Synthesis and conjugation of oligosaccharide fragments related to the immunologically reactive part of the circulating anodic antigen of the parasite *Schistosoma mansoni*

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The immunoreactive part of the circulating anodic antigen (CAA) from the parasite *Schistosoma mansoni* is a threonine-linked polysaccharide consisting of →6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc-(1→ repeating disaccharides. In the framework of an immunochemical project, as a follow-up of earlier synthesized di- to tetrasaccharide CAA fragments, the synthesis of a spacer-containing pentasaccharide fragment, 3-(2-aminoethylthio)propyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(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, is described. Moreover, 1-*O*-[3-(2-aminoethylthio)propyl]-*N*-acetyl-β-D-galactosamine was synthesized. Oxidation steps in the synthesis of tri- to pentasaccharide CAA fragments were performed using pyridinium dichromate and acetic anhydride. TEMPO-catalyzed oxidations were explored in the synthesis of 1-*O*-[6-aminohexyl]-β-D-glucuronicacidand3-aminopropyl(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galactopyranoside, affording short reaction times and high yields. All synthesized compounds, including the earlier described 3-(2-aminoethylthio)propyl-spacered di-, tri-, and tetrasaccharide CAA fragments, were conjugated to BSA using squaric diester chemistry with coupling efficiencies in the range of 30–90%. The efficiency decreased when larger oligosaccharides were coupled to BSA. Finally, conformational analyses of the tri- and tetrasaccharide fragments were performed using Molecular Mechanics (MM) and Molecular Dynamics (MD) calculations.

Introduction

Schistosomiasis (or bilharzia), caused by infection with worms (blood-dwelling flukes) belonging to the class of Trematoda, is one of the most important and widespread parasitic diseases. The number of infected people is estimated to surpass 250 million,[1] and the main species of medical importance to man are *Schistosoma mansoni*, *S. haematobium,* and *S. japonicum*. The life cycle of the parasite involves several parasitic stages, alternated by free-living stages in either intermediate hosts (fresh-water snails) or definitive hosts.[2] Human infection is initiated during exposure to water containing cercariae, free-living mobile stages of the parasite. Since, for cure of the infection, chemotherapy is available, early diagnosis is important. In this context, the development of diagnostic protocols based on the detection of schistosome antigens in the circulatory system of the host is receiving more and more attention. More specifically, in recent years much research is focused on the use of monoclonal antibody-based assays for the detection of highly immunogenic schistosomal antigens in serum and urine.

One of the major antigens of *S. mansoni* is the gut-associated circulating anodic antigen (CAA).[3] The immunologically dominant part of CAA is a threonine-linked polysaccharide consisting of disaccharide repeating units, {→6)-[β-D-Glc*p*A-(1→3)]-β-D-Gal*p*NAc-(1→}$_n$ ($n = \pm 30$), probably connected to the protein *via* an as-yet-unknown, core saccharide with GlcNAc at the reducing end.[4] Recently, we have started a programme to synthesize well defined oligosaccharide fragments of the polysaccharide in order to identify the immunologic epitopes of CAA for the generated anti-CAA monoclonal antibodies, and to investigate the potential of synthetic CAA glycan fragments as diagnostic markers for the detection of human schistosomiasis. As a first result the stereoselective synthesis of di-, tri-, and tetrasaccharide fragments of the CAA polysaccharide (**1**, **2** and **4**, Fig. 1) has been described.[5] Here, we describe the synthesis of other spacer-armed CAA glycan fragments, *i.e*. the tri- (**3**) and pentasaccharide fragment (**5**), and the spacer-armed monosaccharide constituents (**6** and **7**). Compounds **1**–**7** were conjugated to bovine serum albumin (BSA). Additionally, conformational studies were performed on the tri- and tetrasaccharide fragments using molecular mechanics and molecular dynamics calculations. Results of the immunological studies with the neoglycoconjugates will be published elsewhere.

Results and discussion

Synthesis of CAA fragments

The synthesized spacer-containing saccharide fragments of the CAA glycan are shown in Fig. 1. The convergent synthesis of **1**, **2**, and **4**[5] involved the preparation of the allyl glycoside precursors of **1**, **2**, and **4,** followed by an elongation of the allyl functions with 2-aminoethanethiol (cysteamine).[6] Compounds **5** and **6** were synthesized using a similar strategy. In view of the severe problems in realizing a general protocol for the oxidation of Glc to GlcA during the preparation of **1** [oxalyl dichloride–dimethyl sulfoxide (DMSO); $NaClO_2$], **2** [pyridinium dichromate(PDC)–dichloromethane–molecular sieves 4 Å], **4** and **5** (PDC–dichloromethane–acetic anhydride), and structures larger than **5** (see below), an alternative approach using 2,2,6,6-tetramethylpiperidine 1-oxide[7–9] (TEMPO) was

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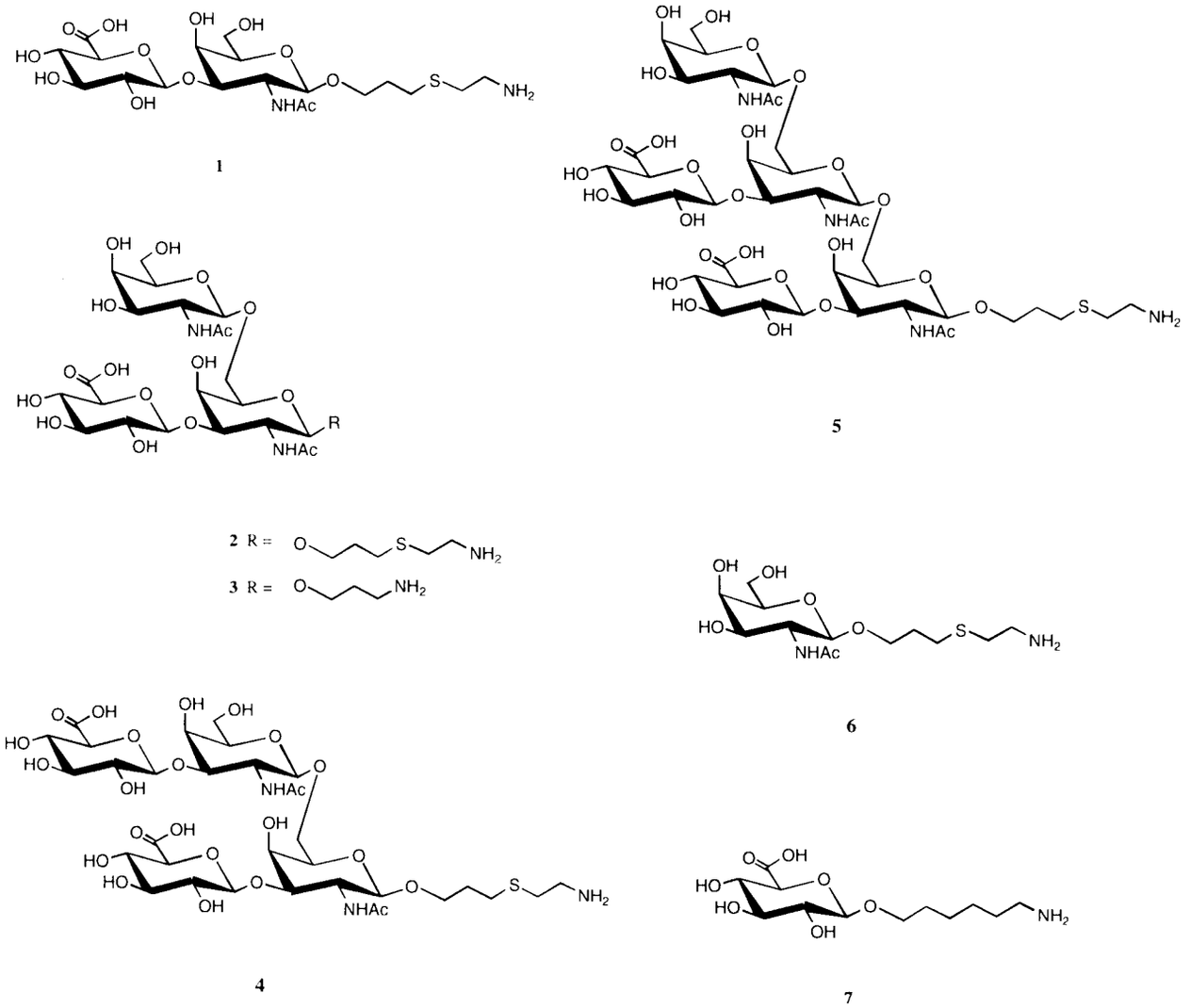


Fig. 1 Structures of the synthesized oligosaccharides representing fragments of the circulating anodic antigen of *Schistosoma mansoni*.

explored. However, the application of TEMPO required the use of other spacer systems than the 3-(2-aminoethylthio)propyl function, as evidenced in the synthesis of compounds **3** and **7**. Compounds **1**–**7** were conjugated to BSA using diethyl squarate; this reagent has been shown to be effective when small quantities of oligosaccharides (≈1 mg) are coupled to carrier proteins.[10–12]

Synthesis of pentasaccharide 5

For the synthesis of **5**, 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-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 **9** was selected as glycosyl acceptor and ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside[5] **10** as glycosyl donor (Scheme 1). The levulinoyl groups at the C-6 positions of the β-D-glucopyranosyl moieties were chosen in view of the proposed oxidation of the primary hydroxy functions after deprotection in the final stage of the synthesis. Compound **9** was prepared from 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[5] **8** by desilylation using toluene-*p*-sulfonic acid in acetonitrile–water (⟶ **9**, 82%). Galactosylation of **9** with donor **10** using *N*-iodosuccinimide (NIS) and silver triflate in toluene[13,14] gave stereospecifically pentamer **11** (66%). Condensation of **9** with **10** conducted in other solvent systems or in the presence of NIS and triflic acid (cat.)[15] did not result in higher yields. Selective delevulinoylation of **11** was performed by treatment with hydrazinium acetate[16] in toluene–ethanol (⟶ **12**, 92%), followed by oxidation of HO-6′, -6″ of **12** using PDC and acetic anhydride in dichloromethane[17] (3.5 h) to give **13** (73%). The complete oxidation of **12** was confirmed by ^{1}H NMR analysis of a minor amount of methyl-esterified **13** (⟶ **13a**, $COOCH_3$: δ 3.64 and 3.67). After deacylation/dephthaloylation using methylamine in ethanol[18] (2 weeks at room temperature), and subsequent *N*-acetylation with acetic anhydride in methanol at 0 °C, compound **14** was isolated in 65% yield. Subsequently, allyl glycoside **14** was treated with cysteamine hydrochloride under radical conditions (UV irradiation) to afford **5** (80%), suitable for conjugation to BSA (*vide infra*). The structures of **14** and **5** were unambiguously ascertained by mass spectrometry and ^{1}H (1D and 2D TOCSY) NMR analysis (Table 1).

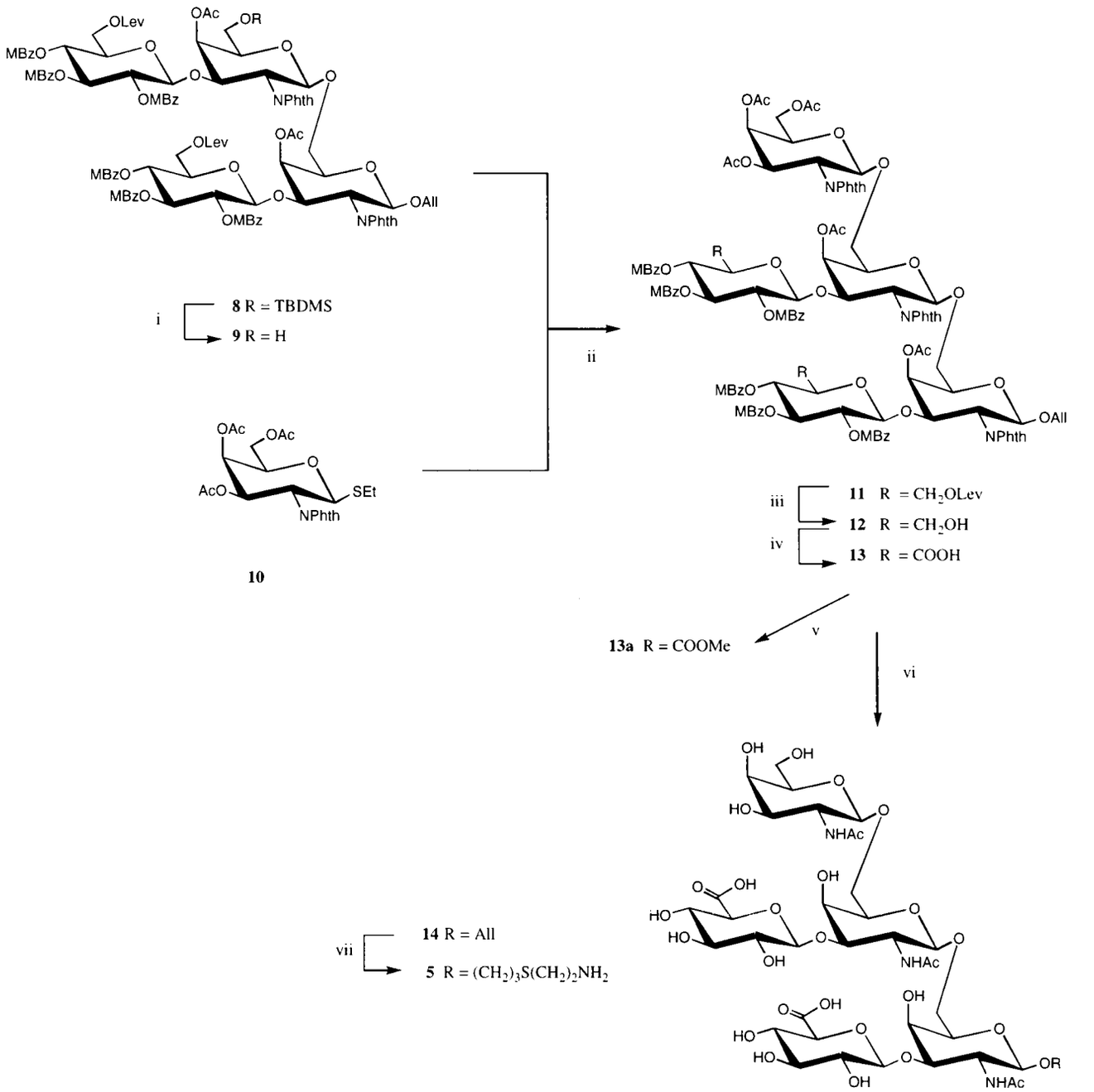
Preparation of spacer containing monosaccharides 6 and 7

For immunological purposes, the preparation of neoglycoconjugates bearing the individual monosaccharide constituents of the repeating disaccharide unit, *i.e.* *N*-acetyl-β-D-galactosamine and β-D-glucuronic acid, was also of interest. The synthesis of 3-(2-aminoethylthio)propyl 2-acetamido-2-deoxy-β-D-galactopyranoside **6** is depicted in Scheme 2. Reaction of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-galactopyranose

Table 1 500 MHz ^{1}H NMR data (1D and 2D TOCSY) of compound **5**

Proton (δ_H)	GalNAc/GalNAc″	GlcA′/GlcA‴	GalNAc⁗
H-1 ($J_{1,2}$)	4.46 and 4.50 (8.5)	4.48 and 4.51 (7.5)	4.47 (8.5)
H-2 ($J_{2,3}$)	3.97 and 4.01 (11.0)	3.33 (9.4)	3.91 (10.5)
H-3 ($J_{3,4}$)	3.83 and 3.84	3.45 (9.0)	3.75 (3.0)
H-4 ($J_{4,5}$)	4.13 and 4.15	3.47 (8.3)	3.94
H-5	n.d.[a]	3.67 and 3.70	n.d.[a]
$OCH_2CH_2CH_2S(CH_2)_2NH_2$	1.87		
$OCH_2CH_2CH_2S(CH_2)_2NH_2$	2.63		
$O(CH_2)_3SCH_2CH_2NH_2$	3.23 and 3.47		
$OCH_2(CH_2)_2SCH_2CH_2NH_2$	3.69 and 3.96		
$NHCOCH_3$	2.00, 2.03 and 2.04		

[a] n.d. = not determined.



