Synthesis of *Streptococcus pneumoniae* Type 3 Neoglycoproteins Varying in Oligosaccharide Chain Length, Loading and Carrier Protein

Dirk J. Lefeber, Johannis P. Kamerling, and Johannes F. G. Vliegenthart*[a]

Abstract: The preparation is described of a range of neoglycoproteins containing synthesised fragments of the capsular polysaccharide of *Streptococcus pneumoniae* type 3, that is β-D-Glc*p*A-$(1\rightarrow4)$-β-D-Glc*p*-$(1\rightarrow O)$-$(CH_2)_3NH_2$ (**1**), β-D-Glc*p*-$(1\rightarrow3)$-β-D-Glc*p*A-$(1\rightarrow4)$-β-D-Glc*p*-$(1\rightarrow O)$-$(CH_2)_3NH_2$ (**2**), and β-D-Glc*p*A-$(1\rightarrow4)$-β-D-Glc*p*-$(1\rightarrow3)$-β-D-Glc*p*A-$(1\rightarrow4)$-β-D-Glc*p*-$(1\rightarrow O)$-$(CH_2)_3NH_2$ (**3**). A blockwise approach was developed for the synthesis of the protected carbohydrate chains, in which the carboxylic groups were introduced prior to deprotection by selective oxidation of HO-6 in the presence of HO-4 by using TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy radical). After deprotection, the 3-aminopropyl spacer of the fragments was elongated with diethyl squarate (3,4-diethoxy-3-cyclobutene-1,2-dione) and the elongated oligosaccharides were conjugated to CRM_{197} (cross-reacting material of diphtheria toxin), KLH (keyhole limpet hemocyanin) or TT (tetanus toxoid). The resulting neoglycoconjugates varied in oligosaccharide chain length, oligosaccharide loading and protein carrier. These well-defined conjugates are ideal probes for evaluating the influence of the different structural parameters in immunological tests.

Keywords: antigens · neoglycoconjugate vaccines · oligosaccharides · oxidation · protecting groups

Introduction

The Gram positive bacterium *Streptococcus pneumoniae* is one of the most prevalent infectious pathogens, which causes life-threatening diseases such as meningitis, pneumonia and otitis media. In the past, these infections could be efficiently treated with antibiotics. However, death rates have not declined and furthermore, resistance to pneumococcal strains is still growing.[1] Immunocompetent people can be protected efficiently by vaccination with the available 23-valent capsular polysaccharide (CPS) vaccines.[2] The highest incidence of pneumococcal infections is, however, in young children, the elderly and immunocompromised patients. People in these groups do not benefit from the mentioned vaccines, since they do not respond adequately to the T-cell independent polysaccharides.[3] However, conjugation of carbohydrate antigens to a protein results in a T-cell dependent neoglycoconjugate antigen that can give an efficient immune response in the population at high risk.[4]

Currently, neoglycoconjugate vaccines are in use against *Haemophilus influenzae* type b,[5] *Neisseria meningitidis* type C,[6] and *Streptococcus pneumoniae* serotypes.[7] These neoglycoproteins are prepared by conjugation of isolated capsular polysaccharides or a mixture of polysaccharide-derived oligosaccharides to a protein carrier. The polysaccharide–protein conjugates have a very complex and undefined structure, whereas the oligosaccharide–protein conjugates contain the carbohydrate as mixtures with respect to chain length and presented epitope. Especially in the case of a pneumococcal conjugate vaccine, wherein many serotypes have to be included, the presence of mixtures complicates the analysis of the products, which is increasingly important for product control. The use of synthetic polysaccharide-related oligosaccharide fragments with a unique structure could help to solve these problems. Furthermore, it creates the opportunity for the preparation of tailor-made neoglycoproteins that can be used for vaccination, for ELISA tests not hindered by bacterial contamination,[8] and for studying the immune response at a molecular level. Although synthetic neoglycopeptides contain a defined carbohydrate and a defined peptide part, the use of neoglycoproteins with a defined carbohydrate part allows a comparison with the currently used vaccines.

Recent studies with *Streptococcus pneumoniae* type 6B neoglycoproteins showed that a synthetic tetrasaccharide fragment, that is one repeating unit of the 6B CPS, coupled to KLH was sufficient to generate a protective antibody response in mice.[9] An overview of the synthetic oligosaccharides from several serotypes of *S. pneumoniae* has been

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given by Kamerling,[10] and an overview of well-defined neoglycoconjugates related to several encapsulated bacteria by Pozsgay.[11]

To obtain well-defined products for the simplification of product control, and to investigate the influence of oligosaccharide chain length, oligosaccharide loading and carrier protein on the immunogenicity of the neoglycoproteins, here we present the development of a versatile synthetic route for the preparation of oligosaccharides related to the CPS of *S. pneumoniae* type 3 and their conjugation to a protein in varying ratios. For the conjugation, the defined coupling chemistry of diethyl squarate (3,4-diethoxy-3-cyclobutene-1,2-dione) was applied, and as carrier proteins were used CRM_{197} (cross-reacting material of diphtheria toxin) and TT (tetanus toxoid), which are relevant for human vaccination, and KLH (keyhole limpet hemocyanin), which is suitable for animal studies.

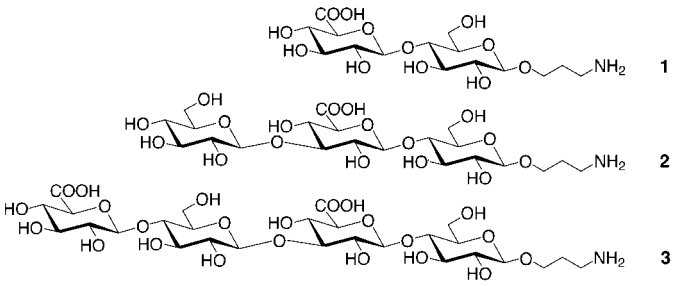
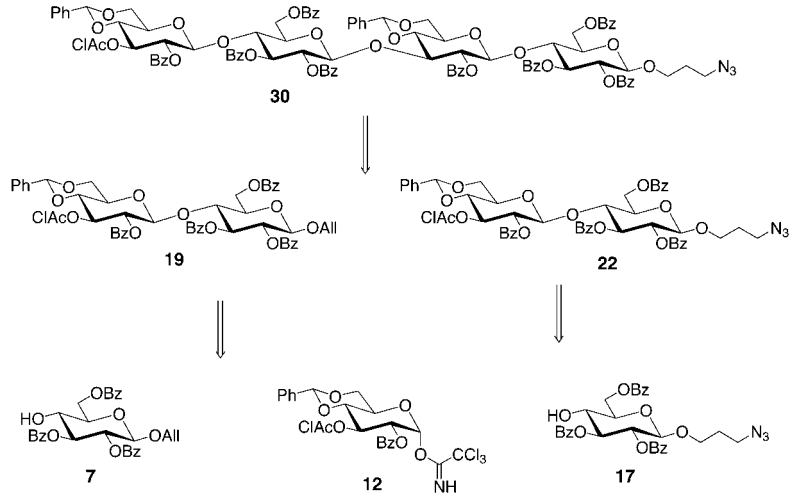


Figure 1. Synthesised oligosaccharide fragments to be coupled to a carrier protein.



Scheme 1. Retrosynthetic strategy.

Results and Discussion

Retrosynthetic strategy: The CPS of *S. pneumoniae* type 3 consists of $\beta(1\rightarrow3)$-linked cellobiuronic acid repeating units. A route for the synthesis of small fragments has been reported by Chernyak et al.,[12] based on 3,6-lactonisation of glucuronic acid containing structures to prepare acceptors with a free HO-3 group for the introduction of the $(1\rightarrow3)$-linkage. However, the yields for lactonisation and opening of the lactone were moderate in the synthesis of the disaccharide acceptor and can be expected to be even lower in the preparation of larger fragments. During the progress of our work, the synthesis of a protected disaccharide was described by Garegg et al.[13] In contrast to these earlier reports, in our approach the carboxylic functions are introduced in a late stage of the synthetic route by regioselective oxidation using TEMPO. This approach avoids elaborate protecting group manipulations and possibly low yielding coupling steps. Furthermore, the blockwise strategy employing disaccharide building blocks paves the way for the versatile preparation of larger fragments.

For the synthesis of the target oligosaccharide fragments **1**, **2** and **3** (Figure 1), three monosaccharide building blocks were designed (see Scheme 1).

Coupling of imidate donor **12** with acceptor **7** or **17** results in the formation of disaccharide **19** or **22**, respectively. Dechloroacetylation of **22** results in an acceptor for the synthesis of the tri- and tetrasaccharide fragments. Disaccharide **19** can be deallylated and imidated to obtain a disaccharide donor, which can be coupled with **22** to give **30**. Introduction of the carboxylic groups is achieved by selective 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) mediated oxidation of the primary hydroxyl groups after liberation of the benzylidene protecting groups. This selective oxidation eliminates the need for protection of HO-4. After debenzoylation, the azide can be hydrogenated and the resulting aminopropyl spacer coupled via diethyl squarate to lysine residues of CRM_{197}, KLH or TT. The tetrasaccharide acceptor can be elongated to obtain longer fragments by coupling with the mentioned disaccharide or other donors.

Synthesis of the saccharide fragments: Monosaccharide acceptor allyl 2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**7**) was prepared as depicted in Scheme 2. Benzoylation of allyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**4**)[14] by using benzoyl chloride ($\rightarrow$**5**, 92%), and subsequent debenzylidenation with trifluoroacetic acid afforded **6** in a yield of 92%. Selective benzoylation of the primary hydroxyl function with benzoyl imidazole[15] gave monosaccharide acceptor **7** in 95% yield.

For the synthesis of imidate donor **12** (Scheme 2), selective protection of allyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**4**) was attempted. However, several methods, for example, using stannylene acetals (Bu_2SnO),[16] benzoyl chloride at $-50\,^{\circ}C$, benzoyl imidazole, heterogeneous catalysis with tetrabutylammonium iodide and potassium carbonate,[17] or silylation,[18] failed to give satisfactory isolated yields of compounds with a protecting group on HO-2 or HO-3. In the related α-glucoside of **4** the acidity of HO-2 is increased by H-bond formation with the anomeric oxygen,[19] thereby increasing the difference in reactivity between HO-2 and HO-3. For this

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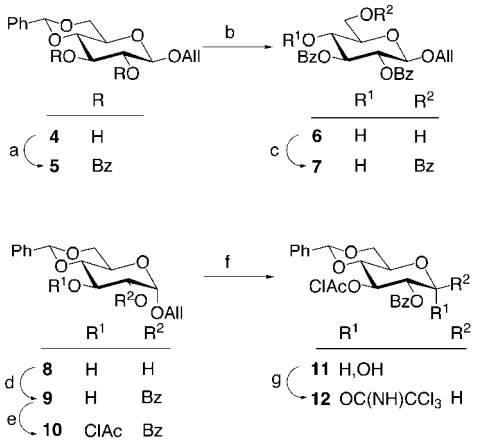
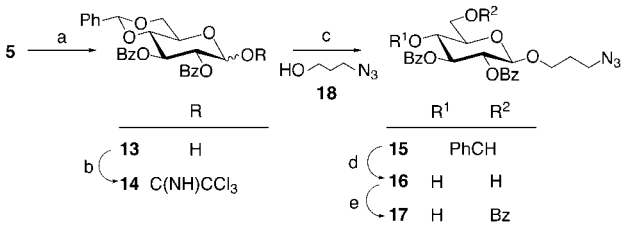


Scheme 2. Synthesis of the monosaccharide building blocks **7** and **12**. a) PhCOCl, C_5H_5N, CH_2Cl_2, 92%; b) CF_3COOH, H_2O, CH_2Cl_2, 92%; c) PhCOCl, imidazole, CH_2Cl_2, 95%; d) PhCOCl, imidazole, CH_2Cl_2, 74%; e) ClAc-Cl, C_5H_5N, CH_2Cl_2, 96%; f) i) $[(PPh_3)_3Rh^ICl]$, $CH_3C_6H_5$/EtOH 9:4, ii) NIS, H_2O, THF, 77%; g) Cl_3CCN, DBU, CH_2Cl_2, 93%.

reason, selective benzoylation of allyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**8**)[20] was performed with benzoyl imidazole, which resulted in the formation of **9** in a good yield of 74%. Subsequent chloroacetylation[21] (→**10**, 96%), deallylation by isomerisation of the double bond with Wilkinson's catalyst[22] and removal of the 1-propenyl group with *N*-iodosuccinimide and water[23] (→**11**, 77%), and trichloroacetimidation[24] afforded monosaccharide donor **12** (93%).

