Application of β-1,4-galactosyltransferase in the synthesis of complex branched-chain oligosaccharide mimics of fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 14

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The chemoenzymic synthesis is described of β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc-(1→O[CH_2]$_3$O→4)-β-D-Glc*p*-(1→OCH_2CH=CH_2) 32 and β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-(1→O[CH_2]$_3$O→4)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc-(1→O[CH_2]$_3$O→4)-β-D-Glc*p*-(1→OCH_2-CH=CH_2) 33, representing hexa- and octasaccharide mimics of fragments of the *Streptococcus pneumoniae* type 14 polysaccharide. In a chemical approach the intermediate linear oligosaccharide mimics 30 and 31 were synthesized, wherein both terminal and non-terminal *N*-acetyl-β-D-glucosamine residues were not yet galactosylated. The alkyl-bridged derivatives were found to be good acceptor substrates for bovine milk β-1,4-galactosyltransferase. Reaction of the anomeric allyl functions with cysteamine under UV-irradiation gave the corresponding 3-(2-aminoethylthio)propyl glycosides 34 and 35, suitable for further coupling of the oligosaccharide mimics to protein carriers.

Introduction

Pneumococcal infections are major causes of bacterial pneumonia, otitis media and meningitis and despite the availability of antibiotic therapy is still a significant cause of mortality throughout the world.[1,2] Owing to the rapid course of the disease and the emergence of antibiotic-resistant strains,[3–5] disease prevention by vaccination is highly desirable. The current polyvalent pneumococcal vaccines Pneumovax 23® (Merck, Sharp and Dohme) and Pnu-Immune 23 (Lederle-Praxis), which contain the capsular polysaccharides of 23 of the most common serotypes,[6] offer 90% protection in immunocompetent adults, but are inadequate in the population at greatest risk for serious pneumococcal infections.[7,8] The immune response to T-cell-independent (TI) polysaccharide antigens is poor in infants and young children up to the age of 2 years. The development of more immunogenic conjugate vaccines for serotypes responsible for most pediatric diseases (*e.g.* 6B, 14, 18C, 19F, and 23F) is thus of great importance.[9,10]

The *Streptococcus pneumoniae* type 14 polysaccharide (Pn 14-PS) consists of a branched tetrasaccharide repeating unit[11] which is structurally identical with the *asialo* core antigen of the type III group B *Streptococcus* (GBS III) capsular polysaccharide:[12]

→6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*(1→
4
↑
1
β-D-Gal*p*

Structural similarities between antigenic determinants of the Pn 14 polysaccharide and human oligosaccharide structures, which may give rise to the induction of autoantibodies and suppression of the immune response, may be responsible for the poor immunogenicity of the Pn 14 polysaccharide among the pneumococcal capsular polysaccharides.[13] Evidence for cross-reactivity with human tissue was found by immunization of both rabbits and mice with Pn 14-PS.[14]

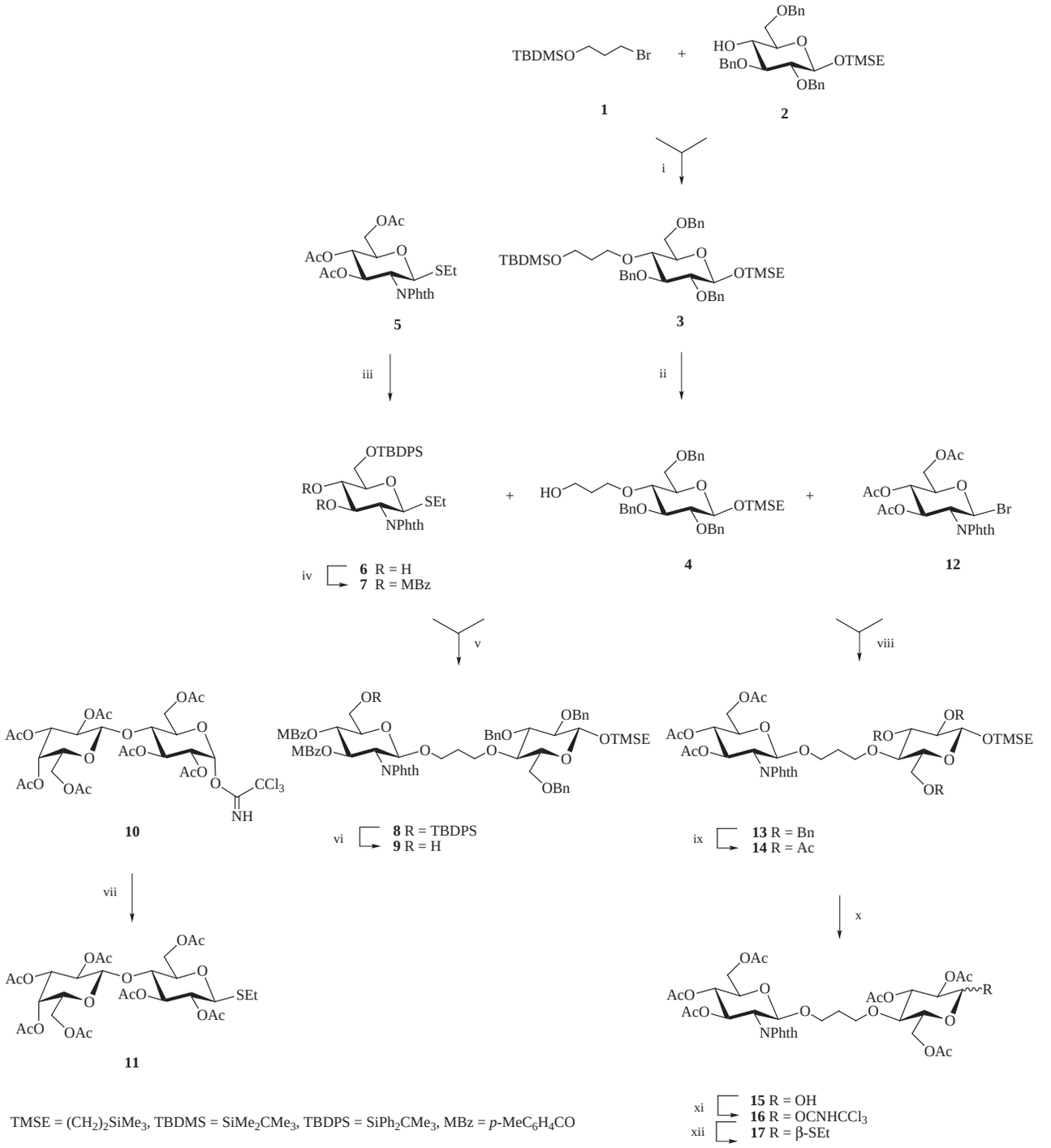
As autoreactive antibodies were preferentially reactive with lactose, we designed mimics of the Pn 14 polysaccharide in which the galactose moiety of the repeating →6)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→ trisaccharide backbone structure is substituted by a flexible acyclic spacer. For an easy access to branched-chain oligosaccharide mimics, also the β-1,4-galactosyltransferase-catalyzed galactosylation of *N*-acetyl-β-D-glucosamine residues of linear oligosaccharide mimics, using uridine-5′-diphosphogalactose (UDP-Gal) as donor, was investigated.

Owing to its availability and flexibility both in donor and acceptor substrate specificity, β-1,4-galactosyltransferase is one of the most extensively studied mammalian glycosyltransferases.[15,16] Two findings stimulated our approach to branched-chain oligosaccharide mimics by enzymic galactosylation. β-1,4-Galactosyltransferase (UDP-Gal: D-glucose β-1,4-galactosyltransferase [EC 2.4.1.22]), which uses D-glucose as the preferred acceptor in the presence of α-lactalbumin, is regarded as transferring *in vivo* galactose to only terminal *N*-acetyl-β-D-glucosamine residues.[17,18] However, 6-*O*-glycosylated *N*-acetyl-β-D-glucosamine derivatives like α-L-Fuc*p*-(1→6)-β-D-Glc*p*NAc,[19] α-Neu*p*5Ac(OMe)-(2→6)-β-D-Glc*p*-NAc,[19] and β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→OAll)[20] were found to be substrates of the enzyme. Furthermore, a large variety of aglycones are readily tolerated by the enzyme, and even increasing galactosylation has been observed for *N*-acetyl-β-D-glucosamine glycosides of hydrophobic aglycones.[21,22] This so called hydrophobic effect was confirmed by studies on recognition- and binding-subsites of the enzyme using diantennary alkyl-bridged oligosaccharides with terminal *N*-acetyl-β-D-glucosamine residues.[23,24]

Here, we report on the chemoenzymic synthesis of alkyl-bridged oligosaccharide mimics of fragments of the *Streptococcus pneumoniae* type 14 polysaccharide containing an aglycone spacer for the subsequent attachment to carrier proteins.

Results and discussion

The convergent synthetic strategy for the preparation of the key linear oligosaccharide mimic derivatives **18** and **19** involves the glycosylation of the common trisaccharide mimic acceptor **9** with either disaccharide **11** or trisaccharide mimic **17** thioethyl glycoside donors. In a series of protecting-group manipulations key compounds **18** and **19** can be converted into the



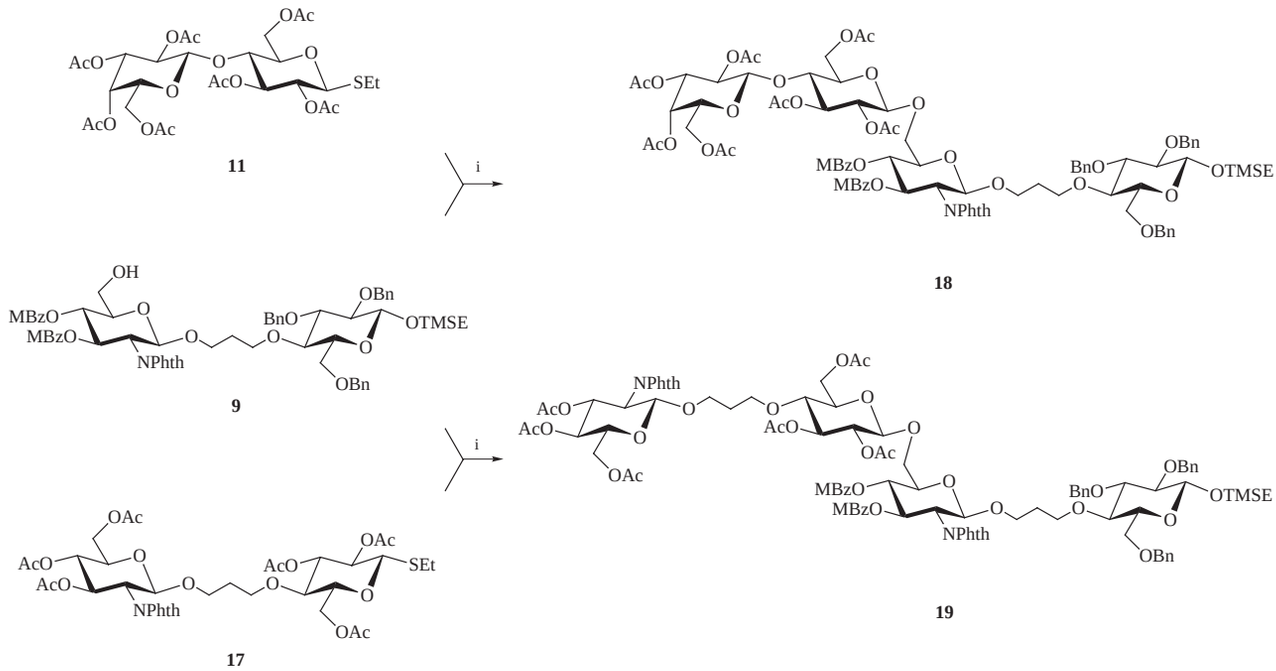
Scheme 1 *Reagents and yields*: i, NaH (96%); ii, *p*-TsOH (97%); iii, (a) NaOMe, MeOH; (b) TBDPSCl (72%); iv, MBzCl (90%); v, NIS, AgOTf (85%); vi, AcCl, MeOH (89%); vii, EtSH, $BF_3{\cdot}Et_2O$ (94%); viii, AgOTf (76%); ix, (a) H_2, Pd–C; (b) Ac_2O, pyridine (84%); x, TFA (96%); xi, CCl_3CN, DBU (89%); xii, EtSH, $BF_3{\cdot}Et_2O$ (87%).

unprotected penta- and hexasaccharide mimics β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→O[CH_2]$_3$O→4)-β-D-Glc*p*-(1→O$CH_2CH{=}CH_2$) **30** and β-D-Glc*p*NAc-(1→O-[CH_2]$_3$O→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→O[CH_2]$_3$O→4)-β-D-Glc*p*-(1→O$CH_2CH{=}CH_2$) **31**, respectively, being the acceptor structures for β-1,4-galactosyltransferase.

