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# Carbohydrates as potentially versatile core subcarriers for multivalent immunogens†

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Synthetic multivalent glycoclusters that carry carbohydrate antigen epitopes have been recognized as promising candidates for the development of carbohydrate based vaccines. Here we describe a convergent strategy for the synthesis of conjugation-ready multivalent glycoclusters using sugars as versatile core subcarriers. D-Glucose and gentiobiose were converted into poly-alkyne functionalized cores which were then decorated with an azide bearing model ligand D-glucose using click chemistry, to form structurally well-defined tetra- and heptavalent glycoclusters. Each cluster was conjugated to a model protein bovine serum albumin (BSA) by squaric acid chemistry. Carbohydrate clusters can be prepared in a variety of sizes and spatial arrangements by altering the structure and configuration of the core, depending on the mono-, or oligosaccharides used for their assembly. It is suggested that the use of carbohydrate as core subcarriers provides an opportunity to tailor the size and topology of antigens and modify multivalent presentation of immunogens in a way to optimize cluster effect for stronger immunoreactivity.

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## Introduction

Binding of carbohydrates to host proteins is a frequent event in biology. Carbohydrate–protein interactions control diverse physiologically important biological processes.[1] To these belongs also the recognition of O-specific polysaccharides (O-SP), which are the protective antigens of Gram-negative bacterial pathogens, by the elements of the immune system of vertebrates. The magnitude of the immune response and, in turn, the spectrum and level of antibodies produced upon infection depend on the strength of these interactions. Nature and evolution prepared us to respond to many infections in close to perfect way. Thus, were it not for some, often tolerable level of toxicity and pyrogenicity, vaccines based on killed or attenuated pathogens would be the best and cheapest means of prevention of infectious diseases. Glycoconjugate vaccines, the man-made constructs from detoxified lipopolysaccharides (LPS) and carrier proteins, are often, although still not entirely, free from the undesirable effects bacterial vaccines often have. The ideal vaccine would contain the pure protective antigen as the sole active antigenic ingredient. Lack of a reliable method for preparation of pure O-SP from LPS led to the idea of using synthetic oligosaccharides that mimic structures of O-SPs as antigenic components of conjugate vaccines. For the clinical use, such constructs would be made from pure components, *i.e.* synthetic oligosaccharide antigens and recombinant proteins. Thus, neoglycoconjugate vaccines, *i.e.* a vaccine from the fully synthetic carbohydrate antigen, would be well defined, easy-to-characterize and reproduce molecules with long shelf life. The carbohydrate and the recombinant protein fields have progressed to the stage that we can make virtually any structure that would be needed. Thus, making such molecules is not the bottleneck responsible for the lack of progress in the synthetic vaccine development and for the fact that to date only one neoglycoconjugate vaccine is known to be clinically useful.[2]

Making vaccines from synthetic oligosaccharides is a very young field and structural/architectural requirements for a potent vaccine are not known. Optimizing such features is difficult because the formation of a glycoconjugate offers virtually an infinite number of configurational choices, as very ably demonstrated by Stein.[3]

When monovalent carbohydrate ligands are involved in interactions with receptors, the binding is often weak. To magnify the recognition, biological systems have circumvented this binding limitation through multivalency. The phenomenon of multivalency (cluster effect) was first described in their seminal work by Lee and coworkers.[4] They observed that not only the number of ligands in the cluster but also the ligand's spatial arrangement affected the ligand's binding affinity to receptors. These phenomena have not been systematically studied in connection with vaccine development from synthetic oligosaccharides.

We have been interested in developing a conjugate vaccine for cholera from synthetic fragments of O-specific polysaccharides (O-SP) of *Vibrio cholerae* O1 for a number of years.[5] When looking at how some variables affect the immunogenicity

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of glycoconjugates, we have examined the size of the oligosaccharide,[6] the length of the spacer (linker)[7] and the carbohydrate–protein ratio.[8] The conjugates used in those works were made according to the single-ended conjugation model,[9] which ensured that the vaccines were well defined, non cross-linked molecules. For making conjugates containing hapten in the form of clusters[10] we designed our own core subcarrier.[11] Its attractive feature was that it allowed to vary the number of ligands in the cluster but had the drawback that its use gave rise to cross-linked, high molecular mass constructs which were difficult to reproduce and characterize. Generally, the cross-linking can be avoided only when the fully assembled clusters are conjugated to the carriers according to the single-ended model.[9] Thus, the synthetic scheme towards a vaccine where the antigen is present in a form of clusters must include a core subcarrier that allows installation of a reasonable and defined number of antigens and be equipped with the spacer bearing only a single reactive group compatible with the intended chemistry of conjugation to carriers. Towards this goal, here we have devised a generally applicable route for the preparation of conjugation-ready multivalent glycoclusters utilizing sugars as versatile core subcarriers. D-Glucose and gentiobiose have been chosen as model sugars for making the poly-alkyne functionalized cores and 6-azidohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside was used as model hapten to be attached to the cores. Cu(I)-catalyzed azide–alkyne "click" cycloaddition (CuAAC)[12] was employed for their coupling to form structurally well defined tetra- and heptavalent glycoclusters. Furthermore, we show how the cluster thus formed can be conjugated to the model protein bovine serum albumin (BSA) using squaric chemistry.[13,14] When a bacterial antigen or a synthetic fragment thereof would be used as hapten in such conjugation, an experimental immunogen (vaccine) would be formed.

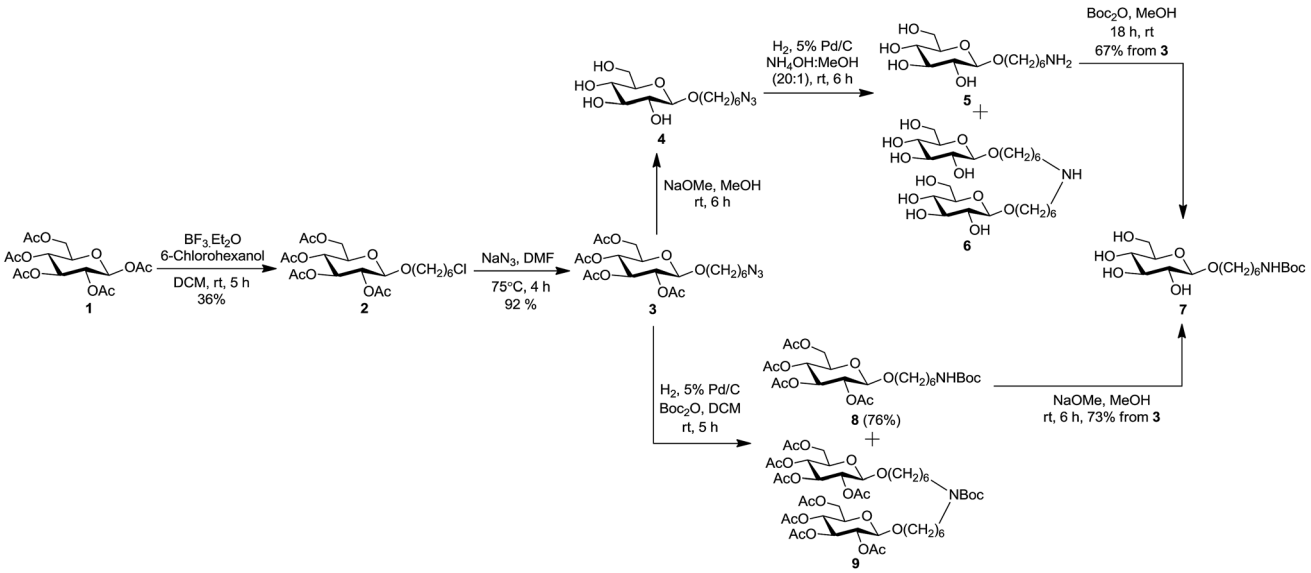
## Results and discussion

When searching for the class of substance that was to become the core to carry our antigen in the future cluster-type immunogen (vaccine), we took several factors into consideration. We wanted the core not only to carry a defined number of haptens but to also allow us to vary the size of the cluster and fine-tune the spatial arrangements of the haptens around it. Carbohydrates are among few classes of compounds that can fulfil these requirements. They are inexpensive commodities, many occur naturally, and methods for orthogonal functionalization of this class of compounds are readily available. The rich structural and configurational diversity of carbohydrates allows them to adopt many forms (different ring size and anomeric varieties) or unusual conformations (into which sugars can be forced by deoxygenation at various positions in the pyranose or furanose ring form). When such structures are utilized as cores, a large number of multivalent glycomimetics with diverse spatial arrangements, number of haptens and degree of freedoms can be constructed. Therefore, depending on the nature of the receptor, the use of carbohydrates as core allows optimization of the glycoside-cluster effect by providing the opportunity to tailor the valency and topology of the clusters formed.

Using carbohydrates as core molecules for creating clusters as probes for many purposes in the life sciences, also as chiral scaffolds,[15] is quite common[16–32] but to our knowledge making carbohydrate clusters by attaching fragments of O-SPs to cores by click chemistry and attaching the resulting cluster of antigens to carrier proteins by squaric acid chemistry to make neoglycoconjugate vaccines for bacterial diseases has not been explored. Here, we propose carbohydrates as convenient starting materials to form tailor-made core subcarriers for making clustered immunogens/vaccines. The synthetic strategy and the methods for characterization of the constructs obtained are generally applicable to making clusters from virtually any appropriately functionalized ligand. Clusters can be obtained in many sizes and shapes when the core results from a rational choice of carbohydrate(s). The newly formed constructs can be used as vaccines when the ligand is a mimic of a bacterial carbohydrate antigen, or tailor-made probes in many branches of the life sciences.

We herein report a straightforward and efficient strategy for the synthesis of two carbohydrate-centered glycoclusters and their conjugation to a model carrier protein BSA by squaric acid chemistry. The latter has become our method of choice for conjugation because of its unsurpassed efficiency and experimental simplicity.[33] For the preparation of poly-alkyne functionalized carbohydrate cores **19** and **22**, we chose simple monosaccharide per-*O*-acetylated β-D-glucose **1**[34] and the disaccharide per-*O*-acetylated β-gentiobiose **10** as starting materials.[35] The synthesis began with the introduction of spacer arm to per-*O*-acetates **1** and **10** by $BF_3 \cdot Et_2O$ catalyzed glycosidation with 6-chlorohexanol, to obtain **2** and **11** in 36% and 64% yield respectively (Scheme 1 and 2). The bromo analog of **2** was previously obtained in the same yield in a similar way by Dubber *et al.*[24] However, the chloro derivatives are preferred intermediates when conversion to the corresponding azides is anticipated because 6-chlorohexanol is a less expensive commodity. The azidation of **2** and **11** was brought about in the subsequent step, by their treatment with $NaN_3$ in DMF at 75 °C to afford corresponding azides **3** and **12** in almost theoretical yields. The starting materials and the products of the aforementioned conversions showed very similar chromatographic mobility in each case. However, the formation of **3** and **12** could be monitored by NMR spectroscopy. It showed confidently that the conversion of chlorides was complete and that these were one-product reactions. In both cases, compared to the $^1H$ spectra of **2** and **11**, the 2-proton triplet for H-6′ of **3** and **12** was shifted upfield by ~0.26 ppm, and the C-6′ signal in $^{13}C$ NMR spectra was shifted downfield by ~6.4 ppm.

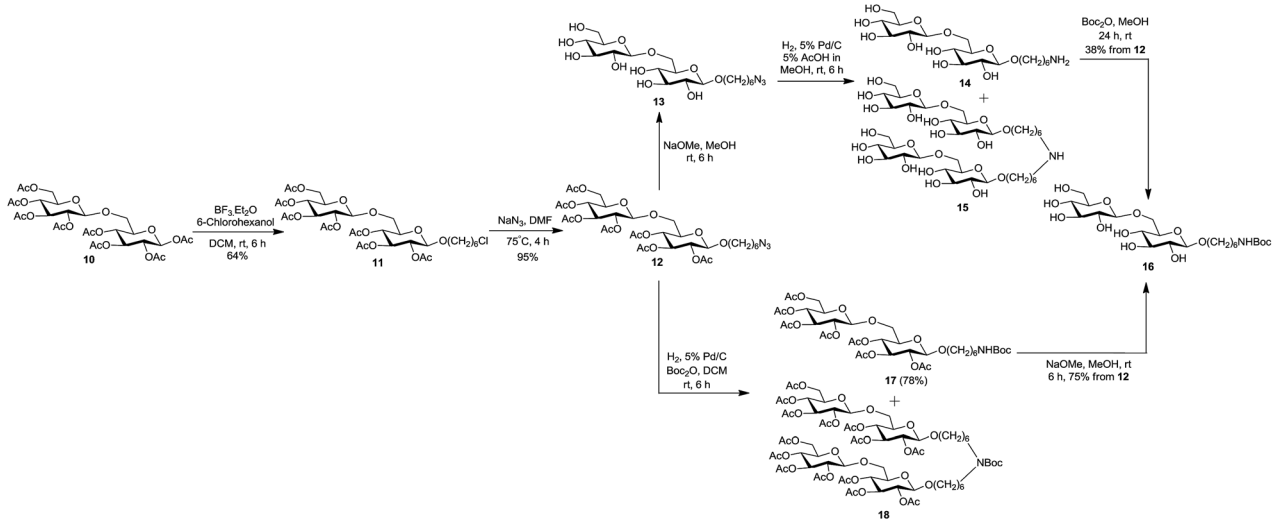
With the acetylated azide armed glycosides **3** and **12** at hand, we intended to convert them to the corresponding polyhydroxylated glycosides **7** and **16** carrying a protected amine-functionalized linker. The route employed was de-*O*-acetylation (Zemplén) of azide **3** to obtain **4**, followed by the reduction of **4** under catalytic hydrogenation conditions ($H_2$, 5% Pd/C, experimental, preparation of **7**, **A**). Careful analysis of the reaction mixture of hydrogenation of **4** by TLC revealed the presence of two poorly resolved major products in ~1 : 1 ratio. A challenging chromatographic separation on a small scale led to




Scheme 1 Synthesis of the intermediate **7**.

products amenable to structural analysis (NMR, MS). The compounds isolated were identified as the expected amine **5** and a by-product. Structure of the by-product **6** was determined on the basis of the spectral data (NMR, MS) and the literature precedence.[36–38] The same holds for deduction of structures of compounds **9**, **15**, and **18** described below. Because the proportion of side product **6** was unacceptably high, more favourable conditions for azide reduction were sought. Compound **5** was formed in a significantly improved amount (**5**/**6**, ~10 : 1) when the reduction of **4** was performed in presence of $NH_4OH$ in MeOH (experimental, preparation of **7**, **B**). On preparative scale, the difficult separation of **5** and **6** was omitted and the crude mixture was directly submitted for *N*H-Boc protection with di-*tert*-butyl dicarbonate in MeOH. In this way the key intermediate *N*H-Boc protected amine **7** was isolated in 67% yield (from **3**), after chromatography.