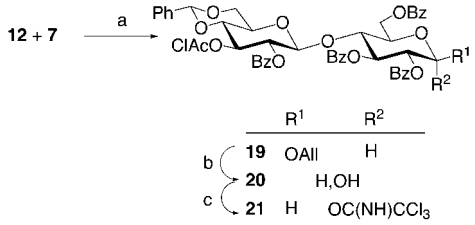
Monosaccharide acceptor **17**, containing a 3-azidopropyl spacer, was prepared from **5** (Scheme 3). Deallylation of **5** by



Scheme 3. Synthesis of the monosaccharide building block **17**. a) i) $[(PPh_3)_3Rh^ICl]$, $CH_3C_6H_5$/EtOH 5:2, ii) NIS, H_2O, THF, 75%; b) Cl_3CCN, DBU, CH_2Cl_2, 82%; c) 8% TMSOTf, CH_2Cl_2, 65%; d) CF_3COOH, H_2O, CH_2Cl_2, 63%; e) PhCOCl, imidazole, CH_2Cl_2, 96%.

using Wilkinson's catalyst and *N*-iodosuccinimide (→**13**, 75%) and subsequent trichloroacetimidation afforded monosaccharide donor **14** (82%). Coupling of **14** with 3-azido-1-propanol (**18**), prepared in 74% from 3-bromo-1-propanol by treatment with sodium azide, was performed in dichloromethane using 8% TMSOTf as a promoter (→**15**, 65%). Debenzylidenation of **15** by using trifluoroacetic acid (→**16**, 63%) and selective benzoylation of HO-6 with benzoyl imidazole afforded acceptor **17** in 96% yield.

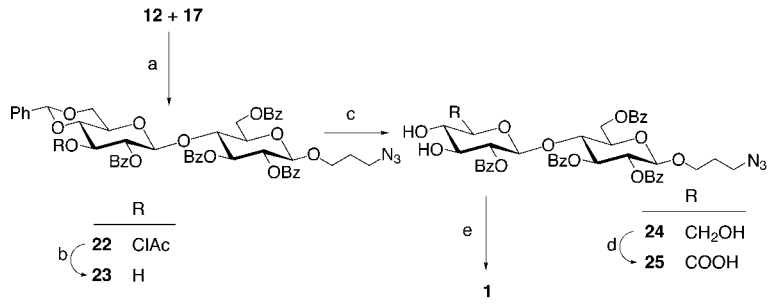
The preparation of disaccharide donor **21** is presented in Scheme 4. Coupling of donor **12** with acceptor **7** using 5% TMSOTf as a promoter afforded disaccharide **19** in an excellent yield of 96%. Deallylation with tris(triphenylphosphine)rhodium(I) chloride and *N*-iodosuccinimide (→**20**, 70%) and subsequent trichloroacetimidation gave disaccharide donor **21** (88%).



Scheme 4. Synthesis of disaccharide donor **21**. a) 5% TMSOTf, CH_2Cl_2, 96%; b) i) $[(PPh_3)_3Rh^ICl]$, DABCO, CH_2Cl_2/$CH_3C_6H_5$/EtOH 0.6:9:4, ii) NIS, H_2O, THF, 70%; c) Cl_3CCN, DBU, CH_2Cl_2, 88%.

In Scheme 5, the strategies for the preparation of the disaccharide acceptor **23** and target disaccharide **1** are shown. Test-oxidations with TEMPO[25] of monosaccharide **6** showed that the allyl group was incompatible with the reaction conditions. Therefore, the 3-azidopropyl spacer was introduced prior to oxidation. Coupling of disaccharide imidate **21** to 3-azido-1-propanol **18** to afford **22** was very troublesome and gave irreproducible yields of around 50% due to orthoester formation ($J_{1,2}=5.1$ Hz). Coupling via the glycosyl bromide, prepared from **20** by reaction with Vilsmeier reagent,[26] gave similar problems. Alternatively, the synthesis of disaccharide **22** was achieved by coupling of donor **12** and the spacer-containing monosaccharide acceptor **17** using 8% TMSOTf as a promoter in a yield of 75%. Subsequent dechloroacetylation with diazabicyclo[2.2.2]octane (DABCO)[27] gave disaccharide acceptor **23** (98%).

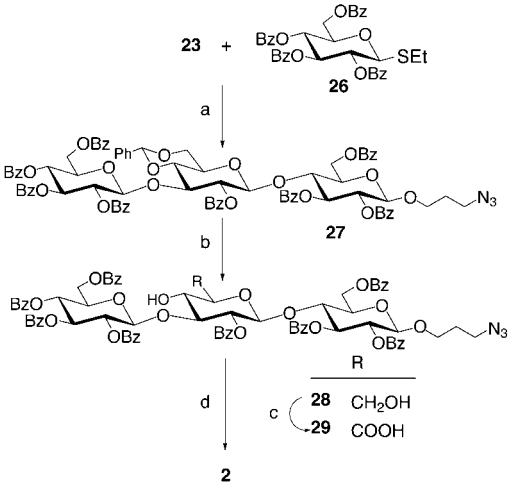
For the synthesis of the target disaccharide fragment **1**, first **23** was debenzylidenated using trifluoroacetic acid (→**24**, 90%). Then, selective oxidation by using TEMPO and aqueous sodium hypochlorite afforded the protected cellobiuronic acid derivative **25** (85%). ^{1}H NMR analysis after methylation by using diazomethane proved the identity of the compound ($\delta=3.37$, $COOCH_3$). Debenzoylation of **25** using sodium methoxide in methanol at pH 11, followed by hydrogenation of the azide should give **1**. However, using the



Scheme 5. Synthesis of disaccharide acceptor **23** and target disaccharide **1**. a) 8% TMSOTf, CH_2Cl_2, 78%; b) 15 equiv DABCO, $CH_3C_6H_5$/EtOH 1:1, 55 °C, 98%; c) CF_3COOH, H_2O, CH_2Cl_2, 90%; d) TEMPO, aqueous NaOCl, KBr, Bu_4NBr, aqueous NaCl, aqueous $NaHCO_3$, CH_2Cl_2, 85%; e) i) NaOMe, MeOH (pH 11), ii) $NaBH_4$, 10% Pd/C, H_2O, 90%.

conventional hydrogenolysis method with 10% Pd/C and hydrogen, a side product in about 20% was generated. ^{1}H NMR analysis showed the removal of some of the spacer signals, but the full identity of the compound could not be disclosed. When using dithiothreitol and DBU,[28] the same side-product was formed. Reduction with sodium borohydride and 10% Pd/C in H_2O afforded disaccharide fragment **1** in 90% yield over two steps with the formation of only small amounts of the mentioned side-product.

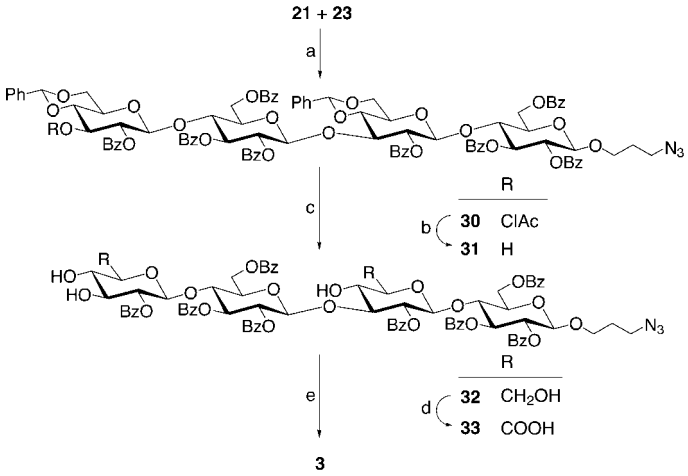
Focusing on target trisaccharide fragment **2**, the synthesis of protected trisaccharide **27** (Scheme 6) was quite troublesome.



Scheme 6. Synthesis of target trisaccharide **2**. a) NIS, TfOH, Et_2O/CH_2Cl_2 1:2, 82%; b) CF_3COOH, H_2O, CH_2Cl_2, 70%; c) TEMPO, aqueous NaOCl, KBr, Bu_4NBr, aqueous NaCl, aqueous $NaHCO_3$, CH_2Cl_2, 76%; d) i) NaOMe, MeOH (pH 11), ii) $NaBH_4$, 10% Pd/C, H_2O, 89%.

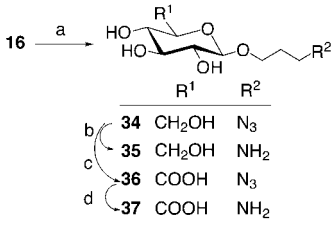
Coupling of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate with **23** using 10% TMSOTf as a promoter gave a complex reaction mixture. The use of the corresponding glucosyl bromide or thioglucoside as a donor resulted in the same complex mixture with one of the main side-products being the orthoester ($J_{1'',2''} = 5.2$ Hz). Coupling of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate with **23** using 10% TMSOTf as a promoter gave trisaccharide **27** in 70% yield. The yield was, however, irreproducible due to the formation of orthoester and some other side-products. Finally, the use of ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**26**) as donor was found to result in an efficient glycosylation of **23**. Activation with triflic acid and *N*-iodosuccinimide gave trisaccharide **27** in a reproducible yield of 82%. Debenzylidenation using trifluoroacetic acid ($\rightarrow$**28**, 70%) and subsequent oxidation of HO-6 using TEMPO and aqueous sodium hypochlorite gave the protected trisaccharide fragment **29** (76%) (^{1}H NMR: $\delta = 3.62$, d, $J_{4',5'} = 9.3$ Hz, 1H; H-5′). Deprotection of **29** as described for **25** afforded trisaccharide fragment **2** in 89% yield over two steps.

In the synthesis of target tetrasaccharide fragment **3** (Scheme 7), as a first step disaccharide donor **21** was coupled with disaccharide acceptor **23** using 10% TMSOTf as a promoter, resulting in **30** (75%). Dechloroacetylation of **30** using thiourea ($\rightarrow$**31**, 84%), followed by debenzylidenation using trifluoroacetic acid ($\rightarrow$**32**, 70%), and selective oxidation using TEMPO and aqueous sodium hypochlorite gave **33** in a yield of 65%. ^{1}H NMR analysis after methylation with diazomethane showed two singlets at $\delta = 3.39$ and 3.34 ($COOCH_3$). Deprotection of **33** as described for **25** gave tetrasaccharide fragment **3** in 60% yield over two steps.



Scheme 7. Synthesis of target tetrasaccharide **3**. a) 10% TMSOTf, CH_2Cl_2, 75%; b) thiourea, $EtOH/C_5H_5N$ 8:1, 90°C, 84%; c) CF_3COOH, H_2O, CH_2Cl_2, 70%; d) TEMPO, aqueous NaOCl, KBr, Bu_4NBr, aqueous NaCl, aqueous $NaHCO_3$, CH_2Cl_2, 65%; e) i) NaOMe, MeOH (pH 11), ii) $NaBH_4$, 10% Pd/C, H_2O, 60%.

In order to make a comparison possible of the immunological studies of the aimed di-, tri- and tetrasaccharide–protein conjugates with relevant blanks, the glucose and glucuronic acid conjugates were also prepared. To this end, 3-aminopropyl glucopyranoside (**35**) and 3-aminopropyl glucopyranosiduronic acid (**37**) (Scheme 8) were prepared from **16**. Debenzoylation of **16** ($\rightarrow$**34**), followed by reduction of the azide with sodium borohydride and 10% Pd/C in water gave **35** in 88% over two steps. Oxidation of **34** with TEMPO and aqueous sodium hypochlorite in water gave **36**, which was purified by conventional acetylation with acetic anhydride and pyridine, column chromatography, and deacetylation using sodium methoxide. Glucuronic acid derivative **37** was obtained after reduction of **36** as described for **34** (80% over three steps).



Scheme 8. Synthesis of 3-aminopropyl glucopyranoside **35** and 3-aminopropyl glucopyranosiduronic acid **37**. a) NaOMe, MeOH (pH 10); b) $NaBH_4$, 10% Pd/C, H_2O, 88%; c) TEMPO, aqueous NaOCl, KBr, H_2O; d) $NaBH_4$, 10% Pd/C, H_2O, 80%.