Scheme 1 *Reagents and yields*: i, *p*-TsOH (82%); ii, NIS, AgOTf (66%); iii, H_2NNH_2·HOAc (92%); iv, PDC, Ac_2O (73%); v, CH_2N_2 (quant.); vi, (a) $MeNH_2$; (b) Ac_2O, MeOH (65%); vii, $HS(CH_2)_2NH_2$ (80%).

15 with hydrazinium acetate in dichloromethane for 1 h at 60 °C furnished **16**, which was imidoylated[19] using trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to yield **17** (56% over two steps). Condensation of **17** with allyl alcohol in dichloromethane in the presence of trimethylsilyl triflate (0.15 equiv. based on **17**) gave **18** (80%).

Although only a few methods can be found in the literature describing successful glycoside bond formation with *N*-acetyl glycosyl donors,[20,21] glycosidation using the trichloroacetimidate *N*-acetyl donor **17** proceeded in high yield and with the exclusive formation of the β-glycosidic linkage. Zemplén de-*O*-acetylation (⟶ **19**, 88%), followed by reaction with cysteamine hydrochloride under radical conditions in an anti-Markownikoff way for 2 h yielded target compound **6** (81%). As the removal of excess of reagent from **6** was rather complicated, only 1 equiv. of cysteamine was used for the reaction.

For the preparation of **7** a TEMPO-mediated oxidation step was used.[7,9] This type of oxidation was explored for reasons mentioned above. It should be noted that structures larger than the precursor of **5** could not be oxidized using PDC, pyridinium chlorochromate,[22] Jones oxidation procedure,[22] or Swern oxidation procedure[23] (data not shown). However, the application of nitroxyl radicals for oxidation in the presence of an allyl group is impossible as the double bond of this group is sensitive to radicals. Furthermore, it was found that oxidation of 3-(2-aminoethylthio)propyl (2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranosyl)-(1→3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranoside (an alternative precursor of disaccharide **1**) resulted in the formation of by-products due to oxidation of the thio function (results not shown). The latter finding is in agreement with recent data indicating that oxidation of alkyl/aryl thioglycosides with TEMPO and sodium hypochlorite leads to mixtures of sulfoxides and sulfones in preference to the oxidation of primary hydroxy groups.[24] For this reason, the anomeric allyl group was changed for a 6-aminohexyl spacer moiety. The preparation of 6-aminohexyl β-D-glucopyranosiduronic acid **7** is outlined in Scheme 2. Condensation of 6-*O*-levulinoyl-2,3,4-tri-*O*-*p*-toluoyl-α-D-glucopyranosyl trichloroacetimidate **20**[5] with 6-azidohexan-1-ol **21** in dichloromethane catalyzed by trimethylsilyl triflate (0.05 equiv. based on **20**) gave **22** (83%). Then, **22** was delevulinoylated with hydrazinium acetate in toluene–ethanol to afford **23** (89%). Oxidation of the primary hydroxy function in **23** in a two-phase system (dichloromethane–saturated aq. sodium bicarbonate) with sodium hypochlorite in the presence of a catalytic amount of TEMPO gave a rapid conversion of **23** into its uronic acid derivative (⟶ **24**, 79%). As the oxidation was performed under alkaline conditions, the reaction time was kept to a minimum in order to avoid cleavage of the base-labile acyl functions (15 min). The presence of a carboxylic function in **24** was confirmed by ^{1}H NMR analysis of methyl-esterified **24** (⟶ **24a**; $COOCH_3$ singlet at δ 3.69). After detoluoylation of **24** using methylamine in ethanol (7 days; ⟶ **25**, 88%), followed by hydrogenation[25] in the presence of Pd-C, compound **7** was obtained in 75% yield.

Synthesis of trisaccharide 3

To explore if nitroxyl radical-catalyzed oxidations could be implemented in synthetic strategies towards the preparation of larger CAA glycan fragments, trisaccharide **3** was assembled having a 3-aminopropyl spacer at its anomeric centre (Scheme 3). Condensation of disaccharide donor **26** with 3-azidopropan-1-ol **27** in dichloromethane in the presence of trimethylsilyl triflate (0.05 equiv. based on **26**) afforded disaccharide derivative **28** in 81% yield. Removal of the silyl ether group under acidic conditions (toluene-*p*-sulfonic acid in acetonitrile–water) gave **29** (67%). During purification, some product was lost due to migration of the acetyl function from O-4 to O-6. Conventional NIS/silver triflate-assisted glycosylation of acceptor **29** with donor **10** in toluene afforded trisaccharide derivative **30** in 42% yield. This rather low yield can probably be assigned to acceptor loss during coupling by *in situ* *O*-acetyl migration from O-4 to O-6, as TLC analysis during the reaction showed the presence of the 6-*O*-acetylated by-product. Delevulinoylation of **30** using hydrazinium acetate in toluene–methanol gave **31** (84%), which was oxidized to afford **32** using the TEMPO-mediated protocol as described for the oxidation of **23** to **24** (⟶ **32**, 75%). Treatment of a small amount of acid **32** with diazomethane in diethyl ether gave ester **32a**; ^{1}H NMR analysis showed the presence of the methyl ester at δ 3.69. Dephthaloylation/deacylation of **32** using methylamine in ethanol (14 days), and subsequent *N*-acetylation with acetic anhydride in methanol at 0 °C, afforded target compound **33** (80%). Finally, hydrogenation of the azido group of **33** was performed using sodium borohydride in the presence of Pd-C (⟶ **3**, 93%). For ^{1}H NMR data of compound **3**, see Table 2. In conclusion, sodium hypochlorite oxidation catalyzed by TEMPO is a promising alternative for oxidation steps in the synthesis of larger CAA glycan fragments.

Table 2 500 MHz ^{1}H NMR data (1D and 2D TOCSY) of compound **3**

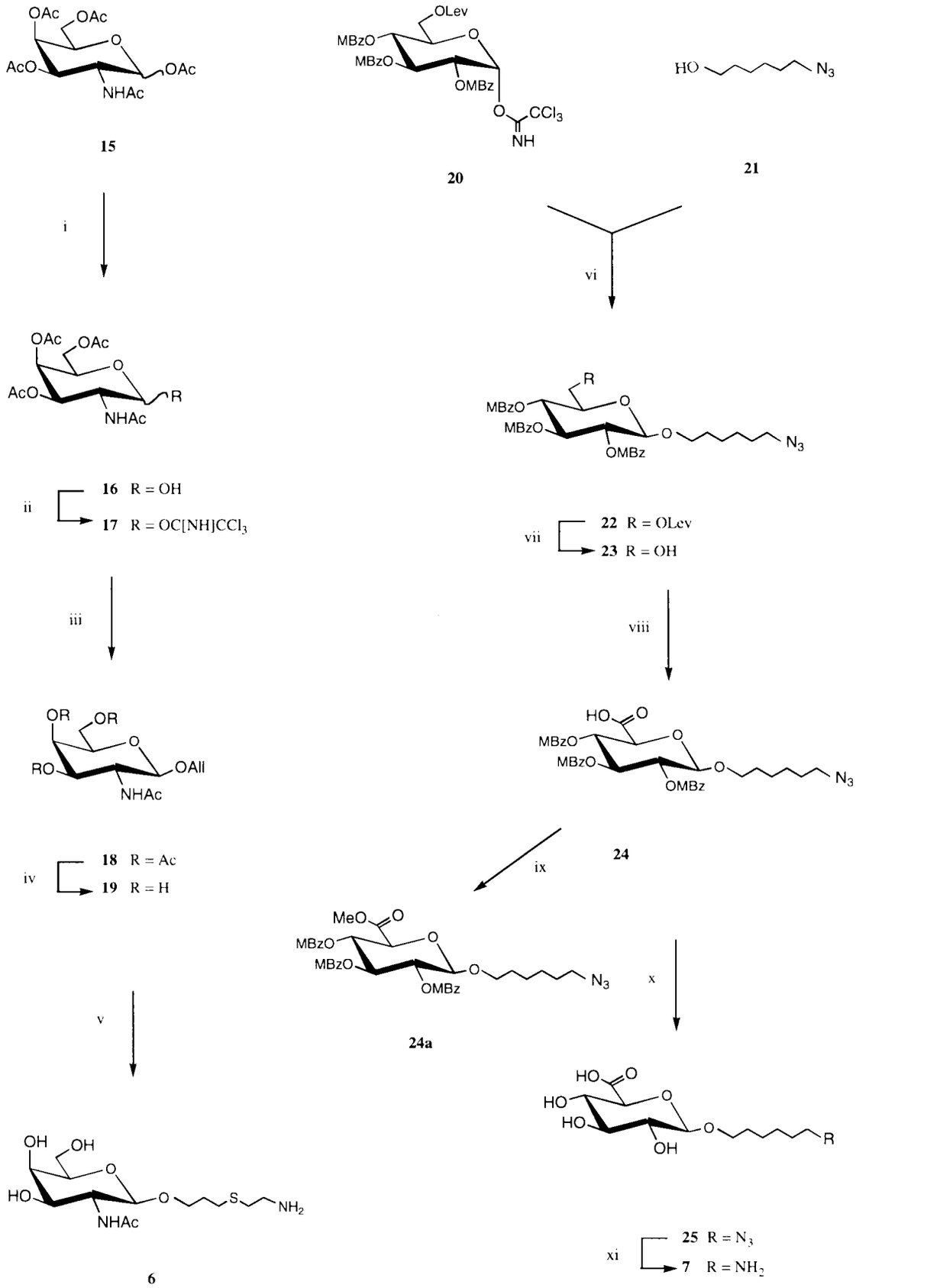
Proton (δ_H)	GalNAc	GlcA	GalNAc′
H-1 ($J_{1,2}$)	4.46 (8.4)	4.53 (7.4)	4.48 (8.8)
H-2 ($J_{2,3}$)	4.03 (10.8)	3.34 (9.3)	3.89 (10.8)
H-3 ($J_{3,4}$)	3.84 (3.9)	3.48 (9.3)	3.73 (3.0)
H-4 ($J_{4,5}$)	4.15 (< 1)	3.48	3.94 (< 1)
$OCH_2CH_2CH_2NH_2$	1.93–2.00		
$O(CH_2)_2CH_2NH_2$	3.10		
$OCH_2(CH_2)_2NH_2$	3.76 and 3.98		
$NHCOCH_3$	2.03		

Conversion of 1–7 into neoglycoconjugates

Reaction of the amine functions of compounds **1**–**7** with diethyl squarate in a phosphate-buffered mixture of water and ethanol at pH 7 afforded the squarate adducts **34**–**40**, respectively (Scheme 4).[26,27] Squarate reactions were performed starting with 1.0–1.5 mg of carbohydrate, and TLC analysis showed in most cases complete reactions without decomposition of the starting compounds. Reactions with larger compounds (**4** and **5**) required relatively long reaction times (up to 1 day). Most products were readily purified by passing the reaction mixtures through a C-18 Sep-Pak cartridge. However, purification of **37** and **38** was complicated, and additional HiTrap gel filtration was needed to isolate the adducts. Purification of **36** was very complicated and isolation using a Sep-Pak cartridge was not successful, most likely due to the shorter spacer-arm of **36** compared with that of other adducts; **36** could only be isolated by HiTrap gel filtration. The isolated products **34**–**40** were immediately used for the subsequent covalent coupling to BSA *via* the ε-amino groups of the lysine residues of the protein. The targeted incorporation onto BSA was in all cases 15 oligosaccharide units per BSA molecule, and **35**–**40** were added to a solution of BSA in bicarbonate buffer (pH 9). After stirring for 3 days, the isolation of the neoglycoconjugates was performed by HiTrap gel filtration. Lyophilization provided the neoglycoproteins **41**–**47** (Scheme 4). The average degree of incorporation was determined by MALDI-TOF MS (Table 3) by determination of the centre of the distribution of the singly charged molecular ion.

It is evident from Table 3 that, in general, coupling efficiencies, based on two reaction steps, decreased when larger oligosaccharides were conjugated to BSA. The low coupling efficiency of the conjugation reaction with **36** is most likely due to the difficult isolation procedure. Therefore, the use of spacer systems longer than the 3-aminopropyl moiety is recommended.