Following the optimized reaction conditions for the preparation of **7**, the synthesis of its disaccharide analogue **16** was attempted from azide **12** (Scheme 2). De-*O*-acetylation of **12** (Zemplén)[39] gave azide **13** in virtually theoretical yield, but hydrogenation of the latter under the conditions applied to **4** was unsuccessful: more than 95% (TLC) of **13** remained unchanged after several hours. The effort to reduce azide **13** under high pressure (200 psi) also failed. The reduction of **13** could be completed when carried out in 5% AcOH in MeOH as solvent. Sadly, though, the desired amine **14** and the side product **15** (deduced from MS and analogy to formation of **6**) were formed in ~1 : 1 ratio (TLC). Because the treatment of such mixture with Boc anhydride furnished **16** in only 38% yield (in 3 steps from **12**), and neither the yield of **7** was considered quite satisfactory, an alternative, more efficient synthetic route for **7** and **16** was sought.

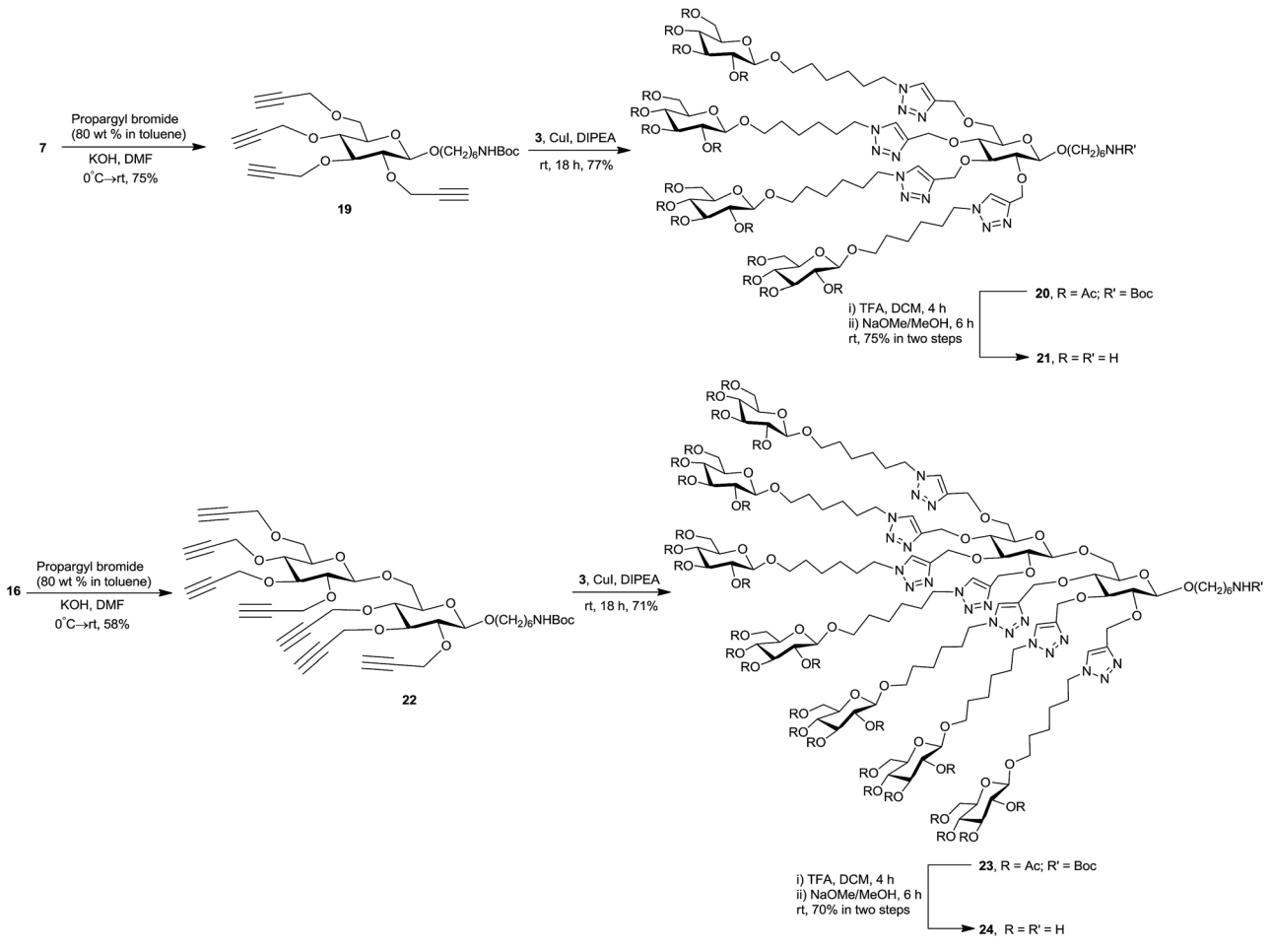



Scheme 2 Synthesis of the key intermediate **16**.

We have previously described a convenient conversion of a sugar azide to the corresponding *N*-acetamido derivative by conducting the catalytic hydrogenation ($H_2$, Pd/C) in presence of $Ac_2O$ using MeOH as solvent.[40] Because in the present situation neither the starting material nor the conversion products contained hydroxyl groups, the use of MeOH as solvent became unimportant. Accordingly, hydrogenation (5% Pd/C) of azide **3** in presence of di-*tert*-butyl dicarbonate using DCM as solvent effected the reduction to amine and simultaneous *N*H-Boc protection in one pot (experimental, preparation of **7**, **C**), to give a mixture of desired amine **8** and side product **9** (Scheme 1). A simple chromatographic separation afforded amine **8** (76%), which was de-*O*-acetylated under Zemplén transesterification conditions, to afford the key intermediate **7** in virtually theoretical yield. Synthesis of disaccharide **16** was achieved from **12** (75%, overall in two steps) in a similar way (Scheme 2).

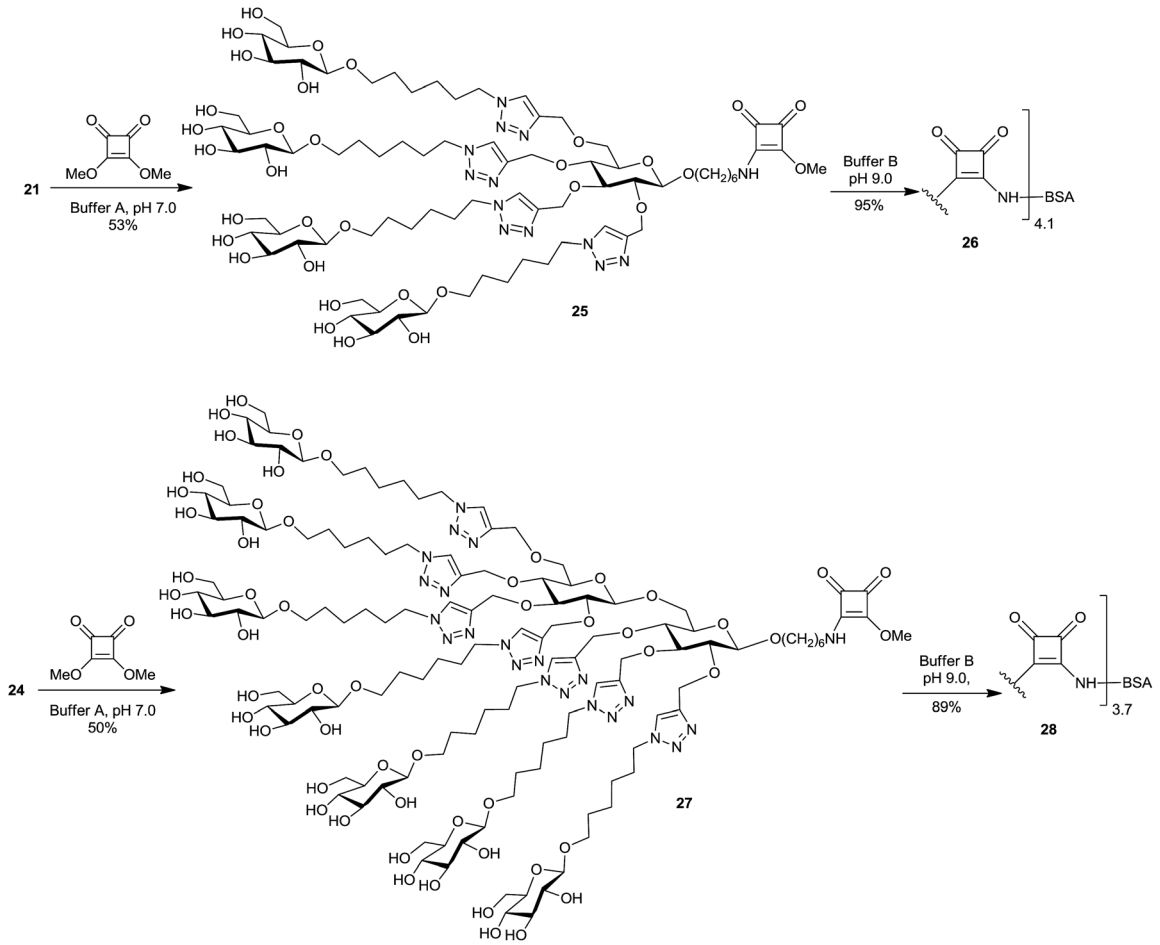
To proceed towards the title cores, glycosides **7** and **16** were treated with propargyl bromide and KOH at 0 °C → rt, to obtain the corresponding alkyne functionalized, clickable intermediates **19** and **22** in 75% and 58% yield, respectively. The structure confirmation for **19** and **22** was provided by spectral analysis (NMR, HRMS). In the $^1$H NMR spectrum of each of **19** and **22**, the characteristic ≡C–*H* protons appeared as multiplet at $\delta$ 2.5 ppm with integral values of four and seven protons respectively. In $^{13}$C NMR spectra, the signals for two acetylenic carbons appeared at $\delta$ ~80.0 ppm (*C*≡C–H) and ~74.0 ppm (C≡*C*–H). The molecular ion peak $[M + Na]^+$ at *m*/*z* 554.2731 for **19** and at 830.3727 for **22** confirmed the complete alkylation in each case.

To demonstrate the feasibility of our synthetic strategy in glycocluster formation, azidosugar **3** was chosen as click synthon to decorate the carbohydrate-centered building blocks **19** and **22** (Scheme 3). A Cu(I) catalyzed click reaction[41–44] was conducted between **19** (1.0 equiv.) and **3** (5.0 equiv.) in presence of CuI and diisopropylethylamine (DIPEA), which afforded **20** (77%). The synthesis of **23** (71%) was achieved in a similar manner through click coupling of **22** (1.0 equiv.) and **3** (8.0 equiv.). The $^1$H NMR spectra of **20** and **23** showed presence of four ($\delta$ 8.16, 7.94, 7.68 ppm in 1 : 2 : 1 ratio) and seven newly formed triazolyl proton singlets ($\delta$ 8.15, 8.04, 7.94, 7.89, 7.77 ppm in 2 : 1 : 2 : 1 : 1 ratio), respectively, and the signals for the alkyne ≡C–*H* protons were absent. This, along with the MS data, confirmed the successful multiple click reactions in both cases. The $^{13}$C NMR spectra of **20** and **23** revealed the presence of characteristic carbon signals at $\delta$ ~145–144 and $\delta$ ~124–123 ppm, respectively, for *C*-4 and *C*-5 of the 1,4-disubstituted 1,2,3-trizole ring. The complete structural




Scheme 3 Synthesis of tetra- and heptavalent glycoclusters **21** and **24** *via* click chemistry.




Scheme 4 Conjugation of the glycoclusters **21** and **24** to BSA by squaric acid chemistry.

assignment of **20** and **23** was achieved with the aid of 2D NMR experiments ($^1$H COSY, TOCSY and $^1$H–$^{13}$C HSQC).

The fully protected clusters **20** and **23** were then treated with TFA in DCM to remove the Boc protecting group, and subsequent de-*O*-acetylation (Zemplén) yielded the conjugation-ready clusters **21** and **24**, respectively, in good yields after purification by reverse-phase chromatography. The structures of **21** and **24** were confirmed by NMR (1D and 2D) and HRMS spectral data.

Conjugation of the synthetic glycoclusters to BSA carrier protein was executed by squaric acid chemistry.[13,14,45,46] Thus, clusters **21** and **24** were treated with 3,4-dimethoxy-3-cyclobutene-1,2-dione (dimethyl squarate) at pH 7 (phosphate buffer) to give the squaric acid monoester derivatives **25** and **27**, respectively, which were readily purified by chromatography (Scheme 4).[46] The formation of **25** and **27** was established by HRMS and NMR spectroscopy. In the $^1$H NMR spectra of each of **25** and **27**, the –OC$H_3$ protons attached to the squaric acid moiety resonated at $\delta$ 4.36 ppm and their $^{13}$C NMR spectra showed –OC$H_3$ carbon as two signals at $\delta$ ~61 ppm. The $^{13}$C spectra of monoesters **25** and **27** exhibited typical splitting of some signals due to the double bond nature of the vinylogous amide group, which is characteristic of squaric acid amide esters.[14]

Each of the monomethyl ester **25** and **27** was next treated with BSA in 0.5 M borate buffer at pH 9 for 2 days, according to the protocol developed in our lab.[45] The conjugations were carried out at a hapten/BSA ratio 6 : 1 and at a hapten concentration ~4.0 mM. After freeze drying, the SELDI MS analysis of the purified conjugates showed average molecular mass 73 700 Da for the conjugate **26** (molar ratio **25** : BSA ~4.1 : 1) and 77 600 Da for the conjugate **28** (molar ratio **27** : BSA ~3.7 : 1).