The ^{1}H NMR data of the target saccharide fragments **1**–**3**, **35** and **37**, derived from two-dimensional TOCSY and ROESY measurements, are presented in Table 1.

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Table 1. 500 MHz ^{1}H NMR data of **1**, **2**, **3**, **35**, and **37** at 300 K (in ppm).[a] $J_{1,2}$ couplings are presented in parentheses.

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
35	4.49 (7.9)	3.29	3.50	3.38	3.48	3.93	3.73
37	4.48 (7.9)	3.33	3.51	3.51	3.72		
1	4.51 (7.7)	3.33	3.66	3.61	3.61	3.98	3.81
1′	4.51 (7.7)	3.36	3.52	3.52	3.75		
2	4.51 (7.7)	3.33	3.66	3.61	3.61	3.98	3.80
2′	4.54 (8.3)	3.57	3.79	n.d.[b]	n.d.		
2″	4.79 (7.7)	3.36	3.51	3.40	3.46	3.92	3.72
3	4.51 (8.2)	3.33	3.65	3.62	n.d.	3.98	3.81
3′	4.54 (7.7)	3.58	3.79	n.d.	n.d.		
3″	4.82 (8.2)	3.38	3.68	3.61	n.d.	3.98	3.82
3‴	4.51 (8.2)	3.36	3.52	3.52	3.76		

[a] The signals for the 3-aminopropyl spacer are similar for all compounds: $\delta = 4.03$ and 3.84 ($OCH_2CH_2CH_2NH_2$), 3.15 ($OCH_2CH_2CH_2NH_2$), 2.00 ($OCH_2CH_2CH_2NH_2$). [b] n.d. = not determined.

Preparation of the protein conjugates: Studying neoglycoconjugates in which only one parameter is varied is important to understand the different factors that influence the immunogenicity of the conjugates. To this end, focusing on CPS-related oligosaccharides from *S. pneumoniae* type 3, neoglycoproteins with a different oligosaccharide chain length, carbohydrate–protein ratio, and protein carrier have been prepared.

The saccharide fragments described above were conjugated to CRM_{197}, TT or KLH, using diethyl squarate as linker (Figure 2).[29, 30] Elongation of the saccharides with diethyl squarate was performed in ethanol/0.1M sodium phosphate (pH 6.9). The reaction products were purified by solid-phase extraction and coupled to protein in 0.1M sodium borate buffer at pH 9.5.

CRM_{197} is a non-toxic product of a single mutation in the diphtheria toxin gene[31] and exists as a pure and well-defined protein that is often used as a carrier in human neoglycoconjugate vaccines.[32] As can be seen from the coupling efficiencies in Table 2, the yield for the conjugation of the saccharides to CRM_{197} decreases when charged structures are coupled (entries 1 and 2), or when the oligosaccharides become larger (entries 1, 3, 5, and 8). The amount of carbohydrate incorporation (Table 2) was measured by MALDI-TOF analysis (see Figure 3 for some representative spectra). It should be noted that analysis of the

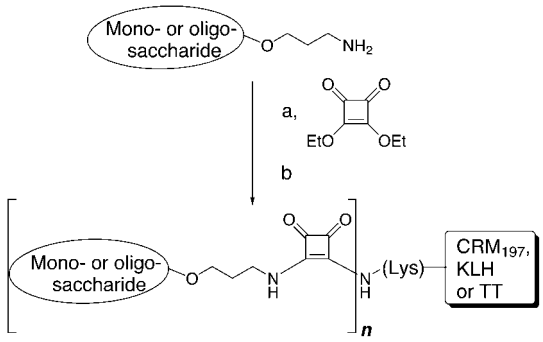

Figure 2. Coupling of saccharide fragments to a protein via diethyl squarate. a) EtOH/sodium phosphate buffer (0.1M, pH 6.9); b) sodium borate buffer (0.1M, pH 9.5); CRM_{197}, KLH or TT.

Table 2. Oligosaccharide loading and coupling efficiency for the conjugation of **1**, **2**, **3**, **35** and **37** to CRM_{197}.

Entry	Glycoconjugate	Oligosaccharide loading [mol mol^{-1}]	Coupling efficiency [%]
1	CRM_{197}–**35**	8.5	77
2	CRM_{197}–**37**	6.6	67
3	CRM_{197}–**1**	3.1	56
4	CRM_{197}–**1**	6.7	52
5	CRM_{197}–**2**	4.9	45
6	CRM_{197}–**2**	6.8	41
7	CRM_{197}–**2**	12.0	54
8	CRM_{197}–**3**	2.9	26
9	CRM_{197}–**3**	6.7	32
10	CRM_{197}–**3**	8.1	37

CRM_{197}-conjugates by MALDI-TOF was possible only when about 10mM sodium borate buffer was still present in the sample. Attempts to measure the molecular mass in water failed.

To investigate the immunological effect of the protein carrier, two other proteins were coupled with the saccharide fragments. Tetanus toxoid (TT), prepared by inactivation of the crude toxin with formalin, is frequently used in human immunisation studies, and KLH is frequently used for immunisation studies in animals. The results for the coupling of the saccharide fragments to TT and KLH are shown in Table 3. Since TT and KLH are heterogeneous protein preparations, the carbohydrate loading of these conjugates could not be derived reliably by MALDI-TOF analysis. This is

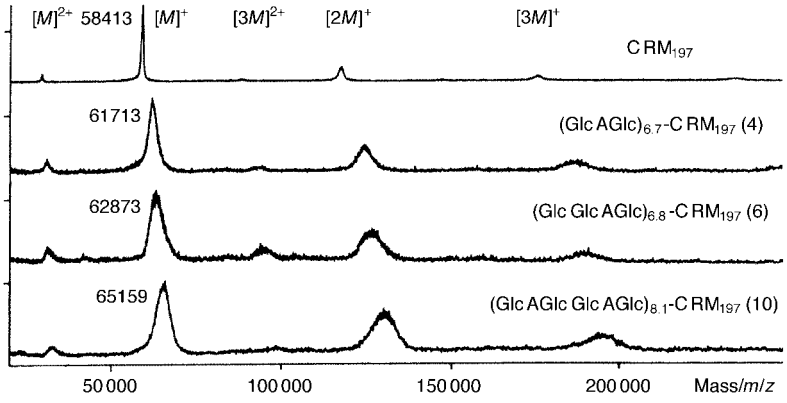

Figure 3. MALDI-TOF spectra of CRM_{197} and entries 4, 6 and 10 (Table 2).

due to their intrinsic heterogeneous nature. Therefore, the loading was determined by analysis of the carbohydrate (Dubois)[33] and protein content (Pierce).[34]

Table 3. Oligosaccharide loading and coupling efficiency for the conjugation of **1**, **2** and **3** to KLH and TT.

Entry	Glycoconjugate	Oligosaccharide loading [μg mg^{-1}]	Coupling efficiency [%]
1	KLH–**1**	14.1	9
2	KLH–**2**	13.3	15
3	KLH–**3**	24.6	9
4	TT–**1**	31.3	16
5	TT–**2**	23.6	11
6	TT–**3**	22.9	8

In conclusion, we have synthesised a range of neoglycoproteins related to *S. pneumoniae* type 3. The immunological properties of these well-defined neoglycoconjugates will be evaluated, in order to gain insight in the structural factors influencing the immune response of this type of neoglycoconjugate vaccines. Preliminary results of the immunisation of mice with the CRM_{197}-conjugates show that they can induce a protective immune response.[35]

Experimental Section

General: All chemicals were of reagent grade, and were used without any further purification. A solution of tetanus toxoid (TT; 6.12 mg mL^{-1}) was obtained from the Dutch National Institute of Health (RIVM, Bilthoven, The Netherlands); a solution of keyhole limpet hemocyanin (KLH; 50 mg mL^{-1}) was obtained from the Eijkman–Winkler Institute for Microbiology (Utrecht University, Utrecht, The Netherlands); a solution of a non-toxic variant of diphtheria toxin (cross-reacting material, CRM_{197}; 61.15 mg mL^{-1}) was obtained from Chiron (Siena, Italy). Reactions were monitored by TLC on plastic silica gel 60 F_{254} (Merck); after examination under UV light, compounds were visualised by heating with 10% (*v*/*v*) ethanolic H_2SO_4, orcinol (2 mg mL^{-1}) in 20% (*v*/*v*) methanolic H_2SO_4, or ninhydrin (1.5 mg mL^{-1}) in BuOH/H_2O/HOAc (38:1.75:0.25). In the work-up procedures of reaction mixtures, organic solutions were washed with appropriate amounts of the indicated aqueous solutions, then dried ($MgSO_4$), and concentrated under reduced pressure at 20–40 °C on a water-bath. Column chromatography was performed on silica gel 60 (Merck, 0.063–0.200 mm). Optical rotations were measured in $CHCl_3$, unless stated otherwise, with a Perkin–Elmer 241 polarimeter, using a 10 cm 1 mL cell. ^{1}H NMR spectra in $CDCl_3$ were recorded at 27 °C with a Bruker AC 300 spectrometer; the δ_H values are given in ppm relative to the signal for internal Me_4Si ($\delta = 0$). ^{13}C (APT, 75 MHz) NMR spectra in $CDCl_3$ were recorded at 27 °C with a Bruker AC 300 spectrometer; indicated ppm values for δ_C are relative to the signal of $CDCl_3$ ($\delta = 76.9$). ^{1}H NMR spectra in D_2O were recorded at 27 °C with a Bruker AMX 500 spectrometer, and the δ_H values are given in ppm relative to the signal for internal acetone ($\delta = 2.225$). Two-dimensional TOCSY and ROESY spectra in D_2O were recorded using a Bruker AMX 500 spectrometer (500 MHz) at 27 °C to assign the spectra of compounds **1**, **2**, **3**, **35** and **37**. Fast-atom-bombardment mass spectrometry (FABMS) was performed on a JEOL JMS SX/SX 102A four-sector mass spectrometer. Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) spectra were obtained on a Voyager-DE mass spectrometer using 2,4-dihydroxybenzoic acid (DHB) in H_2O as a matrix. Elemental analyses were carried out by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany).

Allyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-*β*-D-glucopyranoside (5): Benzoyl chloride (10.3 mL, 88.7 mmol) was added dropwise to a solution of allyl 4,6-*O*-benzylidene-*β*-D-glucopyranoside (**4**)[14] (10.5 g, 34.1 mmol) in CH_2Cl_2 (80 mL) and pyridine (10 mL). After 3 h, the mixture was diluted with CH_2Cl_2, washed twice with 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2) to obtain **5** as a white solid (16.2 g, 92%). $R_f = 0.81$ (CH_2Cl_2/EtOAc 9:1); $[\alpha]_D^{20} = +19$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 7.98–7.94$, 7.39–7.29 (2m, 15H; *Ph*CH, 2*Ph*CO), 5.84–5.70 (m, 1H; $OCH_2CH{=}CH_2$), 5.79 (t, $J_{2,3} = 9.5$, $J_{3,4} = 9.5$ Hz, 1H; H-3), 5.54 (s, 1H; PhC*H*), 5.51 (dd, $J_{1,2} = 7.8$ Hz, 1H; H-2), 5.27–5.10 (m, 2H; $OCH_2CH{=}CH_2$), 4.85 (d, 1H; H-1), 4.43 (dd, $J_{5,6a} = 4.9$, $J_{6a,6b} = 10.3$ Hz, 1H; H-6a), 4.39–4.32, 4.17–4.09 (2m, each 1H; $OCH_2CH{=}CH_2$), 3.94 (t, $J_{4,5} = 9.5$ Hz, 1H; H-4), 3.88 (t, $J_{5,6b} = 10.3$ Hz, 1H; H-6b), 3.69 (ddd, 1H; H-5); ^{13}C NMR ($CDCl_3$): $\delta = 165.4$, 165.0 (2Ph*C*O), 117.6 ($OCH_2CH{=}CH_2$), 101.3 (Ph*C*H), 100.3 (C-1), 78.7, 72.3, 72.0, 66.5 (C-2,3,4,5), 70.1 ($OCH_2CH{=}CH_2$), 68.5 (C-6); elemental analysis calcd (%) for $C_{30}H_{28}O_8$ (516.6): C 69.76, H 5.46; found: C 69.88, H 5.38.

Allyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranoside (6): CF_3COOH (1.64 mL) and H_2O (0.22 mL) were added to a solution of **5** (1.50 g, 2.90 mmol) in CH_2Cl_2 (40 mL). The mixture was stirred for 3 h, diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous $NaHCO_3$ (2×) and 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2/MeOH 98:2) to obtain **6** (1.14 g, 92%). $R_f = 0.15$ (CH_2Cl_2/acetone 9:1); $[\alpha]_D^{20} = +90$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 7.97–7.94$, 7.51–7.26 (2m, 10H; 2*Ph*CO), 5.85–5.72 (m, 1H; $OCH_2CH{=}CH_2$), 5.27–5.10 (m, 2H; $OCH_2CH{=}CH_2$), 4.78 (d, $J_{1,2} = 7.7$ Hz, 1H; H-1), 4.38–4.31, 4.18–4.11 (2m, each 1H; $OCH_2CH{=}CH_2$), 3.58 (ddd, $J_{4,5} = 9.6$, $J_{5,6a}$, $J_{5,6b} = 3.6$, 4.5 Hz, 1H; H-5); ^{13}C NMR ($CDCl_3$): $\delta = 165.2$ (2Ph*C*O), 117.6 ($OCH_2CH{=}CH_2$), 99.8 (C-1), 77.3, 75.7, 71.3, 69.9 (C-2,3,4,5), 70.1 ($OCH_2CH{=}CH_2$), 62.1 (C-6); elemental analysis calcd (%) for $C_{23}H_{24}O_8$ (428.4): C 64.48, H 5.65; found: C 64.32, H 5.64.

Allyl 2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside (7): Benzoyl chloride (0.15 mL, 1.28 mmol) was added dropwise to a solution of imidazole (0.15 g, 2.2 mmol) in CH_2Cl_2 (4 mL). The suspension was filtered, and the filtrate was added dropwise to a solution of **6** (0.50 g, 1.17 mmol) in CH_2Cl_2 (4 mL). After stirring for 36 h, the mixture was diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography (CH_2Cl_2/EtOAc 95:5) to obtain **7** (0.59 g, 95%). $R_f = 0.73$ (CH_2Cl_2/EtOAc 9:1); $[\alpha]_D^{20} = +63$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.09–7.94$, 7.47–7.33 (2m, 15H; 3*Ph*CO), 5.85–5.72 (m, 1H; $OCH_2CH{=}CH_2$), 5.24–5.07 (m, 2H; $OCH_2CH{=}CH_2$), 4.81 (d, $J_{1,2} = 7.6$ Hz, 1H; H-1), 4.75 (dd, $J_{5,6b} = 4.5$, $J_{6a,6b} = 12.1$ Hz, 1H; H-6b), 4.68 (dd, $J_{5,6a} = 2.5$ Hz, 1H; H-6a), 4.38–4.31, 4.18–4.10 (2m, each 1H; $OCH_2CH{=}CH_2$), 3.83 (ddd, $J_{4,5} = 9.7$ Hz, 1H; H-5); ^{13}C NMR ($CDCl_3$): $\delta = 167.1$, 166.8, 165.1 (3Ph*C*O), 117.5 ($OCH_2CH{=}CH_2$), 99.6 (C-1), 76.5, 74.3, 71.4, 69.5 (C-2,3,4,5), 69.9 ($OCH_2CH{=}CH_2$), 63.4 (C-6); elemental analysis calcd (%) for $C_{30}H_{28}O_9$ (532.5): C 67.66, H 5.29; found: C 67.53, H 5.20.

Allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-*α*-D-glucopyranoside (9): Benzoyl chloride (0.68 mL, 5.81 mmol) was added dropwise to a solution of imidazole (0.80 g, 11.73 mmol) in CH_2Cl_2 (10 mL). The suspension was filtered and the filtrate was added dropwise to a solution of allyl 4,6-*O*-benzylidene-*α*-D-glucopyranoside (**8**)[20] (1.50 g, 4.86 mmol) in CH_2Cl_2 (9.5 mL). After stirring at boiling under reflux for 36 h, the mixture was diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography, whereby the 2,3-di-*O*-benzoylated compound was eluted with toluene/EtOAc 95:5, and **9** with toluene/EtOAc 9:1 (1.48 g, 74%). $R_f = 0.61$ (toluene/EtOAc 7:3); $[\alpha]_D^{20} = +99$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.09–8.06$, 7.56–7.35 (2m, 10H; *Ph*CH, *Ph*CO), 5.87–5.74 (m, 1H; $OCH_2CH{=}CH_2$), 5.54 (s, 1H; PhC*H*), 5.30–5.22, 5.15–5.10 (2m, each 1H; $OCH_2CH{=}CH_2$), 5.20 (d, $J_{1,2} = 3.8$ Hz, 1H; H-1), 5.04 (dd, $J_{2,3} = 9.6$ Hz, 1H; H-2), 4.36 (brt, $J_{3,4} = 9.4$ Hz, 1H; H-3), 4.29 (dd, $J_{6a,6b} = 10.2$, $J_{5,6a} = 4.8$ Hz, 1H; H-6a), 3.75 (t, $J_{5,6b} = 10.2$ Hz, 1H; H-6b), 3.59 (t, 1H; H-4), 2.68 (s, 1H; HO-2); ^{13}C NMR ($CDCl_3$): $\delta = 166.0$ (Ph*C*O), 136.9 (*Ph*CH, quaternary C), 129.4 (*Ph*CO, quaternary C), 117.5 ($OCH_2CH{=}CH_2$), 101.8 (Ph*C*H), 95.8 (C-1), 81.3, 73.8, 68.7, 62.2 (C-2,3,4,5), 68.7, 68.6 (C-6, $OCH_2CH{=}CH_2$); elemental analysis calcd (%) for $C_{23}H_{24}O_7$ (412.4): C 66.98, H 5.86; found: C 67.18, H 5.76.

Allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-*α*-D-glucopyranoside (10): Chloroacetyl chloride (70 μL, 0.88 mmol) was added to a cooled (5 °C) solution of **9** (0.35 g, 0.84 mmol) in CH_2Cl_2 (30 mL) and pyridine (1.5 mL). After stirring for 2 h, another portion of chloroacetyl chloride (35 μL, 0.44 mmol) was added, and stirring was continued for 1 h. Then, the

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mixture was co-concentrated with toluene, diluted with CH_2Cl_2, and washed with 10% (*w*/*v*) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 95:5) to afford **10** (0.38 g, 96%). $R_f = 0.50$ (toluene/EtOAc 9:1); $[\alpha]_D^{20} = +104$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.04-8.02$, 7.35–7.48 (2m, 10H; *Ph*CH, *Ph*CO), 5.88–5.75 (m, 1H; $OCH_2CH{=}CH_2$), 5.87 (t, $J_{2,3} = 9.9$, $J_{3,4} = 9.8$ Hz, 1H; H-3), 5.54 (s, 1H; PhC*H*), 5.31–5.23, 5.16–5.12 (2m, each 1H; $OCH_2CH{=}CH_2$), 5.28 (d, $J_{1,2} = 3.8$ Hz, 1H; H-1), 5.12 (dd, 1H; H-2), 4.33 (dd, $J_{6a,6b} = 10.3$, $J_{5,6a} = 4.8$ Hz, 1H; H-6a), 4.00, 3.94 (2d, $J_{gem} = 14.8$ Hz, each 1H; $ClCH_2CO$), 3.81 (t, $J_{5,6b} = 10.3$ Hz, 1H; H-6b), 3.78 (t, $J_{4,5} = 9.6$ Hz, 1H; H-4); ^{13}C NMR ($CDCl_3$): $\delta = 166.3$, 165.7 (Ph*C*O, $ClCH_2CO$), 136.6 (*Ph*CH, quaternary C), 128.7 (*Ph*CO, quaternary C), 117.8 ($OCH_2CH{=}CH_2$), 101.5 (Ph*C*H), 95.7 (C-1), 78.8, 72.0, 70.8, 62.5 (C-2,3,4,5), 68.7, 68.6 (C-6, $OCH_2CH{=}CH_2$), 40.4 ($ClCH_2CO$); elemental analysis calcd (%) for $C_{25}H_{25}ClO_8$ (488.9): C 61.41, H 5.15; found: C 61.22, H 5.03.

2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-D-glucopyranose (11): Tris(triphenylphosphine)rhodium(I) chloride (70 mg) was added to a solution of **10** (0.10 g, 0.21 mmol) in absolute EtOH (20 mL) and toluene (45 mL). After stirring at boiling under reflux for 2.5 h, TLC (toluene/EtOAc 9:1) showed the formation of a new spot ($R_f = 0.52$), and the mixture was concentrated. The residue was dissolved in THF (18 mL), and water (2 mL) and NIS (0.13 g) were added. After 20 min, the mixture was concentrated, diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous $NaHSO_3$ (2×) and 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography. Impurities were eluted with toluene/EtOAc 9:1, and **11** was obtained as a light brown syrup by elution with toluene/EtOAc 8:2 (71 mg, 77%; $\alpha/\beta = 4:1$). $R_f = 0.29$ (toluene/EtOAc 9:1); ^{1}H NMR ($CDCl_3$): $\delta = 8.04-8.02$, 7.46–7.34 (2m, 10H; *Ph*CH, *Ph*CO), 5.54 (s, 0.8H; PhCH^{α}), 5.52 (s, 0.2H; PhCH^{β}), 5.88 (t, $J_{2,3} = 9.9$, $J_{3,4} = 9.8$ Hz, 1H; H-3), 5.57 (d, $J_{1,2} = 3.6$ Hz, 0.8H; H-1$^{\alpha}$), 5.19 (dd, $J_{1,2} = 7.9$ Hz, 0.2H; H-2$^{\beta}$), 5.10 (dd, 0.8H; H-2$^{\alpha}$), 4.92 (d, 0.2H; H-1$^{\beta}$), 4.01, 3.95 (2d, $J_{gem} = 14.8$ Hz, each 1H; $ClCH_2CO$); ^{13}C NMR ($CDCl_3$): $\delta = 166.6$, 165.8 (Ph*C*O, $ClCH_2CO$), 136.6 (*Ph*CH, quaternary C), 101.5 (Ph*C*H), 95.8 (C-1$^{\alpha}$), 90.7 (C-1$^{\beta}$), 68.8 (C-6$^{\alpha}$), 68.3 (C-6$^{\beta}$), 40.4 ($ClCH_2CO^{\alpha}$), 40.3 ($ClCH_2CO^{\beta}$).

2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate (12): Cl_3CCN (2.7 mL) and DBU (87 μL) were added to a solution of **11** (1.06 g, 2.43 mmol) in dry CH_2Cl_2 (28 mL). After 2 h, the mixture was concentrated, and the residue was purified by column chromatography (toluene/EtOAc 95:5) to obtain **12** (1.31 g, 93%). $R_f = 0.49$ (toluene/EtOAc 9:1); $[\alpha]_D^{20} = +67$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.60$ (s, 1H; N*H*), 8.01–7.98, 7.48–7.36 (2m, 10H; *Ph*CH, *Ph*CO), 6.71 (d, $J_{1,2} = 3.8$ Hz, 1H; H-1), 5.94 (t, $J_{2,3} = 9.9$, $J_{3,4} = 9.9$ Hz, 1H; H-3), 5.59 (s, 1H; PhC*H*), 5.40 (dd, 1H; H-2), 4.41 (dd, $J_{5,6a}$ 4.9, $J_{6a,6b} = 10.4$ Hz, 1H; H-6a), 4.21 (dt, $J_{4,5} = 9.9$, $J_{5,6b} = 10.2$ Hz, 1H; H-5), 4.03, 3.97 (2d, $J_{gem} = 14.8$ Hz, each 1H; $ClCH_2CO$), 3.92 (t, 1H; H-4), 3.84 (t, 1H; H-6b); ^{13}C NMR ($CDCl_3$): $\delta = 166.3$, 165.3 (Ph*C*O, $ClCH_2CO$), 160.6 (O*C*(NH)CCl_3), 136.3 (*Ph*CH, quaternary C), 101.6 (Ph*C*H), 93.4 (C-1), 78.1, 70.8, 70.5, 65.1 (C-2,3,4,5), 68.4 (C-6), 40.4 ($ClCH_2CO$); elemental analysis calcd (%) for $C_{24}H_{21}Cl_4NO_8$ (593.1): C 48.59, H 3.56; found: C 48.10, H 3.42.

