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Synthetic Oligosaccharides as Tools to Demonstrate Cross-Reactivity between Polysaccharide Antigens

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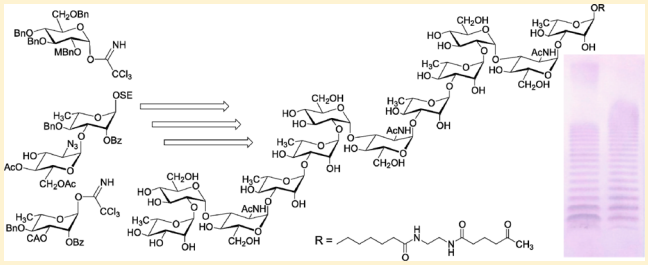
Supporting Information

ABSTRACT: *Escherichia coli* O148 is a nonencapsulated enterotoxigenic (ETEC) Gram negative bacterium that can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in humans. The surface-exposed O-specific polysaccharide (O-SP) of the lipopolysaccharide of this bacterium is considered both a virulence factor and a protective antigen. It is built up of the linear tetrasaccharide repeating unit [3)-α-L-Rha*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-GlcNAc*p*-(1→3)-α-L-Rha*p*-(1→] differing from that of the O-SP of *Shigella dysenteriae* type 1 (*SD*) only in that the latter contains a D-Gal*p* residue in place of the glucose moiety of the former. The close similarity of the O-SPs of these bacteria indicated a possible cross-reactivity. To answer this question we synthesized several oligosaccharide fragments of *E. coli* O148 O-SP, up to a dodecasaccharide, as well as their bovine serum albumin or recombinant diphtheria toxin conjugates. Immunization of mice with these conjugates induced anti-O-SP-specific serum IgG antibody responses. The antisera reacted equally well with the LPSs of both bacteria, indicating cross-reactivity between the *SD* and *E. coli* O148 O-SPs that was further supported by Western-blot and dot-blot analyses, as well as by inhibition of binding between the antisera and the O-SPs of both bacteria.



INTRODUCTION

Escherichia coli O148 is an enterotoxigenic (ETEC) bacterium that has been identified as a cause of enteric infections in children and adults, including dysentery, hemorrhagic colitis, and hemolytic uremic syndrome. This disease is endemic in developing countries and affects travelers to those areas.[1−5] The main carriers of ETEC bacteria are food and water,[5] and their spreading is facilitated by poor sanitary conditions. As has been reported recently regarding the spread of the bacterium *E. coli* O104 in several Western European countries, even the best sanitary conditions cannot always prevent an epidemic. It is likely that infections caused by various ETEC serotypes are under-reported because of insufficient surveillance and difficulties in serotyping. In early 2009, the World Health Organization declared the development of a vaccine against ETEC an urgency.[6,7]

[3)-α-L-Rha*p*-(1→2)-α-D-Glc*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ **A**

[3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*-(1→3)-α-D-Glc*p*NAc-(1→3)-α-L-Rha*p*-(1→]$_n$ **B**

E. coli O148 has been proposed to be a precursor to *Shigella dysenteriae* type 1 (*SD*), the most virulent of all *Shigellae*.[8] Virulence of these two bacteria is related to their O-specific polysaccharide (O-SP): those that lack a fully developed O-SP are not considered virulent.[9] Of the two, *SD* is the more common pathogen. The repeating unit of the O-SP of *E. coli* O148 **A** is similar to the O-SP of *SD* **B**, the only difference being in the chirality of a single carbon atom in the tetrasaccharide repeating unit: the galactose residue in *SD* is replaced by a D-glucose moiety in the *E. coli* repeating unit, while the anomeric configurations and the locations of all of the interglycosidic linkages are preserved. The two bacteria have the same genes for O antigen synthesis, except that in *SD*, a glucosyltransferase gene is interrupted by a deletion, and a galactosyltransferase gene located on a plasmid is responsible for the transfer of galactose to synthesize the O-antigen.[8] The two bacteria also have chemically identical LPS cores and O-chain−core linkage regions.[10]

On the basis of the close similarity of the O-SPs of *E. coli* O148 and *SD*, we hypothesized that they may cross-react; i.e., antibodies raised against one of the O-SPs or their fragments will react (bind) not only with the saccharides of the homologous organism but also with those of the cross-reacting ones. We note, however, that cross-reactivity between similar surface polysaccharides of different bacteria is not obvious. We approached this question by using synthetic oligosaccharides.

We have reported the synthesis of a panel of oligosaccharides related to the O-SP of *SD* up to a tetracosasaccharide[11−13] and demonstrated that the immunogenicity of their BSA conjugates in mice is influenced by the size of the saccharides, their loading on the protein, as well as by the identity of the nonreducing terminus.[12,14]

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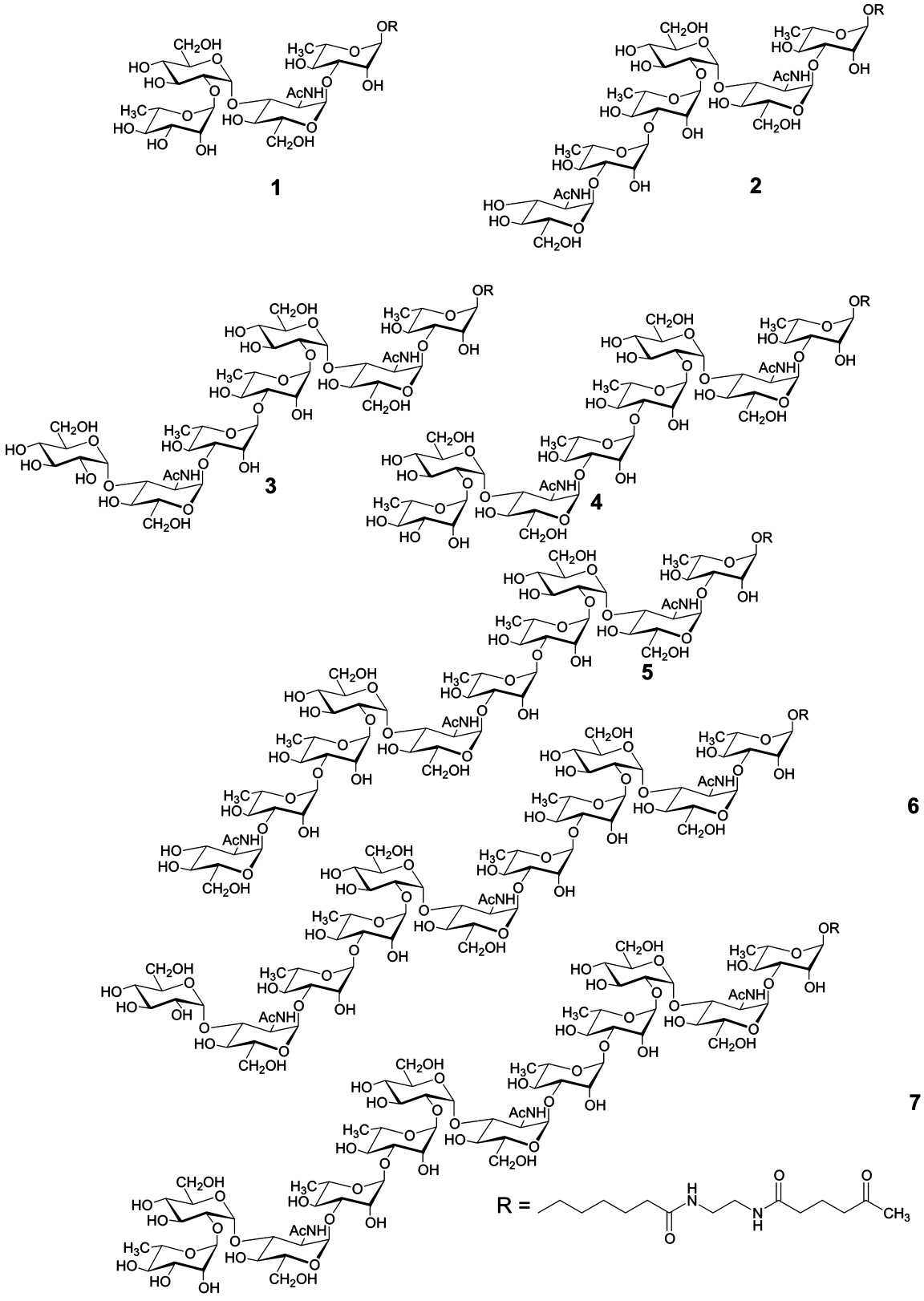

Figure 1. Spacer-equipped oligosaccharide fragments of the O-SP of *E. coli* O148 synthesized in this study.

In this paper, we report our studies that may lead to the development of a single component neoglycoconjugate vaccine against the enteric bacteria *SD* and *E. coli* O148, consisting of a covalent conjugate of an oligosaccharide fragment of the O-specific oligosaccharide of only one of them, covalently attached to an immunogenic protein. The idea behind using a protein conjugate of synthetic or natural oligosaccharides to induce anticarbohydrate serum is not new: it dates back to the early part of the twentieth century when it was shown that a covalent conjugate of the capsular polysaccharide of Type 3 pneumo-

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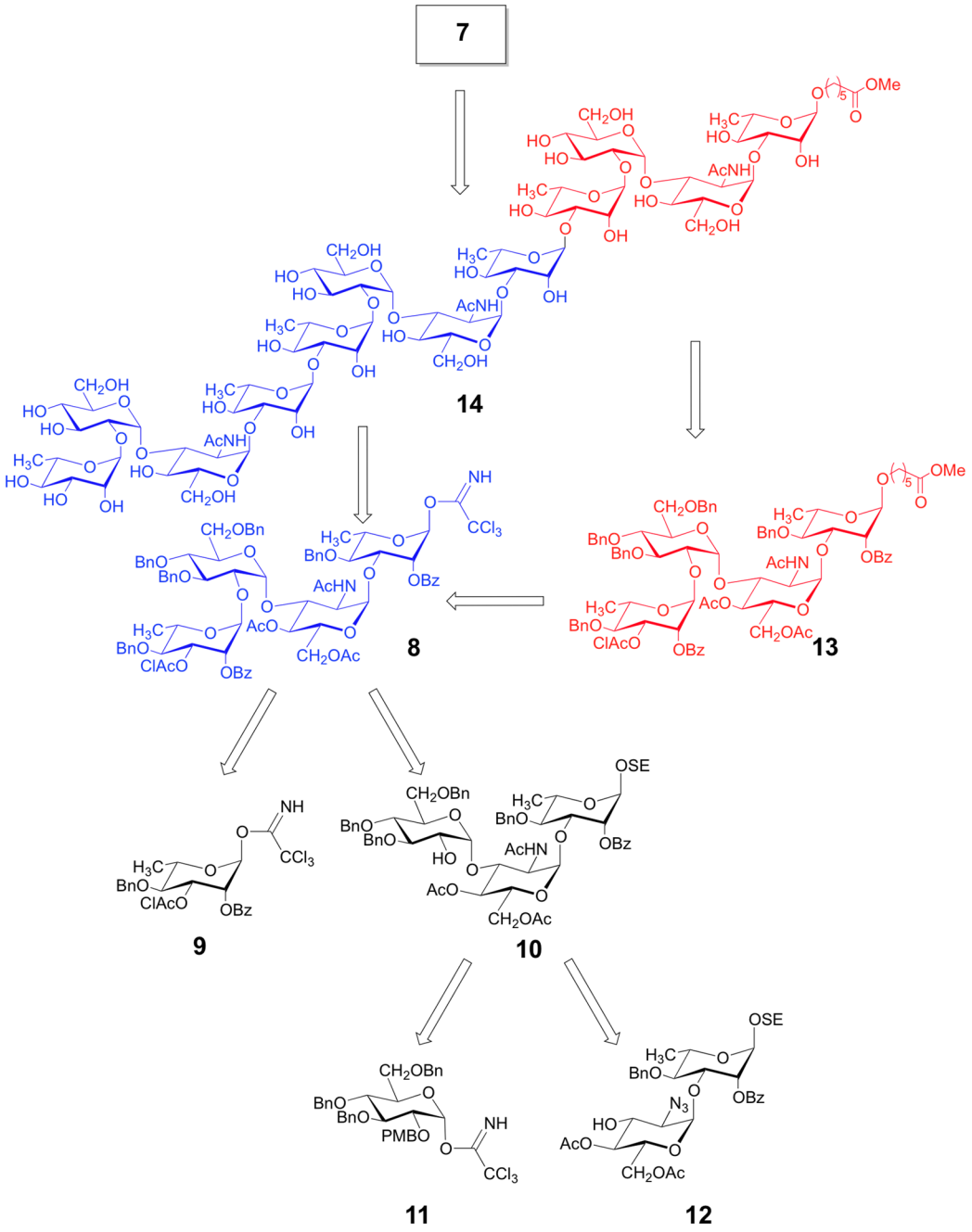

Figure 2. Retrosynthetic strategy toward the targeted oligosaccharides.

coccus with horse serum globulin elicited antipolysaccharide-specific antibodies in rabbits.[15−17] The antisera conferred both active and passive protection against the homologous organism. On the basis of this idea, several polysaccharide−protein conjugate vaccines have been developed for human use, including vaccines against *Haemophilus influenzae* type b, *Neisseria meningitidis* serotypes A, C, Y, W-135, pneumococci, and *Salmonella typhi*.[18] A tetanus toxoid conjugate of synthetic fragments of the capsular material of *H. influenzae* type b is also an efficacious vaccine for both children and adults.[19] The potential of synthetic oligosaccharide fragments of bacterial cell-surface glycans as antibacterial vaccines has generated increasing interest in the field that led to the synthesis of numerous bacterial oligosaccharides[20−24] and improved conjugation methods.[25]

Here, we first report the chemical synthesis of oligosaccharide components **1**−**7** of the O-SP of *E. coli* O148, ranging from tetra- to dodecasaccharides (Figure 1), then test their immunogenicity and cross-reactivity with the O-SP of *SD*. Oligosaccharides **1**−**7** are equipped with a heterobifunctional spacer to allow their one-point conjugation to the proteins bovine serum albumin (BSA) and recombinant diphtheria toxin H21G.[26] This protein is a genetically modified diphtheria toxin in which a histidine residue is replaced by glycine. The toxicity of the resulting protein is greatly reduced,[27] and the recombinant diphtheria toxin H21G has been shown to be an immunogenic carrier suitable for the preparation of conjugate vaccines.[28]

These constructs, injected without an adjuvant at a schedule and dosage compatible with use for humans, were used to evaluate the antibody response to the native O-SPs in mice. We