For uncharged carbohydrate structures, it was claimed[11] that the nature of the carbohydrate will not substantially affect the efficiency of conjugations using squarate chemistry. However, other authors found that the rate and level of incorporation of charged glycosides, *e.g.* sialic acid derivatives,[28] were lower than



Scheme 2 *Reagents and yields*: i, H_2NNH_2·HOAc; ii, CCl_3CN, DBU (56% over i and ii); iii, CH_2=$CHCH_2OH$, TMSOTf (80%); iv, NaOMe (88%); v, $HS(CH_2)_2NH_2$ (81%); vi, TMSOTf (83%); vii, H_2NNH_2·HOAc (89%); viii, TEMPO, KBr, Bu_4NCl, NaOCl (79%); ix, CH_2N_2 (quant.); x, $MeNH_2$ (88%); xi, H_2, Pd–C (75%).

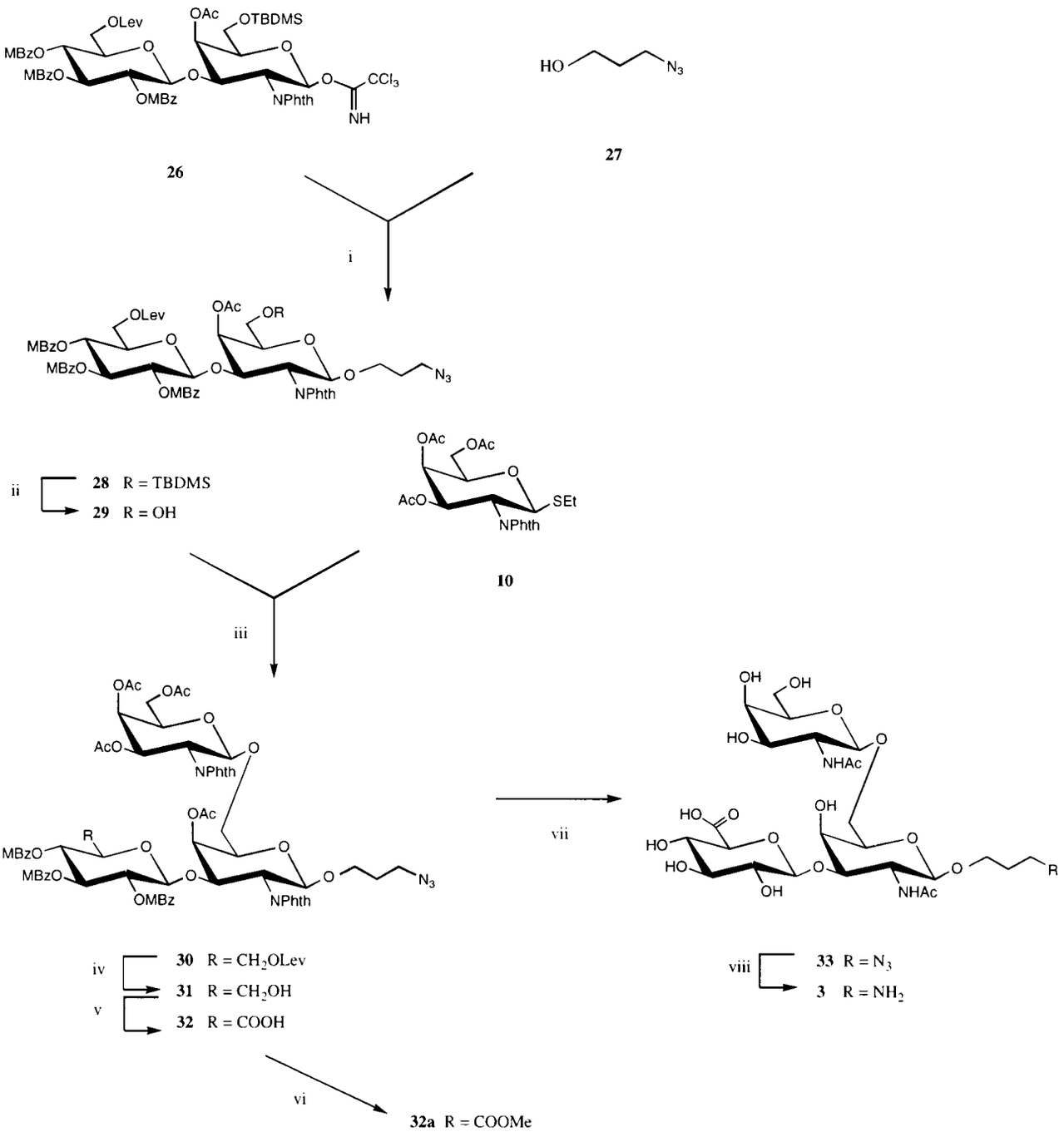
in corresponding reactions with neutral glycosides. The results presented here suggest an influence of size and charge on the efficiency of the conjugation. In terms of charge, this effect can probably be explained by a repulsive build-up of negative charges on the acidic BSA protein.[29]

Conformational studies on structures 2 and 4

In order to get insight into the conformational effect of the additional GalNAc residue in **2** and **5**, compared with **1** and **4**, respectively, the preferred conformations of compounds **2** and **4**

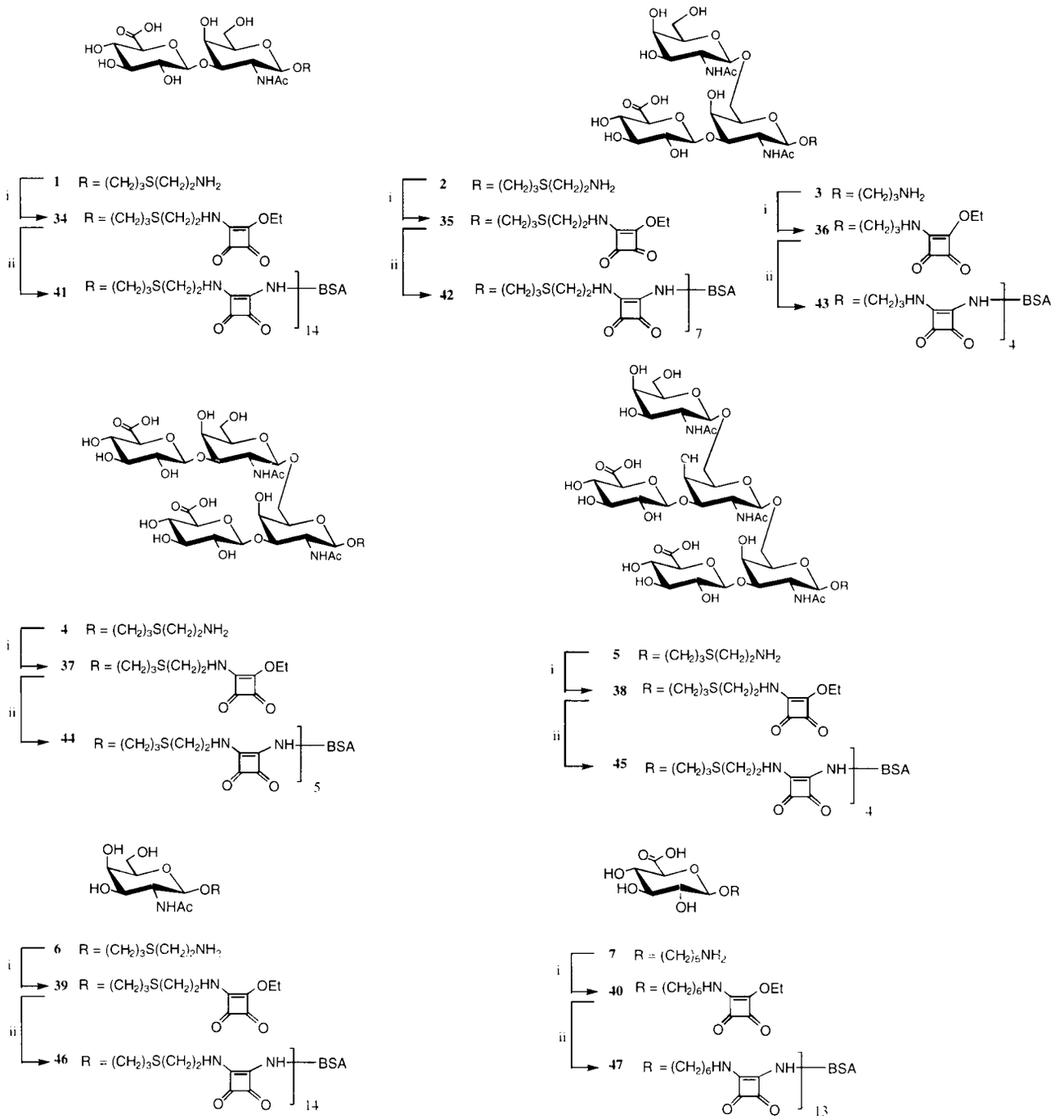
Table 3 Degree of incorporation of **34**–**40** onto BSA

Compound	Oligosaccharide used (mg)	Targeted incorporation (*n*-value)	Product	Achieved incorporation (*n*-value)	Incorporation efficiency (%)
34	1.0	15	**41**	13.5	90
35	1.0	15	**42**	6.5	43
36	1.5	15	**43**	4.4	29
37	1.0	15	**44**	5.4	36
38	1.0	15	**45**	4.5	30
39	1.5	15	**46**	14.2	95
40	1.5	15	**47**	12.8	85



Scheme 3 *Reagents and yields*: i, TMSOTf (81%); ii, *p*-TsOH, water (67%); iii, NIS, AgOTf (42%); iv, H_2NNH_2·HOAc (84%); v, TEMPO, KBr, Bu_4NCl, NaOCl (75%); vi, CH_2N_2 (quant.); vii, (a) $MeNH_2$; (b) Ac_2O, MeOH (80%); viii, $NaBH_4$, Pd–C (93%).

were investigated by performing molecular mechanics and molecular dynamics calculations. First, relaxed energy maps of the methyl glycosides of the constituting disaccharides, β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc (*cf.* structure **1**) and β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc, *in vacuo* were constructed with the CHEAT[30,31] force field. All maps were generated by changing the interresidual torsion angles in steps of 10°, resulting in a 36 × 36 grid. Three dihedral angles per sugar ring and both



Scheme 4 *Reagents and conditions*: i, diethyl squarate, pH 7.0; ii, BSA, pH 9.0 (conversion in Na^+-form).

interglycosidic angles are kept fixed by setting constraints during a first minimization at each grid point. Subsequently, all constraints in the ring were removed and the molecule was minimized again. Contour levels were plotted in steps of 1 kcal mol^{-1}† from the global minimum. In this force field hydroxy groups are treated as united atoms, which prevents the formation of intramolecular hydrogen bonds, and whereby the energies are independent of the hydroxy-group orientation. For β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→OMe) one energy map was generated to study the preferred conformations for the glycosidic linkages φ (O5′–C1′–O3–C3) and ψ (C1′–O3–C3–C2) (Fig. 2a).[32] For β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc-(1→OMe), three φ/ψ energy maps were generated, corresponding to the three staggered conformations around the C5–C6 bond, commonly denoted GG, GT and TG. In this nomenclature G stands for *gauche* (±60°) and T for *trans* (±180°). The torsion angle of the O6–C6–C5–O5 moiety is indicated by the first letter, and the torsion angle ω[32] of the O6–C6–C5–C4 moiety by the second. The three isoenergy contour plots (Fig. 2b for the TG contour plot) show basically the same profile, with the global energy minimum at φ/ψ −65°/−175° (Table 4). A set of three second-lowest energy minima had an energy that was at least 3 kcal mol^{-1} higher than the global minima, and were not further considered. For each energy map, the conformations with the lowest energy were minimized again, but with no constraints on the interglycosidic dihedral angles. The resulting disaccharides were used as building blocks to create the tri- and tetrasaccharide starting structures (*cf.* **2** and **4**) used in the molecular dynamics (MD) calculations.

To consider the conformational aspects in solution, for the methyl glycosides of the tri- and tetrasaccharide, MD runs in water with a duration of 1 ns were performed with the GROMOS force field. All runs were started from the global-

† 1 cal = 4.184 J.

Table 4 Inter-glycosidic dihedral angles of the TG, GG and GT lowest-energy conformations for β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc-(1→OMe)

Conformation	Dihedral angles[a]			Energy[b]
	φ	ψ	ω	
TG	−63	−178	−57	49.76
GG	−64	−179	72	49.65
GT	−66	−172	−175	48.39
TG	51	−172	−59	52.77
GG	36	164	68	52.23
GT	40	179	−173	53.20

[a] Angles are in degrees. [b] Energy is in kcal mol^{-1}.

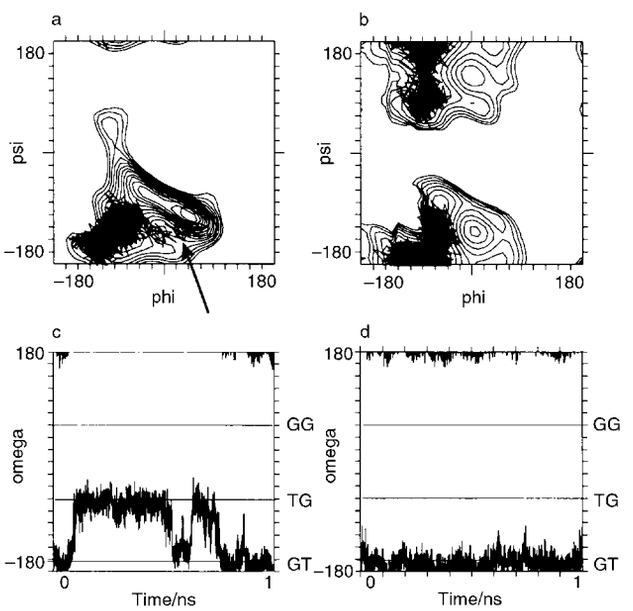


Fig. 2 Selected trajectories of the GROMOS MD runs. Panel a shows the results of the β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc φ/ψ dihedral angles for a 1 ns MD simulation of the trisaccharide. The trajectory is projected on top of the energy-contour map calculated for inter-glycosidic linkages of the β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc disaccharide, and the arrow indicates the global energy minimum. Panel b displays the trajectory of the β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc φ/ψ dihedral angles of the trisaccharide displayed on top of the energy-contour plot of φ/ψ of the β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc disaccharide in the GT conformation. Panels c and d show the MD trajectories of the ω dihedral angle of the tetra- and trisaccharide, respectively.

minimum-energy conformation. For the simulations of both structures, the φ dihedral angle of the glycosidic linkage(s) between GlcA and GalNAc changed rapidly from 40° to somewhere in the region of −100°/−180°; see, for example, Fig. 2d. Further investigation showed that for $\varphi = 40°$, a hydrogen bond is formed between the COO^- group of GlcA and the NH group of GalNAc. During the grid search *in vacuo*, this hydrogen bond is the cause of an unrealistically low energy for this conformation (note that NH is NOT a united atom in CHEAT and that the carboxy group is implemented as a charged group). The β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc interglycosidic dihedral angle ω is shown in Fig. 2c and 2d, for the tetra- and trisaccharide, respectively. The plots show some interchange between the GT and TG conformation for the tetrasaccharide, the trisaccharide existing mostly in the GT conformation.

