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# A Bioactive Synthetic Outer-Core Oligosaccharide Derived from a *Klebsiella pneumonia* Lipopolysaccharide for Bacteria Recognition

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**Abstract:** There is an urgent need for new treatment options for carbapenem-resistant *Klebsiella pneumoniae* (*K. pneumoniae*), which is a common cause of life-threatening hospital- and community-acquired infections. Prophylactic or therapeutic vaccination may offer an approach to control these infections, however, none has yet been approved for human use. Here, we report the chemical synthesis of an outer core tetra- and pentasaccharide derived from the lipopolysaccharide of *K. pneumoniae*. The oligosaccharides were equipped with an aminopentyl linker, which facilitated conjugation to the carrier proteins $CRM_{197}$ and BSA. Mice immunized with the glycoconjugate vaccine candidates elicited antibodies that recognized isolated LPS as well as various strains of *K. pneumoniae*. The successful preparation of the oligosaccharides relied on the selection of monosaccharide building blocks equipped with orthogonal hydroxyl and amino protecting groups. It allowed the differentiation of three types of amines of the target compounds and the installation of a crowded 4,5-branched Kdo moiety.

## Introduction

*Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram-negative encapsulated bacterium that is a common cause of hospital- and community-acquired infections.[1] Although found in normal human intestinal and oropharynx microbiota, it can colonize the mucosa and then invade other tissues causing among others pneumonia, urinary and soft tissue infections, bacteraemia and sepsis. The young and patients with an impaired immune system are most venerable to these infections. Furthermore, infections caused by carbapenem-resistant *K. pneumoniae* are particularly problematic due to limited therapeutic options leading to high mortality rates.[2] Vaccination may offer an attractive approach to control *K. pneumoniae* infections.

Various vaccine strategies for *K. pneumoniae* have been explored including whole cell vaccines, outer membrane vesicles, protein-based formulations, and capsular polysaccharide (CPS) and lipopolysaccharide (LPS).[3] LPS and CPS are virulence factors of *K. pneumoniae*, highly immunogenic and surface exposed making these biomolecules interesting vaccine candidates.[4] Clinically approved CPS-based vaccines have been developed for various pathogenic bacteria, including *Streptococcus pneumoniae*, *Haemophilus influenzae* type b and *Neisseria meningitidis*,[3] providing a further impetus to focus on this class of compounds. To date, 77 capsular serotypes (referred to as K polysaccharides) have been identified for *K. pneumoniae*, which have different geographic distributions making it challenging to develop a broad acting vaccine.[5] LPS is structurally less diverse and 9 different serotypes have been described.[6] Several studies have shown that LPS isolated from various *K. pneumonia* serotypes can induce protective immune responses,[7] however, its high toxicity and T-cell independent properties makes it a challenging vaccine target.

LPS is composed of lipid A, a core oligosaccharide and an O-antigen. The most common O-antigens observed in clinical isolates of *K. pneumoniae* are composed of galactans or mannans repeating units, and classified into four serogroups

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(O1, O2, O3 and O5).[8] O-antigens have been examined as vaccine candidate by for example chemical removal of the lipid A moiety followed by conjugation of the resulting polysaccharides to various carrier proteins.[9] Such conjugates could elicit O-antigen specific antibodies that reduced bacterial burden and protected against fatal intravenous infection.

The core oligosaccharide exhibits even less structural diversity and only two types have been identified (type 1 and 2).[10] Both core-types share the same inner core, which structurally is very similar to that of other *Enterobacteriaceae* (Figure 1). Outer core type 1 is composed of α-GlcN-(1,4)-α-GalA that at the C-6 position is further extended by 3-deoxy-D-manno-octulosonic acid (Kdo) residue. The latter monosaccharide is non-stoichiometrically modified by an α1,4-linked L,D-Hep*p* moiety. Furthermore, a β-GlcNAc residue is present at the C-5 position serving as the anchoring point for the O-antigen. Outer core type 2 has also α-GlcN-(1,4)-α-GalA moiety, however, in this case, the GlcN residue is substituted at the C-4 position by β-Glc(1,6)-α-Glc. Outer core type 1 is observed in most clinical isolates.[10b]

Release of O-antigen from lipid A of *K. pneumoniae* for conjugate vaccine development[9] is performed by treatment with nitrous acid,[6b,11] which results in cleavage of the glycosidic linkage of the α-GlcN residue of the core oligosaccharide, and thus such preparations cannot examine antigenicity of the core region and its potential to be used as a vaccine candidate. Furthermore, there are indications that the outer core of *Enterobacteriaceae* is more accessible for immunological attack than the inner core.[10b] Therefore, we were compelled to investigate antigenic properties of this moiety for vaccine development. To accomplish this goal, we report here the chemical synthesis of pentasaccharide **1** and tetrasaccharide **2** that are derived from the outer core of LPS of *K. pneumoniae*. These compounds are equipped with an aminopentyl linker, which facilitated conjugation to the carrier proteins $CRM_{197}$ and BSA. Mice immunized with the glycoconjugate vaccine candidates elicited IgG antibodies that could recognize LPS isolated from *K. pneumoniae*. FACS experiments using various *K. pneumoniae* strains showed that the antisera can bind whole bacteria providing support for the development of an outer-core based vaccine.

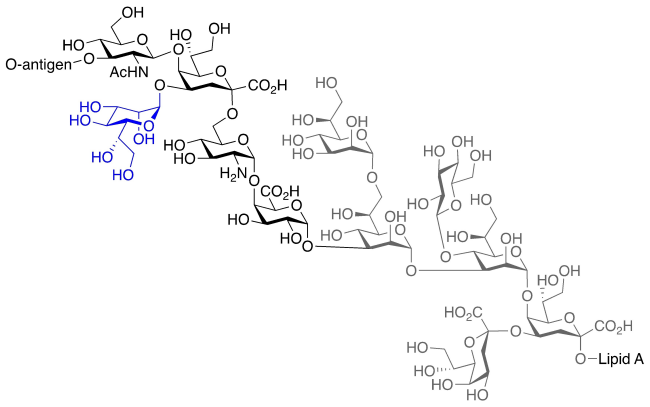



**Figure 1.** Chemical structure of most common LPS core (type 1) of *K. pneumoniae*. L-α-D-heptose (in blue) is expressed nonstoichiometrically on outer core (in black); inner core (in gray).

## Results and Discussion

### Chemical synthesis of outer core oligosaccharides

The preparation of outer core oligosaccharide **1** and **2** of *K. pneumoniae* was challenging due to the need to install glycosidic linkages at a crowded 4,5-branched Kdo.[12] Furthermore, the formation of glycosidic linkages, including α-linked Kdo, α-linked glucosamine and α-galactoside, that usually give mixtures of anomeric products.[13] Furthermore, the target compounds have three types of amino substitutions that required the selection of amino masking or protecting groups that individually could be modulated, and allowed the introduction of glycosidic linkages with appropriate anomeric configuration. Additionally, the free amine together with the two carboxylic acids of the target oligosaccharides made conjugation to carrier proteins challenging.[14]

To assemble the outer core oligosaccharide derived from LPS of *K. pneumoniae*, a strategy was designed employing monosaccharide building blocks **3**, **4**, **5**, **6** and **7** (Figure 2). The C-2 azido moiety of **3**, which does not perform neighboring group participation during glycosylations, allowed the installation of an α-glucosidic linkage, which can then be reduced to an amine and temporarily protected as a trifluoroacetamide. At the very end of the synthetic route, saponification will remove the trifluoroacetyl (TFA) protecting group to provide the required glucosamine moiety. Galactosyl acceptor **4** is equipped with an α-linked aminopentyl moiety that is doubly protected by benzyl and benzyloxycarbonyl (Cbz), which at a late stage of the synthesis can be removed by hydrogenation to give an amine that was modified by a thioacetyl moiety for conjugation to a carrier protein.[15] The 2-methylnaphthyl (Nap) ether of **4** can at an appropriate stage be removed to give an alcohol that can be oxidized to a carboxylic acid. Kdo was prepared from D-mannose by a reported procedure,[16] and subsequently subjected to protecting group manipulation to provide **5** (see Supporting Information for details). The latter compound is

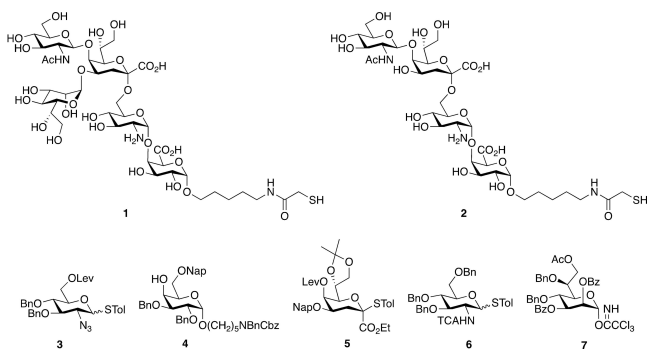
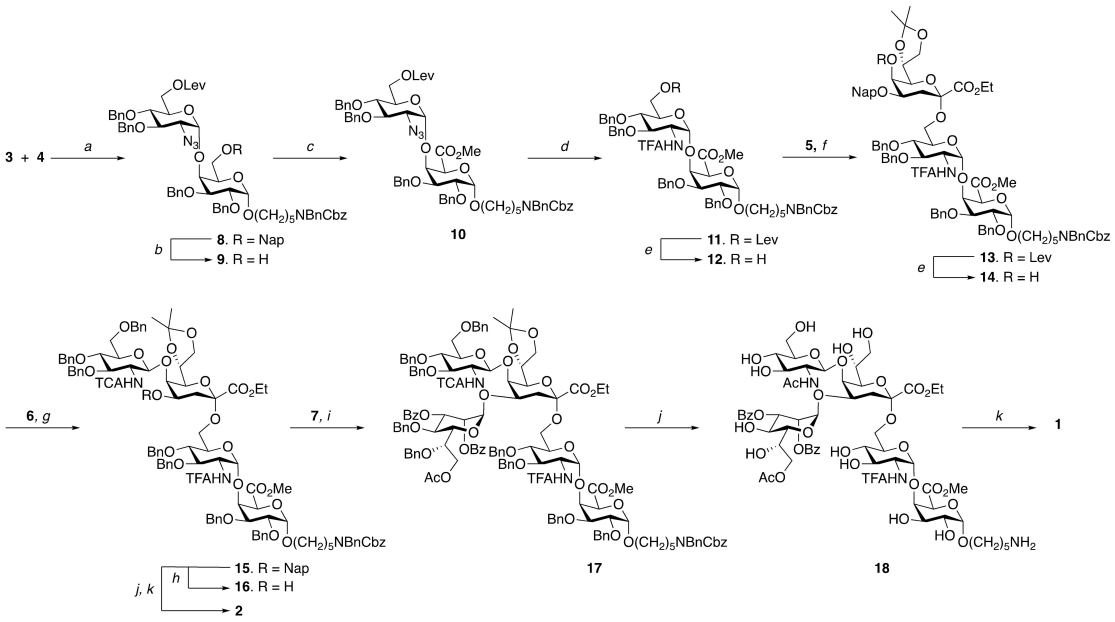



**Figure 2.** Target pentasaccharide **1** and tetrasaccharide **2**, and the monosaccharide building blocks for their assembly.

modified by the orthogonal protecting groups, Nap ether at C-4 and levulinoyl (Lev) ester at C-5, which can be removed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or treatment with hydrazine acetate, respectively to give appropriate glycosyl acceptors. Furthermore, the ester at C-5 of the Kdo donor **5** was expected to improve the α-anomeric selectivity of the glycosylation.[17] The resulting hydroxyls at C-4 and C-5 of Kdo can then be glycosylated with glycosyl donors **7** and **6**, respectively to form the 4,5-branched Kdo. A small number of studies have dealt with the installation of glycosidic bonds at the C-4 as well as the C-5 hydroxyl of Kdo.[13b,18] It was observed that the type of donor and the protecting groups of the donor and acceptor greatly influence the outcome of the glycosylations, and except for one study,[18c] the C-4 hydroxyl was first glycosylated followed by the C-5 hydroxyl. We chose for a strategy to first glycosylate the C-5 hydroxyl of the Kdo moiety of **13** because it would provide access to pentasaccharide **1** as well as tetrasaccharide **2** (Scheme 1).