Both the synthesis of the trisaccharide mimic acceptor **9** and donor **17** required the 2-(trimethylsilyl)ethyl (TMSE) 4-*O*-(3-hydroxypropyl) glycoside **4** as intermediate (Scheme 1). For anomeric protection the 2-(trimethylsilyl)ethyl group was chosen, as TMSE glycosides are compatible with a wide range of different reaction conditions as well as being easy removable by reaction with TFA in dichloromethane without affecting the glycosidic bonds.[25]

The 4-*O*-alkylated glycoside **3** was prepared in 96% yield by reaction of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[25] **2** with 3-(*tert*-butyldimethylsilyloxy)propyl bromide[26] **1** in a 1 : 1 mixture of DMF and THF. Subsequent removal of the acid-sensitive *tert*-butyldimethylsilyl (TBDMS) group with *p*-TsOH in aq. acetonitrile gave glycosyl acceptor **4** in 97% yield.

For the synthesis of the trisaccharide mimic acceptor **9** a combination of *tert*-butyldiphenylsilyl (TBDPS) and *p*-methylbenzoyl (MBz) protecting groups was chosen, which had already successfully been used in the preparation of the β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*NAc-(1→OAll) trisaccharide fragment of the *S. pneumoniae* type 14 polysaccharide.[20] The suitably protected 6-*O*-silylated β-thioglycoside



Scheme 2 *Reagents and yields*: i, NIS, AgOTf (**18**: 42%; **19**: 66%).

donor **7** was prepared in 65% overall yield by deacetylation of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[27] **5**, followed by selective silylation of the primary HO-6 group with *tert*-butyldiphenylsilyl chloride (TBDPSCl) (⟶ **6**) and subsequent *p*-methylbenzoylation of the HO-3 and HO-4 groups with MBzCl. *N*-Iodosuccinimide (NIS) promoted glycosylation of the primary hydroxy group of the 4-*O*-(3-hydroxypropyl) glycoside **4** with β-thioethyl glycoside **7** in the presence of a catalytic amount of silver trifluoromethanesulfonate (AgOTf)[28,29] afforded the desired trisaccharide mimic **8** in 85% yield. Selective desilylation with acetyl chloride in 1 : 1 methanol–toluene[30] gave the desired trisaccharide mimic acceptor **9** in 89% yield.

As the anomeric TMSE group of glycosyl acceptor **9** is not compatible with Lewis acid-induced activation of glycosyl imidates,[31] the β-thioethyl group was chosen for anomeric activation of both disaccharide donor **11** and trisaccharide mimic donor **17**.

As per-acetylated α-lactose, which is formed by acetylation of lactose with acetic anhydride in pyridine, does not form the corresponding thioglycoside upon reaction with ethanethiol in the presence of Lewis acid,[32] the thiolactoside **11** was prepared in 94% yield by reaction of lactosylimidate **10**[33] with ethanethiol in the presence of boron trifluoride–diethyl ether.

For the preparation of the trisaccharide mimic donor **17** first the 4-*O*-(3-hydroxypropyl) glucoside **4** was coupled with glycosyl bromide **12**[34] in the presence of AgOTf to give compound **13** in 76% yield. Hydrogenolytic cleavage of the benzyl groups of compound **13** with 10% Pd–C as catalyst in acetic acid and subsequent *O*-acetylation with acetic anhydride in pyridine gave hexaacetate **14** in 84% overall yield. Then, TMSE glycoside **14** was converted into β-thioglycoside **17** by selective removal of the anomeric 2-(trimethylsilyl)ethyl group with TFA in dichloromethane (⟶ **15**, 96%), followed by imidation with trichloroacetonitrile in the presence of DBU (⟶ **16**, 89%) and subsequent glycosylation with ethanethiol in the presence of $BF_3{\cdot}Et_2O$ (⟶ **17**, 87%).

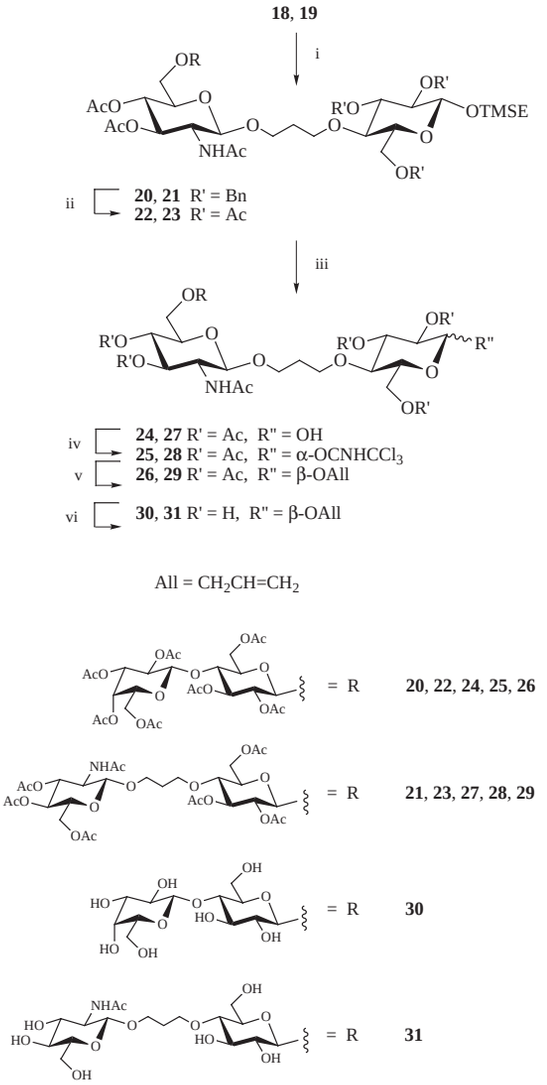
NIS/AgOTf-promoted reactions of glycosyl acceptor **9** with thioglycosides **11** and **17** gave the key 6′-*O*-glycosylated compounds **18** (42%) and **19** (66%), respectively (Scheme 2).

De-*N*-phthaloylation of intermediates **18** and **19** was achieved in high yield by applying a reaction sequence established earlier as a consequence of MBz group migration during de-*N*-phthaloylation.[20] Thus, compounds **18** and **19** were first de-*O*-*p*-methylbenzoylated and de-*O*-acetylated with sodium methoxide in methanol, and subsequently de-*N*-phthaloylated with ethylenediamine in butan-l-ol[35] for 24 h at 80 °C. Re-*N*,*O*-acetylation gave compounds **20** (95%) and **21** (97%), respectively (Scheme 3). Removal of the benzyl groups by hydrogenolysis with 10% Pd–C as catalyst in acetic acid, followed by *O*-acetylation, gave the per-*O*-acetylated 2-(trimethylsilyl)ethyl glycosides **22** and **23** in 72% and 84% yield, respectively.

In subsequent reactions compounds **22** and **23** were converted into allyl glycosides **26** and **29**, respectively, by treatment with TFA in dichloromethane [⟶ hemiacetal sugars **24** (90%) and **27** (84%)], followed by imidation with trichloroacetonitrile in the presence of DBU [⟶ **25** (85%) and **28** (82%)], and subsequent allylation with allyl alcohol and $BF_3{\cdot}Et_2O$ [⟶ **26** (61%) and **29** (59%)]. Deacetylation with sodium methoxide in methanol furnished the linear glycosyl mimics **30** (86%) and **31** (92%), respectively.

The bovine β-1,4-galactosyltransferase-catalyzed syntheses of the branched-chain oligosaccharide mimics **32** and **33** was achieved by transfer of galactosyl groups from UDP-galactose to the *N*-acetyl-β-D-glucosamine residues of intermediates **30** and **31**, respectively (Scheme 4). Initial reaction rates were determined under standard conditions with a coupled enzyme assay for UDP.[36] With *N*-acetyl-D-glucosamine taken as a reference (with an assigned relative rate of 100) the pentasaccharide mimic **30**, containing a non-terminal 6-*O*-substituted *N*-acetyl-β-D-glucosamine residue, showed good acceptor activity (35%). The hexasaccharide mimic **31**, containing both terminal and non-terminal *N*-acetyl-β-D-glucosamine residues, was found to be a weaker acceptor (15%).

Oligosaccharide mimics **32** and **33** were then prepared on a preparative scale. Alkaline phosphatase was added to the incubation mixtures to prevent feedback inhibition by released UDP[37,38] and to promote a high conversion of the acceptor [⟶ **32** (85%) and **33** (80%)]. Fast-atom bombardment (FAB) mass spectrometry confirmed the introduction of one galactosyl residue in compound **32** and the presence of two galactosyl residues in compound **33**. The ^{13}C NMR spectra of compounds **32** and **33** showed the expected



Scheme 3 *Reagents and yields*: i, (a) NaOMe, MeOH; (b) H_2NCH_2-CH_2NH_2, butan-l-ol, 80 °C; (c) Ac_2O, pyridine (**20**: 95%; **21**: 97%); ii, (a) H_2, Pd–C; (b) Ac_2O, pyridine (**22**: 72%; **23**: 84%); iii, TFA (**24**: 90%; **27**: 84%); iv, CCl_3CN, DBU (**25**: 85%; **28**: 82%); v, AllOH, $BF_3{\cdot}Et_2O$ (**26**: 61%; **29**: 59%); vi, NaOMe, MeOH (**30**: 86%; **31**: 92%).

additional anomeric signals, corresponding to mono- (**32**) and di-galactosylation (**33**), respectively. Furthermore, 2D COSY,† TOCSY† and ROESY† spectra confirmed the structures of compounds **32** (Table 1) and **33** (Table 2), with inter-residual nuclear Overhauser effects (NOEs) between 1-H of the transferred galactose and 4-H of terminal and non-terminal *N*-acetylglucosamine residues, respectively.

The allyl glycosides **32** and **33** were then converted by reaction with cysteamine[39] under UV-irradiation into the 3-(2-aminoethylthio)propyl glycosides **34** and **35**, respectively, suitable for conjugation to carrier proteins.

Conjugation of the type 14 oligosaccharide mimics to CRM_{197} (cross-reactive material) and immunological studies are in progress.

Experimental

General procedures

Reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck) with detection either by UV light or charring with either 10% H_2SO_4 in EtOH, 0.2% orcinol in 20% methanolic H_2SO_4, or 1% $KMnO_4$ in 0.2 M aq. Na_2CO_3. Solutions were concentrated under reduced pressure at <40 °C. Column chromatography was performed on Silica Gel 60 (0.063–0.200 mm, Merck). Gel-permeation chromatography was performed on Toyopearl® HW-40S (Supelco) (2.0 × 60 cm). UV-irradiations were performed in quartz vials at 254 nm using a Cole-Parmer® 50 W high-intensity UV lamp. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. $[a]_D$-Values are given in 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectra (300 MHz) were recorded with a Bruker AC 300 spectrometer; only selected NMR data are reported. Two-dimensional double-quantum filtered ^{1}H–^{1}H correlated spectra (2D DQF ^{1}H–^{1}H COSY), two-dimensional TOCSY spectra with 100 ms and 150 ms mixing sequences, and 2D ^{1}H ROESY spectra (300 ms mixing sequence) were recorded at 300 K using a Bruker AMX 500 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si (δ 0, $CDCl_3$) or acetone (δ 2.225, D_2O). *J*-Values are given in Hz. ^{13}C NMR spectra (75.5 MHz) were recorded with a Bruker AC 300 spectrometer; δ_C(ppm)-values are given relative to the signal for $CDCl_3$ (δ_C 76.9) or internal acetone (δ_C 31.08). ^{13}C signals of $SiMe_3$ groups (<0 ppm) are not listed. Owing to overlap not all ^{13}C signals are present in appropriate numbers. Fast-atom bombardment mass spectrometry (FABMS) was performed on a JEOL JMS SX/SX 102A four-sector mass spectrometer, equipped with a JEOL MS-FAB 10 D FAB gun. Elemental analyses were carried out by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany). All compounds for which elemental analytical data are not available were chromatographically homogeneous and NMR and mass spectral data were in full agreement with the assigned structures.

