H,

$OCH_2CH=CH_2$), 4.801 (d, 1 H, H-1′), 4.601 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 2.82–2.62 (m, 4 H, $COCH_2CH_2COCH_3$), 2.333, 2.313, and 2.220 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.210 (s, 3 H, $COCH_2CH_2COCH_3$), 0.909 (s, 9 H, $Si(CH_3)_2C(CH_3)_3$), 0.090 and 0.081 (2 s, each 3 H, $Si(CH_3)_2C(CH_3)_3$); ^{13}C, δ 206.3 ($COCH_2CH_2COCH_3$), 172.1 ($COCH_2CH_2COCH_3$), 165.5, 165.0, and 164.3 (3 $COC_6H_4CH_3$), 130.8 ($OCH_2CH=CH_2$), 117.0 ($OCH_2CH=CH_2$), 101.3 and 96.8 (C-1,1′), 63.0 and 62.5 (C-6,6′), 54.7 (C-2), 37.8, 29.7, and 27.6 ($COCH_2CH_2COCH_3$), 25.8 [$Si(CH_3)_2C(CH_3)_3$], 21.5 (2 C) and 21.4 (3 $COC_6H_4CH_3$), 18.3 [$Si(CH_3)_2C(CH_3)_3$], 5.3 [$Si(CH_3)_2C(CH_3)_3$]. Anal. Calcd for $C_{58}H_{67}NO_{17}Si$: C, 64.61; H, 6.26. Found: C, 65.08; H, 6.37.

*Allyl (6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-galactopyranoside* (**16**).—To a solution of **15** (0.61 g, 0.57 mmol) in CH_2Cl_2 (27 mL), containing pyridine (0.5 mL, 5.7 mmol), was added slowly at 0 °C a solution of trifluoromethanesulfonic anhydride (0.38 mL, 2.26 mmol) in CH_2Cl_2 (5 mL). After stirring for 6 h at 0 °C, TLC (R_f 0.75; 95:5 CH_2Cl_2–acetone) showed the triflation to be complete. The mixture was diluted with EtOAc (150 mL), washed with aq 10% $NaHCO_3$ and aq 10% NaCl, and the organic layer was dried, filtered, concentrated and co-concentrated with toluene. The residue was dissolved in DMF (16 mL) and tetrabutylammonium acetate (0.85 g, 2.8 mmol) was added. The mixture was stirred overnight at room temperature, diluted with EtOAc (150 mL), washed with aq 10% NaCl, dried, filtered, concentrated and co-concentrated with toluene. Column chromatography (97:3 CH_2Cl_2–acetone) of the residue afforded **16**, isolated as a syrup (0.49 g, 78%); $[\alpha]_D$ +8° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.732, 7.557, 7.359, 7.120, 6.979, and 6.879 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.601 (d, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ < 1 Hz, H-4), 5.66–5.53 (m, 1 H, $OCH_2CH=CH_2$), 5.395 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.8 Hz, H-2′), 5.042 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 5.07–4.93 (m, 2 H, $OCH_2CH=CH_2$), 4.833 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-3), 4.799 (d, 1 H, H-1′), 4.526 (dd, 1 H, H-2), 2.83–2.53 (m, 4 H, $COCH_2CH_2COCH_3$), 2.326 and 2.228 (2 s, 6,3 H, 3 $COC_6H_4CH_3$), 2.215 (s, 3 H, $COCH_2CH_2COCH_3$), 2.193 (s, 3 H, Ac), 0.893 (s, 9 H, $Si(CH_3)_2C(CH_3)_3$), 0.064 (s, 6 H, $Si(CH_3)_2C(CH_3)_3$); ^{13}C, δ 206.4 ($COCH_2CH_2COCH_3$), 172.2 ($COCH_2CH_2COCH_3$), 169.9 ($COCH_3$), 165.5, 164.9, and 164.2 (3 $COC_6H_4CH_3$), 130.9 ($OCH_2CH=CH_2$), 117.3 ($OCH_2CH=CH_2$), 101.1 and 97.3 (C-1,1′), 62.2 and 62.1 (C-6,6′), 52.4 (C-2), 37.9, 29.7, and 27.8 ($COCH_2CH_2COCH_3$), 25.6 [$Si(CH_3)_2C(CH_3)_3$], 21.5 (2 C) and 21.4 (3 $COC_6H_4CH_3$), 20.8 ($COCH_3$), 18.1 [$Si(CH_3)_2C(CH_3)_3$], 5.6 and 5.5 [$Si(CH_3)_2C(CH_3)_3$]. FABMS (positive-ion mode; $C_{60}H_{69}NO_{18}Si$): *m/z* 1142 $[M+Na]^+$, 1120 $[M+H]^+$.