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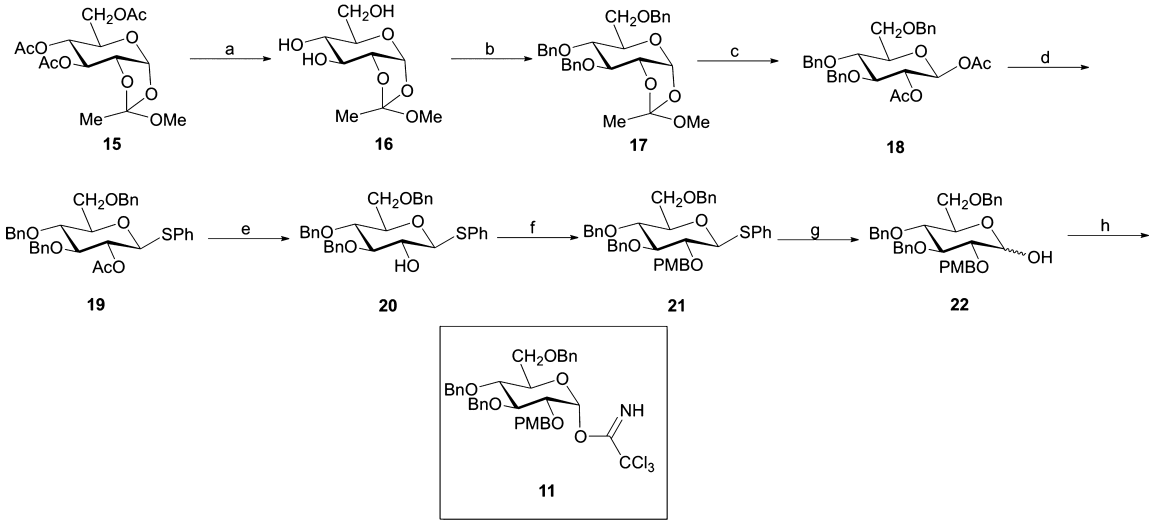
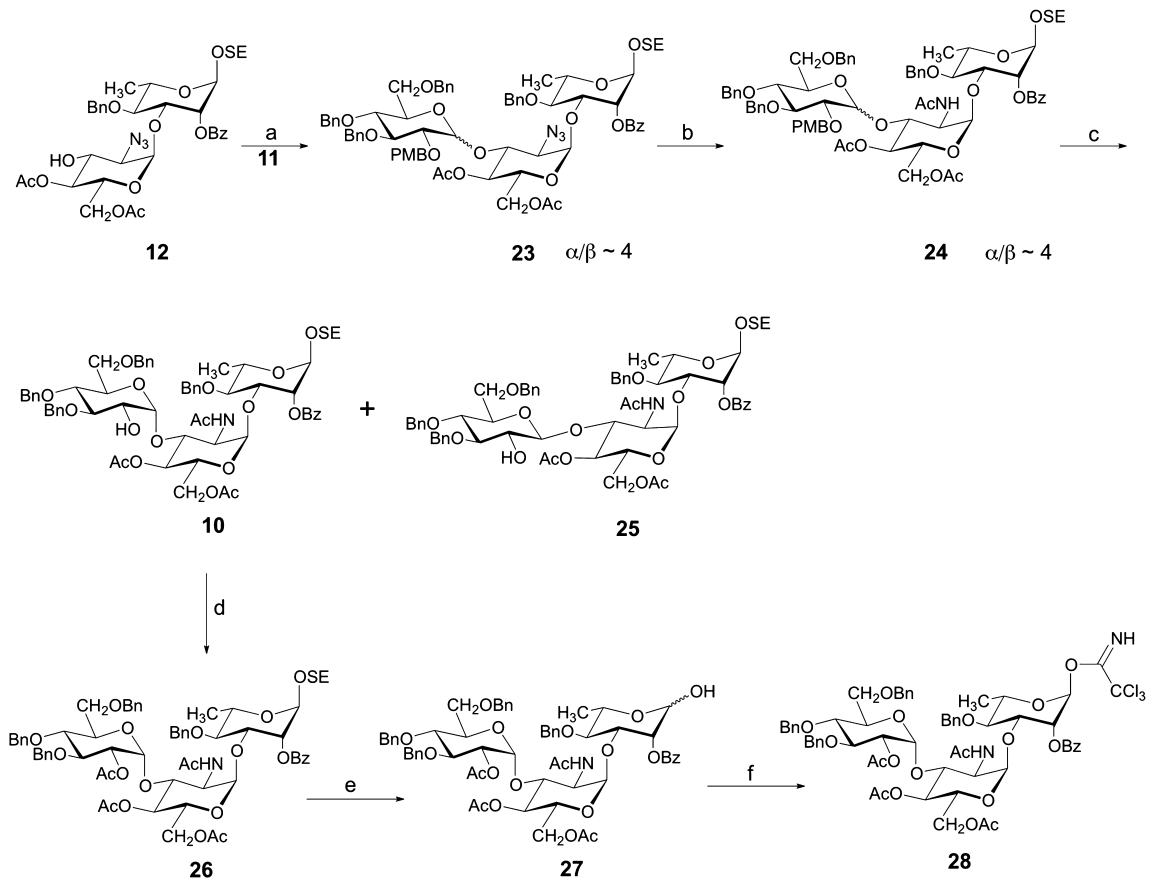
Scheme 1. Synthesis of Glucosyl Trichloroacetimidate 11[a]



[a]Reagents and conditions: (a) NaOMe, MeOH, quant.; (b) BnBr, NaH, DMF, 95%; (c) $AcOSi(CH_3)_3$ (excess), reflux, 2 h, 93%; (d) $PhSSi(CH_3)_3$, BF_3, Et_2O, CH_2Cl_2, 0 °C, 90 min, 84%; (e) MeONa, MeOH, 4 h, 97%; (f) PMBCl, NaH, DMF, 91%; (g) $(CF_3CO_2)_2Hg$, CH_2Cl_2, H_2O, 97%; (h) CCl_3CN, Cs_2CO_3, CH_2Cl_2, quantitative.

Scheme 2. Synthesis of the Trisaccharide Trichloroacetimidate 28[a]



[a]Reagents and conditions: (a) 1.6 equiv of **11**, TMSOTf, −40 → 0 °C, 2 h, 90%; (b) H_2 (200 psi), Pd/C, Et_3N, EtOAc, EtOH, Ac_2O; (c) $Ce(NH_4)_2(NO_3)_6$, CH_3CN, H_2O, yields: 57% for **10**, 11% for **25**; (d) Ac_2O, C_5H_5N, DMAP, CH_2Cl_2, quantitative; (e) TFA, CH_2Cl_2, 80%; (f) CCl_3CN, DBU, CH_2Cl_2, 95%.

also describe the isolation of purified LPSs of *E. coli* O148 and *SD* and report on their binding to mice sera elicited by various protein conjugates of the synthetic oligosaccharides, and to sera generated in rabbits by heat-killed *E. coli* O148 and *SD*. The selection of this panel of oligosaccharides was based on our observations on the immunogenicity of BSA conjugates of *SD*-

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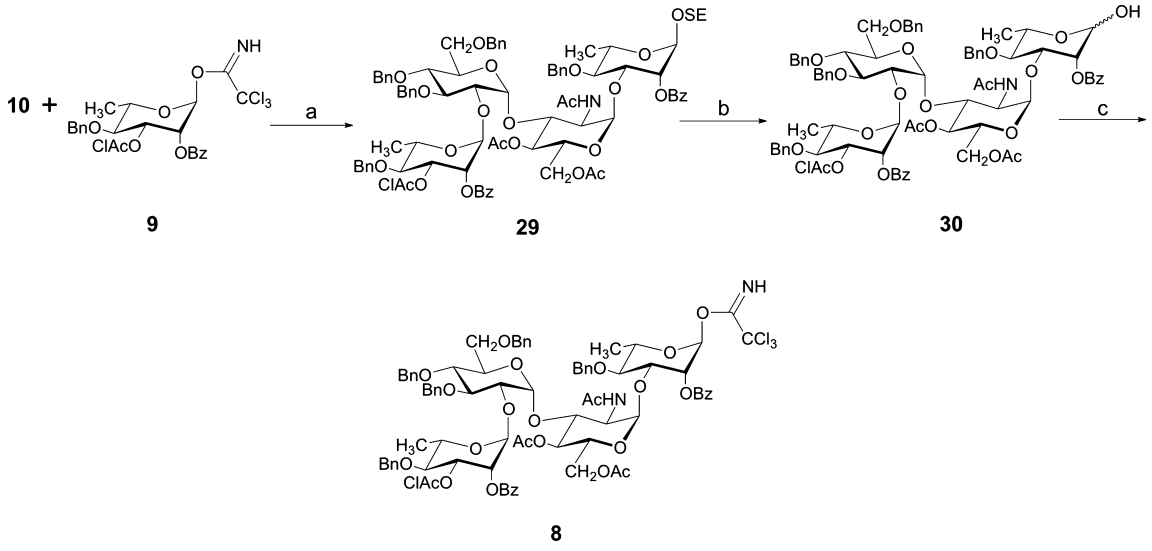
Scheme 3. Synthesis of the Tetrasaccharide Trichloroacetimidate 8[a]



[a]Reagents and conditions: (a) 4.5 equiv of **9**, TMSOTf, CH_2Cl_2, 0 → 23 °C, 3 h, 91%; (b) TFA, CH_2Cl_2, 23 °C, 3 h, 65%; (c) CCl_3CN, DBU, CH_2Cl_2, 0 → 23 °C, 1 h, 78%.

related oligosaccharides in mice.[12,14] In those studies, we found that the conjugates containing an *N*-acetyl-D-glucosamine or a D-galactose residue at the nonreducing end of the oligosaccharide portion induced statistically significantly higher O-SP-specific IgG antibody levels in mice than those having an L-rhamnose moiety at that position.

It is to be expected that mapping the immunogenicity of the protein conjugates of the synthesized oligosaccharides will contribute to the development of oligosaccharide-based semisynthetic vaccines against enteric diseases.

RESULTS

Synthetic Studies. We envisioned a blockwise approach to the target oligosaccharides **1**−**7** that required mono-, di-, tri-, and tetrasaccharide building units (Figure 2). For example, the retrosynthetic analysis of the dodecasaccharide **7** called for the tetrasaccharide donor **8** as the crucial intermediate that was prepared from the rhamnosyl donor **9**[11] and the trisaccharide acceptor **10** that in turn was constructed by condensation of the glucosyl donor **11** and the disaccharide alcohol **12**.[29] The spacer-linked tetrasaccharide **13** was obtained from the imidate **8**. The synthesis of the dodecasaccharide **14** was completed by two iterative sequential chain extension steps using the tetrasaccharide donor **8**. This was followed by global deprotection and installation of the linker to afford **7**, in which the spacer features a keto functionality to be used for the conjugation to proteins, using the oxime methodology.[30] The syntheses of the oligosaccharides **1**−**6** were carried out in a similar fashion, as described in detail below.

The tetrasaccharide block **8** was prepared from the trisaccharide **10**, assembly of which required the D-glucose derivative **11** that was prepared as shown in Scheme 1. The starting material was orthoester **15** that was deacetylated (→ **16**) followed by *O*-benzylation to afford compound **17**.[31] Next, we attempted to convert orthoester **17** into the diacetate **18**, using acetic acid.[32] It has been noted that in such conversions the acetic acid must be meticulously dry, because even traces of water would lead to complete hydrolysis.[31,32] A safer alternative is the use of $AcOSi(CH_3)_3$ instead of acetic acid.[33] Thus, boiling a solution of orthoester **17** in $AcOSi(CH_3)_3$ afforded the diacetate **18** in 93% yield after chromatographic purification. Next, **18** was converted to thioglucoside **19** with trimethylsilylthiophenol[34] in the presence of $BF_3{\cdot}Et_2O$ in 84% yield. Subsequently, the acetyl group in **19** was removed by NaOMe in MeOH to afford the alcohol **20.** The β anomeric configuration was ascertained from the $J_{1,2}$ coupling constant whose value was 9.5 Hz. Next, compound **20** was reacted with 4-methoxybenzyl chloride in the presence of NaH to afford **21** in 91% yield. Subsequent hydrolysis of the thioglucosyl linkage by the action of $(CF_3CO_2)_2Hg$[11,35] in moist CH_2Cl_2 afforded the hemiacetal **22** in 97% yield, from which the Schmidt-type donor **11** was prepared in nearly quantitative yield by treatment with CCl_3CN/Cs_2CO_3.

With the glucosyl imidate **11** in hand, synthesis of the trisaccharide donor **28** was undertaken. (Scheme 2) First, the disaccharide alcohol **12**[29] was glucosylated with compound **11** using TMSOTf activation. Under these conditions, an inseparable 4:1 mixture of the α and β-linked trisaccharides **23** was obtained in a combined yield of 90%. Attempted activation of **11** by other Lewis acids, e.g., BF_3-etherate, failed to improve the anomeric stereoselectivity. Next, the azido group was converted to acetamido by catalytic hydrogenation over palladium-on-charcoal in the presence of Et_3N, followed by reaction with Ac_2O (→ **24**). Subsequent treatment with ammonium ceric nitrate in a mixture of CH_3CN and water removed the methoxybenzyl group. To our delight, the required trisaccharide alcohol **10** could be isolated in a pure form in 57% yield after column chromatographic purification without the β glucosyl-linked minor product **25** that was also recovered in 11% yield. The synthesis of the trisaccharide **28** was completed by (i) acetylation of HO-2 of the glucose residue in **10** (→ **26**, quantitative), (ii) removal of the trimethylsilylethyl group by treatment of compound **26** with trifluoroacetic acid[36] (→ **27**, 80%), and (iii) exposure of the hemiacetal **27** to CCl_3CN/DBU to afford the trisaccharide trichloroacetimidate **28** in 95% yield.

The tetrasaccharide building block **8** was synthesized by coupling the trisaccharide acceptor **10** with the rhamnosyl donor **9**[11] under TMSOTf activation, to afford tetrasaccharide **29** in

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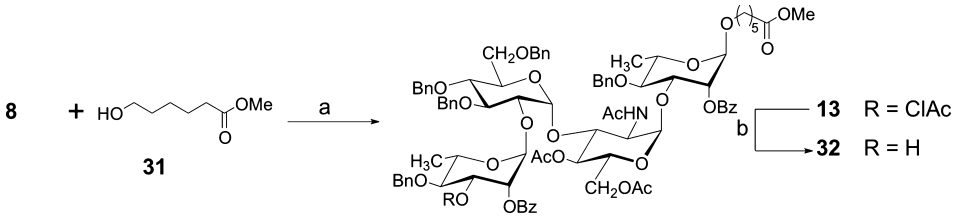
Scheme 4. Synthesis of the Spacer-Linked Tetrasaccharide Acceptor 32[a]



[a]Reagents and conditions: (a) 1.4 equiv of **31**, TMSOTf, CH_2Cl_2, 0 °C, 1 h, 81%; (b) $CS(NH_2)_2$, C_5H_5N, DMF, 23 °C, 12 h, 83%.

91% yield (Scheme 3). Routine removal of the trimethylsilylethyl group with trifluoroacetic acid in CH_2Cl_2[36] (→ **30**, 65%) followed by reaction with CCl_3CN/DBU yielded the tetrasaccharide trichloroacetimidate **8** in 78% yield.

As a prelude to the assembly of higher-membered oligosaccharides, the tetrasaccharide donor **8** was condensed with methoxycarbonylpentanol **31**[37] under TMSOTf promotion, to afford compound **13** in 81% yield (Scheme 4). We favor aglycon **31** as the linker because it is stable under a variety of conditions and can easily be converted to several reactive species for incorporation into proteins. Routine removal of the monochloroacetyl group by thiourea[38] afforded the tetrasaccharide alcohol **32** that was subjected to further chain extension steps with glycosyl donors as presented in Scheme 5. Condensation of the alcohol **32** with the di- **33**,[29] tri- (**28**), and tetrasaccharide (**8**) donors using TMSOTf as the activator in CH_2Cl_2 afforded the fully protected hexa- (**34**), hepta- (**35**), and octasaccharides (**36**) in the range of 60 to 91% yields. For further chain extension, the temporary monochloroacetyl group was removed from the fully protected octasaccharide **36** by treatment with thiourea to afford the alcohol **37** (77%). Glycosylation of the octamer **37** with the di- (**33**), tri- (**28**), and tetrasaccharides (**8**) using TMSOTf as the activator yielded the deca- (**38**), undeca- (**39**), and dodecasaccharides (**40**) (Scheme 5). In these condensations, the donors were used in 2- to 3-fold molar excesses, and the isolated yields were in the 60−91% range. Although the isolated yields of the protected oligosaccharides were, in most cases, acceptable, the procedures were not without the need for repeated column chromatographic purification, steps that undoubtedly contribute to the often moderate yields.

Preparation of the targets **1**−**7** from the fully protected tetra- to dodecasaccharides **13**, **34**, **35**, **36**, and **38**−**40** (denoted **C** in Scheme 6) proceeded in four stages: (i) base-promoted removal of the *O*-acyl protecting groups, (ii) hydrogenolytic removal of the *O*-benzyl groups (→ **D**), (iii) aminolysis with ethylenediamine (→ **E**), and (iv) N-acylation with 5-ketohexanoic anhydride (→ **F**). Removal of all the protecting groups was ascertained by the ^1H and ^{13}C NMR spectra of the oligosaccharides so obtained that were consistent with the proposed structures.

In addition to further transformations as described below, three representative intermediates **14**, **41**, and **42** (Figure 3) were used in detailed NMR studies.

Nuclear Magnetic Resonance Studies. The three methoxycarbonylpentyl glycosides **14**, **41**, and **42** were examined in detail by ^1H and ^{13}C NMR spectroscopy, using 2D COSY-30 and TOCSY to assign the ^1H spectra, and 2D HSQC and HMBC to confirm assignments for the ^{13}C spectra. ^1H chemical shifts for the glycosides **41** and **42** are shown in Table 1, and for **14** in Table 2. Vicinal and geminal $^1H-^1H$ coupling constants are listed for **41** and **42** in Table 3, and for **14** in Table 4. ^{13}C chemical shifts are shown for **41** and **42** in Table 5, and for **14** in Table 6. Finally, values of the $^1J_{C\text{-}1,H\text{-}1}$ coupling constants measured by ^1H-coupled 2D HSQC for **41**, **42**, and **14** may be compared in Table 7. The observation of exclusively large $^1J_{C\text{-}1,H\text{-}1}$ values (168.9−175.8 Hz) by ^1H-coupled 2D HSQC indicates the α anomeric configuration for all linkages in the three oligosaccharide glycosides, as synthetically designed. Similar repeating structural environments in glycosides **42** and **14** cause equivalence or near-equivalence of the chemical shifts of residues in the same immediate environment. These residues are Glc C and G in **42**, and residues Glc C, G and K; GlcNAc F and J; Rha D and H; and Rha E and I, in **14**. For example, the multiplet patterns in the ^1H-coupled 2D HSQC spectra of **42** (Figure 4a) and **14** (Figure 4b) are remarkably similar. However, close inspection of these spectra reveals intensity differences due to the coincidence of resonances of residues in equivalent environments. According to the ^1H chemical shifts of **14** (Table 2), the structural environment of GlcNAc B is observably different to those of GlcNAc F and J, apparently because of the proximity of GlcNAc B to the unique Rha A residue at the glycoside terminus. Characteristic ^{13}C-1 chemical shifts were observed for the different types of residues: GlcNAc at ∼95 ppm, Glc at 98.5 ppm, Rha A at ∼100 ppm, Rha glycosidically attached to Glc at 102.1−102.3 ppm, and Rha glycosidically bound to Rha at 102.7−102.8 ppm. All of the linkage positions in the three oligosaccharide glycosides **41**, **42**, and **14** were confirmed by the observation of specific connectivities in the 2D HMBC spectra, for one or two $^{13}C,H$ pairs per linkage. Interpretation of the $^1H-^1H$ coupling constants (Tables 3 and 4) for the glycosides **41**, **42**, and **14** according to Karplus considerations confirmed the types of sugar residues present and their chair conformations.

