Synthesis of a Dimeric Lewis Antigen and the Evaluation of the Epitope Specificity of Antibodies Elicited in Mice

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Abstract: The Lewis^y–Lewis^x heptasaccharide, modified by an artificial aminopropyl spacer, was synthesized by an approach that employed two orthogonally protected lactosamine building blocks. A *p*-(benzoyl)-benzyl glycoside was used as a novel anomeric protecting group, which could be selectively removed at a late stage in the synthesis, thus offering the benefit of enhanced flexibility. The artificial aminopropyl moiety was modified by a thioacetyl group, which allowed an efficient conjugation to keyhole limpet hemocyanin (KLH) that had been activated with electrophilic 3-(bromoacetamido)-propionyl groups. Mice were immunized with the Le^{y}Le^x–BrAc–KLH antigen. Analysis of the sera by ELISA established that a strong helper T-cell immune response was raised against the Le^{y}Le^x saccharide. Further ELISA analysis showed that the titer for monomeric Le^y tetrasaccharide was tenfold lower whereas recognition of the Le^x trisaccharide was negligible.

Keywords: antigens · antitumor agents · glycoconjugates · oligosaccharides · vaccines

Introduction

In the event of cell malignancies, dramatic changes occur in the nature and abundance of protein- and lipid-linked cell surface oligosaccharides. This abnormal glycosylation is associated with tumor progression and strongly correlates with poor survival rates. Prominent tumor associated antigens are, for example, the Lewis antigens, Lewis^y (Le^y) and sialyl-Lewis^x (SLe^x), and KH-1. In the majority of carcinomas including those of the breast, ovary, pancreas, prostate and colon,[1] Le^y is overexpressed. It has been established that Le^y and SLe^x promote metastasis by binding to endothelial cell-surface proteins.[2,3]

The carbohydrate antigens expressed by tumor cells offer a unique opportunity for the development of anticancer vaccines. Immunization with a tumor-associated antigens may induce an immune response that is directed towards cancer cells.[4,5] The opsonizing or cytotoxic antibodies raised in such a response may be exploited for the treatment of a "minimal residual disease". In this respect, they could target a small number of metastasized cells that may have persisted after primary therapies such as surgery or chemotherapy. This add-on immune therapy could protect cancer patients against a relapse and, thus, enhancing survival rates.

The extreme heterogeneity of cell surface glycosylation makes the isolation of tumor associated carbohydrate antigens in well-defined forms an almost impossible task, thus, presenting a major obstacle for the development of cancer vaccines. This obstacle is being addressed by synthetic organic chemistry, which can provide homogeneous oligosaccharide antigens of high purity in relatively large amounts. However, despite recent advances in the organic synthesis of oligosaccharides, the preparation of these large complex antigens is by no means a trivial matter.

Another obstacle for pursuing cancer vaccines is that tumor-associated antigens are auto-antigens and, thus, are being tolerated by the immune system. The difficult question thus posed is how to trick the immune system to induce a response to these tumor-associated antigens. The inherently T-cell independent nature of oligosaccharides is an additional problem that complicates carbohydrate-based cancer vaccine development. The inability of carbohydrates to activate T-cells results in formation of exclusively low affinity IgM antibodies and a lack of immunological memory.[6–10] The activation of both B-cells and T-cells and their interaction with one another is necessary for an effective immunological reaction.[9,11,12] The helper T-cells are in essence the

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orchestrators of the immune response and direct the activation of cytotoxic T-cells and the antibody producing B-cells.

Fortunately, the T-cell independence and B-cell tolerance can be overcome by conjugation of tumor-associated oligosaccharides to a carrier protein, such as KLH or BSA.[13,14] In elegant studies, Danishefsky, Livingston and co-workers have utilized this approach to develop experimental carbohydrate based anticancer vaccines.[14] For example, immunizations of mice with a conjugate of the tetrasaccharide Lewis^y to the carrier protein KLH in combination with the immuno-adjuvant QS-21, resulted in good titers of both IgM and IgG antibodies.[15] Encouraged by these results a phase I clinical trial with patients with documented ovarian, fallopian tube, or peritoneal cancer[16] was conducted. Although the vaccine did not induce adverse effects related to autoimmunity, the immunizations failed to induce sufficiently strong helper T-cell responses. Obviously, there is a need for alternative strategies for the development of vaccine candidates useful for immunotherapy against cancer.

As part of a program to develop fully synthetic anticancer vaccines, we recently reported[17] a solution-phase synthesis of the tetrasaccharide Le^y. This saccharide was coupled to the protein carrier KLH using several different linkers. The objective was to investigate the influence of the linker on the immunogenicity of the tetrasaccharide. It was found that a highly immunogenic linker such as 4-(maleimidomethyl)-cyclohexane-1-carboxylate, dramatically reduced titers of antibodies against the weakly immunogenic Le^y tetrasaccharide antigen. The use of a less immunogenic linker such as 3-(bromoacetamido) propionate improved the immunological response considerably.

To further investigate the ideal presentation of the Le^y antigen, we chose to pursue the presentation of Le^y in a dimeric form. Naturally occurring Lewis antigens exist not only as positional isomers in monomeric forms, but also as homo- and heterodimers. For example, the Le^y–Le^x heterodimer (Figure 1) is part of the KH-1 antigen that was isolated from human colonic adenocarcinoma cells.[18] This antigen has only been found on the surface of adenocarcinomas cells, and has never been isolated from normal colonic tissue, thus, providing a highly specific marker for colon malignancies.[19,20]

For the safe use of these antigens for active immunotherapy, it is important to investigate the cross-reactivity of antibodies raised against the KH-1 antigen with other Lewis antigens, in particular Le^y and Le^x. In this paper, we report a highly convergent chemical synthesis of the Le^y–Le^x heptasaccharide equipped with an artificial linker for selective conjugation to a carrier protein KLH. IgM and IgG class antibodies were raised when mice were immunized with a KLH conjugate. Investigation of the epitope specificity of the antibodies showed that they recognized the Le^y–Le^x as well as the Le^y antigen. However, reactivity with the Le^y antigen was of a much lower titer. No reactivity with Le^x was observed.

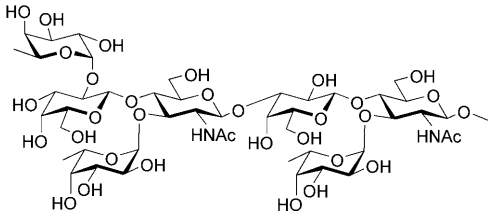


Figure 1. Le^{y}Le^x heptasaccharide.

Results and Discussion

Synthesis: We required substantial quantities of the synthetic Lewis^y–Lewis^x oligosaccharide.[21–24] Also, a reference glycoconjugate of the saccharide linked to a carrier protein was needed. Furthermore, we wanted to investigate the cross-reactivity of antibodies raised against a particular Lewis antigen. Thus, we required a flexible strategy for the preparation of Lewis antigens.

Strategic planning is of highest importance when designing a synthetic route for complex oligosaccharide. The regio- and stereoselective outcome of glycosylations must be taken in consideration and are in essence influenced by the protection group pattern of the glycosyl donors and acceptors. In this respect, a straightforward synthesis that requires minimal protecting group manipulations of expensive building blocks is highly desirable.

It was envisaged that the synthesis of the target heptasaccharide **31** would require only one orthogonally protected lactosamine building block **1** (Figure 2), which could be utilized for the synthesis of both the Le^x acceptor and the Le^y donor. Selective deprotection of the orthogonal protecting groups Lev and Fmoc[25,26] of **1** would allow mono- or difucosylation with **6** to obtain either properly protected Le^x or Le^y. The use of the trichloroethyloxycarbonyl (Troc) group for protection of the amino functionality would ensure compatibility with the removal of the Lev and Fmoc group.[27] The implementation of the novel temporary anomeric protecting group, *p*-(benzoyl)benzyl, which can be removed in a two-step fashion by using hydrogen peroxide followed by DDQ oxidation, would allow transformation into a glycosyl donor at a late stage in the synthesis and thus greatly enhance the flexibility of the synthesis. Furthermore, a silyl protecting group at 3′-OH could be removed to furnish a Le^x acceptor, which can then be coupled with a Le^y donor providing a straightforward synthesis of heptasaccharide Le^y–Le^x **31**. However, attempts to implement this strategy failed, due to an inability to difucosylate a 3,2′-diol lactosamine building block obtained by removal of the Lev and Fmoc group of **1**. Probably the steric hindrance by the 3′-O-TBDPS protecting group obstructs successful glycosylation. Unfortunately, efforts were unsuccessful to vary the bulkiness of the silyl protecting group (**2** and **3**) without affecting its desired stability during protecting group manipulations and glycosylations. In addition, it was found to be difficult to glycosylate the 3′-hydroxyl of a Le^x acceptor that was carrying a Lev group at the 2′-position. It was thus decided to

abandon the initial strategy and to use the two differently protected lactosamine derivatives, **4** and **5**. As can be seen in Figure 2, the 3′-O-silyl protecting group obstructing difucosylation as to obtain a Le^y derivative was replaced by a 3′-O-benzyl ether (**4**). Previous, it was found that a lactosamine building block carrying a 3′-O-benzyl ether could indeed be difucosylated.[17] Building block **5** carrying a 2′-O-benzoyl instead of a Lev group was expected to be a more appropriate substrate for the preparation of a Le^x acceptor.

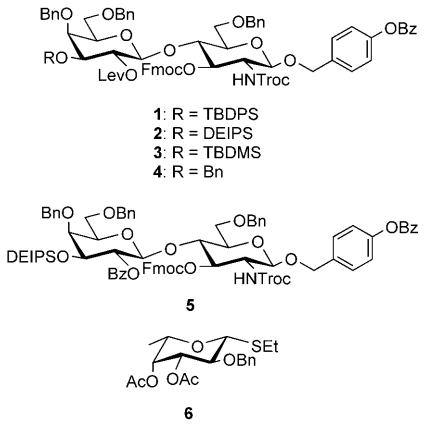
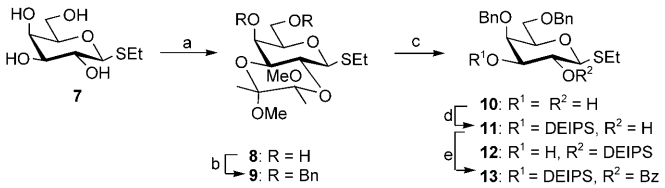


Figure 2.