*Allyl (6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside* (**17**).—To a solution of **16** (0.32 g, 0.28 mmol) in acetonitrile (27 mL), containing water (2.7 mL), was added *p*-toluenesulfonic acid (0.17 g, 1.0 mmol). The mixture was stirred for 2 h, when TLC (R_f 0.33; 8:1 CH_2Cl_2–acetone) showed the reaction to be complete. The mixture was concentrated, diluted with EtOAc (150 mL), washed with aq 10% $NaHCO_3$ and aq 10% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue rendered **17**, isolated as a syrup (0.26 g, 91%); $[\alpha]_D$ +3° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.739, 7.548, 7.322, 7.124, 6.975, and 6.834 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.599 (d, 1 H, $J_{3,4}$ 3.2, $J_{4,5}$ < 1 Hz, H-4), 5.64–5.51 (m, 1 H, $OCH_2CH=CH_2$), 5.314 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.7 Hz, H-2′), 5.062 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 5.06–4.92 (m, 2 H, $OCH_2CH=CH_2$), 4.903 (dd, 1 H, $J_{2,3}$ 11.3 Hz, H-3), 4.871 (d, 1 H, H-1′), 4.571 (dd, 1 H, H-2), 2.88–2.53 (m, 4 H, $COCH_2CH_2COCH_3$), 2.322, 2.309, and 2.225 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.296 (s, 3 H, $COCH_2CH_2COCH_3$), 2.203 (s, 3 H, Ac); ^{13}C, δ 206.5 ($COCH_2CH_2COCH_3$), 172.3 ($COCH_2CH_2COCH_3$), 168.6 ($COCH_3$), 165.3, 164.8, and 163.9 (3 $COC_6H_4CH_3$), 130.7 ($OCH_2CH=CH_2$), 117.2 ($OCH_2CH=CH_2$), 101.4 and 97.5 (C-1,1′), 62.1 and 59.8 (C-6,6′), 52.1 (C-2), 37.7, 29.5, and 27.5 ($COCH_2CH_2COCH_3$), 21.3 (2 C) and 21.1 (3 $COC_6H_4CH_3$), 20.8 ($COCH_3$). Anal. Calcd for $C_{54}H_{55}NO_{18}$: C, 64.47; H, 5.51. Found: C, 64.42; H, 5.62.

*(6-*O*-Levulinoyl-2,3,4-tri-*O*-p-toluoyl-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl trichloroacetimidate* (**18**).—A solution of **16** (0.21 g, 0.19 mmol), tris(triphenylphosphine)rhodium(I) chloride (64 mg, 74 μmol), and a catalytic amount of 1,4-diazabicyclo[2.2.2]octane in EtOH (27 mL) was boiled under reflux for 45 min. The mixture

was cooled to room temperature, diluted with CH_2Cl_2 (100 mL), washed with aq 10% NaCl, dried, filtered, and concentrated. To a solution of the residue in THF (15 mL), containing water (1.5 mL), was added *N*-iodosuccinimide (112 mg, 0.50 mmol). After stirring for 30 min, the mixture was diluted with CH_2Cl_2 (150 mL), washed with aq 10% $NaHSO_3$, aq 10% $NaHCO_3$, and aq 10% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue gave the deallylated intermediate, isolated as a syrup. To a solution of the residue and trichloroacetonitrile (0.14 mL, 1.34 mmol) in CH_2Cl_2 (7 mL) was added at 0 °C 1,8-diazabicyclo[5.4.0]undec-7-ene (4.7 μL, 31 μmol). The mixture was stirred overnight and applied to column chromatography (R_f 0.55; 95:5 CH_2Cl_2–acetone) to yield **18**, isolated as a glass (0.14 g, overall yield 71%); $[\alpha]_D$ +19° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 7.755, 7.572, 7.377, 7.126, 6.986, and 6.882 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 6.334 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 5.705 (d, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ < 1 Hz, H-4), 5.274 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.8 Hz, H-2′), 4.988 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-3), 4.795 (d, 1 H, H-1′), 4.748 (dd, 1 H, H-2), 2.79–2.60 (m, 4 H, $COCH_2CH_2COCH_3$), 2.327 and 2.233 (2 s, 6,3 H, 3 $COC_6H_4CH_3$), 2.258 (s, 3 H, $COCH_2CH_2COCH_3$), 2.209 (s, 3 H, Ac), 0.870 (s, 9 H, $Si(CH_3)_2C(CH_3)_3$), 0.043 and 0.028 (2 s, each 3 H, $Si(CH_3)_2C(CH_3)_3$). Anal. Calcd for $C_{58}H_{65}Cl_3N_2O_{18}Si$: C, 57.45; H, 5.40. Found: C, 57.83; H, 5.29.

*Allyl (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*-galactopyranoside* (**19**).—To a solution of **17** (0.28 g, 0.28 mmol) 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–acetone) showed the acetylation to be complete (**19**; R_f 0.64). The mixture was concentrated and co-concentrated with toluene, EtOH, and CH_2Cl_2 (3×20 mL). Column chromatography (95:5 CH_2Cl_2–acetone) of the residue afforded **19**, isolated as a glass (0.26 g, 88%); $[\alpha]_D$ +8° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.738, 7.562, 7.353, 7.132, 6.990, and 6.871 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.60–5.51 (m, 1 H, $OCH_2CH=CH_2$), 5.266 (dd, 1 H, $J_{1',2'}$ 7.6, $J_{2',3'}$ 9.8 Hz, H-2′), 5.052 (d, 1 H, $J_{1,2}$ 8.7 Hz, H-1), 5.06–4.94 (m, 2 H, $OCH_2CH=CH_2$), 4.862 (dd, 1 H, $J_{2,3}$ 11.3, $J_{3,4}$ 3.3 Hz, H-3), 4.788 (d, 1 H, H-1′), 4.535 (dd, 1 H, H-2), 2.88–2.53 (m, 4 H, $COCH_2CH_2COCH_3$), 2.331, 2.238, 2.208, and 2.090 (4 s, 6,6,3,3 H, 3 $COC_6H_4CH_3$, $COCH_2CH_2COCH_3$, and 2 Ac); ^{13}C, δ 172.2 ($COCH_2CH_2COCH_3$), 170.4 and 169.8 (2 $COCH_3$), 164.8 (2 C) and 164.1 (3 $COC_6H_4CH_3$), 133.1 ($OCH_2CH=CH_2$), 117.4 ($OCH_2CH=CH_2$), 101.1 and 97.9 (C-1,1′), 62.5 (C-6,6′), 52.1 (C-2), 37.8, 29.5, and 27.7 ($COCH_2CH_2COCH_3$), 21.4 ($COC_6H_4CH_3$), 20.8 and 20.1 (2 $COCH_3$).