2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-D-glucopyranose (13): Tris(triphenylphosphine)rhodium(I) chloride (2.1 g) was added to a solution of **5** (3.02 g, 5.84 mmol) in EtOH (100 mL) and toluene (250 mL). After stirring at boiling under reflux for 3 h, TLC (toluene/EtOAc 8:2) showed the formation of a new spot ($R_f = 0.81$), and the mixture was concentrated. The residue was dissolved in THF (250 mL), and water (30 mL) and NIS (2.0 g) were added. After 20 min, the mixture was concentrated, diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous $NaHSO_3$ (2×) and 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography. Impurities were eluted with toluene/EtOAc 9:1, and **13** was obtained as a light brown syrup by elution with toluene/EtOAc 8:2 (2.09 g, 75%; $\alpha/\beta = 3:2$). $R_f = 0.25$ (toluene/EtOAc 8:2); ^{1}H NMR ($CDCl_3$): $\delta = 7.99-7.94$, 7.45–7.25 (2m, 15H, *Ph*CH, 2*Ph*CO), 6.14 (t, $J_{2,3} = 9.8$, $J_{3,4} = 9.8$ Hz, 0.6H; H-3$^{\alpha}$), 5.83 (t, $J_{2,3} = 9.6$, $J_{3,4} = 9.6$ Hz, 0.4H; H-3$^{\beta}$), 5.66 (d, $J_{1,2} = 3.7$ Hz, 0.6H; H-1$^{\alpha}$), 5.54 (s, 0.6H; PhCH^{α}), 5.49 (s, 0.4H; PhCH^{β}), 5.37 (dd, $J_{1,2} = 7.9$ Hz, 0.4H; H-2$^{\beta}$), 5.30 (dd, 0.6H; H-2$^{\alpha}$), 4.99 (d, 0.4H; H-1$^{\beta}$); ^{13}C NMR ($CDCl_3$): $\delta = 166.0$, 165.7 (2Ph*C*O), 101.4 (Ph*C*H$^{\alpha}$), 101.3 (Ph*C*H$^{\beta}$), 96.1 (C-1$^{\alpha}$), 91.0 (C-1$^{\beta}$), 68.3 (C-6$^{\alpha}$), 66.6 (C-6$^{\beta}$); elemental analysis calcd (%) for $C_{27}H_{24}O_8$ (476.4): C 68.06, H 5.08; found: C 68.08, H 5.06.

2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-D-glucopyranosyl trichloroacetimidate (14): Cl_3CCN (4.1 mL) and DBU (0.14 mL) were added at 0°C to a solution of **13** (1.82 g, 3.82 mmol) in dry CH_2Cl_2 (65 mL). After 3 h, the mixture was concentrated, and the residue was purified by column chromatography. Impurities were eluted with toluene/EtOAc 95:5, and **14** was obtained by elution with toluene/EtOAc 9:1 (1.88 g, 82%; $\alpha/\beta = 3:2$). $R_f = 0.68$ (toluene/EtOAc 8:2); ^{1}H NMR ($CDCl_3$): $\delta = 8.72$ (s, 0.4H; NH^{β}), 8.60 (s, 0.6H; NH^{α}), 6.76 (d, $J_{1,2} = 3.8$ Hz, 0.6H; H-1$^{\alpha}$), 6.23 (d, $J_{1,2} = 7.2$ Hz, 0.4H; H-1$^{\beta}$), 6.17 (t, $J_{2,3} = 9.8$, $J_{3,4} = 9.9$ Hz, 0.6H; H-3$^{\alpha}$), 5.84 (dd, $J_{2,3} = 8.2$, $J_{3,4} = 9.1$ Hz, 0.4H; H-3$^{\beta}$), 5.75 (t, 0.4H; H-2$^{\beta}$), 5.60 (s, 0.6H; PhCH^{α}), 5.57 (s, 0.4H; PhCH^{β}), 5.56 (dd, 0.6H; H-2$^{\alpha}$); ^{13}C NMR ($CDCl_3$): $\delta = 165.3$ (2Ph*C*O), 160.7 (O*C*(NH)CCl_3), 101.5 (Ph*C*H), 95.5, 93.6 (C-1$^{\alpha}$,1$^{\beta}$), 68.4 (C-6).

3-Azidopropyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside (15): A solution of 3-azido-1-propanol **18** (1.4 g, 14 mmol) and **14** (1.80 g, 2.90 mmol) in dry CH_2Cl_2 (35 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (42 μL, 0.23 mmol) was introduced, and after 30 min, the mixture was neutralised with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (*w*/*v*) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 95:5) to obtain **15** (1.05 g, 65%). $R_f = 0.70$ (toluene/EtOAc 8:2); $[\alpha]_D^{20} = +19$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 7.98-7.94$, 7.52–7.25 (2m, 15H, *Ph*CH, 2*Ph*CO), 5.78 (t, $J_{2,3} = 9.5$, $J_{3,4} = 9.5$ Hz, 1H; H-3), 5.55 (s, 1H; PhC*H*), 5.46 (dd, $J_{1,2} = 7.8$ Hz, 1H; H-2), 4.79 (d, 1H; H-1), 4.44 (dd, $J_{6a,6b} = 10.3$, $J_{5,6a} = 4.9$ Hz, 1H; H-6a), 3.93 (t, $J_{4,5} = 9.6$ Hz, 1H; H-4), 3.88 (t, $J_{5,6b} = 10.1$ Hz, 1H; H-6b), 3.70 (dt, 1H; H-5), 3.98 (dt, 1H; O*CH*$HCH_2CH_2N_3$), 3.61 (ddd, 1H; OCH*H*$CH_2CH_2N_3$), 3.29–3.17 (m, 2H; $OCH_2CH_2CH_2N_3$), 1.83–1.72 (m, 2H; $OCH_2CH_2CH_2N_3$); ^{13}C NMR ($CDCl_3$): $\delta = 136.6$ (*Ph*CH, quaternary C), 101.7, 101.4 (Ph*C*H, C-1), 78.7, 72.4, 71.9, 66.6 (C-2,3,4,5), 68.5 (C-6), 66.7 ($OCH_2CH_2CH_2N_3$), 47.7 ($OCH_2CH_2CH_2N_3$), 28.9 ($OCH_2CH_2CH_2N_3$); elemental analysis calcd (%) for $C_{30}H_{29}O_8N_3$ (559.5): C 64.39, H 5.22; found: C 64.25, H 5.27.

3-Azidopropyl 2,3-di-*O*-benzoyl-β-D-glucopyranoside (16): CF_3COOH (1.0 mL) and H_2O (0.16 mL) were added to a solution of **15** (1.24 g, 2.21 mmol) in CH_2Cl_2 (40 mL). The mixture was stirred for 3 h, diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous $NaHCO_3$ (2×) and 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2/EtOAc 9:1 → CH_2Cl_2/acetone 6:4) to obtain **16** (0.66 g, 63%). $R_f = 0.07$ (CH_2Cl_2/EtOAc 9:1); $[\alpha]_D^{20} = +37$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.01-7.94$, 7.51–7.29 (2m, 10H, 2*Ph*CO), 4.73 (d, $J_{1,2} = 7.9$ Hz, 1H; H-1), 3.59 (ddd, 1H; OCH*H*$CH_2CH_2N_3$), 3.33–3.17 (m, 2H; $OCH_2CH_2CH_2N_3$), 1.83–1.73 (m, 2H; $OCH_2CH_2CH_2N_3$); ^{13}C NMR ($CDCl_3$): $\delta = 101.0$ (C-1), 77.1, 75.8, 71.3, 69.9 (C-2,3,4,5), 66.3 ($OCH_2CH_2CH_2N_3$), 62.1 (C-6), 47.7 ($OCH_2CH_2CH_2N_3$), 28.9 ($OCH_2CH_2CH_2N_3$); elemental analysis calcd (%) for $C_{23}H_{25}O_8N_3$ (471.4): C 58.59, H 5.34; found: C 58.32, H 5.40.

3-Azidopropyl 2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (17): Benzoyl chloride (0.20 mL, 1.69 mmol) was added dropwise to a solution of imidazole (0.14 g, 2.11 mmol) in CH_2Cl_2 (10 mL). The suspension was filtered, and the filtrate was added dropwise to a solution of **16** (0.66 g, 1.41 mmol) in CH_2Cl_2 (10 mL). After stirring for 18 h, the mixture was diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography (toluene/EtOAc 95:5) to obtain **17** (0.79 g, 96%). $R_f = 0.41$ (toluene/EtOAc 8:2); $[\alpha]_D^{20} = +58$ ($c = 0.8$); ^{1}H NMR ($CDCl_3$): $\delta = 8.09-7.93$, 7.50–7.30 (2m, 15H, 3*Ph*CO), 5.53 (dd, $J_{2,3} = 9.8$, $J_{3,4} = 8.6$ Hz, 1H; H-3), 5.41 (dd, $J_{1,2} = 7.8$ Hz, 1H; H-2), 4.76 (d, 1H; H-1), 3.61 (ddd, 1H; OCH*H*$CH_2CH_2N_3$), 3.26–3.15 (m, 2H; $OCH_2CH_2CH_2N_3$), 1.85–1.68 (m, 2H; $OCH_2CH_2CH_2N_3$); ^{13}C NMR ($CDCl_3$): $\delta = 167.0$, 166.9, 165.2 (3Ph*C*O), 101.0 (C-1), 76.1, 74.3, 71.4, 69.4 (C-2,3,4,5), 66.4 ($OCH_2CH_2CH_2N_3$), 63.3 (C-6), 47.7 ($OCH_2CH_2CH_2N_3$), 28.8 ($OCH_2CH_2CH_2N_3$); elemental analysis calcd (%) for $C_{30}H_{29}O_9N_3$ (575.5): C 62.60, H 5.08; found: C 62.68, H 5.14.

3-Azido-1-propanol (18): Sodium azide (3.58 g, 55.0 mmol) was added to a solution of 3-bromo-1-propanol (1.54 g, 11.1 mmol) in DMF (9 mL). After stirring at boiling under reflux for 36 h, the mixture was filtered, and co-concentrated with toluene. The residue was diluted with CH_2Cl_2 and washed twice with 10% (*w*/*v*) aqueous NaCl. The organic layer was dried, filtered, and concentrated. An analytically pure sample was obtained by distillation under reduced pressure (0.83 g, 74%). n_D (1.4521, 23°C); lit[36]

(1.4569, 28 °C); ^{1}H NMR ($CDCl_3$): $\delta = 3.73$ (t, 2H; HOCH_2CH$_2$CH$_2$N$_3$), 3.44 (t, 2H; HOCH$_2$CH$_2$CH_2N$_3$), 1.87–1.79 (m, 2H; HOCH$_2$CH_2CH$_2$N$_3$); IR (KBr; liquid film): $\tilde{\nu} = 2100\ cm^{-1}$ (N_3).

Allyl (2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (19): A solution of **7** (0.42 g, 0.80 mmol) and **12** (0.85 g, 1.43 mmol) in dry CH_2Cl_2 (13 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (15 μL, 84 μmol) was added. After 30 min, the mixture was neutralised with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (*w*/*v*) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 95:5) to obtain **19** (0.73 g, 96%). $R_f = 0.35$ (toluene/EtOAc 9:1); $[\alpha]_D^{20} = +29$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.01$–7.91, 7.50–7.25 (2m, 25H; *Ph*CH, 4*Ph*CO), 5.77–5.64 (m, 1H; OCH$_2$C*H*=CH$_2$), 5.69 (t, $J_{2,3} = 9.7$ Hz, 1H; H-3), 5.39 (dd, $J_{1,2} = 7.8$ Hz, 1H; H-2), 5.38 (t, $J_{2',3'} = J_{3',4'} = 9.4$ Hz, 1H; H-3′), 5.27 (dd, $J_{1',2'} = 7.6$ Hz, 1H; H-2′), 5.20 (s, 1H; PhC*H*), 5.16–5.10, 5.07–5.03 (2m, each 1H; OCH$_2$CH=CH_2), 4.77, 4.71 (2d, each 1H; H-1,1′), 3.91, 3.85 (2d, J_{gem} = 14.8 Hz, each 1H; ClCH_2CO); ^{13}C NMR ($CDCl_3$): $\delta = 166.3$, 165.6, 165.1 (2C), 164.9 (4Ph*C*O, ClCH$_2$*C*O), 136.2 (*Ph*CH, quaternary C), 117.6 (OCH$_2$CH=*C*H$_2$), 101.5, 101.1, 99.2 (Ph*C*H, C-1,1′), 69.8 (O*C*H$_2$CH=CH$_2$), 67.3 (C-6′), 62.2 (C-6), 40.2 (Cl*C*H$_2$CO); elemental analysis calcd (%) for $C_{52}H_{47}O_{16}Cl$ (963.3): C 64.83, H 4.91; found: C 64.59, H 4.97.