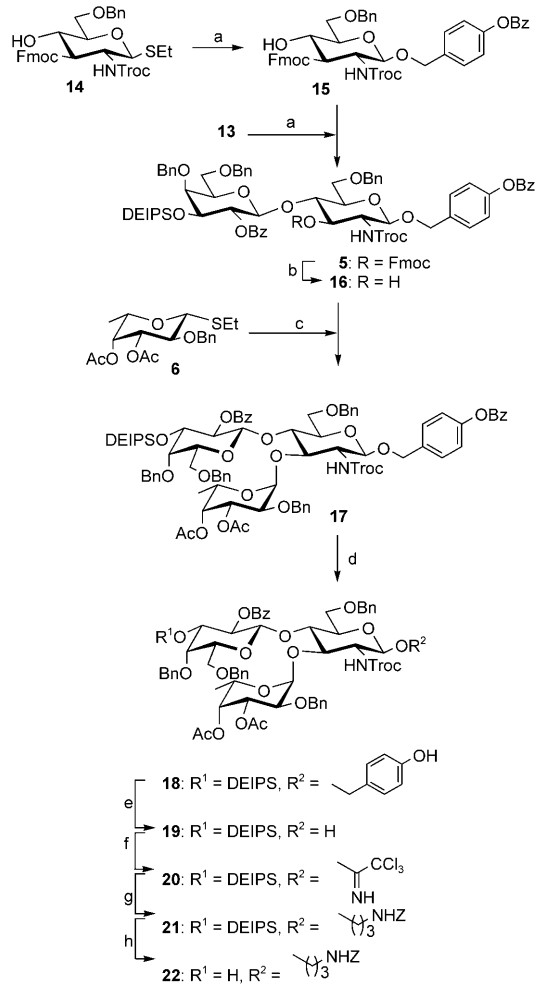
It was envisaged that key building blocks **4** and **5** could be synthesized from monosaccharides **6**, **13** and **15**.

Galactose donor **13**, carrying a silyl-protecting group at C-3 and a 2-O-benzoate, was synthesized in a straightforward manner starting from tetraol **7** (Scheme 1). Selective introduction of a butane diacetal[28] and benzylation of the 4- and 6-hydroxyl groups under standard conditions followed by acetal cleavage proceeded smoothly to give diol **10** in good overall yield. Regioselective silylation by using diethylisopropyl chloride in THF furnished the desired alcohol **11** in a yield of 78%. A small amount (15%) of the 2-O-isomer **12** could easily be separated by silica gel column chromatography, which could be desilyated and recycled. Finally, benzoylation of the C-2 hydroxyl gave thiogalactoside **13**. The H-2 signal in ^{1}H NMR spectrum of compound **13** was shifted down-field to 5.62 ppm, clearly demonstrating the selectivity of the silylation and confirming the structure of **13**.



Scheme 1. Synthesis of galactose donor **13**. a) Butane-2,3-dione, HC(CH$_3$)$_3$, CSA, MeOH, reflux, 78%; b) NaH, BnBr, DMF, 85%; c) TFA/H$_2$O 9:1, 67%; d) DEIPSCl, imidazole, THF, 78%; e) BzCl, TEA, DMAP, CH$_2$Cl$_2$, 89%.

Glucosamine acceptor **15**, carrying the temporary anomeric protecting group *p*-(benzoyl)-benzyl was easily prepared in high yield by coupling *p*-(benzoyl)-benzylalcohol with glycosyl donor **14**[25] by using *N*-iodosuccinimide/trimethylsilyl triflate (NIS/TMSOTf) as the activator (Scheme 2). Compound **15** could immediately be used as an acceptor in a glycosylation with galactosyl donor **13** by using NIS/TMSOTf as the promoter to give the first key lactosamine derivative **5** in a yield of 69%. Selective removal of the Fmoc group of disaccharide **5** was easily achieved by treatment with 20% triethylamine in dichloromethane to afford lactosyl acceptor **16** which was fucosylated with glycosyl donor **6**[29] to afford the fully protected Le^x trisaccharide **17** in 74% yield. As determined by the $^1J_{H,H}$ coupling (J = 3.5 Hz), complete α-selectivity was achieved in the glycosylation. The temporary anomeric protecting group of **17** was then removed by a two-step procedure without affecting any other protecting

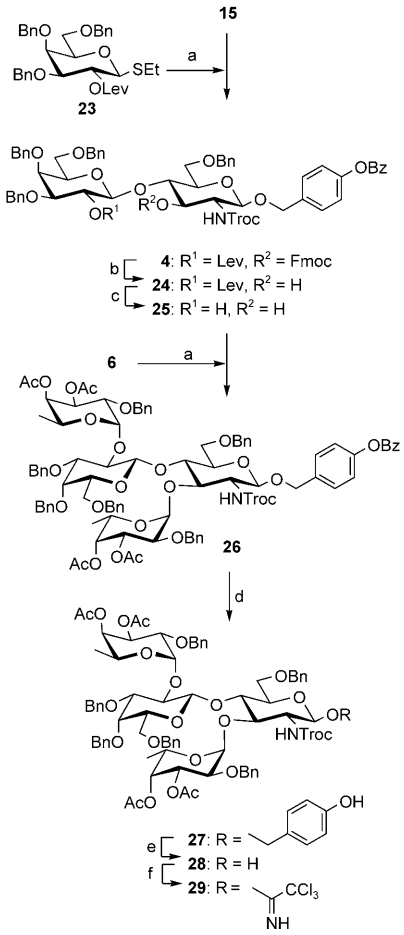


Scheme 2. Synthesis of the Le^x acceptor. a) *p*-benzoyl-benzyl alcohol, NIS, TESOTf, CH$_2$Cl$_2$, 0°C, 86%; b) CH$_2$Cl$_2$/Et$_3$N 5:1, 95%; c) NIS, TESOTf, CH$_2$Cl$_2$, 0°C, 74%; d) H$_2$O$_2$, Et$_3$N, THF, 80%; e) DDQ, CH$_2$Cl$_2$/H$_2$O 95:5, 81%; f) CCl$_3$CN, DBU, CH$_2$Cl$_2$, 90%; g) BF$_3$·Et$_2$O, CH$_2$Cl$_2$, 86%; h) TBAF, HOAc, THF, 82%.

groups. Thus, cleavage of the phenolic benzoate using hydrogenperoxide in a mixture of triethylamine and THF liberated the *p*-hydroxyl **18**, which was immediately subjected to oxidation with DDQ to furnish hemiacetal **19**. Conversion of **19** into a trichloroacetimidate **20** was accomplished using standard conditions[30] and the resulting **20** was glycosylated with 3-[*N*-(benzyloxycarbonyl)amino]-propanol[31] in the presence of boron trifluoride etherate to give the fully protected spacer equipped Le^x derivative **21** in a good overall yield. The 3′-O-DEIPS group (DEIPS: diethylisopropylsilyl) of **21** was easily removed using tetrabutylammonium fluoride (TBAF) in THF buffered with acetic acid to give the $Lewis^X$ glycosyl acceptor **22** in a yield of 82%.

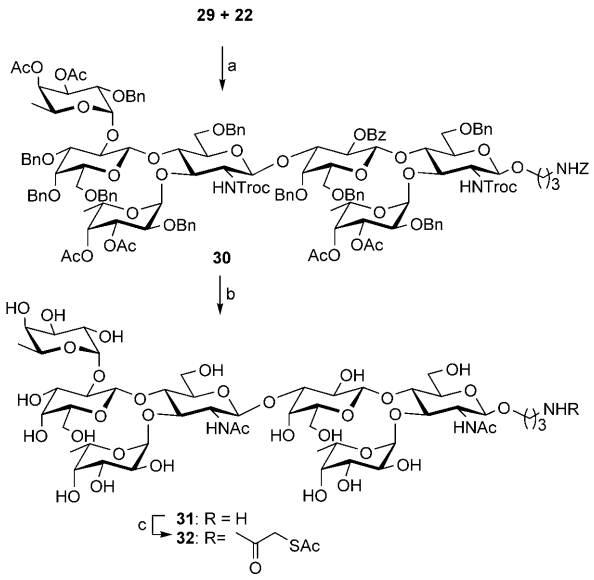
The preparation of the properly protected $Lewis^y$ donor **29** was accomplished by a similar reaction sequence as outlined for the synthesis $Lewis^X$ acceptor **22**. Thus, coupling of galactosyl donor **23**[27] with the C-3 hydroxyl of acceptor **15** gave lactosamine derivative **4** in 81% yield (Scheme 3). Removal of the Fmoc group by using triethylamine in dichloromethane and subsequent treatment with hydrazine acetate to remove the Lev group gave the 3,2′-diol **25**. Difucosylation with glycosyl donor **6** afforded the fully protected tetrasaccharide **26** in a yield of 86%. The fucosylations proceeded with complete α-selectivity, as confirmed by $^1J_{H,H}$ couplings ($J = 3.5$ Hz). The phenolic ester of the anomeric protecting group was cleaved by treatment with hydrogenperoxide in the presence of triethylamine. The so-formed *p*-hydroxybenzyl derivative **27** was oxidized with DDQ to completely remove the temporary anomeric protecting group to give **28**. The hemiacetal **28** was converted into the corresponding trichloroacetimidate **29** by using standard conditions.

The key glycosylation of the assembly of the fully protected heptasaccharide **30** involved a coupling of trisaccharide acceptor **22** with tetrasaccharide donor **29** using tributylsilyl triflate (TBSOTf) in dichloromethane at −30 °C. This glycosylation afforded the fully protected Le^yLe^x heptasaccharide in a yield of 62%. The use of the common promoters[30] such as TMSOTf or TESOTf resulted in lower yields of the heptasaccharide (<40%). Also, the reaction temperature was critical as it was observed that temperatures higher than −20 °C gave inferior result. The heptasaccharide **30** was then deprotected by a four-step procedure. First, the N-Troc groups were converted into 2-acetamido-2-deoxy functionalities by treatment with nano-size zinc in acetic acid followed by acetylation using acetic anhydride in pyridine. It was found that saponification of the ester groups, in particular the 2′-O-benzoate, required prolonged reaction times resulting in partial decomposition. However, improved overall yields were achieved by performing hydrogenolysis of the benzyl ethers prior to ester hydrolysis. Thus, catalytic hydrogenolysis using $Pd(OAc)_2$ to remove the benzyloxycarbonyl moiety and the benzyl ethers followed by base mediated removal of the O-acyl groups gave, after purification by Biogel P2 size-exclusion column chromatography, target compound **31** in an overall yield of 32%.



Scheme 3. Synthesis of the Le^y donor. a) NIS, TESOTf, CH_2Cl_2, 0 °C; b) CH_2Cl_2/Et_3N 5:1, 95%; c) NH_2NH_2-HOAc, MeOH, CH_2Cl_2, 87%; d) H_2O_2, Et_3N, THF, 82%; e) DDQ, CH_2Cl_2/H_2O 95:5, 78%; f) CCl_3CN, DBU, CH_2Cl_2, 91%.

Preparation of carbohydrate–protein conjugates and immunizations: For the immunological evaluation of heptasaccharide **31** (Scheme 4) it was linked to a carrier protein, KLH. In our previous studies,[17] activating KLH with a bromoacetyl linker and reacting it with thiolated oligosaccharides had proven to result in glycoconjugates that gave an antigen focused immune response with low titers of anti-linker antibodies. To this end, the amino functionality of heptasaccharide **31** was derivatized with an acetyl thioacetic acid moiety by reaction with S-acetylthioglycolic acid pentafluorophenyl ester to afford **32**, which, after purification by size-exclusion chromatography, was directly de-S-acetylated by using 7% ammonia (g) in DMF just prior to conjugation. The de-S-acylation was performed under a strict argon atmosphere to prevent formation of the corresponding disulfide. KLH was activated with succinimidyl 3-(bromoacetamido) propionate (SBAP) in a sodium phosphate buffer pH 7.2 containing 0.15 M sodium chloride for 2 h and then purified by centrifugal filters with a nominal molecular-weight limit of 10 kDa. The bromoacetyl activated KLH was subsequently incubated over night at room temperature



Scheme 4. Synthesis of target molecule **28**. a) NIS, TBSOTf, CH_2Cl_2, −30 °C, 62 %; b) 1) Zn, HOAc; 2) Ac_2O, pyridine; 3) $Pd(OAc)_2$, H_2, HOAc/EtOH 1:5, 4) NaOMe, MeOH, pH 10, 52 % over four steps; c) SAMA-OPfp, Et_3N, DMF.

with thiolated heptasaccharide **32** in a 0.1 mM sodium phosphate buffer pH 8.0 containing 0.1 mM ethylenediamine tetraacetate (EDTA). The afforded glycoconjugate carried 1190 copies of Le^yLe^x per KLH molecule as determined by Lowry's protein concentration test[32] and Dubois' phenol sulfuric acid assay.[33] For the ELISAs, the Le^yLe^x–BSA, Le^y–BSA, and Le^x–BSA conjugates were prepared using the 3-(bromoacetamido) propionate linker and procedures similar to the preparation of the Le^yLe^x–KLH conjugate.