*Allyl (2,3,4-tri-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-galactopyranoside* (**20**).—To a solution of **19** (0.16 g, 0.15 mmol) in EtOH (20 mL) and toluene (6 mL) was added NH_2NH_2·HOAc (70 mg, 0.76 mmol). The mixture was stirred for 90 min, when TLC (9:1 CH_2Cl_2–acetone) showed the conversion of **19** into **20** (R_f 0.64), then concentrated. Column chromatography (9:1 CH_2Cl_2–acetone) of the residue yielded **20**, isolated as a glass (136 mg, 94%); $[\alpha]_D$ +7° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.752, 7.585, 7.345, 7.137, 7.006, and 6.901 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.737 (d, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ < 1 Hz, H-4), 5.69–5.61 (m, 1 H, $OCH_2CH=CH_2$), 5.287 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.8 Hz, H-2′), 5.038 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.01–4.94 (m, 2 H, $OCH_2CH=CH_2$), 4.885 (d, 1 H, H-1′), 4.559 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-2), 2.339, 2.292, and 2.229 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.036 (s, 6 H, 2 Ac); ^{13}C, δ 170.4 and 169.8 (2 $COCH_3$), 165.4, 165.0, and 163.9 (3 $COC_6H_4CH_3$), 133.0 ($OCH_2CH=CH_2$), 117.4 ($OCH_2CH=CH_2$), 101.9 and 97.4 (C-1,1′), 52.1 (C-2), 21.4 ($COC_6H_4CH_3$), 20.3 and 20.1 (2 $COCH_3$). Anal. Calcd for $C_{51}H_{51}NO_{17}$: C, 64.48; H, 5.41. Found: C, 64.34; H, 5.50.

*Allyl (2,3,4-tri-*O*-p-toluoyl-*β*-*D*-glucopyranosyl-uronic acid)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-galactopyranoside* (**21**).—To a cold (−78 °C) 2 M solution of oxalyl chloride (0.24 mL, 0.48 mmol) in CH_2Cl_2 (2 mL) was added Me_2SO (73.2 μL, 1.03 mmol), and the mixture was stirred for 10 min. A solution of **20** (47 mg, 50 μmol) in CH_2Cl_2 (2 mL) was added, and the mixture was stirred for 1 h, whereby in 20 min a white precipitate was formed. Diisopropylethylamine (0.36 mL) was added, and after 10 min the mixture was diluted with EtOAc (150 mL), washed with aq 1 M HCl, and the organic layer was dried, filtered, and concentrated. To a solution of the residue in *tert*-BuOH (2 mL) and 2-methyl-2-butene (0.77 mL), containing water (1.27 mL) and NaH_2PO_4 (126 mg), was added $NaClO_2$ (126 mg). The mixture was stirred overnight, when TLC

(10:9:1 CH_2Cl_2–EtOAc–HOAc) showed a complete conversion of **20** into **21** (R_f 0.64). The mixture was concentrated, and a solution of the residue in water was washed with hexane, acidified with aq 1 M HCl, and extracted with EtOAc (3×20 mL), and the organic layer was dried, filtered, and concentrated. Column chromatography (10:9:1 CH_2Cl_2–EtOAc–HOAc) of the residue yielded **21**, isolated as a white solid (37 mg, 83%); $[\alpha]_D$ +3° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.802, 7.590, 7.301, 7.151, 7.007, and 6.777 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.715 (d, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ < 1 Hz, H-4), 5.73–5.66 (m, 1 H, $OCH_2CH=CH_2$), 5.287 (dd, 1 H, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.1 Hz, H-2′), 4.996 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.986 (d, 1 H, H-1′), 4.486 (dd, 1 H, $J_{2,3}$ 11.3 Hz, H-2), 2.099 and 2.072 (2 s, each 3 H, 2 Ac); ^{13}C, δ 165.2, 164.9, and 163.9 (3 $COC_6H_4CH_3$), 133.0 ($OCH_2CH=CH_2$), 117.1 ($OCH_2CH=CH_2$), 101.3 and 97.4 (C-1,1′), 52.1 (C-2), 21.4 and 21.3 ($COC_6H_4CH_3$), 21.0 and 20.5 (2 $COCH_3$). Anal. Calcd for $C_{51}H_{49}NO_{18}$: C, 63.61; H, 5.04. Found: C, 63.28; H, 5.28.

*Allyl (*β-D-*glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy*-β-D-*galactopyranoside* (**1**).—A solution of **21** (17 mg, 18 μmol) in ethanolic 33% $MeNH_2$ (7.5 mL) was stirred for 2 days at room temperature, during which the mixture was once concentrated and new reagent (7.5 mL) added. After concentration, the residue was dissolved in dry MeOH (5 mL), and Ac_2O (100 μL) was added at 0 °C. The mixture was stirred for 2 h, when TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed a complete conversion of **21** into **1** (R_f 0.53). The solution was concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL). Gel filtration over Sephadex G-10 (water) of the residue yielded **1**, isolated after lyophilisation as a white, amorphous powder (7 mg, 88%); NMR (D_2O): ^1H, δ 5.912 (m, 1 H, $OCH_2CH=CH_2$), 5.36–5.23 (m, 2 H, $OCH_2CH=CH_2$), 4.544 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.501 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.027 (dd, 1 H, $J_{2,3}$ 10.9 Hz, H-2), 3.831 (dd, 1 H, $J_{3,4}$ 3.2 Hz, H-3), 3.330 (t, 1 H, $J_{2',3'}$ 8.0 Hz, H-2′), 2.116 (s, 3 H, $NHCOCH_3$); ^{13}C, δ 175.4 (*C*OOH), 174.6 ($NHCOCH_3$), 133.4 ($OCH_2CH=CH_2$), 118.3 ($OCH_2CH=CH_2$), 103.8 and 100.2 (C-1,1′), 60.9 (C-6), 51.1 (C-2), 22.1 ($NHCOCH_3$). FABMS (positive-ion mode; $C_{17}H_{27}NO_{12}$): *m/z* 460 $[M+Na]^+$.