Conjugation of the Oligosaccharides to Proteins. Oligosaccharides do not elicit an immune response when injected into mammals. However, they can be converted to immunogens when covalently linked to immunogenic proteins. Of the numerous possible approaches, selective oxime formation between a keto-derivatized carbohydrate and an aminooxy-equipped protein counterpart stands out with its simple operation, mild conditions, and applicability to a variety of saccharide haptens, ranging from mono- to polysaccharides.[14,30,39] In our approach (Scheme 7), the spacer-equipped oligosaccharides **1**−**7** (**F**) were combined with the aminooxy-derivatized proteins **I** to afford the conjugates **J**, at pH 7.2 or below.

The aminooxy moiety was introduced into the protein carriers in a two-step process. First, the proteins BSA or recombinant diphtheria toxin (**G**) were reacted with succinimidyl (3-bromoacetamido)propionate to afford structure **H** featuring a labile bromine moiety that, upon reacting with 2-aminooxypropanethiol,[40] allowed the formation of a stable thioether linkage connecting the derivatized protein and the aminooxy

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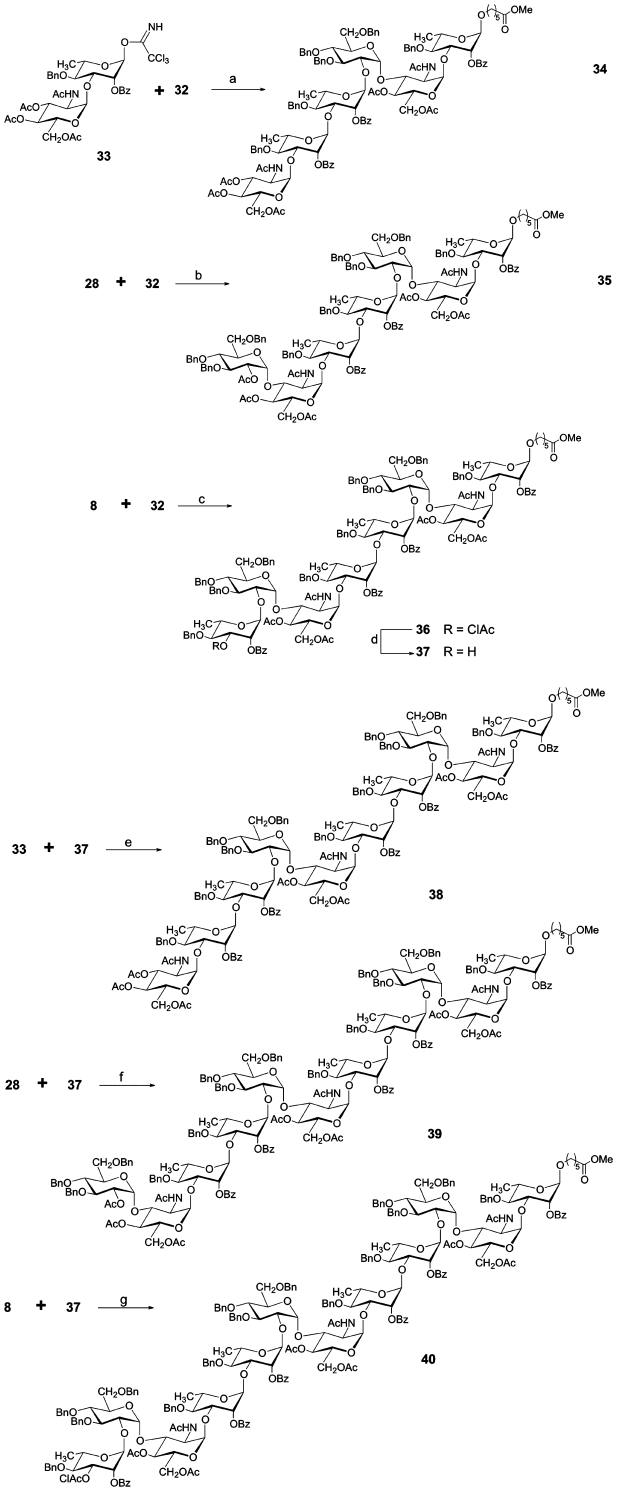
Scheme 5. Synthesis of the Higher-Membered Oligosaccharides 34−40[a]



[a]Reagents and conditions: (a) 2.3 equiv of **33**, TMSOTf, CH_2Cl_2, 0 °C, 45 min, 91%; (b) 2.3 equiv of **28**, TMSOTf, CH_2Cl_2, 0 °C, 40 min, 60%; (c) 2.1 equiv of **8**, TMSOTf, CH_2Cl_2, 0 → 23 °C, 1 h, 88%; (d) $CS(NH_2)_2$, C_5H_5N, DMF, 23 °C, 16 h, 77%; (e) 5.2 equiv of **33**, TMSOTf, CH_2Cl_2, 0 → 23 °C, 1 h, 88%; (f) 3.5 equiv of **28**, TMSOTf, CH_2Cl_2, 0 → 23 °C, 1 h, 86%; (g) 1.9 equiv of **8**, TMSOTf, CH_2Cl_2, 0 → 23 °C, 1 h, 64%.

moiety (→ **I**). Depending on the ratio of the bromoacyl derivatized protein and the aminooxypropanethiol, up to 35 aminooxy moieties were attached to the proteins. In order to avoid overderivatization that might reduce immunogenicity, the number of the carbohydrate chains were limited to an average of 20 or less. The conjugates were purified through a Sephadex G-50 column to afford neoglycoproteins having an average of up to 15 oligosaccharide chains, as determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). For the details of the determination of the average number of carbohydrate chains, see ref 30.

Serum Antibody Responses. The immunogenicity of the conjugates containing the *E. coli* O148 oligosaccharides and those made from oligosaccharide portions related to the LPS of *SD*[14] (Table 8) was estimated in groups of 10 mice (Table 9). Three subcutaneous injections containing 2.5 μg of saccharides in the form of conjugates in phosphate buffered saline (PBS) were given without adjuvant at 2 week intervals. We note that almost all of the synthetic oligosaccharide-based carbohydrate−protein conjugates reported in the literature have been administered in Freund's complete adjuvant followed by two injections in Freund's incomplete adjuvant. Since Freund's adjuvant cannot be given to humans, the extrapolation of the experimental data for human use is at least questionable. All of the conjugates tested induced significantly higher IgG antibody levels against both LPSs than the control group that received PBS at the same schedule (not shown, $p < 0.0001$). There were no statistical differences between the anti-*SD* IgG levels induced either by the *SD* or by the *E. coli* oligosaccharide conjugates (data in column 5, Table 9) with one exception: the conjugate of the *E. coli* 10mer **5** (item no. 5) induced a statistically lower anti-SD level than did the conjugate of the *SD* 10mer (**44**) conjugate (item no. 11, 4.6 vs 11.8 EU, $p = 0.03$). Similarly, there were no statistical differences between the anti-*E. coli* O148 levels induced by any of the conjugates (column 6, Table 9). The highest antibody levels against both LPSs were induced by the BSA conjugate of the *E. coli* octasaccharide **4** (item no. 4). Some of the differences, e.g., the higher anti-*SD* response by the conjugate of the *E. coli* 8-mer **4** versus that of the *SD* octasaccharide **43** (item 10), may be a consequence of different loadings on the protein. In general, the conjugates that induced high anti-*SD* IgG levels also induced high anti-*E. coli* O148 IgG levels. These data support the assumption that immunity to either of these bacteria would protect the host against both of them.

Immunoblotting. Western blot assays were performed to evaluate if sera of immunized animals cross-react with LPSs of both bacteria. Sera used in these experiments were induced either in rabbits by heat-killed bacteria or in mice by the conjugates of synthetic oligosaccharides corresponding to their O-SP subunits: compound **7** (12mer) in the case of *E. coli* O148 and compounds **44**, **45**, **46**, and **47** (10, 11, 12, and 13mer) (Table 9) in the case of *SD*.[14] The results presented in Figure 5 show that all sera reacted with both LPSs with similar intensity and in both directions.

In a dot-blot experiment, BSA conjugates of the monosaccharides galactose and glucose containing an average of 22 monosaccharide residues per protein (not described in the experimental) did not bind to sera raised to either bacterium. The BSA conjugate of compound **1** (4mer), representing one repeating unit of *E. coli* O148 O-SP, bound to sera raised by either bacterium less strongly than did conjugates of compounds **2**−**7**, which underwent binding with approximately the same intensity.

Competitive Inhibition of Binding. In order to corroborate the results of the immunoblotting experiments and

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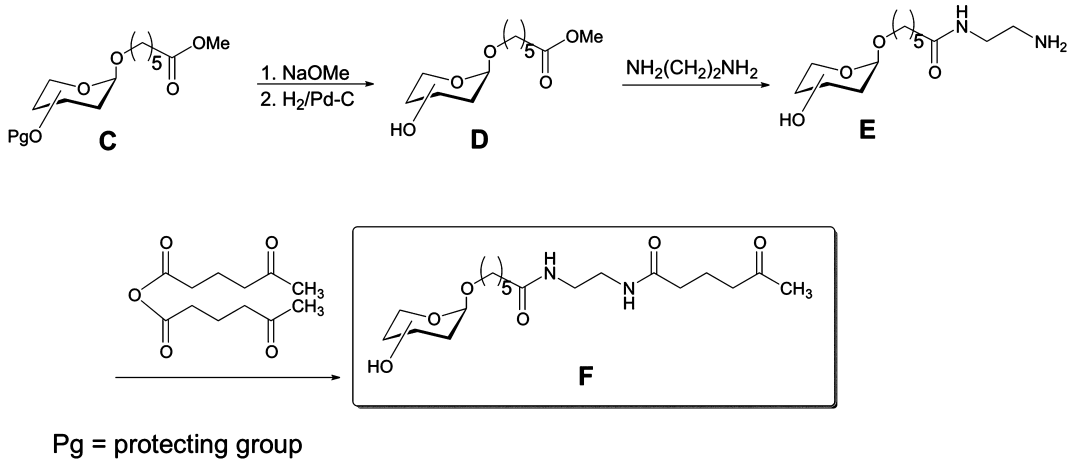
Scheme 6. Removal of the Protecting Groups and Attachment of the Linker Moiety



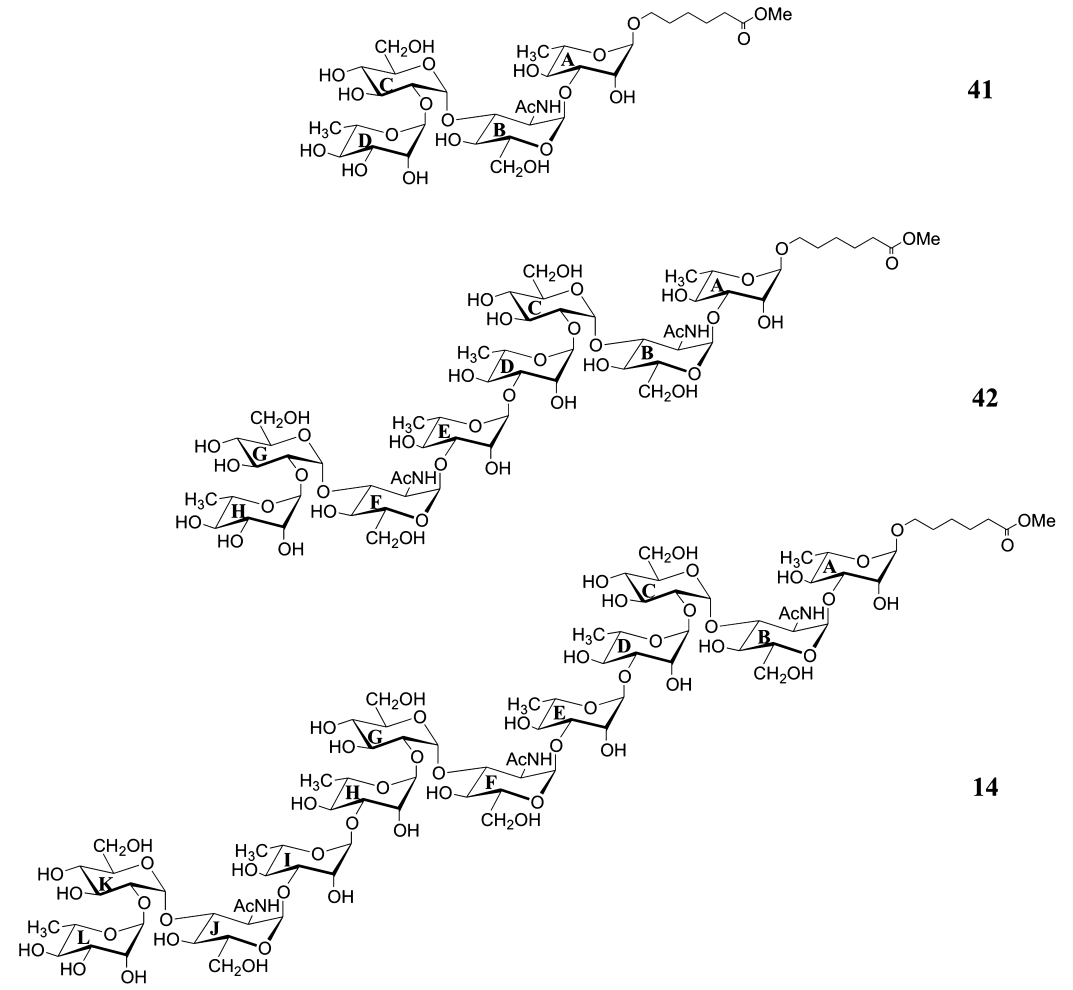


Figure 3. Tetra- **41**, octa- (**42**), and dodecasaccharide (**14**) methoxycarbonylpentyl glycosides.

demonstrate further that the cross-reactivity between *SD* and *E. coli* O148 is due to the O-SP portion of their respective lipopolysaccharides, we analyzed the inhibition of binding between sera induced by DT conjugates of *SD* 12mer (compound **46**) and *E. coli* O148 12mer (compound **7**) and their respective LPSs (Tables 10 and 11), using the O-SP of the unrelated bacterium *Bordetella bronchiseptica* as the control. The data in Tables 10 and 11 demonstrate dose-related inhibition of binding within similar ranges, thus confirming that the cross reactivity between *SD* and *E. coli* O148 is, indeed, due to their respective O-SPs.

■ DISCUSSION

As part of our program directed toward the development of carbohydrate-based conjugate vaccines against human pathogenic bacteria, we were intrigued as to whether there exists cross-

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Table 1. ^{1}H NMR Chemical Shifts (ppm) of the *E. coli* O148 Tetra- (41) and Octasaccharide (42) 5-Methoxycarbonylpentyl Glycosides

	tetrasaccharide **41**					octasaccharide **42**								
	A	B	C	D	MeOCO	A	B	C	D	E	F	G	H	MeOCO
	Rha	GlcNAc	Glc	Rha	Pentyl	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Pentyl
H-1	4.806	4.981	5.534	5.106	3.702	4.801	4.980	5.564	5.103	5.123	5.038	5.564	5.127	3.695
H-1′	–	–	–	–	3.527	–	–	–	–	–	–	–	–	3.512
H-2	4.067	4.128	3.660	4.069	1.627	4.066	4.136	3.662	4.182	4.247	4.140	3.662	4.077	1.612
H-3	3.810	4.032	3.730	3.788	1.377	3.825	4.067	3.754	3.886	3.968	4.081	3.754	3.802	1.377
H-4	3.525	3.785	3.493	3.471	1.627	3.547	3.803	3.488	3.572	3.576	3.794	3.486	3.490	1.612
H-5	3.722	~4.012	~3.639	3.840	2.404	3.717	~4.041	3.658	3.901	3.915	~4.053	3.658	3.857	2.391
H-6	1.312	3.808	3.852	1.293	–	1.314	3.841	3.876	1.303	1.349	3.845	3.868	1.303	–
H-6′	–	3.808	3.802	–	–	–	3.841	3.796	–	–	3.804	3.796	–	–
MeO	–	–	–	–	3.690	–	–	–	–	–	–	–	–	3.685
NAc	–	2.041	–	–		–	2.053[a]	–	–	–	2.061[a]	–	–	–

[a]Interchangeable.