The C-2 amine of glycosyl donor **6** is modified as trichloroacetamide because during glycosylation it will control the anomeric selectivity by neighboring group participation to provide a 1,2-*trans*-glycoside without causing oxazoline formation. The C-3, C-4 and C-6 hydroxyls of **6** are protected as benzyl ethers which at the end of the synthesis can easily be removed by hydrogenation with concomitant reduction of the trichloroacetyl (TCA) moiety to give the required *N*-acetyl-glucosamine residue. Finally, the heptosyl donor **7** contains benzoyl esters at C-2 which will perform neighboring group participation during glycosylation to provide the required α-anomer.

The assembly of the pentasaccharide **1** started with a glycosylation of 2-azidoglucosyl donor **3** with galactosyl acceptor **4** in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[19] to give disaccharide **8** as only the α-anomer ($\delta_{Glc\,H\text{-}1}$ 4.99, $J_{Glc\,H1\text{-}H2} = 3.7$ Hz) in a yield of 55%. The Nap ether of **8** could easily be removed by treatment with DDQ in wet DCM[20] to afford alcohol **9**, which was oxidized to a carboxylic acid using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)benzene (BAIB).[21] Subsequent treatment with (diazomethyl)trimethylsilane ($TMSCHN_2$) in THF gave methyl ester **10**. The azido moiety of the latter compound was reduced with zinc in a mixture of acetic acid and THF to give an amine that was protected as trifluoroacetamide by reaction with trifluoroacetic anhydride providing **11**. Disaccharide acceptor **12** was obtained by treatment of **11** with hydrazine acetate in DCM and MeOH resulting in clean removal of the Lev ester. Next, coupling of the Kdo donor **5** with **12** using NIS and trifluoromethanesulfonic acid (TfOH)[17b] as the promoter system and acetonitrile as the solvent resulted in the formation of trisaccharide **13** in yield of 71% as mainly the α-anomer (α:β = 4:1; configuration conformed by coupling constant between C-1 and H-$3_{ax}$ of Kdo,[13b,18b,22] $J_{\alpha Kdo\,C1\text{-}H3ax} < 1$ Hz) that was readily separable from the unwanted β-anomer. We chose for a strategy to first glycosylate the axial C-5 hydroxyl of the Kdo moiety of **13** because it has a lower glycosyl accepting reactivity compared to the equatorial hydroxyl at C-4. Furthermore, this strategy provided access to pentasaccharide **1** as well as tetrasaccharide **2**.




**Scheme 1.** Synthesis of pentasaccharide **1** and tetrasaccharide **2**. Conditions and reagents: *a*) NIS, TMSOTf, DCM, 0 °C, 4 h: 55%. *b*) DDQ, DCM/$H_2O$, rt, 3 h: 76%. *c*) i. TEMPO, BAIB, AcOH, DCM/$H_2O$, rt, 6 h, then ii. $TMSCHN_2$, THF, 0 °C, 15 min: 80%. *d*) i. Zn, AcOH/THF, rt, 16 h, then ii. $(CF_3CO)_2O$, THF, 0 °C, 1 h: 79%. *e*) $NH_2NH_2 \cdot HOAc$, DCM/MeOH, rt, 2 h: **12**, 83%; **14** was used immediately in the next synthetic step. *f*) TfOH, NIS, MeCN, 0 °C, 2 h: α/β = 4/1, 71% of α-anomer. *g*) TfOH, NIS, DCM/MeCN, 0 °C, 6 h: 53% over two steps starting from **13**. *h*) DDQ, β-pinene, DCM/$H_2O$, rt, 2 h: 72%. *i*) TMSOTf, DCM, 0 °C, 6 h: 70%. *j*) i. TFA/DCM/$H_2O$, 0 °C, 30 min, then ii. $Pd(OH)_2$/C, $H_2$, *t*-BuOH/$H_2O$, rt, 24 h. *k*) i. SAMA-OPfp, DIPEA, DMF, rt, 3 h, then ii. 0.2 M NaOH/MeOH = 1/1, 0 °C, 3 h: **1**, 42% over four steps; **2**, 30% over four steps.

Thus, treatment of **13** with hydrazine acetate resulted in removal of the Lev ester to provide trisaccharide acceptor **14** which was immediately used in a glycosylation with thioglycosyl donor **6** using TfOH/NIS as the promotor system[23] in a mixture of DCM and MeCN at 0 °C to afford tetrasaccharide **15** in yield of 53% over two steps. Only the β-anomer was obtained due to neighboring group participation of the TCA protecting group. Next, the Nap ether of **15** was oxidatively cleaved with DDQ using β-pinene as the proton scavenger[24] to give tetrasaccharide **16** in 72% yield (configuration confirmed by $^1$H NMR, $\delta_{\beta GlcH1} = 5.04$, d, $J_{\beta GlcH1\text{-}H2} = 7.8$ Hz). The glycosylation of acceptor **16** with heptosyl donor **7** was mediated by a catalytic amount of TMSOTf in DCM at 0 °C resulting in the formation of pentasaccharide **17** in a yield of 70%, and the anomeric configuration of this compound was confirmed using coupling constants between C-1 and H-1 ($J_{\alpha HepC1\text{-}H1} = 174$ Hz, $J_{\alpha GalAC1\text{-}H1} = 176$ Hz, $J_{\alpha GlcNHTFAC1\text{-}H1} = 177$ Hz, $J_{\beta GlcNHTCAC1\text{-}H1} = 162$ Hz).

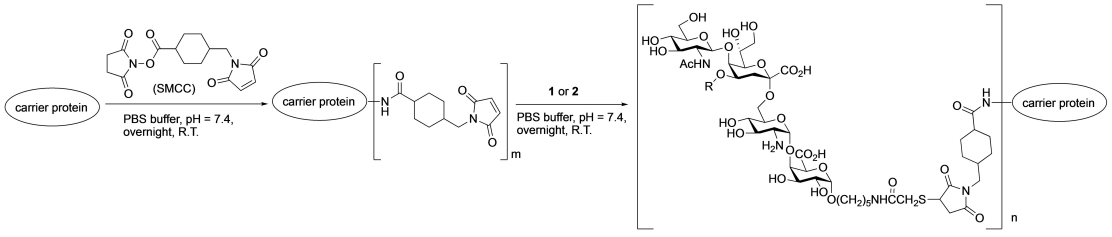
Deprotection of pentasaccharide **17** started with cleavage of the isopropylidene acetal using TFA in wet DCM which was followed by hydrogenation using $Pd(OH)_2/C$ in a mixture of *t*-BuOH/$H_2O$ to remove the benzyl ethers and the protecting groups of the amino pentyl linker. Under these conditions, the trichloroacetamide was reduced to *N*-acetyl moiety to give, after filtration through a pad of celite, pentasaccharide **18** that was used immediately in next step. The aminopentyl linker of **18** was activated with perfluorophenyl 2-(acetylthio)acetate (SAMA-OPfp) in the presence of *N,N*-diisopropylethylamine (DIPEA) in dry DMF which was followed by saponification of the benzoyl, acetyl, methyl, ethyl esters and trifluoroacetamide with aqueous NaOH in MeOH to give, after purification by Bio-Gel P2 size exclusion column chromatography, the target compound **1** in a yield of 42% over four steps. Installation of a thiol containing linker followed by saponification of esters and amides made it possible to prepare a compound in which the amine of the linker and glucosamine could be differentiated. Tetrasaccharide **2** was obtained by deprotection of compound **15** under aforementioned conditions in a yield of 30% over four steps.

## Conjugation of outer core oligosaccharides to carrier proteins

$CRM_{197}$ is widely employed as a carrier protein for conjugate vaccine development because of its favorable immunogenicity, safety and tolerability.[25] It is derived from *C. diphtheriae* toxin having a single amino acid mutation (glycine 52→glutamic acid) which greatly reduces its toxicity. BSA is a protein that has also been used as carrier for glycoconjugate vaccine development. Thus, BSA and $CRM_{197}$ were activated by treatment with succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) in PBS buffer (pH 7.4) to give, after purification by ultrafiltration, maleimide-modified proteins. The thiol-containing pentasaccharide **1** and tetrasaccharide **2** were reacted with the maleimide-modified carrier proteins in PBS buffer (pH 7.4) to give the responding BSA and $CRM_{197}$ conjugates BSA-**1**, CRM-**1**, BSA-**2** and CRM-**2** (Scheme 2). An excess of oligosaccharide (5 equiv. of glycan per modified amine on the carrier proteins) was employed to accomplish a high level of saccharide conjugation. Additionally, an excess was also important because the thiols had a propensity to dimerize by disulfide formation blocking the conjugation abilities. The glycan content of the conjugates was determined by MALDI-TOF mass spectrometry and the following average loading were determined: 4.9 for BSA-**1**; 4.5 for CRM-**1**; 7.0 for BSA-**2** and 4.0 for CRM-**2** (saccharides per protein molecule, Supporting Information).

## Mouse immunization studies

Mice were immunized with BSA-**1**, CRM-**1**, BSA-**2** and CRM-**2**. For each glycoconjugate, two wild type female C57BL/6J mice were immunized intramuscularly on day 0 with 50 μg of immunogen formulated with Complete Freund's Adjuvant. On days 18 and 32 booster doses of 50 μg were given with the same immunogen but formulated with Incomplete Freund's Adjuvant. On day 40, mice were euthanized and serum was collected. The resulting antisera was examined for the ability to bind to LPS isolated from *K. pneumoniae* strains K1, K2 and C4 (ST-258) by ELISA (Figure 3A). Thus, microtiter plates were coat with LPS and then exposed to the antisera and detection of IgG antibody binding was accomplished using mouse IgG HRP conjugate. Each glycoconjugate had elicited IgG antibodies that can recognize LPS isolated from the various of *K. pneumoniae* strains indicating that the synthetic outer core oligosaccharides had elicited anti-LPS antibody (Figure 3). IgG antibodies elicited by the pentasaccharide-containing conjugates (BSA-**1** and CRM-**1**) showed somewhat stronger binding compared to the corresponding tetrasaccharide-containing conjugates (BSA-**2** and CRM-**2**) indicating the L-α-D-heptose moiety of **1** may play a role in generating antibodies that can recognize isolated LPS.