Table 1 ^{1}H NMR data (COSY, TOCSY, ROESY) of compound **32**

Proton (δ_H)	Glc[a]	GlcNAc	Glc[b]	Gal[a]	Gal[b]
H-1[c]	4.47	4.54	4.56	4.45	4.54
H-2	3.29	3.73	3.39	3.55	3.54
H-3	3.43	3.69	3.68	3.67	3.66
H-4	3.73	3.83	3.63	3.92	3.92
H-5	3.56	3.71	3.60	3.74	3.72
H^a-6	3.91	4.28	3.98	*d*	*d*
H^b-6	3.89	3.96	3.82	*d*	*d*
$OCH_2CH{=}CH_2$	4.37, 4.21 (2 m, each 1 H)				
$OCH_2CH{=}CH_2$	5.98 (m, 1 H)				
$OCH_2CH{=}CH_2$	5.40–5.27 (m, 2 H)				
$NHCOCH_3$	2.05 (s, 3 H)				
$OCH_2CH_2CH_2O$	1.87–1.82 (m, 2 H)				

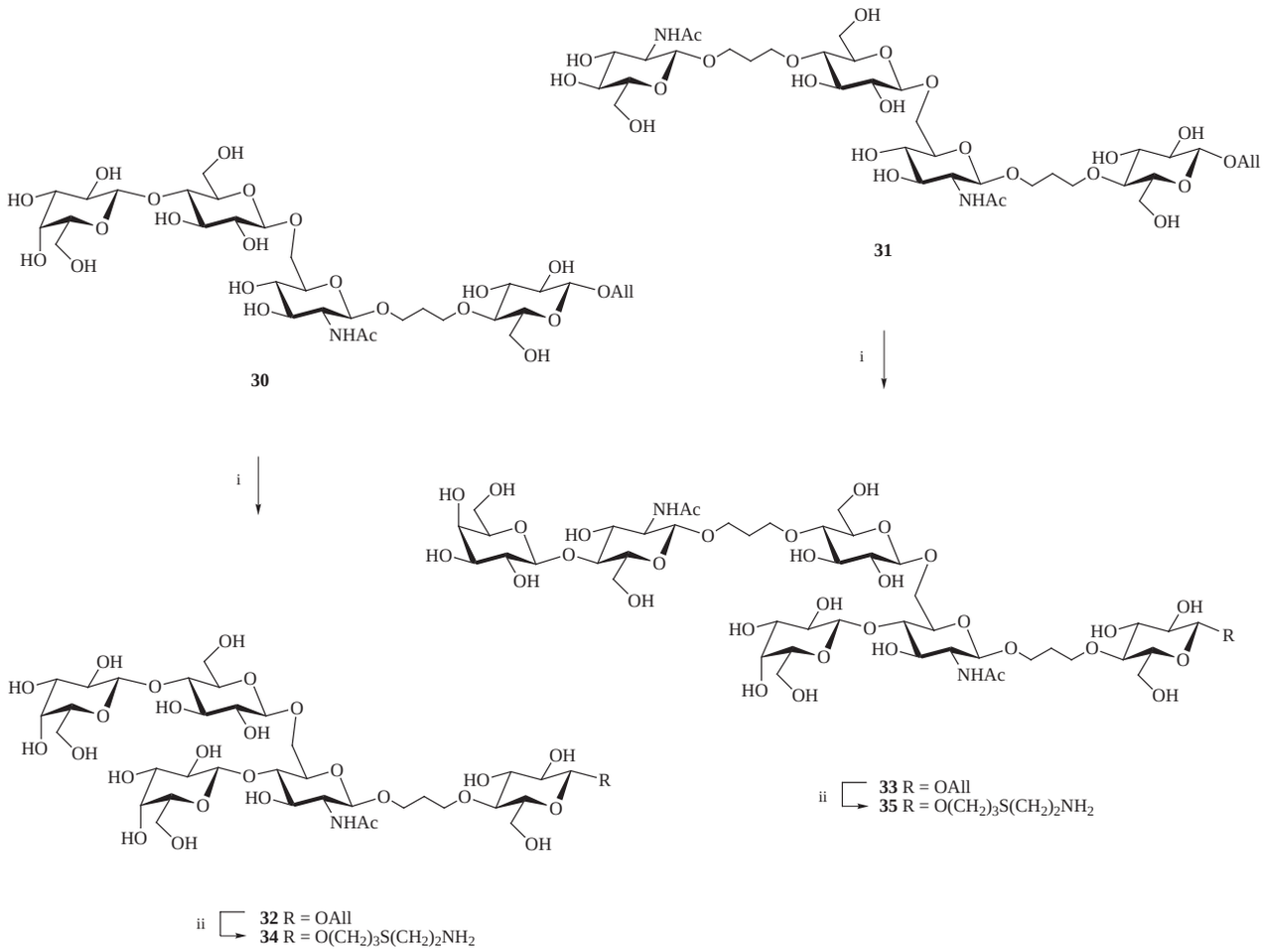
[a,b] Glc[a]: Glcβ(1-OAll). Glc[b]: Glc(β1→6)GlcNAc. Gal[a]: Gal(β1→4)-Glc. Gal[b]: Gal(β1→)GlcNAc. [c] $J_{1,2}$ Coupling constants were >7 Hz, indicating β-configuration for all monosaccharide residues. [d] Not determined.

Table 2 ^{1}H NMR data (COSY, TOCSY, ROESY) of compound **33**

Proton (δ_H in ppm)	Glc[a]	GlcNAc[a]	Glc[b]	GlcNAc[b]	Gal[a]	Gal[b]
H-1[c]	4.47	4.53	4.49	4.53	4.47	4.53
H-2	3.29	3.73	3.31	3.73	3.54	3.54
H-3	3.43	3.69	3.46	*d*	3.68	*d*
H-4	*d*	3.83	3.68	3.74	3.93	3.93
H-5	3.57	3.72	3.58	*d*	3.74	*d*
H^a-6	*d*	4.28	3.98	*d*	*d*	*d*
H^b-6	*d*	3.94	3.82	*d*	*d*	*d*
$OCH_2CH{=}CH_2$	4.39, 4.22 (2 m, each 1 H)					
$OCH_2CH{=}CH_2$	5.98 (m, 1 H)					
$OCH_2CH{=}CH_2$	5.40–5.27 (m, 2 H)					
$NHCOCH_3$	2.05 (s, 6 H)					
$OCH_2CH_2CH_2O$	1.90–1.80 (m, 4 H)					

[a,b] Glc[a]: Glcβ(1-OAll). Glc[b]: Glc(β1→6)GlcNAc[a]. Gal[a]: Gal(β1→4)-GlcNAc[b]. Gal[b]: Gal(β1→)GlcNAc[a]. [c,d] As in Table 1.

† 2D COSY: 2-dimensional chemical-shift–correlation spectroscopy; TOCSY: phase-sensitive 2-dimensional total correlation spectroscopy; ROESY: rotating-frame nuclear Overhauser enhancement spectroscopy.



Scheme 4 *Reagents and yields*: i, UDP-Gal, β-1,4-galactosyltransferase (**32**: 85%; **33**: 80%); ii, cysteamine hydrochloride, *hv* (**34**: 78%; **35**: 85%).

Materials

Bovine milk β-1,4-galactosyltransferase (EC 2.4.1.22),‡ UDP-galactose, β-nicotinamide adenine dinucleotide (reduced form; β-NADH), phospho(enol)pyruvate, pyruvate kinase (EC 2.7.1.40, type III from rabbit muscle),§ L-lactic dehydrogenase (EC 1.1.1.27, type XI from rabbit muscle),¶ and alkaline phosphatase (EC 3.1.3.1, type I from bovine intestine)‖ were obtained from Sigma.

Measurement of galactosyltransferase activity

Initial reaction rates were determined under standard conditions at 20 °C in 500 μl sodium cacodylate buffer (100 mM, pH 7.5) containing 10 mM $MnCl_2$, 50 mM KCl, 0.2 mM UDP-galactose, 1 mM phospho(enol)pyruvate, 0.3 mM β-NADH, 25 U pyruvate kinase, 25 U L-lactic dehydrogenase, 10 mM acceptor, and 20 U β-1,4-galactosyltransferase. Formation of UDP was followed by monitoring the decrease in absorbance at 340 nm.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[3-(*tert*-butyldimethylsilyloxy)propyl]-β-D-glucopyranoside 3

To a suspension of sodium hydride (60% dispersion in oil; 0.4 g) in dry DMF (10 ml) was added at 0 °C under argon a solution of 3-(*tert*-butyldimethylsilyloxy)propyl bromide[26] **1** (1.00 g, 3.9 mmol) and 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside[25] **2** (1.28 g, 2.3 mmol) in dry THF (10 ml). The solution was stirred for 3 h at 0 °C. MeOH (3 ml) was added and the solution was poured onto ice–water, then extracted with CH_2Cl_2, and the organic layer was washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (toluene–EtOAc, 40 : 1) of the residue gave title compound **3** (1.60 g, 96%); TLC (toluene–EtOAc, 10 : 1) R_f 0.35 (**2**), 0.57 (**3**); $[\alpha]_D$ +4 (*c* 1, in $CHCl_3$) (Found: C, 68.2; H, 8.6. $C_{41}H_{62}O_7Si_2$ requires C, 68.1; H, 8.6%); δ_H(300 MHz; $CDCl_3$) 7.34–7.23 (15 H, m, 3 × Ph), 4.94, 4.87, 4.75 and 4.71 (4 H, 2 AB systems, $J_{A,B}$ 11.0, 2 × PhCH_2O), 4.64 and 4.56 (2 H, AB system, $J_{A,B}$ 12.2, PhCH_2O), 4.38 (1 H, d, $J_{1,2}$ 7.7, 1-H), 1.70–1.69 (2 H, m, $OCH_2CH_2CH_2O$), 1.08–1.02 (2 H, m, CH_2Si), 0.86 [9 H, s, $C(CH_3)_3$], 0.03 [9 H, s, $Si(CH_3)_3$] and 0.01 [6 H, s, $Si(CH_3)_2$]; δ_C (75.5 MHz; $CDCl_3$) 138.6–138.2 and 128.2–127.4 (Ar-C), 103.0 (C-1), 84.5, 82.2, 78.3 and 75.0 (C-2, -3, -4, -5), 75.4, 74.7, 73.4, 69.8, 69.2, 67.3 and 60.0 (C-6, 3 × PhCH_2O, O$CH_2CH_2CH_2$O and OCH_2CH_2Si), 33.6 ($OCH_2CH_2CH_2O$), 25.8 [C(CH_3)$_3$], 18.4 [$C(CH_3)_3$ and CH_2Si]; FABMS of $C_{41}H_{62}O_7Si_2$ (M, 722.4) *m*/*z* 721.5 $(M - H)^-$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-hydroxypropyl)-β-D-glucopyranoside 4

To a solution of compound **3** (0.90 g, 1.2 mmol) in CH_3CN (80 ml) were added water (9 ml) and *p*-TsOH monohydrate (50 mg). The solution was stirred for 1.5 h at rt, neutralized with triethylamine, and concentrated. To the residue were added CH_2Cl_2 and water, and the organic layer was separated, washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (toluene–EtOAc, 4 : 1) of the residue gave title compound **4** (0.71 mg, 97%) isolated as a syrup; TLC (toluene–EtOAc, 1 : 1) R_f 0.92 (**3**), 0.69 (**4**); $[\alpha]_D$ +9 (*c* 1, $CHCl_3$) (Found:

‡ (1 unit will transfer 1.0 μmol of galactose from UDP-galactose to glucose per min at pH 8.4 at 30 °C in the presence of 0.2 mg α-lactalbumin).
§ (1 unit will convert 1.0 μmol of phospho(enol)pyruvate to pyruvate per min at pH 7.6 at 37 °C).
¶ (1 unit will reduce 1.0 μmol of pyruvate to L-lactate per min at pH 7.5 at 37 °C).
‖ (1 unit will hydrolyse 1.0 μmol of *p*-nitrophenyl phosphate per min at pH 10.4 at 37 °C).