Table 2. ^{1}H NMR Chemical Shifts (ppm) of the *E. coli* Dodecasaccharide 5-Methoxycarbonylpentyl Glycoside 14

	dodecasaccharide residues												
	A	B	C	D	E	F	G	H	I	J	K	L	MeOCO
	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Pentyl
H-1	4.801	4.980	5.581	5.121	5.136	5.044	5.580	5.118	5.136	5.044	5.578	5.139	3.692
H-1′	–	–	–	–	–	–	–	–	–	–	–	–	3.506
H-2	4.065	4.145	3.662	4.191	4.258[a]	4.150	3.662	4.189	4.261[a]	4.150	3.662	4.083	1.612
H-3	3.833	4.088	3.767	3.896	3.987	4.102	3.767	3.895	3.987	4.102	3.767	3.810	1.380
H-4	3.560	3.811	3.483	3.586	3.590	3.797	3.483	3.585	3.590	3.797	3.481	3.501	1.612
H-5	3.713	4.052	3.661	~3.905	3.928	4.064	3.661	~3.904	3.590	4.064	3.661	3.865	2.386
H-6	1.315	3.838	3.890	1.311	1.356	3.860	3.890	1.308	1.356	3.860	3.881	1.308	–
H-6′	–	3.814	3.793	–	–	3.804	3.793	–	–	3.804	3.793	–	–
MeO	–	–	–	–	–	–	–	–	–	–	–	–	3.684
NAc	–	2.061[b]	–	–	–	2.072[b]	–	–	–	2.070[b]	–	–	–

[a,b]Interchangeable within each letter group.

Table 3. ^{1}H−^{1}H Coupling Constants (Hz) of the *E. coli* O148 Tetra- (41) and Octasaccharide (42) 5-Methoxycarbonylpentyl Glycosides

	tetrasaccharide residues				octasaccharide residues							
	A	B	C	D	A	B	C	D	E	F	G	H
	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha
$J_{1,2}$	2.1	3.6	3.6	1.8	1.7	3.6	3.6	1.7	~2.1	3.6	3.6	1.8
$J_{2,3}$	3.3	10.6	10.0	3.6	3.6	10.7	9.8	3.2	3.3	10.7	9.8	3.6
$J_{3,4}$	10.1	8.8	8.9	10.0	10.2	8.7	9.1	9.6	10.6	8.7	8.8	10.1
$J_{4,5}$	9.8	10.2	10.1	9.8	9.8	10.0	10.2	9.4	9.7	9.8	10.0	9.8
$J_{5,6}$	6.3	nr[a]	2.3	6.3	6.2	nr	2.3	6.2	6.2	2.0	2.1	6.4
$J_{5,6'}$	–	nr	4.2	–	–	nr	4.6	–	–	3.9	4.6	–
$J_{6,6'}$	–	nr	12.3	–	–	nr	12.2	–	–	12.4	12.0	–

[a]nr, not resolved.

reactivity between the O-SPs of *SD* and *E. coli* O148 based on their close structural similarity. We hypothesized that cross reactivity between the O-SPs may serve as a basis for protection against both bacteria by a single monovalent vaccine and decided to explore cross-reactivity by using oligosaccharide fragments of the O-SPs of both bacteria, in the form of covalent conjugates with immunogenic proteins. Such oligosaccharides are difficult to isolate in homogeneous form from the native O-SP because of the difficulty of its site-specific fragmentation by either chemical or enzymatic processes. Therefore, we prepared the required oligosaccharides by chemical syntheses as described in detail in the Experimental Section.

The synthetic oligosaccharides were equipped with a linking arm featuring an oxo function for covalent attachment to immunogenic proteins, including bovine serum albumin and recombinant diphtheria toxin, by using the oxime method.[30] The level of incorporation was determined by MALDI-TOF mass spectrometry and reached up to an average of 15 oligosaccharide chains per protein molecule. The neoglycoconjugates, subcutaneously injected in groups of 10 mice, induced IgG antibodies with O-SP specificities shown by immunochemical experiments.

Western-blot analysis demonstrated that sera raised against heat-killed *SD* or *E. coli* O148 bacteria in rabbits or against the synthetic oligosaccharide−protein conjugates in mice reacted

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Table 4. $^1H-^1H$ Coupling Constants (Hz) of the *E. coli* O148 Dodecasaccharide 5-Methoxycarbonylpentyl Glycoside **14**

	dodecasaccharide residues											
	A	B	C	D	E	F	G	H	I	J	K	L
	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha
$J_{1,2}$	1.8	3.6	3.8	1.8	1.5	3.6	3.7	1.7	2.0	3.6	3.7	1.8
$J_{2,3}$	3.1	10.6	10.0	3.1	3.4	10.5	10.0	3.1	3.3	10.5	10.0	3.3
$J_{3,4}$	9.9	8.5	9.1	9.7	9.9	8.6	9.1	9.8	9.9	8.6	9.1	10.1
$J_{4,5}$	9.6	10.2	10.0	9.6	9.7	9.9	10.0	9.7	9.7	9.9	10.1	9.7
$J_{5,6}$	6.2	3.0	2.2	6.2	6.2	2.5	2.2	6.3	6.2	2.5	2.1	6.2
$J_{5,6'}$	–	4.3	5.1	–	–	4.9	5.1	–	–	4.9	5.1	–
$J_{6,6'}$	–	12.8	12.3	–	–	~11.7	12.3	–	–	~11.7	12.2	–

with the lipopolysaccharides of either *SD* or of *E. coli* O148, in both directions. The dot blot assays indicate the importance of the length of the oligosaccharide chains. BSA conjugates of the monosaccharides galactose and glucose failed to react with either of the antisera, and the conjugate of the tetrasaccharide **1** reacted weakly as compared with the conjugates of the hexa- to dodecasaccharides **2**–**7**, which reacted with comparable intensities. These qualitative observations support the view that a chain length exceeding a complete repeating unit is necessary for specific antigenic recognition by either *SD* or *E.coli* O148 antibodies. Further proof for the cross-reactivity was provided by competitive inhibition of binding between sera raised by the neoglycoconjugates and their respective homologous LPS. The observed cross-reactivity suggests that anti-O-SP-based immunity against one of these organisms would provide protection to both, an observation that should be conducive to a single monovalent vaccine against the two organisms.

EXPERIMENTAL SECTION

General Chemical Synthesis. All chemicals were commercial grade and used without purification. Solvents for chromatography were distilled prior to use. All glycosylation reactions were carried out after drying the reacting partners at 10 μm or less for 12 h except for the reaction with imidate **11** that was used immediately after its preparation. Glycosylation reactions as well as preparation of trichloroacetimidates were carried out under argon. Column chromatography was performed on silica gel 60 (0.040–0.063 mm), and thin layer chromatography was performed on glass-supported silica gel layers or on HPTLC plates. Visualization was carried out by inspection under UV light (254 nm), by iodine adsorption, and by charring using a solution of ammonium cerium(IV) sulfate and ammonium molybdate in sulfuric acid. Column chromatography was performed on silica gel 60 (230–400 mesh). Routine ^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, using $CDCl_3$ as the solvent unless indicated otherwise. Chemical shifts are recorded in ppm relative to internal references. For ^1H: 0.00 for $(CH_3)_4Si$, 3.30 for CD_2HOD, or 2.225 for acetone. For ^{13}C: 48.90 for CD_3OD, 31.00 for acetone, or 77.00 for $CDCl_3$. Glycoside **41** was examined as its solution in D_2O, but for **42** and **14**, the D_2O lock signals were found to be insensitive to shimming. Therefore, spectra for **41** and **42** were recorded for solutions in D_2O with 20% v/v of acetone-d_6 present. The ^1H and ^{13}C NMR spectra of oligosaccharides **1**–**7** were measured in D_2O. The spectra of **2**, **3**, **5**, and **6** for which partial assignments are presented were measured in D_2O/acetone-d_6 (4:1 v/v). Interchangeable assignments are denoted with an asterisk. The monosaccharide residues are denoted with subscripted capital letters starting with A for the reducing end unit. Because of the instability of the acetone-d_6 lock signal in the presence of a strong D_2O resonance, the best procedure found was to shim on acetone-d_6, then lock on D_2O for data acquisition. The methods used for 1D and 2D NMR data acquisition were similar to those reported by us recently for a set of glycolipids.[41] Atmospheric pressure electrospray ionization mass spectrometry (API-ES-MS) was carried out on an LC/MSD SL spectrometer.

General Procedure for the Deprotection of Oligosaccharides 13, 34–36, and 38–40. The protected oligosaccharide **C** (Scheme 6) was dissolved in anhydrous $CHCl_3$ to which anhydrous MeOH was added. To the resulting solution was added a solution of NaOMe in MeOH (25 wt %) at room temperature until the pH of the solution reached approximately 12 as estimated with a moistened indicator paper. After 24 h, the solution was neutralized with Dowex (H^+) resin. Next, the resin was removed by filtration, and the volatiles were removed under reduced pressure. To a solution of the residue so obtained in EtOH was added Pd/C, and the resulting mixture was stirred under hydrogen (200 psi) for 24 h. Removal of the catalyst by filtration through a layer of Celite followed by concentration afforded the deprotected oligosaccharides as 5-methoxycarbonyl glycosides **D**. In addition to further transformations as described below, compounds **14**, **41**, and **42** (Figure 3) were used in NMR studies.

General Procedure for the Preparation of the Spacer-Linked Oligosaccharides 1–7. The deprotected oligosaccharide was dissolved in 1,2-diaminoethane. After 24 h at room temperature, the solution was diluted with water followed by evaporation of the volatiles by freeze-drying. The residue was purified by chromatography through a Bio-Gel P-2 column, using 0.02 M pyridine acetate in water as the eluant. The fractions containing carbohydrates as determined by the phenol-sulfuric acid assay were combined, and the solution was freeze-dried. This cycle was repeated two more times to afford intermediate **E** (Scheme 6) as a white amorphous solid. To a stirred solution of derivative **E** in MeOH were added triethylamine and 5-ketohexanoic anhydride.[30] After 10 min, the solution was concentrated under reduced pressure. To the residue was added water, and the mixture was stirred with a magnetic stirring bar. The flask containing the solution was immersed in ice–water. The clear solution was siphoned off with a Pasteur pipet, the tip of which was covered with tissue paper. The solution so obtained was freeze-dried. To the residue was added 0.02 M pyridine–acetate buffer. The mixture was filtered through a pad of tissue paper to remove residual solid particles. The clear solution was passed through a Biogel *P*-4 column using 0.02 M pyridine-acetate as the eluant. The fractions containing carbohydrates were pooled and freeze-dried. The residue was dissolved in water, and the solution was freeze-dried. This cycle was repeated two more times to afford compound **F** as a white amorphous solid.

Synthesis and/or Characterization of the New Compounds Described in This Paper. *5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (1).* ^1H NMR (D_2O, partial) δ 5.53 (d, 1H, J = 3.6 Hz), 5.11 (br s, 1H), 4.98 (d, 1H, J = 3.6 Hz), 4.80 (br s, 1H), 4.13 (dd, 1H, J = 3.6 Hz, J = 10.4 Hz), 3.30 (br s, 4H), 2.58 (t, 2H, J = 7.4 Hz), 2.25–2.24 (m, 4H), 2.20, 2.04 (2 s, 2 × 3H), 1.83–1.77 (m, 2H), 1.64–1.54 (m, 4H), 1.40–1.37 (m, 2H), 1.31, 1.29 (2 d, 2 × 3H); ^{13}C NMR (D_2O) δ 216.2, 177.9, 177.0, 174.8, 102.2, 100.2, 98.5, 94.9, 77.1, 75.89, 75.85, 73.1, 72.9, 72.6, 72.5, 71.7, 71.0, 70.7, 70.5, 70.0, 69.8, 69.4, 68.4, 67.4, 60.8, 60.7, 52.6, 42.8, 39.3, 39.2, 36.5, 35.6, 30.0, 28.9, 25.8, 25.7, 22.9, 20.33, 20.28, 17.4, 17.3. HRMS m/z calcd for $[C_{40}H_{69}N_3O_{22}]H^+$: 944.4451. Found: 944.4449.

5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-

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Table 5. ^{13}C NMR Chemical Shifts (ppm) of the *E. coli* O148 tetra- (41) and Octasaccharide (42) 5-Methoxycarbonylpentyl Glycosides

	tetrasaccharide residues					octasaccharide residues								
	A	B	C	D	MeOCO	A	B	C	D	E	F	G	H	MeOCO
	Rha	GlcNAc	Glc	Rha	Pentyl	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Pentyl
C-1	100.23	94.94	98.49	102.25	68.52	100.32	94.98	98.47	102.14	102.73	94.88	98.47	102.26	68.39
C-2	67.48	52.65	77.15	70.58	24.74[a]	67.54	52.66	77.30	70.37	67.49	52.67	77.12	70.65	24.84[b]
C-3	75.91	75.98	73.14	70.78	25.66	76.01	76.22	73.33[c]	78.90	75.67	75.82	73.18[c]	70.87	25.76
C-4	71.00	71.69	69.81	72.62	28.86[a]	71.03	71.87	69.99	72.01	71.08	71.87	70.07	72.68	28.99[b]
C-5	69.41	72.49	72.95	69.99	34.33	69.39	72.55	72.99	69.99	69.99	72.63	72.99	69.95	34.31
C-6	17.42	60.71	60.88	17.34	–	17.53	60.86	61.08	17.47[d]	17.68	60.95	61.08	17.58[d]	–
MeO	–	–	–	–	52.85	–	–	–	–	–	–	–	–	52.74
NAc	–	22.89	–	–	–	–	22.93[e]	–	–	–	22.95[e]	–	–	–
NC═O	–	174.81	–	–	–	–	174.58	–	–	–	174.49	–	–	–
OC═O	–	–	–	–	178.33	–	–	–	–	–	–	–	–	177.77

[a,b,c,d,e]Interchangeable within each letter group.

Table 6. ^{13}C NMR Chemical Shifts (ppm) of the *E. coli* O148 Dodecasaccharide 5-Methoxycarbonylpentyl Glycoside 14

	dodecasaccharide residues												
	A	B	C	D	E	F	G	H	I	J	K	L	MeOCO
	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Pentyl
C-1	100.38	95.00	98.49	102.15	102.77[a]	94.87	98.51[b]	102.15	102.73[a]	94.87	98.46[b]	102.25	68.31
C-2	67.58	52.74	77.29	70.41[c]	67.50[d]	52.66	77.29	70.40[c]	67.49[d]	52.66	77.11	70.69	24.91[e]
C-3	76.08	76.19[f]	73.27[g]	78.98[h]	75.70	75.78[e]	73.23[g]	78.96[h]	75.70	76.19[f]	73.23[g]	70.94	25.82
C-4	71.05	71.97	70.12	72.02[i]	71.11	71.97	70.12	72.03[i]	71.11	71.97	70.12	72.72	29.09[e]
C-5	69.37	72.62	73.02	70.05	69.92	72.66[j]	73.02	69.92	69.92	72.70[j]	73.02	69.92	34.31
C-6	17.68	60.97	61.23[k]	17.60	17.75	61.09[l]	61.21[k]	17.60	17.75	61.05[l]	61.21[k]	17.55	–
MeO	–	–	–	–	–	–	–	–	–	–	–	–	52.66
NAc	–	22.98[m]	–	–	–	22.98[m]	–	–	–	22.96[m]	–	–	–
NC═O	–	174.46	–	–	–	174.41[n]	–	–	–	174.33[n]	–	–	–
OC═O	–	–	–	–	–	–	–	–	–	–	–	–	177.38

[a,b,c,d,e,f,g,h,i,j,k,l,m,n]Interchangeable within each letter group.

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Table 7. $^1J_{C\text{-}1,H\text{-}1}$ Coupling Constants (Hz) of the *E. coli* O148 Tetra- (41), Octa- (42), and Dodecasaccharide 14 5-Methoxycarbonylpentyl Glycosides

	tetrasaccharide residues				octasaccharide residues							
	A	B	C	D	A	B	C	D	E	F	G	H
	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha
$^1J_{C\text{-}1,H\text{-}1}$	170.0	171.6	175.8	172.6	170.0	171.0	174.4	173.0	170.6	172.0	174.4	172.4
	dodecasaccharide residues											
	A	B	C	D	E	F	G	H	I	J	K	L
	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha	Rha	GlcNAc	Glc	Rha
$^1J_{C\text{-}1,H\text{-}1}$	169.9	168.9	175.3	171.8	171.1	168.9	175.3	171.8	171.1	168.9	175.3	172.8

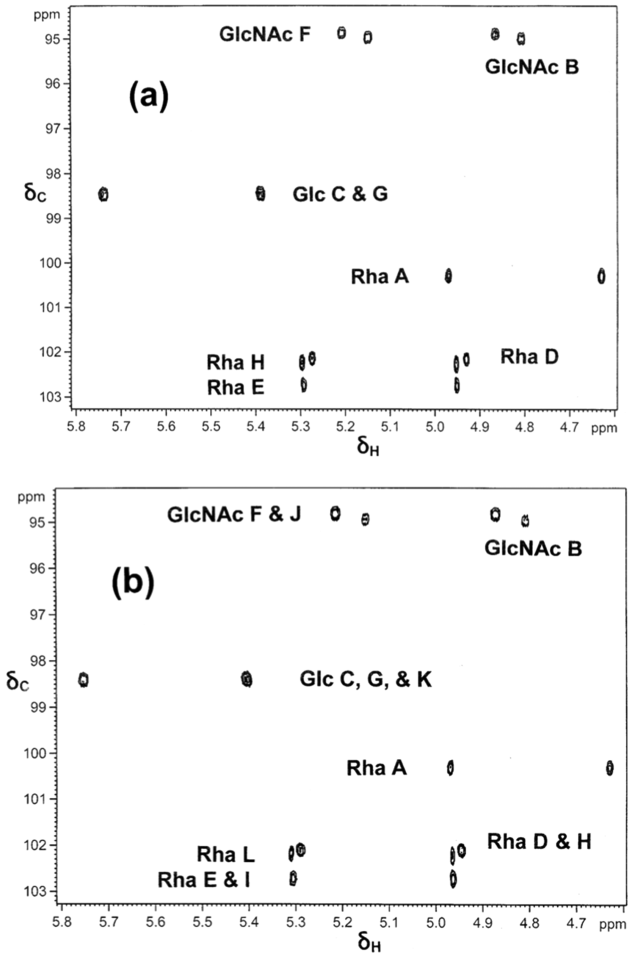

Figure 4. ^{1}H-coupled 2D HSQC spectrum of (a) octasaccharide **42** and (b) dodecasaccharide **14**.