*Allyl (3,4,6-tri-*O-*acetyl-2-deoxy-2-phthalimido-*β-D-*galactopyranosyl)-(1→6)-[(6-*O-*levulinoyl-2,3,4-tri-*O-p-*toluoyl-*β-D-*glucopyranosyl)-(1→3)]-4-*O-*acetyl-2-deoxy-2-phthalimido-*β-D-*galactopyranoside* (**23**).—To a solution of **17** (85 mg, 84 μmol) and ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (**22**; 85 mg, 0.17 mmol) in CH_2Cl_2 (5.5 mL), containing 4 Å molecular sieves (0.2 g), was added dropwise at −20 °C a solution of *N*-iodosuccinimide (24 mg, 0.11 mmol) and HOTf (106 μL, 11 μmol) in dry CH_2Cl_2 (2 mL). After stirring for 30 min, TLC (9:1 CH_2Cl_2–acetone) showed the disappearance of **17** and the formation of a new product (R_f 0.61). The mixture was neutralised with Et_3N, diluted with CH_2Cl_2 (100 mL), washed with aq 5% $NaHSO_3$, aq 5% $NaHCO_3$, and aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (94:6 CH_2Cl_2–acetone) of the residue gave **23**, isolated as a white solid (96 mg, 80%); $[\alpha]_D$ +13° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.724, 7.536, 7.314, 7.120, 6.971, and 6.851 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.807 (dd, 1 H, $J_{2',3'}$ 11.5, $J_{3',4'}$ 3.4 Hz, H-3′), 5.487 (d, 1 H, $J_{4',5'}$ < 1 Hz, H-4′), 5.453 (d, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ < 1 Hz, H-4), 5.39–5.28 (m, 1 H, $OCH_2CH=CH_2$), 5.353 and 4.863 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.4 and 8.5 Hz, H-1,1′), 5.218 (dd, 1 H, $J_{1'',2''}$ 7.8, $J_{2'',3''}$ 9.8 Hz, H-2″), 4.98–4.86 (m, 2 H, $OCH_2CH=CH_2$), 4.746 (dd, 1 H, $J_{2,3}$ 11.2 Hz, H-3), 4.726 (d, 1 H, H-1″), 4.526 and 4.409 (2 dd, each 1 H, H-2,2′), 2.335, 2.325, and 2.237 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.206 (s, 3 H, $COCH_2CH_2COCH_3$), 2.203, 2.179, 2.099, and 1.835 (4 s, each 3 H, 4 Ac); ^{13}C, δ 172.1 ($COCH_2CH_2COCH_3$), 170.2, 170.0 (2 C), and 169.5 (4 $COCH_3$), 165.3, 164.8, and 164.0 (3 $COC_6H_4CH_3$), 134.0 ($OCH_2CH=CH_2$), 117.4 ($OCH_2CH=CH_2$), 101.0, 98.1, and 96.7 (C-1,1′,1″), 52.2 and 51.2 (C-2,2′), 37.7, 29.6, and 27.7 ($COCH_2CH_2COCH_3$), 21.4 and 21.3 (2 C) (3 $COC_6H_4CH_3$), 20.7, 20.5 (2 C), and 20.3 (4 $COCH_3$). Anal. Calcd for $C_{74}H_{74}N_2O_{27}$: C, 62.44; H, 5.24. Found: C, 62.28; H, 5.36.

*Allyl (3,4,6-tri-*O-*acetyl-2-deoxy-2-phthalimido-*β-D-*galactopyranosyl)-(1→6)-[(2,3,4-tri-*O-p-*toluoyl-*β-D-*glucopyranosyl)-(1→3)]-4-*O-*acetyl-2-deoxy-2-phthalimido-*β-D-*galactopyranoside* (**24**).—To a solution of **23** (96 mg, 67 μmol) in EtOH (11 mL) and toluene (5 mL) was added NH_2NH_2·HOAc (61 mg, 67 μmol). The mixture was stirred for 2 h, when TLC (9:1 CH_2Cl_2–acetone) showed the conversion of **23** into **24** (R_f 0.61), then concentrated. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue afforded **24**, isolated as a glass (84 mg, 91%); $[\alpha]_D$ +1° (*c* 1); NMR

($CDCl_3$): ^1H, δ 7.734, 7.516, 7.279, 7.131, 6.956, and 6.777 (6 d, each 2 H, 3 $COC_6H_4CH_3$), 5.803 (dd, 1 H, $J_{2',3'}$ 11.5, $J_{3',4'}$ 3.3 Hz, H-3′), 5.586 (d, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ < 1 Hz, H-4), 5.478 (d, 1 H, $J_{4',5'}$ < 1 Hz, H-4′), 5.346 and 4.867 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.6 and 8.5 Hz, H-1,1′), 5.237 (dd, 1 H, $J_{1'',2''}$ 7.8, $J_{2'',3''}$ 9.8 Hz, H-2″), 4.96–4.82 (m, 2 H, $OCH_2CH=CH_2$), 4.812 (d, 1 H, H-1″), 4.733 (dd, 1 H, $J_{2,3}$ 11.1 Hz, H-3), 4.534 and 4.421 (2 dd, each 1 H, H-2,2″), 2.332, 2.276, and 2.248 (3 s, each 3 H, 3 $COC_6H_4CH_3$), 2.213, 2.087, and 1.840 (3 s, 6,3,3 H, 4 Ac); ^{13}C, δ 170.2, 170.1, 169.5, and 168.5 (4 $COCH_3$), 165.3, 165.0, and 163.9 (3 $COC_6H_4CH_3$), 130.6 ($OCH_2CH=CH_2$), 117.5 ($OCH_2CH=CH_2$), 101.7, 97.8, and 96.8 (C-1,1′,1″), 52.1 and 51.1 (C-2,2′), 21.4, 21.2, 20.9, 20.5, and 20.3 ($COC_6H_4CH_3$ and $COCH_3$). Anal. Calcd for $C_{69}H_{68}N_2O_{25}$: C, 62.53; H, 5.17. Found: C, 62.39; H, 5.31.

*Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-galactopyranosyl)-(1→6)-[(2,3,4-tri-*O*-p-toluoyl-*β*-*D*-glucopyranosyluronic acid)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-galactopyranoside* (**25**).—To a solution of **24** (45 mg, 34 μmol) in dry CH_2Cl_2 (5 mL), containing 4 Å powdered molecular sieves (300 mg), was added pyridinium dichromate (62 mg, 161 μmol). After stirring for 48 h, TLC (8:4:1 CH_2Cl_2–EtOAc–HOAc) showed the conversion of **24** into **25** (R_f 0.68). After the addition of EtOAc (25 mL), the suspension was filtered through Celite and applied to column chromatography (8:4:1 CH_2Cl_2–EtOAc–HOAc) to yield **25**, isolated as a glass (39 mg, 86%); $[\alpha]_D$ +4° (*c* 1); ^{13}C NMR ($CDCl_3$): δ 170.2, 170.1, 169.5, and 168.5 (4 $COCH_3$), 165.3, 165.0, and 163.9 (3 $COC_6H_4CH_3$), 130.6 ($OCH_2CH=CH_2$), 117.5 ($OCH_2CH=CH_2$), 101.7, 97.8, and 96.8 (C-1,1′,1″), 52.1 and 51.1 (C-2,2′), 21.4, 21.2, 20.9, 20.5, and 20.3 ($COC_6H_4CH_3$ and $COCH_3$). Anal. Calcd for $C_{69}H_{66}N_2O_{26}$: C, 61.88; H, 4.97. Found: C, 61.66; H, 5.12.

*Allyl (2-acetamido-2-deoxy-*β*-*D*-galactopyranosyl)-(1→6)-[(*β*-*D*-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-*β*-*D*-galactopyranoside* (**3**).—A solution of **25** (21 mg, 15 μmol) in ethanolic 33% $MeNH_2$ (7.5 mL) was stirred for 5 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), and Ac_2O (100 μL) was added at 0 °C. The mixture was stirred for 2 h, when TLC (4:2:2:0.5 1-BuOH–EtOH-H_2O–HOAc) showed the formation of a new product (R_f 0.38). The solution was concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL). Gel filtration over Sephadex G-10 (water) of the residue yielded **3**, isolated after lyophilisation as a white, amorphous powder (7 mg, 74%); NMR (D_2O): ^1H, δ 5.914 (m, 1 H, $OCH_2CH=CH_2$), 5.34–5.27 (m, 2 H, $OCH_2CH=CH_2$), 4.553 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.542 and 4.475 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.6 and 8.5 Hz, H-1,1′), 4.110 and 3.939 (2 d, each 1 H, $J_{3,4/3',4'}$ 2.8 and 2.7 Hz, $J_{4,5/4',5'}$ < 1 Hz, H-4,4′), 4.019 and 3.899 (2 dd, each 1 H, $J_{2,3/2',3'}$ 11.2 and 10.7 Hz, H-2,2′), 3.356 (dd, 1 H, $J_{2'',3''}$ 8.4 Hz, H-2″), 2.025 and 2.012 (2 s, each 3 H, 2 $NHCOCH_3$); ^{13}C, δ 177.6 and 177.4 (2 $NHCOCH_3$), 175.4 (*C*OOH), 135.9 ($OCH_2CH=CH_2$), 121.2 ($OCH_2CH=CH_2$), 107.0, 104.6, and 102.8 (C-1,1′,1″), 55.2 and 53.8 (C-2,2′), 25.1 and 25.0 ($NHCOCH_3$). FABMS (positive-ion mode; $C_{25}H_{40}N_2O_{17}$): *m/z* 663 $[M+Na]^+$.

*Allyl (6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-2-deoxy-2-phthalimido-*β*-*D*-galactopyranosyl)-(1→6)-[(6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-*β*-*D*-glucopyranosyl)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthalimido-*β*-*D*-galactopyranoside* (**26**).—To a solution of **17** (94 mg, 94 μmol) and **18** (187 mg, 150 μmol) in CH_2Cl_2 (5 mL), containing 4 Å molecular sieves (0.3 g), was added Me_3SiOTf (0.8 μL, 4.3 μmol). After stirring for 10 min, TLC (9:1 CH_2Cl_2–acetone) showed the disappearance of **17** and the formation of a new product (R_f 0.66). The mixture was neutralised with Et_3N, diluted with CH_2Cl_2 (100 mL), washed with aq 5% NaCl, and the organic layer was dried, filtered, and concentrated. Column chromatography (95:5 CH_2Cl_2–acetone) of the residue afforded **26**, isolated as a glass (141 mg, 73%); $[\alpha]_D$ −1° (*c* 1); NMR ($CDCl_3$): ^1H, δ 7.736, 7.708, 7.549, 7.521, 7.472, 7.331, 7.278, 7.114, 6.980, 6.952, 6.870, and 6.843 (12 d, each 2 H, 6 $COC_6H_4CH_3$), 5.659 and 5.403 (2 d, each 1 H, $J_{3,4/3',4'}$ 3.2 and 3.1 Hz, $J_{4,5/4',5'}$ < 1 Hz, H-4,4′), 5.29–5.21 (m, 1 H, $OCH_2CH=CH_2$), 5.237 and 5.193 (2 dd, each 1 H, $J_{1'',2''/1''',2'''}$ 7.8 and 7.9 Hz, $J_{2'',3''/2''',3'''}$ 9.8 and 9.9 Hz, H-2″,2‴), 5.031 and 4.731 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.4 and 8.5 Hz, H-1,1′), 4.752 and 4.682 (2 d, each 1 H, H-1″,1‴), 4.465 and 4.347 (2 dd, each 1 H, $J_{2,3/2',3'}$ 11.2 and 11.3 Hz, H-2,2′), 2.336, 2.309, 2.222, 2.198, and 2.138 (5 s, each 6 H, 6 $COC_6H_4CH_3$, 2 $COCH_2CH_2COCH_3$, and 2 Ac), 0.907 (s, 9 H, $Si(CH_3)_2C(CH_3)_3$), 0.770 (s, 6 H, $Si(CH_3)_2C(CH_3)_3$); ^{13}C, δ