## Conclusions

We propose that carbohydrates can serve as versatile subcarriers for clusters of a variety of ligands whose valences and spatial arrangement can be predetermined by judicious choice of natural or chemically modified carbohydrates. Following that rational, two structurally well defined tetra- and heptavalent glycoclusters bearing amino-functionalized linker were synthesized by the Cu(I)-catalyzed click coupling of polyalkyne-functionalized D-glucose and gentiobiose cores and azide-equipped ligand. Squaric acid chemistry was successfully applied for conjugation of the obtained amino group-containing, synthetic, multivalent glycoclusters to the carrier

protein BSA. The synthetic materials, many of which were obtained in the analytically pure state, were characterized by physical constants (mp, $[\alpha]_D$) and spectral data (NMR, HRMS).

## Experimental section

### General methods

Melting points were determined with a Kofler hot stage. Optical rotations were measured at ambient temperature for solutions in $CHCl_3$ and MeOH with a Perkin–Elmer automatic polarimeter, Model 341. HPLC grade solvents were used, and reactions requiring anhydrous conditions were carried out under nitrogen or argon. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 glass slides. Spots were visualized by charring with $H_2SO_4$ in EtOH (5% v/v). Column chromatography was performed with Biotage Isolera chromatograph equipped with a Varian Evaporative Light-Scattering Detector. Nuclear magnetic resonance (NMR) spectra were measured at 600 MHz for $^1H$, and 150 MHz for $^{13}C$ for solutions in $CDCl_3$, MeOD and $D_2O$ with Bruker Avance spectrometer. Chemical shifts were reported relative to TMS ($^1H$: $\delta$ 0.00 ppm), $CHCl_3$ ($^{13}C$: $\delta$ 77.00 ppm), MeOH ($^1H$: $\delta$ 3.31 ppm; $^{13}C$: $\delta$ 49.00 ppm) or $H_2O$ ($\delta$ 4.79 ppm). Assignments of NMR signals were aided by 1D and 2D experiments (APT, COSY, TOCSY, HSQC, HMQC, and HMBC). When reporting assignment of NMR signals, nuclei associated with the subcarrier spacer (linker) are denoted with a prime and those associated with the ligand spacer are denoted with a double prime. Sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycone, and are identified by a Roman numeral superscript in listings of signal assignments. Liquid Chromatography-Electron Spray-Ionization Mass Spectrometry (ESI-MS) was performed with a Hewlett–Packard 1100 MSD spectrometer. Conjugation of carbohydrates was monitored by the BioRad Protein Chip SELDI system using NP-20 chip arrays. 3,5-Dimethoxy-4-hydroxycinnamic acid (sinapinic acid) was used as matrix. Attempts have been made to obtain correct combustion analysis data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtained within ±0.3%. The palladium-on-charcoal catalyst used was purchased from Engelhard Industries (Escat 103, Lot# FC96303). BSA was purchased from Sigma Chemical Company: A. Cat. Number A-0281; B. Fraction V, Sigma Cat. no. A-4503, from which fatty acids were removed by charcoal treatment.[47] Buffers used were as follows; A, BuffAR pH 7.0 Reference solution (Macron, Cat. no. 0031-04), concentrated to 1/10 volume; B, 0.5 M borate buffer pH 9, made in house (1 L) from boric acid (30.9 g), KCl (26.1 g), and KOH (8.42 g), and final adjustment to pH 9.0 by addition of solid KOH. During squaric acid monoester formation reaction, pH of the reaction mixture was monitored intermittently with a Corning, Model 440 pH meter equipped with model MI-410 microelectrode (Microelectrodes, Inc. Bedford, NH, USA) and when necessary, pH was maintained at 7 by addition of buffer salts, obtained by freeze-drying Buffer A. Solutions in organic solvents were dried with anhydrous $Na_2SO_4$, and concentrated at <40 °C/2 kPa.

**6-Chlorohexyl 2,3,4,6-tetra-*O*-acetyl β-D-glucopyranoside (2).** To a solution of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (8.0 g, 20.5 mmol) and 6-chlorohexanol (3.0 mL, 22.5 mmol) in anhydrous DCM (200 mL), $BF_3$ $Et_2O$ (7.6 mL, 61.5 mmol) was added drop wise and the mixture was stirred at room temperature for 5 h. The mixture was diluted with DCM, neutralized with aqueous $NaHCO_3$, and washed with brine (3 × 200 mL). The organic layer was concentrated and the residue was crystallized from EtOH. The crystals were washed with cold EtOH (twice) and with hexane to afford **2** (3.44 g, 36%). Recrystallized **2** (EtOH) showed mp 98–99 °C, $R_f$ 0.5 (2 : 1 hexane–acetone), and $[\alpha]_D^{23}$ −17.8 (*c* 0.7, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 600 MHz): $\delta$ 5.20 (t, 1H, *J* 9.5 Hz, H-3), 5.09 (t, 1H, *J* 9.7 Hz, H-4), 4.98 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.49 (d, 1H, H-1), 4.26 (dd, 1H, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.3 Hz, H-$6_a$), 4.14 (dd, 1H, $J_{5,6b}$ 2.5 Hz, H-$6_b$), 3.87 (dt, 1H, *J* 6.3, 9.6 Hz, H-$1'_a$), 3.69 (ddd, 1H, $J_{5,4}$ 10.0 Hz, H-5), 3.53 (t, 2H, *J* 6.7 Hz, H-$6'_{a,b}$), 3.48 (dt, 1H, *J* 6.6 Hz, H-$1'_b$), 2.09, 2.04, 2.03, 2.00 (4 s, 12H, 4 COC$H_3$), 1.76 (m, 2H, H-$5'_{a,b}$), 1.59 (m, 2H, H-$2'_{a,b}$), 1.44 (m, 2H, H-$4'_{a,b}$), 1.36 (m, 2H, H-$3'_{a,b}$) ppm; $^{13}C$ NMR ($CDCl_3$, 150 MHz): $\delta$ 170.67, 170.30, 169.34, 169.26 (4 *C*OCH$_3$), 100.80 (C-1), 72.85 (C-3), 71.76 (C-5), 71.34 (C-2), 69.89 (C-1′), 68.47 (C-4), 61.98 (C-6), 44.92 (C-6′), 32.45 (C-5′), 29.21 (C-2′), 26.48 (C-4′), 25.11 (C-3′), 20.72, 20.64, 20.60, 20.58 (4 CO*C*H$_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{35}NO_{10}Cl$ [M + $NH_4$]$^+$: 484.1949; found: 484.1953; anal. calcd for $C_{20}H_{31}ClO_{10}$: C, 51.45; H, 6.69; found: C, 51.35; H, 6.62.

**6-Azidohexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (3).** A mixture of acetate **2** (4.66 g, 10.0 mmol) and $NaN_3$ (2.6 g, 40.0 mmol) in DMF (15 mL) was stirred at 75 °C. For monitoring the progress of reaction by $^1H$ NMR, the solvent was removed from a few drops of the reaction mixture with strong stream of air, the residue was triturated with $CDCl_3$, and the extract was filtered directly into the NMR tube. When the spectrum showed complete disappearance of the starting material (~4 h), the mixture was concentrated and the residue was partitioned between $CHCl_3$ and brine. After concentration of the organic phase, pure azide **3** was obtained by crystallization of the residue from EtOH as described for **2**. Two more crops were obtained in the same way from the mother liquor (total yield, 4.35 g, 92%). The recrystallized (EtOH) azide **3** showed mp 79–80 °C, $R_f$ 0.5 (2 : 1 hexane–acetone) and $[\alpha]_D^{23}$ −18.9 (*c* 0.7, $CHCl_3$). $^1H$ NMR ($CDCl_3$, 600 MHz): $\delta$ 5.20 (t, 1H, *J* 9.6 Hz, H-3), 5.09 (t, 1H, *J* 9.8 Hz, H-4), 4.98 (dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.49 (d, 1H, H-1), 4.26 (dd, 1H, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.3 Hz, H-$6_a$), 4.14 (dd, 1H, $J_{5,6b}$ 2.5 Hz, H-$6_b$), 3.87 (dt, 1H, *J* 6.3, 9.6 Hz, H-$1'_a$), 3.69 (ddd, 1H, $J_{5,4}$ 10.0 Hz, H-5), 3.48 (dt, 1H, *J* 6.7 Hz, H-$1'_b$), 3.26 (t, 2H, *J* 7.0, H-$6'_{a,b}$), 2.09, 2.04, 2.03, 2.01 (4 s, 12H, 4 COC$H_3$), 1.59 (m, overlapped, 4H, H-$2'_{a,b}$, $5'_{a,b}$), 1.37 (m, overlapped, 4H, H-$3'_{a,b}$, $4'_{a,b}$) ppm; $^{13}C$ NMR ($CDCl_3$, 150 MHz): $\delta$ 170.68, 170.31, 169.40, 169.26 (4 *C*OCH$_3$), 100.81 (C-1), 72.85 (C-3), 71.77 (C-5), 71.35 (C-2), 69.89 (C-1′), 68.48 (C-4), 61.99 (C-6), 51.33 (C-6′), 29.24, 28.75, 26.38, 25.40 (C-2′, C-3′, C-4′, C-5′), 20.73, 20.64, 20.61, 20.59 (4 CO*C*H$_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{20}H_{35}N_4O_{10}$ [M + $NH_4$]$^+$: 491.2353; found: 491.2351; anal. calcd for $C_{20}H_{31}N_3O_{10}$: C, 50.74; H, 6.60; N, 8.87; found: C, 50.63; H, 6.53; N, 8.85.

**6-*N*-(*tert*-Butyloxycarbonyl)hexyl β-D-glucopyranoside (7)**

***A.*** To a solution of acetate **3** (500 mg, 1.05 mmol) in MeOH (6 mL), a solution of NaOMe in MeOH was added until strong alkalinity and the mixture was kept at room temperature until the reaction was complete (~6 h). The mixture was diluted with MeOH, neutralized with Amberlite IR 120H$^+$, filtered and concentrated. Chromatography (4 : 3 : 0.2 DCM–acetone–MeOH) afforded 6-azidohexyl β-D-glucopyranoside **4** (310 mg, 96%) as colorless syrup. $R_f$ 0.25 (4 : 3 : 0.2 DCM–acetone–MeOH). $^1$H NMR (MeOD, 600 MHz): $\delta$ 4.24 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 3.91 (dt, 1H, $J$ 6.7, 9.6 Hz, H-1′$_a$), 3.86 (dd, 1H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 12.0 Hz, H-6$_a$), 3.66 (dd, 1H, $J_{5,6b}$ 5.3 Hz, H-6$_b$), 3.55 (dt, 1H, $J$ 6.7 Hz, H-1′$_b$), 3.34 (t, 1H, $J$ 8.8 Hz, H-3), 3.29–3.24 (m, overlapped, 4H, H-6′$_{a,b}$, H-4, H-5), 3.17 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 1.62 (m, overlapped, 4H, H-2′$_{a,b}$, 5′$_{a,b}$), 1.42 (m, overlapped, 4H, H-3′$_{a,b}$, 4′$_{a,b}$) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 104.37 (C-1), 78.14 (C-3), 77.92 (C-5), 75.14 (C-2), 71.69 (C-4), 70.67 (C-1′), 62.79 (C-6), 52.40 (C-6′), 30.63 (C-2′), 29.86 (C-5′), 27.62, 26.64 (C-3′, C-4′) ppm. HRMS (ESI): $m/z$ calcd for $C_{12}H_{23}N_3O_6Na$ [M + Na]$^+$: 328.1485; found: 328.1479.

A stirred solution of azido sugar **4** (310 mg, 1.01 mmol) in MeOH (~5 mL) was treated with hydrogen in presence of 5% Pd/C (60 mg) at room temperature overnight. TLC (1 : 2 : 0.06 DCM–MeOH–25% $NH_4OH$) showed that two major products were formed in an approximate ratio of 1 : 1 ($R_f$ ~0.1, partially overlapping). After filtration through Celite pad, the filtrate was concentrated. For identification, a small amount of the mixture was chromatographed to obtain the major products, which were sufficiently pure for spectral identification. Eluted first was material identified as **5** (white solid), $^1$H NMR (MeOD, 600 MHz): $\delta$ 4.24 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.91 (dt, 1H, $J$ 6.7, 9.6 Hz, H-1′$_a$), 3.86 (m, 1H, 12.0 Hz, H-6$_a$), 3.66 (dd, 1H, $J_{6a,6b}$ 12.0 Hz, $J_{5,6b}$ 5.3 Hz, H-6$_b$), 3.55 (dt, 1H, H-1′$_b$), 3.34 (m, 1H, H-3), 3.29–3.24 (m, overlapped, 2H, H-4, H-5), 3.16 (m, 1H, H-2), 2.67 (t, 2H, $J$ 7.2 Hz, H-6′$_{a,b}$), 1.64 (m, 2H, $CH_2$ linker), 1.50 (m, 2H, $CH_2$ linker), 1.39 (m, overlapped, 4H, 2 × $CH_2$ linker) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 104.37 (C-1), 78.15 (C-3), 77.93 (C-5), 75.13 (C-2), 71.68 (C-4), 70.73 (C-1′), 62.77 (C-6), 42.19 (C-6′), 32.92, 30.64, 27.66, 26.86 (4 × $CH_2$ linker) ppm. HRMS (ESI): $m/z$ calcd for $C_{12}H_{25}NO_6Na$ [M + Na]$^+$: 302.1580; found: 302.1584. Later eluted was the side product **6** (white solid), $^1$H NMR (MeOD, 600 MHz): $\delta$ 4.24 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.91 (dt, 1H, $J$ 6.7, 9.6 Hz, H-1′$_a$), 3.86 (m, 1H, H-6$_a$), 3.66 (dd, 2H, $J_{6a,6b}$ 11.8 Hz, $J_{5,6b}$ 5.2 Hz, H-6$_b$), 3.55 (dt, 2H, H-1′$_b$), 3.34 (m, 2H, H-3), 3.29–3.24 (m, overlapped, 4H, H-4, H-5), 3.16 (t, 2H, $J$ 8.2 Hz, H-2), 2.61 (t, 4H, $J$ 7.6 Hz, H-6′$_{a,b}$), 1.64 (m, 4H, 2 × $CH_2$ linker), 1.54 (m, 4H, 2 × $CH_2$ linker), 1.40 (m, overlapped, 8H, 4 × $CH_2$ linker) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 104.36 (C-1), 78.13 (C-3), 77.91 (C-5), 75.12 (C-2), 71.67 (C-4), 70.74 (C-1′), 62.77 (C-6), 50.43 (C-6′), 30.63, 29.96, 28.11, 26.93 (4 × $CH_2$ linker) ppm. HRMS (ESI): $m/z$ calcd for $C_{24}H_{48}NO_{12}$ [M + H]$^+$: 542.3177; found: 542.3183. Because **5**, the intended intermediate to **7**, was not the major reaction product, this route to **7** was not pursued further.