2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**2**). ^{1}H NMR (D_2O, partial) δ 5.55 (d, 1H, J = 3.6 Hz, H-1$_C$), 5.11 (d, 1H, J = 1.4 Hz, H-1$_E$), 5.10 (d, 1H, J = 1.2 Hz, H-1$_D$), 5.06 (d, 1H, J = 3.7 Hz, H-1$_F$), 4.98 (d, 1H, J = 3.7 Hz, H-1$_B$), 4.80 (d, 1H, J = 1.5 Hz, H-1$_A$), 3.30 (br, 4H), 2.57 (t, 2H, J = 7.3 Hz), 2.23, 2.21 (2 t, 2 × 2H, J ~ 7 Hz), 2.19, 2.04, 2.03 (3 s, 3 × 3H), 1.80 (m, 2H), 1.63−1.54 (m, 4H), 1.38−1.27 (m, 11H); ^{13}C NMR (D_2O) δ 216.2, 177.9, 177.0, 175.2, 174.8, 102.70 ($J_{C\text{-}1,H\text{-}1}$ = 171.9 Hz, C-1$_E$), 102.09 ($J_{C\text{-}1,H\text{-}1}$ = 173.1 Hz, C-1$_D$), 100.23 ($J_{C\text{-}1,H\text{-}1}$ = 171.3 Hz, C-1$_A$), 98.45 ($J_{C\text{-}1,H\text{-}1}$ = 177.8 Hz, C-1$_C$), 94.98 ($J_{C\text{-}1,H\text{-}1}$ = 172.9 Hz, C-1$_F$), 94.90 ($J_{C\text{-}1,H\text{-}1}$ = 173.6 Hz, C-1$_B$), 78.8, 77.3, 76.3, 76.0, 75.9, 73.1, 73.0, 72.6, 72.5, 72.0, 71.8, 71.7, 71.1, 71.0, 70.5, 70.3, 70.1, 70.0, 69.8, 69.4, 68.5, 67.6, 67.5, 61.0, 60.9, 60.8, 54.4, 52.7, 42.9, 39.4, 39.3, 36.5, 35.6, 30.1, 29.0, 25.9, 25.7, 22.9, 22.7, 20.4, 17.6, 17.5, 17.4. HRMS m/z calcd for $[C_{54}H_{91}N_4O_{31}]H^+$: 1315.5643. Found: 1315.5658.

5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**3**). ^{1}H NMR (D_2O, partial) δ 5.56 (d, 1H, J = 3.7 Hz, H-1$_C$), 5.39 (d, 1H, J = 3.9 Hz, H-1$_G$), 5.12 (d, 1H, J = 1.6 Hz, H-1$_E$), 5.10 (d, 1H, J = 1.5 Hz, H-1$_D$), 5.05 (d, 1H, J = 3.6 Hz, H-1$_F$), 4.98 (d, 1H, J = 3.7 Hz, H-1$_B$), 4.80 (d, 1H, J = 1.7 Hz, H-1$_A$), 4.22, 4.16 (2 br, 2 × 1H), 3.30 (br, 4H), 2.57 (t, 1H, J = 7.3 Hz), 2.24−2.20 (m, 4H), 2.19 (s, 3H), 2.03 (s, 6H), 1.80 (m, 2H), 1.65−1.54 (m, 4H), 1.39−1.26 (m, 11H); ^{13}C NMR (D_2O) δ 216.3, 178.0, 177.1, 174.9, 174.8, 102.72 ($J_{C\text{-}1,H\text{-}1}$ = 175.1 Hz, C-1$_E$), 102.15 ($J_{C\text{-}1,H\text{-}1}$ = 174.3 Hz, C-1$_D$), 100.33 ($J_{C\text{-}1,H\text{-}1}$ = 178.1 Hz, C-1$_G$), 100.28 ($J_{C\text{-}1,H\text{-}1}$ = 170.7 Hz, C-1$_A$), 98.49 ($J_{C\text{-}1,H\text{-}1}$ = 177.3 Hz, C-1$_C$), 95.11 ($J_{C\text{-}1,H\text{-}1}$ = 172.9 Hz, C-1$_F$), 94.95 ($J_{C\text{-}1,H\text{-}1}$ = 173.3 Hz, C-1$_B$), 78.9, 78.6, 77.4, 76.3, 76.0, 75.9, 73.6, 73.2, 73.02, 72.98, 72.5, 72.4, 72.1, 71.8, 71.2, 71.14, 71.08, 70.4, 70.2, 70.2, 69.9, 69.8, 69.5, 68.6, 67.6, 67.5, 60.93, 60.90, 60.83, 52.7, 43.0, 39.4, 39.3, 36.6, 35.7, 30.1, 29.0, 25.9, 25.8, 23.0, 20.4, 17.7, 17.54, 17.50. HRMS m/z calcd for $[C_{60}H_{102}N_4O_{36}]H^+$: 1455.6334. Found: 1455.6341.

5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**4**). ^{1}H NMR (D_2O, partial) δ 5.53 (br, 2H), 5.10 (br, 2H), 5.08 (br, 1H), 5.02 and 4.97 (2 d, 2 × 1H, J ~ 3.6 Hz), 4.79 (br, 1H), 4.22, 4.16, 4.13, 4.11 (4 br, 4 × 1H), 4.07 (br, 2H), 3.93 (dd, 1H, J = 3.3 Hz, J = 10.0 Hz), 3.30 (m, 4H); ^{13}C NMR (D_2O) δ 216.3, 178.0, 177.1, 174.9, 174.8, 1s02.7, 102.3, 102.2, 100.2, 98.5, 95.0, 94.9, 78.8, 77.3, 77.2, 76.3, 75.93, 75.91, 75.7, 73.2, 73.1, 73.0, 72.6, 72.5, 74.5, 72.0, 71.8, 71.7, 71.1, 71.0, 70.8, 70.6, 70.3, 70.1, 70.0, 69.8, 69.4, 68.5, 67.5, 60.9, 60.7, 42.9, 39.4, 39.3, 36.5, 35.6, 30.0, 29.9, 25.9, 25.7, 22.9, 20.4, 17.6, 17.5, 17.4, 17.3. HRMS m/z calcd for $[C_{66}H_{112}N_4O_{40}]H^+$: 1601.6931. Found: 1601.7007.

5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl 2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**5**). ^{1}H NMR (D_2O, partial) δ 5.561 (d, 1H, J = 3.6 Hz, H-1$_C$), 5.559 (d, 1H, J = 3.6 Hz, H-1$_G$), 5.118 and 5.114 (2d, 2 × 1H, J ~ 2.8 and 1.8 Hz, H-1$_{E,I}$), 5.102 and 5.099 (2d, 2 × 1H, J ~ 1.7 and 1.5 Hz, H-1$_{D,H}$), 5.06 (d, 1H, J = 3.8 Hz, H-1$_J$), 5.04 (d, 1H, J = 3.6 Hz, H-1$_F$), 4.98 (d, 1H, J = 3.7 Hz, H-1$_B$), 4.80 (d, 1H, J = 1.7 Hz, H-1$_A$), 4.98 (d, 1H, J = 3.7 Hz, H-1$_B$), 4.80 (d, 1H, J = 1.7 Hz, H-1$_A$), 4.23, 4.17 (2 br s, 2 × 1H), 4.17 (br s, 2H), 3.30 (s, 4H), 2.58 (t, 2H, J ~ 7.1 Hz), 2.38 (t, J ~ 7.0 Hz), 2.24, 2.22 (2 t, 2 × 1H. J ~ 7.0 Hz), 2.20, 2.05, 2.04, 2.03 (4 s, 4 × 3H), 1.83−1.77 (m, 2H), 1.65−1.55 (m, 4H), 1.34 (d, 6H, J ~ 6.3 Hz), 1.31 (d, 3H, J = 6.3 Hz), 1.30 (d, 6H, J ~ 6.3 Hz); ^{13}C NMR (D_2O) δ 216.2, 177.9, 177.0, 175.2, 174.80, 174.78, 102.74 ($J_{C\text{-}1,H\text{-}1}$ = 172.2 Hz, C-1$_{E,I}$), 102.13 ($J_{C\text{-}1,H\text{-}1}$ = 172.3 Hz, C-1$_{D,H}$), 100.26 ($J_{C\text{-}1,H\text{-}1}$ = 169.6 Hz, C-1$_A$), 98.49 ($J_{C\text{-}1,H\text{-}1}$ = 175.3 Hz, C-1$_{C,G}$), 95.01 ($J_{C\text{-}1,H\text{-}1}$ = 170.8 Hz, C-1$_J$), 94.93* ($J_{C\text{-}1,H\text{-}1}$ = 170.9 Hz, C-1$_F$), 94.88* ($J_{C\text{-}1,H\text{-}1}$ = 174.1 Hz, C-1$_B$), 78.8, 77.3, 76.26, 76.0, 75.9, 75.6, 73.1, 73.0, 72.6, 72.5, 72.0, 71.8, 71.7, 71.13, 71.08, 71.0, 70.5, 70.3, 70.1, 70.0, 69.96, 69.8, 69.4, 68.5, 67.6,

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Scheme 7. Synthesis of Neoglycoproteins by Oxime-Conjugation between Carbohydrates and Proteins

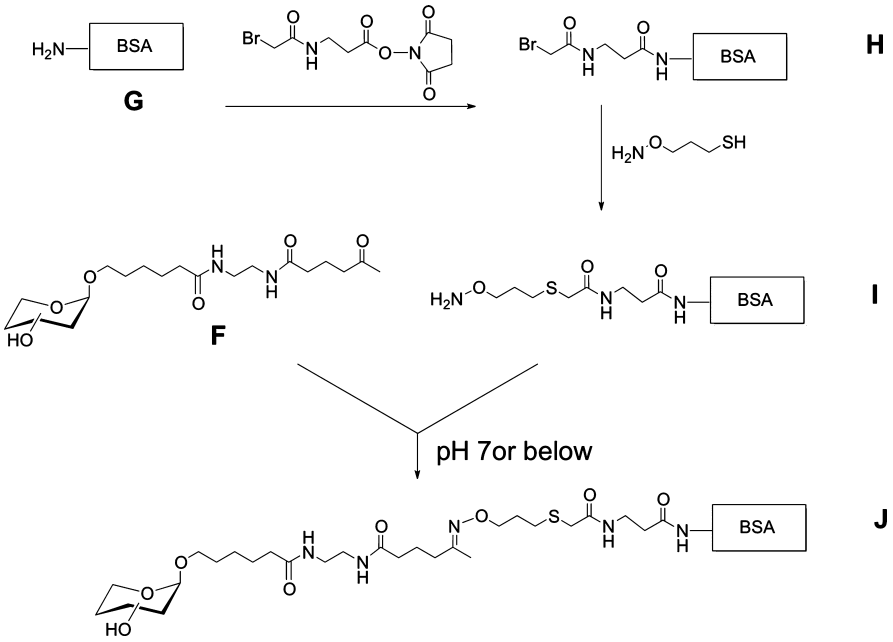
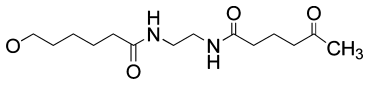


Table 8. Oligosaccharides Related to *S. dysentariae* Type 1 Lipopolysaccharide That Were Used in This Study

R=



[α-L-**Rhap**-1,2-α-D-**Galp**-1,3-α-D-**Glc*p*NAc**-1,3-α-L-**Rhap**]$_2$-R	43
α-D-**Glc*p*NAc**-1,3-α-L-**Rha*p***-1,3-[α-L-**Rhap**−1,2-α-D-**Galp**-1,3-α-D-**Glc*p*NAc**-1,3-α-L-**Rhap**]$_2$-R	44
α-D-**Galp**-1,3-α-D-**Glc*p*NAc**-1,3-α-L-**Rhap**-1,3-[α-L-**Rhap**−1,2-α-D-**Galp**-1,3-α-D-**Glc*p*NAc**-1,3-α-L-**Rhap**]$_2$-R	45
[α-L-**Rhap**-1,2-α-D-**Galp**-1,3-α-D-**Glc*p*NAc**-1,3-α-L-**Rhap**]$_3$-R	46
α-L-**Rhap**-1,3-[α-L-**Rhap**−1,2-α-D-**Galp**-1,3-α-D-**Glc*p*NAc**-1,3-α-L-**Rhap**]$_3$-R	47

67.5, 61.0, 60.9, 54.4, 52.7, 42.9, 39.4, 39.3, 36.5, 35.6, 30.1, 29.0, 25.9, 25.7, 22.92, 22.91, 22.7, 20.4, 17.6, 17.5, 17.4. HRMS *m*/*z* calcd for $[C_{80}H_{135}N_5O_{49}]H^+$: 1950.8304. Found: 1950.8314.

*5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**6**).* ^{1}H NMR (D_2O, partial) δ 5.56 (br d, 2H, $J \sim 2.6$ and 2.8 Hz, H-$1_{C,G}$), 5.39 (d, 1H, $J = 3.9$ Hz, H-1_K), 5.12 (br s, 2H, H-$1_{E,I}$), 5.10 (br s, 2H, H-$1_{D,H}$), 5.05 (d, 1H, $J = 3.8$ Hz, H-1_J), 5.04 (d, 1H, $J \sim 3.9$ Hz, H-1_F), 4.98 (d, 1H, $J = 3.6$ Hz, H-1_B), 4.80 (d, 1H, $J = 1.5$ Hz, H-1_A), 4.23, 4.17 (2 br s, 2 × 2H), 3.30 (s, 4H), 2.23, 2.22 (2 t, 2 × 1H, $J \sim 7$ Hz), 2.19, 2.04, 2.03 (3 s, 3 × 3H), 1.83−1.77 (m, 2H), 1.64−1.53 (m, 4H), 1.33 (d, 6H, $J \sim 6.3$ Hz), 1.30 (d, 3H, $J = 6.3$ Hz), 1.29 (d, 6H, $J = 6.3$ Hz); ^{13}C NMR (D_2O) δ 216.3, 178.0, 177.1, 174.9, 174.83, 174.81,102.69 ($J_{C\text{-}1,H\text{-}1} = 170.7$ Hz, C-$1_{E,I}$), 102.13 ($J_{C\text{-}1,H\text{-}1} = 172.7$ Hz, C-$1_{D,H}$), 100.32 ($J_{C\text{-}1,H\text{-}1} = 172.5$ Hz, C-1_K), 100.26 ($J_{C\text{-}1,H\text{-}1} = 172.1$ Hz, C-1_A), 98.48 ($J_{C\text{-}1,H\text{-}1} = 174.9$ Hz, C-$1_{C,G}$), 95.09 ($J_{C\text{-}1,H\text{-}1} = 172.5$ Hz, C-1_J), 94.93*($J_{C\text{-}1,H\text{-}1} = 172.4$ Hz, C-1_F), 94.88*($J_{C\text{-}1,H\text{-}1} = 172.4$ Hz, C-1_B), 78.8, 78.5, 77.3, 76.3, 75.9, 73.6, 73.2, 73.0, 72.9, 72.5, 72.4, 72.0, 71.8, 71.2, 71.1, 71.0, 70.3, 70.1, 70.0, 69.8, 69.7, 69.4, 68.5, 67.6, 67.5, 60.87, 60.86, 60.8, 52.7, 42.9, 39.4, 39.3, 36.5, 35.6, 30.1, 29.0, 25.9, 25.7, 22.9, 20.4, 17.6, 17.5, 17.4. HRMS *m*/*z* calcd for $[C_{86}H_{145}N_5O_{54}]H^+$: 2112.8832. Found: 2112.8816.