Groups of five mice were immunized with the Le^yLe^x–KLH conjugate together with the immunoadjuvant QS-21 (Antigenics Inc., Lexington, MA.). The mice received 24 μg carbohydrate and 10 μg QS-21 in each boost. The immunizations were repeated three times at weekly intervals and sera were collected seven days after the last boost. Titers of anti-Le^yLe^x antibodies were determined by ELISA by the addition of serial dilutions of sera to microtiter plates coating with Le^yLe^x–BSA. An anti-mouse IgM (μ-chain specific) or IgG (heavy chain specific) antibody labeled with alkaline phosphatase was employed as a secondary antibody for detection purposes. As can be seen in entry 1 of Table 1, the

Table 1. ELISA antibody titers[a] after four immunizations with Le^yLe^x–KLH.

	Coating	IgM Titers	IgG Titers
1	Le^yLe^x–BSA	1060	31 645
2	Le^y–BSA	< 120	3115
3	Le^x–BSA	n.d.	500

[a] All titers are medians for a group of five mice. Titers were determined by regression analysis, plotting $\log_{10}$ dilution vs absorbance. The titers were calculated to be the highest dilution that gave three times the absorbance of normal saline mouse sera diluted 1:120.

Le^yLe^x conjugate raised significant IgG anti-Le^yLe^x titers indicating a proper helper T-cell response. It should be noted that the epitope density of the glycoconjugate was high (> 1000), which may have facilitated the high titers of elicited IgG antibodies. Reports of immunizations with the KH-1 antigen show that not only the nature of the glycoconjugate, but also the epitope density influences the immunological response.[34] This notion is also supported by our own observations of immunizations with the Le^y antigen[17] as well as reports from other groups.[35,36]

An important aspect of active immunotherapy against cancer is that the antibodies are specific for a particular antigen. Due to the structural similarities of the Lewis antigens, it is important to examine cross-reactivity with other Lewis antigens. For example, an antibody raised against a dimeric structure may only bind terminal saccharides and as a result will cross react with a corresponding monomeric antigen. On the other hand, an antibody raised against a dimeric structure may recognize internal saccharides and hence be more specific for dimeric Lewis antigens. In addition, large oligosaccharides may have different conformational properties compared to smaller fragments and this may also affect antibody selectivity.

The Le^y antigen is expressed predominantly during embryogenesis. In normal adult tissue expression of Le^y and Le^x is mainly restricted to granulocytes and epithelial surfaces.[37] The Le^x antigen is, however, also expressed by neutrophils (PMNs).[38,39] Thus, the safe use of Lewis antigens in vaccine development requires a detailed knowledge of the cross-reactivity of a particular antibody with respect to other Lewis antigens.

In order to investigate the cross-reactivity of the elicited antibodies with other Lewis antigens, in particular Le^y and Le^x, ELISA by using microtiters plates coated with Le^y–BSA and Le^x–BSA was performed. As can be seen in entry 2 of Table 1, the IgM and IgG antibodies do recognize the Le^y tetrasaccharide albeit with significantly lower titers (ten-fold) compared with that of the heptasaccharide Le^yLe^x. The substantially lower reactivity clearly indicates that the antibodies recognize an epitope spanning both the Le^y and Le^x monomers. The titer of IgG antibodies against the Le^x monomer was very low (entry 3). This result is important but perhaps not surprising, since it is known that when a large oligosaccharide is presented to the immune system, the more accessible terminal ends become the major epitope.[40] The finding of low cross-reactivity and thus high specificity of the antibodies raised against the Le^yLe^x dimer is of great importance for the safe use of an anticancer vaccine, especially considering the distribution of Le^x in a healthy environment.

Conclusion

We report here an efficient synthesis of the complex dimeric $Lewis^y$–$Lewis^x$ oligosaccharide based on two different orthogonally protected lactosamine building blocks. This ap-

proach provided easy access to a Le^y glycosyl donor and a Le^x glycosyl acceptor that could be coupled in one key glycosylation to provide a hetero dimeric Lewis antigen. The use of a *p*-(benzoyl)-benzyl glycoside as a temporary anomeric protecting group offered additional flexibility because it was stable to conditions used to remove the Fmoc, Lev and DEIPS protecting group but could be selectively cleaved at a late stage in the synthesis without an adverse effect on the complex structure. The anomeric aminopropyl spacer was employed for selective conjugation to carrier proteins. Immunizations of the conjugate of Le^yLe^x to KLH in combination with the immuno-adjuvant QS-21 evoked a strong helper T-cell immune response. Further studies of antibody cross-reactivity revealed that the antibodies recognized a monomeric Le^y tetrasaccharide, albeit with a tenfold lower titer. This clearly demonstrated that the raised antibodies have high specificity for the Le^yLe^x heptasaccharide, indicating that the recognized epitope is spanning the two Lewis antigen monomers. Importantly, it was determined that the antibody recognition of the internal $Lewis^x$ trisaccharide was very low. Thus, the results reported here supports the notion that it may be possible to develop a tumor specific carbohydrate-based anticancer vaccine.

Experimental Section

General methods: Succinimidyl 3-(bromoacetamido) propionate (SBAP), sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA-MI) were purchased from Pierce Endogen, Rockford, IL. BSA was purchased from Sigma. NIS was purchased from Fluka and recrystallized from dioxane/CCl_4. All other chemicals were purchased from Aldrich, Acros, and Fluka and used without further purification. Molecular sieves were activated at 145 °C for 10 h. All solvents employed were of reagent grade and dried by refluxing over appropriate drying agents. TLC was performed by using Kieselgel 60 F_{254} (Merck) glass/alumiunium/plastic plates, with detection by UV light (254 nm) and/or by charring with 8% sulfuric acid in ethanol. Column chromatography was performed on silica gel (Merck, mesh 70–230). Extracts were concentrated under reduced pressure at $\leq$ 40 °C (water bath). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova300 spectrometer, a Varian Inova500 spectrometer and a Varian Inova800 spectrometer equipped with Sun workstations. ^{1}H spectra recorded in $CDCl_3$ were referenced to residue $CHCl_3$ at 7.26 ppm or TMS, and ^{13}C spectra to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made using standard 1D and gCOSY, gHSQC and TOCSY 2D experiments. Positive ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument by using gentisic acid as a matrix. Centrifugal filter devices were purchased from Millipore Inc. The immunoadjuvant QS-21 was a gift from Antigenics Inc., Lexington MA. ELISA plates Immulon II Hb was purchased from Fisher Scientific Inc.

(2′*R*,3′*R*)-Ethyl 2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thiol-β-D-galactopyranoside (8): Galactoside **7** (14.7 g, 65.7 mmol), butane-2,3-dione (6.9 mL, 78.8 mmol), trimethylorthoformate (23 mL, 197 mmol) and camphorsulfonic acid (1.5 g, 6.5 mmol) in methanol (200 mL) were heated under reflux for 16 h. The reaction mixture was cooled to room temperature and triethylamine (2 mL) was added to quench the reaction. After the solution was concentrated to dryness, the residue was purified by column chromatography (silica gel, hexane/EtOAc 2:3) to furnish diol **8** (17.2 g, 78%) as a white foam. $[\alpha]_D = 147.1$ (c = 1.0 in $CHCl_3$); $R_f = 0.35$ (hexane/EtOAc 2:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 4.57$ (d, $J = 9.6$ Hz, 1H, H-1), 4.10 (t, $J = 9.6$ Hz, 1H, H-2), 4.00 (d, $J = 2.1$ Hz, 1H, H-4), 3.93 (dd, $J = 5.5$, 11.8 Hz, 1H, H-6), 3.79 (dd, $J = 5.5$, 11.8 Hz, 1H, H-6), 3.74 (dd, $J = 2.1$, 9.6 Hz, 1H, H-3), 3.61 (t, $J = 5.5$ Hz, 1H, H-5), 3.28 (s, 3H, OCH_3-BDA), 3.26 (s, 3H, OCH_3-BDA), 2.75 (q, $J = 1.32$, 2H, 7.42 Hz, SCH_2CH_3), 1.32 (s, 3H, CH_3-BDA), 1.31 (s, 3H, CH_3-BDA), 1.30 ppm (t, $J = 7.42$ Hz, 3H, SCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 100.51$, 100.48 (2×C-BDA), 83.4 (C-1), 78.9 (C-5), 71.9 (C-3), 68.2 (C-4), 66.3 (C-2), 62.3 (C-6), 48.3 (2×C, OCH_3-BDA), 24.7 (SCH_2CH_3), 17.9 (CH_3-BDA), 17.7 (CH_3-BDA), 14.7 ppm (SCH_2CH_3); HR-MALDI-TOF MS: m/z: calcd for $C_{14}H_{26}O_7S$: 338.1399; found: 361.1307 $[M+Na]^+$.

(2′*R*,3′*R*)-Ethyl 4,6-di-*O*-benzyl-2,3-*O*-(2′,3′-dimethoxybutane-2′,3′-diyl)-1-thiol-β-D-galactopyranoside (9): A mixture of compound **8** (5 g, 14.8 mmol) and sodium hydride (0.78 g, 32.5 mmol) in dry *N*,*N*-dimethylformamide (30 mL) was cooled to 0 °C with an ice-bath. Benzyl bromide (3.87 mL, 32.5 mmol) was added dropwise, the ice-bath was removed and the reaction mixture was stirred at ambient temperature for 2 h after which, methanol (3 mL) was added to quench the reaction. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, hexane/EtOAc 2:3) to give compound **9** (6.5 g, 85%) as a white foam. $[\alpha]_D = 147.1$ (c = 1.0 in $CHCl_3$); $R_f = 0.88$ (hexane/EtOAc 2:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.42$–7.26 (m, 10H, Ar-H), 4.96 (d, $J = 11.6$ Hz, 1H, $ArCH_2$), 4.59 (d, $J = 11.6$ Hz, 1H, $ArCH_2$), 4.48 (q, $J = 11.8$, 8.0 Hz, 2H, $ArCH_2$), 4.26 (d, $J = 9.4$ Hz, 1H, H-1), 4.07 (t, $J = 9.4$ Hz, 1H, H-2), 3.81 (m, 2H, H-6), 3.75 (d, $J = 2.1$ Hz, 1H, H-3), 3.68–3.63 (m, 2H, H-4, H-5), 3.30 (s, 3H, OCH_3-BDA), 3.27 (s, 3H, OCH_3-BDA), 2.78–2.66 (m, 2H, SCH_2CH_3), 1.29 (t, $J = 7.4$ Hz, 3H, SCH_2CH_3), 1.28 (s, 3H, CH_3-BDA), 1.27 ppm (s, 3H, CH_3-BDA); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 139.2$–127.5 (12C, Ar-C), 100.2, 100.0 (2×C-BDA), 83.6 (C-1), 78.2 (C-4), 74.2 (C-3), 73.9 ($ArCH_2$), 73.8 ($ArCH_2$), 73.5 (C-5), 69.2 (C-6), 67.0 (C-2), 48.2 (2×C, OCH_3-BDA), 24.7 (SCH_2CH_3), 17.9 (CH_3-BDA), 17.7 (CH_3-BDA), 15.3 ppm (SCH_2CH_3); HR-MALDI-TOF MS: m/z: calcd for $C_{28}H_{38}O_7S$: 518.2338; found: 541.2315 $[M+Na]^+$.