***B.*** Compound **3** (500 mg, 1.05 mmol) was deacetylated as described in **A** and a solution of azido sugar **4** formed was hydrogenated in 1 : 20 25% $NH_4OH$–MeOH (~5 mL) in presence of 5% Pd/C (60 mg) at room temperature overnight. After conventional processing, the crude mixture of **5** and **6** (281 mg) obtained was dissolved in MeOH (5 mL) and treated overnight with di-*tert*-butyl dicarbonate (330 mg, 1.50 mmol). After concentration, the residue was chromatographed (8 : 1 $CHCl_3$–MeOH) to yield **7** as white foam (310 mg, 67%, in three steps from **3**); $R_f$ 0.5 (6 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{23}$ −30.7 ($c$ 0.8, $CHCl_3$). $^1$H NMR (MeOD, 600 MHz): $\delta$ 4.24 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.90 (dt, 1H, $J$ 6.8, 9.5 Hz, H-1′$_a$), 3.86 (dd, 1H, $J_{5,6a}$ 2.1 Hz, $J_{6a,6b}$ 11.9 Hz, H-6$_a$), 3.66 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6$_b$), 3.54 (dt, 1H, H-1′$_b$), 3.34 (t, 1H, $J$ 8.8 Hz, H-3), 3.28 (t, overlapped, 1H, H-4), 3.25 (m, overlapped, 1H, H-5), 3.16 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 3.02 (t, 2H, $J$ 7.0 Hz, H-6′$_{a,b}$), 1.63 (m, 2H, H-2′$_{a,b}$), 1.49–1.38 (m, overlapped, H-5′$_{a,b}$, H-3′$_{a,b}$), 1.43 (s, overlapped, NHCOOC(C$H_3$)$_3$), 1.34 (m, 2H, H-4′$_{a,b}$) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 158.57 (NH*C*OOC(CH$_3$)$_3$), 104.37 (C-1), 79.78 (NHCOO*C*(CH$_3$)$_3$), 78.14 (C-3), 77.92 (C-5), 75.13 (C-2), 71.68 (C-4), 70.75 (C-1′), 62.78 (C-6), 41.28 (C-6′), 30.90 (C-5′), 30.69 (C-2′), 28.80 (NHCOOC(*C*H$_3$)$_3$), 27.65 (C-4′), 26.76 (C-3′) ppm. HRMS (ESI): $m/z$ calcd for $C_{17}H_{33}NO_8Na$ [M + Na]$^+$: 402.2104; found: 402.2111; anal. calcd for $C_{17}H_{33}NO_8$: C, 53.81; H, 8.77; N, 3.69; found: C, 53.73; H, 8.85; N, 3.65.

***C.*** A suspension of 5% Pd/C (50 mg) in DCM (2 mL) was added to the solution of azide **3** (250 mg, 0.53 mmol) and di-*tert*-butyl dicarbonate (230 mg, 1.05 mmol) in DCM (4 mL) and the mixture was stirred at room temperature under $H_2$ atmosphere for 5 h, when TLC showed complete consumption of starting material. After conventional processing, as above, and concentration, the residue was chromatographed (4 : 1 hexane–acetone) to give 6-*N*-(*tert*-butyloxycarbonyl)hexyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **8** (218 mg, 76%), $R_f$ 0.4 (2 : 1 hexane–acetone). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 5.20 (t, 1H, $J$ 9.5 Hz, H-3), 5.07 (t, 1H, $J$ 9.7 Hz, H-4), 4.97 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.57–4.48 (bs, N*H*), 4.49 (d, 1H, H-1), 4.26 (dd, 1H, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.3 Hz, H-6$_a$), 4.14 (dd, 1H, $J_{5,6b}$ 2.5 Hz, H-6$_b$), 3.85 (dt, 1H, $J$ 6.3, 9.6 Hz, H-1′$_a$), 3.69 (ddd, 1H, $J_{5,4}$ 9.9 Hz, H-5), 3.47 (dt, 1H, $J$ 6.7 Hz, H-1′$_b$), 3.09 (t, 2H, $J$ 6.9 Hz, H-6′$_{a,b}$), 2.08, 2.03, 2.02, 2.00 (4 s, 12H, 4 COC$H_3$), 1.56 (m, 2H, H-2′$_{a,b}$), 1.46 (m, overlapped, H-5′$_{a,b}$), 1.44 (s, overlapped, NHCOOC(C$H_3$)$_3$), 1.32 (m, overlapped, 4H, H-3′$_{a,b}$, 4′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.56, 170.20, 169.33, 169.19 (4 *C*OCH$_3$), 155.97 (NH*C*OOC(CH$_3$)$_3$), 100.82 (C-1), 79.03 (NHCOO*C*(CH$_3$)$_3$), 72.92 (C-3), 71.82 (C-5), 71.44 (C-2), 69.95 (C-1′), 68.61 (C-4), 62.05 (C-6), 40.51 (C-6′), 29.99 (C-5′), 29.29 (C-2′), 28.41 (NHCOOC(*C*H$_3$)$_3$), 26.41 (C-4′), 25.50 (C-3′), 20.65, 20.58, 20.53, 20.51 (4 CO*C*H$_3$) ppm. HRMS (ESI): $m/z$ calcd for $C_{25}H_{41}NO_{12}Na$ [M + Na]$^+$: 570.2526; found: 570.2526. Eluted later was the side product **9**. $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 5.20 (t, 2H, $J$ 9.5 Hz, H-3), 5.07 (t, 2H, $J$ 9.7 Hz, H-4), 4.97 (dd, 2H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.49 (d, 2H, H-1), 4.26 (dd, 2H, $J_{5,6a}$ 4.8 Hz, $J_{6a,6b}$ 12.2 Hz, H-6$_a$), 4.14 (dd, 2H, $J_{5,6b}$ 2.5 Hz, H-6$_b$), 3.85 (dt, 2H, $J$ 6.5, 9.6 Hz, H-1′$_a$), 3.69 (ddd, 2H, $J_{5,4}$ 10.0 Hz, H-5), 3.47 (dt, 2H, $J$ 6.7 Hz, H-1′$_b$), 3.13 (t, 4H, $J$ 7.1 Hz, H-6′$_{a,b}$), 2.08, 2.03, 2.02, 2.00 (4 s, 24H, 2 × 4 COC$H_3$), 1.57 (m, 4H, 2 × $CH_2$ linker), 1.48 (m, 4H, 2 × $CH_2$ linker), 1.44 (s, 9H, NHCOOC(C$H_3$)$_3$), 1.39–1.24 (m, overlapped, 8H, 4 × $CH_2$ linker) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.59, 170.24, 169.36, 169.19 (2 × 4 *C*OCH$_3$), 155.58

(NH*C*OOC($CH_3$)$_3$), 100.85 (C-1), 78.98 (NHCOO*C*($CH_3$)$_3$), 72.95 (C-3), 71.84 (C-5), 71.47 (C-2), 70.02 (C-1′), 68.63 (C-4), 62.08 (C-6), 46.96 (C-6′), 29.42 ($CH_2$ linker), 28.50 (overlapped, NHCOOC($CH_3$)$_3$, $CH_2$ linker), 26.67 ($CH_2$ linker), 25.63 ($CH_2$ linker), 20.65, 20.60, 20.56, 20.54 (4 CO*C*$H_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{45}H_{71}NO_{22}Na$ [M + Na]$^+$: 1000.4365; found: 1000.4359.

A solution of **8** (218 mg, 0.40 mmol) in MeOH (4 mL), was treated with NaOMe in MeOH as described for the preparation of **4**. Chromatography of the residue (8 : 1 $CHCl_3$–MeOH) afforded **7** (146 mg, 73% in two steps from **3**) as white foam, which was in all aspects identical with **7** described in **B**.

**6-Chlorohexyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside [6-chlorohexyl 2,3,4,2′,-3′,4′,6′-hepta-*O*-acetyl-β-gentiobioside] (11).** To a stirred solution of 1,2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-β-gentiobiose (**10**, 2.0 g, 2.95 mmol)[35] and 6-chlorohexanol (0.48 mL, 3.54 mmol) in dry DCM (40 mL), $BF_3$ $Et_2O$ (1.09 mL, 8.84 mmol) was added slowly at room temperature and the mixture was stirred for 5 h. The mixture was diluted with DCM, neutralized with aqueous $NaHCO_3$ and washed with brine (3 × 75 mL). The organic layer was dried, concentrated and chromatography (10 : 1 toluene–acetone) of the residue furnished **11** (1.44 g, 64%) as white solid. A portion of **11** was crystallized (EtOH) to give material, mp 148–149 °C; $R_f$ 0.4 (4 : 1 toluene–acetone); $[\alpha]_D^{23}$ −23.5 (*c* 0.8, $CHCl_3$). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 5.19 (t, overlapped, *J* 9.5 Hz, H-3$^I$), 5.18 (t, overlapped, *J* 9.5 Hz, H-3$^{II}$), 5.07 (t, 1H, *J* 9.7 Hz, H-4$^{II}$), 4.99 (dd, 1H, $J_{1^{II},2^{II}}$ 8.0 Hz, $J_{2^{II},3^{II}}$ 9.6 Hz, H-2$^{II}$), 4.93 (dd, 1H, $J_{1^{I},2^{I}}$ 8.0 Hz, $J_{2^{I},3^{I}}$ 9.7 Hz, H-2$^I$), 4.88 (t, 1H, *J* 9.7 Hz, H-4$^I$), 4.60 (d, 1H, H-1$^{II}$), 4.46 (d, 1H, H-1$^I$), 4.27 (dd, 1H, $J_{5^{II},6a^{II}}$ 4.8 Hz, $J_{6a^{II},6b^{II}}$ 12.3 Hz, H-6$_a^{II}$), 4.13 (dd, 1H, $J_{5^{II},6b^{II}}$ 2.3 Hz, H-6$_b^{II}$), 3.88 (dt, overlapped, *J* 6.2, 9.6 Hz, H-1′$_a$), 3.85 (dd, overlapped, $J_{5^{I},6a^{I}}$ 1.6 Hz, $J_{6a^{I},6b^{I}}$ 11.1 Hz, H-6$_a^I$), 3.70–3.65 (m, overlapped, 2H, H-5$^{II}$, H-5$^I$), 3.62 (m, 1H, 6$_b^I$), 3.53 (t, 2H, *J* 6.7 Hz, H-6′$_{a,b}$), 3.47 (dt, 1H, *J* 6.6 Hz, H-1′$_b$), 2.09, 2.04, 2.03, 2.02, 2.00, 1.99 (s, overlapped, 21H, 7 COC*H*$_3$), 1.77 (m, 2H, H-5′$_{a,b}$), 1.59 (m, 2H, H-2′$_{a,b}$), 1.45 (m, 2H, H-4′$_{a,b}$), 1.37 (m, 2H, H-3′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.59, 170.22, 170.17, 169.60, 169.39, 169.26, 169.18 (7 *C*OCH$_3$), 100.79 (C-1$^{II}$), 100.55 (C-1$^I$), 73.36 (C-5$^I$), 72.78, 72.74 (C-3$^{II}$, C-3$^I$), 71.96 (C-5$^{II}$), 71.37 (C-2$^I$), 71.11 (C-2$^{II}$), 69.75 (C-1′), 69.17 (C-4$^I$), 68.30 (C-6$^I$), 68.27 (C-4$^{II}$), 61.81 (C-6$^{II}$), 44.92 (C-6′), 32.46 (C-5′), 29.21 (C-2′), 26.52 (C-4′), 25.16 (C-3′), 20.71, 20.64, 20.60, 20.59, 20.57 (overlapped, 7 CO*C*H$_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{32}H_{47}ClO_{18}Na$ [M + Na]$^+$: 777.2349, found: 777.2344; anal. calcd for $C_{32}H_{47}ClO_{18}$: C, 50.90; H, 6.27, found: C, 50.61; H, 6.18.