To estimate the rotamer population distribution of ω, the method of adaptive umbrella sampling of the potential of mean force[33] was used. With this method it is possible to obtain free-energy differences between conformers, and as a consequence from this information the distribution. Umbrella sampling runs, with a duration of 15 ns, were performed for the

Table 5 Probability distributions of conformations of the β-D-galactopyranoside hydroxymethyl group,[34] and distributions around C5–C6 of the β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc part in **2** and **4**

	Gal[34]	Trisaccharide (**2**)	Tetrasaccharide (**4**)
TG	25	18	31
GG	5	2	3
GT	70	80	66

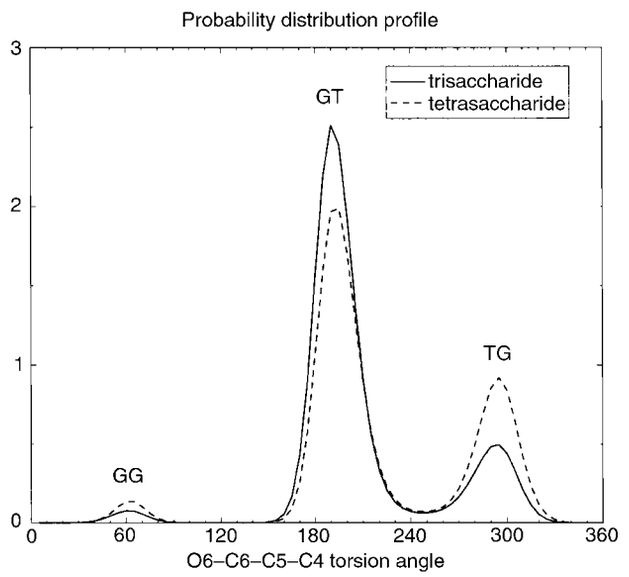


Fig. 3 Probability distribution profiles of the ω dihedral angle orientation in the tri- and the tetrasaccharide.

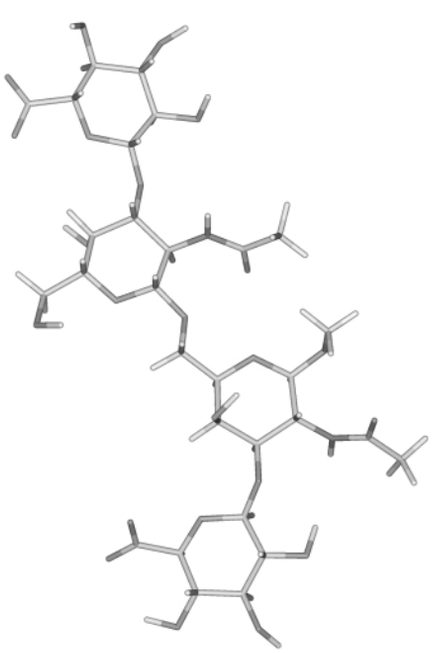


Fig. 4 GT Conformation of tetrasaccharide **4** as its methyl glycoside.

dihedral angle ω. The distribution profiles for the tri- and tetrasaccharide (displayed in Fig. 3) show that the GT conformation is predominant, but also that the TG conformation contributes significantly to the overall conformations of both the tri- and tetrasaccharide.

In summary, the results of the GG, GT and TG distributions around C5–C6 of β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc correspond with those found for the hydroxymethyl distribution for Gal[34] (for comparison see Table 5). However, no striking differences are observed between the tri- and tetrasaccharide

fragments. Interestingly, the resulting conformations show no interactions between the residues, except over the glycosidic linkages. The low free-energy barrier between the GT and TG conformations, which the umbrella sampling method estimated at 2 kcal mol^{-1} for both the tri- and tetrasaccharide, suggests a flexible polysaccharide molecule. In Fig. 4 the tetrasaccharide **4** in the GT conformation is represented.

Experimental

General procedures

In the work-up procedures of reaction mixtures, organic solutions were washed with appropriate amounts of indicated aqueous solutions, then dried ($MgSO_4$), and concentrated under reduced pressure at 40 °C (water-bath). Reactions were monitored by TLC on Silica Gel 60 F_{254} (Merck) with detection by UV light, then charring with either 10% H_2SO_4 in EtOH or 0.2% orcinol in 20% methanolic H_2SO_4. Column chromatography was performed on Silica Gel 60 F_{254} (Merck, 0.063–0.200 mm). Gel-permeation chromatography was carried out on Sephadex LH-20 or Toyopearl® HW-40S (Supelco) (2.0 × 60 cm). ^{1}H NMR spectra (300 MHz) were recorded at 25 °C with a Bruker AC 300 spectrometer. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si for solutions in $CDCl_3$ (δ 0), or by reference to acetone (δ 2.225) for solutions in D_2O. *J*-Values are given in Hz. Two-dimensional double-quantum filtered ^{1}H–^{1}H correlated spectra (2D DQF ^{1}H–^{1}H COSY spectra with various mixing times) were recorded at 300 K using a Bruker AMX 500 spectrometer. ^{13}C NMR spectra (75.5 MHz) were recorded with a Bruker AC 300 spectrometer; δ_C-values are given in ppm relative to the signal for $CDCl_3$ (δ_C 76.9). Optical rotations were determined for solutions in $CHCl_3$, unless otherwise stated, at 20 °C with a Perkin-Elmer 241 polarimeter, using a 10-cm 1-ml cell. $[\alpha]_D$-Values are given in 10^{-1} deg cm^2 g^{-1}. UV irradiations for synthetic purposes were performed in quartz vials at 254 nm using a Cole-Parmer®. 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. MALDI-TOF mass spectra were recorded on a Voyager-DE (PerSeptive Biosystems) instrument using sinapinic acid as the matrix; proteins were analyzed in the linear mode at an acceleration voltage of 22.5 kV. Samples for MALDI-TOF analysis were prepared as follows. First, an aliquot of 1 µl of the matrix solution was placed on the target stage of the mass spectrometer. After complete evaporation of the solvent, a 1-µl droplet of a 7.5 pmol ml^{-1} sample solution in acetonitrile–water (1:1) acidified with 0.1% trifluoroacetic acid was applied to the thin matrix film. Spectra were obtained by summing positive-ion signals of 183 to 188 laser shots. GLC was performed using a CP-Sil5 CB WCOT fused-silica capillary column (25 m × 0.34 mm, Chrompack). Native SDS-PAGE (8% acrylamide) was run on a Pharmacia Phast System according to the manufacturer's instructions. Bovine serum albumin (BSA) together with pre-treated BSA were used as reference and the gel was stained by a standard Coomassie technique. 3-Azidopropan-1-ol and 6-azidohexan-1-ol were prepared from the commercially available 3-bromopropan-1-ol and 6-bromohexan-1-ol, respectively. Crystalline BSA was obtained from Bayer Corporation.

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-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 9

To a solution of 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[5] **8** (272 mg, 0.13 mmol) in acetonitrile (12 ml) and water (1.3 ml) was added *p*-TsOH monohydrate (83 mg, 0.44 mmol). The mixture was stirred for 20 min at rt, then neutralized with triethylamine, and the solution was diluted with CH_2Cl_2 (250 ml), washed successively with 10% aq. $NaHCO_3$ (same vol., 1 ×) and 5% aq. NaCl (half vol., 1 ×), dried, filtered, and concentrated. Column chromatography (CH_2Cl_2–acetone, 9:1; 0.1% triethylamine) of the residue gave **9**, isolated as a colourless glass (208 mg, 82%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.94 (**8**), 0.22 (**9**); $[\alpha]_D$ + 1 (*c* 1, $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 2.15, 2.20, 2.21 (2 ×), 2.29 (2 ×), 2.30 (2 ×) and 2.31 (2 ×) [30 H, 6 s, 6 × $COC_6H_4CH_3$, 2 × $CO(CH_2)_2COCH_3$ and 2 × $COCH_3$], 2.68 [8 H, m, 2 × $CO(CH_2)_2COCH_3$], 4.35 and 4.51 (2 H, 2 dd, $J_{2,3}$ 11.2, $J_{2'',3''}$ 11.2, 2- and 2″-H), 4.71 and 4.86 (2 H, 2 d, 1′- and 1‴-H), 4.70 and 4.91 (2 H, 2 dd, $J_{3,4}$ 3.3, $J_{3'',4''}$ 3.3, 3- and 3″-H), 4.78 and 5.05 (2 H, 2 d, $J_{1,2}$ 8.5, $J_{1'',2''}$ 8.5, 1- and 1″-H), 5.20 and 5.29 (2 H, 2 dd, $J_{1',2'}$ 7.8, $J_{1''',2'''}$ 7.8, $J_{2',3'/2''',3'''}$ 9.8/10.0, 2′- and 2‴-H), 5.29 (1 H, m, $OCH_2CH{=}CH_2$), 5.37 (2 H, br t, 3′- and 3‴-H), 5.43 and 5.62 (2 H, 2 d, 4- and 4″-H), 5.59 and 5.64 (2 H, 2 br t, 4′- and 4‴-H), 6.82, 6.84, 6.96 (2 ×), 7.11, 7.12, 7.29, 7.30, 7.53 (2 ×), 7.72 and 7.74 (24 H, 10 d, 6 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 20.6 and 20.7 (2 × $COCH_3$), 21.3 (2 C) and 21.4 (4 C) (6 × $COC_6H_4CH_3$), 27.6, 27.7 and 37.8 (2 C) [2 × $CO(CH_2)_2COCH_3$], 29.5 and 29.6 [2 × $CO(CH_2)_2COCH_3$], 52.1 and 52.2 (C-2, -2″), 60.0, 62.0, 62.2, 64.1 and 68.8 (C-6, -6′, -6″, -6‴ and $OCH_2CH{=}CH_2$), 96.7 and 98.4 (C-1, -1″), 101.0 and 101.4 (C-1′, -1‴), 117.4 ($OCH_2CH{=}CH_2$), 164.0 (2 C), 164.8, 164.9 and 165.4 (2 C) (6 × $COC_6H_4CH_3$), 166.6, 168.4 (2 C) and 170.1 (2 × $COCH_3$ and 2 × COPhth), 172.2 and 172.3 [2 × $CO(CH_2)_2COCH_3$]; FABMS of $C_{105}H_{104}N_2O_{35}$ (M, 1954.0) *m/z* 1976.8 $(M + Na)^+$.

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-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 11

A mixture of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside[5] **10** (56 mg, 0.12 mmol) and **9** (115 mg, 59 µmol) in dry toluene (2 ml), containing molecular sieves 4 Å (0.2 g), was stirred under Ar for 2 h at rt. Then, NIS (16 mg, 72 µmol) and silver trifluoromethanesulfonate (15 mg, 58 µmol) were added, and the resulting suspension was stirred for 2.5 h at rt. After neutralization with pyridine, the mixture was diluted with CH_2Cl_2 (200 ml), and filtered over Celite. The organic phase was washed successively with 10% aq. $NaHSO_3$ (half vol., 2 ×), 10% aq. $NaHCO_3$ (same vol., 1 ×) and 5% aq. NaCl (half vol., 1 ×), dried, and concentrated. Sephadex LH-20 chromatography (CH_2Cl_2–MeOH, 1:1), followed by column chromatography (CH_2Cl_2–acetone, 92:8) of the residue yielded **11**, isolated as a colourless glass (92 mg, 66%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.67 (**10**), 0.54 (**11**); $[\alpha]_D$ +80 (*c* 1, $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.84, 2.11, 2.15 (2 ×), 2.16, 2.17, 2.21 (3 ×) and 2.31 (4 ×) [39 H, 7 s, 6 × $COC_6H_4CH_3$, 2 × $CO(CH_2)_2COCH_3$ and 5 × $COCH_3$], 2.60 [8 H, m, 2 × $CO(CH_2)_2COCH_3$], 3.28 and 3.50 (2 H, 2 m, $OCH_2CH{=}CH_2$), 4.40, 4.41 and 4.47 (3 H, 3 dd, $J_{1,2/1'',2''/1''',2'''}$ 8.4/8.5/8.5, $J_{2,3}$ 11.3, $J_{2'',3''}$ 11.3, $J_{2''',3'''}$ 11.3, 2-, 2″- and 2‴-H), 4.64 and 4.79 (2 H, 2 d, 1′- and 1‴-H), 4.69 and 4.74 (2 H, 2 dd, $J_{3,4}$ 3.4, $J_{3'',4''}$ 3.4, 3- and 3″-H), 4.76 and 4.94 (2 H, 2 d, 1- and 1″-H), 5.22 (1 H, m, $OCH_2CH{=}CH_2$), 5.18 and 5.22 (2 H, 2 dd, $J_{1',2'}$ 7.8, $J_{1''',2'''}$ 7.8, $J_{2',3'}$ 9.8, $J_{2''',3'''}$ 9.8, 2′- and 2‴-H), 5.36 and 5.40 (2 H, 2 br t, 3′- and 3‴-H), 5.46, 5.52 and 5.58 (3 H, 3 d, 4-, 4″- and 4‴-H), 5.55 and 5.60 (2 H, 2 t, $J_{4',5'}$ 9.6, $J_{4''',5'''}$ 9.6, 4′- and 4‴-H), 5.64 (1 H, d, $J_{1''',2'''}$ 8.4, 1‴-H), 5.93 (1 H, dd, $J_{3''',4'''}$ 3.5, 3‴-H), 6.85 (2 ×), 6.95 (2 ×), 7.09, 7.11, 7.29

(2 ×), 7.51 (2 ×), 7.69 and 7.70 (24 H, 8 d, 6 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 20.4 and 20.7 (4 C) (5 × $COCH_3$), 21.4 (2 C) and 21.5 (4 C) (6 × $COC_6H_4CH_3$), 27.7 (2 C) and 37.8 (2 C) [2 × $CO(CH_2)_2COCH_3$], 29.5 and 29.6 [2 × $CO(CH_2)_2COCH_3$], 52.1 (3 C) (C-2, -2″, -2‴), 96.7, 96.8 and 98.2 (C-1, -1″, -1‴), 100.9 and 101.0 (C-1′, -1‴), 117.4 ($OCH_2CH{=}CH_2$), 164.1 (2 C), 164.8 (2 C) and 165.4 (2 C) (6 × $COC_6H_4CH_3$), 169.6, 169.8, 170.2, 170.5 and 170.7 (5 × $COCH_3$), 172.1 and 172.2 [2 × $CO(CH_2)_2COCH_3$], 192.9 and 193.0 [2 × $CO(CH_2)_2COCH_3$]; FABMS of $C_{125}H_{123}N_3O_{44}$ (M, 2371.3) *m*/*z* 2394.0 $(M + Na)^+$.

Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galacto-pyranosyl)-(1→6)-[(2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranosyl)-(1→3)]-(4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galacto-pyranosyl)-(1→6)-[(2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranosyl)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyrano-side 12

To a solution of compound **11** (39 mg, 16 μmol) in 2:1 EtOH–toluene (4 ml) was added hydrazinium acetate (15 mg, 0.16 mmol). The mixture was stirred for 20 min at rt, then concentrated. Column chromatography (CH_2Cl_2–acetone, 9:1) of the residue gave **12**, isolated as a colourless glass (32 mg, 92%); TLC (toluene–EtOAc, 1:1) R_f 0.47 (**11**), 0.46 (**12**); $[a]_D$ +4 (*c* 1, $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.85, 2.12, 2.21 (3 ×), 2.22, 2.25, 2.27 (2 ×) and 2.32 (2 ×) (33 H, 7 s, 6 × $COC_6H_4CH_3$ and 5 × $COCH_3$), 3.17 and 3.37 (2 H, 2 m, $OCH_2CH{=}CH_2$), 4.38, 4.42 and 4.47 (3 H, 3 dd, $J_{1,2/1'',2''/1''',2'''}$ 8.4, $J_{2,3/2'',3''/2''',3'''}$ 11.1/11.2/11.3, 2-, 2″- and 2‴-H), 4.74 and 4.94 (2 H, 2 d, 1- and 1″-H), 4.74 and 4.85 (2 H, 2 d, $J_{1',2'/1'',2''}$ 7.8, 1′- and 1‴-H), 5.18 (1 H, m, $OCH_2CH{=}CH_2$), 5.30 and 5.32 (2 H, 2 t, $J_{2',3'/2'',3''}$ 9.6, $J_{3',4'/3'',4'''}$ 9.6, 3′- and 3‴-H), 5.55, 5.60 and 5.70 (3 H, 3 d, $J_{4,5/4'',5''/4''',5'''}$ 3.4/3.7, 4-, 4″- and 4‴-H), 5.59 and 5.63 (2 H, 2 t, 4′- and 4‴-H), 5.92 (1 H, dd, $J_{3''',4'''}$ 3.6, 3‴-H), 6.77 (2 ×), 6.94 (2 ×), 7.11 (2 ×), 7.24, 7.27, 7.49, 7.50, 7.72 and 7.73 (24 H, 9 d, 6 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 20.4, 20.7 (2 C) and 20.9 (2 C) (5 × $COCH_3$), 21.3 (2 C) and 21.5 (4 C) (6 × $COC_6H_4CH_3$), 52.0 and 52.2 (2 C) (C-2, -2″, -2‴), 61.0 (2 C) (C-6, -6″), 62.0 (C-6‴), 65.7 and 66.0 (C-6′, -6‴), 96.2, 96.8 and 98.2 (C-1, -1″, -1‴), 101.7 (C-1′, -1‴), 117.5 ($OCH_2CH{=}CH_2$), 163.9 (2 C), 165.0 (2 C), 166.5 (2 C) (6 × $COC_6H_4CH_3$), 166.7, 168.3 and 168.5 (3 × COPhth), 169.6, 170.2, 170.8, 171.3 and 172.4 (5 × $COCH_3$); FABMS of $C_{115}H_{111}N_3O_{40}$ (M, 2175.1) *m*/*z* 2197.8 $(M + Na)^+$.

Allyl (3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galacto-pyranosyl)-(1→6)-[(2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranos-yluronic acid)-(1→3)]-(4-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-(1→6)-[(2,3,4-tri-*O*-*p*-toluoyl-β-D-gluco-pyranosyluronic acid)-(1→3)]-4-*O*-acetyl-2-deoxy-2-phthal-imido-β-D-galactopyranoside 13

To a solution of compound **12** (30 mg, 14 μmol) in dry CH_2Cl_2 (1 ml) were added PDC (21 mg, 84 μmol), acetic anhydride (14 μl, 0.15 mmol) and a catalytic amount of dry pyridine. The reaction mixture was stirred for 3.5 h at rt, then EtOAc (1 ml) was added. Column chromatography (EtOAc ⟶ EtOAc–HOAc, 98:2) of the suspension yielded **13** (22 mg, 73%); TLC (CH_2Cl_2–acetone–HOAc, 18:2:1) R_f 0.19 (**13**); $[a]_D$ +3 (*c* 1, $CHCl_3$). A solution of **13** in 5:1 MeOH–CH_2Cl_2 (5 ml) was stirred with Dowex-50 (Na^+) for 30 min, filtered, concentrated, and analyzed by ^{13}C NMR: δ_C (75.5 MHz; $CDCl_3$) 20.4 and 20.6 (4 C) (5 × $COCH_3$), 21.3 (2 C) and 21.5 (4 C) (6 × $COC_6H_4CH_3$), 51.3 and 52.4 (2 C) (C-2, -2″, -2‴), 62.7 (C-6, -6″, -6‴), 97.1, 97.4 and 97.5 (C-1, -1″, -1‴), 100.9 and 101.0 (C-1′, -1‴), 117.1 ($OCH_2CH{=}CH_2$), 164.3, 165.4, 166.0 (2 C) and 166.5 (2 C) (6 × $COC_6H_4CH_3$), 169.8 (3 C) and 170.4 (2 C) (5 × $COCH_3$).

A small amount of acid **13** was esterified with diazomethane in diethyl ether (**13a**), and analyzed by ^1H NMR: δ_H (300 MHz; $CDCl_3$) 1.84, 2.10, 2.15, 2.17, 2.20, 2.21, 2.23 (2 ×), 2.33 (2 ×) and 2.34 (33 H, 9 s, 6 × $COC_6H_4CH_3$ and 5 × $COCH_3$), 3.64 and 3.67 (6 H, 2 s, 2 × $COOCH_3$), 4.66, 4.80, 4.85 and 4.91 (4 H, 4 d, $J_{1,2/1'',2''/1''',2'''}$ 7.7/8.6/7.7/8.4, 1-, 1″-, 1‴- and 1⁗-H), 5.24 (1 H, m, $OCH_2CH{=}CH_2$), 5.42 and 5.55 (2 H, 2 br t, $J_{3',4'/3''',4'''}$ 9.6, 3′- and 3‴-H), 5.44, 5.49 and 5.67 (3 H, 3 d, 4-, 4″- and 4⁗-H), 5.93 (1 H, dd, 3⁗-H), 6.90, 6.98, 7.11, 7.29, 7.54 and 7.71 (24 H, 6 dd, 6 × $COC_6H_4CH_3$); for acid **13**: FABMS of $C_{115}H_{109}N_3O_{42}$ (M, 2203.1) *m*/*z* 2225.8 $(M + Na)^+$.

Allyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(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 14

A solution of compound **13** (27 mg, 12 μmol) in ethanolic 33% $MeNH_2$ (15 ml) was stirred for 2 weeks at rt, during which the mixture was concentrated repeatedly and new reagent (8 × 10 ml) added. After concentration, the residue was dissolved in dry MeOH (3.6 ml), and acetic anhydride (100 μl) was added at 0 °C. The mixture was stirred for 2 h, then concentrated and co-concentrated with 1:1 toluene–MeOH (3 × 10 ml). The residue was purified on Toyopearl HW-40S (5 mM aq. NH_4HCO_3) to give, after lyophilization, **14** (8 mg, 65%); TLC (butan-1-ol–MeOH–water–HOAc, 8:4:4:1) R_f 0.30 (**14**); $[a]_D$ −14 (*c* 0.8, water); δ_H (500 MHz; D_2O; TOCSY) 2.00, 2.02 and 2.03 (9 H, 3 s, 3 × $NHCOCH_3$), 3.33 and 3.34 (2 H, 2 dd, $J_{1',2'/1'',2''}$ 8.0, $J_{2',3'/2'',3''}$ 9.2, 2′- and 2‴-H), 3.45 (2 H, br t, $J_{3',4'/3'',4'''}$ 9.2, 3′- and 3‴-H), 3.48 (2 H, t, $J_{4',5'/4''',5'''}$ 8.3, 4′- and 4‴-H), 3.69 (2 H, d, 5′- and 5‴-H), 3.76 (1 H, dd, $J_{3''',4'''}$ 3.0, 3⁗-H), 3.82 and 3.86 (2 H, 2 dd, 3- and 3″-H), 3.93 (1 H, dd, 2⁗-H), 3.95 (1 H, dd, $J_{4''',5'''}$ < 1, 4⁗-H), 3.98 and 4.01 (2 H, 2 dd, 2- and 2″-H), 4.13 and 4.15 (2 H, 2 d, 4- and 4″-H), 4.48 (1 H, d, 1⁗-H), 4.50 and 4.51 (2 H, 2 d, 1′- and 1‴-H), 4.51 and 4.53 (2 H, 2 d, $J_{1,2/1'',2''}$ 8.6, 1- and 1″-H), 5.27–5.39 (2 H, m, $OCH_2CH{=}CH_2$), 5.91 (1 H, m, $OCH_2CH{=}CH_2$); FABMS of $C_{39}H_{61}N_3O_{28}$ (M, 1019.6) *m*/*z* 1042.4 $(M + Na)^+$.

3-(2-Aminoethylthio)propyl (2-acetamido-2-deoxy-β-D-galacto-pyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-gluco-pyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galacto-pyranoside 5

Allyl glycoside **14** (2.5 mg, 2.5 μmol) was dissolved in aq. cysteamine hydrochloride (1.5 mg, 13.2 μmol in 250 μl), and the solution was irradiated with UV light for 4.5 h at rt. The product was purified by HiTrap gel filtration (5 mM aq. NH_4HCO_3) to afford, after lyophilization, the title compound **5** (2.2 mg, 80%). TLC (butan-1-ol–water–HOAc, 2:1:1) R_f 0.53 (**14**), 0.10 (**5**); $[\alpha]_D$ −15 (*c* 0.2, water); ^1H NMR data are given in Table 1; FABMS of $C_{41}H_{68}N_4O_{28}S$ (M, 1096.1) *m*/*z* 1094.9 $(M - H)^-$.

Allyl 2-acetamido-2-deoxy-β-D-galactopyranoside 19

To a solution of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-galactopyranose **15** (85 mg, 0.22 mmol) in dry CH_2Cl_2 (1 ml) was added $NH_2NH_2{\cdot}HOAc$ (26 mg, 0.28 mmol). The mixture was stirred for 1 h at 60 °C, when TLC (CH_2Cl_2–acetone, 9:1) showed the complete conversion of **15** into **16**. The mixture was concentrated, and the material was directly used for the next reaction.

To a solution of the residue in dry CH_2Cl_2 (2.2 ml) and trichloroacetonitrile (0.2 ml, 2.0 mmol) at 0 °C was added DBU (10 μl). The mixture was stirred overnight, then concentrated. Column chromatography (CH_2Cl_2–acetone, 9:1) of the residue furnished **17**, isolated as a syrup (61 mg, 56% over two steps).

A solution of **17** (50 mg, 0.10 mmol) and allyl alcohol (88 μl, 1.0 mmol) in dry CH_2Cl_2 (0.75 ml) containing molecular sieves 4 Å (10 mg) was stirred for 1 h under Ar. Then, Me_3SiOTf (2.7 μl, 14 μmol) was added and the mixture was stirred for 30 min.

After neutralization with pyridine, dilution with CH_2Cl_2 (80 ml), and filtration, the solution was washed with 5% aq. NaCl (half vol., 1 ×), dried, filtered, and concentrated. Column chromatography (CH_2Cl_2–acetone, 95:5) of the residue gave **18**, isolated as a syrup (30 mg, 80%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.72 (**15**), 0.22 (**16**), 0.60 (**17**), 0.55 (**18**); $[\alpha]_D$ −17 (*c* 1, $CHCl_3$) (Found: C, 52.58; H, 6.51. $C_{17}H_{25}NO_9$ requires C, 52.71; H, 6.46%); δ_H (300 MHz; $CDCl_3$) 1.95, 2.00, 2.05 and 2.15 (12 H, 4 s, 4 × $COCH_3$), 3.99 (1 H, m, $J_{1,2}$ 8.5, $J_{2,3}$ 11.1, 2-H), 4.75 (1 H, d, $J_{1,2}$ 8.4, 1-H), 5.20 and 5.28 (2 H, 2 m, $OCH_2CH{=}CH_2$), 5.31 (1 H, dd, $J_{3,4}$ 3.4, 3-H), 5.37 (1 H, dd, $J_{4,5}$ 1.0, 4-H), 5.88 (1 H, m, $OCH_2CH{=}CH_2$); δ_C (75.5 MHz; $CDCl_3$) 20.6 ($COCH_3$), 23.4 ($NHCOCH_3$), 51.7 (C-2), 61.4 (C-6), 99.8 (C-1), 117.8 ($OCH_2CH{=}CH_2$), 133.5 ($OCH_2CH{=}CH_2$), 170.2 (2 C), 170.3 and 170.4 (4 × $COCH_3$); FABMS of $C_{17}H_{25}NO_9$ (M, 387.1) *m*/*z* 388.1 (M + H)$^+$, 410.1 (M + Na)$^+$.