(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-D-glucopyranose (20): A catalytic amount of DABCO and tris(triphenylphosphine)rhodium(I) chloride (0.82 g) were added to a solution of **19** (0.74 g, 0.78 mmol) in absolute EtOH (20 mL), toluene (45 mL), and CH_2Cl_2 (3 mL). After stirring at boiling under reflux for 2.5 h, TLC (toluene/EtOAc 9:1) showed the formation of a new spot ($R_f = 0.38$), and the mixture was concentrated. The residue was dissolved in THF (55 mL), and water (8 mL) and NIS (0.33 g) were added. After 20 min, the mixture was concentrated, diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous $NaHSO_3$ (2×) and 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography. Impurities were eluted with toluene/EtOAc 9:1, and **20** was obtained as a light brown syrup by elution with toluene/EtOAc 8:2 (0.50 g, 70%; $\alpha/\beta = 4{:}1$). $R_f = 0.29^{\alpha}/0.34^{\beta}$ (toluene/EtOAc 8:2); ^{1}H NMR ($CDCl_3$): $\delta = 8.06$–7.93, 7.50–7.31 (2m, 25H; *Ph*CH, 4*Ph*CO), 6.04 (dd, $J_{2,3} = 10.2$, $J_{3,4} = 9.2$ Hz, 0.8H; H-3$^{\alpha}$), 5.48 (d, $J_{1,2}$ = 3.5 Hz, 0.8H; H-1$^{\alpha}$), 5.42 (t, $J_{2',3'} = J_{3',4'} = 9.4$ Hz, 1H; H-3′), 5.30 (dd, $J_{1',2'} = 7.6$ Hz, 1H; H-2′), 5.24 (s, 0.8H; PhC*H*$^{\alpha}$), 5.22 (s, 0.2H; PhC*H*$^{\beta}$), 5.14 (dd, 0.8H; H-2$^{\alpha}$), 4.89, 4.81 (2d, $J_{1,2} \approx 7.8$ Hz, each 0.2H; H-1$^{\beta}$,1′$^{\beta}$), 4.85 (d, 0.8H; H-1′$^{\alpha}$), 3.93, 3.87 (2d, $J_{gem} = 14.9$ Hz, each 1H; ClCH_2CO); ^{13}C NMR ($CDCl_3$): $\delta = 166.7$, 166.4, 165.8, 165.0 (2C) (4Ph*C*O, ClCH$_2$*C*O), 136.3 (*Ph*CH, quaternary C), 101.6, 101.2 (Ph*C*H, C-1′), 95.4 (C-1$^{\beta}$), 90.0 (C-1$^{\alpha}$), 67.4 (C-6′), 62.0 (C-6), 40.3 (Cl*C*H$_2$CO); elemental analysis calcd (%) for $C_{49}H_{43}O_{16}Cl$ (923.3): C 63.74, H 4.69; found: C 63.44, H 4.73.

(2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (21): Cl_3CCN (0.60 mL) and DBU (18 μL) were added to a solution of **20** (0.40 g, 0.44 mmol) in dry CH_2Cl_2 (9 mL). After 2 h, the mixture was concentrated and the residue was purified by column chromatography (toluene/EtOAc 95:5) to yield **21** (0.41 g, 88%). $R_f = 0.32$ (toluene/EtOAc 9:1); ^{1}H NMR ($CDCl_3$): $\delta = 8.54$ (s, 1H; N*H*), 8.05–7.90, 7.49–7.25 (2m, 25H; 4*Ph*CO, *Ph*CH), 6.67 (d, $J_{1,2} = 3.7$ Hz, 1H; H-1), 6.08 (dd, $J_{2,3} = 10.1$, $J_{3,4} = 8.8$ Hz, 1H; H-3), 5.46 (dd, 1H; H-2), 5.41 (t, $J_{2',3'} = 9.2$ Hz, 1H; H-3′), 5.23 (dd, $J_{1',2'} = 7.6$ Hz, 1H; H-2′), 5.28 (s, 1H; PhC*H*), 4.87 (d, 1H; H-1′), 3.91, 3.85 (2d, $J_{gem} = 14.8$ Hz, each 1H; ClCH_2CO); ^{13}C NMR ($CDCl_3$): $\delta = 166.3$, 165.4, 165.3, 164.9, 164.8 (4Ph*C*O, ClCH$_2$*C*O), 160.4 (O*C*(NH)CCl$_3$), 136.3 (*Ph*CH, quaternary C), 101.7, 101.2 (Ph*C*H, C-1′), 92.8 (C-1), 67.5 (C-6′), 61.6 (C-6), 40.2 (Cl*C*H$_2$CO).

3-Azidopropyl (2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (22): A solution of **12** (0.50 g, 0.84 mmol) and **17** (0.28 g, 0.49 mmol) in dry CH_2Cl_2 (10 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (11.5 μL, 63 μmol) was added. After 30 min, the mixture was neutralised with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (*w*/*v*) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 95:5) to obtain **22** (0.38 g, 78%). $R_f = 0.39$ (toluene/EtOAc 9:1); $[\alpha]_D^{20} = +42$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.06$–7.91, 7.48–7.26 (2m, 25H; *Ph*CH, 4*Ph*CO), 5.69 (t, $J \approx 9.3$ Hz, 1H; H-3), 5.38 (t, $J \approx 9.5$ Hz, 1H; H-3′), 5.35, 5.27 (2 dd, $J_{1,2}$, $J_{1',2'} = 7.6$, 7.8 Hz, each 1H; H-2,2′), 5.20 (s, 1H; PhC*H*), 4.78, 4.65 (2d, each 1H; H-1,1′), 4.07, 3.53 (2t, $J \approx 9.4$ Hz, each 1H; H-4,4′), 3.91, 3.86 (2d, each 1H; ClCH_2CO), 3.16 (dt, 1H; OCH$_2$CH$_2$CH_2N$_3$), 1.77–1.61 (m, 2H; OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 166.3$, 165.5, 165.1, 164.9, 164.8 (4Ph*C*O, ClCH$_2$*C*O), 136.3 (*Ph*CH, quaternary C), 101.5, 101.2, 100.7 (Ph*C*H, C-1,1′), 67.4 (C-6′), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.1 (C-6), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 40.2 (Cl*C*H$_2$CO), 28.7 (OCH$_2$*C*H$_2$CH$_2$N$_3$); IR (KBr, liquid film): $\tilde{\nu} = 2096\ cm^{-1}$ (N_3); elemental analysis calcd (%) for $C_{52}H_{48}O_{16}ClN_3$ (1006.4): C 62.05, H 4.80; found: C 62.15, H 4.86.

3-Azidopropyl (2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (23): DABCO (0.43 g, 3.8 mmol) was added to a solution of **22** (0.26 g, 0.26 mmol) in toluene (14 mL) and ethanol (14 mL). After 2.5 h at 55 °C, the mixture was diluted with CH_2Cl_2, washed with aqueous 0.05 M HCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 9:1) to afford **23** (0.23 g, 98%). $R_f = 0.25$ (toluene/EtOAc 9:1); $[\alpha]_D^{20} = +31$ ($c = 1$); ^{1}H NMR ($CDCl_3$): $\delta = 8.08$–7.91, 7.50–7.29 (2m, 25H; *Ph*CH, 4 *Ph*CO), 5.68 (t, $J_{2,3} = 9.8$ Hz, 1H; H-3), 5.36 (dd, $J_{1,2} = 7.9$ Hz, 1H; H-2), 5.22 (s, 1H; PhC*H*), 5.17 (dd, $J_{1',2'}$ = 8.0, $J_{2',3'} = 9.0$ Hz, 1H; H-2′), 4.67, 4.62 (2d, each 1H; H-1,1′), 4.05, 3.36 (2t, $J \approx 9.3$ Hz, each 1H; H-4,4′), 3.87 (t, 1H; H-3′), 3.19–3.11 (m, 2H; OCH$_2$CH$_2$CH_2N$_3$), 1.74–1.60 (m, 2H; OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 165.8$, 165.3, 165.1, 165.0 (4Ph*C*O), 136.6 (*Ph*CH, quaternary C), 101.5, 101.4, 100.7 (Ph*C*H, C-1,1′), 67.5 (C-6′), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.4 (C-6), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.7 (OCH$_2$*C*H$_2$CH$_2$N$_3$); elemental analysis calcd (%) for $C_{50}H_{47}O_{15}N_3$ (929.9): C 64.58, H 5.09; found: C 64.65, H 5.20.

3-Azidopropyl (2-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (24): CF_3COOH (60 μL) and H_2O (8 μL) were added to a solution of **23** (80 mg, 87 μmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 3 h, then diluted with CH_2Cl_2, washed with 10% (*w*/*v*) aqueous $NaHCO_3$ (2×) and 10% (*w*/*v*) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 8:2→toluene/EtOAc 6:4) to obtain **24** (70 mg, 90%). $R_f = 0.04$ (toluene/EtOAc 6:4); $[\alpha]_D^{20} = +28$ (c = 1); ^{1}H NMR ($CDCl_3$): $\delta = 8.04$–7.90, 7.61–7.32 (2m, 20H; 4 *Ph*CO), 5.66 (t, $J_{2,3} = 9.7$ Hz, 1H; H-3), 5.38 (dd, $J_{1,2} = 7.8$ Hz, 1H; H-2), 4.97 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 9.5$ Hz, 1H; H-2′), 4.67, 4.65 (2d, each 1H; H-1,1′), 3.52 (ddd, 1H; OC*H*HCH$_2$CH$_2$N$_3$), 3.19–3.14 (m, 2H; OCH$_2$CH$_2$CH_2N$_3$), 1.79–1.64 (m, 2H; OCH$_2$CH_2CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 165.9$ (3C), 165.2 (4Ph*C*O), 100.7 (C-1,1′), 66.4 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.6, 61.3 (C-6,6′), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.8 (OCH$_2$*C*H$_2$CH$_2$N$_3$); elemental analysis calcd (%) for $C_{43}H_{43}O_{15}N_3$ (841.3): C 61.35, H 5.14; found: C 61.59, H 5.22.

3-Azidopropyl (2-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (25): A catalytic amount of TEMPO, and a solution (0.14 mL) of KBr (7.8 mg) and Bu_4NBr (10.4 mg) in saturated aqueous $NaHCO_3$ (1.4 mL) were added to a solution of **24** (65 mg, 0.072 mmol) in CH_2Cl_2 (0.65 mL). The mixture was stirred vigorously at 0 °C, when a solution of saturated aqueous NaCl (0.14 mL), saturated aqueous $NaHCO_3$ (78 μL), and aqueous NaOCl (13% Cl active; 0.18 mL) was added dropwise. After 45 min, the mixture was acidified with 4 M HCl (pH 4), diluted with CH_2Cl_2, and the organic layer was washed with 10% (*w*/*v*) aqueous NaCl, dried, filtered, and concentrated. The residue was purified by column chromatography. Impurities were eluted with CH_2Cl_2/acetone 8:2, and **25** (55 mg, 85%) was eluted with CH_2Cl_2/acetone/HOAc 8:2:0.5. $R_f = 0.30$ (CH_2Cl_2/acetone/HOAc 8:2:1); ^{13}C NMR ($CDCl_3$): $\delta = 100.6$, 100.4 (C-1,1′), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.3 (C-6), 47.6 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.7 (OCH$_2$*C*H$_2$CH$_2$N$_3$).

A small amount was methylated with diazomethane and acetylated with Ac_2O and pyridine for analysis. ^{1}H NMR ($CDCl_3$): $\delta = 7.94$–7.89, 7.50–7.26 (2m, 20H; 4*Ph*CO), 4.87, 4.65 (2d, $J_{1,2}$, $J_{1',2'} = 7.4$, 7.7 Hz, each 1H; H-1,1′), 3.73 (d, $J_{4',5'} = 9.9$ Hz, 1H; H-5′), 3.37 (s, 3H; COOCH_3), 3.16–3.13 (m, 2H; OCH$_2$CH$_2$CH_2N$_3$), 1.93, 1.84 (2s, each 3H; CH_3CO), 1.75–1.57 (m, 2H; OCH$_2$CH_2CH$_2$N$_3$); elemental analysis calcd (%) for $C_{48}H_{47}O_{18}N_3$ (953.9): C 60.44, H 4.97; found: C 60.65, H 5.03.