**Scheme 2.** Preparation of glycoconjugates: BSA-**1**, CRM-**1**, BSA-**2** and CRM-**2**. BSA-**1**: m = 27, n = 4.9, R = L,D-α-Hep; CRM-**1**: m = 11, n = 4.5, R = L,D-α-Hep; BSA-**2**: m = 17, n = 7.0, R = H; CRM-**2**: m = 15, n = 4.0, R = H.

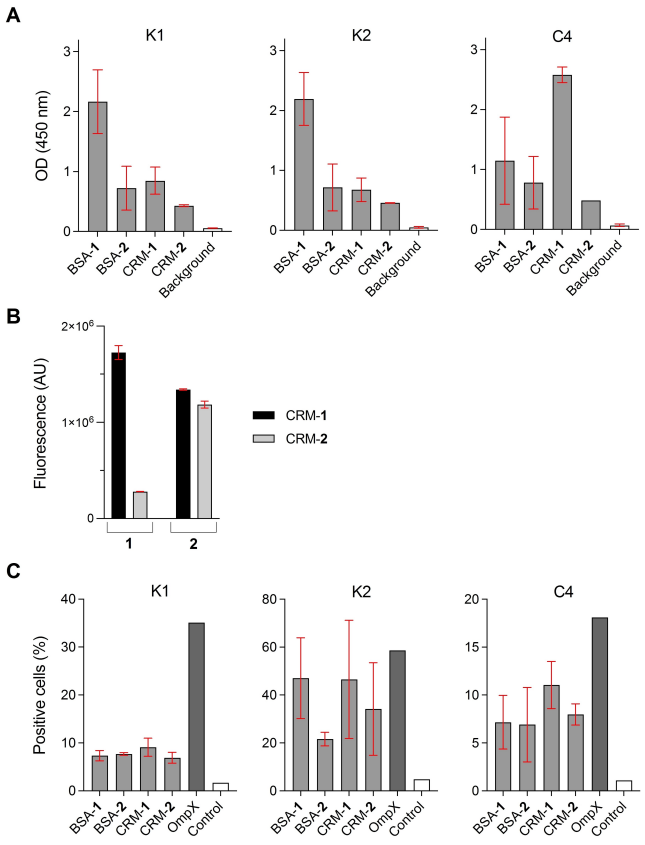



**Figure 3.** Intramuscular immunization of glycoconjugates elicits serum IgG responses. (**A**) ELISA binding of IgG serum antibodies of mice immunized with the glycoconjugates to LPS isolated from *K. pneumoniae* strains K1, K2, and C4. Background control is without serum addition (n = 4). (**B**) Microarray binding of synthetic outer core oligosaccharides **1** and **2** to IgG serum antibodies produced by glycoconjugate immunized mice. (**C**) Bacterial FACS analysis of glycoconjugate immunized mice serum with *K. pneumoniae* strains K1, K2, and C4. OmpX is positive control (polyclonal mouse anti-OmpX serum; n = 1);[27] Control is unimmunized serum (n = 1). All data bars of serum IgG responses represent the mean with range (n = 2).

A surprising observation was that antisera of mice immunized with CRM-**1** exhibited strong recognition of C4. LPS is heterogenous and antibody responses are polyclonal making it difficult to rationalize such observations.

To further examine the selectivity of the antibody responses, thio-containing compounds **1** and **2** were printed on maleimide activated glass slides, and the resulting microarray was probed for binding to the antisera. As can be seen in Figure 3B, IgG antibodies elicited by pentasaccharide **1** recognized both the penta- and tetrasaccharide whereas the antibodies raised by tetrasaccharide **2** preferentially bound to the parent compound and had reduced binding to **1**.

To explore whether the antisera can recognize intact bacteria, *K. pneumoniae* strains K1, K2 and C4 were exposed to the antisera and binding was analyzed by FACS (Figure 3C). Polyclonal mouse anti-outer membrane proteins X (OmpX) serum was included as a positive control. The various antisera recognized the three bacterial strains, and the strongest binding was observed for K2 for which it is known that its LPS is cell surface exposed.[26] Although the various antisera exhibited differences in recognition of isolated LPS (Figure 3A), these were not directly apparent in binding of intact bacteria (Fig 3B). Possibly cell surface exposure of LPS is the most important determinant for recognition of intact bacteria, however, this notion requires more detailed studies using larger numbers of mice. Collectively, the immunization studies show that synthetic outer core oligosaccharides of *K. pneumonia* can elicit IgG antibodies that recognize intact LPS expressed by clinically relevant strains. These observations warrant further examinations to establish if the semi-synthetic glycoconjugate vaccine candidates can provide protection in animal challenge studies.

## Conclusion

In summary, pentasaccharide **1** and tetrasaccharide **2** derived from the outer core of *K. pneumoniae* LPS have been synthesized using monosaccharide building blocks modified with appropriate amino- and hydroxyl protecting groups. These outer core oligosaccharides are unusual by the presence of a Kdo and L-α-D-heptoside residue which usually are only found in the inner core portion of LPS. The use of TFA, Cbz and azide as amino protecting or masking groups made it possible to install an acetamide and free amine and allowed selective conjugation of the oligosaccharides to carrier proteins. The use of Nap and Lev as orthogonal hydroxyl protecting groups made it possible to introduce a crowded 4,5-branched Kdo moiety by sequential glycosylations in appropriate order. These protecting groups were also employed for subsequent acceptor synthesis. Thiol-maleimide coupling chemistry was employed for conjugation of the oligosaccharides to BSA and $CRM_{197}$. Mouse immunization studies show the conjugates can elicit LPS-specific antibody responses. Furthermore, these antibodies could recognize clinically relevant intact bacteria providing a prospect that they may exhibit neutralizing properties. Future studies will focus on examining the semi-synthetic glycoconjugates in mouse protection studies.

## Experimental Section

**General procedures and monosaccharide building blocks**: All chemicals were purchased from Sigma-Aldrich, Fisher Scientific or Biosynth Carbosynth. BSA was purchased from Sigma-Aldrich and $CRM_{197}$ was purchased from Scarab Genomics LLC. NMR spectra, including $^{1}H$, $^{13}C$, COSY, HSQC, HMBC, EXSIDE, TOCSY and bsHSQCAD, were recorded on Agilent 400-MR or Bruker 600 MHz with chemical shifts reported in part per million (ppm) relative to $CDCl_3$ or $D_2O$. $^{1}H$ NMR data are presented in the order: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet) and coupling constants (J) are reported in Hertz (Hz). Mass spectra of proteins were recorded on a Kratos Analytical Maxima-CFR MALDI-TOF system with sinapinic acid in acetonitrile/water (0.3/0.7, v/v) as matrix, and High-Resolution Mass Spectrometry (HRMS) was recorded on an Agilent Technologies 6560 Ion mobility Q-TOF. Chromatographic purifications were performed on silica gel G60 (Silicycle 60–200 μm, 60 Å), and size exclusion chromatography was performed on Bio-Gel P-2 (45–90 μm) by using 5% *n*-butanol in water as eluent. TLC analysis was