C, 69.2; H, 7.9. $C_{35}H_{48}O_7Si$ requires C, 69.05; H, 7.95%); δ_H(300 MHz; $CDCl_3$) 7.36–7.17 (15 H, m, 3 × Ph), 4.96 and 4.92 (2 H, AB system, $J_{A,B}$ 10.9, PhCH_2O), 4.75 and 4.71 (2 H, AB system, $J_{A,B}$ 11.0, PhCH_2O), 4.64 and 4.58 (2 H, AB system, $J_{A,B}$ 12.1, PhCH_2O), 4.39 (1 H, d, $J_{1,2}$ 7.7, 1-H), 2.37 (s, OH), 1.70–1.69 (2 H, m, OCH$_2$CH_2CH$_2$O), 1.08–1.02 (2 H, m, CH_2Si), 0.03 [9 H, s, Si(CH_3)$_3$]; δ_C(75.5 MHz; $CDCl_3$) 138.5–138.0 and 128.2–127.4 (Ar-C), 103.0 (C-1), 84.4, 82.2, 78.4 and 74.8 (C-2, -3, -4, -5), 75.3, 74.6, 73.4, 71.4, 68.9, 67.3 and 61.0 (C-6, 3 × PhCH_2O, OCH_2CH_2CH_2O and OCH_2CH_2Si), 32.5 (OCH$_2$CH_2CH$_2$O) and 18.4 (CH_2Si); FABMS of $C_{35}H_{48}O_7Si$ (M, 608.3) *m*/*z* 631.4 $(M + Na)^+$; 607.4 $(M - H)^-$.

Ethyl 6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3,4-di-*O*-(*p*-methylbenzoyl)-2-phthalimido-1-thio-β-D-glucopyranoside 7

To a solution of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[27] **5** (1.00 g, 2.1 mmol) in MeOH (20 ml) was added 0.2 M NaOMe in MeOH (0.6 ml). After being stirred at rt for 2 h, the solution was neutralized with Dowex 50X8 (H^+-form), filtered, and concentrated. The residue was dissolved in dry pyridine (10 ml) and after addition of DMAP (30 mg), triethylamine (160 µl) and *tert*-butylchlorodiphenylsilane (TBDPSCl) (0.8 ml, 3.1 mmol) was stirred overnight at rt. The solution was poured onto ice–water, extracted with CH_2Cl_2, and the organic layer was washed with saturated aq. $NaHCO_3$, dried ($MgSO_4$), and concentrated. Column chromatography (toluene–EtOAc, 2:1) of the residue gave compound **6** (0.89 g, 72%); TLC (toluene–EtOAc, 1:1) R_f 0.58 (**6**).

To a solution of compound **6** (0.89 g, 1.5 mmol) in dry pyridine (5 ml) was added dropwise at 0 °C a solution of MBzCl (0.5 ml, 3.8 mmol) in dry CH_2Cl_2 (5 ml). The solution was stirred overnight at rt, diluted with CH_2Cl_2, poured onto ice–water, extracted with CH_2Cl_2, and the organic layer was washed with saturated aq. $NaHCO_3$, dried ($MgSO_4$), and concentrated. Column chromatography (toluene–EtOAc, 50:1) gave amorphous title compound **7** (1.12 g, 90%); TLC (toluene–EtOAc, 10:1) R_f 0.71 (**7**); $[\alpha]_D$ +18 (*c* 1, $CHCl_3$) (Found: C, 69.7; H, 5.6. $C_{48}H_{49}NO_8SSi$ requires C, 69.6; H, 5.95%); δ_H(300 MHz; $CDCl_3$) 7.87–7.04 (22 H, m, Phth, 2 × COC$_6H_4$CH$_3$ and 2 × Ph), 6.23 (1 H, dd, $J_{2,3}$ 10.4, $J_{3,4}$ 9.4, 3-H), 5.64 (1 H, t, $J_{4,5}$ 9.6, 4-H), 5.63 (1 H, d, $J_{1,2}$ 10.5, 1-H), 4.62 (1 H, t, 2-H), 3.97 (1 H, dt, $J_{5,6}$ 3.5, 5-H), 3.87–3.86 (2 H, m, 6-H_2), 2.80–2.59 (2 H, m, SCH_2CH$_3$), 2.34 and 2.27 (each 3 H, 2 s, 2 × COC$_6$H$_4$CH_3), 1.26 (3 H, t, *J* 7.4, SCH$_2$CH_3) and 1.04 [9 H, s, C(CH_3)$_3$]; δ_C(75.5 MHz; $CDCl_3$) 165.7 and 164.9 (2 *C*OC$_6$H$_4$CH$_3$), 143.7 and 135.5–123.5 (Ar-C), 80.6, 79.2, 72.1 and 69.3 (C-1, -3, -4, -5), 62.9 (C-6), 54.0 (C-2), 26.5 [C(*C*H$_3$)$_3$], 23.7 (S*C*H$_2$CH$_3$), 21.5 and 21.4 (2 × COC$_6$H$_4$*C*H$_3$), 19.0 [*C*(CH$_3$)$_3$] and 14.9 (SCH$_2$*C*H$_3$); FABMS of $C_{48}H_{49}NO_8SSi$ (M, 827.3) *m*/*z* 850.5 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-{3-[6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-3,4-di-*O*-(*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyloxy]propyl}-β-D-glucopyranoside 8

Compounds **4** (0.56 g, 0.9 mmol) and **7** (1.04 g, 1.3 mmol) were dissolved in dry toluene (15 ml) and stirred under argon with powdered molecular sieves 4 Å (5 g) for 1 h at rt. At −40 °C were added NIS (340 mg, 1.5 mmol) and silver trifluoromethanesulfonate (30 mg, 0.12 mmol) and the suspension was stirred at 0 °C for 1 h. Pyridine (1 ml) was added, the suspension was diluted with CH_2Cl_2, filtered over Celite, washed successively with 10% aq. $Na_2S_2O_3$ and saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated. Column chromatography (toluene–EtOAc, 25:1 to 10:1) of the residue gave amorphous title compound **8** (1.07 g, 85%); TLC (toluene–EtOAc, 5:1) R_f 0.14 (**4**), 0.77 (**7**), 0.66 (**8**); $[\alpha]_D$ +2 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.76–7.04 (37 H, m, Phth, 2 × COC$_6H_4$CH$_3$ and 5 × Ph), 6.16 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.3, 3′-H), 5.58 (1 H, t, $J_{4',5'}$ 9.5, 4′-H), 5.47 (1 H, d, $J_{1',2'}$ 8.4, 1′-H), 4.91 and 4.68 (2 H, AB system, $J_{A,B}$ 11.1, PhCH_2O), 4.78 and 4.63 (2 H, AB system, $J_{A,B}$ 11.0, PhCH_2O), 4.56 and 4.53 (2 H, AB system, $J_{A,B}$ 12.2, PhCH_2O), 4.50 (1 H, dd, 2′-H), 4.29 (1 H, d, $J_{1,2}$ 7.6, 1-H), 2.37 and 2.28 (each 3 H, 2 s, 2 × COC$_6$H$_4$CH_3), 1.71–1.64 (2 H, m, OCH$_2$CH_2CH$_2$O), [1.06 (9 H, s, C(CH_3)$_3$], 1.06–1.02 (2 H, m, CH_2Si) and 0.03 [9 H, s, Si(CH_3)$_3$]; δ_C(75.5 MHz; $CDCl_3$) 165.7 and 164.9 (2 × *C*OC$_6$H$_4$CH$_3$), 143.7 and 138.5–125.9 (Ar-C), 102.9 (C-1), 97.9 (C-1′), 84.2, 82.0, 78.1, 75.0, 74.7, 71.2 and 69.6 (C-2, -3, -4, -5, -3′, -4′, -5′), 75.3, 74.5, 73.1, 69.2, 68.8, 67.2, 66.5 and 62.8 (C-6, -6′, 3 × PhCH_2O, OCH_2CH_2CH_2O and OCH_2CH$_2$Si), 55.0 (C-2′), 30.2 (OCH$_2$CH_2CH$_2$O), 26.5 [C(*C*H$_3$)$_3$], 21.5 and 21.4 (2 × COC$_6$H$_4$*C*H$_3$), 19.0 [*C*(CH$_3$)$_3$] and 18.4 (CH_2Si); FABMS of $C_{81}H_{91}NO_{15}Si_2$ (M, 1373.6) *m*/*z* 1396.7 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-{3-[2-deoxy-3,4-di-*O*-(*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyloxy]-propyl}-β-D-glucopyranoside 9

To a solution of acetyl chloride (3.3 ml) in dry MeOH (50 ml) was added at rt a solution of compound **8** (1.07 g, 0.8 mmol) in dry toluene (50 ml). The solution was stirred overnight at rt, then neutralized with triethylamine, and concentrated. The residue was dissolved in EtOAc and the solution was washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (toluene–EtOAc, 5:1 to 3:1) of the residue gave amorphous title compound **9** (0.81 g, 89%); TLC (toluene–EtOAc, 5:1) R_f 0.70 (**8**), 0.27 (**9**); $[\alpha]_D$ −7 (*c* 1, $CHCl_3$) (Found: C, 68.8; H, 6.5. $C_{65}H_{73}NO_{15}Si$ requires C, 68.7; H, 6.5%); δ_H(300 MHz; $CDCl_3$) 7.83–7.03 (27 H, m, Phth, 2 × COC$_6H_4$CH$_3$ and 3 × Ph), 6.24 (1 H, dd, $J_{2',3'}$ 10.8, $J_{3',4'}$ 9.2, 3′-H), 5.48 (1 H, d, $J_{1',2'}$ 8.4, 1′-H), 5.44 (1 H, t, $J_{4',5'}$ 9.5, 4′-H), 4.92 and 4.68 (2 H, AB system, $J_{A,B}$ 11.1, PhCH_2O), 4.81 and 4.63 (2 H, AB system, $J_{A,B}$ 11.0, PhCH_2O), 4.62 and 4.58 (2 H, system, $J_{A,B}$ 12.2, PhCH_2O), 4.48 (1 H, dd, 2′-H), 4.33 (1 H, d, $J_{1,2}$ 7.7, 1-H), 2.34 and 2.27 (each 3 H, 2 s, 2 × COC$_6$H$_4$CH_3), 1.67–1.59 (2 H, m, OCH$_2$CH_2CH$_2$O), 1.06–1.00 (2 H, m, CH_2Si), 0.03 [9 H, s, Si(CH_3)$_3$]; δ_C(75.5 MHz; $CDCl_3$) 165.8 and 165.6 (2 × *C*OC$_6$H$_4$CH$_3$), 144.2–123.4 (Ar-C), 102.9 (C-1), 98.1 (C-1′), 84.3, 82.1, 78.1, 75.1, 74.4, 70.7 and 69.8 (C-2, -3, -4, -5, -3′, -4′, -5′), 75.3, 74.6, 73.4, 68.9, 68.9, 67.2, 66.6 and 61.2 (C-6, -6′, 3 × PhCH_2O, OCH_2CH_2CH_2O and OCH_2CH$_2$Si), 54.8 (C-2′), 30.1 (OCH$_2$CH_2CH$_2$O), 21.5 and 21.4 (2 × COC$_6$H$_4$CH_3) and 18.4 (CH_2Si); FABMS of $C_{65}H_{73}NO_{15}Si$ (M, 1135.5) *m*/*z* 1158.6 $(M + Na)^+$.

Ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside 11

To a solution of (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate[33] **10** (195 mg, 0.25 mmol) in dry CH_2Cl_2 (2 ml) was added ethanethiol (50 µl, 0.67 mmol) and powdered molecular sieves 4 Å (600 mg) and the suspension was stirred under argon for 1 h at rt. Then BF_3·Et_2O (250 µl, 2.0 mmol) was added and the mixture was stirred for 2.5 h at rt. The suspension was neutralized with triethylamine, diluted with CH_2Cl_2, filtered over Celite, and concentrated. Column chromatography (toluene–EtOAc, 2:1) of the residue gave amorphous title compound **11** (159 mg, 94%); TLC (toluene–EtOAc, 1:1) R_f 0.45 (**10**), 0.47 (**11**); $[\alpha]_D$ −4 (*c* 1, $CHCl_3$) (Found: C, 49.45; H, 6.0. $C_{28}H_{40}O_{17}S$ requires C, 49.40; H, 5.9%); δ_H(300 MHz; $CDCl_3$) 5.35 (1 H, dd, $J_{3',4'}$ 3.3, $J_{4',5'}$ 0.9, 4′-H), 5.21 (1 H, t, $J_{1,2}$ 9.5, $J_{2,3}$ 9.2, 2-H), 5.11 (1 H, dd, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5, 2′-H), 4.95 (1 H, dd, 3′-H), 4.94 (1 H, t, $J_{3,4}$ 9.8, 3-H), 3.87 (1 H, dt, $J_{5',6'}$ 7.0, 5′-H), 3.78 (1 H, t, $J_{4,5}$ 9.7, 4-H), 3.62 (1 H, ddd, $J_{5,6a}$ 2.3, 5-H), 2.70–2.65 (2 H, m, SCH_2CH$_3$), 2.15–1.96 (21 H, m, 7 × COCH$_3$) and 1.26 (3 H, t, *J* 7.4, SCH$_2$CH_3); δ_C(75.5 MHz; $CDCl_3$) 100.9 (C-1′), 83.3 (C-1), 76.6, 76.1, 73.7, 70.9, 70.6, 70.2, 69.0 and 66.5 (C-2, -3, -4, -5, -2′, -3′, -4′, -5′), 62.2 and 60.7 (C-6, -6′), 24.3 (S*C*H$_2$CH$_3$), 20.7–20.3 (CO*C*H$_3$) and 14.8

(SCH_2CH_3); FABMS of $C_{28}H_{40}O_{17}S$ (M, 680.2) *m/z* 703.4 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[3-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 13

To a solution of silver trifluoromethanesulfonate (52 mg, 0.20 mmol) and tetramethylurea (45 mg, 0.39 mmol) in dry CH_2Cl_2 (2 ml) was added compound **4** (62 mg, 0.1 mmol), and the solution was stirred under argon in the presence of powdered molecular sieves 4 Å (600 mg) for 1 h at rt. Then a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-bromide[34] **12** (74 mg, 0.15 mmol) in CH_2Cl_2 (1 ml) was added dropwise at −30 °C, and the mixture was stirred overnight at rt, diluted with CH_2Cl_2, filtered over Celite, and concentrated. Purification twice by column chromatography (toluene–EtOAc, 8:1, then heptane–EtOAc, 2:1) gave title disaccharide **13** (78 mg, 76%); TLC (toluene–EtOAc, 1:1) R_f 0.53 (**4**), 0.52 (**12**), 0.59 (**13**); TLC (hexane–EtOAc, 1:1) R_f 0.51 (**4**), 0.40 (**12**), 0.56 (**13**); $[\alpha]_D$ +7 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.79 and 7.67 (each 2 H, 2 m, Phth), 7.35–7.18 (15 H, m, 3 Ph), 5.77 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.0, 3′-H), 5.32 (1 H, d, $J_{1',2'}$ 8.4, 1′-H), 5.15 (1 H, dd, $J_{4',5'}$ 10.0, 4′-H), 4.91 and 4.67 (2 H, AB system, $J_{A,B}$ 11.0, PhCH_2O), 4.78 and 4.60 (2 H, AB system, $J_{A,B}$ 11.0, PhCH_2O), 4.57 and 4.53 (2 H, AB system, $J_{A,B}$ 12.2, PhCH_2O), 4.31 (1 H, d, $J_{1,2}$ 7.7, 1-H), 4.30 (1 H, dd, 2′-H), 2.08, 2.03 and 1.86 (each 3 H, 3 s, 3 × COCH_3), 1.61–1.57 (2 H, m, OCH$_2$CH_2CH$_2$O), 1.06–1.00 (2 H, m, CH_2Si) and 0.03 [9 H, s, Si$(CH_3)_3$]; δ_C(75.5 MHz; $CDCl_3$) 170.6, 170.0 and 169.3 (3 × *C*OCH$_3$), 138.5–123.5 (Ar-C), 102.9 (C-1), 97.9 (C-1′), 84.3, 82.1, 78.6, 74.8, 71.7, 70.7 and 68.9 (C-2, -3, -4, -5, -3′, -4′, -5′), 75.2, 74.5, 73.2, 69.0, 68.4, 67.3, 66.9 and 61.9 (C-6, -6′, 3 × PhCH_2O, OCH_2-CH$_2$CH_2O and OCH_2CH$_2$Si), 54.5 (C-2′), 30.0 (OCH$_2$CH_2-CH$_2$O), 20.6, 20.5 and 20.3 (3 × CO*C*H$_3$) and 18.4 (CH_2Si); FABMS of $C_{55}H_{67}NO_{16}Si$ (M, 1025.4) *m/z* 1048.6 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[3-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 14

A solution of compound **13** (37 mg, 0.036 mmol) in acetic acid (1 ml) was stirred in the presence of 10% Pd–C (20 mg) under H_2 overnight at rt. TLC (EtOAc) R_f 0.69 (debenzylated compound).

The solution was diluted with EtOAc, filtered over Celite, and co-concentrated with toluene. The residue was dissolved in dry pyridine (2 ml), acetic anhydride (1 ml) was added, and the mixture was stirred overnight at rt . The solution was co-concentrated with toluene, and column chromatography (toluene–EtOAc, 2:1) of the residue gave amorphous title compound **14** (26 mg, 84%); TLC (toluene–EtOAc, 1:1) R_f 0.55 (**14**); $[\alpha]_D$ −1 (*c* 1, in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.86 and 7.76 (each 2 H, 2 m, Phth), 5.76 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.1, 3′-H), 5.33 (1 H, d, $J_{1',2'}$ 8.5, 1′-H), 5.17 (1 H, t, $J_{4',5'}$ 9.3, 4′-H), 5.03 (1 H, t, $J_{2,3}$ 9.6, $J_{3,4}$ 9.4, 3-H), 4.77 (1 H, dd, 2-H), 4.40 (1 H, d, $J_{1,2}$ 7.9, 1-H), 4.28 (1 H, dd, 2′-H), 3.93 and 3.52 (each 1 H, m, OCH_2CH$_2$Si), 3.22 (1 H, t, $J_{4,5}$ 9.4, 4-H), 2.12, 2.10, 2.03, 2.01, 1.97 and 1.89 (each 3 H, 6 s, 6 × COCH_3), 0.96–0.82 (2 H, m, CH_2Si) and 0.03 [9 H, s, Si$(CH_3)_3$]; δ_C(75.5 MHz; $CDCl_3$) 99.8 and 98.0 (C-1, -1′), 76.5, 74.7, 72.6, 71.7, 71.6, 70.6 and 68.8 (C-2, -3, -4, -5, -3′, -4′, -5′), 69.3, 67.2, 66.4, 62.5 and 61.8 (C-6, -6′, OCH$_2$CH$_2$*C*H$_2$O and OCH$_2$CH$_2$Si), 54.4 (C-2′), 30.0 (OCH$_2$*C*H$_2$CH$_2$O), 20.6–20.3 (CO*C*H$_3$) and 17.7 (CH_2Si); FABMS of $C_{40}H_{55}NO_{19}Si$ (M, 881.3) *m/z* 904.5 $(M + Na)^+$.

Ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[3-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy)propyl]-1-thio-β-D-glucopyranoside 17

Compound **14** (359 mg, 0.41 mmol), as in a mixture of dry CH_2Cl_2 (5 ml) and TFA (10 ml), was stirred for 40 min under argon at rt. Propyl acetate (30 ml) and toluene (60 ml) were added, and the solution was concentrated. Column chromatography (toluene–EtOAc, 2:3) gave hemiacetal **15** (299 mg, 96%); TLC (toluene–EtOAc, 1:2) R_f 0.70 (**14**), 0.44 (**15**).

To a solution of compound **15** (299 mg, 0.39 mmol) in dry CH_2Cl_2 (5 ml) was added trichloroacetonitrile (200 µl, 2.0 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (10 µl). After being stirred under argon for 2 h at rt, the solution was concentrated. Column chromatography (toluene–EtOAc, 3:2) of the residue gave the imidate **16** (314 mg, 89%); TLC (toluene–EtOAc, 1:1) R_f 0.20 (**15**), 0.47 (**16**); δ_H(300 MHz; $CDCl_3$) 8.61 (1 H, s, C=NH), 7.87 and 7.75 (each 2 H, 2 m, Phth), 6.44 (d, $J_{1,2}$ 3.2, 1-H), 5.75 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.1, 3′-H), 5.46 (1 H, t, $J_{2,3}$ 10.2, $J_{3,4}$ 9.8, 3-H), 5.34 (1 H, d, $J_{1',2'}$ 8.5, 1′-H), 5.16 (1 H, dd, $J_{4',5'}$ 10.1, 4′-H), 4.95 (1 H, dd, 2-H), 4.27 (1 H, dd, 2′-H), 3.36 (1 H, t, $J_{4,5}$ 9.7, 4-H), 2.11, 2.09, 2.06, 2.04, 2.00 and 1.97 (each 3 H, 6 s, 6 × COCH_3).

To a solution of compound **16** (314 mg, 0.34 mmol) in dry CH_2Cl_2 (3 ml) were added ethanethiol (70 µl, 0.95 mmol) and powdered molecular sieves 4 Å (900 mg), and the suspension was stirred under argon for 1 h at rt. $BF_3{\cdot}Et_2O$ (340 µl, 2.7 mmol) was added at 0 °C and the mixture stirred for 2.5 h at rt, then neutralized with triethylamine, diluted with CH_2Cl_2, filtered over Celite, and concentrated. Column chromatography (toluene–EtOAc, 3:2) of the residue gave amorphous title compound **17** (245 mg, 87%); TLC (toluene–EtOAc, 1:1) R_f 0.48 (**17**); $[\alpha]_D$ 0 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.86 and 7.76 (each 2 H, 2 m, Phth), 5.75 (1 H, dd, $J_{3',4'}$ 9.1, 3′-H), 5.33 (1 H, d, $J_{1',2'}$ 8.5, 1′-H), 5.16 (1 H, t, $J_{4',5'}$ 9.3, 4′-H), 5.06 (1 H, t, $J_{2,3}$ 9.7, $J_{3,4}$ 9.3, 3-H), 4.81 (1 H, t, 2-H), 4.40 (1 H, d, $J_{1,2}$ 10.0, 1-H), 3.20 (1 H, t, $J_{4,5}$ 9.4, 4-H), 2.70–2.63 (2 H, m, SCH_2CH$_3$), 2.11, 2.08, 2.03, 2.02, 1.97 and 1.85 (each 3 H, 6 s, 6 × COCH_3) and 1.24 (3 H, t, *J* 7.4, SCH_2CH_3); δ_C(75.5 MHz; $CDCl_3$) 170.5, 170.4, 170.0, 169.8, 169.5 and 169.3 (6 × *C*OCH$_3$), 98.0 (C-1′), 83.1 (C-1), 76.3, 76.2, 75.7, 71.8, 70.7, 70.3 and 68.9 (C-2, -3, -4, -5, -3′, -4′, -5′), 69.3, 66.5, 62.7 and 61.9 (C-6, -6′ and OCH$_2$CH$_2$*C*H$_2$O), 54.5 (C-2′), 30.0 (OCH$_2$*C*H$_2$CH$_2$O), 24.2 (S*C*H$_2$CH$_3$), 20.6–20.3 (CO*C*H$_3$) and 14.8 (SCH_2CH_3); FABMS of $C_{37}H_{47}NO_{18}S$ (M, 825.2) *m/z* 848.4 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-{3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-deoxy-3,4-di-*O*-(*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyloxy]propyl}-β-D-glucopyranoside 18