**6-Azidohexyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside [6-azidohexyl 2,3,4,2′,-3′,4′,6′-hepta-*O*-acetyl-β-gentiobioside] (12).** A mixture of **11** (2.53 g, 3.35 mmol) and $NaN_3$ (0.87 g, 13.4 mmol) in DMF (10 mL) was stirred at 75 °C until $^1$H NMR spectrum showed complete disappearance of the starting material (∼4 h; for monitoring of the conversion, see preparation of **3**). The mixture was concentrated and the residue was partitioned between $CHCl_3$ and brine (3 × 75 mL). Chromatography of the residue obtained upon concentration of the organic phase (10 : 1 toluene–acetone) gave **12** (2.44 g, 95%) as white solid. A portion of **12** when recrystallized (EtOH) as described for azide **3** showed mp 129–130 °C; $R_f$ 0.4 (4 : 1 toluene–acetone); $[\alpha]_D^{23}$ −22.3 (*c* 0.9, $CHCl_3$). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 5.19 (t, overlapped, *J* 9.5 Hz, H-3$^I$), 5.18 (t, overlapped, *J* 9.5 Hz, H-3$^{II}$), 5.07 (t, 1H, *J* 9.7 Hz, H-4$^{II}$), 4.99 (dd, 1H, $J_{1^{II},2^{II}}$ 8.0 Hz, $J_{2^{II},3^{II}}$ 9.6 Hz, H-2$^{II}$), 4.93 (dd, 1H, $J_{1^{I},2^{I}}$ 8.0 Hz, $J_{2^{I},3^{I}}$ 9.7 Hz, H-2$^I$), 4.88 (t, 1H, *J* 9.7 Hz, H-4$^I$), 4.59 (d, 1H, H-1$^{II}$), 4.45 (d, 1H, H-1$^I$), 4.27 (dd, 1H, $J_{5^{II},6a^{II}}$ 4.9 Hz, $J_{6a^{II},6b^{II}}$ 12.3 Hz, H-6$_a^{II}$), 4.13 (dd, 1H, $J_{5^{II},6b^{II}}$ 2.3 Hz, H-6$_b^{II}$), 3.88 (dt, overlapped, *J* 6.3, 9.6 Hz, H-1′$_a$), 3.85 (dd, overlapped, $J_{5^{I},6a^{I}}$ 2.0 Hz, $J_{6a^{I},6b^{I}}$ 11.0 Hz, H-6$_a^I$), 3.70–3.66 (m, overlapped, H-5$^{II}$, H-5$^I$), 3.62 (m, 1H, 6$_b^I$), 3.47 (dt, 1H, *J* 6.6 Hz, H-1′$_b$), 3.27 (t, 2H, *J* 6.9 Hz, H-6′$_{a,b}$), 2.09, 2.04, 2.03, 2.02, 2.00, 1.99 (s, overlapped, 7 COC*H*$_3$), 1.59 (m, overlapped, 4H, H-2′$_{a,b}$, H-5′$_{a,b}$), 1.38 (m, overlapped, 4H, H-3′$_{a,b}$, H-4′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.59, 170.21, 170.17, 169.60, 169.39, 169.26, 169.18 (7 *C*OCH$_3$), 100.79 (C-1$^{II}$), 100.55 (C-1$^I$), 73.34 (C-5$^I$), 72.78, 72.73 (C-3$^{II}$, C-3$^I$), 71.96 (C-5$^{II}$), 71.36 (C-2$^I$), 71.11 (C-2$^{II}$), 69.75 (C-1′), 69.17 (C-4$^I$), 68.31 (C-6$^I$), 68.27 (C-4$^{II}$), 61.82 (C-6$^{II}$), 51.32 (C-6′), 29.23, 28.74 (C-5′,C-2′), 26.40, 25.44 (C-4′, C-3′), 20.71, 20.60, 20.63, 20.60, 20.58, 20.57, 20.56 (overlapped, 7 CO*C*H$_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{32}H_{47}N_3O_{18}Na$ [M + Na]$^+$: 784.2752; found: 784.2765; anal. calcd for $C_{32}H_{47}N_3O_{18}$: C, 50.46; H, 6.22; N, 5.52; found: C, 50.36; H, 6.15; N, 5.48.

**6-*N*-(*tert*-Butyloxycarbonyl)hexyl 6-*O*-(β-D-glucopyranosyl)-β-D-glucopyranoside [6-*N*-(*tert*-butyloxycarbonyl)hexyl β-gentiobioside] (16)**

***A***. Acetate **12** (0.78 g, 1.02 mmol) in MeOH (5 mL), was treated with a solution of NaOMe in MeOH as described for preparation of **4** to afford 6-azidohexyl β-gentiobioside **13** (0.46 g, 95%) as white foam. $R_f$ 0.46 (1 : 1 DCM–MeOH); $^1$H NMR (MeOD, 600 MHz): $\delta$ 4.38 (d, 1H, $J_{1^{II},2^{II}}$ 7.8 Hz, H-1$^{II}$), 4.26 (d, 1H, $J_{1^{I},2^{I}}$ 7.8 Hz, H-1$^I$), 4.14 (dd, 1H, $J_{5^{I},6a^{I}}$ 2.0 Hz, $J_{6a^{I},6b^{I}}$ 11.5 Hz, H-6$_a^I$), 3.89 (dt, overlapped, *J* 6.6, 9.6 Hz, H-1′$_a$), 3.87 (dd, overlapped, H-6$_a^{II}$), 3.78 (dd, 1H, $J_{5^{I},6a^{I}}$ 5.6 Hz, H-6$_b^I$), 3.67 (dd, 1H, $J_{5^{II},6a^{II}}$ 5.5 Hz, $J_{6a^{II},6b^{II}}$ 11.8 Hz, H-6$_b^{II}$), 3.56 (dt, 1H, *J* 6.6 Hz, H-1′$_b$), 3.44 (m, 1H, H-5$^I$), 3.37–3.33 (m, overlapped, 6H, H-3$^I$, H-3$^{II}$, H-4$^I$), 3.30–3.25 (m, overlapped, 4H, H-4$^{II}$, H-6′$_{a,b}$, H-5$^{II}$), 3.22 (dd, 1H, $J_{2^{II},3^{II}}$ 9.1 Hz, H-2$^{II}$), 3.18 (m, 1H, H-2$^I$), 1.62 (m, overlapped, 4H, H-2′$_{a,b}$, H-5′$_{a,b}$), 1.42 (m, overlapped, 4H, H-3′$_{a,b}$, H-4′$_{a,b}$) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 104.82 (C-1$^{II}$), 104.39 (C-1$^I$), 78.01, 77.97 (C-5$^I$, C-3$^I$, C-3$^{II}$), 77.01 (C-5$^I$), 75.06 (C-2$^I$, C-2$^{II}$), 71.57 (C-4$^{II}$), 71.46 (C-4$^I$), 70.84 (C-1′), 69.76 (C-6$^I$), 62.74 (C-6$^{II}$), 52.39 (C-6′), 30.64, 29.85, 27.61, 26.64 (C-2′, C-5′, C-4′, C-3′) ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{33}N_3O_{11}Na$ [M + Na]$^+$: 490.2013, found: 490.2011.

A solution of the foregoing azido sugar **13** (0.46 g, 0.98 mmol) in 5% AcOH in MeOH (4 mL) was treated overnight with hydrogen in presence of 5% Pd/C (90 mg). The mixture was filtered through Celite pad, the pad was washed with MeOH, and the filtrate was concentrated. The residue was a mixture of **14** and **15** (MALDI-TOF MS: **14**, $C_{18}H_{35}NO_{11}Na$ [M + Na]$^+$: calcd 464.21; found 464.22 and **15**, $C_{36}H_{67}NO_{22}Na$ [M + Na]$^+$: calcd 888.40; found 888.39), analogs of **5** and **6**.

The foregoing mixture was dissolved in MeOH (3 mL) and treated overnight with di-*tert*-butyl dicarbonate (0.32 g, 1.47 mmol). After concentration, the residue was chromatographed

(5 : 1 $CHCl_3$–MeOH) to yield **16** as white foam (0.22 g, 38% in three steps from **12**). $R_f$ 0.3 (5 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{23}$ −32.9 (*c* 0.8, MeOH). $^1$H NMR (MeOD, 600 MHz): $\delta$ 4.38 (d, 1H, $J_{1^{II},2^{II}}$ 7.8 Hz, H-1$^{II}$), 4.25 (d, 1H, $J_{1^{I},2^{I}}$ 7.8 Hz, H-1$^{I}$), 4.14 (dd, 1H, $J_{5^{I},6a^{I}}$ 2.0 Hz, $J_{6a^{I},6b^{I}}$ 11.5 Hz, H-6$_a^{I}$), 3.88 (dt, overlapped, H-1′$_a$), 3.87 (dd, overlapped, H-6$_a^{II}$) 3.78 (dd, 1H, $J_{5^{I},6b^{I}}$ 5.6 Hz, H-6$_b^{I}$), 3.67 (dd, 1H, $J_{5^{II},6b^{II}}$ 5.4 Hz, $J_{6a^{II},6b^{II}}$ 11.8 Hz, H-6$_b^{II}$), 3.55 (dt, 1H, *J* 6.6, 9.6 Hz, H-1′$_b$), 3.44 (m, 1H, H-5$^{I}$), 3.37–3.33 (m, overlapped, H-3$^{I}$, H-3$^{II}$, H-4$^{I}$), 3.29–3.24 (m, overlapped, H-4$^{II}$, H-5$^{II}$), 3.22 (dd, 1H, $J_{2^{II},3^{II}}$ 9.1 Hz, H-2$^{II}$), 3.17 (m, 1H, H-2$^{I}$), 3.03 (t, 2H, *J* 7.1 Hz, H-6′$_{a,b}$), 1.63 (m, 2H, H-2′$_{a,b}$), 1.49–1.38 (m, overlapped, H-3′$_{a,b}$, H-5′$_{a,b}$), 1.43 (s, overlapped, NHCOOC(C*H*$_3$)$_3$), 1.35 (m, 2H, H-4′$_{a,b}$) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 158.55 (NH*C*OOC(CH$_3$)$_3$), 104.81 (C-1$^{II}$), 104.38 (C-1$^{I}$), 79.79 (NHCOO*C*(CH$_3$)$_3$), 78.00, 77.96 (C-5$^{I}$, C-3$^{I}$, C-3$^{II}$), 77.02 (C-5$^{I}$), 75.06 (C-2$^{I}$, C-2$^{II}$), 71.57 (C-4$^{II}$), 71.46 (C-4$^{I}$), 70.93 (C-1′), 69.74 (C-6$^{I}$), 62.74 (C-6$^{II}$), 41.29 (C-6′), 30.90 (C-5′), 30.70 (C-2′), 28.80 (NHCOOC(*C*H$_3$)$_3$), 27.65 (C-4′), 26.76 (C-3′) ppm. HRMS (ESI): *m*/*z* calcd for $C_{23}H_{43}NO_{13}Na$ [M + Na]$^+$: 564.2641; found: 564.2632.