172.0 ($COCH_2CH_2COCH_3$), 169.7 and 169.4 (2 $COCH_3$), 166.5, 165.3 (2 C), 164.8, and 164.0 (2 C) (6 $COC_6H_4CH_3$), 133.2 ($OCH_2CH=CH_2$), 100.9 (2 C), 96.6, and 95.1 (C-1,1′,1″,1‴), 52.3 and 52.0 (C-2,2′), 37.7, 29.5, and 27.7 ($COCH_2CH_2COCH_3$), 25.6 [$Si(CH_3)_2C(CH_3)_3$], 21.3 and 21.2 ($COC_6H_4CH_3$), 20.6 ($COCH_3$), 17.9 [$Si(CH_3)_2C(CH_3)_3$], 5.3 and 5.1 [$Si(CH_3)_2C(CH_3)_3$].

*Allyl (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*-galactopyranosyl)-(1→6)-[(6-*O*-levulinoyl-2,3,4-tri-*O*-p-toluoyl-β-*D*-glucopyranosyl)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**27**).—To a solution of **26** (69 mg, 33 μmol) in acetonitrile (3 mL), containing water (0.3 mL), was added *p*-toluenesulfonic acid (21 mg, 0.12 mmol). The mixture was stirred for 30 min, when TLC (R_f 0.07; 8:1 CH_2Cl_2–acetone) showed the desilylation to be complete. The mixture was concentrated, diluted with EtOAc (100 mL), washed with aq 10% $NaHCO_3$ and aq 10% NaCl, and the organic layer was dried, filtered, and concentrated. To a solution of the residue in pyridine (5 mL) were added Ac_2O (5 mL) and a catalytic amount of 4-dimethylaminopyridine. The solution was stirred overnight when TLC (R_f 0.69; 9:1 CH_2Cl_2–acetone) showed a complete conversion. The mixture was concentrated and co-concentrated with toluene, EtOH, and CH_2Cl_2 (3×20 mL). Column chromatography (93:7 CH_2Cl_2–acetone) of the residue gave **27**, isolated as a glass (57 mg, 90%); $[\alpha]_D$ +4° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.733, 7.718, 7.541, 7.526, 7.323, 7.296, 7.113, 7.107, 6.961, 6.952, 6.845, and 6.832 (12 d, each 2 H, 6 $COC_6H_4CH_3$), 5.635 and 5.429 (2 d, each 1 H, $J_{3,4/3',4'}$ 3.6 and 3.5 Hz, $J_{4,5/4',5'}$ < 1 Hz, H-4,4′), 5.33–5.22 (m, 1 H, $OCH_2CH=CH_2$), 5.246 and 5.213 (2 dd, each 1 H, $J_{1'',2''/1''',2'''}$ 7.8 and 7.9 Hz, $J_{2'',3''}=J_{2''',3'''}=9.7$ Hz, H-2″,2‴), 5.040 and 4.777 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.5 and 8.8 Hz, H-1,1′), 4.779 and 4.722 (2 d, each 1 H, H-1″,1‴), 4.487 and 4.364 (2 dd, each 1 H, $J_{2,3/2',3'}$ 11.2 and 11.1 Hz, H-2,2′), 2.309, 2.295, 2.241, 2.208, 2.203, 2.198, 2.144, and 2.121 (8 s, 6,6,3,6,3,3,3,3 H, 6 $COC_6H_4CH_3$, 2 $COCH_2CH_2COCH_3$, and 3 Ac); ^{13}C, δ 172.0 and 171.9 (2 $COCH_2CH_2COCH_3$), 170.4, 169.8, and 169.7 (3 $COCH_3$), 165.2, 164.7, and 163.9 ($COC_6H_4CH_3$), 130.6 ($OCH_2CH=CH_2$), 117.3 ($OCH_2CH=CH_2$), 101.0 (2 C), 98.2, and 96.6 (C-1,1′,1″,1‴), 52.0 (C-2,2′), 37.7, 29.5, and 27.6 ($COCH_2CH_2COCH_3$), 21.3 and 21.1 ($COC_6H_4CH_3$), 20.6 (2 C) and 20.4 (3 $COCH_3$). FABMS (positive-ion mode; $C_{107}H_{106}N_2O_{36}$): *m*/*z* 2017 $[M+Na]^+$, 1995 $[M+H]^+$.