A mixture of compounds **9** (234 mg, 0.21 mmol) and **11** (168 mg, 0.25 mmol) in dry toluene (5 ml) containing powdered molecular sieves 4 Å (1.5 g) was stirred under argon for 1 h at rt. Then, NIS (67 mg, 0.30 mmol) and silver trifluoromethanesulfonate (7 mg, 0.027 mmol) were added, and the suspension was stirred for 3 h at rt. Pyridine (1 ml) was added, and the suspension was diluted with CH_2Cl_2, and filtered over Celite. The solution was washed successively with 10% aq. $Na_2S_2O_3$ and saturated aq. $NaHCO_3$, dried ($MgSO_4$), and concentrated. Purification twice by column chromatography (toluene–EtOAc, 2:1, then heptane–EtOAc, 1:1) gave title compound **18** (154 mg, 42%); TLC (toluene–EtOAc, 2:1) R_f 0.73 (**9**), 0.26 (**11**), 0.39 (**18**); $[\alpha]_D$ −3 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.80–7.03 (27 H, m, Phth, 2 × COC$_6H_4$CH$_3$ and 3 × Ph), 6.15 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.2, 3′-H), 5.43 (1 H, d, $J_{1',2'}$ 8.4, 1′-H), 5.34 (1 H, t, $J_{4',5'}$ 9.4, 4′-H), 5.33 (1 H, d, $J_{3''',4'''}$ 3.6, 4‴-H), 5.15 (1 H, t, $J_{1'',2''}$ 9.0, $J_{2'',3''}$ 9.0, 2″-H), 5.09 (1 H, dd, $J_{1''',2'''}$ 7.8, $J_{2''',3'''}$ 10.3, 2‴-H), 4.53 and 4.44 (each 1 H, 2 d, *J* 7.7 and 7.8, 1″- and 1‴-H), 4.44 (1 H, dd, 2′-H), 4.31 (1 H, d, $J_{1,2}$ 7.6, 1-H), 2.35 and 2.28 (each 3 H, 2 s, 2 × COC$_6$H$_4$CH_3), 2.14–1.96 (21 H, m, 7 × COCH_3), 1.68–1.60 (2 H, m, OCH$_2$CH_2CH$_2$O), 1.05–1.00 (2 H, m, CH_2Si) and 0.03 [9 H, s, Si$(CH_3)_3$]; δ_C(75.5 MHz; $CDCl_3$) 165.5 and 165.1 (2 × *C*OC$_6$H$_4$CH$_3$), 144.2–125.8 (Ar-C), 102.8, 100.9, 100.4 and 97.8 (C-1, -1′, -1″, -1‴), 84.2, 81.9, 78.2, 76.2, 74.7, 73.5, 72.6, 72.3, 71.3, 70.8, 70.5, 69.8, 68.9 and 66.4 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -2‴, -3‴, -4‴, -5‴), 75.2, 74.4, 73.2, 69.0, 67.2 and 60.8 (C-6, -6′, -6″, -6‴, OCH$_2$CH$_2$*C*H$_2$O

and OCH_2CH_2Si), 54.7 (C-2′), 30.0 (O$CH_2CH_2CH_2O$), 21.5 and 21.4 (2 × $COC_6H_4CH_3$), 20.6–20.3 (COCH_3) and 18.3 (CH_2Si); FABMS of $C_{91}H_{107}NO_{32}Si$ (M, 1753.7) *m/z* 1776.8 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-{2,3,6-tri-*O*-acetyl-4-*O*-[3-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranosyl}-(1→6)-[2-deoxy-3,4-di-*O*-(*p*-methylbenzoyl)-2-phthalimido-β-D-glucopyranosyloxy]propyl)-β-D-glucopyranoside 19

A mixture of compounds **9** (275 mg, 0.24 mmol) and **17** (200 mg, 0.24 mmol) in dry toluene (5 ml) containing powdered molecular sieves 4 Å (1.5 g) was stirred under argon for 2 h at rt. Then, NIS (72 mg, 0.32 mmol) and silver trifluoromethanesulfonate (6 mg, 0.023 mmol) were added, and the mixture was stirred for 1 h at rt. Additional portions of NIS (55 mg, 0.24 mmol) and silver trifluoromethanesulfonate (10 mg, 0.039 mmol) were added, and the mixture was stirred for 2 h. Work-up as described for compound **18** and column chromatography (toluene–EtOAc, 2:1) of the residue gave amorphous title compound **19** (301 mg, 66%); TLC (toluene–EtOAc, 3:2) R_f 0.72 (**9**), 0.32 (**17**), 0.52 (**19**); $[\alpha]_D$ −4 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.80–7.03 (31 H, m, 2 × Phth, 2 × $COC_6H_4CH_3$ and 3 × Ph), 6.15 (1 H, dd, $J_{2',3'}$ 10.7, $J_{3',4'}$ 9.2, 3′-H), 5.75 (1 H, dd, $J_{2''',3'''}$ 10.6, $J_{3''',4'''}$ 9.1, 3‴-H), 5.43 (1 H, d, $J_{1',2'}$ 8.4, 1′-H), 5.34 (1 H, t, $J_{4',5'}$ 9.6, 4′-H), 5.32 (1 H, d, $J_{1''',2'''}$ 8.6, 1‴-H), 5.16 (1 H, t, $J_{4''',5'''}$ 9.5, 4‴-H), 4.46 (1 H, d, $J_{1'',2''}$ 8.1, 1″-H), 4.31 (1 H, d, $J_{1,2}$ 7.7, 1-H), 2.35 and 2.28 (each 3 H, 2 s, 2 × $COC_6H_4CH_3$), 1.71-1.59 (4 H, m, 2 × $OCH_2CH_2CH_2O$), 2.14–1.96 (18 H, m, 6 × $COCH_3$), 1.05–1.00 (2 H, m, CH_2Si) and 0.03 [9 H, s, $Si(CH_3)_3$]; δ_C(75.5 MHz; $CDCl_3$) 170.5, 170.3, 170.0, 169.7, 169.4 and 169.3 (6 × $COCH_3$), 165.5 and 165.1 (2 × $COC_6H_4CH_3$), 144.1–123.5 (Ar-C), 102.8, 100.6, 98.0 and 97.8 (C-1, -1′, -1″, -1‴), 84.2, 82.0, 78.2, 76.3, 74.8, 74.5, 73.6, 72.7, 71.8, 71.5, 70.8, 70.7, 69.9 and 68.9 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴), 75.2, 74.5, 73.2, 69.4, 69.0, 68.4, 67.2, 66.6, 66.5, 62.5, 61.9 and 60.8 (C-6, -6′, -6″, -6‴, 3 × $PhCH_2O$, 2 × $OCH_2CH_2CH_2O$ and OCH_2CH_2Si), 54.7 and 54.4 (C-2′, -2‴), 21.5 and 21.4 (2 × $COC_6H_4CH_3$), 20.6–20.3 (COCH_3) and 18.4 (CH_2Si); FABMS of $C_{100}H_{114}N_2O_{33}Si$ (M, 1898.7) *m/z* 1921.9 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-[3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 20

To a solution of compound **18** (107 mg, 0.061 mmol) in MeOH (15 ml) was added a 0.2 M solution of NaOMe in MeOH (2 ml), and the mixture was stirred for 4 h at rt. The solution was neutralized with Dowex 50X8 (H^+-form), filtered, and concentrated. The residue was dissolved in butan-1-ol (15 ml)–ethylenediamine (3 ml), and the mixture was stirred for 24 h at 80 °C, then co-concentrated with toluene. A solution of the residue in dry pyridine (30 ml) containing acetic anhydride (15 ml) was stirred overnight at rt, then co-concentrated with toluene. Column chromatography (CH_2Cl_2–acetone, 3:1) of the residue gave title product **20** (87 mg, 95%); TLC (CH_2Cl_2–acetone, 3:1) R_f 0.63 (**20**); $[\alpha]_D$ −7 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 7.35–7.26 (15 H, m, 3 × Ph), 5.34 (1 H, d, $J_{3''',4'''}$ 2.9, 4‴-H), 4.63 and 4.38 (each 2 H, 2 d, *J* 7.7 and 8.1, 1-, 1′-, 1″- and 1‴-H), 2.14–1.96 (30 H, m, 10 × $COCH_3$) and 1.05–1.00 (2 H, m, CH_2Si); δ_C(75.5 MHz; $CDCl_3$) 138.8–138.3 and 128.4–127.5 (Ar-C), 103.0, 101.0, 100.3 and 99.6 (C-1, -1′, -1″,1‴), 78.2, 76.1, 75.1, 72.8, 72.7, 72.6, 72.0, 71.3, 70.8, 70.6, 69.3, 69.0 and 66.5 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -2‴, -3‴, -4‴, -5‴), 75.3, 74.6, 73.4, 68.8, 68.2, 67.3, 65.7, 61.8 and 60.6 (C-6, -6′, -6″, -6‴, O$CH_2CH_2CH_2O$ and OCH_2CH_2Si), 54.9 (C-2′), 29.9 (O$CH_2CH_2CH_2O$), 23.0 (NHCOCH_3), 20.7–20.3 (COCH_3) and 18.4 (CH_2Si); FABMS of $C_{73}H_{99}NO_{31}Si$ (M, 1513.6) *m/z* 1536.8 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzyl-4-*O*-(3-{2,3,6-tri-*O*-acetyl-4-*O*-[3-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranosyl}-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)-propyl)-β-D-glucopyranoside 21

Treatment of compound **19** (138 mg, 0.072 mmol) according to the procedure described for the preparation of analogue **20** followed by column chromatography (CH_2Cl_2–acetone, 2:1 to 1:1) gave title product **21** (110 mg, 97%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.73 (**21**); $[\alpha]_D$ −11 (*c* 1, $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 5.82 (1 H, d, N*H*COCH$_3$), 5.43 (1 H, d, $J_{1',2'}$ 8.4, 1′-H) and 4.31 (1 H, d, $J_{1,2}$ 7.6, 1-H); δ_C(75.5 MHz; $CDCl_3$) 138.8–138.2 and 128.4–127.6 (Ar-C), 103.0, 100.4, 99.8 and 99.5 (C-1, -1′, -1″, -1‴), 84.1, 82.1, 78.1, 76.1, 75.0, 74.5, 72.8, 71.9, 71.6, 71.3, 69.3 and 68.6 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴), 75.4, 74.6, 73.4, 69.4, 68.9, 68.0, 67.3, 65.6, 65.4, 62.8 and 62.0 (C-6, -6′, -6″, -6‴, 3 × $PhCH_2O$, 2 × $OCH_2CH_2CH_2O$ and OCH_2CH_2Si), 55.1 and 54.9 (C-2′, 2‴), 29.8 and 29.7 (2 × O$CH_2CH_2CH_2O$), 23.2 and 23.0 (2 × NHCOCH_3), 20.8–20.5 (COCH_3) and 18.4 (CH_2Si); FABMS of $C_{76}H_{106}N_2O_{31}Si$ (M, 1570.7) *m/z* 1593.8 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 22

A solution of compound **20** (87 mg, 0.057 mmol) in HOAc (2 ml) was stirred in the presence of 10% Pd–C (40 mg) under H_2 overnight at rt. The suspension was diluted with MeOH and filtered over Celite. After co-concentration with toluene, a solution of the residue in dry pyridine (5 ml) containing acetic anhydride (2.5 ml) was stirred overnight at rt. Co-concentration with toluene and column chromatography (CH_2Cl_2–acetone, 2:1) of the residue gave amorphous title compound **22** (56 mg, 72%); TLC (CH_2Cl_2–acetone, 3:1) R_f 0.38 (**22**); $[\alpha]_D$ −18 (*c* 1, $CHCl_3$); δ_C(75.5 MHz; $CDCl_3$) 101.0, 100.4, 99.9 and 99.6 (C-1, -1′, -1″, -1‴), 76.3, 76.1, 74.8, 73.0, 72.7, 72.6, 72.0, 71.8, 71.4, 70.9, 70.6, 69.4, 69.0 and 66.5 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, 5 -″, -2‴, -3‴, -4‴, -5‴), 68.4, 68.2, 67.3, 65.3, 63.1, 61.8 and 60.6 (C-6, -6′, -6″, -6‴, O$CH_2CH_2CH_2O$ and OCH_2CH_2Si), 55.3 (C-2′), 29.6 (O$CH_2CH_2CH_2O$), 23.2 (NHCOCH_3), 20.8–20.3 (COCH_3) and 17.8 (CH_2Si); FABMS of $C_{58}H_{87}NO_{34}Si$ (M, 1369.5) *m/z* 1392.7 $(M + Na)^+$.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-[3-{2,3,6-tri-*O*-acetyl-4-*O*-[3-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranosyl}-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)-propyl]-β-D-glucopyranoside 23

Treatment of compound **21** (91 mg, 0.058 mmol) according to the procedure for the preparation of analogue **22** followed by column chromatography (CH_2Cl_2–acetone, 1:1) gave amorphous title product **23** (70 mg, 84%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.46 (**23**); $[\alpha]_D$ −24 (*c* 1, $CHCl_3$); δ_C(75.5 MHz; $CDCl_3$) 100.5, 100.0, 99.9 and 99.6 (C-1, -1′, -1″, -1‴), 76.4, 76.2, 74.8, 74.5, 73.0, 72.9, 72.6, 72.0, 71.8, 71.6, 71.5, 69.4 and 68.7 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴), 68.5, 68.4, 67.3, 65.5, 65.2, 63.1, 62.7 and 62.0 (C-6, -6′, -6″, -6‴, 2 × O$CH_2CH_2CH_2O$ and OCH_2CH_2Si), 55.1 and 55.0 (C-2′, -2‴), 29.8 (2 × O$CH_2CH_2CH_2O$), 23.1 (2 × NHCOCH_3), 20.7–20.5 (COCH_3) and 17.8 (CH_2Si); FABMS of $C_{61}H_{94}N_2O_{34}Si$ (M, 1426.5) *m/z* 1449.7 $(M + Na)^+$.