conducted on Silica gel 60 F254 (EMD Chemicals Inc.) and detected by using UV light (254 nm), staining by 5% sulfuric acid in ethanol, *p*-anisaldehyde solution or an aqueous solution of $Ce(NH_4)_2(NO_3)_6$/$(NH_4)_6MoO_{24}\cdot 4H_2O$ in 5% sulfuric acid. 4 Å molecular sieves were flame-activated under vacuum before use. Carrier proteins were purified by ultrafiltration (Amicon Ultra-0.5 centrifugal filter unit, 10 K) before and after reactions. Monosaccharide building blocks **3**–**7** were prepared by reported procedures[16–17,28] combined with standard protecting group manipulations and details are provided in the Supporting Information.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-azido-6-O-levulinoyl-2-O-deoxy-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-2,3-di-O-benzyl-6-O- (2-naphthyl) methyl-α-D-galactopyranoside (8)**: Donor **3** (2.3 g, 2.8 mmol) and galactosyl acceptor **4** (1.1 g, 1.89 mmol) were co-evaporated with toluene (3×10 mL), and then dissolved in DCM (10 mL) and placed under an atmosphere of Argon. *N*-iodosuccinimide (NIS, 1 g, 4.72 mmol) and 4 Å molecular sieves (flame activated) were added to the solution. The resulting mixture was cooled to 0 °C and stirred at this temperature for 15 min, which was followed by the addition of trimethylsilyl trifluoromethanesulfonate (85.5 µL, 0.47 mmol). The reaction mixture was warmed slowly to room temperature and stirred for another 4 h, and then quenched with triethylamine (1 mL). After removing all solvents under reduced pressure, the resulting residue was dissolved in DCM (200 mL) and washed with brine and water. The organic layer was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give the disaccharide **8** (1.32 g, 55%, α only) as a light-yellow oil. $^1$H NMR (400 MHz, Chloroform-*d*) δ 7.88–7.76 (m, 4H, Ar), 7.53–7.44 (m, 3H, Ar), 7.43–7.11 (m, 28H, Ar), 5.20 (d, *J* = 8.4 Hz, 2H, $\underline{CH_2}$-Cbz), 4.99 (d, *J* = 3.7 Hz, 1H, Glc-H-1), 4.93–4.68 (m, 6H, Gal-H-1, Ar$\underline{CH_2}$×2, ArCH$\underline{H}$), 4.77–4.69 (m, 4H, ArC$\underline{H}$H, Ar$\underline{CH_2}$, ArCH$\underline{H}$), 4.55 (d, *J* = 10.8 Hz, 1H, ArC$\underline{H}$H), 4.49 (d, *J* = 11.1 Hz, 2H, Ar$\underline{CH_2}$), 4.39 (dd, *J* = 10.2, 2.3 Hz, 1H, Glc-H-5), 4.24 (d, *J* = 2.8 Hz, 1H, Gal-H-4), 3.99–3.85 (m, 6H, Gal-H-2, Gal-H-3, Gal-H-5, Gal-H-6a, Glc-H-3, Glc-H-6a), 3.74 (dd, *J* = 12.6, 2.3 Hz, 1H, Glc-H-6b), 3.67–3.50 (m, 3H, Gal-H-6b, Glc-H-4, CH$\underline{H}$-Linker), 3.47–3.34 (m, 1H, C$\underline{H}$H-Linker), 3.30 (dd, *J* = 10.3, 3.6 Hz, 1H, Glc-H-2), 3.27–3.13 (m, 2H, $\underline{CH_2}$-Linker), 2.80–2.64 (m, 2H, $\underline{CH_2}$-Lev), 2.58–2.47 (m, 2H, $\underline{CH_2}$-Lev), 2.15 (s, 3H, $\underline{CH_3}$-Lev), 1.59 (m, 4H, $\underline{CH_2}$-Linker×2), 1.39–1.23 (m, 2H, $\underline{CH_2}$-Linker). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 206.30, 172.27, 138.43, 138.38, 138.08, 137.99, 135.16, 133.30, 133.14, 128.62, 128.60, 128.58, 128.54, 128.51, 128.48, 128.47, 128.44, 128.36, 128.16, 128.10, 128.09, 127.97, 127.91, 127.85, 127.82, 127.80, 127.67, 127.52, 127.35, 127.23, 126.91, 126.32, 126.14, 125.91, 98.20 (Glc-C-1, $J_{C1\text{-}H1}$ = 175.4 Hz), 97.30 (Gal-C-1, $J_{C1\text{-}H1}$ = 170.4 Hz), 80.29 (Glc-C3), 77.99 (Glc-C4), 76.90 (Gal-C3), 75.66 (Gal-C2), 75.46, 75.02, 74.83 (Gal-C4), 73.69, 73.35, 72.83, 69.18 (Glc-C5), 68.94 (Gal-C5), 68.17, 67.42 (Gal-C6), 67.23, 64.10 (Glc-C2), 62.37 (Glc-C6), 50.25, 47.22, 46.22, 37.90, 29.87, 29.78, 29.16, 27.84, 23.48. ESI HRMS (m/z): [M + $Na^+$] calcd for $C_{76}H_{82}N_4NaO_{14}$, 1297.5720 found 1297.5722.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-azido-6-O-levulinoyl-2-O-deoxy-3, 4-di-O-benzyl-α-D–glucopyranosyl-(1→4)-2,3-di-O-benzyl-α-D-galactopyranoside (9)**: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 703 mg, 3.10 mmol) was added to a cooled (0 °C) two-phase system of **8** (3.3 g, 2.58 mmol) in DCM/$H_2O$ (45 mL/4.5 mL), and the reaction mixture was stirred for 3 h at room temperature under exclusion of light. After TLC analysis showed consumption of the starting material, the reaction mixture was diluted with DCM (50 mL) and was washed with sat. aq. $NaHCO_3$ (3×50 mL). The organic layer was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (PE/EA = 1/1, v/v) to give the product **9** (2.3 g, 76%) as a colorless oil. $^1$H NMR (400 MHz, Chloroform-*d*) δ 7.46–7.13 (m, 30H, Ar), 5.24–5.08 (m, 3H, $\underline{CH_2}$-Cbz, Glc-H-1), 4.97–4.89 (m, 2H, Ph$\underline{CH_2}$), 4.88–4.68 (m, 7H, Gal-H-1, Ph$\underline{CH_2}$×3), 4.71 (d, *J* = 11.9 Hz, 2H, Ph$\underline{CH_2}$) 4.61–4.52 (m, 2H, Ph$\underline{CH_2}$), 4.48–4.24 (m, 2H, Gal-H-4, Glc-H-5), 4.02–3.75 (m, 9H, Gal-H-2, Glc-H-3, Gal-H-5, Gal-H-3, Glc-H-6a/b, Gal-H-6a/b, Gal-6-OH), 3.63 (m, 2H, Glc-H-4, CH$\underline{H}$-Linker), 3.50–3.32 (m, 2H, C$\underline{H}$H-Linker, CH$\underline{H}$-Linker), 3.43 (dd, *J* = 10.3, 3.5 Hz, 1H, Glc-H-2), 3.26–3.06 (m, 1H, C$\underline{H}$H-Linker), 2.80–2.59 (m, 2H, $\underline{CH_2}$-Lev), 2.56–2.47 (m, 2H, $\underline{CH_2}$-Lev), 2.16 (s, 3H, $\underline{CH_3}$-Lev), 1.53 (m, 4H, $\underline{CH_2}$-Linker×2), 1.41–1.32 (m, 2H, $\underline{CH_2}$-Linker). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 206.36, 172.32, 138.45, 138.37, 137.96, 137.77, 128.66, 128.59, 128.56, 128.53, 128.50, 128.46, 128.19, 128.13, 128.10, 127.99, 127.92, 127.87, 127.83, 127.63, 127.48, 127.45, 127.26, 98.28 (Glc-C-1), 97.36 (Gal-C-1), 80.35 (Glc-C3), 78.06 (Glc-C4), 75.75 (Gal-C3), 75.53 (Gal-C2), 75.05, 74.73 (Gal-C4), 73.35, 72.94, 70.87 (Gal-C5), 69.24 (Glc-C5), 67.49, 67.22, 64.28 (Glc-C2), 62.41 (Glc-C6), 60.14 (Gal-C6), 50.40, 47.54, 37.90, 29.88, 28.97, 27.86, 27.10, 23.43, 14.28. ESI HRMS (m/z): [M + $Na^+$] calcd for $C_{65}H_{74}N_4NaO_{14}$, 1157.5094 found 1157.5094.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-azido-6-O-levulinoyl-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-O-benzyl)-α-D-galactopyranosyluronate (10)**: Compound **9** (1.8 g, 1.6 mmol) was dissolved in a two-phase solvent of DCM (12 mL) and $H_2O$ (6 mL), which was followed by the addition of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, 50 mg, 0.32 mmol) and (diacetoxyiodo)benzene (BAIB, 1.28 g, 4.0 mmol). After stirring for 6 h at room temperature, the reaction was quenched with aq. $Na_2S_2O_3$ (20%, 40 mL). The two-phase mixture was diluted with DCM (30 mL) and separated. The organic layer was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was co-evaporated 3 times with toluene before being dissolved in THF (16 mL). The solution was cooled to 0 °C and was stirred for 15 min at this temperature, after which trimethylsilyldiazomethane ($TMSCHN_2$, 2.0 M in ether, 4 mL, 8 mmol) was added dropwise via syringe over 15 min. After TLC analysis showed consumption of the starting material, the reaction was quenched with AcOH (5% in MeOH, 10 mL). The reaction mixture was concentrated *in vacuo*, and the resulting residue was purified by flash chromatography over silica gel (PE/EA = 1/1, v/v) to give **10** (1.5 g, 80% over 2 steps) as a light-yellow oil. $^1$H NMR (400 MHz, Chloroform-*d*) δ 7.46–7.14 (m, 30H, Ar), 5.20 (d, *J* = 9.0 Hz, 2H, $\underline{CH_2}$-Cbz), 4.97 (d, *J* = 3.6 Hz, 1H, Glc-H-1), 4.92 (bs, 1H, GalA-H-1), 4.88 (d, *J* = 2.5 Hz, 2H, Ph$\underline{CH_2}$), 4.85 (d, *J* = 3.4 Hz, 1H, PhCH$\underline{H}$), 4.82–4.79 (m, 3H, C$\underline{H}$H, Ph$\underline{CH_2}$), 4.71 (d, *J* = 12.2 Hz, 1H, PhCH$\underline{H}$), 4.57 (d, *J* = 10.6 Hz, 1H, PhC$\underline{H}$H), 4.55–4.48 (m, 3H, GalA-H-4, Ph$\underline{CH_2}$), 4.33 (split by Cbz, 1H, GalA-H-5), 4.31–4.24 (m, 1H, Glc-H-5), 4.06 (dd, *J* = 12.3, 2.3 Hz, 1H, Glc-H-6a), 3.94–3.88 (m, 3H, GalA-H-3, GalA-H-2, Glc-H-3), 3.85 (s, 3H, GalA-$CO_2\underline{CH_3}$), 3.77 (d, *J* = 12.3 Hz, 1H, Glc-H-6b), 3.61 (t, *J* = 9.5 Hz, 1H, Glc-H-4), 3.68–3.54 (m, 1H, CH$\underline{H}$-Linker), 3.45 (q, *J* = 7.0, 6.5 Hz, 1H, C$\underline{H}$H-Linker), 3.39 (dd, *J* = 10.3, 3.5 Hz, 1H, Glc-H-2), 3.26–3.12 (m, 2H, $\underline{CH_2}$-Linker), 2.71 (qt, *J* = 18.3, 6.4 Hz, 2H, $\underline{CH_2}$-Lev), 2.53 (td, *J* = 6.4, 2.1 Hz, 2H, $\underline{CH_2}$-Lev), 2.17 (s, 3H, $\underline{CH_3}$-Lev), 1.63–1.44 (m, 4H, $\underline{CH_2}$-Linker×2), 1.40–1.19 (m, 2H, $\underline{CH_2}$-Linker). $^{13}$C NMR (101 MHz, cdcl$_3$) δ 206.17, 172.18, 169.06, 138.04, 137.99, 137.92, 137.89, 128.53, 128.48, 128.47, 128.45, 128.43, 128.35, 128.24, 128.04, 128.02, 127.97, 127.93, 127.90, 127.88, 127.82, 127.75, 127.52, 127.29, 127.15, 99.00 (Glc-C1), 97.45 (GalA–C1), 80.29 (Glc-C3), 77.70 (Gal-C4), 77.20 (Glc-C4), 76.20(Gal-C3), 75.42, 75.03, 74.44 (Gal-C2), 73.16, 73.05, 70.06 (Gal-C5), 69.51(Glc-C5), 68.65, 67.15, 63.90 (Glc-C2), 62.15 (Glc-C6), 52.58, 50.47, 50.22, 47.08, 46.11, 37.79, 29.79, 29.01, 27.74, 23.32. ESI HRMS (m/z): [M + $Na^+$] calcd for $C_{66}H_{74}N_4NaO_{15}$, 1185.5043 found 1185.5050.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trifluoroacetamido-6-O-levulinoyl-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-O-benzyl)-α-D-galactopyranosyluronate (11)**: To a solution of **10** (315 mg, 0.27 mmol) in THF/AcOH