3-Aminopropyl (β-D-glucopyranosyluronic acid)-(1→4)-β-D-glucopyranoside (1): NaOMe was added until pH 11 to a solution of **25** (90 mg, 0.11 mmol) in MeOH (9 mL). After stirring for 1.5 h, TLC (EtOAc/MeOH/

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H_2O 12:5:3) showed the formation of a new spot ($R_f = 0.37$), and the mixture was neutralised with Dowex H^+, filtered, and concentrated. A solution of the residue in water was washed with CH_2Cl_2 (3 ×), and the aqueous layer was concentrated. MALDI-TOF analysis of the residue showed a peak at m/z 462 $[M+Na]^+$. Then, a solution of the residue in 0.1 M NaOH (1.1 mL) was added dropwise to a suspension of 10% Pd/C (2 mg) and $NaBH_4$ (8.7 mg) in bidistilled water (0.55 mL). After 45 min, when TLC (EtOAc/MeOH/H_2O 12:5:3) showed the formation of a ninhydrin-positive spot on the baseline, the mixture was acidified with Dowex H^+ (pH 4), then loaded on a short column of Dowex 50 W × 2 (H^+, 200–400 mesh). After elution of contaminants with water, elution with 1% NH_4OH afforded **1** after concentration and co-concentration with water (2 ×) (39 mg, 90%). $[\alpha]_D^{20} = -92$ ($c = 0.6$, H_2O); MS (MALDI-TOF): m/z: 414 $[M+H]^+$, 436 $[M+Na]^+$. For ^1H NMR data, see Table 1.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (27): A solution of **23** (53 mg, 57 μmol) and ethyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**26**; 62 mg, 96 μmol) in dry CH_2Cl_2 (0.26 mL), containing 4 Å molecular sieves, was stirred under Ar for 30 min at 0 °C. Then, 0.48 mL of a solution of NIS (420 mg, 1.87 mmol) and TfOH (12 μL) in Et_2O/CH_2Cl_2 (1:1) (9.6 mL) was added. After 5 min, the mixture was neutralised with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (w/v) aqueous $NaHSO_3$ (2 ×) and 10% (w/v) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 9:1) to afford **27** (70 mg, 82%). $R_f = 0.41$ (toluene/EtOAc 85:15); $[\alpha]_D^{20} = +27$ ($c = 1$); ^1H NMR ($CDCl_3$): $\delta = 5.64$, 5.56 (2t, $J_{2'',3''} = 9.3$, $J_{3'',4''} = 9.4$ Hz, each 1H; H-3'',4''), 5.62 (dd, $J_{2,3} = 9.8$, $J_{3,4} = 9.0$ Hz, 1H; H-3), 5.39, 5.31 (2dd, $J_{1,2}$, $J_{1'',2''} = 7.8$ Hz, each 1H; H-2,2''), 5.29 (s, 1H; PhC*H*), 5.27 (dd, $J_{1',2'} = 7.8$, $J_{2',3'} = 9.0$ Hz, 1H; H-2'), 4.91 (d, 1H; H-1''), 4.59, 4.58 (2d, each 1H; H-1,1'), 3.47 (ddd, 1H; OC*H*HCH$_2$CH$_2$N$_3$), 3.13 (dt, 2H; OCH$_2$CH$_2$C*H*$_2$N$_3$), 1.72–1.58 (m, 2H; OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 165.9$, 165.1, 164.9, 164.8 (2C), 164.6, 164.0 (2C) (8Ph*C*O), 136.6 (*Ph*CH, quaternary C), 101.5, 101.2, 100.5 (2C) (Ph*C*H, C-1,1',1''), 67.5 (C-6'), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.8 (2C) (C-6,6''), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.7 (OCH$_2$*C*H$_2$CH$_2$N$_3$); MS (MALDI-TOF): m/z: 1530 $[M+Na]^+$.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (28): CF_3COOH (60 μL) and H_2O (8 μL) were added to a solution of **27** (0.13 g, 82 μmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 3 h, diluted with CH_2Cl_2, washed with 10% (w/v) aqueous $NaHCO_3$ (2 ×), and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 8:2 → toluene/EtOAc 7:3) to obtain **28** (86 mg, 70%). $R_f = 0.37$ (toluene/EtOAc 7:3); $[\alpha]_D^{20} = +14$ ($c = 1$); ^1H NMR ($CDCl_3$): $\delta = 5.76$, 5.54, 5.53 (3t, $J \approx 9.5$ Hz, each 1H; H-3,3'',4''), 5.46 (dd, $J_{1'',2''} = 7.9$, $J_{2'',3''} = 9.8$ Hz, 1H; H-2''), 5.35 (dd, $J_{1,2} = 7.7$, $J_{2,3} = 9.6$ Hz, 1H; H-2), 5.14 (dd, $J_{1',2'} = 8.0$, $J_{2',3'} = 9.2$ Hz, 1H; H-2'), 4.81 (d, 1H; H-1''), 4.56 (d, 1H; H-1), 4.51 (d, 1H; H-1'), 3.14 (dt, 2H; OCH$_2$CH$_2$C*H*$_2$N$_3$), 1.75–1.62 (m, 2H; OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 165.9$, 165.5, 165.1 (2C), 164.9, 164.7, 163.8, 160.2 (8Ph*C*O), 101.5, 100.8 (2C) (C-1,1',1''), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.5, 62.2 (2C) (C-6,6',6''), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.8 (OCH$_2$*C*H$_2$CH$_2$N$_3$); MS (MALDI-TOF): m/z: 1442 $[M+Na]^+$.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (29): TEMPO (catalytic amount), and a solution (70 μL) of KBr (3.6 mg) and Bu_4NBr (4.9 mg) in saturated aqueous $NaHCO_3$ (0.7 mL) were added to a solution of **28** (50 mg, 34 μmol) in CH_2Cl_2 (0.6 mL). The mixture was stirred vigorously at 0 °C, when a solution of saturated aqueous NaCl (70 μL), saturated aqueous $NaHCO_3$ (33 μL), and aqueous NaOCl (13% Cl active; 90 μL) was added dropwise. After 45 min, the mixture was acidified with 4 M HCl (pH 4), diluted with CH_2Cl_2, and the organic layer was washed with 10% (w/v) aqueous NaCl, dried, filtered, and concentrated. The residue was purified by column chromatography. Impurities were eluted with CH_2Cl_2/acetone (8:2), and **29** (38 mg, 76%) was eluted with CH_2Cl_2/acetone/HOAc (8:2:0.5). $R_f = 0.65$ (CH_2Cl_2/acetone/HOAc 8:2:1); $[\alpha]_D^{20} = +25$ ($c = 1$); ^1H NMR ($CDCl_3$): $\delta = 5.76$, 5.64, 5.53 (3t, $J_{2,3} = 9.6$, $J_{2'',3''} = 9.8$, $J_{3'',4''} = 9.3$ Hz, each 1H; H-3,3'',4''), 5.45 (dd, $J_{1'',2''} = 7.9$ Hz, 1H; H-2''), 5.27 (dd, $J_{1,2} = 7.7$ Hz, 1H; H-2), 5.18 (dd, $J_{1',2'} = 7.9$ Hz, 1H; H-2'), 4.86 (d, 1H; H-1''), 4.68 (d, 1H; H-1'), 4.58 (d, 1H; H-1), 3.62 (d, $J_{4',5'} = 9.3$ Hz, 1H; H-5'), 3.12 (dt, 2H; OCH$_2$CH$_2$C*H*$_2$N$_3$), 1.71–1.59 (m, 2H; OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 169.1$ (C-6'), 166.0, 165.7, 165.5, 165.3, 165.1, 164.9, 164.7, 163.8 (8Ph*C*O), 101.4, 100.7, 100.6 (C-1,1',1''), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.5, 62.1 (C-6,6''), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.8 (OCH$_2$*C*H$_2$CH$_2$N$_3$); MS (MALDI-TOF): m/z: 1456 $[M+Na]^+$.

3-Aminopropyl (β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyluronic acid)-(1→4)-β-D-glucopyranoside (2): NaOMe was added until pH 11 to a solution of **29** (50 mg, 33 μmol) in MeOH (3 mL). After stirring for 16 h, TLC (EtOAc/MeOH/H_2O 12:5:2) showed the formation of a new spot ($R_f = 0.27$), and the mixture was neutralised with Dowex H^+, filtered, and concentrated. A solution of the residue in water was washed with CH_2Cl_2 (3 ×), and the aqueous layer was concentrated. MALDI-TOF analysis of the residue showed a peak at m/z 624 $[M+Na]^+$. Then, a solution of the residue in 0.1 M NaOH (0.33 mL) was added dropwise to a suspension of 10% Pd/C (0.6 mg) and $NaBH_4$ (2.7 mg) in bidistilled water (0.17 mL). After 45 min, when TLC (EtOAc/MeOH/H_2O 12:5:3) showed the formation of a ninhydrin-positive spot on the baseline, the mixture was acidified with Dowex H^+ (pH 4), then loaded on a short column of Dowex 50 W × 2 (H^+, 200–400 mesh). After elution of contaminants with water, elution with 1% NH_4OH afforded **2** (17 mg, 89%), after concentration and co-concentration with water (2 ×). $[\alpha]_D^{20} = -18$ ($c = 0.8$, H_2O); MS (MALDI-TOF): m/z: 575 $[M+H]^+$, 597 $[M+Na]^+$. For ^1H NMR data, see Table 1.

3-Azidopropyl (2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (30): A solution of **21** (0.50 g, 0.47 mmol) and **23** (0.27 g, 0.29 mmol) in dry CH_2Cl_2 (4.4 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (8.5 μL, 47 μmol) was added. After 15 min, the mixture was neutralised with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (w/v) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 95:5) to obtain **30** (0.39 g, 75%). $R_f = 0.36$ (toluene/EtOAc 85:15); $[\alpha]_D^{20} = +34$ ($c = 1$); ^1H NMR ($CDCl_3$): $\delta = 5.58$, 5.40, 5.25 (3t, $J \approx 9.5$ Hz, each 1H; H-3,3'',3'''), 5.17, 5.11 (2s, each 1H; 2 PhC*H*), 4.72, 4.55, 4.53, 4.53 (4d, $J \approx 7.7$ Hz, each 1H; H-1,1',1'',1'''), 3.87, 3.81 (2d, $J_{gem} = 14.9$ Hz, each 1H; ClC*H*$_2$CO), 1.79–1.65 (m, 2H; OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta =$ 136.4, 136.2 (2*Ph*CH, quaternary C), 101.4 (2C), 101.1 (2C), 100.6, 99.8 (2Ph*C*H, C-1,1',1'',1'''), 67.5, 67.3 (C-6',6'''), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.0, 61.9 (C-6,6''), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 40.2 (Cl*C*H$_2$CO), 28.7 (OCH$_2$*C*H$_2$CH$_2$N$_3$); MS (FAB): m/z: 1834 $[M+H]^+$, 1856 $[M+Na]^+$.

3-Azidopropyl (2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (31): Thiourea (50 mg) was added to a solution of **30** (0.39 g, 0.22 mmol) in ethanol (5 mL) and pyridine (0.66 mL). After stirring for 3 h at 90 °C, the mixture was cooled to room temperature, co-concentrated with toluene, and the residue was purified by column chromatography (toluene/EtOAc 95:5 → toluene/EtOAc 9:1) to afford **31** (0.32 g, 84%). $R_f = 0.25$ (toluene/EtOAc 8:2); $[\alpha]_D^{20} = +52$ ($c = 1$); ^1H NMR ($CDCl_3$): $\delta = 5.58$, 5.39 (2t, $J \approx 9.5$ Hz, each 1H; H-3,3''), 5.17, 5.13 (2s, each 1H; 2 PhC*H*), 4.73, 4.54 (2H), 4.49 (3d, $J \approx 7.8$ Hz, 4H; H-1,1',1'',1'''), 3.11 (br t, 2H; OCH$_2$CH$_2$C*H*$_2$N$_3$), 1.70–1.59 (m, 2H; OCH$_2$C*H*$_2$CH$_2$N$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 165.9$, 165.6, 165.2, 165.1, 164.8 (2C), 164.7, 163.9 (8Ph*C*O), 136.4 (2*Ph*CH, quaternary C), 101.4 (3C), 101.1, 100.6, 100.0 (2Ph*C*H, C-1,1',1'',1'''), 67.4 (2C) (C-6',6'''), 66.3 (O*C*H$_2$CH$_2$CH$_2$N$_3$), 62.1 (2C) (C-6,6''), 47.7 (OCH$_2$CH$_2$*C*H$_2$N$_3$), 28.7 (OCH$_2$*C*H$_2$CH$_2$N$_3$); MS (FAB): m/z: 1758 $[M+H]^+$, 1780 $[M+Na]^+$.