Ethyl 4,6-di-*O*-benzyl-1-thiol-β-D-galactopyranoside (10): A mixture of **9** (5 g, 9.65 mmol) and TFA/H_2O (200 mL, 9:1) was stirred for 2 min, the solvent removed under reduced pressure and the remaining solid was purified by column chromatography (silica gel, hexane/EtOAc 2:3) to provide diol **10** (4.2 g, 10.4 mmol, 67%) as a white foam. $[\alpha]_D = 147.1$ (c = 1.0 in $CHCl_3$); $R_f = 0.40$ (hexane/EtOAc 2:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.36$–7.26 (m, 10H, Ar-H), 4.71 (q, $J = 11.8$, 12.4 Hz, 2H, $ArCH_2$), 4.48 (q, $J = 11.8$, 8.0 Hz, 2H, $ArCH_2$), 4.29 (d, $J = 9.4$ Hz, 1H, H-1), 3.90 (d, $J = 3.0$ Hz, 1H, H-4), 3.74–3.58 (m, 5H, H-2, H-3, H-5, H-6), 2.78–2.66 (m, 2H, SCH_2CH_3), 1.29 ppm (t, $J = 7.4$ Hz, 3H, SCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 138.6$–118.0 (12C, Ar-C), 86.4 (C-1), 77.8 (C-2), 76.4 (C-4), 75.6 (C-3), 75.4 ($ArCH_2$), 73.8 ($ArCH_2$), 71.1 (C-5), 68.7 (C-6), 24.7 (SCH_2CH_3), 15.5 ppm (SCH_2CH_3); HR-MALDI-TOF MS: m/z: calcd for $C_{22}H_{28}O_5S$: 404.1657; found: 427.1620 $[M+Na]^+$.

Ethyl 3-*O*-diethylisopropylsilyl-4,6-di-*O*-benzyl-1-thiol-β-D-galactopyranoside (11): DEIPSCl (2.63 mL, 9.90 mmol) was added dropwise to a solution of diol **10** (4.0 g, 9.90 mmol) and imidazole (0.67 g, 100 mmol) in dry THF (5 mL). After stirring for 4 h at room temperature, methanol (2 mL) was added to quench the reaction. The mixture was diluted with Et_2O (30 mL), washed with water and dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc 9:1) to furnish silyl ether **11** (4.05 g, 78%) as a white foam. $[\alpha]_D = (c$ = 1.0 in $CHCl_3)$; $R_f = 0.58$ (hexane/EtOAc 11:2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.34$–7.30 (m, 10H, Ar-H), 5.04 (d, $J = 11.3$ Hz, 1H, $ArCH_2$), 4.58 (d, $J = 11.3$ Hz, 1H, $ArCH_2$), 4.25 (q, $J = 6.6$, 9.8 Hz, 2H, $ArCH_2$), 4.31 (d, $J = 9.4$ Hz, 1H, H-1), 3.85 (dd, $J = 5.7$, 2.0 Hz, 1H, H-3), 3.78 (d, $J = 2.0$ Hz, 1H, H-4), 3.74 (dd, $J = 9.4$, 5.7 Hz, 1H, H-2), 3.65 (m, 3H, H-5, H-6), 2.78 (m, 2H, SCH_2CH_3), 1.30 (t, $J = 7.4$ Hz, 3H, SCH_2CH_3), 1.05 (m, 12H, CH_3-DEIPS), 0.75 ppm (m, 5H, CH_2, CH-DEIPS); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 139.3$–127.6 (12C, Ar-C), 86.8 (C-1), 77.7 (C-3), 77.4 (C-6), 76.9 (C-2), 75.3 (CH_2Ph), 73.8 (CH_2Ph), 70.7 (C-5), 69.2 (C-6), 24.5 (SCH_2CH_3), 17.7 (SCH_2CH_3), 17.7, 15.6, 13.3, 7.5, 7.4 (5C, CH, CH_3-DEIPS), 4.24 ppm (2C, CH_2-DEIPS); HR-MALDI-TOF MS: m/z: calcd for $C_{29}H_{44}O_5Ssi$: 532.2679; found 555.2696 $[M+Na]^+$.