To a solution of **18** (25 mg, 65 μmol) in 5:1 MeOH–CH_2Cl_2 (3 ml) was added NaOMe to pH 10, and the mixture was stirred overnight. After neutralization with Dowex-50 (H^+), the mixture was filtered and concentrated. Column chromatography (EtOAc–MeOH, 3:1) of the residue gave **19**, isolated as a colourless glass (15 mg, 88%); TLC (EtOAc–MeOH, 3:1) R_f 0.29 (**19**); $[\alpha]_D$ −45 (*c* 0.6, water); δ_H (300 MHz; D_2O) 2.04 (3 H, s, $NHCOCH_3$), 3.72 (1 H, dd, $J_{2,3}$ 10.8, 3-H), 3.90 (1 H, dd, 2-H), 3.93 (1 H, d, $J_{3,4}$ 3.3, $J_{4,5}$ < 1, 4-H), 4.50 (1 H, d, $J_{1,2}$ 8.5, 1-H), 5.26 and 5.31 (2 H, 2 m, $OCH_2CH{=}CH_2$), 5.91 (1 H, m, $OCH_2CH{=}CH_2$); FABMS of $C_{11}H_{19}NO_6$ (M, 261.3) *m*/*z* 262.1 (M + H)$^+$.

3-(2-Aminoethylthio)propyl 2-acetamido-2-deoxy-β-D-galactopyranoside 6

Allyl glycoside **19** (3 mg, 11 μmol) was dissolved in aq. cysteamine hydrochloride (1.3 mg, 11 μmol in 130 μl), and the solution was irradiated with UV light for 2 h at rt. After concentration of the mixture, column chromatography (MeOH–water, 9:1) of the residue afforded **6**, isolated as a colourless glass (3 mg, 81%); TLC (butan-1-ol–MeOH–water–HOAc, 4:2:2:1) R_f 0.57 (**19**), 0.32 (**6**); $[\alpha]_D$ −1 (*c* 0.2, water); δ_H (300 MHz; D_2O) 1.81–1.90 [2 H, m, $OCH_2CH_2CH_2S(CH_2)_2NH_2$], 2.05 (3 H, s, $NHCOCH_3$), 2.63 and 3.03 [4 H, 2 t, $O(CH_2)_2CH_2SCH_2$-CH_2NH_2], 3.41 (2 H, t, $SCH_2CH_2NH_2$), 3.72 (1 H, dd, $J_{2,3}$ 10.9, 3-H), 3.87 (1 H, dd, 2-H), 3.94 (1 H, d, $J_{3,4}$ 3.3, $J_{4,5}$ < 1, 4-H), 4.44 (1 H, d, $J_{1,2}$ 8.6, 1-H); FABMS of $C_{13}H_{26}N_2O_6S$ (M, 338.4) *m*/*z* 339.1 (M + H)$^+$.

6-Azidohexyl 6-*O*-levulinoyl-2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranoside 22

A solution of 6-*O*-levulinoyl-2,3,4-tri-*O*-*p*-toluoyl-α-D-glucopyranosyl trichloroacetimidate[5] **20** (135 mg, 0.19 mmol) and 6-azidohexan-1-ol **21** (140 mg, 0.97 mmol) in dry CH_2Cl_2 (2 ml), containing molecular sieves 4 Å (10 mg), was stirred for 1 h under Ar. Then, Me_3SiOTf (1.7 μl, 8.8 μmol) was added and the mixture was stirred for 15 min. After neutralization with pyridine, dilution with CH_2Cl_2 (150 ml) and filtration, the solution was washed with 5% aq. NaCl (half vol., 1 ×), dried, filtered, and concentrated. Column chromatography (CH_2Cl_2–acetone, 95:5) of the residue gave **22**, isolated as a syrup (119 mg, 83%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.59 (**20**), 0.65 (**22**); $[\alpha]_D$ −7 (*c* 1, $CHCl_3$) (Found: C, 64.76; H, 6.22. $C_{41}H_{47}N_3O_{11}$ requires C, 64.98; H, 6.25%); δ_H (300 MHz; $CDCl_3$) 1.18–1.26 [4 H, m, $O(CH_2)_2(CH_2)_2(CH_2)_2N_3$], 1.32–1.38 [2 H, m, $O(CH_2)_4CH_2CH_2N_3$], 1.45–1.54 [2 H, m, $OCH_2CH_2(CH_2)_4N_3$], 2.26, 2.32, 2.33 and 2.35 [12 H, 4 s, $CO(CH_2)_2COCH_3$ and 3 × $COC_6H_4CH_3$], 2.55–2.74 [4 H, m, $CO(CH_2)_2COCH_3$], 3.06 [2 H, t, $O(CH_2)_5CH_2N_3$], 3.54 and 3.94 [2 H, 2 m, OCH_2-$(CH_2)_5N_3$], 4.78 (1 H, d, $J_{1,2}$ 7.9, 1-H), 5.46 (1 H, dd, $J_{2,3}$ 9.8, 2-H), 5.51 (1 H, t, $J_{3,4}$ 9.7, 3-H), 5.84 (1 H, t, $J_{4,5}$ 9.7, 4-H), 7.05, 7.14, 7.17, 7.71, 7.80 and 7.84 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 21.4 ($COC_6H_4CH_3$), 25.2, 26.1, 28.4 and 29.0 [$OCH_2(CH_2)_4CH_2N_3$], 27.7 and 37.7 [$CO(CH_2)_2COCH_3$], 29.6 [$CO(CH_2)_2COCH_3$], 51.0 [$O(CH_2)_5CH_2N_3$], 62.7 (C-6), 69.7 [$OCH_2(CH_2)_5N_3$], 101.1 (C-1), 164.8, 165.0 and 165.5 (3 × $COC_6H_4CH_3$), 172.1 [$CO(CH_2)_2COCH_3$]; FABMS of $C_{41}H_{47}N_3O_{11}$ (M, 757.8) *m*/*z* 758.5 (M + H)$^+$, 780.5 (M + Na)$^+$.

6-Azidohexyl 2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranoside 23

To a solution of **22** (107 mg, 0.14 mmol) in 2:1 EtOH–toluene (14 ml) was added NH_2NH_2·HOAc (60 mg, 0.65 mmol). The mixture was stirred for 45 min, then concentrated. Column chromatography (CH_2Cl_2–acetone, 95:5) of the residue furnished **23**, isolated as a colourless glass (82 mg, 89%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.76 (**22**), 0.55 (**23**); $[\alpha]_D$ −5 (*c* 0.7, $CHCl_3$) (Found: C, 65.63; H, 6.32. $C_{36}H_{41}N_3O_9$ requires C, 65.54; H, 6.26%); δ_H (300 MHz; $CDCl_3$) 1.18–1.58 [8 H, m, $O(CH_2)(CH_2)_4(CH_2)N_3$], 2.27, 2.34 and 2.35 (9 H, 3 s, 3 × $COC_6H_4CH_3$), 3.06 [2 H, t, $O(CH_2)_5CH_2N_3$], 3.53 and 3.95 [2 H, 2 m, $OCH_2(CH_2)_5N_3$], 4.79 (1 H, d, $J_{1,2}$ 7.9, 1-H), 5.47 (1 H, dd, $J_{2,3}$ 9.9, 2-H), 5.44 (1 H, t, $J_{3,4}$ 9.7, 3-H), 5.90 (1 H, t, $J_{4,5}$ 9.7, 4-H), 7.06, 7.16, 7.17, 7.74, 7.83 and 7.85 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 21.4 and 21.5 (2 C) (3 × $COC_6H_4CH_3$), 25.2, 26.1, 28.4 and 29.1 [$OCH_2(CH_2)_4$-CH_2N_3], 51.0 [$O(CH_2)_5CH_2N_3$], 61.3 (C-6), 69.7 [$OCH_2(CH_2)_5$-N_3], 101.2 (C-1), 164.8, 165.7 and 166.0 (3 × $COC_6H_4CH_3$); FABMS of $C_{36}H_{41}N_3O_9$ (M, 659.7) *m*/*z* 660.5 (M + H)$^+$, 682.4 (M + Na)$^+$.

6-Azidohexyl 2,3,4-tri-*O*-*p*-toluoyl-β-D-glucopyranosiduronic acid 24

To a solution of **23** (42 mg, 64 μmol) in CH_2Cl_2 (0.3 ml) were added a catalytic amount of TEMPO, KBr (740 μg, 6.2 μmol), Bu_4NCl (1.7 mg, 6.2 μmol), and saturated aq. $NaHCO_3$ (0.2 ml). The suspension was cooled to 0 °C and under vigorously stirring a mixture of 0.35 M aq. NaOCl (84 μl, 79 μmol), saturated aq. $NaHCO_3$ (108 μl), and saturated aq. NaCl (138 μl) were added dropwise. The mixture was stirred for 15 min, then acidified (pH 2) by addition of 4 M aq. HCl and mixed with CH_2Cl_2 (100 ml). The organic layer was washed with 10% aq. NaCl (half vol., 1 ×), dried, filtered, and concentrated. Column chromatography (CH_2Cl_2–acetone, 8:2 ⟶ CH_2Cl_2–acetone–HOAc, 8:2:0.5) of the residue afforded **24** (34 mg, 79%); TLC (CH_2Cl_2–acetone–HOAc, 9:1:0.5) R_f 0.37 (**24**); $[\alpha]_D$ −1 (*c* 1, $CHCl_3$) (Found: C, 63.89; H, 6.08. $C_{36}H_{39}N_3O_{10}$ requires C, 64.08; H, 5.98%); δ_H (300 MHz; $CDCl_3$) 1.18–1.29 [4 H, m, $O(CH_2)_2(CH_2)_2(CH_2)_2N_3$], 1.31–1.40 [2 H, m, $O(CH_2)_4CH_2$-CH_2N_3], 1.45–1.60 [2 H, m, $OCH_2CH_2(CH_2)_4N_3$], 2.29, 2.34 and 2.37 (9 H, 3 s, 3 × $COC_6H_4CH_3$), 3.07 [2 H, t, $O(CH_2)_5$-CH_2N_3], 3.53 and 3.97 [2 H, 2 m, $OCH_2(CH_2)_5N_3$], 4.37 (1 H, d, $J_{4,5}$ 9.2, 5-H), 4.85 (1 H, d, $J_{1,2}$ 7.3, 1-H), 5.48 (1 H, dd, $J_{2,3}$ 9.1, 2-H), 5.69 (1 H, t, $J_{3,4}$ 9.2, 3-H), 5.86 (1 H, t, 4-H), 7.08, 7.13, 7.17, 7.74, 7.80 and 7.84 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 21.5 ($COC_6H_4CH_3$), 25.2, 26.1, 28.5 and 29.0 [$OCH_2(CH_2)_4CH_2N_3$], 51.1 [$O(CH_2)_5CH_2N_3$], 70.2 [OCH_2-$(CH_2)_5N_3$], 101.0 (C-1), 164.9, 165.3 and 165.5 (3 × $COC_6H_4CH_3$), 170.1 (*C*OOH).

A small amount of acid **24** was esterified with diazomethane in diethyl ether (**24a**), and analyzed by ^{1}H NMR: δ_H (300 MHz; $CDCl_3$) 1.18–1.60 [8 H, m, $OCH_2(CH_2)_4(CH_2)N_3$], 2.30, 2.36 and 2.37 (9 H, 3 s, 3 × $COC_6H_4CH_3$), 3.08 [2 H, t, $O(CH_2)_5$-CH_2N_3], 3.52 and 3.97 [2 H, 2 m, $OCH_2(CH_2)_5N_3$], 3.69 (3 H, s, $COOCH_3$), 4.31 (1 H, d, $J_{4,5}$ 9.6, 5-H), 4.81 (1 H, d, $J_{1,2}$ 7.5, 1-H), 5.49 (1 H, dd, $J_{2,3}$ 9.5, 2-H), 5.64 (1 H, t, $J_{3,4}$ 9.5, 3-H), 5.87 (1 H, t, 4-H), 7.09, 7.17, 7.18, 7.75, 7.81 and 7.84 (12 H, 6 d, 3 × $COC_6H_4CH_3$); for acid **24**: FABMS of $C_{36}H_{39}N_3O_{10}$ (M, 673.7) *m*/*z* 674.4 (M + H)$^+$, 696.4 (M + Na)$^+$.

6-Azidohexyl β-D-glucopyranosiduronic acid 25

A solution of compound **24** (22 mg, 32 μmol) in ethanolic 33%

$MeNH_2$ (5 ml) was stirred for 7 days at rt, during which the mixture was concentrated repeatedly and new reagent (3 × 5 ml) was added. Column chromatography (EtOAc–MeOH–H_2O, 10:5:1) of the residue gave **25** (9 mg, 88%); TLC (butan-1-ol–MeOH–water–HOAc, 8:4:4:1) R_f 0.65 (**25**); $[a]_D$ −30 (*c* 1, water); δ_H (300 MHz; D_2O) 1.35–1.44 and 1.56–1.69 [8 H, 2 m, $OCH_2(CH_2)_4CH_2N_3$], 3.16 (1 H, dd, 2-H), 3.32 [2 H, t, $O(CH_2)_5CH_2N_3$], 3.51 (1 H, d, $J_{4,5}$ 8.9, 5-H), 3.64 (1 H, t, $J_{2,3}$ 9.5, $J_{3,4}$ 9.8, 3-H), 3.67 and 3.93 [2 H, 2 m, $OCH_2(CH_2)_5N_3$], 3.70 (1 H, t, 4-H), 4.45 (1 H, d, $J_{1,2}$ 8.0, 1-H); FABMS of $C_{12}H_{21}N_3O_7$ (M, 319.3) *m*/*z* 318.1 $(M - H)^-$.