*Allyl (2,3,4-tri-*O*-p-toluoyl-β-*D*-glucopyranosyl)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-(1→6)-[(2,3,4-tri-*O*-p-toluoyl-β-*D*-glucopyranosyl)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**28**).—To a solution of **27** (90 mg, 45 μmol) in EtOH (7 mL) and toluene (3 mL) was added NH_2NH_2·HOAc (39 mg, 0.45 mmol). The mixture was stirred for 2 h, then concentrated. The residue was applied to column chromatography (9:1 CH_2Cl_2–acetone) to yield **28**, isolated as a glass (79 mg, 98%); $[\alpha]_D$ +1.4° (*c* 1); NMR ($CDCl_3$): ^{1}H, δ 7.750, 7.745, 7.508, 7.270, 7.264, 7.128, 6.952, 6.777, and 6.764 (9 d, 2,2,4,2,2,4,4,2,2 H, 6 $COC_6H_4CH_3$), 5.730 and 5.531 (2 d, each 1 H, $J_{3,4/3',4'}$ 3.4 and 3.6 Hz, $J_{4,5/4',5'}$ < 1 Hz, H-4,4′), 5.263 and 5.217 (2 dd, each 1 H, $J_{1'',2''/1''',2'''}$ 7.8 and 7.9 Hz, $J_{2'',3''}=J_{2''',3'''}=10.0$ Hz, H-2″,2‴), 5.027 and 4.775 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.5 and 8.6 Hz, H-1,1′), 4.866 and 4.793 (2 d, each 1 H, H-1″,1‴), 4.515 and 4.361 (2 dd, each 1 H, $J_{2,3/2',3'}$ 11.0 and 11.1 Hz, H-2,2′), 2.329, 2.313, 2.269, and 2.213 (4 s, 6,3,6,3 H, 6 $COC_6H_4CH_3$), 2.207 and 2.107 (2 s, 6,3 H, 3 Ac); ^{13}C, δ 171.5, 171.4, and 170.4 (3 $COCH_3$), 166.6, 166.5, 165.3 (2 C), 164.9, and 163.9 (6 $COC_6H_4CH_3$), 132.5 ($OCH_2CH=CH_2$), 117.6 ($OCH_2CH=CH_2$), 101.8, 101.6, 98.0, and 96.6 (C-1,1′,1″,1‴), 51.9 (C-2,2′), 21.3 and 21.2 ($COC_6H_4CH_3$), 20.9 (2 C) and 20.4 (3 $COCH_3$). FABMS (positive-ion mode; $C_{97}H_{94}N_2O_{32}$): *m*/*z* 1821 $[M+Na]^+$, 1799 $[M+H]^+$.

*Allyl (2,3,4-tri-*O*-p-toluoyl-β-*D*-glucopyranosyluronic acid)-(1→3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranosyl)-(1→6)-[(2,3,4-tri-*O*-p-toluoyl-β-*D*-glucopyranosyluronic acid)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-galactopyranoside* (**29**).—To a solution of **28** (23 mg, 13 μmol) in dry CH_2Cl_2 (3 mL), containing Ac_2O (129 μL, 130 μmol), was added pyridinium dichromate (48 mg, 130 μmol). After stirring for 4.5 h, TLC (8:2:1 CH_2Cl_2–EtOAc–HOAc) showed the conversion of **28** into **29** (R_f 0.13). After the addition of EtOAc (25 mL), the suspension was applied to column chromatography (8:4:1 CH_2Cl_2–EtOAc–HOAc) to afford **29**, isolated as a glass (14 mg, 64%); $[\alpha]_D$ +6° (*c* 0.5); NMR ($CDCl_3$): ^{1}H, δ 7.750, 7.744, 7.508, 7.269, 7.262, 7.129, 6.936, 6.777, and 6.764 (9 d, 2,2,4,2,2,4,4,2,2 H, 6 $COC_6H_4CH_3$), 5.728 and 5.530 (2 d, each 1 H, $J_{3,4/3',4'}$ 2.9 and 3.8 Hz, $J_{4,5/4',5'}$ < 1 Hz, H-4,4′),

5.261 and 5.214 (2 dd, each 1 H, $J_{1'',2''/1''',2'''}$ 7.9 and 7.8 Hz, $J_{2'',3''/2''',3'''}$ 9.9 and 10.0 Hz, H-2″,2‴), 5.025 and 4.791 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.5 and 8.6 Hz, H-1,1′), 4.868 and 4.774 (2 d, each 1 H, H-1″,1‴), 4.513 and 4.358 (2 dd, each 1 H, $J_{2,3/2',3'}$ 11.3 and 11.4 Hz, H-2,2′), 2.333, 2.272, and 2.218 (3 s, each 6 H, 6 $COC_6H_4CH_3$), 2.315, 2.207, and 2.108 (3 s, each 3 H, 3 Ac); ^{13}C, δ 171.5, 171.2, and 170.6 (3 $COCH_3$), 168.4, 166.5, 165.1, 164.8, and 163.8 ($COC_6H_4CH_3$), 132.7 ($OCH_2CH=CH_2$), 117.5 ($OCH_2CH=CH_2$), 101.3 (2 C), 97.9, and 96.9 (C-1,1′,1″,1‴), 52.1 (C-2,2′), 21.4, 21.3, and 20.8 ($COC_6H_4CH_3$), 20.6, 20.5, and 19.2 (3 $COCH_3$).