Ethyl 2-*O*-benzoyl-3-*O*-diethylisopropylsilyl-4,6-di-*O*-benzyl-1-thiol-β-D-galactopyranoside (13): Triethylamine (0.38 mL, 2.7 mmol), benzoylchloride (0.32 mL, 2.7 mmol), and DMAP (671 mg, 5.5 mmol) were added to a solution of alcohol **11** (309 mg, 0.58 mmol), in dichloromethane (2 mL) at room temperature. The mixture was stirred at room temperature for 20 h after which it was poured into EtOAc (50 mL). The organic phase was washed with 1 M HCl, sat. aqueous sodium bicarbonate and water. The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. Purification of the residue by column chromatography (silica gel, hexane/EtOAc 2:3) yielded **13** (328 mg, 89 %) as a white foam. $[\alpha]_D = (c = 1.0$ in $CHCl_3)$; $R_f = 0.48$ (hexane/EtOAc 2:3); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 8.05 (d, $J = 7.2$ Hz, 2H, Ar-H), 7.57–7.26 (m, 13H, Ar-H), 5.62 (t, $J =$ 8.8 Hz, 1H, H-2), 5.04 (d, $J = 11.6$ Hz, 1H, $ArCH_2$), 4.86 (d, $J = 11.6$ Hz, 1H, $ArCH_2$), 4.25 (m, 2H, $ArCH_2$), 4.20 (d, $J = 9.6$ Hz, 1H, H-1), 4.01 (dd, $J = 5.8$, 1.2 Hz, 1H, H-3), 3.83 (d, $J = 1.2$ Hz, 1H, H-4), 3.65 (q, $J =$ 3.2 Hz, 1H, H-5), 3.61 (m, 2H, H-6), 2.68 (m, 2H, SCH_2CH_3), 1.19 (t, $J =$ 7.4 Hz, 3H, SCH_2CH_3), 0.95 (m, 12H, CH_3-DEIPS), 0.54 ppm (m, 5H, CH_2, CH-DEIPS); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.7$ (COAr), 139.2–125.6 (18C, Ar-C), 84.0 (C-1), 77.9 (C-5), 77.8 (C-4), 76.1 (C-3), 75.5 ($ArCH_2$), 73.8 ($ArCH_2$), 71.5 (C-2), 68.9 (C-6), 23.7 (SCH_2CH_3), 17.5, 17.4, 15.1, 13.1 (4C, CH_3-DEIPS), 17.7 (SCH_2CH_3), 7.3, 4.3, 4.0 ppm (3C, CH_2, CH-DEIPS); HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{36}H_{48}O_6SSi$: 636.2941; found: 661.2965 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2,-trichloroethoxy)carbonyl]amino]-3-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranoside (15)**: A solution of thioglycoside **14** (4.77 g, 6.73 mmol) and *p*-benzoyl-benzyl alcohol (3.07 g, 13.47 mmol) in dry dichloromethane (20 mL) was dried azeotropically with toluene (Na-dried) and then subjected to high vacuum for 2 h. The compounds were dissolved in dry dichloromethane (30 mL) under argon atmosphere and the mixture was stirred at room temperature in the presence of activated 3 Å molecular sieves for 30 min. The mixture was then cooled to 0 °C and treated with NIS (1.67 g, 7.41 mmol) and TESOTf (0.15 mL, 0.67 mmol). After stirring for 30 min TLC showed full conversion of the donor. The solution was diluted by dichloromethane and the molecular sieves were removed by filtering through Celite. The filtrate was washed with 15 % aqueous sodium thiosulfate and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give the product **15** as a white powder (5.05 g, 86 %). $[\alpha]_D = -20.6$ ($c = 1.0$, CH_2Cl_2); $R_f = 0.28$ (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.20$ (d, $J = 7.5$ Hz, 2H, Ar-H), 7.76–7.09 (m, 20H, Ar-H), 5.49 (d, $J = 8.3$ Hz, 1H, NH), 4.92 (d, $J = 8.8$ Hz, 1H, H-1), 4.86 (t, $J =$ 9.4 Hz, 1H, H-3), 4.66–4.54 (m, 6H, $ArCH_2$, Troc), 4.38 (d, $J = 6.3$ Hz, 2H, Fmoc-CH_2), 4.22 (t, $J = 6.3$ Hz, 1H, Fmoc-CH), 3.85–3.80 (m, 4H, H-6, H-4, H-2), 3.65–3.52 ppm (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.6$ (ArCO), 156.0 ($CHCH_2OCO$, Fmoc), 154.7 (Ar-C), 150.6 (NHCO), 150.7–120.3 (29C, Ar-C), 99.2 (C-1), 95.9 (CCl_3), 79.6 (C-3), 74.7 (C-5), 74.5 (OCH_2Cl_3, Troc), 73.9 (OCH_2PhOBz), 70.7 (C-4), 70.1 (OCH_2Ph), 69.9 ($CHCH_2OCO$, Fmoc), 64.8 (C-6), 56.1 (C-2), 46.4 ppm ($CHCH_2OCO$, Fmoc); HR-MALDI-TOF MS *m*/*z*: calcd for $C_{45}H_{40}Cl_3NO_{11}$: 875.1667; found: 898.1659 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-3-*O*-(9-fluorenylmethoxycarbonyl)-4-*O*-(2-*O*-benzoyl-3-*O*-diethylisopropylsilyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (5)**: A solution of the glycosyl donor **13** (150 mg, 0.24 mmol) and the glycosyl acceptor **15** (175 mg, 0.2 mmol) in dry dichloromethane (2 mL) was stirred at room temperature under argon in the presence of activated molecular sieves for 30 min. The mixture was then cooled to 0 °C and NIS (594 mg, 0.26 mmol) was added followed by TESOTf (5.5 μL, 0.024 mmol). After 40 min, the reaction mixture was diluted with dichloromethane (50 mL) and filtered through a plug of Celite. The filtrate was washed with 15 % aqueous sodium thiosulfate and sat. aqueous sodium bicarbonate. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give the disaccharide **5** as a white powder (200 mg, 69 %). $[\alpha]_D = -28.9$ ($c = 1.0$, CH_2Cl_2); $R_f =$ 0.57 (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.20$ (d, $J =$ 7.7 Hz, 2H, Ar-H), 8.05 (d, $J = 7.7$ Hz, 2H, Ar-H), 7.77–7.13 (m, 33H, Ar-H), 5.57 (t, $J = 8.8$ Hz, 1H, H-2′), 5.19 (d, $J = 9.4$ Hz, 1H, N-H), 5.01 (t, $J = 9.4$ Hz, 1H, H-3), 4.98 (d, $J = 11.6$ Hz, 1H, Bn), 4.85 (d, $J =$ 11.6 Hz, 1H, Bn), 4.68 (d, $J = 8.6$ Hz, 1H, H-1), 4.60–4.31 (m, 8H, 2×Bn, Troc, CH_2PhOBz), 4.52 (d, $J = 8.9$ Hz, 1H, H-1′), 4.35 (d, $J = 6.0$ Hz, 2H, Fmoc-CH_2), 4.18 (t, $J = 6.0$ Hz, 1H, Fmoc-CH), 4.10 (dd, $J = 5.9$, 1.6 Hz, 1H, H-3′), 4.08–3.81 (m, 4H, H-2, H-4, H-6), 3.80 (d, $J = 1.6$ Hz, 1H, H-4′), 3.69–3.51 (m, 3H, H-5′, H-6′), 3.61 (d, $J = 9.1$ Hz, 1H, H-5), 0.95 (m, 12H, CH_3-DEIPS), 0.54 ppm (m, 5H, CH_2, CH-DEIPS); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 165.3$ (COPh), 165.1 (ArCO), 154.9 ($CHCH_2OCO$, Fmoc), 154.4 (Ar-C), 150.7 (NHCO), 143.7–12.2 (47C, Ar-C), 101.4 (C-1′), 100.0 (C-1), 95.6 (CCl_3), 77.2 (C-3′), 77.1 (C-4′), 75.7 (C-3), 75.5, 74.7, 73.8, 73.4, 73.3 (4C, 3×CH_2Ph, Troc, CH_2PhOBz), 73.6 (2C, C-2′, C-4), 73.5 (C-5′), 70.2 (CH_2-Fmoc), 68.2, 68.0 (2C, C-6, C-6′), 60.6 (C-2), 46.8 (CH-Fmoc), 17.5, 17.3, 14.5, 13.0 (4C, CH_3-DEIPS), 7.3 (CH-DEIPS), 4.3, 4.0 ppm (2C, CH_2-DEIPS); HR-MALDI-TOF MS *m*/*z*: calcd for $C_{79}H_{86}Cl_3NO_{17}Si$: 1449.4418; found: 1472.4530 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-4-*O*-(2-*O*-benzoyl-3-*O*-diethylisopropylsilyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (16)**: Compound **5** (100 mg, 0.07 mmol) was dissolved in dichloromethane (4 mL) and triethylamine (1 mL) was added. The reaction mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give **16** as a white powder (80 mg, 95 %). $[\alpha]_D = -26.4$ ($c = 1.0$, CH_2Cl_2); $R_f = 0.32$ (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta =$ 8.21 (d, $J = 7.7$ Hz, 2H, Ar-H), 8.07 (d, $J = 7.7$ Hz, 2H, Ar-H), 7.67–7.03 (m, 25H, Ar-H), 5.60 (t, $J = 8.5$ Hz, 1H, H-2′), 5.20 (d, $J = 9.0$ Hz, 1H, NH), 5.08 (d, $J = 11.6$ Hz, 2H, $ArCH_2$), 4.82–4.06 (m, 10H, 3×ArCH_2, CH_2PhOBz, Troc), 4.38 (d, $J = 8.9$ Hz, 1H, H-1), 4.28 (d, $J = 8.7$ Hz, 1H, H-1′), 4.08 (dd, $J = 1.6$, 8.0 Hz, 1H, H-3′), 3.80 (d, $J = 2.0$ Hz, 1H, H-4), 3.79–3.74 (m, 3H, H-2, H-6), 3.62 (m, 1H, H-3), 3.60 (d, $J = 1.6$ Hz, 1H, H-4′), 3.59–3.51 (m, 2H, H-5′, H-6′), 3.41–3.36 (m, 2H, H-5, H-6′), 0.98 (m, 12H, CH_3-DEIPS), 0.64 ppm (m, 5H, CH_2, CH-DEIPS); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 165.7$ (COPh), 165.1 (ArCO), 154.4 (Ar-C), 150.7 (NHCO), 138.7–121.2 (35C, Ar-C), 101.7 (C-1′), 99.9 (C-1), 95.8 (CCl_3), 81.0 (C-3′), 75.5 (C-4′), 74.7 (C-3), 74.8, 74.6, 72.6, 72.2 (4C, 3×CH_2Ph, Troc, CH_2PhOBz), 74.2 (C-2′), 73.3 (C-5′), 70.3 (C-4), 68.8, 68.6 (2C, C-6, C-6′), 57.9 (C-2), 17.5, 17.4, 17.3, 14.4 (4C, CH_3-DEIPS), 7.4 (CH-DEIPS), 4.4, 4.0 ppm (2C, CH_2-DEIPS); HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{64}H_{72}Cl_3NO_{15}Si$: 1227.3737; found: 1250.3685 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-3-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2-*O*-benzoyl-3-*O*-diethylisopropylsilyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (17)**: A solution of the glycosyl donor **6** (53.5 mg, 0.14 mmol) and the glycosyl acceptor **16** (80 mg, 0.07 mmol) was dissolved in dry dichloromethane (3 mL) and the mixture was stirred at room temperature under argon in the presence of activated molecular sieves for 30 min. The mixture was cooled to 0 °C and NIS (34.7 mg, 0.15 mmol) was added followed by TESOTf (3.3 μL, 0.015 mmol). After the donor was fully converted, the reaction mixture was diluted with dichloromethane (50 mL) and the molecular sieves were filtered off through a plug of Celite. The organic layer was washed with 15 % aqueous sodium thiosulfate and brine, dried by $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (silica gel, hexane/EtOAc 2:1) furnished the fully protected trisaccharide **17** as a white powder (80 mg, 74 %). $[\alpha]_D = -42.6$ ($c = 1.0$, CH_2Cl_2); $R_f =$ 0.54 (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.20$ (d, $J =$ 7.4 Hz, 2H, Ar-H), 8.07 (d, $J = 7.4$ Hz, 2H, Ar-H), 7.66–7.05 (m, 30H, Ar-H), 5.45 (t, $J = 8.5$ Hz, 1H, H-2), 5.33 (d, $J = 8.9$ Hz, 1H, N-H), 5.25 (dd, $J = 10.6$, 3.3 Hz, 1H, H-3″), 5.17 (d, $J = 3.6$ Hz, 1-H, H-1″), 5.14 (d, $J = 3.3$ Hz, 1H, H-4″), 4.88 (q, $J = 6.0$ Hz, 1-H, H-5″), 4.84 (d, $J = 8.6$ Hz, 1H, H-1), 4.76–4.37 (m, 12H, 4×Bn, Troc, CH_2PhOBz), 4.62 (d, $J =$ 9.0 Hz, 1H, H-1′), 4.10 (d, $J = 3.6$ Hz, 1H, H-4′), 4.01 (t, $J = 9.0$ Hz, 1H, H-3), 3.85–3.67 (m, 6H, H-4, H-6a, H-3′, H-6′, H-2″), 3.58 (d, $J = 8.5$ Hz, 1H, H-6b), 3.44 (q, $J = 3.6$ Hz, 1H, H-5′), 3.19–3.05 (m, 2H, H-5, H-2), 2.08 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 0.97 (d, $J = 6.0$ Hz, 3H, H-6″), 0.95 (m, 12H, CH_3-DEIPS), 0.54 ppm (m, 5H, CH_2, CH-DEIPS); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.6$, 169.7 (2C, 2×CH_3CO), 165.3 (COPhOBz), 165.0 (ArCO), 153.7 (Ar-C), 150.6 (NHCO), 138.9–121.7 (41C, Ar-C), 100.1 (C-1), 99.0 (C-1), 97.5 (C-1″), 95.6 (CCl_3), 77.5 (C-3′),

75.6 (C-5), 75.3 (C-4′), 75.1, 74.3, 73.3, 72.6, 70.7 (6C, 4×CH_2Ph, Troc, CH_2PhOBz), 74.6 (C-5′), 74.3 (C-3), 73.7 (C-2″), 73.6 (C-2′), 73.5 (C-4), 73.4 (C-4″), 70.7, 70.6 (2C, C-6, C-6′), 70.5 (C-3″), 64.9 (C-5″), 59.1 (C-2), 21.2, 21.0 (2C, 2×CH_3CO), 17.6 (C-6″), 17.5, 17.4, 15.3, 13.0 (4C, CH_3-DEIPS), 7.3 (*CH*-DEIPS), 4.3, 4.0 ppm (2C, CH_2-DEIPS); HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{81}H_{92}Cl_3NO_{21}Si$: 1547.4997; found: 1570.4900 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-3-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2-*O*-benzoyl-3-*O*-diethylisopropylsilyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (21): Triethylamine (2.5 mL) and H_2O_2 (30% in water, 0.13 mL) were added to a stirred solution of compound **17** (80 mg, 0.05 mmol) in THF (5 mL). After stirring the reaction mixture at room temperature for 30 min, it was concentrated under reduced pressure. The obtained residue was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give compound **18** (60 mg, 80%).

DDQ (9 mg, 0.04 mmol) was then added to a solution of **18** (60 mg, 0.04 mmol) in dichloromethane/water (4 mL, 95:5). The reaction mixture was stirred in the dark at room temperature for 1 h, diluted with dichloromethane (50 mL) and was washed with aqueous sodium hydrogen carbonate and brine. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. Purification by column chromatography (silica gel, hexane/EtOAc 3:2) furnished hemiacetal **19** (45 mg, 81%).

A stirred solution of **19** in dichloromethane (5 mL) was treated with trichloroacetonitrile (0.5 mL) and DBU (5 μL). After stirring for 5 min under argon atmosphere, the solution was concentrated to dryness and the residue was purified by column chromatography (silica gel, hexane/EtOAc/triethylamine 1:1:0.01) to give the imidate **20** as a colorless syrup (45 mg, 90%).