***B.*** A solution of azide **12** (1.0 g, 1.31 mmol) and di-*tert*-butyl dicarbonate (0.57 g, 2.62 mmol) in DCM (4 mL) was mixed with a suspension of 5% Pd/C (0.20 g) in DCM (3 mL) and the mixture was stirred at room temperature under $H_2$ atmosphere until TLC showed complete consumption of the starting material (∼6 h). After conventional work-up, as described above, chromatography (2 : 1 toluene–EtOAc) afforded 6-*N*-(*tert*-butyloxycarbonyl) hexyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside [6-*N*-(*tert*-butyloxycarbonyl) hexyl 2,3,4,2′,3′,4′,6′-hepta-*O*-acetyl-β-gentiobioside] **17** (0.867 g, 78%) as white solid. Crystalline **17** showed mp 126–127 °C (EtOH), $R_f$ 0.4 (4 : 1 toluene–acetone) and $[\alpha]_D^{23}$ −16.2 (*c* 0.7, $CHCl_3$). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 5.18 (t, overlapped, *J* 9.5 Hz, H-3$^{I}$), 5.17 (t, overlapped, *J* 9.5 Hz, H-3$^{II}$), 5.07 (t, 1H, *J* 9.6 Hz, H-4$^{II}$), 4.99 (dd, 1H, $J_{1^{II},2^{II}}$ 8.0 Hz, $J_{2^{II},3^{II}}$ 9.6 Hz, H-2$^{II}$), 4.93 (dd, 1H, $J_{1^{I},2^{I}}$ 8.0 Hz, $J_{2^{I},3^{I}}$ 9.6 Hz, H-2$^{I}$), 4.88 (t, 1H, *J* 9.6 Hz, H-4$^{I}$), 4.60 (d, overlapped, H-1$^{II}$), 4.58 (broad s, overlapped, N*H*), 4.45 (d, 1H, H-1$^{I}$), 4.27 (dd, 1H, $J_{5^{II},6a^{II}}$ 4.9 Hz, $J_{6a^{II},6b^{II}}$ 12.3 Hz, H-6$_a^{II}$), 4.13 (dd, 1H, $J_{5^{II},6b^{II}}$ 2.3 Hz, H-6$_b^{II}$), 3.86 (dt, overlapped, H-1′$_a$), 3.85 (dd, overlapped, 6$_a^{I}$), 3.68 (m, overlapped, 2H, H-5$^{II}$, H-5$^{I}$), 3.62 (m, overlapped, 6$_b^{I}$), 3.46 (dt, 1H, *J* 6.6, 9.5 Hz, H-1′$_b$), 3.10 (m, 2H, H-6′$_{a,b}$), 2.09, 2.04, 2.03, 2.02, 2.00, 1.99 (7 s, overlapped, 21H, 7 COC*H*$_3$), 1.57 (m, 2H, H-2′$_{a,b}$), 1.45 (m, overlapped, H-5′$_{a,b}$), 1.44 (s, overlapped, NHCOOC(C*H*$_3$)$_3$), 1.33 (m, overlapped, 4H, H-3′$_{a,b}$, H-4′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.60, 170.21, 170.20, 169.61, 169.39, 169.29, 169.20 (7 *C*OCH$_3$), 155.96 (NH*C*OOC(CH$_3$)$_3$), 100.78 (C-1$^{II}$), 100.54 (C-1$^{I}$), 79.01 (NHCOO*C*(CH$_3$)$_3$), 73.34 (C-5$^{I}$), 72.78, 72.75 (C-3$^{II}$, C-3$^{I}$), 71.94 (C-5$^{II}$), 71.36 (C-2$^{I}$), 71.10 (C-2$^{II}$), 69.89 (C-1′), 69.18 (C-4$^{I}$), 68.30 (C-6$^{I}$), 68.27 (C-4$^{II}$), 61.81 (C-6$^{II}$), 40.46 (C-6′), 29.98 (C-5′), 29.27 (C-2′), 28.41 (NHCOOC(*C*H$_3$)$_3$), 26.48, 25.57 (C-4′, C-3′), 20.71, 20.64, 20.60, 20.58, 20.57 (overlapped, 7 COC*H*$_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{32}H_{57}NO_{20}Na$ [M + Na]$^+$: 858.3372; found: 858.3373; anal. calcd for $C_{32}H_{57}NO_{20}$: C, 53.17; H, 6.87; N, 1.68; found: C, 53.24; H, 6.79; N, 1.72. The side product **18** was subsequently eluted from the column and obtained as white solid. $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 5.18 (t, overlapped, *J* 9.5 Hz, H-3$^{I}$), 5.17 (t, overlapped, *J* 9.5 Hz, H-3$^{II}$), 5.07 (t, 2H, *J* 9.6 Hz, H-4$^{II}$), 4.99 (dd, 2H, $J_{1^{II},2^{II}}$ 8.0 Hz, $J_{2^{II},3^{II}}$ 9.6 Hz, H-2$^{II}$), 4.93 (dd, 2H, $J_{1^{I},2^{I}}$ 8.0 Hz, $J_{2^{I},3^{I}}$ 9.6 Hz, H-2$^{I}$), 4.88 (t, 2H, *J* 9.6 Hz, H-4$^{I}$), 4.50 (d, 2H, H-1$^{II}$), 4.45 (d, 2H, H-1$^{I}$), 4.27 (dd, 2H, $J_{5^{II},6a^{II}}$ 4.9 Hz, $J_{6a^{II},6b^{II}}$ 12.3 Hz, H-6$_a^{II}$), 4.13 (dd, 2H, $J_{5^{II},6b^{II}}$ 2.3 Hz, H-6$_b^{II}$), 3.90–3.85 (m, overlapped, 4H, H-1′$_a$, 6$_a^{I}$), 3.68 (m, overlapped, 4H, H-5$^{II}$, H-5$^{I}$), 3.62 (m, 2H, 6$_b^{I}$), 3.45 (dt, 2H, *J* 6.6, 9.5 Hz, H-1′$_b$), 3.13 (m, 4H, H-6′$_{a,b}$), 2.09, 2.04, 2.03, 2.02, 2.00, 1.99 (7 s, overlapped, 42H, 7 COC*H*$_3$), 1.56 (m, 4H, H-2′$_{a,b}$), 1.48 (m, overlapped, H-5′$_{a,b}$), 1.44 (s, overlapped, NHCOOC(C*H*$_3$)$_3$), 1.33 (m, 4H, 2 × $CH_2$ linker), 1.26 (m, 4H, 2 × $CH_2$ linker) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.57, 170.20, 170.15, 169.60, 169.38, 169.23, 169.18 (7 *C*OCH$_3$), 155.51 (NH*C*OOC(CH$_3$)$_3$), 100.75 (C-1$^{II}$), 100.53 (C-1$^{I}$), 78.94 (NHCOO*C*(CH$_3$)$_3$), 73.22 (C-5$^{I}$), 72.78, 72.73 (C-3$^{II}$, C-3$^{I}$), 71.93 (C-5$^{II}$), 71.35 (C-2$^{I}$), 71.07 (C-2$^{II}$), 69.90 (C-1′), 69.18 (C-4$^{I}$), 68.32 (C-6$^{I}$), 68.25 (C-4$^{II}$), 61.78 (C-6$^{II}$), 46.86 (C-6′), 29.37 ($CH_2$ linker), 28.46 (overlapped, NHCOOC(*C*H$_3$)$_3$, $CH_2$ linker), 26.67, 25.67 (each $CH_2$ linker), 20.69, 20.62, 20.61, 20.59, 20.57, 20.55 (overlapped, 7 COC*H*$_3$) ppm. HRMS (ESI): *m*/*z* calcd for $C_{69}H_{103}NO_{38}Na$ [M + Na]$^+$: 1576.6056; found: 1576.6051.

Deacetylation of **17** (0.45 g, 0.54 mmol) was performed with NaOMe in MeOH (4 mL) as described above, followed by chromatography (5 : 1 $CHCl_3$–MeOH) to afford **16** (0.28 g, 96%) as white foam, which was in all aspects identical with the material described above.

**6-*N*-(*tert*-Butyloxycarbonyl)hexyl 2,3,4,6-tetra-*O*-[{1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxyhexyl)-1,2,3-triazol-4-yl} methyl]-β-D-glucopyranoside (20).** Propargyl bromide (0.40 mL, 3.64 mmol) was added drop wise, at 0 °C under inert atmosphere, to a stirred suspension of **7** (115 mg, 0.30 mmol) and KOH (136 mg, 2.42 mmol) in DMF (5 mL). The mixture was allowed to attain room temperature and stirred overnight. After concentration the residue was partitioned between EtOAc and brine. The organic layer was dried with $Na_2SO_4$, concentrated and the residue was chromatographed (15 : 1 toluene–EtOAc) to give 6-*N*-(*tert*-butyloxycarbonyl)hexyl 2,3,4,6-tetra-*O*-propargyl-β-D-glucopyranoside **19** (120 mg, 75%) as colorless syrup, $R_f$ 0.3 (7 : 1 toluene–EtOAc). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 4.55–4.36 (m, 7H, 3 OC*H*$_2$C≡CH, N*H*), 4.26 (d, overlapped, $J_{1,2}$ 7.9 Hz, H-1), 4.26–4.18 (m, overlapped, OC*H*$_2$C≡CH), 3.88 (dt, 1H, *J* 6.5, 9.5 Hz, H-1′$_a$), 3.84 (dd, 1H, $J_{5,6a}$ 1.9 Hz, $J_{6a,6b}$ 10.8 Hz, H-6$_a$), 3.76 (dd, 1H, $J_{5,6b}$ 4.9 Hz, H-6$_b$), 3.54 (t, 1H, *J* 8.9 Hz, H-3) 3.47 (m, overlapped, H-1′$_b$), 3.44 (m, overlapped, H-4), 3.38 (ddd, 1H, $J_{5,4}$ 9.8 Hz, H-5), 3.31 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 8.9 Hz, H-2), 3.10 (m, 2H, *J* 6.0 Hz, H-6′$_{a,b}$), 2.46 (m, 4H, 4 OCH$_2$C≡C*H*), 1.60 (m, 2H, H-2′$_{a,b}$), 1.49–1.44 (m, overlapped, H-5′$_{a,b}$), 1.44 (s, overlapped, NHCOOC(C*H*$_3$)$_3$), 1.37 (m, overlapped, H-3′$_{a,b}$), 1.33 (m, overlapped, H-4′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 155.92 (NH*C*OOC(CH$_3$)$_3$), 102.98 (C-1), 83.31 (C-3), 81.33 (C-2), 80.02, 79.93, 79.80, 79.52 (4 OCH$_2$*C*≡CH), 78.98 (NHCOO*C*(CH$_3$)$_3$), 76.14 (C-4), 74.62, 74.33, 74.24, 74.23 (4 OCH$_2$C≡*C*H), 74.05 (C-5), 69.90 (C-1′), 68.47 (C-6), 60.20, 59.94, 59.29, 58.61 (4 O*C*H$_2$-C≡CH), 40.47 (C-6′), 29.95 (C-5′), 29.46 (C-2′), 28.38 (NHCOOC(*C*H$_3$)$_3$), 26.47 (C-4′), 25.67 (C-3′) ppm. HRMS (ESI): *m*/*z* calcd for $C_{29}H_{41}NO_8Na$ [M + Na]$^+$: 554.2730; found: 554.2731.

To a solution of sugar alkyne **19** (120 mg, 0.23 mmol) and sugar azide **3** (534 mg, 1.13 mmol) in dry DCM (6.0 mL), CuI (43 mg, 0.23 mmol) and DIPEA (39 μL, 0.23 mmol) were added

and the mixture was stirred at room temperature under inert atmosphere overnight. The mixture was concentrated and the residue was partitioned between brine and EtOAc. The organic layer was dried with $NaSO_4$, concentrated and chromatography (2 : 1 $CHCl_3$–acetone) followed by freeze-drying yielded **20** as white solid (426 mg, 77%), $R_f$ 0.2 (2 : 1 $CHCl_3$–acetone); $[\alpha]_D^{23}$ −18.0 (*c* 0.7, $CHCl_3$). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 8.10 (s, 1H, H-triazole), 7.90 (s, overlapped, 2H, H-triazole), 7.67 (s, 1H, H-triazole), 5.20 (t, 4H, *J* 9.5 Hz, 4 H-3$^{II}$), 5.08 (t, 4H, *J* 9.7 Hz, 4 H-4$^{II}$), 5.04–4.89 (m, overlapped, OC$H_2$-triazole), 4.97 (t, overlapped, *J* 8.8 Hz, 4 H-2$^{II}$), 4.84–4.67 (m, overlapped, OC$H_2$-triazole), 4.69 (bs, overlapped, N*H*), 4.48 (d, overlapped, 4H, $J_{1^{II},2^{II}}$ 8.0 Hz, 4 H-1$^{II}$), 4.35 (m, overlapped, 4 H-6″$_{a,b}$), 4.32 (d, overlapped, $J_{1^I,2^I}$ 7.9 Hz, H-1$^I$), 4.27 (dd, 4H, $J_{5^{II},6a^{II}}$ 4.7 Hz, $J_{6a^{II},6b^{II}}$ 12.2 Hz, 4 H-6$_a^{II}$), 4.13 (dd, 4H, $J_{5^{II},6b^{II}}$ 2.2 Hz, 4 H-6$_b^{II}$), 3.91 (dt, 1H, *J* 6.6, 9.4 Hz, H-1′$_a$), 3.87–3.82 (m, 4H, 4 H-1″$_a$), 3.79 (m, 2H, H-6$_{a,b}^I$), 3.69 (ddd, 4H, $J_{4^{II},5^{II}}$ 9.9 Hz, 4 H-5$^{II}$), 3.51 (m, overlapped, H-3$^I$, H-4$^I$, H-1′$_b$), 3.49–3.44 (m, 4H, 4 H-1″$_b$), 3.37 (m, 1H, H-5$^I$), 3.33 (m, 1H, H-2$^I$), 3.10 (m, 2H, H-6′$_{a,b}$), 2.08 (s, 12H, 4 COC$H_3$), 2.03, 2.02 (s, overlapped, 24H, 8 COC$H_3$), 2.00 (s, 12H, 4 COC$H_3$), 1.91 (m, 8H, 4 H-5″$_{a,b}$), 1.65 (m, overlapped, H-2′$_{a,b}$), 1.56 (m, overlapped, 4 H-2″$_{a,b}$), 1.49 (m, overlapped, H-5′$_{a,b}$), 1.43 (s, overlapped, NHCOOC(C$H_3$)$_3$), 1.42–1.30 (m, 4 H-3″$_{a,b}$, 4 H-4″$_{a,b}$, H-3′$_{a,b}$, H-4′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.61, 170.23, 169.37, 169.23, (4 *C*OCH$_3$), 156.01 (NH*C*OOC(CH$_3$)$_3$), 144.78, 144.72, 144.53, 144.31 (4 triazole C-4), 124.30, 123.73, 123.35, 122.84 (4 triazole C-5), 103.42 (C-1$^I$), 100.77 (C-1$^{II}$), 83.85 (C-3$^I$), 81.67 (C-2$^I$), 78.91 (NHCOO*C*(CH$_3$)$_3$), 77.49 (C-4$^I$), 74.45 (C-5$^I$), 72.80 (C-3$^{II}$), 71.73 (C-5$^{II}$), 71.30 (C-2$^{II}$), 69.94 (C-1′), 69.84–69.81 (C-1″), 69.05 (C-6$^I$), 68.44 (C-4$^{II}$), 66.28, 65.76, 65.72, 64.91 (4 OC$H_2$-triazole), 61.93 (C-6$^{II}$), 50.43, 50.30, 50.22, 50.19 (C-6″), 40.47 (C-6′), 30.13 (C-5″), 29.94 (C-5′), 29.56 (C-2′), 29.15 (C-2″), 28.41 (NHCOOC(*C*H$_3$)$_3$), 26.49 (C-4′), 26.20–26.14 (C-4″), 25.72 (C-3′), 25.28 (C-3″), 20.71, 20.66, 20.58, 20.56 (4 CO*C*H$_3$) ppm; HRMS (ESI): *m*/*z* calcd for $C_{109}H_{166}N_{13}O_{48}$ [M + H]$^+$: 2425.0948; found: 2425.0972; anal. calcd for $C_{109}H_{165}N_{13}O_{48}$: C, 53.98; H, 6.86; N, 7.51; found: C, 53.85; H, 6.87; N, 7.40.