Allyl 2,3,6-tri-*O*-acetyl-4-*O*-[3-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 26

To a solution of compound **22** (80 mg, 0.058 mmol) in dry CH_2Cl_2 (1 ml) was added TFA acid (2 ml), and the mixture was

stirred under argon for 1 h at rt. Propyl acetate (6 ml) and toluene (12 ml) were added and the solution was co-concentrated with toluene. Column chromatography (CH_2Cl_2–acetone, 2:1 to 1:3) of the residue gave compound **24** (67 mg, 90%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.53 (**22**), 0.26 (**24**).

To a solution of compound **24** in dry CH_2Cl_2 (1 ml) was added trichloroacetonitrile (100 µl), and at 0 °C DBU (10 µl), and the mixture was stirred for 3 h at rt. Column chromatography (CH_2Cl_2–acetone, 2:1) gave compound **25** (64 mg, 85%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.64 (**25**); δ_H(300 MHz; $CDCl_3$) 8.66 (1 H, s, C=NH), 6.49 (1 H, d, $J_{1,2}$ 3.6, 1-H) and 6.00 (1 H, d, *NH*COCH$_3$).

To a solution of compound **25** in dry CH_2Cl_2 (0.6 ml) containing molecular sieves 4 Å (150 mg) was added allyl alcohol (15 µl), and the mixture was stirred for 1 h at rt. Then, at −40 °C, was added $BF_3{\cdot}Et_2O$ (5 µl), and the mixture was stirred for 1 h at −40 °C, followed by 1 h at −20 °C. The solution was neutralized with triethylamine, filtered over Celite, and concentrated. Column chromatography (CH_2Cl_2–acetone, 2:1 to 1:2) of the residue gave title compound **26** (36 mg, 61%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.64 (**25**), 0.71 (**26**); $[\alpha]_D$ −18 (*c* 1, $CHCl_3$); δ_C(75.5 MHz; $CDCl_3$) 133.3 (OCH$_2$*C*H=CH$_2$), 117.4 (OCH$_2$CH=*C*H$_2$), 101.0, 100.4, 99.6 and 99.3 (C-1, -1′, -1″, -1‴), 76.3, 76.1, 74.6, 73.0, 72.7, 72.6, 71.9, 71.7, 71.3, 70.8, 70.5, 69.3, 69.0 and 66.5 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -2‴, -3‴, -4‴, -5‴), 69.9, 68.4, 68.3, 65.3, 63.0, 61.8 and 60.6 (C-6, -6′, -6″, -6‴, OCH$_2$CH$_2$*C*H$_2$O and OCH$_2$CH=CH$_2$), 55.2 (C-2′), 29.8 (OCH$_2$*C*H$_2$CH$_2$O), 23.2 (NHCO*C*H$_3$) and 20.7–20.3 (CO*C*H$_3$); FABMS of $C_{56}H_{79}NO_{34}$ (M, 1309.4) *m*/*z* 1310.4 (M + H)$^+$; *m*/*z* 1332.4 (M + Na)$^+$.

Allyl 2,3,6-tri-*O*-acetyl-4-*O*-[3-{2,3,6-tri-*O*-acetyl-4-*O*-[3-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)-propyl]-β-D-glucopyranosyl}-(1→6)-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 29

To a solution of compound **23** (99 mg, 0.069 mmol) in dry CH_2Cl_2 (1 ml) was added TFA (2 ml), and the mixture was stirred under argon for 1 h at rt. Propyl acetate (6 ml) and toluene (12 ml) were added and the solution was co-concentrated with toluene. Column chromatography (CH_2Cl_2–acetone, 1:1 to 1:2) of the residue gave compound **27** (76 mg, 84%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.46 (**23**), 0.26 (**27**).

To a solution of compound **27** in dry CH_2Cl_2 (1 ml) was added trichloroacetonitrile (100 µl), and at 0 °C DBU (10 µl), and the mixture was stirred for 2 h at rt, then concentrated. Column chromatography (CH_2Cl_2–acetone, 1:1) of the residue gave compound **28** (69 mg, 82%); TLC (CH_2Cl_2–acetone, 1:1) R_f 0.54 (**28**); δ_H(300 MHz; $CDCl_3$) 8.66 (1 H, s, C=NH), 6.49 (1 H, d, $J_{1,2}$ 3.6, 1-H), 5.94 and 5.89 (each 1 H, 2 d, N*H*CONCH$_3$), 4.75 and 4.72 (each 1 H, 2 d, *J* 8.6 and 8.8, 1′- and 1‴-H) and 4.51 (1 H, d, $J_{1'',2''}$ 7.9, 1″-H).

To a solution of compound **28** in dry CH_2Cl_2 (0.6 ml) containing molecular sieves 4 Å (150 mg) was added allyl alcohol (15 µl), and the mixture was stirred under argon for 1 h at rt. Then, at −40 °C, was added $BF_3{\cdot}Et_2O$ (5 µl), and the mixture was stirred for 1 h at −40 °C, followed by 1 h at −20 °C. The solution was neutralized with triethylamine, filtered over Celite, and concentrated. Column chromatography (CH_2Cl_2–acetone, 2:1 to 1:1) of the residue afforded title compound **29** (38 mg, 59%); TLC (1:1 CH_2Cl_2–acetone) R_f 0.52 (**29**); $[\alpha]_D$ −20 (*c* 1, $CHCl_3$); δ_C(75.5 MHz; $CDCl_3$) 133.3 (OCH$_2$*C*H=CH$_2$), 117.4 (OCH$_2$CH=*C*H$_2$), 100.5, 99.9, 99.6 and 99.3 (C-1, -1′, -1″, -1‴), 76.3, 76.2, 74.6, 74.5, 73.0, 72.9, 72.7, 72.0, 71.6, 71.4, 69.3 and 68.6 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴), 69.9, 68.6, 68.4, 65.5, 65.2, 63.0, 62.8 and 62.0 (C-6, -6′, -6″, -6‴, 2 × OCH$_2$CH$_2$*C*H$_2$O, OCH$_2$CH=CH$_2$), 55.2 and 55.1 (C-2′, -2‴), 29.8 (2 × OCH$_2$*C*H$_2$CH$_2$O), 23.2 (2 × NHCO*C*H$_3$) and 20.8–20.5 (*C*OCH$_3$); FABMS of $C_{59}H_{86}N_2O_{34}$ (M, 1366.5) *m*/*z* 1367.4 (M + H)$^+$; *m*/*z* 1389.4 (M + Na)$^+$.

Allyl 4-*O*-{3-[β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy]-propyl}-β-D-glucopyranoside 30

A solution of compound **26** (20 mg, 0.015 mmol) in 0.1 M methanolic NaOMe (5 ml) was stirred overnight at rt. Water (1 ml) was added, and the solution was stirred for 24 h at rt, neutralized with Dowex 50X8 (H^+-form), filtered, and concentrated. Purification of the residue on Toyopearl HW-40S with water as eluent gave title compound **30** (10.4 mg, 86%); TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.26 (**30**); $[\alpha]_D$ −13 (*c* 0.6, water); δ_C(75.5 MHz; $CDCl_3$) 175.3 (NH*C*OCH$_3$), 134.2 (OCH$_2$-*C*H=CH$_2$), 119.6 (OCH$_2$CH=*C*H$_2$), 103.8, 103.5, 102.0 and 101.9 (C-1, -1′, -1″, -1‴), 79.3, 79.0, 76.4, 76.2, 75.9, 75.7, 75.6, 75.1, 74.6, 74.0, 73.6, 73.4, 71.8, 70.5 and 69.4 (C-2, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -2‴, -3‴, -4‴, -5‴), 71.5, 70.4, 69.4, 67.8, 61.8, 61.4 and 60.9 (C-6, -6′, -6″, -6‴, OCH$_2$CH$_2$*C*H$_2$O and OCH$_2$CH=CH$_2$), 56.4 (C-2′), 30.3 (OCH$_2$*C*H$_2$CH$_2$O) and 23.0 (NHCO*C*H$_3$); FABMS of $C_{32}H_{55}NO_{22}$ (M, 805.3) *m*/*z* 806.3 (M + H)$^+$; *m*/*z* 828.3 (M + Na)$^+$.

Allyl 4-*O*-[3-{4-*O*-[3-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranosyl}-(1→6)-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 31

Treatment of compound **29** (38 mg, 0.028 mmol) according to the procedure described for the preparation of analogue **30** gave title compound **31** (23.3 mg, 92%); TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.31 (**31**); $[\alpha]_D$ −18 (*c* 0.6, water); δ_C(75.5 MHz; $CDCl_3$) 175.3 (NH*C*OCH$_3$), 134.0 (OCH$_2$*C*H=CH$_2$), 119.4 (OCH$_2$CH=*C*H$_2$), 103.4, 101.8, 101.7 and 101.6 (C-1, -1′, -1″, -1‴), 78.8, 76.5, 76.3, 76.1, 75.8, 75.5, 74.5, 74.4, 73.9, 73.8, 70.6 and 70.3 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴), 71.2, 70.2, 69.2, 67.6, 67.4, 61.4 and 61.2 (C-6, -6′, -6″, -6‴, 2 × OCH$_2$CH$_2$*C*H$_2$O, OCH$_2$CH=CH$_2$), 56.2 (C-2′, -2‴), 30.0 (2 × OCH$_2$*C*H$_2$CH$_2$O) and 22.9 (2 × NHCO*C*H$_3$); FABMS of $C_{37}H_{64}N_2O_{23}$ (M, 904.4) *m*/*z* 905.4 (M + H)$^+$.

Allyl 4-*O*-{3-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[β-D-galactopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl}-β-D-glucopyranoside 32

To a solution of compound **30** (9.6 mg, 11.9 µmol) in sodium cacodylate buffer (50 mM, pH 7.5; 5 mM $MnCl_2$) containing bovine serum albumin (0.7 mg) and NaN_3 (0.02%) were added alkaline phosphatase (6 U), UDP-galactose (9 mg, 14.7 µmol) and β-1,4-galactosyltransferase (2 U). The reaction mixture (600 µl) was incubated for 3 h at 37 °C. Then water (100 ml) was added, and UDP-galactose was removed using a Dowex 1X8 (Cl^--form) column with water as eluent. The eluate was concentrated, applied on a Toyopearl HW-40S column, and eluted with 5 mM aq. NH_4HCO_3 at a flow rate of 13 ml h^{-1}. The appropriate fractions were freeze-dried to give title compound **32** (9.8 mg, 85%); TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.26 (**30**), 0.15 (**32**); $[\alpha]_D$ −15 (*c* 0.4, water); ^{1}H NMR data are given in Table 1; δ_C(75.5 MHz; $CDCl_3$) 175.3 (NH*C*OCH$_3$), 134.2 (OCH$_2$*C*H=CH$_2$), 119.6 (OCH$_2$CH=*C*H$_2$), 103.8, 103.6, 103.3, 102.0 and 101.9 (C-1, -1′, -1″, -1‴, -1⁗), 79.4, 79.1, 78.7, 76.4, 76.2, 76.1, 75.9, 75.6, 75.1, 74.3, 74.0, 73.5, 73.4, 73.2, 71.8 and 69.4 (C-2, -3, -4, -5, -3′, -4′ -,5′, -2″, -3″, -4″, -5″, -2‴, -3‴, -4‴, -5‴, -2⁗, -3⁗, -4⁗, -5⁗), 71.5, 70.4, 68.2, 67.9, 61.9, 61.4 and 60.9 (C-6, -6′, -6″, -6‴, -6⁗, OCH$_2$CH$_2$*C*H$_2$O, OCH$_2$-CH=CH$_2$), 55.9 (C-2′), 30.3 (OCH$_2$*C*H$_2$CH$_2$O) and 23.0 (NHCO*C*H$_3$); FABMS of $C_{38}H_{65}NO_{27}$ (M, 967.4) *m*/*z* 968.3 (M + H)$^+$; *m*/*z* 990.3 (M + Na)$^+$.