Compound **20** and 3-[(*N*-benzyloxycarbonyl)amino]propanol (11 mg, 0.06 mmol) was dissolved in dry dichloromethane (2 mL) and stirred under argon atmosphere for 30 min in the presence of activated molecular sieves. $BF_3{\cdot}Et_2O$ (1 μL) was added and the reaction mixture was kept at room temperature for 10 min where after the reaction was quenched by the addition of triethylamine (20 μL), diluted with dichloromethane (60 mL) and filtered through Celite. The filtrate was washed with brine, dried over $MgSO_4$ and concentrated to dryness. The residue was subjected to column chromatography (silica gel, hexane/EtOAc 1:1) to give **21** as a syrup (40 mg, 86%). $[\alpha]_D = -51.2$ (c = 1.0, CH_2Cl_2); $R_f = 0.49$ (hexane/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.88$ (d, $J = 7.8$ Hz, 2H, Ar-H), 7.50–7.12 (m, 28H, Ar-H), 5.50 (d, $J = 8.7$ Hz, 1H, N*H*), 5.35 (t, $J = 9.3$ Hz, 1H, H-2′), 5.25 (dd, $J = 8.8$, 2.9 Hz, 1H, H-3″), 5.10 (d, $J = 3.5$ Hz, 1-H, H-1″), 5.04 (d, $J = 2.9$ Hz, 1H, H-4″), 4.97 (s, 2H, CH_2NHCOOCH_2Ph), 4.80 (q, $J = 6.5$ Hz, 1-H, H-5″), 4.64 (d, $J = 9.0$ Hz, 1H, H-1), 4.76–4.37 (m, 10H, 4×Bn, Troc), 4.58 (d, $J = 8.6$ Hz, 1H, H-1′), 3.85 (d, $J = 3.6$ Hz, 1H, H-4′), 3.83 (m, 1H, H-3), 3.80–3.65 (m, 6H, H-4, H-6a, H-6′, H-3′, H-2″), 3.58 (d, $J = 8.5$ Hz, 2H, OCH_2CH$_2$CH$_2$N), 3.44 (d, $J = 8.8$ Hz, 1H, H-6b), 3.38 (q, $J = 3.8$ Hz, 1H, H-5′), 3.30–3.20 (m, 1H, OCH$_2$CH$_2$CH_{2a}N), 3.19–3.10 (m, 1H, OCH$_2$CH$_2$CH_{2b}N), 3.09–3.01 (m, 2H, H-5, H-2), 2.00 (s, 3H, COCH_3), 1.88 (s, 3H, COCH_3), 1.19 (s, 2H, OCH$_2$CH_2CH$_2$N), 0.87 (d, $J = 6.5$ Hz, 3H, H-6″), 0.85 (m, 12H, CH_3-DEIPS), 0.54 ppm (m, 5H, CH_2, C*H*-DEIPS); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.6$, 169.6 (2C, 2×CH_3*C*O), 165.0 (Ar*C*O), 156.7 (NH*C*OOCH$_2$Ph), 154.6 (NHCO), 138.9–127.7 (36C, Ar-C), 100.0 (C-1), 97.7 (C-1′), 96.5 (C-1″), 95.4 (CCl_3), 77.5 (C-3′), 75.8 (C-5), 75.5 (C-4′), 75.0, 74.3, 73.3, 72.6, 70.7 (6C, 4×CH_2Ph, Troc, NHCOO*C*H$_2$Ph), 74.7 (C-5′), 74.6 (C-3), 73.7 (C-2″), 73.6 (C-2′), 73.5 (C-4), 73.4 (C-4″), 73.3 (C-3″), 68.3, 68.2 (2C, C-6, C-6′), 66.9 (O*C*H$_2$CH$_2$CH$_2$NH), 65.0 (C-5″), 59.0 (C-2), 37.8 (OCH$_2$CH$_2$CH$_2$NH), 30.0 (OCH$_2$*C*H$_2$CH$_2$NH), 21.2, 21.0 (2C, 2×CH_3CO), 17.5 (C-6″), 17.5, 17.3, 15.3, 13.0 (4C, CH_3-DEIPS), 7.3 (*CH*-DEIPS), 4.3, 4.0 ppm (2C, CH_2-DEIPS); HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{78}H_{95}Cl_3N_2O_{21}Si$: 1528.5262; found: 1551.5194 $[M+Na]^+$.

3-[(*N*-Benzyloxycarbonyl)amino]propyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-3-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (22): Compound **21** (40 mg, 0.03 mmol) was dissolved in dry THF (2 mL), and then acetic acid (0.2 mL) and a solution of TBAF in THF (1M, 0.2 mL) were added. After stirring at room temperature for 2 d, the reaction mixture was diluted with EtOAc (50 mL) and washed with water (10 mL), sat. aqueous sodium hydrogen carbonate and brine. The organic phase was dried over $MgSO_4$, filtered and then concentrated in vacuo. The obtained residue was purified by column chromatography (silica gel, hexane/EtOAc 1:1) to yield the alcohol **22** as a white powder (30 mg, 82%). $[\alpha]_D = -49.3$ (c = 1.0, CH_2Cl_2); $R_f = 0.38$ (hexane/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.92$ (d, $J = 7.8$ Hz, 2H, Ar-H), 7.65–7.10 (m, 28H, Ar-H), 5.61 (d, $J = 9.0$ Hz, 1H, N-H), 5.38 (t, $J = 8.0$ Hz, 1H, H-2), 5.28 (dd, $J = 9.6$, 3.0 Hz, 1H, H-3″), 5.18 (d, $J = 3.5$ Hz, 1-H, H-1″), 5.09 (d, $J = 3.1$ Hz, 1H, H-4″), 5.01 (s, 2H, CH_2NHCOOCH_2Ph), 4.82 (q, $J = 6.5$ Hz, 1-H, H-5″), 4.59 (d, $J = 8.9$ Hz, 1H, H-1), 4.76–4.37 (m, 10H, 4×Bn, Troc), 4.40 (d, $J = 9.2$ Hz, 1H, H-1′), 4.10 (d, $J = 3.8$ Hz, 1H, H-4′), 4.03 (m, 1H, H-3), 4.02–3.95 (m, 5H, H-4, H-6′, H-3′, H-2″), 3. 85 (m, 2H, OCH_2CH$_2$CH$_2$N), 3.64 (d, $J = 3.8$ Hz, 2H, H-6), 3.58 (q, $J = 4.0$ Hz, 1H, H-5′), 3.30 (m, 1H, OCH$_2$CH$_2$CH_{2a}N), 3.19–3.10 (m, 3H, OCH$_2$CH$_2$CH_{2b}N, H-5, H-2), 2.20 (s, 3H, COCH_3), 2.00 (s, 3H, COCH_3), 1.29 (s, 2H, OCH$_2$CH_2CH$_2$N), 1.15 ppm (d, $J = 6.5$ Hz, 3H, H-6″); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.8$, 169.8 (2C, 2×CH_3*C*O), 166.1 (Ar*C*O), 156.7 (NH*C*OOCH$_2$Ph), 154.0 (NHCO), 138.6–127.9 (36C, Ar-C), 100.3 (C-1), 98.0 (C-1′), 95.7 (C-1″), 95.4 (CCl_3), 77.4 (C-3′), 76.4 (C-5), 75.9 (C-4′), 75.7, 74.8, 74.7, 72.5, 70.8 (6C, 4×CH_2Ph, Troc, NHCOO*C*H$_2$Ph), 74.3 (C-5′), 74.3 (C-3), 73.3 (C-2″), 72.6 (C-2′), 73.3 (C-4), 72.6 (C-4″), 71.7 (C-3″), 67.8, 66.9 (2C, C-6, C-6′), 66.8 (O*C*H$_2$CH$_2$CH$_2$NH), 65.0 (C-5″), 59.4 (C-2), 37.8 (OCH$_2$CH$_2$*C*H$_2$NH), 30.0 (OCH$_2$*C*H$_2$CH$_2$NH), 21.2, 21.0 (2C, 2×CH_3CO), 15.8 ppm (C-6″); HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{71}H_{79}Cl_3N_2O_{21}$: 1400.4241; found: 1423.4268 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-3-*O*-(9-fluorenylmethoxycarbonyl)-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-levulinoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (4)**: A solution of compound **15** (4.0 g, 4.57 mmol) and donor **23** (4.06 g, 6.86 mmol) in dry dichloromethane (20 mL) was dried azeotropically with toluene (Na-dried) and then subjected to high vacuum for 2 h. The compounds were dissolved in dry dichloromethane (25 mL) and the mixture was stirred at room temperature under argon in the presence of activated 3 Å molecular sieves for 30 min. The mixture was cooled to 0°C and reacted with NIS (1.69 g, 7.54 mmol) and TESOTf (0.16 mL, 0.69 mmol). After 30 min the solution was diluted by dichloromethane and the molecular sieves were removed by filtering through Celite. The filtrate was washed with 15% aqueous sodium thiosulfate and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give the disaccharide **4** as a white powder (5.19 g, 81%). $[\alpha]_D = -64.2$ (c = 1.0, CH_2Cl_2); $R_f = 0.38$ (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.20$ (d, $J = 8.4$ Hz, 2H, Ar-H), 7.75–7.12 (m, 35H, Ar-H), 5.34 (t, $J = 9.2$ Hz, 1H, H-2), 5.18 (d, $J = 9.8$ Hz, 1H, N-H), 4.96 (t, $J = 9.4$ Hz, 1H, H-3), 4.88 (d, $J = 12.0$ Hz, 1H, ArCH_2), 4.85 (d, $J = 12.0$ Hz, 1H, ArCH_2), 4.61 (d, $J = 8.2$ Hz, 1H, H-1), 4.60–4.30 (m, 10H, 3×ArCH_2, Troc, CH_2PhOBz), 4.44 (d, $J = 7.7$ Hz, 1H, H-1′), 4.28 (d, $J = 6.8$ Hz, 2H, Fmoc-CH_2), 4.12 (t, $J = 6.0$ Hz, 1H, Fmoc-CH), 4.02–3.71 (m, 4H, H-4, H-4′, H-6), 3.60–3.51 (m, 3H, H-2, H-5, H-6a′), 3.69–3.51 (m, 2H, H-5′, H-6b′), 3.44 (dd, $J = 9.2$, 1.6 Hz, 1H, H-3′), 3.36 (d, $J = 9.1$ Hz, 1H, H-5′), 2.69–2.36 (m, 4H, CH_2CH_2, Lev), 2.13 ppm (s, 3H, COCH_3, Lev); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 206.6$ (CH_3*C*OCH$_2$, Lev), 171.5 (O*C*OCH$_2$CH$_2$, Lev), 165.3 (ArCO), 155.0 (CHCH$_2$*C*O, Fmoc), 154.3 (Ac-C), 150.7 (NH*C*O), 146.0–120.1 (47C, Ar-C), 100.5 (C-1′), 100.0 (C-1), 95.6 (CCl_3), 80.5 (C-5′), 77.3 (C-3), 75.4 (C-4), 75.0 (C-5), 74.7, 73.9, 72.0 (6C, 4×O*C*H$_2$Ph, O*C*H$_2$CCl$_3$, O*C*H$_2$-PhOBz), 73.5 (C-3′), 72.6 (C-4′), 72.1 (C-2′), 70.2 (CH*C*H$_2$CO, Fmoc), 68.2, 67.9 (2C, C-6, C-6′), 56.5 (C-2), 46.8 (*C*HCH$_2$CO, Fmoc), 38.0 (OCO*C*H$_2$CH$_2$, Lev), 30.1 (CH$_2$CO*C*H$_3$, Lev), 28.1 ppm (OCO*C*H$_2$C*H*$_2$, Lev); HR-MALDI-TOF: *m*/*z*: calcd for $C_{74}H_{77}Cl_3N_2O_{18}$: 1405.3972; found: 1428.4265 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-levulinoyl-β-D-galactopyranosyl)-β-D-glucopyranoside (24)**: Compound **4** (500 mg, 0.36 mmol) was dissolved in 20% triethylamine solution in dichloromethane (5 mL). The solution was stirred at room temperature under argon for 18 h and concentrated to dryness under reduced pressure. The residue was purified by