*5-{2-[(5′-Oxohexanoyl)amino]ethyl-amino}carbonylpentyl α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**7**).* ^{1}H NMR (D_2O, partial) δ 5.57 (br s, 3H), 5.13 (br s, 2H), 5.06 (br d, 2H, $J = 3.5$ Hz), 5.00 (br s, 1H, $J = 3.5$ Hz), 4.83 (br s, 1H), 4.26, 4.19 (2 br s, 2 × 2H), 3.33 (br s, 4H), 2.16 (t, 2H, $J = 7.3$ Hz), 2.27, 2.26 (2 t, 2 × 2H, $J \sim 7$ Hz), 2.10−2.04 (m, 9H), 1.86−1.80 (m, 2H), 1.67−1.57 (m, 4H), 1.42−1.30 (m, 18H); ^{13}C NMR (D_2O) δ 216.2, 177.9, 177.1, 174.84, 174.81, 174.8, 102.72, 102.69, 102.3, 102.2, 102.1, 100.2, 98.5, 94.95, 94.92, 78.8, 77.3, 77.1, 76.3, 75.92, 75.90, 75.6, 73.20, 73.16, 73.02, 73.01, 72.96, 72.93, 72.92, 72.91, 72.6, 72.52, 72.48, 72.04, 72.02, 71.99, 71.8, 71.7, 71.10, 71.07, 71.06, 71.02, 70.99, 70.8, 70.6, 70.3, 70.2, 70.12, 70.08, 70.07, 70.06, 70.05, 70.0, 69.9, 69.85, 69.80, 69.4, 68.5, 67.5, 67.4, 60.93, 60.92, 60.91, 60.87, 60.86, 60.83, 60.76, 60.75, 60.73, 60.72, 60.70, 52.7, 42.9, 39.39, 39.37, 39.3, 36.5, 35.6, 30.1, 29.0, 25.9, 25.7, 22.9, 20.4, 17.62, 17.59, 17.51, 17.47, 17.43, 17.40, 17.39, 17.37. HRMS *m*/*z* calcd for $[C_{92}H_{155}N_5O_{58}]H^+$: 2258.9411. Found: 2258.9670.

*(2-O-Benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranose trichloroacetimidate (**8**).* To a solution of **30** (13.0 g, 8.7 mmol) in CH_2Cl_2 (50 mL) were added CCl_3CN (15 mL) and DBU (0.3 mL) under cooling in ice−water. The mixture was allowed to reach room temperature. After 1 h the solution was concentrated, and the residue purified by column chromatography using hexanes−EtOAc 3:2 containing 0.1% Et_3N to afford **8** (11.1 g, 78%) as an amorphous material: ^{1}H NMR ($CDCl_3$, partial) δ 8.75 (s, 1H), 8.15, 8.13, 8.00, 7.96 (4 s, 4 × 1H), 7.67−7.59 (m, 2H), 7.55−7.49 (m 2H), 7.37−7.02 (m, 24H), 6.29 (d, 1H, $J = 11.7$ Hz), 5.75 (d, 1H, $J = 9.5$ Hz), 5.63−5.60 (2 br s, 2 × 1 H), 5.40 (dd, 1H, $J = 3.5$ Hz, $J = 9.2$ Hz), 5.19 (t, 1H, $J = 9.6$ Hz), 5.16 (br s, 1H), 4.93 (d, 1H, $J = 3.4$ Hz each), 4.79 (d, 1H, $J = 3.2$ Hz), 4.74, 4.69, 4.66, 4.62, 5.53 (5 d, 5 × 1H, $J \sim 11$ Hz), 4.52 (d, 2H, $J \sim 11$ Hz), 4.45 (dt, 1H, $J = 3.4$ Hz, $J = 10.0$ Hz), 4.35, 4.34, 4.30 (3 d, 3 × 1H, $J \sim 11$ Hz), 4.24 (dd, 1H, $J = 3.3$ Hz, $J = 10.0$ Hz), 3.49 (t, 1H, $J = 9.6$ Hz), 2.13, 2.00, 1.79 (3 s, 3 × 3H), 1.40, 1.09 (2 d, 2 × 3H, $J = 6.3$ each); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.4, 169.4, 165.9, 165.5, 165.2, 160.1, 138.2, 137.9, 137.7, 137.6, 137.1, 134.2, 133.4, 129.84, 129.80, 129.77, 129.2, 128.9, 128.7, 128.44, 128.39, 128.3, 128.35, 128.3, 128.25, 128.18, 128.1, 128.05, 128.02, 127.98, 127.94, 127.8, 127.7, 127.54, 127.48, 127.35, 127.32, 99.6, 99.4, 96.8, 94.8, 90.6, 80.9, 78.8, 78.42, 78.38, 78.0, 77.6, 75.7, 75.44, 75.37, 74.8, 74.4, 73.9,

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Table 9. Composition of Protein Conjugates of Synthetic Oligosaccharides Related to *S. dysenteriae* Type 1 and *E. coli* O148, and the Geometric Means of Their Anti-LPS Serum IgG[a,b,c,d]

item no.	conjugate/ OS size	nonreducing terminus	average molecular mass of conjugate [kDa]	average number of OS chains per protein	protein sugar ratio (w/w)	anti-SD LPS IgG GM[EU][e]	anti-Ec148 LPS IgG GM [EU][e]
1	**1**-EC-BSA/4-mer	Rha(1)	92	20	4.9	ND	ND
2	**2**-EC-BSA/ 6mer	GlcNAc	95	18	3.6	ND	ND
3	**3**-EC-BSA/ 7mer	Glc	94	15	3.7	ND	ND
4	**4**-EC-BSA/8-mer	Rha(2)	95	14	3.6	33.3	155.6
5	**5**-EC-BSA/ 10-mer	GlcNAc	102	15	2.6	4.6	14.7
6	**6**-EC-BSA/ 11mer	Glc	98	12	3.0	ND	ND
7	**6**-EC-DT/ 11mer	Glc	86	11	2.9	12.9	39.8
8	**7**-EC-BSA/ 12mer	Rha(2)	102	13	2.6	11.2	56.4
9	**7**-EC-DT/ 12mer	Rha(2)	90	12	2.8	14.5	45.7
10	**43**-SD-DT/ 8mer	Rha(2)	74	7	6.6	14.4	ND
11	**44**-SD-DT/ 10mer	GlcNAc	86	12	2.9	11.8	33.7
12	**45**-SD-DT/ 11mer	Gal	86	11	2.9	9.5	48.0
13	**46**-SD-DT/ 12mer	Rha(2)	79	7	4.1	17.9	ND
14	**47**-SD-DT/ 13mer	Rha(1)	80	7	3.9	13.5	ND

[a]The immune responses to the BSA conjugates of oligosaccharides **1**, **2**, **3**, and **6** were not investigated. These conjugates were tested for their antigenicity as reported in the Immunoblotting section. [b]Molecular weights: BSA, 66.5 kDa; rDT, 58.3 kDa; aminooxy-derivatized BSA, 73 kDa; aminooxy-derivatized rDT, 63 kD. [c]Average numbers of aminooxy groups in the derivatized BSA: 28; in the derivatized rDT: 20. [d]Abbreviations: BSA, bovine serum albumin; DT, recombinant diphtheria toxin; EC, *Escherichia coli*; EU, elisa unit; GM, geometric mean; IgG, immunoglobulin G; LPS, lipopolysaccharide; ND, not determined; Rha(1), the rhamnose unit to which GlcNAc is attached; Rha(2), the rhamnose unit which is linked to either Gal or Glc; OS, oligosaccharide; SD, *Shigella dysenteriae* type 1. [e]All groups vs control: $p < 0.001$.

73.5, 71.5, 71.4, 70.1, 68.7, 68.6, 68.3, 68.0, 67.8, 60.9, 51.9, 40.4, 23.1, 21.0, 20.7, 18.2, 17.4.

*2-(Trimethylsilyl)ethyl (3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**10**).* A mixture of **24** (approximately 35 g, 27.0 mmol) in CH_3CN (350 mL) and water (60 mL) was treated with ammonium cerium(IV)nitrate (34 g, 62 mmol) in portions. The mixture was cooled to 0 °C and then was decolorized with 10% aq $NaHSO_3$. The solution was then extracted with saturated aq $NaHCO_3$. The mixture was filtered through a Celite layer, and the solids were washed with $CHCl_3$. The combined organic layers were washed with brine several times. Concentration of the organic layer yielded a syrupy residue, which was chromatograped through silica gel using hexanes−EtOAc 5:1 → 2:1 as the eluant to afford **10** (17.8 g, 15 mmol) as a syrupy material: ^{1}H NMR ($CDCl_3$, partial) δ 8.12−8.05 (m, 2H), 7.58−7.53 (m, 1H), 7.43−7.41 (m, 2H), 7.38−7.19 (m, 16H), 7.19−7.15 (m, 2H), 7.10−7.07 (m, 2H), 5.46 (m, 1H), 5.14 (t, 1H, J = 9.9 Hz), 5.11 (d, 1H, J = 3.6 Hz), 4.87 (d, 1H, J = 3.6 Hz), 4.86 (d, 1H, J = 11.0 Hz), 4.84 (d, 1H, J = 12 Hz), 4.81 (d, 1H, J = 11.8 Hz), 4.76 (d, 1H, J = 11.5 Hz), 4.73 (d, 1H, J = 11.6 Hz), 4.68 (d, 1H, J = 11.0 Hz), 4.30 (d, 1H, J = 12.0 Hz), 4.20 (d, 1H, J = 12.0 Hz), 3.36 (dd, 1H, J = 6.2 Hz, J = 10.0 Hz), 3.29 (dd, 1H, J = 8.6 Hz, J = 10.0 Hz), 2.04, 1.90, 1.61 (3 s, 3 × 3H), 1.44 (d, 3H, J = 6.3 hz), 1.00−0.88 (m, 2H), 0.03 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.1, 169.8, 165.2, 138.4, 138.0, 137.7, 137.3, 133.5, 129.7−126.9, 99.7, 96.9, 93.9, 82.8, 79.7, 77.1, 75.41, 75.39, 75.1, 74.3, 73.2, 72.9, 72.7, 70.6, 70.4, 68.8, 68.7, 68.0, 67.8, 65.4, 61.1, 51.4, 22.5, 20.7, 20.6, 18.0, 17.8, −1.5. HRMS m/z calcd for $[C_{64}H_{79}NO_{18}Si]H^+$: 1178.5145. Found: 1178.5162.

*3,4,6-Tri-O-benzyl-2-O-(4-methoxybenzyl)-α,β-D-glucopyranosyl trichloroacetimidate (**11**).* To a stirred mixture of compound **22** (12 g, 21 mmol) and Cs_2CO_3 (1.2 g, 3.7 mmol) in anhydrous CH_2Cl_2 (100 mL) was added Cl_3CCN (25 mL, 243 mmol) under ice-cooling. After 30 min at room temperature, the mixture was extracted with brine 3 times followed by drying (Na_2SO_4). Removal of the volatiles under reduced pressure afforded a syrup, which was used in the next step without further purification.

*5-(Methoxycarbonyl)pentyl (2-O-benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**13**).* To a stirred solution of compounds **8** (3.0 g, 1.83 mmol) and **31** (375 mg, 2.5 mmol) in CH_2Cl_2 (35 mL) was added TMSOTf (15 μL) under external ice−water cooling. After 1 h the solution was treated with Et_3N (~0.1 mL) followed by removal of the volatiles under vacuum. Column chromatography of the residue using hexanes−EtOAc 2:1 → 3:2 as the eluant gave **13** (2.4 g, 81%) as an amorphous substance: ^{1}H NMR ($CDCl_3$, partial) δ 8.13−8.11 (m, 2H), 7.98−7.97 (m, 2H), 7.66−7.59 (m, 2H), 7.52−7.45 (m, 4H), 7.35−7.15 (m, 19H), 7.09−7.02 (m, 6H), 5.72 (d, 1H, J = 5.7 Hz), 5.60 (dd, 1H, J = 1.7 Hz, J = 3.5 Hz), 5.40 (dd, 1H, J = 3.6 Hz, J = 10.3 Hz), 5.39 (br s, 1H), 5.19 (t, 1H, J = 9.9 Hz), 5.14 (br d, 1H, J ~ 1.8 Hz), 4.90 (d, 1H, J = 3.5 Hz), 4.80 (d, 1H J = 1.9 Hz), 4.78 (d, 1H, J = 3.3 Hz), 4.74 (d, 1H, J = 10.5 Hz), 4.43 (dt, 1H, J = 3.5 Hz, J = 10.2 Hz), 4.17 (dd, 1H, J = 2.9 Hz, J = 9.7 Hz), 3.67 (s, 3H), 3.37 (t, 1H, J = 9.6 Hz), 2.34 (t, 1H, J = 7.6 Hz), 2.14, 2.01,1.75 (3 s, 3 × 3H), 1.69−1.55 (m, 4H), 1.42−1.37 (m, 2H), 1.36, 1.08 (2 d, 2 × 3H, J = 6.3 Hz each); ^{13}C NMR ($CDCl_3$) δ 174.0, 170.7, 170.5, 169.4, 166.0, 165.7, 165.3, 138.3, 138.0, 137.8, 137.5, 137.4, 133.9, 133.4, 129.8−127.3, 99.8, 99.5, 97.4, 96.5, 80.9, 79.5, 78.8, 78.4, 78.0, 77.9, 75.7, 75.4, 75.2, 75.1, 74.4, 73.9, 73.5, 71.4, 70.4, 70.1, 68.58, 68.55, 68.1, 98.0, 67.9, 67.7, 60.8, 51.9, 51.4, 40.4, 33.8, 29.0, 25.6, 24.6, 23.0, 21.1, 20.7, 18.2, 17.4. HRMS m/z calcd for $[C_{88}H_{100}ClNO_{26}]H^+$: 1622.6300. Found: 1622.6238.

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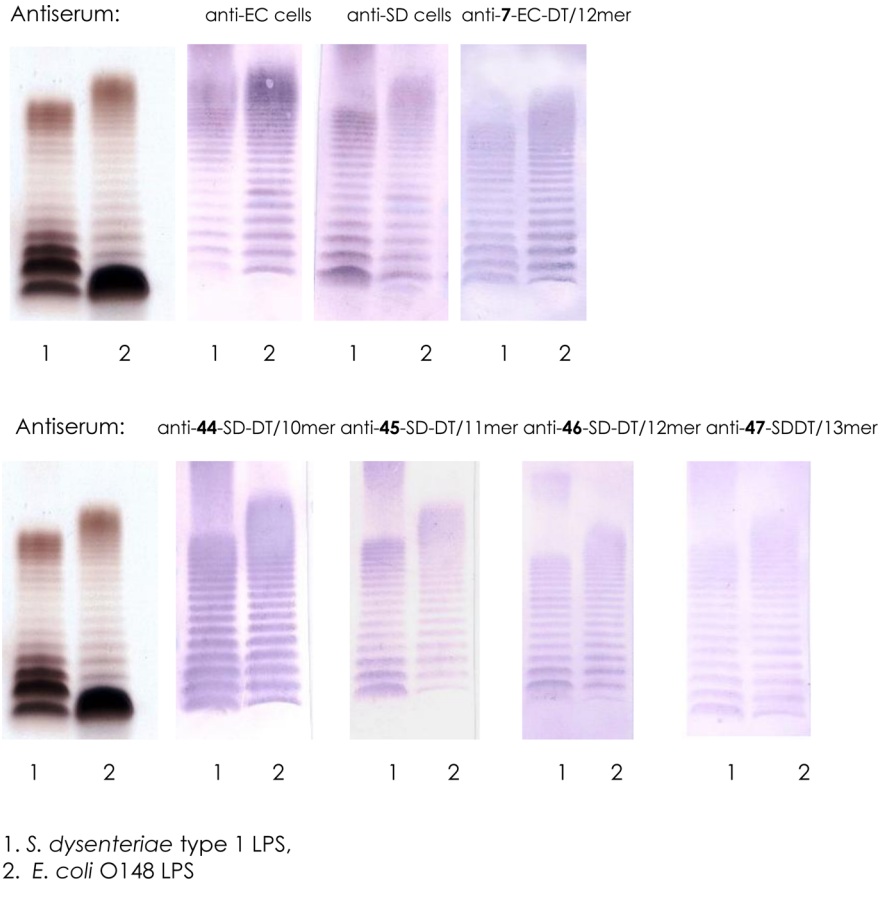

Figure 5. Western immunoblot of *S. dysenteriae* type 1 and *E. coli* O148 LPS with sera induced by whole bacteria or synthetic oligosaccharides bound to the recombinant diphtheria toxin (DT). The terminal sugars are as follows: 10mer, GlcNAc; 11mer, Gal; 12 and 13mers, Rha. Abbreviations: EC, *E. coli* O148; SD, *S. dysenteriae* type 1.

Table 10. Competitive Inhibition of Anti-*S. dysenteriae* Type 1 Serum (Induced By rDT/Sd1-12mer) Binding to the Homologous LPS by Different Dosages of *S. dysenteriae* Type 1, *E. coli* O148, or *B. bronchiseptica* RB50 O-SPs

	inhibition (%)			
	inhibitor per well (μg)			
inhibitor	0.04	0.2	5	80
S. dysenteriae type 1 O-SP	23	39	69	75
E. coli O148 O-SP	21	37	71	79
B. bronchiseptica O-SP	5	9	14	25

*Methoxycarbonylpentyl α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranoside (**14**).* For ^{1}H and ^{13}C NMR data, see Tables 2, 4, 6, and 7.