(8 mL/2 mL) was added Zn (washed by 1.0 M HCl, $H_2O$ and acetone sequentially, 0.5 g). The reaction suspension was stirred overnight at room temperature, after which it was filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was co-evaporated with toluene (4×5 mL), and then was dissolved in dry THF (5 mL). The solution was cooled to 0 °C, and then added trifluoroacetic anhydride (375 μL, 2.71 mmol). After stirring for 1 h at 0 °C, the reaction mixture was diluted with toluene (20 mL) and was concentrated under reduced pressure. The resulting residue was dissolved in EtOAc (30 mL) and washed with aq. sat. $NaHCO_3$ (10 mL), the organic layer was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated *in vacuo*. The crude product was purified by flash chromatography over silica gel (PE/EA = 1.5/1, v/v) to give **11** (264 mg, 79%) as a colorless oil. $^1$H NMR (600 MHz, Chloroform-*d*) δ 7.44–7.09 (m, 30H), 6.62 (t, *J* = 10.2 Hz, 1H, $CF_3CO\underline{NH}$), 5.16 (d, *J* = 18.4 Hz, 2H, $\underline{CH_2}$-Cbz), 4.89 (bs, 1H, GalA-H-1), 4.84–4.66 (m, 7H, Ph$\underline{CH_2}$×3, Glc-H-1), 4.61 (dd, *J* = 11.0, 4.6 Hz, 1H, PhCH$\underline{H}$), 4.54 (dd, *J* = 10.6, 4.2 Hz, 1H, PhC$\underline{H}$H), 4.47 (d, *J* = 15.5 Hz, 2H, Ph$\underline{CH_2}$), 4.40 (s, 1H, GalA-H-4), 4.32–4.25 (m, 2H, GalA-H-5, Glc-H-5), 4.25–4.18 (m, 1H, Glc-H-2), 3.99–3.93 (m, 1H, Glc-H-6a), 3.89 (d, *J* = 11.5 Hz, 1H, GalA-H-3), 3.84–3.78 (m, 2H, GalA-H-2, Glc-H-6b), 3.74–3.65 (m, 2H, Glc-H-3, Glc-H-4), 3.65 (s, 3H, $CO_2\underline{CH_3}$), 3.63–3.50 (m, 1 H, CH$\underline{H}$-Linker), 3.46–3.33 (m, 1H, C$\underline{H}$H-Linker), 3.25–3.12 (m, 2H, $\underline{CH_2}$-Linker), 2.71 (qt, *J* = 18.3, 6.4 Hz, 2H, $\underline{CH_2}$-Lev), 2.53 (q, *J* = 6.5 Hz, 2H, $\underline{CH_2}$-Lev), 2.16 (s, 3H, $\underline{CH_3}$-Lev), 1.61–1.45 (m, 4H, $\underline{CH_2}$-Linker×2), 1.32–1.13 (m, 2H, $\underline{CH_2}$-Linker). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 206.33, 172.38, 138.10, 137.93, 137.65, 128.67, 128.61, 128.57, 128.36, 128.09, 128.06, 128.04, 128.00, 127.95, 127.67, 127.28, 98.10 (Glc-C1), 97.51 (Gal-C1), 79.29 (Glc-C3), 77.83 (Glc-C4), 76.42 (Gal-C4), 76.14 (Gal-C3), 75.55 (Gal-C2), 75.20, 73.62, 73.10, 70.17 (Gal-C5), 69.47 (Glc-C5), 68.97, 67.30, 62.05 (Glc-C6), 53.39 (Glc-C2), 52.46, 37.94, 29.94, 29.15, 27.90, 23.42. ESI HRMS (m/z): [M + $Na^+$] calcd for $C_{68}H_{75}F_3N_2NaO_{16}$, 1255.4961 found 1255.4958.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-2-deoxy-2-trifluoroacetamido-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-O-benzyl)-α-D-galactopyranosyl uronate (12)**: Hydrazine acetate (35 mg, 0.374 mmol) was added to the solution of **11** (230 mg, 0.187 mmol) in DCM/MeOH (2 mL/0.2 mL), and the mixture was stirred for 2 h at room temperature. After TLC analysis showed consumption of the starting material, the reaction mixture was concentrated *in vacuo*. The resulting residue was dissolved in DCM (50 mL) and washed with water (3×30 mL), and the organic phase was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated *in vacuo*. The resulting crude product was purified by silica gel column chromatography (DCM/acetone, 15/1, v/v) to give the disaccharide acceptor **12** (175 mg, 83%) as a colorless oil. $^1$H NMR (600 MHz, Chloroform-*d*) δ 7.30 (m, 30H, Ar), 6.69 (d, *J* = 10.2 Hz, 1H, TFA$\underline{NH}$), 4.88 (bs, GalA-H-1), 4.85–4.77 (m, 5H, Glc-H-1, $\underline{CH_2}$-Cbz, Ph$\underline{CH_2}$), 4.73 (d, *J* = 11.9 Hz, 1H, PhCH$\underline{H}$), 4.67 (t, *J* = 14.0 Hz, 2H, PhC$\underline{H}$H, PhCH$\underline{H}$), 4.61 (d, *J* = 11.0 Hz, 1H, PhC$\underline{H}$H), 4.48 (d, *J* = 15.2 Hz, 2H, Ph$\underline{CH_2}$), 4.40 (bs, 1H, GalA-H-4), 4.30 (split by Cbz, GalA-H-5), 4.19 (td, *J* = 9.6, 3.4 Hz, 1H, Glc-H-2), 4.12 (d, *J* = 8.0 Hz, 1H, Glc-H-5), 3.87 (td, *J* = 9.4, 2.8 Hz, 1H, GalA-H-3), 3.82 (dd, *J* = 10.2, 2.8 Hz, 1H, GalA-H-2), 3.66 (s, 3H, $CO_2\underline{CH_3}$), 3.68–3.66 (m, 2H, Glc-H-3, Glc-H-4), 3.64–3.51 (m, 1H, CH$\underline{H}$-Linker), 3.46–3.33 (m, 3H, Glc-H-6a/b, C$\underline{H}$H-Linker), 3.28–3.12 (m, 2H, $CH_2$-Linker), 1.60–1.48 (m, 4H, $\underline{CH_2}$-Linker×2), 1.29–1.22(m, 2H, $\underline{CH_2}$-Linker). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 169.15, 138.09, 137.79, 128.68, 128.66, 128.64, 128.61, 128.57, 128.22, 128.14, 128.08, 128.04, 127.95, 127.60, 127.48, 127.28, 98.22 (Glc-C1), 97.58 (Gal-C1), 79.18 (Glc-C3), 77.91 (Glc-C4), 76.44 (2 C, Gal-C4, Gal-C3), 75.45 (Gal-C2), 75.31, 75.14, 73.60, 73.22, 72.38 (Glc-C5), 69.56 (Gal-C5), 68.98, 67.30, 60.95 (Glc-C6), 53.64 (Glc-C2), 52.55, 29.16, 27.59, 23.45. ESI HRMS (m/z): [M + $Na^+$] calcd for $C_{63}H_{69}F_3N_2NaO_{14}$, 1157.4593 found 1157.4594.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-O-(2-methylnaphthyl)-5-O-levulinoyl-7,8-O-isopropylidene-3-deoxy-α-D-manno-oct-2-ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2, 3-di-O-benzyl)-α-D-galactopyranosyl urinate (13)**: Disaccharide acceptor **12** (471 mg, 0.41 mmol) and Kdo donor **5** (540 mg, 0.82 mmol) were co-evaporated with toluene (3×5 mL) and were dissolved in dry acetonitrile (5 mL) and the solution was placed under an atmosphere of Argon. *N*-iodosuccinimide (284 mg, 1.26 mmol) and flame activated 4 Å M.S. were then added. The mixture was stirred for 15 min at 0 °C, after which trifluoromethanesulfonic acid (TfOH, 7.3 μL, 0.082 mmol) was added, and the reaction was stirred for 2 h at 0 °C. After TLC analysis showed consumption of disaccharide acceptor, the reaction was quenched with $Et_3N$ (0.5 mL). The molecular sieves were filtered off, and the solution was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (toluene/EA = 3/1, v/v) to give **13** (487 mg, 71% of α-anomer) as an oil. The α-anomer was confirmed by NMR ($J_{H3ax\text{-}C1} < 1$ Hz) $^1$H NMR (600 MHz, $CDCl_3$) δ 7.83–7.69 (m, 4H, Ar), 7.42–7.16 (m, 31H, Ar), 7.09 (dd, *J* = 7.4, 2.2 Hz, 2H, Ar), 5.52 (dd, *J* = 2.7, 1.4 Hz, 1H, Kdo-H-5), 5.16 (d, *J* = 15.7 Hz, 2H, $CH_2$-Cbz), 4.84 (bs, 1H, GalA-H-1), 4.78 (dd, *J* = 12.0, 6.7 Hz, 2H, Ph$\underline{CH_2}$), 4.74–4.67 (m, 3H, GlcNTFA-H-1, Ph$\underline{CH_2}$), 4.67–4.63 (m, 1H, PhCH$\underline{H}$), 4.60 (d, *J* = 12.5 Hz, 1H, PhC$\underline{H}$H), 4.55 (dd, *J* = 13.0, 11.4 Hz, 2H, Ph$\underline{CH_2}$), 4.51 (d, *J* = 11.0 Hz, 1H, PhCH$\underline{H}$), 4.49–4.44 (m, 2H, PhC$\underline{H}$H, PhCH$\underline{H}$), 4.33–4.29 (m, 2H, GalA-H-4, PhC$\underline{H}$H), 4.27–4.20 (m, 2H, GlcNTFA-H-5, GalA-H-5), 4.19 (dt, *J* = 6.8, 2.6 Hz, 1H, GlcNTFA-H-2), 4.17–4.12 (m, 2H, $CO_2\underline{CH_2}CH_3$), 4.10 (dt, *J* = 7.4, 5.9 Hz, 1H, Kdo-H-7), 4.01 (dd, *J* = 8.6, 6.1 Hz, 1H, Kdo-H-8a), 3.87 (ddd, *J* = 11.7, 4.8, 2.8 Hz, 1H, Kdo-H-4), 3.79–3.68 (m, 3H, Kdo-H-8b, GalA-H-2, GalA-H-3), 3.65 (s, 3H, $CO_2\underline{CH_3}$), 3.65–3.50 (m, 5H, GlcNTFA-H-3, GlcNTFA-H-4, GlcNTFA-H-6a, Kdo-H-6, CH$\underline{H}$-Linker), 3.41 (m, 2H, GlcNTFA-H-6b, C$\underline{H}$H-Linker), 3.25–3.13 (m, 2H, $CH_2$-Linker), 2.78–2.60 (m, 4H, $\underline{CH_2CH_2}$-Lev), 2.29 (ddd, *J* = 13.1, 4.9, 1.2 Hz, 1H, Kdo-$H_{eq}$-3), 2.11 (s, 3H, $\underline{CH_3}$-Lev), 1.96 (dd, *J* = 13.2, 11.9 Hz, 1H, Kdo-$H_{ax}$-3), 1.58–1.43 (m, 4H, $CH_2$-Linker×2), 1.31 (s, 3H, C($\underline{CH_3}$)$_2$), 1.29–1.22 (m, 10H, $\underline{CH_2}$-Linker, $CO_2CH_2\underline{CH_2}$, C($\underline{CH_3}$)$_2$). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 206.32, 171.96, 167.23 (Kdo-C1), 138.09, 137.70, 135.38, 133.37, 133.12, 128.69, 128.67, 128.63, 128.61, 128.58, 128.36, 128.12, 128.02, 127.97, 127.82, 127.79, 127.70, 127.35, 126.75, 126.25, 126.01, 125.83, 109.44, 98.93 (Kdo-C2), 97.60 (Gal-C1), 97.44 (Glc-C1), 79.09 (Glc-C3), 78.31 (Glc-C4), 76.38 (Gal-C4), 75.44 (Gal-C3), 75.07 (Gal-C2), 74.82, 73.51 (Kdo-C7), 73.20, 72.85, 72.58 (Kdo-C6), 71.40 (Kdo-C4), 70.83 (Glc-C5), 70.56, 69.49, 69.30 (Gal-C5), 67.31, 66.98 (Kdo-C8), 65.32 (Kdo-C5), 62.45 (Glc-C6), 61.68, 53.42 (Glc-C2), 52.53, 47.33, 38.18, 33.34 (Kdo-C3), 29.91, 29.71, 28.21, 26.75, 25.58, 25.53, 22.77, 14.25, 11.93. HRMS (m/z): [M + $Na^+$] calcd for $C_{92}H_{103}F_3N_2NaO_{23}$, 1683.6796 found 1683.6800.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl-ethyl (4-O-(2-methylnaphthyl) −7,8-O-isopropylidene-3-deoxy-α-D-manno-oct-2-ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3,4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2, 3-di-O-benzyl)-α-D-galactopyranosyl urinate (14)**: Trisaccharide **13** (398 mg, 0.24 mmol) and $NH_2NH_2$·HOAc (44 mg, 0.48 mmol) were dissolved in a mixture of DCM/MeOH (10 mL/1 mL), and the reaction was stirred for 3 h at room temperature. After TLC analysis showed consumption of starting material, the reaction mixture was concentrated *in vacuo*. The obtained residue was dissolved in DCM (20 mL) and washed with $H_2O$ (10 mL). The organic layer was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was passed through a silica gel pad (DCM/acetone = 15/1, v/v) to give the crude trisaccharide acceptor **14** as a light-yellow oil, which was used for the next step without additional purification. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.4 Hz, 1H, Ar), 7.76–7.70 (m, 3H, Ar), 7.42 (dd, *J* = 8.4, 1.7 Hz, 1H, Ar), 7.40–7.13 (m, 30H), 7.09 (dd, *J* = 7.3, 2.2 Hz, 2H, Ar), 5.16 (d, *J* = 16.5 Hz, 2H, $CH_2$-