column chromatography (silica gel, hexane/EtOAc 2:1) to give the product **24** as a white powder (400 mg, 95%). $[\alpha]_D = -27.7$ (c = 1.0, CH_2Cl_2); $R_f = 0.21$ (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.21$ (d, $J = 7.4$ Hz, 2H, Ar-H), 7.54–7.16 (m, 27H, Ar-H), 5.38 (dd, $J = 10.1$, 8.3 Hz, 1H, H-2′), 5.08 (d, $J = 8.5$ Hz, 1H, N-H), 4.89 (dd, $J = 8.8$, 2.1 Hz, 2H, CH_2Ar), 4.59 (d, $J = 8.2$ Hz, 1H, H-1), 4.80–4.23 (m, 10H, 3×CH_2Ar, Troc, CH_2PhOBz), 4.40 (d, $J = 7.7$ Hz, 1H, H-1′), 3.84 (d, $J = 2.4$ Hz, 1H, H-4′), 3.89–3.73 (m, 3H, H-3, H-6′), 3.70–3.62 (m, 2H, H-6a, H-4), 3.60 (dd, $J = 9.0$, 5.1 Hz, H-5), 3.58–3.49 (m, 3H, H2, H-5′, H-6b), 3.46 (dd, $J = 8.9$, 5.2 Hz, 1H, H-3′), 2.87–2.21 (m, 4H, OCOCH_2CH_2, Lev), 2.10 ppm (s, 3H, CH_2COCH_3, Lev); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 206.4 (CH_2 *C*OCH$_3$, Lev), 171.6 (O*C*OCH$_2$CH$_2$, Lev), 165.3 (Ar*C*O), 154.4 (Ar-C), 150.7 (NH*C*O), 138.7–121.9 (35C, Ar-C), 101.7 (C-1′), 100.0 (C-1), 95.8 (CCl_3), 81.2 (C-4), 80.4 (C-3′), 74.8, 74.7, 73.9, 73.8, 72.5, 72.4 (6C, 4×OCH_2Ph, OCH_2CCl$_3$, OCH_2PhOBz), 74.5 (C-5), 74.0 (C-5′), 72.6 (C-3), 72.3 (C-4′), 71.7 (C-2′), 70.3, 68.5 (2C, C-6, C-6′), 57.9 (C-2), 37.9 (OCO*C*H$_2$CH$_2$, Lev), 30.1 (CH$_2$CO*C*H$_3$, Lev), 28.1 ppm (OCOCH$_2$*C*H$_2$, Lev); HR-MALDI-TOF: m/z: calcd for $C_{59}H_{67}Cl_3N_2O_{16}$: 1183.3291; found: 1206.3286 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy) carbonyl]amino]-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (25)**: Methanolic hydrazine acetate (12 mL, 0.5 M) was added to a solution of compound **24** (200 mg, 0.17 mmol) in dichloromethane (10 mL). After stirring at ambient temperature for 2 h, the reaction was quenched by adding acetonylacetone (0.8 mL), and diluted by dichloromethane (40 mL). The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification of the crude product by column chromatography (silica gel, hexane/EtOAc 2:1) gave diol **25** as a white powder (160 mg, 87%). $[\alpha]_D = -26.5$ (c = 1.0, CH_2Cl_2); $R_f = 0.30$ (hexane/EtOAc 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.22$ (d, $J = 7.8$ Hz, 2H, Ar-H), 7.67 (d, $J = 1.5$ Hz, Ar-H), 7.64 (d, $J =$ 1.5 Hz, Ar-H), 7.54–7.17 (m, 25H, Ar-H), 5.28 (d, $J = 8.5$ Hz, 1H, N-H), 4.89 (dd, $J = 9.8$, 3.1 Hz, 2H, ArCH_2), 4.85 (d, $J = 8.2$ Hz, 1H, H-1), 4.82–4.43 (m, 10H, 3×ArCH_2, Troc, CH_2PhOBz), 4.42 (d, $J = 7.7$ Hz, 1H, H-1′), 3.94 (t, $J = 2.4$ Hz, 1H, H-4′), 3.86–3.80 (m, 3H, H-3, H-6′), 3.82 (dd, $J = 10.2$, 8.6 Hz, 1H, H-2′), 3.70–3.62 (m, 4H, H-6a, H-2, H-4), 3.60 (dd, $J = 8.6$, 5.1 Hz, 1H, H-5′), 3.58–3.49 (m, 2H, H-5, H-6b), 3.39 ppm (dd, $J = 9.9$, 2.2 Hz, 1H, H-3′); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 167.3$ (Ar*C*O), 156.7 (Ar-C), 153.2 (NH*C*O), 138.2–121.8 (35C, Ar-C), 104.7 (C-1′), 100.0 (C-1), 96.8 (CCl_3), 82.2 (C-4), 82.0 (C-3′), 75.2, 74.9, 73.8, 73.6, 72.4, 72.0 (6C, 4×OCH_2Ar, OCH_2CCl$_3$, OCH_2PhOBz), 74.3 (C-5), 73.9 (C-5′), 72.8 (C-3), 71.9 (C-4′), 71.4 (C-2′), 70.3, 68.7 (2C, C-6, C-6′), 57.9 ppm (C-2); HR-MALDI-TOF: m/z: calcd for $C_{59}H_{67}Cl_3N_2O_{16}$: 1085.2923; found: 1108.2845 $[M+Na]^+$.

***p*-(Benzoyl)-benzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy) carbonyl]amino]-3-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranoside (26)**: A solution of diol **25** (120 mg, 0.11 mmol) and thiofucoside **6** (126 mg, 0.33 mmol) in dry dichloromethane (2 mL) was stirred under argon with 3 Å molecular sieves for 30 min, the temperature was cooled to 0°C and NIS (81.7 mg, 0.36 mmol) and TESOTf (7.5 μL, 0.03 mmol) were added. After stirring for 30 min the solution was diluted by dichloromethane (60 mL) and the molecular sieves were removed by filtration. The filtrate was washed with 15% aqueous sodium thiosulfate and brine, dried over $MgSO_4$, filtered and concentrated. Purification of the crude compound by column chromatography (silica gel, hexane/EtOAc 2:1) yielded tetrasaccharide **26** as a white powder (164 mg, 86%). $[\alpha]_D = -82.7$ (c = 1.0, CH_2Cl_2); $R_f = 0.48$ (hexane/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 8.20$ (d, $J = 7.7$ Hz, 1H, Ar-H), 7.53–6.93 (m, Ar-H, 37H, 6×OCH_2Ph, OCH_2PhOBz), 5.65 (d, $J = 3.0$ Hz, 1H, H-1‴), 5.49 (dd, $J = 10.6$, 2.7 Hz, 1H, H-3″), 5.43 (dd, $J = 10.8$, 2.7 Hz, 1H, H-3‴), 5.42–5.20 (m, 3H, H-4″, H-4‴, N*H*), 5.18 (d, $J = 3.9$ Hz, 1H, H-1″), 5.07 (q, $J = 6.0$ Hz, 1H, H-5″), 4.76 (d, $J = 9.0$ Hz, 1H, H-1), 4.80–4.15 (m, 20H, 6×OCH_2Ph, OCH_2PhOH, OCH_2CCl$_3$, H-5‴, H-1′, H-4, H-3), 4.05 (m, 2H, H-6), 3.96 (t, $J = 8.2$ Hz, 1H, H-2′), 3.95 (d, $J = 3.1$ Hz, 1H, H-4′), 3.88–3.84 (m, 2H, H-6), 3.84 (dd, $J = 10.8$, 3.0 Hz, 1H, H-2‴), 3.76 (dd, $J = 10.6$, 3.9 Hz, 1H, H-2″), 3.38 (dd, $J = 8.2$, 3.1 Hz, 1H, H-3′), 3.25–3.26 (m, 2H, H-5′, H-5), 3.09 (dd, 1H, H-2), 2.20 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 1.17 (d, $J = 6.3$ Hz, 3H, H-6‴), 0.92 ppm (d, $J = 6.0$ Hz, 3H, H-6″); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.8$, 170.5, 170.4, 169.7 (4C, 4×CH$_3$*C*O), 165.3 (1C, Ar-C), 153.6 (NH*C*O), 150.7 (Ph*C*O), 138.8–121.8 (47C, Ar-C), 99.7 (C-1′), 98.8 (C-1″), 98.3 (C-1‴), 97.6 (C-1), 95.6 (CCl_3), 83.8 (C-3′), 75.4 (C-5), 75.2 (C-3), 74.7, 73.9, 73.7, 73.6, 73.3, 73.1 73.0, 72.9 (8C, 6×OCH_2Ph, OCH_2PhOBz, OCH_2CCl$_3$), 73.4 (C-2‴), 73.4 (C-2′), 72.9 (C-5′), 72.6 (C-4), 72.2 (C-2″), 72.0 (2C, 4″, C-4‴), 71.4 (C-4′), 70.9 (C-3‴), 70.7 (C-3″), 68.1 (2C, C-6, C-6′), 64.9 (C-5″), 64.8 (C-5‴), 59.7 (C-2), 21.2, 21.1, 20.9, 20.8 (4C, 4×*C*H$_3$CO), 15.7 (C-6‴), 15.6 ppm (C-6″); HR-MALDI-TOF MS: m/z: calcd for $C_{91}H_{98}Cl_3NO_{26}$: 1725.5443; found: 1748.6041 $[M+Na]^+$.