Table 11. Competitive Inhibition of Anti-*E. coli* O148 Serum (Induced By rDT/EcO148-12mer) Binding to the Homologous LPS by Different Dosages of *S. dysenteriae* Type 1, *E. coli* O148, or *B. bronchiseptica* RB50 O-SPs

	inhibition (%)			
	inhibitor per well (μg)			
inhibitor	0.04	0.2	5	80
S. dysenteriae type 1 O-SP	23	54	58	80
E. coli O148 O-SP	30	42	72	83
B. bronchiseptica O-SP	8	9	8	24

*1,2-Di-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranose (**18**).* A stirred solution of compound **17** (5.0 g) in Me_3SiOAc (120 mL) was refluxed for 2 h. The solution was concentrated, and the residue purified by column chromatography using hexanes−EtOAc (2:1) to afford **18** (4.6 g, 93%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 7.41−7.12 (m, 15H), 5.61 (d, 1H, J = 7.8 Hz), 5.11 (dd,1 H, J = 8.3 Hz, J = 9,4 Hz), 4.80, 4.77, 4.67, 4.62, 4.54, 4.49 (6 d, $J \sim$ 11 Hz each), 3.82 (t, 1H, J = 9.2 Hz), 3.77−3.69 (m, 3H), 3.61−3.57 (m, 1H), 2.07, 1.92 (2 s, 2 × 3H); ^{13}C NMR ($CDCl_3$) δ 169.4, 138.0−127.7, 92.2, 82.7, 75.7, 75.1, 75.0, 73.5, 72.1, 68.0, 20.9, 20.7. HRMS m/z calcd for $[C_{31}H_{34}O_8]NH_4^+$: 552.2597. Found: 552.2593.

*Phenyl 2-O-acetyl-3,4,6-tri-O-benzyl-1-thio-β-D-glucopyranoside (**19**).* To a stirred solution of diacetate **18** (2.4 g, 4.5 mmol), $PhSSiMe_3$ (2.0 mL) in CH_2Cl_2 (15 mL) was added $BF_3{\cdot}Et_2O$ (0.8 mL) under cooling with ice−water. The cooling bath was then removed, and the solution was allowed to reach room temperature. After 90 min total reaction time, to the solution was added Et_3N (2 mL) followed by removal of the volatiles under reduced pressure to yield a solid, which was stirred in MeOH for 14 h. Filtration followed by drying afforded crystalline **19** (2.2 g, 84%): mp 114−116 °C; ^{1}H NMR ($CDCl_3$) δ 7.52−7.48 (m, 2H), 7.33−7.19 (m, 18H), 5.02 (m, 2H), 4.80−4.77 (m, 2H), 4.67 (d, 1H, J = 11.0 Hz), 4.61 (d, 1H, J = 10.0 Hz), 4.59 (d, 1H, J = 12.0 Hz), 4.57 (d, 1H, J = 11.0 Hz), 4.53 (d, 1H, J = 12.0 Hz), 3.79 (dd, 1H, J = 1.8 Hz, J = 11.0 Hz), 3.72 (dd, 1H, J = 4.8 Hz, J = 11.0 Hz), 3.70−3.65 (m, 2H), 3.54 (m, 1H), 1.99 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.4, 138.1, 138.0, 137.8, 132.9, 132.2, 128.7−127.5, 85.9, 84.3, 79.3, 77.7, 75.2, 75.0, 73.3, 71.7, 68.8, 20.9. HRMS m/z calcd for $[C_{35}H_{36}O_6S]NH_4^+$: 602.2576. Found: 602.2556.

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*Phenyl 3,4,6-tri-O-benzyl-1-thio-β-D-glucopyranoside (**20**).* To a solution of **19** (38 g) in CH_2Cl_2 (150 mL) were added sequentially MeOH (50 mL) and NaOMe (10 mL of a 25% solution of NaOMe in MeOH) at room temperature. After 4 h the solution was treated with Dowex50 (H^+) until its pH dropped to approximately 3. The solids were removed by filtration, and the solution so obtained was extracted with 5% aq $NaHCO_3$ and then washed with H_2O twice. The organic layers were combined and concentrated. Hexanes were added to and evaporated from the residue. The solids so obtained were triturated with hexanes followed by filtration to afford **20** (34 g, 97%) as a crystalline solid: mp 74−76 °C; ^{1}H NMR ($CDCl_3$) δ 7.61−7.51 (m, 2H), 7.39−7.11 (m, 18H), 4.90, 4.83, 4.81, 4.60, 4.56, 4.53 (6 d, 6 × 1H, J ∼ 11 Hz), 4.49 (d, 1H, J = 9.5 Hz), 3.78 (dd, 1H, J = 10.4 Hz), 3.72 (dd, 1H, J = 4.2 Hz, J = 10.4 Hz), 3.62−3.57 (m, 2H), 3.55−3.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 138.4, 138.2, 138.0, 132.8, 131.8, 128.9−127.5, 88.0, 85.9, 79.3, 77.3, 75.2, 75.0, 73.3, 72.5, 68.9 . HRMS m/z calcd for $[C_{33}H_{34}O_5S]NH_4^+$: 560.2471. Found: 560.2458.

*Phenyl 3,4,6-tri-O-benzyl-2-O-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside (**21**).* To a stirred solution of **20** (33.5 g, 62 mmol) in DMF (150 mL) was added NaH (5.0 g, ∼125 mmol of a 60% suspension in mineral oil) in portions under ice−water cooling. After 15 min, MBnCl (11 mL, 81 mmol) was added dropwise. The mixture was allowed to reach room temperature in approximately 30 min. To the stirred mixture was added MeOH (excess) under cooling with ice−water. The volatiles were removed by distillation under reduced pressure, and the residue was equilibrated between $CHCl_3$ and water. The organic layer was concentrated. Trituration of the residue in hexanes followed by filtration afforded **21** (38.4 g, 91%) as a colorless crystalline solid: mp 86−87 °C; ^{1}H NMR ($CDCl_3$) δ 7.76−7.73 (m, 2H), 7.47−7.26 (m, 20H), 6.99−6.92 (m, 2H), 5.02, 4.95 (2 d, 2 × 1 H, J = 11.3 Hz each), 4.94, 4.91 (2 br s, 2 × 1H), 4.77, 4.76 (2 d, 2 × 1H, J = 10.2 Hz), 4.70 (d, 1H, J = 12.0 Hz), 4.69 (d, 1H, J = 10.7 Hz), 4.63 (d, 1H, J = 12.0 Hz), 3.86 (s, 3H), 3.89−3.73 (m, 4H), 3.67−3.54 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.3, 138.4, 138.2, 138.0, 133.8, 131.8, 130.1−127.3, 113.8, 87.4, 86.7, 80.5, 79.0, 77.7, 75.7, 74.97, 74.95, 73.3, 68.9, 55.2. HRMS m/z calcd for $[C_{41}H_{42}O_6S]NH_4^+$: 680.3046. Found: 680.3057.

*3,4,6-Tri-O-benzyl-2-O-(4-methoxybenzyl)-α,β-D-glucopyranose (**22**).* To a stirred mixture of **21** (33.0 g, 48 mmol), CH_2Cl_2 (500 mL), and water (2 mL) was added $(CF_3CO_2)_2Hg$ (33.0 g, 77 mmol) under cooling in ice−water. After 5 min the mixture was treated with saturated aqueous KI (∼50 mL). The organic layer was separated and washed with water. The residue obtained after removal of the volatiles was purified by column chromatography using hexanes−EtOAc 2:1 → 3:2 as the eluant to afford **22** (27.7 g, 97%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, partial) δ 7.36−7.22 (m, 15H), 7.16−7.09 (m, 2H), 6.85−6.80 (m, 2H), 5.15 (d, J = 3.6 Hz), 4.93, 4.82, 4.81, 4.69, 4.61, 4.58 (6 d, 6 × 1H, J ∼ 11 Hz), 3.94 (t, J = 9.0 Hz), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$, partial) δ 159.4, 138.7, 138.2, 137.8, 129.9−127.6, 113.9, 91.3, 81.7, 79.6, 77.6, 75.6, 75.0, 73.4, 72.8, 70.2, 68.6, 55.2. HRMS m/z calcd for $[C_{35}H_{38}O_7]NH_4^+$: 588.2961. Found: 588.2961.

*2-(Trimethylsilyl)ethyl [3,4,6-tri-O-benzyl-2-O-(4-methoxybenzyl)-α,β-D-glucopyranosyl]-(1→3)-(4,6-di-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**23**).* To a stirred solution of **11** prepared from hemiacetal **22** (15.0 g, 26.3 mmol) and **12** (12.0 g, 16.4 mmol) in CH_2Cl_2 (100 mL) was added TMSOTf (50 μL) at approximately −40 °C, and then the solution was allowed to reach 0 °C in 2 h. The solution was extracted with aq 5% $NaHCO_3$, dried, and concentrated. Column chromatographic purification of the residue using hexanes−EtOAc 10:1 → 2:1 as the eluant afforded **23** (19.0 g, 90%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, partial) δ 8.17−8.12 (m, 4H), 7.60−7.07 (m, 25H), 6.80−6.70 (m, 4H), 5.56 (m), 5.35 (d, J = 3.6 Hz), 5.15 (t, J = 10.0 Hz), 5.00 (d, J = 3.6 Hz), 3.70 (s, 3H), 2.06, 1.76 (2 s), 1.40 (d, J = 6.3 Hz), 1.02−0.88 (m), 0.03 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.7, 169.3, 165.9, 159.4, 138.8−127.6, 98.6, 97.0, 93.0, 81.4, 79.5, 79.0, 77.3, 75.7, 75.4, 74.6, 73.3, 72.9, 72.7, 71.3, 69.3, 67.7, 67.4, 67.3, 65.2, 62.4, 55.0, 20.6, 17.9, 17.8, −1.5. HRMS m/z calcd for $[C_{70}H_{83}N_3O_{18}Si]NH_4^+$: 1299.5785. Found: 1299.5830.

*2-(Trimethylsilyl)ethyl [3,4,6-tri-O-benzyl-2-O-(4-methoxybenzyl)-α,β-D-glucopyranosyl]-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**24**).* A mixture consisting of **23** (33 g, 10.9 mmol), Et_3N (7.5 mL), EtOAc (30 mL), EtOH (140 mL), and 10% palladium-on-charcoal (5.5 g) was stirred under hydrogen at 200 psi at room temperature for 1 h. The mixture was treated with Ac_2O (11 mL) and then was filtered through a Celite layer followed by concentration. A solution of the residue in $CHCl_3$ was washed with water, dried (Na_2SO_4), and concentrated. The material so obtained was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, partial) δ 8.11−8.06 (m, 2H), 7.62−7.55 (m, 1H), 7.49−7.43 (m, 2H), 7.41−7.36 (m, 2H), 7.35−7.28 (m, 4H), 7.27−7.17 (m, 12H), 7.16−7.10 (m, 2H), 7.08−7.02 (m, 2H), 6.76−6.69 (m, 2H), 5.57 (d, 1H, J = 9.8 Hz), 5.42 (m, 1H), 5.19 (t, 1H, J = 9.8 Hz), 5.03 (d, 1H, J = 3.5 Hz), 3.71 (s, 3H), 2.02, 1.71, 1.52 (3 s, 3 × 3H), 1.41 (d, 1H, J = 6.3 Hz), 0.99−0−86 (m, 2H), 0.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.8, 170.0, 169.2, 165.6, 159.3, 138.8, 138.5, 137.8, 137.7, 130.4−127.4, 113.8, 113.6, 99.2, 97.0, 95.2, 81.5, 80.1, 79.9, 77.5, 75.6, 75.4, 74.7, 73.9, 73.5, 72.9, 71.3, 69.5, 69.4, 68.1, 65.6, 55.3, 52.0, 22.9, 20.9, 20.8, 18.2, 18.0, −1.3. HRMS m/z calcd for $[C_{72}H_{87}NO_{19}Si]H^+$: 1299.5720. Found: 1299.5760.

*2-(Trimethylsilyl)ethyl (3,4,6-tri-O-benzyl-β-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**25**).* Compound **25** (approximately 3.5 g, 3 mmol) was obtained after the isomer **10** was eluted as described above: ^{1}H NMR ($CDCl_3$, partial) δ 8.16−8.09 (m, 2H), 7.69−7.63 (m, 1H), 7.58−7.50 (m, 1H), 7.39−7.17 (m, 27H), 7.16−7.13 (m, 2H), 7.12−7.06 (m, 2H), 5.81 (d, 1H, J = 8.9 Hz), 5.44 (br, 1H), 2.01, 1.91, 1.66 (3 s, 3 × 3H), 1.47 (d, 3H, J = 6.3 Hz), 1.00−0.88 (m, 3H), 0.03 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.0, 170.7, 169.6, 166.8, 165.8, 138.8, 138.3, 138.2,137.9, 137.6, 137.5, 137.4, 134.0, 129.8−127.4, 104.1, 96.9, 94.7, 85.3, 84.3, 79.9, 79.7,78.6, 76.7, 76.6, 75.5, 75.3, 74.9, 74.8, 74.7, 74.5, 73.7, 73.6, 73.3, 73.2, 69.4, 69.0, 68.2, 68.09, 68.06, 67.6, 65.5, 61.4, 52.2, 42.5, 22.8, 20.7, 20.5,18.2, 17.9, −1.4. API-ES-MS m/z calcd for $[C_{64}H_{79}NO_{18}Si]H^+$: 1178.5145. Found: 1178.5162.

*2-(Trimethylsilyl)ethyl (2-O-acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**26**).* To a solution of compound **10** (4.3 g, 3.6 mmol) in CH_2Cl_2 (20 mL) were added C_5H_5N (5 mL), Ac_2O (5 mL), and a catalytic amount of 4-dimethylaminopyridine. The solution was kept at ∼45 °C for 1 h followed by removal of the volatiles under reduced pressure. Toluene was added to and removed from the residue several times to afford **26** (quant.) that was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, partial) δ 8.11−8.08 (m, 2H), 7.59−7.56 (m, 1H), 7.45−7.42 (m, 2H), 7.36−7.18 (m, 16H), 7.15−7.14 (m. 2H), 7.11−7.07 (m, 2H), 5.89 (d, 1H, J = 9.5 Hz), 5.47 (m, 1H), 5.14 (t, 1H, J = 9.7 Hz), 5.12, 5.08 (2d, 2 × 1H, J ∼ 3.6 Hz), 4.82 (d, 1H, J = 1.8 Hz), 4.76 (dd, 1H, J = 7.4 Hz, J = 11.0 Hz), 4.61 (d, 1H, J = 11.5 Hz), 4.45 (dd, 1H, J = 3.5 Hz, J = 10.3 Hz), 4.28−4.25 (m 2H), 4.02 (dd, 1H, J = 9.3 Hz, J = 10.8 Hz), 3.54−3.46 (m, 3H), 3.34 (dd, 1H, J = 6.8 Hz, J = 9.5 Hz), 3.30 (dd, 1H, J = 9.5 Hz, J = 10.0 Hz), 2.04, 1.97, 1.81, 1.60 (4 s, 4 × 3H), 1.42 (d, 3H, J = 6.3 Hz), 1.00−0.88 (m, 2H), 0.02 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.0, 170.6, 169.9, 169.2, 165.2, 138.3, 138.0, 137.7, 137.4, 133.6, 129.7−126.9, 97.0, 95.5, 93.6, 79.9, 79.8, 77.5, 75.5, 76.2, 74.4, 73.3, 73.0, 72.8, 72.7, 70.8, 70.3, 68.8, 68.7, 68.0, 67.7, 65.4, 61,2, 51.1, 22.6, 20.75, 20.68, 20.64, 18.0, 17.8, −1.5. HRMS m/z calcd for $[C_{66}H_{81}NO_{19}Si]H^+$: 1220.5250. Found: 1220.5238.

*(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-L-rhamnopyranose (**27**).* A solution of **26** (4.5 g, 4.1 mmol) in a mixture of CH_2Cl_2 (20 mL) and CF_3CO_2H (100 mL) was allowed to stand at rt for 4 h. Removal of the volatiles under reduced pressure followed by column chromatography (hexanes−EtOAc 2:1 → 1:1) of the resulting syrup afforded **27** (3.3 g, 80%) as an amorphous material: ^{1}H NMR (D_2O) δ 8.05−8.01 (m, 2H), 7.54−7.50 (m, 1H), 7.39−7.17 (m, 18H), 7.13−7.06 (m, 4H), 4.01 (dd, 1H, J = 9.0 Hz, J = 10.6 Hz), 3.83 (t, 1H, J ∼ 10.3 Hz), 3.80 (d, 1H, J = 3.3 Hz), 3.49, 3.45 (2 d, 2 × 1H, J ∼ 9.6 Hz), 2.02, 1.95, 11.78, 1.65 (4 s, 4 × 3H), 1.41 (d, 3H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.7, 170.6, 169.3, 165.2, 138.2,

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137.9, 137.7, 137.2, 133.5−126.9, 95.5, 93.4, 92.1, 80.0, 79.8, 77.5, 75.6, 75.1, 74.4, 73.2, 72.9, 72.7, 72.3, 70.9, 70.3, 69.3, 68.7, 68.0, 67.7, 61.3, 51.3, 22.6, 20.72, 20.67, 18.1. HRMS m/z calcd for $[C_{61}H_{69}NO_{19}]H^+$: 1120.4542. Found: 1120.4515.