**6-Aminohexyl 2,3,4,6-tetra-*O*-[{1-(β-D-glucopyranosyloxyhexyl)-1,2,3-triazol-4-yl}methyl]-β-D-glucopyranoside (21).** TFA (320 μL) was added drop wise at 0 °C to a solution of **20** (180 mg, 0.074 mmol) in dry DCM (4.0 mL). The cooling was removed, and the mixture was stirred at room temperature under inert atmosphere for 4 h, when TLC (8 : 1 $CHCl_3$–MeOH) showed complete disappearance of **20**. After concentration, the residue was deacetylated under conditions described above, and the crude product was subjected to solid-phase extraction (C18 5 g Waters SepPack cartridge, 1 : 2 → 4 : 1 MeOH–water) to afford **21** (92 mg, 75%) as white foam, after concentration and freeze drying. $R_f$ 0.3 (C18-silica, 1 : 1 MeOH–water); $^1$H NMR ($D_2O$, 600 MHz): $\delta$ 8.17, 8.12, 8.04, 8.01 (4 s, 4H, 4 H-triazole), 5.06 (m, 1H, OC$H_2$-triazole), 4.96–4.74 (m, overlapped, 6H, 3 OC$H_2$-triazole), 4.63 (d, overlapped, $J_{1^I,2^I}$ 7.6 Hz, H-1$^I$), 4.62 (m, overlapped, OC$H_2$-triazole), 4.56–4.47 (m, overlapped, 12H, 4 H-1$^{II}$, 4 H-6″$_{a,b}$), 4.04 (m, overlapped, 4 H-6$_a^{II}$), 4.01 (m, overlapped, H-1′$_a$), 4.00–3.95 (m, overlapped, 4 H-1″$_a$), 3.87 (m, overlapped, H-6$_a^I$), 3.85 (m, overlapped, 4 H-6$_b^{II}$), 3.82 (m, overlapped, H-6$_b^I$), 3.77 (m, overlapped, H-1′$_b$), 3.76–3.72 (m, overlapped, 4 H-1″$_b$), 3.74 (m, overlapped, H-3$^I$), 3.66 (m, overlapped, H-5$^I$), 3.64 (m, overlapped, H-4$^I$), 3.62 (m, overlapped, 4 H-3$^{II}$), 3.57 (m, 4H, 4 H-5$^{II}$), 3.51 (m, overlapped, 4 H-4$^{II}$), 3.48 (m, overlapped, H-2$^I$), 3.39 (m, 4H, 4 H-2$^{II}$), 3.13 (t, 2H, *J* 7.6 Hz, H-6′$_{a,b}$), 1.98 (m, 8H, H-5″$_{a,b}$), 1.79 (m, overlapped, H-5′$_{a,b}$), 1.74 (m, overlapped, H-2′$_{a,b}$), 1.68 (m, overlapped, 4 H-2″$_{a,b}$), 1.53–1.43 (m, overlapped, 12H, H-4′$_{a,b}$, H-3′$_{a,b}$, 4 H-3″$_{a,b}$), 1.36 (m, 8H, 4 H-4″$_{a,b}$) ppm; $^{13}$C NMR ($D_2O$, 150 MHz): $\delta$ 144.84, 144.34 (4 triazole C-4), 125.73, 125.42, 125.31, 125.28 (4 triazole C-5), 103.07 (C-1$^I$), 102.88 (C-1$^{II}$), 83.54 (C-3$^I$), 81.70 (C-2$^I$), 77.53 (C-4$^I$), 76.57, 76.55 (overlapped, C-3$^{II}$, C-5$^{II}$), 74.04 (C-5$^I$), 73.84 (C-2$^{II}$), 71.26 (C-1′), 70.92–70.89 (C-1″), 70.38 (C-4$^{II}$), 68.39 (C-6$^I$), 66.09, 65.60, 65.32, 63.75 (4 OC$H_2$-triazole), 61.50 (C-6$^{II}$), 50.98–50.93 (C-6″), 40.10 (C-6′), 29.94–29.85 (C-5″), 29.21–29.16 (C-2″, C-2′), 27.24 (C-5′), 25.96–25.89, 25.35 (C-4″, C-3′, C-4′) 25.11–25.04 (C-3″) ppm; HRMS (ESI): *m*/*z* calcd for $C_{72}H_{126}N_{13}O_{30}$ [M + H]$^+$: 1652.8734; found: 1652.8705.

**6-*N*-(*tert*-Butyloxycarbonyl)hexyl 2,3,4,2′,3′,4′,6′-hepta-*O*-[{1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxyhexyl)-1,2,3-triazol-4-yl}methyl]-β-gentiobioside (23).** To a stirred suspension of **16** (135 mg, 0.25 mmol) and KOH (196 mg, 3.49 mmol) in DMF (5 mL), propargyl bromide (0.45 mL, 3.98 mmol) was added drop wise at 0 °C under inert atmosphere. The mixture was allowed to attain room temperature and stirred overnight. After concentration, the residue was dissolved in EtOAc and washed with brine (3 × 75 mL), the organic layer was dried ($Na_2SO_4$) and concentrated. The residue was chromatographed (10 : 1 toluene–EtOAc) to obtain 6-*N*-(*tert*-butyloxycarbonyl)hexyl 2,3,4,2′,3′,4′,6′-hepta-*O*-propargyl-β-gentiobioside **22** (120 mg, 58%) as colorless syrup; $R_f$ 0.4 (5 : 1 toluene–EtOAc). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 4.55–4.35 (m, overlapped, N*H*, 6 OC$H_2$-C≡CH), 4.40 (d, overlapped, H-1$^{II}$), 4.27 (d, overlapped, $J_{1^I,2^I}$ 7.9 Hz, H-1$^I$), 4.26–4.18 (m, overlapped, OC$H_2$C≡CH), 4.23 (m, overlapped, H-6$_a^I$), 3.87 (dt, overlapped, 1H, *J* 6.4, 9.6 Hz, H-1′$_a$), 3.84 (dd, overlapped, $J_{5^{II},6a^{II}}$ 1.9 Hz, $J_{6a^{II},6b^{II}}$ 10.8 Hz, H-6$_a^{II}$), 3.76 (dd, 1H, $J_{5^{II},6b^{II}}$ 4.9 Hz, H-6$_b^{II}$), 3.66 (dd, 1H, $J_{5^I,6b^I}$ 7.5 Hz, $J_{6a^I,6b^I}$ 11.4 Hz, H-6$_b^I$), 3.54 (t, overlapped, *J* 9.0 Hz, H-3$^I$), 3.51 (t, overlapped, *J* 8.8 Hz, H-3$^{II}$), 3.48–3.42 (m, overlapped, H-4$^{II}$, H-5$^I$, H-1$_b$′), 3.38 (dd, 1H, $J_{4^{II},5^{II}}$ 9.8 Hz, H-5$^{II}$), 3.34 (dd, overlapped, $J_{1^{II},2^{II}}$ 7.9 Hz, $J_{2^{II},3^{II}}$ 9.0 Hz, H-2$^{II}$), 3.32–3.29 (m, overlapped, H-2$^I$, H-4$^I$), 3.10 (m, 2H, H-6′$_{a,b}$), 2.46 (m, 7H, 7 OCH$_2$C≡C*H*), 1.60 (m, 2H, H-2′$_{a,b}$), 1.46 (m, overlapped, H-5′$_{a,b}$), 1.44 (s, overlapped, NHCOOC(C$H_3$)$_3$), 1.35 (m, overlapped, 4H, H-3′$_{a,b}$, H-4′$_{a,b}$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 155.94 (NH*C*OOC(CH$_3$)$_3$), 103.32 (C-1$^{II}$), 102.82 (C-1$^I$), 83.42 (C-3$^I$), 83.35 (C-3$^{II}$), 81.41 (C-2$^I$), 81.23 (C-2$^{II}$), 80.04, 79.99, 79.96, 79.91, 79.86, 79.80, 79.62 (7 OCH$_2$-*C*≡CH), 79.03 (NHCOO*C*(CH$_3$)$_3$), 76.38 (C-4$^I$), 76.04 (C-4$^{II}$), 74.77, 74.68, 74.44, 74.37, 74.33, 74.31, 74.27 (7 OCH$_2$C≡*C*H), 74.39 (C-5$^I$), 74.13 (C-5$^{II}$), 69.97 (C-1′), 69.05 (C-6$^I$), 68.44 (C-6$^{II}$), 60.23, 60.20, 59.99, 59.86, 59.26, 58.68 (7 OCH$_2$C≡CH), 40.53 (C-6′), 30.02 (C-5′), 29.54 (C-2′), 28.42 (NHCOOC(*C*H$_3$)$_3$), 26.57, 25.79 (C-4′, C-3′) ppm. HRMS (ESI): *m*/*z* calcd for $C_{44}H_{57}NO_{13}Na$ [M + Na]$^+$: 830.3728; found: 830.3727.

To a solution of the foregoing sugar alkyne **22** (120 mg, 0.14 mmol) and sugar azide **3** (560 mg, 1.19 mmol) in dry DCM, CuI (28 mg, 0.14 mmol) and DIPEA (26 μL, 0.14 mmol) were added

and the mixture was stirred at room temperature overnight under inert atmosphere. The mixture was concentrated and the residue was partitioned between brine and DCM. Concentration of the organic phase, chromatography (1 : 1 $CHCl_3$–acetone) and freeze-drying (benzene) afforded **23** (430 mg, 71%) as white solid; $R_f$ 0.3 (1 : 1 $CHCl_3$–acetone); $[\alpha]_D^{23}$ −18.9 (*c* 0.8, $CHCl_3$). $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 8.15, 8.04, 7.94, 7.89, 7.77 (s, 7H, H-triazole), 5.20 (7 t, overlapped, 7H, *J* 9.5 Hz, 7 H-3$^{III}$), 5.08 (7 t, overlapped, 7H, *J* 9.7 Hz, 7 H-4$^{III}$), 5.06–4.66 (m, overlapped, 14H, 7 OC$H_2$-triazole), 4.97 (m, overlapped, 7 H-2$^{III}$), 4.49 (7 d, overlapped, $J_{1^{III},2^{III}}$ 8.0 Hz, 7 H-1$^{III}$), 4.47 (m, overlapped, H-1$^{II}$), 4.38–4.31 (m, overlapped, 7 H-6″$_{a,b}$), 4.36 (m, overlapped, H-1$^{I}$), 4.27 (7 dd, overlapped, 7H, $J_{5^{III},6a^{III}}$ 4.5 Hz, $J_{6a^{III},6b^{III}}$ 12.2 Hz, 7 H-6$_a^{III}$), 4.21 (m, 1H, H-6$_a^{I}$), 4.13 (7 dd, overlapped, 7H, 7 H-6$_b^{III}$), 3.89–3.82 (m, overlapped, 8H, H-1′$_a$, 7 H-1″$_a$), 3.80 (m, 2H, H-6$_{a,b}^{II}$), 3.75 (m, 1H, H-6$_b^{I}$), 3.70 (m, 7H, 7 H-5$^{III}$), 3.54 (m, overlapped, H-3$^{I}$), 3.51 (m, overlapped, H-5$^{I}$), 3.50 (m, overlapped, H-3$^{II}$, H-4$^{II}$), 3.46 (m, overlapped, H-1′$_b$, 7 H-1″$_b$), 3.39 (m, overlapped, H-4$^{I}$), 3.38 (m, overlapped, H-5$^{II}$), 3.35 (m, overlapped, H-2$^{II}$), 3.33 (m, overlapped, H-2$^{I}$), 3.06 (m, 2H, H-6′$_{a,b}$), 2.08–2.00 (m, overlapped, 84H, 28 COC$H_3$), 1.95–1.86 (m, 7 H-5″$_{a,b}$), 1.61–1.51 (m, overlapped, 7 H-2″$_{a,b}$), 1.46–1.27 (m, 7 H-3″$_{a,b}$, 7 H-4″$_{a,b}$) 1.42 (s, overlapped, NHCOOC(C$H_3$)$_3$) ppm; $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 170.63, 170.24, 169.40, 169.25 (28 *C*OCH$_3$), 156.05 (NH*C*OOC(CH$_3$)$_3$), 144.76, 144.70, 144.56, 144.52, 144.50, 144.31, 144.25 (7 triazole C-4), 124.29, 123.80, 123.72, 123.35, 123.14 (7 triazole C-5), 103.71 (C-1$^{II}$), 103.23 (C-1$^{I}$), 100.77 (C-1$^{III}$), 83.96 (C-3$^{I}$), 83.90 (C-3$^{II}$), 81.74 (C-2$^{I}$), 81.40 (C-2$^{II}$), 78.83 (NHCOO*C*(CH$_3$)$_3$), 78.18 (C-4$^{I}$), 77.36 (C-4$^{II}$), 74.54 (C-5$^{I}$), 74.47 (C-5$^{II}$), 72.81 (7 C-3$^{III}$), 71.73 (7 C-5$^{III}$), 71.30 (7 C-2$^{III}$), 69.86 (7 C-1″), 68.99 (C-6$^{II}$), 68.73 (C-6$^{I}$), 68.44 (7 C-4$^{III}$), 66.34, 65.92, 65.70, 64.83 (7 O*C*H$_2$-triazole), 61.94 (7 C-6$^{III}$), 50.39–50.23 (7 C-6″), 40.44 (C-6′), 30.20 (7 C-5″), 29.88, 29.66, 29.50, 29.18 (7 C-2″), 28.43 (NHCOOC(*C*H$_3$)$_3$), 26.47, 26.24, 26,21, 26.18, 25.70, 25.29 ppm. HRMS (ESI): *m*/*z* calcd for $C_{184}H_{274}N_{22}O_{83}$ $[M]^+$: 4119.7896; found: 4119.7900; anal. calcd for $C_{184}H_{274}N_{22}O_{83}$: C, 53.61; H, 6.70; N, 7.48; found: C, 53.84; H, 6.81; N, 7.37.