***p*-Hydroxybenzyl 6-*O*-benzyl-2-deoxy-2[[(2,2,2-trichloroethoxy)carbonyl]amino]-3-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-4-*O*-(3,4,6-tri-*O*-benzyl-2-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranosyl)-β-D-glucopyranoside (27)**: Triethylamine (400 μL) and H_2O_2 (50% in water, 200 μL) were added to a solution of compound **26** (160 mg, 0.093 mmol) in THF (8 mL), and the mixture was stirred under argon atmosphere for 18 h where after it was concentrated in vacuo. Purification of the residue by column chromatography (silica gel, hexane/EtOAc 2:1) furnished **27** as a white powder (123 mg, 82%). $[\alpha]_D = -83.9$ (c = 1.0, CH_2Cl_2); $R_f = 0.31$ (hexane/EtOAc 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.37$–6.78 (m, Ar-H, 34H, 6×OCH$_2$Ph, OCH$_2$PhOH), 5.68 (d, $J = 2.9$ Hz, 1H, H-1‴), 5.29 (dd, $J = 10.8$, 2.9 Hz, 1H, H-3″), 5.23 (dd, $J = 11.0$, 2.9 Hz, 1H, H-3‴), 5.22–5.20 (m, 3H, H-4″, H-4‴, N*H*), 5.12 (d, $J = 3.9$ Hz, 1H, H-1″), 5.03 (q, $J = 6.3$ Hz, 1H, H-5″), 4.76 (d, $J = 8.2$ Hz, 1H, H-1), 4.76–4.12 (m, 20H, 6×OCH_2Ph, OCH_2PhOH, OCH_2CCl$_3$, H-5‴, H-1′, H-4, H-3), 4.05 (dd, $J = 10.1$, 9.3 Hz, 1H, H-6a), 3.96 (dd, $J =$ 10.5, 8.2 Hz, 1H, H-2′), 3.90 (d, $J = 2.8$ Hz, 1H, H-4′), 3.88–3.84 (m, 3H, H-6′, H-6b), 3.84 (dd, $J = 11.0$, 2.9 Hz, 1H, H-2‴), 3.76 (dd, $J = 10.8$, 3.9 Hz, 1H, H-2″), 3.38 (dd, $J = 10.5$, 2.8 Hz, 1H, H-3′), 3.25 (dd, $J = 8.6$, 2.8 Hz, 1H, H-5′), 3.26 (d, $J = 9.3$ Hz, 1H, H-5), 3.09 (ddd, 1H, H-2), 2.10 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.00 (s, 6H, 2×CH_3CO), 1.14 (d, $J = 6.7$ Hz, 3H, H-6‴), 0.92 ppm (d, $J = 6.3$ Hz, 3H, H-6″); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 171.4$, 170.7, 170.6, 169.6 (4C, 4×CH$_3$*C*O), 156.2 (1C, Ar-C), 153.5 (NH*C*O), 138.6–115.6 (41C, Ar-C), 99.6 (C-1′), 98.1 (C-1″), 97.4 (C-1‴), 97.3 (C-1), 95.5 (CCl_3), 83.7 (C-3′), 75.5 (C-5), 75.3 (C-3), 74.7, 73.9, 73.7, 73.6, 73.3, 73.1 72.9, 73.6, 72.7, 72.4, 71.8, 71.1, 70.9, 70.7 (16 C, 6×OCH_2Ph, OCH_2PhOH, OCH_2CCl$_3$, C-2‴, C-2′, C-5′, C-4, C-2″, 4″, C-4‴, C-4′), 70.8 (C-3‴), 70.1 (C-3″), 68.0 (2C, C-6, C-6′), 64.7 (C-5″), 64.6 (C-5‴), 59.7 (C-2), 21.6, 21.2, 21.1, 20.9 (4C, 4×*C*H$_3$CO), 15.6 (C-6‴), 15.5 ppm (C-6″); HR-MALDI-TOF MS: m/z: calcd for $C_{84}H_{94}Cl_3NO_{25}$: 1621.5181; found: 1644.5204 $[M+Na]^+$.

Trichloroacetimidate (29): DDQ (40 mg, 0.136 mmol) was added to a stirred mixture of compound **27** (110 mg, 0.068 mmol) in dichloromethane (3.8 mL) and water (0.2 mL). The mixture was stirred in the dark for 1 h, diluted by dichloromethane, washed with brine, dried over $MgSO_4$, filtered and concentrated. Purification of the residue by column chromatography (silica gel, hexane/EtOAc 2:1) gave hemiacetal **26** as a colorless syrup (80 mg, 78.0%). Compound **26** (80 mg, 0.053 mmol) was dissolved in dry dichloromethane (5 mL) and CCl_3CN (0.5 mL) and DBU (5 μL) were added. After stirring under argon for 5 min at ambient temperature, the solution was concentrated to dryness. Purification by column chromatography (silica gel, hexane/EtOAc/triethylamine 1:1:0.01) yielded imidate **29** as a colorless syrup (80 mg, 91%). MALDI-TOF MS: m/z: calcd for $C_{79}H_{88}Cl_6N_2O_{24}$: 1662.29; found: 1686.10 $[M+Na]^+$.

Fully protected heptasaccharide 30: Glycosyl donor **29** (45 mg, 0.027 mmol) and glycosyl acceptor **22** (35 mg, 0.025 mmol) were dissolved in dry dichloromethane (1 mL) and activated molecular sieves were added. After stirring at room temperature for 30 min, the mixture was cooled to −30°C and TBSOTf (1.0 μL) was added. After the donor was fully converted, the reaction mixture was diluted with dichloromethane (50 mL) and the molecular sieves were removed by filtration. The solution was washed with water and sat. aqueous sodium hydrogen carbonate, dried by $MgSO_4$, filtered and concentrated to dryness under vacuum. Purification by column chromatography (silica gel, hexane/EtOAc 1:1) afforded heptasaccharide **30** as a white powder (36 mg, 62%). $[\alpha]_D = -104.3$ (c = 1.0, CH_2Cl_2); $R_f = 0.28$ (hexane/EtOAc 1:1).

Table 2. ^{1}H NMR chemical shifts for heptasaccharide **30**.

	Gal′	Fuc″	GlcNAc′	Fuc′	Gal	GlcNAc	Fuc
H-1	4.67	5.64	5.02	5.10	4.50	4.31	5.18
H-2	5.36	3.76	3.20	3.66	5.38	2.81	3.80
H-3	3.89	5.23	4.04	4.95	3.76	4.06	5.14
H-4	4.01	5.20	3.89	4.90	4.05	3.89	5.18
H-5	3.67	4.69	3.10	4.90	3.76	3.09	4.75
H-6	4.68	1.02	3.50	0.81	4.62	3.52	0.98

The ^{1}H NMR (800 MHz, $CDCl_3$) spectral data for compound **30** are listed as following in Table 2.

HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{148}H_{165}Cl_6N_3O_{44}$: 2897.8897; found: 2920.9002 $[M+Na]^+$.

Heptasaccharide 31: Zinc (10 mg, 0.15 mmol, nano-size powder) was added to a stirred solution of heptasaccharide **30** (20 mg, 0.02 mmol) in acetic acid (2 mL). After 20 min, the zinc dust was removed by filtration through Celite and the filtrate was concentrated to dryness. The residue was dissolved in pyridine (2 mL) and acetic anhydride (1 mL) and the mixture was stirred at room temperature over night. After quenching by addition of methanol (2 mL), the mixture was diluted by dichloromethane (60 mL) and was washed successively with 1 M HCl solution, sat. aqueous sodium hydrogen bicarbonate, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was hydrogenolyzed over $Pd(AcO)_2$ (20 mg) in a solution of ethanol and acetic acid (5:1, 3 mL). After 24 h the mixture was filtered through Celite to remove the catalyst and concentrated to dryness under reduced pressure. The obtained residue was dissolved by methanol (5 mL) and sodium methoxide (1 M in methanol) was added until pH 10. The solution was stirred at room temperature for 24 h, neutralized with Dowex 50 H^+ resin, diluted by methanol (50 mL), filtered and concentrated. The residue was purified by size exclusion column chromatography (Biogel P2 column, eluted with H_2O containing 1% *n*BuOH) to give the product **31** as a white powder (4 mg, 52%). $[\alpha]_D = -99.4$ (c = 1.0, MeOH); ^{1}H NMR (800 MHz, D_2O, selected data): $\delta = 5.42$ (d, $J = 3.5$ Hz, 1H), 5.05 (d, $J = 4.0$ Hz, 1H), 5.04 (d, $J = 3.7$ Hz, 1H), 4.83 (q, $J = 6.5$ Hz, 1H), 4.67 (q, $J = 6.5$ Hz, 1H), 4.64 (d, $J = 7.6$ Hz, 1H), 4.45 (d, $J = 8.0$ Hz, 2H), 4.41 (d, $J = 7.8$ Hz, 1H), 4.22 (q, $J = 6.5$ Hz, 1H), 2.01 (s, 6H, $2\times NHCOCH_3$), 1.21, 1.22, 1.24 ppm (3d, $J = 6.5$ Hz, 9H, Fuc); ^{13}C NMR (D_2O, 200 MHz, selected data): $\delta = 105.1$, 104.2, 103.4, 103.0, 102.5, 101.0 ppm (7C, anomeric C); HR-MALDI-TOF MS: *m*/*z*: calcd for $C_{49}H_{85}N_3O_{33}$: 1243.5065; found: 1266.5523 $[M+Na]^+$.

Conjugation of Le^y–Le^x to KLH: This was accomplished as described earlier. In short, compound **31** (4 mg) was slurried in dry DMF and SAMA-OPfp (2 equiv) and triethylamine (2 equiv) were added. After stirring for 2 h, the mixture was evaporated and the residue was purified using a Biogel P-2 column, eluted with H_2O containing 1% *n*-butanol to give, after lyophilization, thioacetate **32** as a white powder. MALDI-TOF MS *m*/*z*: calcd for $C_{49}H_{85}N_3O_{33}$: 1243.5065; found: 1266.5523 $[M+Na]^+$.

De-S-acetylation just prior to conjugation was achieved by stirring a mixture of the thioacetate **32** (1.5 mg), H_2O (20 μL), and 7% NH_3 in DMF (75 μL) under argon atmosphere for 45 min. The mixture was evaporated and co-evaporated twice with toluene. The liberated thiol was dried under high vacuum for 30 min and then used immediately for conjugation without further purification.

A solution of SBAP (2.5 mg) in DMF (80 μL) was added to a solution of KLH (5.75 mg) in 0.1 M sodium phosphate buffer pH 7.2 containing 0.15 M sodium chloride (500 μL). The mixture was incubated at room temperature for 2 h and then purified by using centrifugal filters with a molecular cut-off of 10 KDa. All centrifugations were performed at 15 °C for 20 min spinning at 13 *g*. The reaction mixture was centrifuged off and the residue was washed with conjugation buffer (2×200 μL). The activated protein was retrieved by spinning at 13 g for 20 min at 15 °C and taken up in 0.1 mM sodium phosphate buffer pH 8.0 containing 0.1 mM EDTA (600 μL). A solution of the thiolated Le^{y}Le^x dimer in the conjugation buffer (200 μL) was added to the activated protein and the mixture was incubated at room temperature over night. Purification was achieved as described above using centrifugal filters. The glycoconjugate was taken up in 10 mM Hepes buffer pH 6.5 (1 mL). This gave a glycoconjugate with 1190 copies of Le^{y}Le^x per KLH molecule as determined by phenol-sulfuric acid total carbohydrate assay and Lowry's protein concentration test.

Immunizations: Groups of five mice (female BALB/c, 8 weeks) were immunized subcutaneously on days 0, 7, 14 and 21 with carbohydrate(24 μg) and the adjuvant QS-21 (10 μg) in each boost. The mice were bled on day 28 (leg-vein) and the sera were tested for the presence of antibodies.

ELISA: 96-well plates were coated over night at 4 °C with Le^{y}Le^x–BSA, Le^y–BSA, or Le^x–BSA (2.5 μg mL^{-1}) in 0.2 M borate buffer (pH 8.5) containing 75 mM sodium chloride (100 μL) per well). The plates were washed three times with 0.01 M Tris buffer containing 0.5% Tween 20% and 0.02% sodium azide. Blocking was achieved by incubating the plates 1 h at room temperature with 1% BSA in 0.01 M phosphate buffer containing 0.14 M sodium chloride. Next, the plates were washed and then incubated for 2 h at room temperature with serum dilutions in phosphate buffered saline. Excess antibody was removed and the plates were washed three times. The plates were incubated with rabbit anti-mouse IgG Fcγ fragment specific alkaline phosphatase conjugated antibodies (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for 2 h at room temperature. Then, after the plates were washed, enzyme substrate (*p*-nitrophenyl phosphate) was added and allowed to react for 30 min before the enzymatic reaction was quenched by addition of 3 M aqueous sodium hydroxide and the absorbance read at dual wavelengths of 405 and 490 nm. Antibody titers were determined by regression analysis, with $\log_{10}$ dilution plotted against absorbance. The titers were calculated to be the highest dilution that gave three times the absorbance of normal mouse sera diluted 1:120.

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