Cbz), 4.85 (bs, 1H, GalA-H-1), 4.79 (d, $J$ = 12.1 Hz, 1H, PhCHH), 4.75–4.72 (m, 2H, GlcNTFA-H-1, PhCHH), 4.71 (m, 2H, $PhCH_2$), 4.69–4.63 (m, 3H, $PhCH_2$, PhHH), 4.58 (d, $J$ = 11.0 Hz, 1H, PhCHH), 4.53 (d, $J$ = 11.0 Hz, 1H, PhHH), 4.47 (m, 2H, PhCHH, PhCHH), 4.35 (m, 3H, Kdo-H-7, GalA-H-4, PhCHH), 4.27–4.20 (m, 3H, GlcNTFA-H-2, GlcNTFA-H-5, GalA-H-5), 4.16 (q, $J$ = 7.1 Hz, 2H, $CO_2CH_2CH_3$), 4.06–4.01 (m, 2H, Kdo-H-5, Kdo-H-8a), 3.86–3.79 (m, 2H, Kdo-H-4, GalA-H-3), 3.74 (m, 2H,Kdo-H-8b, GalA-H-2), 3.65 (s, 3H, $CO_2CH_3$), 3.64–3.56 (m, 2H, GlcNTFA-H-3, GlcNTFA-H-4), 3.56–3.48 (m, 1H, GlcNTFA-H-6a), 3.45–3.34 (m, 4H, Kdo-H-6, GlcNTFA-H-6b, $CH_2$-Linker), 3.26–3.13 (m, 2H, $CH_2$-Linker), 2.32 (dd, $J$ = 13.2, 4.9 Hz, 1H, Kdo-$H_{eq}$-3), 2.00 (t, $J$ = 13.0, 1H, Kdo-$H_{ax}$-3), 1.59–1.44 (m, 4H, $CH_2$-Linker ×2), 1.34 (s, 3H, $C(CH_3)_2$), 1.31–1.20 (m, 11H, $CH_2$-Linker, $CO_2CH_2CH_3$, $C(CH_3)_2$). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 177.01, 169.21, 167.35 (Kdo-C1), 138.24, 138.08, 137.74, 135.11, 133.34, 133.18, 128.69, 128.67, 128.63, 128.57, 128.11, 128.04, 128.01, 127.97, 127.94, 127.84, 127.81, 127.66, 127.41, 126.68, 126.45, 126.20, 125.68, 109.30, 99.02, 97.59 (2C, Gal-C1, Glc-C1), 79.18 (Glc-C3), 78.24 (Glc-C4), 76.35 (Gal-C3), 75.48 (Gal-C4), 75.20 (Gal-C2), 75.04, 74.78, 73.77 (Kdo-C7), 73.36 (Kdo-C6), 73.20, 72.90 (Kdo-C4), 72.59, 70.90 (Glc-C5), 70.30, 69.36 (Gal-C5), 69.00 (Glc-C6), 67.33 (Kdo-C8), 64.41 (Kdo-C5), 62.29, 61.64, 53.43 (Glc-C2), 52.51, 31.72 (Kdo-C3), 29.19, 26.71, 25.49, 23.44, 14.26. HRMS (m/z): [M + $Na^+$] calcd for $C_{87}H_{97}F_3N_2NaO_{21}$, 1585.6428 found 1585.6429.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl- 2-deoxy-2-trichloroacetamido-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→5)-ethyl (4-O-(2-methylnaphthyl)-7, 8-O-isopropylidene-3-deoxy-α-D-manno-oct-2-ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-O-benzyl)-α-D-galactopyranosyl urinate (15)**: Trisaccharide acceptor **14** (374 mg, 0.24 mmol) and monosaccharide donor **6** (248 mg, 0.36 mmol) were co-evaporated with toluene (5 mL ×3) and were dissolved in DCM/MeCN (2 mL/0.5 mL) under an atmosphere of Argon. NIS (112.5 mg, 0.5 mmol) and flame activated 4 Å M.S. were added. The suspension was stirred for 15 min at 0 °C, after which was added TfOH (3.0 μL, 0.034 mmol), and the reaction mixture was stirred for 6 h at 0 °C. The reaction was quenched with $Et_3N$ (0.5 mL), and the molecular sieves were filtered off. The obtained solution was concentrated *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 3/1, v/v) to give **15** (274 mg, 53 % over two steps) as an oil. Labeling monosaccharides for NMR assignment: GlcNTCA as **A**; Kdo as **B**, GlcNTFA as **C**; GlcA as **D**. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.84–7.69 (m, 4H, Ar), 7.43–7.10 (m, 48H, Ar), 6.86 (d, $J$ = 8.2 Hz, 1H, NHTCA), 6.60 (s, 1H, NHTFA), 5.17 (d, $J$ = 16.4 Hz, 2H, $CH_2$-Cbz), 4.89–4.85 (bs, 1H, D-H-1), 4.83–4.77 (m, 3H, A-H-1, C-H-1, PhCHH), 4.77–4.73 (m, 2H, PhCHH), 4.72–4.64 (m, 4H, $PhCH_2$ ×2), 4.62–4.51 (m, 6H, $PhCH_2$ ×3), 4.51–4.45 (m, 2H, $PhCH_2$), 4.40 (d, $J$ = 10.8 Hz, 1H, PhCHH), 4.37–4.33 (m, 2H, D-H-4, PhCHH), 4.31 (dd, $J$ = 6.8, 5.1 Hz, 1H, C-H-5), 4.25 (m, 3H, A-H-5, C-H-2, D-H-5), 4.14–4.06 (m, 3H, B-H-8a, $CO_2CH_2CH_3$), 4.00–3.96 (m, 1H, B-H-5), 3.95–3.89 (m, 1H, B-H-8b), 3.89–3.80 (m, 3H, A-H-2, B-H-4, D-H-3), 3.77 (dd, $J$ = 10.1, 3.4 Hz, 1H, D-H-2), 3.74–3.69 (m, 2H, A-H-6a/b), 3.67 (s, 3H, $CO_2CH_3$), 3.66–3.60 (m, 5H, B-H-6, C-H-3, C-H-4, C-H-6a, A-H-4), 3.59–3.55 (m, 2H, C-H-6b, CHH-Linker), 3.49 (ddd, $J$ = 9.5, 4.2, 2.6 Hz, 1H, B-H-7), 3.38 (m, 2H, A-H-3, CHH-Linker), 3.25–3.14 (m, 2H, $CH_2$-Linker), 2.40–2.33 (m, 1H, B-$H_{eq}$-3), 2.07 (t, $J$ = 12.3 Hz, 1H, B-$H_{ax}$-3), 1.61–1.44 (m, 4H, $CH_2$-Linker ×2), 1.32 (s, 3H, $C(CH_3)_2$), 1.30–1.13 (m, 11H, $CH_2$-Linker, $CO_2CH_2CH_3$, $C(CH_3)_2$). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 169.23, 167.25 (B–C1), 161.51, 157.73, 138.32, 138.22, 138.12, 138.07, 137.92, 137.73, 134.82, 133.26, 128.88, 128.65, 128.64, 128.62, 128.59, 128.56, 128.53, 128.51, 128.40, 128.09, 128.06, 128.00, 127.96, 127.95, 127.91, 127.80, 127.75, 127.74, 127.71, 127.43, 127.27, 127.06, 126.77, 126.48, 125.78, 116.79, 114.88, 108.56, 100.93 (A–C1), 98.91 (C–C1), 97.77 (D–C1), 97.55 (B–C2), 92.71, 83.03 (A–C3), 79.30 (C–C3), 78.29 (C–C4), 78.17 (A–C4), 76.06 (D–C3), 75.82, 75.52 (D–C4), 75.26, 75.12 (C–C5), 75.01(B–C7), 74.84 (D–C2), 74.80, 74.00 (B–C4), 73.76, 73.12, 72.99 (B–C6), 72.86, 72.42 (B–C5), 71.10, 70.94 (A–C5), 69.39 (D–C5), 69.24 (A–C6), 68.95 (C–C6), 67.29, 65.91 (B–C8), 62.23, 61.66, 57.39 (A–C2), 53.45 (C–C2), 52.50, 50.62, 50.33, 47.19, 46.18, 32.31(B–C3), 29.15, 26.24, 26.17, 23.40, 14.20. HRMS (m/z): [M + $NH_4^+$] calcd for $C_{116}H_{129}Cl_3F_3N_4O_{26}$, 2155.7907 found 2155.7880.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl- 2-deoxy-2-trichloroacetamido-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→5)-ethyl (7, 8-O-isopropylidene-3-deoxy-α-D-manno-oct-2-ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3, 4-di-O-benzyl-α-D–glucopyranosyl-(1→4)-methyl (2,3-di-O-benzyl)-α-D-galactopyranosyl urinate (16)**: β-Pinene (37 μL, 0.234 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 27 mg, 0.117 mmol) were added to a mixture of **15** (148 mg, 0.069 mmol) in DCM/$H_2O$ (8 mL/0.4 mL) under the exclusion of light. The reaction mixture was stirred for 2 h at room temperature, after which it was diluted with DCM (20 mL) and was quenched with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried ($Na_2SO_4$), filtered, and the filtrate was concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (PE/EA = 2/1, v/v) to give the tetrasaccharide acceptor **16** (100 mg, 72 %) as a white amorphous solid. Labeling monosaccharides for NMR assignment: GlcNTCA as **A**; Kdo as **B**, GlcNTFA as **C**; GlcA as **D**. $^1$H NMR (600 MHz, $CDCl_3$) δ 7.46–7.12 (m, 52H, Ar), 6.64 (s, 1H, NHTFA), 5.19 (d, $J$ = 17.4 Hz, 2H, $CH_2$-Cbz), 5.04 (d, $J$ = 7.8 Hz, 1H, A-H-1), 4.91 (bs, 1H, D-H-1), 4.86–4.80 (m, 4H, C-H-1, $PhCH_2$, PhCHH), 4.79–4.74 (m, 4H, PhCHH, $PhCH_2$, PhCHH), 4.69 (m, 2H, PhCHH, PhCHH), 4.63–4.58 (m, 4H, PhCHH, $PhCH_2$, PhCHH), 4.55 (d, $J$ = 12.0 Hz, 1H, PhCHH), 4.49 (d, $J$ = 14.3 Hz, 2H, $PhCH_2$), 4.45–4.43 (m, 1H, D-H-4), 4.38–4.30 (m, 2H, A-H-5, B-H-7), 4.27 (td, $J$ = 4.5, 1.6 Hz, 1H, D-H-5), 4.22 (td, $J$ = 10.1, 3.5 Hz, 1H, C-H-2), 4.17–4.09 (m, 3H, $CO_2CH_2CH_3$, B-H-8a), 4.08–4.00 (m, 1H, B-H-4), 3.96 (dd, $J$ = 8.8, 6.2 Hz, 1H, B-H-8b), 3.94–3.90 (m, 2H, B-H-5, C-H-4), 3.88 (d, $J$ = 9.5 Hz, 1H, D-H-3), 3.81 (dd, $J$ = 9.8, 3.5 Hz, 1H, D-H-2), 3.79–3.68 (m, 5H, A-H-2, A-H-4, B-H-6, C-H-3), 3.67 (m, 5H, A-H-6a/b, $CO_2CH_3$), 3.66–3.54 (m, 5H, A-H-3, C-H-5, C-H-6a, $CH_2$-Linker), 3.47–3.37 (m, 1H, C-H-6b), 3.29–3.15 (m, 2H, $CH_2$-Linker), 2.57–2.48 (m, 1H, -OH), 2.00 (dd, $J$ = 13.0, 4.5 Hz, 1H, B-$H_{eq}$-3), 1.88 (t, $J$ = 12.3 Hz, 1H, B-$H_{ax}$-3), 1.64–1.46 (m, 4H, $CH_2$-Linker ×2), 1.35–1.17 (m, 11H, $CH_2$-Linker, $CO_2CH_2CH_3$, $C(CH_3)_2$). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 176.52, 169.30, 169.28, 167.41 (B–C1), 161.90, 138.31, 138.26, 138.23, 137.94, 137.92, 137.71, 137.65, 128.69, 128.66, 128.62, 128.59, 128.56, 128.09, 128.06, 128.02, 128.01, 127.98, 127.97, 127.95, 127.83, 127.81, 127.70, 127.44, 114.87, 99.78 (A–C1), 99.65 (B–C2), 98.73 (D–C1), 97.46 (C–C1), 92.65, 80.78 (C–C4), 79.10 (C–C3), 78.87 (A–C3), 77.78 (A–C4), 76.16 (D–C3), 75.43 (D–C2), 75.24 (C–C5), 75.19 (B–C7), 75.12 (D–C4), 74.88, 74.68, 73.75 (B–C5), 73.11, 72.85 (B–C6), 71.09 (A–C5), 70.68 (A–C6), 69.48, 69.34 (D–C5), 69.11 (C–C6), 66.81 (B–C4), 66.16 (B–C8), 63.17, 62.69, 61.54, 57.49 (A–C2), 53.52 (C–C2), 52.54, 35.37 (B–C3), 32.06, 29.83, 29.71, 29.17, 26.51, 26.13, 22.83, 22.76, 14.26, 14.21, 14.09, 11.93. HRMS (m/z): [M + $Na^+$] calcd for $C_{105}H_{117}Cl_3F_3N_3NaO_{26}$, 2020.6835 found 2020.6885.