6-Aminohexyl β-D-glucopyranosiduronic acid 7

A solution of **25** (5.0 mg, 16 μmol) in MeOH (0.5 ml) and HOAc (50 μl) was hydrogenolyzed in the presence of 10% Pd–C (6.4 mg) under H_2 for 2 h at rt. Then, the mixture was filtered and concentrated. Column chromatography (EtOAc–MeOH–water, 7:5:1) of the residue, followed by lyophilization from water, afforded **7**, isolated as a white powder (3.5 mg, 75%); TLC (EtOAc–MeOH–H_2O, 10:5:1) R_f 0.54 (**25**), 0.05 (**7**); $[a]_D$ −17 (*c* 0.4, water); δ_H (300 MHz; D_2O) 1.32–1.46 and 1.58–1.70 [8 H, 2 m, $OCH_2(CH_2)_4CH_2NH_2$], 2.93 [2 H, t, $O(CH_2)_5CH_2NH_2$], 3.17 (1 H, dd, 2-H), 3.50 (1 H, d, $J_{4,5}$ 8.8, 5-H), 3.66 and 3.92 [2 H, 2 m, $OCH_2(CH_2)_5NH_2$], 3.66 (1 H, t, $J_{2,3}$ 9.2, $J_{3,4}$ 9.2, 3-H), 3.68 (1 H, t, 4-H), 4.45 (1 H, d, $J_{1,2}$ 7.9, 1-H); FABMS of $C_{12}H_{23}NO_7$ (M, 293.3) *m*/*z* 294.1 $(M + H)^+$.

3-Azidopropyl (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 28

To a solution of (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[5] **26** (0.14 g, 0.12 mmol) and 3-azidopropan-1-ol **27** (53 mg, 0.52 mmol) in dry CH_2Cl_2 (3.2 ml), containing molecular sieves 4 Å (0.1 g), was added at rt Me_3SiOTf (1.1 μl, 5.7 μmol). After stirring for 15 min, the mixture was neutralized with triethylamine, and CH_2Cl_2 (150 ml) was added. The organic layer was washed with 5% aq. NaCl (half vol., 1 ×), dried, filtered, and concentrated. Column chromatography (CH_2Cl_2–acetone, 96:4) of the residue gave **28** (0.11 g, 81%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.75 (**26**), 0.80 (**28**); $[a]_D$ +7 (*c* 1, $CHCl_3$) (Found: C, 62.11; H, 6.15. $C_{60}H_{70}N_4O_{18}Si$ requires C, 61.91; H, 6.02%); δ_H (300 MHz; $CDCl_3$) 0.06 and 0.07 [6 H, 2 s, $Si(CH_3)_2C(CH_3)_3$], 0.89 [9 H, s, $Si(CH_3)_2C(CH_3)_3$], 1.50–1.75 (2 H, m, $OCH_2CH_2CH_2N_3$), 2.20 (3 H, s, $COCH_3$), 2.22 (2 ×), 2.32 and 2.33 [12 H, 3 s, $CO(CH_2)_2COCH_3$ and 3 × $COC_6H_4CH_3$], 2.60 and 2.76 [4 H, 2 m, $CO(CH_2)_2COCH_3$], 2.98–3.09 (2 H, m, $OCH_2CH_2CH_2N_3$), 3.43 and 3.79 (2 H, 2 m, $OCH_2CH_2CH_2N_3$), 4.48 (1 H, dd, $J_{1,2}$ 8.5, $J_{2,3}$ 11.2, 2-H), 4.78 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 4.84 (1 H, dd, $J_{3,4}$ 3.3, 3-H), 5.00 (1 H, d, 1-H), 5.26 (1 H, dd, $J_{2',3'}$ 9.8, 2′-H), 5.44 (1 H, t, $J_{3',4'}$ 9.7, 3′-H), 5.61 (1 H, d, $J_{4,5}$ <1, 4-H), 5.64 (1 H, t, 4′-H), 6.88, 6.98, 7.12, 7.37, 7.56 and 7.74 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 18.0 [$Si(CH_3)_2C(CH_3)_3$], 20.7 ($COCH_3$), 21.2 and 21.4 (2 C) (3 × $COC_6H_4CH_3$), 25.6 [$Si(CH_3)_2C(CH_3)_3$], 27.7 and 37.8 [$CO(CH_2)_2COCH_3$], 29.6 [$CO(CH_2)_2COCH_3$], 28.5, 47.7 and 65.8 ($OCH_2CH_2CH_2N_3$), 52.4 (C-2), 62.1 (2 C) (C-6, -6′), 98.4 and 101.0 (C-1, -1′), 122.7, 123.1, 130.7 and 133.5 (Phth), 164.2, 164.8 and 165.4 (3 × $COC_6H_4CH_3$), 169.8 ($COCH_3$), 172.1 [$CO(CH_2)_2COCH_3$]; FABMS of $C_{60}H_{70}N_4O_{18}Si$ (M, 1162.8) *m*/*z* 1185.5 $(M + Na)^+$.

3-Azidopropyl (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 29

To a solution of **28** (96 mg, 85 μmol) in acetonitrile (7.9 ml), containing water (0.9 ml), was added *p*-TsOH monohydrate (30 mg, 0.16 mmol). The mixture was stirred for 45 min, then diluted with CH_2Cl_2 (150 ml). The organic layer was washed successively with 10% aq. $NaHCO_3$ (same vol., 1 ×) and 5% aq. NaCl (half vol., 1 ×), dried, filtered, and concentrated. Column chromatography (CH_2Cl_2–acetone, 96:4; 0.1% triethylamine) of the residue gave **29** (59 mg, 67%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.16 (**29**); $[a]_D$ +4 (*c* 1, $CHCl_3$) (Found: C, 61.20; H, 5.03. $C_{54}H_{56}N_4O_{18}$ requires C, 61.83; H, 5.34%); δ_H (300 MHz; $CDCl_3$) 1.63 (2 H, m, $OCH_2CH_2CH_2N_3$), 2.21 (3 H, s, $COCH_3$), 2.23, 2.30, 2.31 and 2.32 [12 H, 4 s, $CO(CH_2)_2COCH_3$ and 3 × $COC_6H_4CH_3$], 2.51–2.87 [4 H, m, $CO(CH_2)_2COCH_3$], 3.41 and 3.82 (2 H, 2 m, $OCH_2CH_2CH_2N_3$), 4.53 (1 H, dd, $J_{1,2}$ 8.5, $J_{2,3}$ 11.2, 2-H), 4.88 (1 H, d, $J_{1',2'}$ 7.9, 1′-H), 4.91 (1 H, dd, $J_{3,4}$ 3.4, 3-H), 5.02 (1 H, d, 1-H), 5.32 (1 H, dd, $J_{2',3'}$ 9.9, 2′-H), 5.40 (1 H, t, $J_{3',4'}$ 9.8, 3′-H), 5.60 (1 H, d, $J_{4,5}$ <1, 4-H), 5.68 (1 H, t, $J_{4',5'}$ 9.7, 4′-H), 6.84, 6.98, 7.13, 7.33, 7.55 and 7.74 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 20.4 ($COCH_3$), 21.4 ($COC_6H_4CH_3$), 27.7 and 37.8 [$CO(CH_2)_2COCH_3$], 29.9 [$CO(CH_2)_2COCH_3$], 28.6, 47.6 and 66.0 ($OCH_2CH_2CH_2N_3$), 52.3 (C-2), 59.9 and 62.2 (C-6, -6′), 98.6 (C-1), 101.5 (C-1′), 122.6, 123.1, 130.7 and 133.4 (Phth), 164.1, 165.0 and 165.4 (3 × $COC_6H_4CH_3$), 172.4 [$CO(CH_2)_2COCH_3$]; FABMS of $C_{54}H_{56}N_4O_{18}$ (M, 1048.8) *m*/*z* 1071.4 $(M + Na)^+$.

3-Azidopropyl (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 30

A mixture of **10** (52 mg, 0.11 mmol) and **29** (54 mg, 51 μmol) in dry toluene (1.5 ml) containing molecular sieves 4 Å (0.1 g) was stirred under Ar for 2 h at rt. Then, NIS (15 mg, 67 μmol) and silver trifluoromethanesulfonate (14 mg, 55 μmol) were added, and the resulting suspension was stirred for 2 h at rt. After neutralization with pyridine, the mixture was diluted with CH_2Cl_2 (100 ml), filtered over Celite, washed successively with 10% aq. $NaHSO_3$ (half vol., 3 ×), 10% aq. $NaHCO_3$ (same vol., 1 ×) and 5% aq. NaCl (half vol., 1 ×), dried, and concentrated. The residue was purified by sequential Sephadex LH-20 chromatography (CH_2Cl_2–MeOH, 1:1) and Silica Gel column chromatography (CH_2Cl_2 ⟶ CH_2Cl_2–acetone, 95:5) to yield **30**, isolated as a colourless glass (33 mg, 42%); TLC (CH_2Cl_2–acetone, 9:1) R_f 0.65 (**10**), 0.40 (**30**); $[a]_D$ +4 (*c* 1, $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.29–1.48 (2 H, m, $OCH_2CH_2CH_2N_3$), 1.83, 2.09, 2.18 and 2.20 (12 H, 4 s, 4 × $COCH_3$), 2.20, 2.23, 2.31 and 2.33 [12 H, 4 s, $CO(CH_2)_2COCH_3$ and 3 × $COC_6H_4CH_3$], 2.59 and 2.78 [4 H, 2 m, $CO(CH_2)_2COCH_3$], 2.85–2.90 (2 H, m, $OCH_2CH_2CH_2N_3$), 3.12 and 3.42 (2 H, 2 m, $OCH_2CH_2CH_2N_3$), 4.37 (1 H, dd, $J_{1'',2''}$ 8.5, $J_{2'',3''}$ 11.2, 2″-H), 4.52 (1 H, dd, $J_{1,2}$ 8.5, $J_{2,3}$ 11.5, 2-H), 4.72 (1 H, d, $J_{1',2'}$ 7.7, 1′-H), 4.73 (1 H, dd, $J_{3,4}$ 3.4, 3-H), 4.82 (1 H, d, 1-H), 5.22 (1 H, dd, $J_{2',3'}$ 9.8, 2′-H), 5.36 (1 H, d, 1″-H), 5.39 (1 H, t, $J_{3',4'}$ 9.8, 3′-H), 5.46 (1 H, d, $J_{4,5}$ <1, 4-H), 5.48 (1 H, d, $J_{4'',5''}$ <1, 4″-H), 5.60 (1 H, t, $J_{4',5'}$ 9.7, 4′-H), 5.80 (1 H, dd, $J_{3'',4''}$ 3.4, 3″-H), 6.86, 6.97, 7.12, 7.32, 7.54 and 7.73 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 20.3, 20.5 (2 C) and 20.7 (4 × $COCH_3$), 21.4 ($COC_6H_4CH_3$), 27.7 and 37.8 [$CO(CH_2)_2COCH_3$], 28.6, 47.6 and 66.5 ($OCH_2CH_2CH_2N_3$), 51.3 and 52.2 (C-2, -2″), 98.1 (2 C) (C-1, -1‴), 101.1 (C-1′), 123.3, 130.7 and 133.5 (Phth); FABMS of $C_{74}H_{75}N_5O_{27}$ (M, 1465.0) *m*/*z* 1488.5 $(M + Na)^+$.

3-Azidopropyl (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 31

To a solution of compound **30** (25 mg, 18 μmol) in 2:1 MeOH–toluene (2.8 ml) was added hydrazinium acetate (17 mg, 0.18 mmol). The mixture was stirred for 20 min at rt, then concentrated. Column chromatography (CH_2Cl_2–acetone, 93:7) of the residue gave **31** (20 mg, 84%); TLC (CH_2Cl_2–acetone, 9:1) R_f

0.48 (**31**); $[\alpha]_D$ −1 (*c* 1, $CHCl_3$) (Found: C, 60.18; H, 4.92. $C_{69}H_{69}N_5O_{25}$ requires C, 60.55; H, 5.04); δ_H (300 MHz; $CDCl_3$) 1.84, 2.09, 2.21 and 2.22 (12 H, 4 s, 4 × $COCH_3$), 2.25, 2.28 and 2.33 (9 H, 3 s, 3 × $COC_6H_4CH_3$), 2.84–2.90 (2 H, m, $OCH_2CH_2CH_2N_3$), 3.15 and 3.41 (2 H, 2 m, $OCH_2CH_2CH_2N_3$), 4.38 (1 H, dd, $J_{1'',2''}$ 8.4, $J_{2'',3''}$ 11.5, 2″-H), 4.53 (1 H, dd, $J_{1,2}$ 8.5, $J_{2,3}$ 11.4, 2-H), 4.73 (1 H, dd, $J_{3,4}$ 3.4, 3-H), 4.81 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 4.82 (1 H, d, 1-H), 5.24 (1 H, dd, $J_{2',3'}$ 9.9, 2′-H), 5.33 (1 H, t, $J_{3',4'}$ 9.7, 3′-H), 5.36 (1 H, d, 1″-H), 5.48 (1 H, d, $J_{4,5}$ <1, 4-H), 5.59 (1 H, d, $J_{4'',5''}$ <1, 4″-H), 5.64 (1 H, t, $J_{4',5'}$ 9.8, 4′-H), 5.79 (1 H, dd, $J_{3'',4''}$ 3.3, 3″-H), 6.78, 6.96, 7.13, 7.29, 7.52 and 7.75 (12 H, 6 d, 3 × $COC_6H_4CH_3$); δ_C (75.5 MHz; $CDCl_3$) 20.4 and 20.6 ($COCH_3$), 21.0, 21.3 and 21.4 (3 × $COC_6H_4CH_3$), 28.4, 47.6 and 65.4 ($OCH_2CH_2CH_2N_3$), 51.2 and 52.2 (C-2, -2″), 97.8 and 98.2 (C-1, -1‴), 101.8 (C-1′), 165.1, 165.4 and 166.7 (3 × $COC_6H_4CH_3$), 169.6, 170.2, 170.3 and 171.5 (4 × $COCH_3$); FABMS of $C_{69}H_{69}N_5O_{25}$ (M, 1367.3) *m*/*z* 1390.4 $(M + Na)^+$.