3-Azidopropyl (2-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (32): CF_3COOH (0.12 mL) and H_2O (10 μL) were added to a solution of **31** (0.20 g, 0.11 mmol) in CH_2Cl_2 (6.5 mL). The mixture was stirred for 2.5 h, diluted with CH_2Cl_2, washed with 10% (w/v) aqueous $NaHCO_3$ (2 ×) and 10% (w/v) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene/EtOAc 1:1 → toluene/EtOAc 3:7) to obtain **32** (0.12 g, 70%). $R_f = 0.14$ (toluene/EtOAc 1:1); $[\alpha]_D^{20} = +36$ ($c = 1$); ^1H NMR ($CDCl_3$): $\delta =$ 5.52 (br t, $J \approx 9.3$ Hz, 2H; H-3,3''), 5.31, 5.29, 5.05, 4.91 (4dd, each 1H;

H-2,2′,2″,2‴), 4.63, 4.61, 4.54, 4.45 (4d, $J \approx 7.5$ Hz, each 1H; H-1,1′,1″,1‴), 1.68–1.56 (m, 2H; $OCH_2CH_2CH_2N_3$); ^{13}C NMR ($CDCl_3$): $\delta = 165.9$, 165.8, 165.7, 165.1 (2C), 165.0, 164.9, 163.9 (8Ph*C*O), 101.2, 100.7 (2C), 100.6 (C-1,1′,1″,1‴), 66.3 ($OCH_2CH_2CH_2N_3$), 62.3 (2C), 62.0, 61.3 (C-6,6′,6″,6‴), 47.7 ($OCH_2CH_2CH_2N_3$), 28.8 ($OCH_2CH_2CH_2N_3$); MS (FAB): *m/z*: 1582 $[M+H]^+$, 1604 $[M+Na]^+$.

3-Azidopropyl (2-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (33): TEMPO (catalytic amount), and a solution (0.12 mL) of KBr (6.6 mg) and Bu_4NBr (8.7 mg) in saturated aqueous $NaHCO_3$ (1.2 mL) were added to a solution of **32** (95 mg, 57 μmol) in CH_2Cl_2 (0.6 mL). The mixture was stirred vigorously at 0 °C, then a mixture of saturated aqueous NaCl (0.12 mL), saturated aqueous $NaHCO_3$ (0.06 mL), and aqueous NaOCl (13% Cl active; 0.15 mL) was added dropwise. After 45 min, the mixture was acidified with 4M HCl (pH 4), diluted with CH_2Cl_2, and the organic layer was washed with 10% (*w/v*) aqueous NaCl, dried, filtered, and concentrated. The residue was purified by column chromatography. Impurities were eluted with CH_2Cl_2/acetone (8:2), and **33** (64 mg, 65%) was eluted with CH_2Cl_2/acetone/HOAc (8:2:0.5). $R_f = 0.41$ (CH_2Cl_2/acetone/HOAc 8:2:1).

A small sample was methylated with diazomethane for analysis. ^1H NMR ($CDCl_3$): $\delta = 5.56$ (br t, $J \approx 9.3$ Hz, 2H; H-3,3″), 5.36, 5.28, 5.22, 5.13 (4dd, each 1H; H-2,2′,2″,2‴), 4.84, 4.66, 4.58, 4.54 (4d, $J \approx 7.8$ Hz, each 1H; H-1,1′,1″,1‴), 3.39, 3.34 (2s, each 3H; 2COO*C*H$_3$), 3.11 (dt, 2H; $OCH_2CH_2CH_2N_3$), 1.68–1.56 (m, 2H; $OCH_2CH_2CH_2N_3$); MS (MALDI-TOF): *m/z*: 1610 $[M+H]^+$, 1632 $[M+Na]^+$.

3-Aminopropyl (β-D-glucopyranosyluronic acid)-(1→4)-(β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyluronic acid)-(1→4)-β-D-glucopyranoside (3): NaOMe was added until pH 11 to a solution of **33** (75 mg, 43 μmol) in MeOH (3 mL). After stirring for 16 h, TLC (EtOAc/MeOH/H_2O 12:5:3) showed the formation of a new spot ($R_f = 0.30$), and the mixture was neutralised with Dowex H^+, filtered, and concentrated. A solution of the residue in water was washed with CH_2Cl_2 (3×), and the aqueous layer was concentrated. MALDI-TOF analysis of the residue showed a peak at *m/z* 800 $[M+Na]^+$. Then, a solution of the residue in 0.1M NaOH (0.43 mL) was added dropwise to a suspension of 10% Pd/C (0.8 mg) and $NaBH_4$ (3.5 mg) in bidistilled water (0.22 mL). After 45 min, when TLC (EtOAc/MeOH/H_2O 12:5:3) showed the formation of a ninhydrin-positive spot on the baseline, the mixture was acidified with Dowex H^+ (pH 4), then loaded on a short column of Dowex 50 W×2 (H^+, 200–400 mesh). After elution of contaminants with water, elution with 1% NH_4OH afforded **3** after concentration and co-concentration with water (2×) (19 mg, 60%). $[\alpha]_D^{20} = +124$ ($c = 0.7$, H_2O); MS (MALDI-TOF): *m/z*: 752 $[M+H]^+$, 774 $[M+Na]^+$. For ^1H NMR data, see Table 1.

3-Aminopropyl β-D-glucopyranoside (35): NaOMe was added until pH 10 to a solution of **16** (81 mg, 0.12 mmol) in MeOH (3 mL). After stirring for 2 h, when TLC (EtOAc/MeOH/H_2O 6:3:1) showed the formation of **34** ($R_f = 0.63$), the mixture was neutralised with Dowex H^+, filtered, and concentrated. A solution of the residue in water was washed with CH_2Cl_2 (3×), then the aqueous layer was concentrated, and a solution of the residue (crude **34**) in 0.1M NaOH (1.1 mL) was added dropwise to a suspension of 10% Pd/C (2 mg) and $NaBH_4$ (9.7 mg) in bidistilled water (0.5 mL). After 45 min, when TLC (EtOAc/MeOH/H_2O 12:5:3) showed the formation of **35** as a ninhydrin-positive spot on the baseline, the mixture was acidified with Dowex H^+ (pH 4) and loaded on a short column of Dowex 50 W×2 (H^+, 200–400 mesh). Contaminants were eluted with water, and after elution with 1% NH_4OH, **35** was obtained after concentration and co-concentration with water (2×) (25 mg, 88%). $[\alpha]_D^{20} = +6$ ($c = 0.2$, H_2O); MS (MALDI-TOF): *m/z*: 238 $[M+H]^+$, 260 $[M+Na]^+$. For ^1H NMR data, see Table 1.

3-Aminopropyl β-D-glucopyranosiduronic acid (37): TEMPO (0.12 mg) and KBr (5.6 mg) were added to a cooled solution (5 °C) of **34** (30 mg, 0.11 mmol) in H_2O (3 mL). Aqueous NaOCl (13% Cl active; 0.37 mL) was brought to pH 10 by addition of 4M HCl and cooled to 5 °C. The two solutions were combined and pH 10 was maintained by addition of 0.5M NaOH. After stirring for 3 h at 5 °C, the mixture was neutralised with 4M HCl and concentrated. Desalting of the mixture could not be achieved by size-exclusion chromatography. Therefore, the crude residue was acetylated by stirring with Ac_2O (1 mL) and pyridine (1 mL) for 4 h, and after concentration, the residue was purified by column chromatography (toluene/EtOAc 7:3). To a solution of the residue in MeOH (1 mL) and H_2O (0.5 mL) was added 0.5M NaOH until pH 10 was reached. After stirring overnight, Dowex H^+ was added until pH 6, and the mixture was filtered and concentrated to obtain **36**. A solution of the residue in 0.1M NaOH (1 mL) was added dropwise to a suspension of 10% Pd/C (2 mg) and $NaBH_4$ (8.1 mg) in H_2O (0.5 mL). After 30 min, when TLC (EtOAc/MeOH/H_2O 12:5:2) showed the formation of **37** as a ninhydrin-positive spot on the baseline, the mixture was acidified with Dowex H^+ (pH 4) and loaded on a short column of Dowex 50 W×2 (H^+, 200–400 mesh). Contaminants were eluted with water, and after elution with 1% NH_4OH, **37** was obtained after concentration and co-concentration with water (2×) (22 mg, 80%). $[\alpha]_D^{20} = -25$ ($c = 1$, H_2O); MS (MALDI-TOF): *m/z*: 252 $[M+H]^+$, 274 $[M+Na]^+$. For ^1H NMR data, see Table 1.

General procedure for the elongation of 1, 2, 3, 35 and 37 with diethyl squarate: A solution of diethyl squarate (1 μmol) in EtOH (76.2 μL, stock solution of 24.9 μL diethyl squarate in 12.8 mL EtOH) was added to a solution of 3-aminopropyl glycoside (1 μmol) in 0.1M sodium phosphate (40 μL for **35** and **37**, 75 μL for **1**–**3**; pH 6.9). After stirring for 16 h, EtOH was evaporated by flushing with N_2. To the water layer was added H_2O (**35**, 4 mL) or aqueous HOAc pH 4 (**1**–**3**, **37**, 6 mL), and the mixture was loaded on a C18 Bakerbond spe column (500 mg). Rinsing with H_2O (10 mL) eluted the starting material, and elution with MeOH (4 mL) and evaporation of the solvent by flushing with N_2, afforded the pure elongated fragment that was used for the conjugation reaction.

General procedure for the conjugation of the elongated saccharides to a protein carrier

CRM_{197}-conjugates: For a targeted oligosaccharide incorporation of about 11 mol mol^{-1}, the elongated saccharide fragment (≈1 μmol) was dissolved in 0.1M sodium borate buffer (400 μL; pH 9.5), and a solution of CRM_{197} (61.15 mg mL^{-1}; 86 μL, 0.09 μmol) was added. After stirring for two to three days, the mixture was diluted with H_2O to a protein concentration of 1 mg mL^{-1}, samples were taken for MALDI-TOF analysis, and the mixture was dialysed against 50mM sodium phosphate buffer (pH 7.2). To obtain different oligosaccharide loadings, the amount of elongated saccharide was varied. For MALDI-TOF analysis, samples were mixed on the target plate in a ratio of 1:1 (*v/v*) with the matrix 3,5-dimethoxy-4-hydroxycinnamic acid in 30% aqueous CH_3CN containing 0.3% CF_3COOH.

KLH-conjugates: The elongated saccharide fragment (≈1 μmol) was dissolved in 0.1M sodium borate buffer (400 μL; pH 9.5), and a solution of KLH (50 mg mL^{-1}; 75 μL for **1**, 60 μL for **2**, and 40 μL for **3**) was added. After stirring for two to three days, the mixture was diluted with 50mM sodium phosphate buffer (pH 7.2) and dialysed against the same buffer.

TT-conjugates: The elongated saccharide fragment (≈1 μmol) was dissolved in 0.1M sodium borate buffer (300 μL; pH 9.5), and a solution of TT (6.12 mg mL^{-1}; 0.61 mL for **1**, 0.49 mL for **2**, and 0.33 mL for **3**) was added. The pH was adjusted to 9.5 by addition of 0.1M NaOH. After stirring for two to three days, the reaction mixture was diluted with 50mM sodium phosphate buffer (pH 7.2) and dialysed against the same buffer.

The protein concentrations of all conjugates were determined by the Pierce assay[34] and the carbohydrate content by MALDI-TOF (CRM_{197}) or Dubois (KLH and TT) analysis.[33] Coupling efficiencies (Tables 2 and 3) were expressed as percentage of the targeted incorporation.

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