***N*-(benzyl)-*N*-benzyloxycarbonyl-5-aminopentyl- [2,3-di-O-benzoyl-4,6-di-O-benzyl-7-O-acetyl-L-*glycero*-α-D-manno-heptopyranosyl]-(1→4)-[2-deoxy-2-trichloroacetamido-3,4,6-tri-Obenzyl-β-D-glucopyranosyl]-(1→5)-ethyl (7, 8-O-isopropylidene-3-deoxy-α-D-manno-oct-2-ulopyranoside)onate(2→6)-2-deoxy-2-trifluoroacetamido-3, 4-di-O-benzyl-α-D-glucopyranosyl-(1→4)-methyl (2,3-di-O-benzyl)-α-D-galactopyranosyl urinate (17)**: Tetrasaccharide acceptor **16** (100 mg, 0.05 mmol) and heptosyl donor **7** (79 mg, 0.10 mmol) were co-evaporated with toluene (5 mL×3) and were dissolved in dry DCM (3 mL) and the solution was placed under an atmosphere of Argon. Flame activated 4 Å M.S. were added, and the obtained suspension was stirred for 15 min at 0 °C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 1.8 μL,

0.01 mmol) was then added, and the reaction mixture was stirred for 6 h at 0 °C. The reaction was quenched with $Et_3N$ (0.5 mL), and the molecular sieves were filtered off. The solvents were removed *in vacuo*, and the resulting residue was purified by silica gel column chromatography (PE/EA = 2.5/1, v/v) to give pentasaccharide **17** (92 mg, 70 %) as a white amorphous solid. NMR data confirmed the heptoside is α-linked ($J_{C1\text{-}H1}$ = 174 Hz). Labeling monosaccharides for NMR assignment: GlcNTCA as **A**; L,D-Hepp as **B**; Kdo as **C**, GlcNTFA as **D**; GlcA as **E**. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.03–7.94 (m, 2H, Ar), 7.81–7.76 (m, 2H, Ar), 7.71 (d, *J* = 9.7 Hz, 1H, Ar), 7.58 (t, *J* = 7.4 Hz, 1H, Ar), 7.50 (t, *J* = 7.2 Hz, 1H, Ar), 7.42 (d, *J* = 6.6 Hz, 2H, Ar), 7.38–7.14 (m, 54H, Ar), 7.11–7.06 (m, 2H, Ar), 6.37 (d, *J* = 9.5 Hz, 1H, NHTFA), 5.72 (dd, *J* = 9.7, 2.8 Hz, 1H, B-H-3), 5.54 (t, *J* = 2.5 Hz, 1H, B-H-2), 5.16 (d, *J* = 15.3 Hz, 2H, $CH_2$-Cbz), 5.11 (d, *J* = 2.1 Hz, 1H, B-H-1), 5.06 (d, *J* = 10.8 Hz, 1H, PhCHH), 4.93 (d, *J* = 10.9 Hz, 1H, PhCHH), 4.84 (m, 3H, E-H-1, $PhCH_2$), 4.81–4.56 (m, 13H, A-H-1, D-H-1, B-H-7a, $PhCH_2$ ×5), 4.53 (d, *J* = 11.7 Hz, 2H, $PhCH_2$), 4.50–4.40 (m, 4H, B-H-4, $PhCH_2$, PhCHH), 4.35 (m, 2H, E-H-4, PhCHH), 4.32–4.16 (m, 7H, A-H-2, B-H-7b, C-H-7, C-H-8a, D-H-2, E-H-5, $CO_2CHHCH_3$), 4.15–4.02 (m, 4H, B-H-6, C-H-4, C-H-6, $CO_2CHHCH_3$), 4.03–3.94 (m, 2H, A-H-3, B-H-5), 3.90 (t-like, *J* = 1.6 Hz, 1H, C-H-5), 3.84 (d, *J* = 7.1 Hz, 1H, E-H-3), 3.80–3.66 (m, 6H, A-H-5, A-H-6a/b, C-H-6, D-H-5, E-H-2), 3.65 (s, 4H, $CO_2CH_2$, CHH-Linker), 3.64–3.50 (m, 4H, A-H-4, D-H-3, D-H-6a, CHH-Linker), 3.43–3.31 (m, 1H, D-H-6b), 3.25–3.13 (m, 2H, $CH_2$-Linker), 2.28–2.17 (m, 2H, C-H-$3_{eq/ax}$), 2.13 (s, 3H, OAc), 1.60–1.43 (m, 2H, $CH_2$-Linker), 1.37–1.13 (m, 13H, $CH_2$-Linker ×2, $CO_2CH_2CH_3$, $C(CH_3)_2$). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 170.85, 169.11, 167.22 (C–C1), 167.18, 165.68, 161.96, 138.48, 138.30, 137.98, 137.73, 133.71, 130.05, 129.76, 129.24, 129.10, 128.73, 128.69, 128.64, 128.63, 128.61, 128.57, 128.51, 128.49, 128.45, 128.11, 128.06, 128.02, 127.98, 127.92, 127.82, 127.77, 127.67, 127.63, 127.53, 127.50, 116.80, 108.37, 101.82 (A–C1), 98.78 (C–C2), 97.63 (D–C1), 97.55 (E–C1), 97.21 (B–C1), 93.17, 82.67 (A–C3), 79.14 (A–C4), 78.58 (D–C4), 78.32 (E–C2), 77.97, 76.06 (E–C3), 75.90 (C–C7), 75.84 (E–C4), 75.68 (E–C4), 75.27 (2 C, C–C4, D–C5), 75.09 (D–C3), 74.99, 74.88, 74.81, 74.02 (B–C3), 73.56 (C–C6), 73.17, 72.84, 72.73 (A–C5), 72.54, 72.46 (B–C4), 72.00 (B–C5), 70.91 (B–C6), 70.60 (C–C5), 70.23 (B–C2), 69.38 (E–C5), 69.05 (A–C6), 68.82 (D–C6), 67.30, 65.57 (C–C8), 61.63, 61.12 (B–C7), 57.58 (A–C2), 53.15 (D–C2), 52.48, 33.77 (C–C3), 32.08, 29.85, 29.51, 29.15, 26.36, 26.11, 23.41, 22.84, 21.02, 14.27, 14.18. HRMS (m/z): [M + $Na^+$] calcd for $C_{142}H_{151}Cl_3F_3N_3NaO_{35}$, 2642.9038 found 2642.9104.

***N*-(2-mercaptoacetamide)-5-carbonylamino-pentyl-[L-*glycero*-α-D-manno-heptopyranosyl]-(1→4)-[2-deoxy-2-acetamine-β-D-gluco-pyranosyl]-(1→5)-3-deoxy-α-D-manno-oct-2-ulopyranosid-(2→6)-2-deoxy-2-amino-α-D-glucopyranosyl-(1→4)-α-D-galactopyrano-syluronic acid (1)**: A solution of pentasaccharide **17** (41 mg, 0.015 mmol) in DCM/TFA/$H_2O$ (1.8 mL/0.18 mL/0.02 mL) was stirred for 30 min at 0 °C. After TLC analysis showed consumption of staring material, the reaction was diluted with toluene (10 mL) and concentrated *in vacuo*. The obtained residue was dissolved in *t*-BuOH/$H_2O$ (4 mL/1 mL), and $Pd(OH)_2$/C (20 mg) and AcOH (20 μL) were added. The reaction was stirred for 24 h at room temperature under an atmosphere of $H_2$. The reaction mixture was filtered through celite and was concentrated *in vacuo* to give crude product **18**, which was used for the next step directly. A solution of pentasaccharide **18** in dry DMF (1.0 mL) was added S-acetylthioglycolic acid pentafluorophenyl ester (SAMA-OPfp, 9.4 mg, 0.031 mmol) and *N,N*-diisopropylethylamine (DIPEA, 5.4 μL, 0.031 mmol). The reaction was stirred for 3 h at room temperature, after which it was quenched with $H_2O$ (1 mL). The reaction mixture was concentrated in *in vacuo*, and the resulting residue was purified by size-exclusion chromatography (bio-gel P2, eluent: 5 % *n*-BuOH in $H_2O$) to give the corresponding pentasaccharide (14.4 mg, 49 %) was a white solid. The pentasaccharide (10 mg, 6.4 μmol) was dissolved in MeOH (1 mL), and the solution was cooled to 0 °C. NaOH (aqueous, 0.2 M, 1 mL) was then added, and the reaction mixture was stirred for 3 h at 0 °C. After LC–MS analysis showed complete conversion of starting material, the reaction was neutralized with Amberlite ($H^+$, washed with MeOH, aq. 1 M NaOH, $H_2O$, aq. 1 M HCl, $H_2O$ and MeOH sequentially) till pH = 7. The resin was filtered off, and the filtrate was treated with D,L-dithiothreitol (DTT, 0.2 mg). The obtained solution was stirred for 30 min at 40 °C, after which it was concentrated under reduced pressure. The resulting residue was purified by Bio-Gel P2 column using 5 % *n*-BuOH in $H_2O$ (deoxygenated before use) as eluent to give the final product **1** (7.1 mg, quantitive yield, 42 % yield from compound **17**) as white powder. HRMS (m/z): [M + $H^+$] calcd for $C_{42}H_{72}N_3O_{30}S$, 1130.3916 found 1130.3934. For $^1$H NMR data, see Table 1.