3-Azidopropyl (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 32

To a solution of **31** (17 mg, 13 μmol) in CH_2Cl_2 (0.3 ml) were added a catalytic amount of TEMPO, KBr (160 μg, 1.3 μmol), Bu_4NCl (181 μg, 0.66 μmol), and saturated aq. $NaHCO_3$ (0.3 ml). The suspension was cooled to 0 °C and under vigorously stirring a mixture of 0.35 M aq. NaOCl (17 μl, 16 μmol), saturated aq. $NaHCO_3$ (22 μl), and saturated aq. NaCl (28 μl) was added dropwise. The mixture was stirred for 10 min, and thereafter the solution was acidified (pH 2) by adding 4 M aq. HCl. Then, CH_2Cl_2 (50 ml) was added and the organic layer was washed with 10% aq. NaCl (half vol., 1 ×), dried, filtered and concentrated. Column chromatography (CH_2Cl_2–acetone, 8:2 ⟶ CH_2Cl_2–acetone–HOAc, 8:2:0.5) of the residue furnished **32** (13 mg, 75%); TLC (CH_2Cl_2–acetone–HOAc, 9:1:0.5) R_f 0.37 (**32**); $[\alpha]_D$ +4 (*c* 1, $CHCl_3$). A solution of **32** in 5:1 MeOH–CH_2Cl_2 (3 ml) was stirred with Dowex-50 (Na^+) for 30 min, filtered, concentrated and analyzed by ^{13}C NMR: δ_C (75.5 MHz; $CDCl_3$) 20.4 and 20.6 ($COCH_3$), 21.1, 21.4 and 21.5 (3 × $COC_6H_4CH_3$), 29.6, 47.6 and 65.6 ($OCH_2CH_2CH_2N_3$), 51.2 and 52.2 (C-2, -2″), 97.6 and 98.3 (C-1, -1‴), 101.4 (C-1′), 164.0, 165.2 and 166.6 (3 × $COC_6H_4CH_3$), 168.8, 169.7 and 170.3 (2 C) (4 × $COCH_3$).

A small amount of acid **32** was esterified with diazomethane in diethyl ether (**32a**), and analyzed by ^1H NMR: δ_H (300 MHz; $CDCl_3$) 1.83, 2.09, 2.18 and 2.20 (12 H, 4 s, 4 × $COCH_3$), 2.24, 2.33 and 2.34 (9 H, 3 s, 3 × $COC_6H_4CH_3$), 2.85–2.91 (2 H, m, $OCH_2CH_2CH_2N_3$), 3.69 (3 H, s, $COOCH_3$), 4.39 and 4.52 (2 H, 2 dd, 2- and 2″-H), 4.72 (1 H, dd, 3-H), 4.75 (1 H, d, $J_{1',2'}$ 7.8, 1′-H), 4.81 (1 H, d, $J_{1,2}$ 8.4, 1-H), 5.27 (1 H, dd, 2′-H), 5.35 (1 H, d, $J_{1'',2''}$ 8.6, 1″-H), 5.41 (1 H, d, $J_{3,4}$ 3.5, 4-H), 5.48 (1 H, d, 4″-H), 5.52 (1 H, t, $J_{2',3'/3',4'}$ 9.7, 3′-H), 5.65 (1 H, t, 4′-H), 5.79 (1 H, dd, $J_{2'',3''}$ 11.3, $J_{3'',4''}$ 3.3, 3″-H), 6.88, 7.00, 7.14, 7.31, 7.57 and 7.74 (12 H, 6 d, 3 × $COC_6H_4CH_3$); FABMS of $C_{69}H_{67}N_5O_{26}$ (M, 1381.4) *m*/*z* 1404.4 $(M + Na)^+$.

3-Azidopropyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galactopyranoside 33

A solution of compound **32** (24 mg, 18 μmol) in ethanolic 33% $MeNH_2$ (5 ml) was stirred for 2 weeks at rt, during which the mixture was concentrated repeatedly and new reagent (3 × 5 ml) added. After concentration, the residue was dissolved in dry MeOH (3 ml) and acetic anhydride (100 μmol) was added at 0 °C. The mixture was stirred for 2 h, then concentrated and co-concentrated with 1:1 toluene–MeOH (3 × 10 ml). Purification of the residue on Toyopearl HW-40S with 5 mM aq. NH_4HCO_3 gave, after lyophilization, **33** (9.9 mg, 80%); TLC (butan-1-ol–water–HOAc, 2:1:0.5) R_f 0.35 (**33**); $[\alpha]_D$ −10 (*c* 1, water); δ_H (500 MHz; D_2O; TOCSY) 1.82–1.89 (2 H, m, $OCH_2CH_2CH_2N_3$), 2.03 (6 H, s, 2 × $NHCOCH_3$), 3.33 (1 H, dd, $J_{1',2'}$ 8.0, 2′-H), 3.39 [2 H, t, $O(CH_2)_2CH_2N_3$], 3.47 (1 H, t, $J_{2',3'}$ 9.3, $J_{3',4'}$ 9.3, 3′-H), 3.50 (1 H, t, 4′-H), 3.68 (1 H, d, $J_{4',5'}$ 8.3, 5′-H), 3.74 (1 H, dd, $J_{3'',4''}$ 3.1, 3″-H), 3.84 (1 H, dd, $J_{3,4}$ 3.1, 3-H), 3.89 (1 H, dd, $J_{1'',2''}$ 8.6, $J_{2'',3''}$ 10.7, 2″-H), 3.94 (1 H, d, $J_{4'',5''}$ <1, 4″-H), 4.00 (1 H, dd, $J_{1,2}$ 8.6, $J_{2,3}$ 10.7, 2-H), 4.16 (1 H, d, $J_{4,5}$ <1, 4-H), 4.47 (1 H, d, 1-H), 4.49 (1 H, d, 1″-H), 4.50 (1 H, d, 1′-H); FABMS of $C_{25}H_{41}N_5O_{17}$ (M, 683.0) *m*/*z* 682.0 $(M - H)^-$.

3-Aminopropyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galactopyranoside 3

To a solution of **33** (5 mg, 7.3 μl) in 0.05 M aq. NaOH were added 10% Pd–C (2.5 mg) and a catalytic amount of $NaBH_4$, and the mixture was stirred for 2 h at rt. Then, the mixture was filtered and concentrated. Column chromatography (MeOH–H_2O, 2:1) of the residue gave **3** (4.5 mg, 93%); TLC (MeOH–H_2O, 2:1) R_f 0.56 (**3**); $[\alpha]_D$ −130 (*c* 0.1, water); ^1H NMR data are given in Table 2; FABMS of $C_{25}H_{43}N_3O_{17}$ (M, 657.3) *m*/*z* 658.3 $(M + H)^+$.

3-{2-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]ethylthio}-propyl (β-D-glucopyranosyluronic acid)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside 34

To a solution of compound **1**[5] (1.0 mg, 1.9 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (100 μl) was added a solution of 3,4-diethoxycyclobut-3-ene-1,2-dione (diethyl squarate; 0.27 μl, 1.86 μmol) in EtOH (100 μl). The mixture was stirred for 4 h at rt, when TLC (butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5) showed an incomplete conversion into a higher moving spot. Again, diethyl squarate (0.1 μl, 0.69 μmol) was added and the mixture was stirred overnight. After concentration, a solution of the crude residue in water (1 ml) was loaded on a C-18 Sep-Pak cartridge. The column was washed with water (2 ml, 3 ×), then the product was eluted with MeOH (2 ml, 2 ×). The MeOH phase was evaporated, and a solution of the residue in water (2 ml) was concentrated to yield **34** as a colourless glass; TLC (butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5) R_f 0.22 (**1**), 0.46 (**34**). The material was directly used for the preparation of neoglycoconjugate **41**.

3-{2-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]ethylthio}-propyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galactopyranoside 35

A similar protocol as described for **34** was followed: compound **2**[5] (1.0 mg, 1.4 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (100 μl); diethyl squarate (0.18 μl, 1.26 μmol) in EtOH (100 μl); reaction time, overnight at rt; TLC with butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5. Product **35** was isolated as a colourless glass; TLC (butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5) R_f 0.16 (**2**), 0.35 (**34**). The material was directly used for the preparation of neoglycoconjugate **42**.

3-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]propyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(β-D-glucopyranosyluronic acid)-(1→3)]-2-acetamido-2-deoxy-β-D-galactopyranoside 36

A similar protocol as described for **34** was followed: compound **3** (1.0 mg, 1.5 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (100 μl); diethyl squarate (0.19 μl, 1.35 μmol) in EtOH (100 μl); reaction time, overnight at rt; TLC with butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5. Product **36** was purified by HiTrap gel filtration, and isolated as a colourless glass; TLC (butan-1-ol–MeOH–water–HOAc, 3:3:3:1) R_f 0.10 (**3**), 0.23 (**36**). The material was directly used for the preparation of neoglycoconjugate **43**.

3-{2-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]ethylthio}-propyl (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 37

A similar protocol as described for **34** was followed: compound **4**[5] (1.0 mg, 1.1 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (100 μl); diethyl squarate (0.15 μl, 1.0 μmol) in EtOH (80 μl); reaction time, 4.5 h at rt; further diethyl squarate (0.07 μl, 0.5 μmol); reaction time, overnight at rt; TLC with butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5. Product **37** was isolated, after an additional chromatographic purification on HiTrap (5% aq. NH_4HCO_3), as a colourless glass; TLC (butan-1-ol–MeOH–water–HOAc, 3:3:3:1) R_f 0.34 (**4**), 0.47 (**37**). The material was directly used for the preparation of neoglycoconjugate **44**.

3-{2-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]ethylthio}-propyl (2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→6)-[(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 38

A similar protocol as described for **34** was followed: compound **5** (0.78 mg, 0.71 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (50 μl); diethyl squarate (0.1 μl, 0.7 μmol) in EtOH (60 μl); reaction time, 16 h at rt; TLC with butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5. Product **38** was isolated, after an additional chromatographic purification on HiTrap (5% aq. NH_4HCO_3), as a colourless glass; TLC (butan-1-ol–MeOH–water–HOAc, 4:2:2:0.5) R_f 0.09 (**5**), 0.20 (**38**). The material was directly used for the preparation of neoglycoconjugate **45**.

3-{2-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]ethylthio}-propyl 2-acetamido-2-deoxy-β-D-galactopyranoside 39

A similar protocol as described for **34** was followed: compound **6** (3.0 mg, 8.9 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (150 μl); diethyl squarate (1.3 μl, 8.9 μmol) in EtOH (150 μl); reaction time, overnight at rt; TLC with EtOAc–MeOH–water, 10:5:1. Product **39** was isolated as a colourless glass; TLC (MeOH–water, 9:1) R_f 0.19 (**6**), 0.72 (**39**). The material was directly used for the preparation of neoglycoconjugate **46**.

6-[*N*-(2-Ethoxy-3,4-dioxocyclobut-1-enyl)amino]hexyl-β-D-glucopyranosiduronic acid 40

A similar protocol as described for **34** was followed: compound **7** (1.5 mg, 34.5 μmol) in 75 mM sodium phosphate buffer (pH 7.0) (130 μl); diethyl squarate (0.63 μl, 4.28 μmol) in EtOH (100 μl); reaction time, 2.5 h at rt; TLC with EtOAc–MeOH–water, 10:5:1. Product **40** was isolated as a colourless glass; TLC (EtOAc–MeOH–water, 10:5:1) R_f 0.46. The material was directly used for the preparation of neoglycoconjugate **47**.

Pretreatment of bovine serum albumin (BSA)

BSA (40 mg ml^{-1}) was stirred in 0.1 M NaOAc buffer (pH 4.5) containing 10 mM $NaIO_4$ for 1.5 h at rt to oxidize carbohydrate of glycoprotein contaminants.[35] Excess of periodate was destroyed by adding glycerol to a final concentration of 10 mM. The solution was dialyzed against water (three changes; Milli Q), followed by lyophilization. After treatment of the material with aq. $NaBH_4$ (catalytic amount) for 1 h at rt, the solution was diluted with water (1 ml), and neutralized with 4 M HOAc. After lyophilization, the quality of pretreated BSA was checked by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. To verify the complete removal of carbohydrate contaminants with GLC, a small amount of the protein material (1 mg) was subjected to methanolysis (1.0 M methanolic HCl, 24 h, 85 °C) followed by re-*N*-acetylation and trimethylsilylation.[36]

Preparation of BSA-glycoconjugates 41–47

Pretreated BSA (25 mg ml^{-1}) was dissolved in 0.1 M $NaHCO_3$ buffer (pH 9.0) and the solution was stirred for 30 min. Then, the oligosaccharide–squarate adduct (**34**–**40**) in water (0.5 mg ml^{-1}) was added to the pretreated-BSA solution (15 mequiv. based on BSA) and the resulting mixture was stirred for 3 days at rt. The mixture was purified by HiTrap gel filtration (5% aq. NH_4HCO_3) to afford, after lyophilization from water, the neoglycoconjugate (**41**–**47**). The degree of incorporation of **34**–**40** onto BSA was determined by MALDI-TOF MS (Table 3).

Molecular mechanics calculations

Minimum-energy calculations *in vacuo* were performed with CHEAT,[30,31] a CHARMm-based force field for carbohydrates wherein OH groups are represented by extended atoms to prevent intramolecular hydrogen-bond formation. Carboxylic and *N*-acetyl parameters were taken from the CHARMm force field.

Molecular dynamics calculations

Molecular dynamics simulations were carried out using the GROMOS program package and the updated carbohydrate force field for GROMOS[34] on Silicon Graphics O2 computers. Each molecule was surrounded by SPC/E[37] water molecules and placed in a truncated octahedral periodic box. All bond lenghts were kept fixed using the SHAKE procedure.[38] A cut-off radius of 0.8 nm and a time step of 2 fs was used. Simulations were performed with loose coupling to a pressure bath at 1 atm and a temperature bath at 300 K[39] with time constants of 0.5 and 0.1 ps, respectively.

Potential of mean force calculations on the β-D-Gal*p*NAc-(1→6)-β-D-Gal*p*NAc rotamer distribution

To calculate the free-energy differences of the GG, GT and TG conformations around the GalNAc–GalNAc glycosidic ω angle, potential-of-mean-force calculations[33] were run with the GROMOS force field for the methyl glycoside analogues of compounds **2** and **4**. All simulations were divided into jobs of 10 ps. The φ-values were collected into 72 classes, each with a width of $\Delta\varphi = 5°$. The derivatives were fitted to a 12-term Fourier series. The first 0.2 ps of each job was discarded.

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