A small amount of **29** was esterified with diazomethane in ether, and analysed by ^1H NMR ($CDCl_3$): δ 7.746, 7.735, 7.565, 7.553, 7.309, 7.286, 7.130, 6.992, 6.984, 6.883, and 6.858 (11 d, 2,2,2,2,2,2,4,2,2,2,2 H, 6 $COC_6H_4CH_3$), 5.583 and 5.365 (2 d, each 1 H, $J_{3,4/3',4'}$ 3.1 and 3.9 Hz, $J_{4,5/4',5'} < 1$ Hz, H-4,4′), 5.282 and 5.244 (2 dd, each 1 H, $J_{1'',2''/1''',2'''}$ 7.7 and 7.8 Hz, $J_{2'',3''/2''',3'''}$ 9.5 and 9.6 Hz, H-2″,2‴), 5.009 and 4.767 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.5 and 8.4 Hz, H-1,1′), 4.817 and 4.742 (2 d, each 1 H, H-1″,1‴), 4.521 and 4.382 (2 dd, each 1 H, $J_{2,3}=J_{2',3'}=11.2$ Hz, H-2,2′), 4.216 and 4.157 (2 d, each 1 H, $J_{4'',5''/4''',5'''}$ 9.6 and 9.7 Hz, H-5″,5‴), 3.708 and 3.676 (2 s, each 3 H, 2 $COOCH_3$), 2.333, 2.314, and 2.237 (3 s, each 6 H, 6 $COC_6H_4CH_3$), 2.221, 2.132, and 2.123 (3 s, each 3 H, 3 Ac). FABMS (positive-ion mode; $C_{99}H_{94}N_2O_{34}$): m/z 1877 $[M+Na]^+$, 1855 $[M+H]^+$.

Allyl (β-D-glucopyranosyluronic acid)-(1→3)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galactopyranoside (**5**).—A solution of **29** (15 mg, 8.5 μmol) in ethanolic 33% $MeNH_2$ (7.5 mL) was stirred for 7 days at room temperature, during which the mixture was concentrated repeatedly and new reagent (7×7.5 mL) added. After concentration, the residue was dissolved in dry MeOH (5 mL), and Ac_2O (100 μL) was added at 0 °C. The mixture was stirred for 2 h, when TLC (4:2:2:0.5 1-BuOH–EtOH–H_2O–HOAc) showed the formation of a major spot (R_f 0.21). The solution was concentrated and co-concentrated with 1:1 toluene–MeOH (3×10 mL). Gel filtration over Sephadex G-10 (water) of the residue yielded **5**, isolated after lyophilisation as a white, amorphous powder (4.5 mg, 63%); $[\alpha]_D$ −10° (*c* 0.4, H_2O); NMR (D_2O): ^1H, δ 5.914 (m, 1 H, $OCH_2CH=CH_2$), 5.34–5.27 (m, 2 H, $OCH_2CH=CH_2$), 4.528 and 4.511 (2 d, each 1 H, $J_{1,2/1',2'}$ 8.4 and 8.2 Hz, H-1,1′), 4.506 and 4.490 (2 d, each 1 H, $J_{1'',2''/1''',2'''}$ 8.0 and 7.9 Hz, H-1″,1‴), 4.182 and 4.154 (2 d, each 1 H, $J_{3,4}=J_{3',4'}=3.3$ Hz, $J_{4,5/4',5'} < 1$ Hz, H-4,4′), 2.012 and 2.006 (2 s, each 3 H, 2 $NHCOCH_3$). FABMS (positive-ion mode; $C_{31}H_{48}N_2O_{23}$): m/z 839 $[M+Na]^+$.

*General protocol for the preparation of the 3-(2-aminoethylthio)propyl adducts of **1**, **3**, and **5***.—The allyl glycoside and cysteamine hydrochloride (3 eq based on the allyl glycoside) were dissolved in water (50 μL/μmol allyl glycoside) and the solution was irradiated for 8–16 h in quartz with an UV lamp. Excess of cysteamine hydrochloride was removed by HiTrap gel filtration (aq 5% NH_4HCO_3) to afford a mixture of the desired 3-(2-aminoethylthio)propyl adducts and a minor amount of the starting material. Separation was achieved by column chromatography (1:1:0.2 CH_2Cl_2–MeOH–HOAc) to yield the pure 3-(2-aminoethylthio)propyl glycosides. The products **2** {$[\alpha]_D$ −17° (*c* 0.9, H_2O)}, **4** {$[\alpha]_D$ +3° (*c* 0.3, H_2O)}, and **6** {$[\alpha]_D$ −10° (*c* 0.5, H_2O)} were obtained in a

Table 1
^1H Chemical shifts (δ, ppm) and coupling constants (Hz; in brackets) of the 3-(2-aminoethylthio)propyl adducts **2**, **4**, and **6**

Protons	Compound								
	2		**4**			**6**[b]			
	GalNAc	GlcA′	GalNAc	GalNAc′	GlcA″	GalNAc	GalNAc′	GlcA″	GlcA‴
H-1	4.485 (8.5)	4.506 (7.8)	4.454 (8.6)	4.482 (9.3)	4.499 (8.0)	4.454 (8.5)	4.542 (8.4)	4.507 (7.8)	4.498 (7.6)
H-2	3.98 (10.9)	3.336 (8.3)	3.98	3.90	3.331	3.98	4.00	3.188	3.188
H-3	3.833 (2.9)	3.470 (9.1)	3.841 (2.8)	3.75 (2.7)	3.470	3.82 (2.8)	3.85 (2.8)	3.47	3.47
H-4	4.175 (<1)	n.d[a]	4.154	3.942	n.d[a]	4.153 (<1)	4.179 (<1)	n.d.[a]	n.d.[a]
$O(CH_2)_3S(CH_2)_2NH_2$	1.86		1.87			1.88			
	2.63		2.63			2.63			
	3.71		3.70			3.68			
	3.95		4.04			3.96			

[a]n.d., not determined.
[b]GlcA″ is terminal β-D-glucuronic acid residue; GlcA‴ is branching β-D-glucuronic acid residue.

yield of 85, 63, and 78%, respectively. For ^{1}H NMR data of the products, see Table 1.

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