Allyl 4-*O*-[3-{4-*O*-[3-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranosyl}-(1→6)-[β-D-galactopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 33

To a solution of compound **31** (9.4 mg, 10.4 µmol) in sodium cacodylate buffer (50 mM, pH 7.5; 5 mM $MnCl_2$) containing bovine serum albumin (0.7 mg) and NaN_3 (0.02%) were added

alkaline phosphatase (7 U), UDP-galactose (20.6 mg, 34.0 μmol), and galactosyltransferase (2 U). The reaction mixture (800 μl) was incubated for 7 h at 37 °C. Work-up as described for compound **32** and purification twice on Toyopearl HW-40S gave title compound **33** (10.2 mg, 80%); TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.31 (**31**), 0.17 (**33**); $[\alpha]_D$ −10 (*c* 0.4, water); ^{1}H NMR data are given in Table 2; δ_C(75.5 MHz; $CDCl_3$) 175.3 ($NHCOCH_3$), 134.2 ($OCH_2CH{=}CH_2$), 119.6 ($OCH_2CH{=}CH_2$), 103.7, 103.6, 103.4, 102.0, 101.9 and 101.8 (C-1, -1′, -1″, -1‴, -1⁗, -1⁗′;), 79.4 79.0, 78.7, 76.4, 76.3, 76.2, 76.1, 76.0, 75.9, 75.6, 74.3, 74.0, 73.9, 73.4, 73.3, 73.2, 71.8 and 69.4 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴, -2⁗, -3⁗, -4⁗,-5⁗, -2⁗′;, -3⁗′, -4⁗′, -5⁗′), 71.4, 70.4, 68.2, 67.8, 67.7, 61.9, 61.4 and 60.9 (C-6, -6′, -6″, -6‴, -6⁗, -6⁗′, 2 × OCH_2-CH_2CH_2O, $OCH_2CH{=}CH_2$), 55.9 (C-2′, -2‴), 30.2 (2 × OCH_2-CH_2CH_2O), 23.1 (2 × $NHCOCH_3$); FABMS of $C_{49}H_{84}N_2O_{33}$ (M, 1228.5) *m*/*z* 1229.4 $(M + H)^+$; *m*/*z* 1251.3 $(M + Na)^+$.

3-(2-Aminoethylthio)propyl 4-*O*-{3-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[β-D-galactopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl}-β-D-glucopyranoside 34

The allyl glycoside **32** (11.1 mg, 11.5 μmol) was dissolved in aq. cysteamine hydrochloride (6.6 mg, 57.5 μmol in 300 μl), and the mixture was irradiated under UV-light for 3 h at rt. The product was purified by size-exclusion chromatography on a Toyopearl HW-40S column, eluted with 0.1 M aq. NH_4OAc at a flow rate of 13 ml h^{-1}. Product-containing fractions were lyophilized and deionized on a Dowex 1X8 (OH^--form) column with water as eluent to give title glycoside **34** (9.4 mg, 78%); TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.17 (**32**), 0.08 (**34**); δ_H(300 MHz; $CDCl_3$) 4.57–4.52 (3 H, 1′-, 1″- and 1‴-H), 4.45 and 4.43 (each 1 H, 2 d, *J* 7.4 and 7.7, 1- and 1‴-H), 4.29 (1 H, d, *J* 10.5, 6′-H), 3.09, 2.80 and 2.69 (each 2 H, 3 t, $OCH_2CH_2CH_2SCH_2$-CH_2NH_2) and 2.05 (3 H, s, $NHCOCH_3$); δ_C(75.5 MHz; $CDCl_3$) 176.2 ($NHCOCH_3$), 104.6, 104.4, 104.1, 103.9 and 102.8 (C-1, -1′, -1″, -1‴, -1⁗), 80.1, 79.9, 79.4, 77.2, 77.0, 76.9, 76.8, 76.4, 76.0, 75.1, 74.9, 74.3, 74.2, 74.0, 72.6 and 70.2 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -2‴, -3‴, -4‴, -5‴, -2⁗, -3⁗, -4⁗, -5⁗), 71.3, 70.4, 69.0, 68.6, 62.7, 62.7, 62.2 and 61.8 (C-6, -6′, -6″, -6‴, -6⁗ $OCH_2CH_2CH_2O$ and $OCH_2CH_2CH_2S$), 56.8 (C-2′), 40.4, 31.7, 30.5, 30.4 and 28.8 ($OCH_2CH_2CH_2O$ and $OCH_2CH_2CH_2SCH_2CH_2NH_2$) and 23.2 ($NHCOCH_3$); FABMS of $C_{40}H_{72}N_2O_{27}S$ (M, 1044.4) *m*/*z* 1045.3 $(M + H)^+$.

3-(2-Aminoethylthio)propyl 4-*O*-[3-{4-*O*-[3-(β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)oxypropyl]-β-D-glucopyranosyl}-(1→6)-[β-D-galactopyranosyl-(1→4)]-(2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl]-β-D-glucopyranoside 35

Treatment of compound **33** (28.9 mg, 23.5 μmol) according to the procedure described for the preparation of analogue **34** gave title glycoside **35** (26.1 mg, 85%); TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.15 (**33**), 0.06 (**35**); δ_C(75.5 MHz; $CDCl_3$) 175.3 (2 × $NHCOCH_3$), 103.7, 103.6, 103.4, 103.0, 101.9 and 101.8 (C-1, -1′, -1″, -1‴, -1⁗, -1⁗′), 79.3, 79.0, 78.6, 76.4, 76.3, 76.2, 76.1, 76.0, 75.9, 75.6, 74.3, 74.0, 73.9, 73.3, 73.2, 71.8 and 69.3 (C-2, -3, -4, -5, -3′, -4′, -5′, -2″, -3″, -4″, -5″, -3‴, -4‴, -5‴, -2⁗,-3⁗, -4⁗, -5⁗, -2⁗′, -3⁗′ -4⁗′, -5⁗′), 70.4, 69.5, 68.1, 67.8, 67.7, 61.9, 61.3 and 60.9 (C-6, -6′, -6″, -6‴, -6⁗, -6⁗′, 2 × OCH_2-CH_2CH_2O, $OCH_2CH{=}CH_2$), 55.9 (C-2′, -2‴), 39.1, 29.5, 29.0 and 27.9 ($OCH_2CH_2CH_2SCH_2CH_2NH_2$), 30.2 (2 × OCH_2CH_2-CH_2O) and 23.0 (2 × $NHCOCH_3$); FABMS of $C_{51}H_{91}N_3O_{33}S$ (M, 1305.5) *m*/*z* 1306.4 $(M + H)^+$.

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References

1 T. J. Mitchell and P. W. Andrew, in *Molecular and Clinical Aspects of Bacterial Vaccine Development*, ed. D. A. A. Ala'Aldeen and C. E. Hormaeche, Wiley, Chichester, 1995, p. 93.
2 D. L. Klein and R. W. Ellis, in *New Generation Vaccines*, ed. M. M. Levine, G. C. Woodrow, J. B. Kaper and G. S. Cobon, Marcel Dekker Inc., NewYork, 1997, p. 503.
3 R. Austrian, *Rev. Infect. Dis.*, 1989, **11**, S598.
4 P. C. Appelbaum, *Clin. Infect. Dis.*, 1992, **15**, 77.
5 R. F. Breiman, J. C. Butler, F. C. Tenover, J. A. Elliot and R. R. Facklam, *JAMA*, 1994, **271**, 1831.
6 J. B. Robbins, R. Austrian, C.-J. Lee, S. C. Rastogi, G. Schiffman, J. Henrichsen, P. H. Mäkela, C. V. Broome, R. R. Facklam, R. H. Tiesjema and J. C. Parke, Jr., *J. Infect. Dis.*, 1983, **148**, 1136.
7 D. M. Musher, *Clin. Infect. Dis.*, 1992, **14**, 801.
8 E. A de Velasco, A. F. M. Verheul, J. Verhoef and H. Snippe, *Microbiol. Rev.*, 1995, **59**, 591.
9 J. B. Robbins and R. Schneerson, *J. Infect. Dis.*, 1990, **161**, 821.
10 G. T. Rijkers, E. A. M. Sanders, M. A. Breukels and B. J. M. Zegers, *Rev. Med. Microbiol.*, 1996, **7**, 3.
11 B. Lindberg, J. Lönngren and D. A. Powell, *Carbohydr. Res.*, 1977, **58**, 177.
12 M. R. Wessels, V. Pozsgay, D. L. Kasper and H. J. Jennings, *J. Biol. Chem.*, 1987, **262**, 8262.
13 S. H. Landesman and G. Schiffman, *Rev. Infect. Dis.*, 1981, **3**, S184.
14 R. G. Colling, T. C. Pearson and J. C. Brown, *Infect. Immun.*, 1983, **41**, 205.
15 J. Thiem, *FEMS Microbiol. Rev.*, 1995, **16**, 193.
16 C.-H. Wong, R. L. Halcomb, Y. Ichikawa and T. Kajimoto, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 521.
17 T. A. Beyer, J. E. Sadler, J. I. Rearick, J. C. Paulson and R. L. Hill, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1981, **52**, 23.
18 S. David, C. Augé and C. Gautheron, *Adv. Carbohydr. Chem. Biochem.*, 1991, **49**, 175.
19 M. M. Palcic, O. P. Srivastava and O. Hindsgaul, *Carbohydr. Res.*, 1987, **159**, 315.
20 J. Niggemann, J. P. Kamerling and J. F. G. Vliegenthart, *Bioorg. Med. Chem.*, 1998, in the press.
21 C. R. Geren, S. C. Magee and K. E. Ebner, *Arch. Biochem. Biophys.*, 1976, **172**, 149.
22 M. M. Palcic, L. D. Heerze, M. Pierce and O. Hindsgaul, *Glycoconjugate J.*, 1988, **5**, 49.
23 S.-C. Ats, J. Lehmann and S. Petry, *Carbohydr. Res.*, 1992, **233**, 141.
24 S.-C. Ats, J. Lehmann and S. Petry, *Carbohydr. Res.*, 1994, **252**, 325.
25 K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg and G. Magnusson, *J. Org. Chem.*, 1988, **53**, 5629.
26 A. G. Kalivretenos and K. Nakanishi, *J. Org. Chem.*, 1993, **58**, 6596.
27 H. Lönn, *Carbohydr. Res.*, 1985, **139**, 105.
28 P. Konradsson, U. E. Udodong and B. Fraser-Reid, *Tetrahedron Lett.*, 1990, **31**, 4313.
29 K. Takeo, K. Nagayoshi, K. Nishimura and S. Kitamura, *J. Carbohydr. Chem.*, 1994, **13**, 1159.
30 E. M. Nashed and C. P. J. Glaudemans, *J. Org. Chem.*, 1987, **52**, 5255.
31 R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21.
32 H. Paulsen and M. Paal, *Carbohydr. Res.*, 1984, **135**, 53.
33 F. A. W. Koeman, J. W. G. Meissner, H. R. P. van Ritter, J. P. Kamerling and J. F. G. Vliegenthart, *J. Carbohydr. Chem.*, 1994, **13**, 1.
34 R. U. Lemieux, T. Takeda and B. Y. Chung, in *Synthetic Methods for Carbohydrates*, ed. H. S. El Khadem, ACS Symposium Series 39, American Chemical Society, Washington, DC, 1976, p. 90.
35 O. Kanie, S. C. Crawley, M. M. Palcic and O. Hindsgaul, *Carbohydr. Res.*, 1993, **243**, 139.
36 U. Brodbeck and K. E. Ebner, *J. Biol. Chem.*, 1966, **241**, 762.
37 H. Babad and W. Z. Hassad, *J. Biol. Chem.*, 1966, **241**, 2672.
38 C. Unverzagt, H. Kunz and J. C. Paulson, *J. Am. Chem. Soc.*, 1990, **112**, 9308.
39 R. T. Lee and Y. C. Lee, *Carbohydr. Res.*, 1974, **37**, 193.

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