$^{13}$C NMR (151 MHz, $D_2O$) δ 102.07, 98.20, 97.56, 96.27, 79.51, 75.32, 72.74, 71.94, 70.97, 70.65, 70.49, 70.33, 70.17, 70.00, 69.52, 69.04, 68.55, 68.39, 67.91, 66.14, 63.72, 63.56, 62.75, 60.98, 60.34, 55.99, 54.05, 41.00, 39.71, 35.04, 34.39, 33.43, 27.95, 24.89, 22.79, 22.31, 19.73.

Compound **2** was prepared from **15** by a similar procedure as for the conversion of **17** into **1** and details are provided in the Supporting Information.

**General procedure for the conjugation 1 and 2 to carrier proteins**: Carrier protein (450 μL, 2.5 mg/mL in pH 7.4 HEPES buffer for $CRM_{197}$; 450 μL, 2.5 mg/mL in pH 7.4 PBS buffer for BSA) was purified by ultrafiltration (Amicon Ultra-0.5 Centrifugal Filter Unit, 10 K) 3 times use pH 7.4 PBS buffer as eluent. The concentrated protein was dissolved in PBS buffer (pH 7.4, 0.5 mL), and succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, 1.5 mg) was added, and the reaction mixture was shaken overnight at room temperature. The suspension was then centrifuged, and the supernatant was transferred to a centrifugal filter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10 K). After purified 5 times with PBS buffer (pH 7.4) as eluent, the concentrated protein was diluted with PBS buffer (pH 7.4) till ~10 mg/mL (concentration detected by nano-drop). Pentasaccharide **1** (1.9 mg for BSA, 0.92 mg for CRM) or tetrasaccharide **2** (1.1 mg for BSA, 1.1 mg for CRM) in water (100 μL) was added to the solution, and the reaction mixture was shaken overnight at room temperature. The solution was then transferred to a centrifugal filter (Amicon Ultra-0.5 Centrifugal Filter Unit, 10 K), and purified 5 times using PBS buffer (pH 7.4) as eluent to give the

**Table 1.** $^1$H NMR data of **1**.

| | H1 | H2 | H3 | H4 | H5 | H6(a) | H7(6b) | H8(Ac) |
|---|---|---|---|---|---|---|---|---|
| βGlcNAc | 4.59, d, 7.4 Hz | 3.68 | 3.56 | 3.47 | 3.51 | 3.96 | 3.76 | 2.01 |
| αKdo | – | – | 1.85/2.07 | 4.19 | 4.38 | 3.61 | 3.81 | 3.58/3.97 |
| αGlcN | 5.19, d, 3.4 Hz | 3.39 | 3.85 | 3.55 | 4.32 | 3.62 | 3.29 | – |
| αGalA | 5.01, d, 3.0 Hz | 3.85 | 4.05 | 4.36 | 4.21 | – | – | – |
| αHep | 5.10, s | 4.00 | 3.82 | 3.88 | 3.57 | 4.02 | 3.72 | – |
| Linker | 1.25×2, 1.30×2, 1.47, 1.55, 2.20/2.28, 3.17, 3.47 | | | | | | 3.38×2 ($CH_2SH$) | |

*During NMR measurements compound **1** had dimerized by disulfide formation.

glycoconjugate, which was diluted to ~1 mg/mL with pH 7.4 PBS buffer.

Sinapinic acid (5 mg) was dissolved in a mixture of acetonitrile/water/trifluoroacetic acid (3/7/0.01, v/v/v), and the suspension was treated by ultrasonification, then filtered through a PTFE syringe filter (Acrodisc, 0.2 μm), and the filtrate was used as a matrix for MALDI-TOF measurement.[29] The proteins (BSA, $CRM_{197}$, maleimide-modified BSA and maleimide-modified $CRM_{197}$) and glycoconjugate (BSA-**1**, CRM-**1**, BSA-**2** and CRM-**2**) were desalted with C18 ZipTip (Millipore, 10 μL) followed manufacturer's protocol, and the concentration was adjusted to ~1 mg/mL with milli-Q water. 1 μL of the desalted protein solution and 2 μL of the fresh prepared matrix were mixed, and 1 μL of the mixture were loaded on MALDI plate. The sample spots were dried by air, and then measured by MALDI-TOF using Linear positive mode. The results are provided in Figure S1 and S2 and Table S1 and S2.

**Mouse immunization**: Wild type female C57BL/6 J mice were housed under pathogen free conditions at Tulane University and the experiments were performed as per Institutional Animal Care and Use Committee of Tulane University. Two (8 to 12 weeks old) mice for each glycoconjugate were immunized intramuscularly on D0 with 50 μg of immunogen formulated with Complete Freund's Adjuvant (CFA; BD™ Difco™ Adjuvants, 263810). On D18, the mice received the 1st booster dose and on D32 the 2nd booster with the same immunogen but formulated with Incomplete Freund's Adjuvant (IFA; BD™ Difco™ Adjuvants, 263910). On D40 mice were euthanized and blood was collected by cardiac puncture. Serum was isolated and stored at −80 °C until further use.

**Bacterial FACS analysis for serum IgG binding**: Bacterial binding was performed as previously described[27] with minor changes. Briefly, *K. pneumoniae* 396 (K1 strain), ATCC 43816 (K2 strain), and ST258 (C4 strain) were grown in 3 mL tryptic LB Broth, Miller (Fisher BioReagents, BP1426-2) for 18 h at 37 °C. Cultures were then diluted 1:100 and grown for an additional 2 h to reach the early logarithmic phase. The concentration of *K. pneumoniae* was determined by measuring the absorbance at 600 nm. Bacteria were pelleted by centrifugation and washed twice in cold phosphate-buffered saline (PBS), resuspended in PBS and plated in 96-well plates at a density of $10^6$ cells/well. Bacteria were pelleted by centrifugation, then 25 μL of diluted immunized serum (10% in PBS) was added, suspensions were mixed and incubated at 4 °C overnight with gentle rocking. Next, bacteria were pelleted and washed twice as described above, pelleted again followed by addition of 25 μL of 1:100 diluted anti-mouse IgG conjugated to FITC (BioLegend, 406001). Suspensions were mixed and incubated for 30 min at room temperature with gentle rocking. Next cells were washed twice with PBS, fixated in 1% paraformaldehyde, washed again and resuspended in 200 μL PBS. Flow cytometry was performed (Cytek Aurora, Cytek Biosciences) and acquisition data were analyzed by FlowJo software and reported as % positive cells.

**Lipopolysaccharide (LPS) isolation from bacteria**: Similar as above, bacterial strains were grown to the early logarithmic phase. Bacteria were pelleted and re-suspended in sterile PBS to obtain an optical density (OD) of 0.5. Next, 4.5 mL of OD 0.5 adjusted bacterial suspension was incubated for 15 min at 95 °C and vortexed vigorously for 5 min to liberate LPS. The cells were pelleted by centrifugation and supernatant was transferred into a fresh 50-mL conical tube and 4 volumes of ice-cold 95% ethanol were added, mixed and incubated overnight at −20 °C for precipitation. Precipitates were pelleted by centrifuging at 2,500 x g for 10 min at 4 °C and air dried. Pellets were dissolved in 0.5 mL sterile PBS and DNAse (100 μg/mL; MilliporeSigma, DN25) and RNAse (100 μg/mL; ThermoFisher Scientific, EN053) were added for digestion and incubated overnight at 37 °C. Followed by digestion with pronase (100 μg/mL; MilliporeSigma, 10165921001) for 2 h at 56 °C. Post-digestion, aliquots were heated for 5 min at 95 °C, cooled down immediately on ice, and diluted further in coating buffer for ELISA.

**Serum IgG ELISA for LPS binding**: ELISA 96-well plates were coated with LPS diluted 1:100 in carbonate coating buffer (100 μL/well) overnight at 4 °C. Coated plates were rinsed with washing buffer (0.05% Tween 20 in PBS) and incubated for 2 h with blocking buffer (1% bovine serum albumin (BSA) and 0.1% Tween 20 in PBS). After washing, serum diluted 1:100 in assay diluent (0.5% BSA and 0.05% Tween 20 in PBS) was added and incubated for 1 h at 37 °C. After washing, goat anti-mouse IgG conjugated with horseradish peroxidase (Southern Biotech, 1030-05) 1:4,000 diluted in assay diluent was added and incubated for 1 h at room temperature. After washing, 100 μL TMB substrate solution (Thermo Scientific, N301) was added to each well and incubated in the dark for color development. The reaction was stopped by adding 100 μL stop solution and absorbance was measured at 450 nm using a microplate reader (BioTek).

**Microarray printing and screening**: The target compounds bearing a thiol-containing linker were dissolved in degassed and deionized water to make a 1 mM stock solution. To the solution (10 μL) was added immobilized TCEP (50 μL; Thermo Scientific, 77712) and PBS (40 μL). The mixture was vortexed for 5 min. LC/MS was used to confirm that the compounds has a free thiol. If needed, the reaction mixtures were gently warmed (~40 °C) to speed-up the reaction. The sample tubes were centrifuged to remove immobilized TCEP. An aliquot of each sample was then taken off the top and used for printing. All compounds were printed on maleimide activated glass slides (PolyAn GmbH, Germany) using a Scienion sciFLEXARRAYER S3 non-contact microarray printer equipped with a Scienion PDC80 nozzle (Scienion Inc.) The compounds were printed in replicates of 6 with spot volume ~400 pL at 20 °C and 50% humidity. Each slide has 24 subarrays in a 3×8 layout. After printing, slides were incubated in a humidity chamber for 8 h and then blocked with a 50 mM aqueous solution of mercaptoethanol for 1 h. Blocked slides were rinsed with DI water, spun dry, and kept in a desiccator at room temperature for future use. Printed glass slide was pre-blocked with a solution of 1x TSM binding buffer (20 mM Tris·HCl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$, 0.05% Tween-20, 1% BSA) for 30 min and the blocking solution was discarded. The sera (1:100 diluted in TSM binding buffer) were incubated on the glass slide for 1 h at room temperature, followed by washing and incubation with a goat anti-mouse IgG antibody conjugated to Alexa Fluor 633 (10 μg/mL; Invitrogen, A21050) for 30 min. The slide was sequentially washed with TSM wash buffer (20 mM Tris·HCl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$, 0.05% Tween-20), TSM buffer (20 mM Tris·HCl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$) and water. The slides were scanned using a GenePix 4000B microarray scanner (Molecular Devices) at the appropriate excitation wavelength with a resolution of 5 μM. Various gains and PMT values were employed in the scanning to ensure that all the signals were within the liner range of the scanner's detector and there was no saturation of signals. The image was analyzed using GenePix Pro 7 software (Molecular Devices, version 7.2.29.2). The data were analyzed with an Excel macro.[30] The highest and the lowest value of the total fluorescence intensity of the six replicates spots were removed, and the 4 middle values were used to provide the mean value and standard deviation.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** conjugation · glycoconjugate · glycosylation · oligosaccharides · vaccine

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