**6-Aminohexyl 2,3,4,2′,3′,4′,6′-hepta-*O*-[{1-(β-D-glucopyranosyloxyhexyl)-1,2,3-triazol-4-yl}methyl]-β-gentiobioside (24).** TFA (300 μL) was added drop wise at 0 °C to a solution of **23** (150 mg, 0.036 mmol) in dry DCM (3.0 mL) and the mixture was stirred at room temperature under inert atmosphere for 4 h, when TLC (8 : 1 $CHCl_3$–MeOH) showed complete disappearance of **23**. After concentration, the residue (145 mg, 0.036 mmol) was deacetylated (Zemplén condition) and solid-phase extraction (C18 5 g Waters SepPack cartridge, 1 : 2 → 4 : 1 MeOH/water) afforded **24** (72 mg, 70%) as white foam, after freeze drying. $R_f$ 0.3 (C18, 1 : 1 MeOH–water); $^1$H NMR ($D_2O$, 600 MHz): $\delta$ 8.24, 8.21, 8.20, 8.18, 8.15, 8.13, 8.12 (7 s, overlapped, 7H, 7 H-triazole), 5.17 (m, 2H, OC$H_2$-triazole), 5.07–4.75 (m, 12H, 6 OC$H_2$-triazole), 4.76 (m, overlapped, H-1$^{II}$), 4.69 (d, 1H, $J_{1^I,2^I}$ 8.0 Hz, H-1$^{I}$), 4.63 (m, overlapped, 7 H-1$^{III}$), 4.60 (m, overlapped, 7 H-6″$_{a,b}$), 4.33 (m, 1H, H-6$_a^{I}$), 4.13 (m, 7H, 7 H-6$_a^{III}$), 4.10–4.03 (m, 7H, 7 H-1″$_a$), 3.99 (m, overlapped, H-6$_a^{II}$), 3.97 (m, overlapped, H-1′$_a$), 3.95 (m, overlapped, 7 H-6$_b^{III}$), 3.94 (m, overlapped, H-6$_b^{I}$), 3.93 (m, overlapped, H-6$_b^{II}$), 3.89 (m, overlapped, H-3$^{I}$), 3.85 (m, overlapped, H-3$^{II}$), 3.83 (m, overlapped, 7 H-1″$_b$, H-5$^{I}$), 3.75 (m, overlapped, H-5$^{II}$, H-4$^{II}$), 3.74 (m, overlapped, H-1′$_b$), 3.71 (m, 7 H-3$^{III}$, H-4$^{I}$), 3.65 (m, overlapped, 7 H-5$^{III}$), 3.63 (m, overlapped, 7 H-4$^{III}$), 3.62 (m, overlapped, H-2$^{II}$), 3.60 (m, overlapped, H-2$^{I}$), 3.49 (m, 7 H-2$^{III}$), 3.11 (t, *J* 7.4 Hz, 2H, H-6′$_{a,b}$), 2.06 (m, 14H, H-5″$_{a,b}$), 1.83–1.72 (m, 16H, H-5′$_{a,b}$, 7 H-2″$_{a,b}$), 1.70 (m, overlapped, H-2′$_{a,b}$), 1.62–1.39 (m, 32H, H-3′$_{a,b}$, H-4′$_{a,b}$, 7 H-3″$_{a,b}$, 7 H-4″$_{a,b}$) ppm; $^{13}$C NMR ($D_2O$, 150 MHz): $\delta$ 145.02, 144.96, 144.92, 144.90, 144.57, 144.45 (7 triazole C-4), 125.57, 125.33, 125.29, 125.24 (7 triazole C-5), 103.40 (C-1$^{II}$), 103.07 (C-1$^{I}$), 102.95 (C-1$^{III}$), 83.82 (C-3$^{I}$), 83.72 (C-3$^{II}$), 81.84 (C-2$^{I}$), 81.57 (C-2$^{II}$), 78.25 (C-4$^{I}$), 77.55 (C-4$^{II}$), 76.63, 76.61 (C-3$^{III}$, C-5$^{III}$), 74.36, 74.32 (C-5$^{I}$, C-5$^{II}$), 73.90 (C-2$^{III}$), 71.11 (C-1′), 70.88 (C-1″), 70.46 (C-4$^{III}$), 69.09 (C-6$^{I}$), 68.73 (C-6$^{II}$), 66.22, 66.16, 65.69, 65.65, 65.62, 65.36, 64.11 (7 O*C*H$_2$-triazole), 61.59 (C-6$^{III}$), 50.99–50.94 (C-6″), 40.32 (C-6′), 30.01–29.91 (C-5″), 29.28–29.24 (C-2″), 28.19 (C-5″), 26.10–25.97 (C-4″) 25.44, 25.14 (C-3″). HRMS (ESI): *m*/*z* calcd for $C_{123}H_{210}N_{22}O_{53}$ $[M]^+$: 2843.4414; found: 2843.4429.

**6-(2-Methoxycyclobutene-3,4-dione)aminohexyl 2,3,4,6-tetra-*O*-[{1-(β-D-glucopyranosyloxyhexyl)-1,2,3-triazol-4-yl}methyl]-β-D-glucopyranoside (25).** 3,4-Dimethoxy-3-cyclobutene-1,2-dione (13 mg, 0.090 mmol) was added to the solution of **21** (30 mg, 0.018 mmol) in 1.13 mL of pH 7 phosphate buffer (0.5 M) and the solution was kept with gentle motion at room temperature for 18 h. The mixture was concentrated and the residue was chromatographed (2 : 1 $CHCl_3$–MeOH) to give **25** (17 mg, 53%); $R_f$ 0.13 (2 : 1 $CHCl_3$–MeOH). $^1$H NMR (MeOD, 600 MHz): $\delta$ 8.04, 8.02, 8.00, 7.97 (s, 4H, 4 H-triazole), 4.98 (m, 1H, OC$H_2$-triazole), 4.89–4.78 (m, overlapped, 4H, OC$H_2$-triazole), 4.68–4.58 (m, overlapped, 4H, OC$H_2$-triazole, N*H*), 4.41–4.35 (m, overlapped, 4 H-6″$_{a,b}$, OC$H_3$), 4.38 (m, overlapped, H-1$^{I}$), 4.24 (4 d, overlapped, $J_{1^{II},2^{II}}$ 7.8 Hz, 4H, 4 H-1$^{II}$), 3.88 (m, overlapped, H-1′$_a$), 3.87 (m, overlapped, 4 H-1″$_a$), 3.86 (m, overlapped, 4 H-6$_a^{II}$), 3.71 (m, 2H, H-6$_{a,b}^{I}$), 3.66 (dd, 4H, $J_{5^{II},6b^{II}}$ 5.2 Hz, $J_{6a^{II},6b^{II}}$ 11.9 Hz, 4 H-6$_b^{II}$), 3.58 (m, overlapped, H-6′$_a$), 3.55 (m, overlapped, H-1′$_b$), 3.54 (m, overlapped, H-3$^{I}$), 3.52 (m, overlapped, 4 H-1″$_b$), 3.46 (m, overlapped, H-4$^{I}$), 3.40 (m, overlapped, H-5$^{I}$), 3.39 (m, overlapped, H-6′$_b$), 3.35 (m, overlapped, 4 H-3$^{II}$), 3.29–3.24 (m, overlapped, 9H, 4 H-4$^{II}$, 4 H-5$^{II}$, H-2$^{I}$), 3.17 (4 t, overlapped, *J* 8.5 Hz, 4H, 4 H-2$^{II}$), 1.89 (m, 8H, 4 H-5″$_{a,b}$), 1.65–1.55 (m, overlapped, 12H, 4 H-2″$_{a,b}$), 1.46–1.37 (m, overlapped, 12H, 4 H-3″$_{a,b}$), 1.31 (m, overlapped, 8H, 4 H-4″$_{a,b}$) ppm; $^{13}$C NMR (MeOD, 150 MHz): $\delta$ 190.03 (d, C=O), 184.82 (d, C=O), 177.80 (d, NHC=*C*OCH$_3$), 174.49 (d, NH*C*=COCH$_3$), 146.29, 146.20, 145.87, 145.79 (4 triazole C-4), 125.38, 125.29, 125.22, 125.17 (4 triazole C-5), 102.61 (C-1$^{I}$), 102.35 (C-1$^{II}$), 84.88 (C-3$^{I}$), 82.97 (C-2$^{I}$), 78.48 (C-4$^{I}$), 78.12 (C-3$^{II}$), 77.91 (C-5$^{II}$), 75.62 (C-5$^{I}$), 75.13 (C-2$^{II}$), 71.68 (C-4$^{II}$), 70.79 (C-1′), 70.57 (C-1″), 70.07 (C-6$^{I}$), 67.08, 66.34, 65.32 (overlapped, 4 OC$H_2$-triazole), 62.79 (C-6$^{II}$), 61.19, 61.07 (two signals, O*C*H$_3$), 51.30 (C-6″), 45.58–45.28 (d, C-6′), 31.39, 31.21 (C-5″), 30.60, 30.50 (C-2″), 27.22 (C-4″), 27.08, 26.77, 26.44 (C-3″) ppm. HRMS (ESI): *m*/*z* calcd for $C_{77}H_{128}N_{13}O_{33}$ $[M + H]^+$: 1762.8738; found: 1762.8719.

**Conjugation of 25 to BSA.** BSA (18.86 mg, 0.00028 mmol) and methyl squarate derivative **25** (3.00 mg, 0.0017 mmol) were weighed into a 1 mL V-shaped reaction vessel and 425 μL of 0.5 M pH 9 borate buffer was added (to form ∼4.0 mM solution with respect to cluster **25**, cluster–BSA 6 : 1). The mixture was gently stirred at room temperature for 48 h, when SELDI-TOF

showed the ratio of hapten–BSA to be 4.1 : 1 (conjugation efficiency, 68%). The mixture was transferred into an Amicon Ultra (0.5 mL, 30 K cutoff) centrifuge tube and dialyzed (centrifugation at 4 °C, 7 min, 8 times) against 10 mM aqueous $(NH_4)_2CO_3$. After lyophilization, 19.87 mg (94%, based on BSA) of conjugate **26** was obtained as white solid.

**6-(2-Methoxycyclobutene-3,4-dione)aminohexyl 2,3,4,2′,3′,4′,6′-hepta-*O*-[{1-(β-D-glucopyranosyloxyhexyl)-1,2,3-triazol-4-yl}methyl]-β-gentiobioside (27).** 3,4-Dimethoxy-3-cyclobutene-1,2-dione (13 mg, 0.090 mmol) was added to the solution of **24** (25 mg, 0.009 mmol) in 2.2 mL of pH 7 phosphate buffer (0.5 M) and stirred at room temperature for 18 h. The mixture was concentrated and the residue was chromatographed (1 : 1 EtOAc–MeOH) to yield **27** (13 mg, 50%); $R_f$ 0.2 (1 : 1 EtOAc–MeOH). $^1$H NMR (MeOH, 600 MHz): $\delta$ 8.07, 8.05, 8.04, 8.03, 7.99 (s, 7H, H-triazole), 5.02–4.79 (m, overlapped, 10H, 5 OC$H_2$-triazole), 4.69–4.61 (m, overlapped, 4H, 2 OC$H_2$-triazole), 4.47 (m, 1H, H-1$^{II}$), 4.40–4.34 (m, overlapped, 7 H-6″$_{a,b}$, OC$H_3$), 4.39 (m, overlapped, H-1$^{I}$), 4.24 (7 d, overlapped, 7H, 7 H-1$^{III}$), 4.07 (m, 1H, H-6$_a^{I}$), 3.90–3.84 (m, overlapped, 7 H-6$_a^{III}$, 7 H-1″$_a$), 3.81 (m, overlapped, H-1′$_a$), 3.73 (m, 2H, H-6$_{a,b}^{II}$), 3.66 (7 dd, overlapped, 2H, $J_{5^{III},6a^{III}}$ 5.0 Hz, $J_{6a^{III},6b^{III}}$ 12.0 Hz, 7 H-6$_b^{III}$), 3.63 (m, overlapped, H-6$_b^{I}$), 3.60–3.45 (m, overlapped, H-3$^{I}$, H-6′$_a$, H-3$^{II}$, 7 H-1″$_b$, H-5$^{I}$, H-1′$_b$, H-4$^{II}$), 3.45–3.38 (m, overlapped, H-4$^{I}$, H-5$^{II}$), 3.37 (m, overlapped, H-6′$_b$), 3.35 (m, overlapped, 7 H-3$^{III}$), 3.32 (m, overlapped, H-2$^{I}$), 3.30 (m, overlapped, H-2$^{II}$), 3.28 (m, overlapped, 7 H-4$^{III}$), 3.26 (m, overlapped, 7 H-5$^{III}$), 3.17 (7 t, overlapped, 7 H-2$^{III}$), 1.89 (m, 14H, 7 H-5″$_{a,b}$), 1.63–1.53 (m, 18H, 7 H-2″$_{a,b}$), 1.46–1.38 (m, 14H, 7 H-3″$_{a,b}$), 1.35–1.27 (m, 18H, 7 H-4″$_{a,b}$) ppm; $^{13}$C NMR (MeOH, 150 MHz): $\delta$ 190.01, 184.87, 178.01, 174.47 (4 d), 146.30, 146.22, 146.19, 146.14, 145.82 (overlapped, 7 triazole C-4), 125.43–125.17 (overlapped, 7 triazole C-5), 104.81 (C-1$^{II}$), 104.51 (C-1$^{I}$), 104.35 (C-1$^{III}$), 85.08 (C-3$^{I}$, C-3$^{II}$), 83.05 (C-2$^{I}$), 82.78 (C-2$^{II}$), 78.95 (C-4$^{I}$), 78.45 (C-4$^{II}$), 78.13 (C-3$^{III}$), 77.91 (C-5$^{III}$), 75.72 (C-5$^{II}$), 75.53 (C-5$^{I}$), 75.13 (C-2$^{III}$), 71.68 (C-4$^{III}$), 70.83 (C-1′), 70.58 (C-1″), 70.15 (C-6$^{II}$), 69.69 (C-6$^{I}$), 67.20, 67.16, 66.45, 66.33, 65.42 (overlapped, 7 OC$H_2$-triazole), 62.80 (C-6$^{III}$), 61.30, 61.12 (two signals, OC$H_3$), 51.30 (C-6″), 45.57, 45.28 (d, C-6′), 31.88, 31.40, 31.23 (C-5″), 30.78, 30.52 (C-2″), 27.26 (C-4″), 27.07, 27.04, 26.69, 26.46 (C-3″) ppm. HRMS (ESI): *m*/*z* calcd for $C_{128}H_{212}N_{22}O_{56}$ $[M]^+$: 2953.4418; found: 2953.4443.

**Conjugation of 27 to BSA.** The protocol described above for the conjugation of **25** was applied to the methyl squarate derivative **27** (2.00 mg, 0.00068 mmol), BSA (7.49 mg, 0.00011 mmol) and 169 μL of pH 9 borate buffer (to form ∼4.0 mM solution with respect to cluster **27**). After 30 h, the mixture was processed as described above, to give, after lyophilization 8.06 mg (89%, based on BSA) of the conjugate **28** as white solid. SELDI-TOF showed the ratio of hapten–BSA to be 3.7 : 1 (conjugation efficiency, 62%).

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