*(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl trichloroacetimidate (**28**).* To a stirred solution of compound **27** (3.0 g, 2.7 mmol) in anhydrous CH_2Cl_2 (50 mL) were added CCl_3CN (3 mL) and DBU 0.9 mL) cooled at 0 °C. The solution was allowed to reach room temperature in approximately 1 h. the solution was concentrated, and the residue chromatographed through silica gel using hexanes−EtOAc 3:2 as the eluant, containing 0.1% Et_3N to afford **28** (3.2 g, 95%) as an amorphous material: ^{1}H NMR (D_2O) δ 8.70 (s, 1H), 8.10 (d, 2H, J = 7.8 Hz), 7.60 (t, 1H, J = 7.3 Hz), 7.50 (t, 2H, J = 7.9 Hz), 7.36−7.18 (m, 16H), 7.13, 7.09 (m, 2 × 2H), 6.27 (d, 1H, J = 1.7 Hz), 5.95 (d, 1H, J = 10.0 Hz), 5.71 (m, 1H), 5.16−5.10 (m, 3H), 4.78, 4.25 (2 × 1H, J = 11.5 Hz), 4.71−4.66 (m, 3H), 4.59 (d, 1H, J = 11.5 Hz), 4.45 (dt, 1H, J = 3.8 Hz, J = 10.6 Hz), 4.39 (d, 1H, J = 11.5 Hz), 4.32 (dd, 1H, J = 3.2 Hz, J = 9.8 Hz), 4.32 (d, 1H, J = 12.5 Hz), 4.22 (d, 1H, J = 12.5 Hz), 4.08−3.98 (m, 3H), 3.91 (m, 1H), 3.84 (dd, 1H, J = 9.0 Hz, J = 10.0 Hz), 3.81 (d, 2H, J = 3.1 Hz), 3.57 (t, 1H, J = 9.6 Hz), 3.48 (dd, 1H, J = 1.5 Hz, J = 10.2 Hz), 3.28 (t, 1H, J = 9.5 Hz), 3.21 (dd, 1H, J = 7.0 Hz, J = 10.1 Hz), 2.03, 1.97, 1.82, 1.64, (4 s, 4 × 3H), 1.46 (d, 3H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.6, 170.0, 169.3, 165.0, 164.9, 160.0, 138.3, 138.0, 137.4, 137.3, 133.9, 138.3, 138.0, 137.4, 137.3, 133.9, 129.9, 128.9−127.4, 95.6, 95.0, 94.0, 90.6, 80,0, 79.1, 77.6, 75.6, 75.5, 74.5, 73.3, 73.0, 72.7, 72.5, 71.1, 70.8, 70.3, 68.9, 68.1, 66.9, 61.3, 51.1, 22.7, 20.8, 20.7, 18.1, 14.2.

*2-(Trimethylsilyl)ethyl (2-O-benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**29**).* To a stirred solution of compounds **9** (36 g, 62.1 mmol) and **10** (16.4 g, 13.9 mmol) in CH_2Cl_2 (180 mL) was added TMSOTf (60 μL) under cooling in ice−water. The solution was allowed to reach room temperature. After 3 h the solution was treated with Et_3N (0.5 mL). The solution was washed sequentially with 2% aq $NaHCO_3$ and water. The solution was concentrated, and the residue purified by column chromatography using hexanes−EtOAc 5:1 → 2:1 as the eluant to afford **29** (20.0 g, 91%) as an amorphous solid: ^{1}H NMR ($CDCl_3$, partial) δ 8.09 (m, 2H), 7.94 (m, 2H), 7.62−7.56 (m, 2H), 7.48−7.41 (m, 4H), 7.32−6.99 (m, 25H), 5.69 (d, 1H, J = 9.2 Hz), 5.57 (dd, 1H, J = 1.7 Hz, J = 3.4 Hz), 5.37 (dd, 1H, J = 3.5 Hz, J = 9.2 Hz), 5.35 (m, 1H), 5.16 (dd, 1H, J = 9.2 Hz, J = 10.3 Hz), 5.12 (d, 1H, J = 1.6 Hz), 4.86 (d, 1H, J = 3.4 Hz), 4.81 (d, 1H, J = 1.9 Hz), 4.76 (d, 1H, J = 3.5 Hz), 4.71 (d, 1H, J = 10.8 Hz), 4.64 (d, 1H, J = 11.3 Hz), 4.63 (d, 1H, J = 11.0 Hz), 4.59 (d, 1H, J = 11.6 Hz), 4.51 (d, 1H, J = 11.3 Hz), 4.47 (d, 1H, J = 11.3 Hz), 4.39 (dt, 1H, J = 3.3 Hz, J = 10.5 Hz), 4.18 (dd, 1H, J = 3.3 Hz, J = 10.5 Hz), 4.00 (m, 1H), 3.35 (t, 1H, J = 9.8 Hz), 2.12, 2.01, 1.74 (3 s, 3 × 3H), 1.37 (d, 3H, J = 6.3 Hz), 1.09 (d, 1H, J = 6.3 Hz), 1.03−0.89 (m, 2H), 0.03 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 170.7, 170.4, 169.4, 165.9, 165.7, 165.3, 139.8−137.3, 133.9, 133.4, 99.8, 99.4, 96.8, 96.3, 80.9, 79.6, 78.7, 78.0, 77.8, 77.3, 75.7, 75.3, 75.2, 75.0, 74.4, 73.9, 73.5, 71.4, 70.5, 70.2, 68.7, 68.5, 68.1, 68.0, 67.8, 65.4, 60.9, 51.9, 40.4, 23.0, 21.1, 20.7, 18.2, 17.9, 17.4, −1.4. HRMS m/z calcd for $[C_{86}H_{100}ClNO_{24}Si]H^+$: 1594.6171. Found: 1594.6184.

*(2-O-Benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranose (**30**).* To a solution of compound **29** (21.5 g, 13.4 mmol) in CH_2Cl_2 (100 mL) was added trifluoroacetic acid (400 mL) at room temperature. After 3 h the volatiles were removed under reduced pressure. Toluene was added to and evaporated from the residue four times followed by column chromatographic purification using hexanes−EtOAc 1:1 → 2:3 as the eluant to afford **30** (13.0 g, 65%) as a solid material: ^{1}H NMR ($CDCl_3$, partial) δ 8.11−7.92 (m, 4H), 7.61 (t, 2H, J = 7.2 Hz), 7.46 (t, 4H, J = 7.7 Hz), 7.35−7.01 (m, 25H), 5.81 (d, 1H, J = 9.8 Hz), 5.59, 5.46 (2 br, 2 × 1H), 5.39 (dd, 1H, J = 3.6 Hz, 9.5 Hz), 5.24 (br, 1H), 5.15 (m, 2H), 2.11, 2.01, 1.76 (3 s, 3 × 3H), 1.35, 1.09 (2 d, 2 × 3H, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 170.9, 170.7, 169.4, 166.0, 165.7, 165.3, 138.3, 137.9, 137.8, 137.6, 133.8, 133.4, 129.9−127.4, 99.7, 99.2, 95.9, 92.1, 80.9, 79.5, 78.5, 78.4, 78.0, 75.7, 75.3, 75.1, 74.6, 74.5, 73.9, 73.5, 71.4, 70.7, 70.1, 68.6, 68.5, 68.0, 67.8, 60.9, 51.8, 40.4, 23.0, 21.0, 20.7, 18.30, 18.27, 17.4. HRMS m/z calcd for $[C_{81}H_{88}ClNO_{24}]H^+$: 1494.5463. Found: 1494.5516.

*5-(Methoxycarbonyl)pentyl (2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**32**).* A mixture consisting of compound **13** (3.3 g, 2.0 mmol), thiourea (3 g, 39.5 mmol), C_5H_5N (4 mL), and DMF (35 mL) was stirred at room temperature for 12 h. The volatiles were removed by distillation under reduced pressure. The residue was stirred in $CHCl_3$ (50 mL) followed by removal of the solids by filtration. Concentration afforded a syrup that was purified by column chromatography using hexanes−EtOAc 2:1 → 3:2 as the eluant to afford **32** (2.6 g, 83%) as an amorphous substance: ^{1}H NMR ($CDCl_3$, partial) δ 8.16, 8.00 (2 m, 2 × 2H), 7.68−7.59 (m, 2H), 7.55−7.45 (m, 4H), 7.41−7.18 (m, 1pH), 7.13−7.03 (m, 6H), 5.69 (d, 1H, J = 9.9 Hz), 5.42 (br s, 2H), 5.24 (br s, 1H), 5.19 (t, 1H, J = 9.8 Hz), 4.96 (d, 1H, J = 3.6 Hz), 4.77, 4.73, 4.69, 4.64, 4.56, 4.55 (6 d, 6 × 1H, J ∼ 11 Hz each), 4.44 (dt, 1H, J = 3.4 Hz, J = 10.0 Hz), 3.69 (s, 3H), 2.36 (t, 2H, J = 7.6) 2.05, 2.03, 1.70 (3 s, 3 × 3H), 1.69−1.59 (m, 4H), 1.46−1.39 (m, 2H), 1.38, 1.28 (2 d, 2 × 3 H, J = 6.3 Hz each); ^{13}C NMR ($CDCl_3$) δ 174.1, 170.8, 170.5, 169.0, 166.2, 165.7, 138.333, 138.327, 138.2, 137.74, 137.67, 134.0, 133.4, 129.9−127.4, 99.4, 98.5, 97.5, 95.7, 81.5, 81.4, 79.6, 78.0, 75.9, 75.7, 75.4, 75.3, 74.5, 74.4, 73.6, 73.3, 71.5, 70.6, 70.0, 69.3, 68.5, 68.4, 68.1, 68.0, 61.0, 51.9, 51.5, 33.9, 29.1, 25.7, 24.6, 23.0, 21.1, 20.8, 18.1, 17.9. HRMS m/z calcd for $[C_{86}H_{99}NO_{25}]H^+$: 1546.6584. Found: 1546.6604.

*5-(Methoxycarbonyl)pentyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**34**).* To a stirred solution of imidate **33**[29] (820 mg, 0.98 mmol) and compound **32** (650 mg, 0.42 mmol) in CH_2Cl_2 (10 mL) was added TMSOTf (8 μL) under external cooling with ice−water. After 45 min the cooling bath was removed, and the solution was treated with Et_3N (∼0.1 mL). Concentration under reduced pressure followed by column chromatographic purification of the residue using hexanes−EtOAc 3:2 → 2:3 as the eluant afforded **34** (0.85 g, 91%) as an amorphous substance: ^{1}H NMR ($CDCl_3$, partial) δ 8.12−8.07 (m, 2H), 8.03−7.99 (m, 4H), 7.67−7.57 (m, 2H), 7.53−7.44 (m, 6H), 7.37−7.10 (m, 29H), 7.04−6.97 (m, 2H), 5.75 (d, 1H, J = 9.7 Hz), 5.57 (dd, 1H, J = 1.5 Hz, J = 3.4 Hz), 5.41, 5.38 (2 br m, 2 × 1H), 5.36 (d, 1H, J = 9.7 Hz), 5.21, 5.20 (2 d, 2 × 1H, J = 1.5 Hz each), 5.06, 4.98 (2 d, 2 × 1H, J = 9.7 Hz each), 5.02 (d, 1H, J = 7 Hz), 4.91 (d, 1H, J = 3.4 Hz), 4.15, 4.10 (2 dd, 2 × 1 H, J = 3.0, J = 9.9 Hz each), 3.66 (s, 3H), 2.33 (t, 2H, J = 7.4 Hz), 1.98, 1.96, 1.91, 1.87, 1.77, 1.69, 1.45 (7 s, 7 × 3H), 1.66−1.57 (m, 4H), 1.43−1.40 (m, 2H), 1.37, 1.24, 1.11 (3 d, 3 × 3H, J = 6.3 Hz each); ^{13}C NMR ($CDCl_3$) δ 174.0, 171.1, 170.6, 170.5, 170.3, 169.9, 169.0, 168.9, 165.6, 165.3, 165.0, 138.3, 138.1, 137.7, 137.6, 137.3, 133.9, 133.8, 133.3, 129.8−126.8, 99.3, 99.2, 98.5, 97.4, 95.8, 93.7, 81.1, 80.1, 79.5, 78.8, 77.9, 75.70, 75.6, 75.2, 74.5, 74.4, 73.4, 72.9, 72.4, 71.5, 71.2, 70.0, 69.4, 69.1, 68.5, 68.4, 68.1, 67.94, 67.85, 67.3, 67.1, 60.9, 60.7, 51.5, 51.4, 50.9, 33.8, 29.0, 25.6, 24.6, 22.9, 22.4, 21.2, 20.61, 20.59, 20.5, 20.4, 18.18, 18.16, 17.9, 17.5. TOF-MS-ES$^+$ m/z calcd for $[C_{120}H_{138}N_2O_{38}]NH_4^+$: 2232.9. Found: 2232.9.

*5-(Methoxycarbonyl)pentyl (2-O-acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**35**).* To a solution of compounds **28** (1.19 g, 0.9 mmol) and **32** (650 mg, 0.4 mmol) in CH_2Cl_2 (10 mL) was added TMSOTf (2 μL) under ice-cooling. After 40 min the solution was allowed to reach room temperature in approximately 40 min. The solution was treated with Et_3N (0.1 mL). The syrupy residue obtained after removal of the volatiles under reduced pressure was purified by

column chromatography using hexanes−EtOAc 2:1 → 4:3 as the eluant afforded unreacted **32** (344 mg) followed by **35** (320 mg, 60% based on recovered **32**: ^{1}H NMR ($CDCl_3$, partial) δ 8.15−7.95 (m, 7H), 7.65−7.45 (m, 10H), 7.30−6.95 (m, 43H), 5.77, 5.70 (2 d, 2 × 1H, $J \sim 9.7$ Hz), 5.62 (br s, 2H), 5.42 (br m, 2H), 5.30 (dd, 1H, $J = 3.6$ Hz, $J = 9.5$ Hz), 5.24 (t, 1H, $J = 9.6$ Hz), 4.86 (d, 1H, $J = 3.5$ Hz), 3.65 (s, 3H), 2.35 (t, 2H, $J = 7.5$ Hz), 2.05, 1.98, 1.96, 1.93, 1.88, 1.77 (6 s, 6 × 3H); ^{13}C NMR ($CDCl_3$) δ 174.0,173.9, 171.2, 171.0, 170.6, 170.1, 169.9, 169.2, 168.8, 165.6, 165.4, 165.2, 138.3, 138.2, 138.0, 137.9, 137.8, 137.6, 137.5, 137.3, 137.1, 133.9, 133.7, 133.2, 129.8−126.5, 100.0, 99.4, 98.7, 98.5, 97.6, 96.7, 94.9, 81.3, 80.1, 80.0, 79.6, 79.5, 78.8, 77.9, 75.7, 75.6, 75.2, 75.0, 74.7, 74.5, 74.4, 74.1, 73.9, 73.5, 72.8, 72.6, 72.4, 71.5, 71.2, 70.1, 69.4, 69.1, 68.5, 68.4, 68.1, 67.94, 67.85, 67.3, 67.1, 60.8, 51.6, 51.4, 50.9, 33.8, 29.0, 25.6, 24.6, 22.9, 22.4, 21.2, 20.61, 20.59, 20.5, 20.4, 18.2, 17.9, 17.5. TOF-MS-ES$^+$ m/z calcd for $[C_{147}H_{166}N_2O_{43}]NH_4^+$: 2665.1. Found: 2665.1.

*5-(Methoxycarbonyl)pentyl) (2-O-benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**36**).* TMSOTf (7.5 μL) was added to a stirred solution of compounds **8** (2.6 g, 1.6 mmol) and **32** (1.3 g, 0.84 mmol) in CH_2Cl_2 (15 mL) under ice−water cooling. After 5 min the cooling bath was removed, and the solution was allowed to reach room temperature. After 1 h Et_3N (0.2 mL) was added followed by removal of the volatiles. The residue was purified by column chromatography (hexanes−EtOAc 2:1 → 3:2) to give **36** (2.2 g, 88%) as an amorphous solid: ^{1}H NMR ($CDCl_3$, partial) δ 8.11, 8.09, 8.05, 8.03, 8.01, 7.99, 7.97, 7.95 (8 s, 8 × 1H), 7.66−7.57 (m, 4H), 7.52−7.43 (m, 8H), 7.32−6.94 (m, 50H), 5.79, 5.75 (2 d, 2 × 1H, $J = 9.9$ Hz each), 5.60−5.54 (br m, 2H), 5.40 (br s, 1H), 5.37 (dd, 1H, $J = 3.5$ Hz, $J = 9.2$ Hz), 5.3, 5.28 (1 br s, 2 × 1H), 5.22 (t, 1H, $J = 9.7$ Hz), 5.20 (br s, 1H), 5.11−5.07 (m, 2H), 4.95, 4.89 (2 d, 2 × 1H, $J \sim 3$ Hz), 4.92 (d, 1H, $J = 11.0$ Hz), 4.82 (d, 1H, $J = 11.0$ Hz), 4.81 (br s, 1H), 4.17 (dd, 1H, $J = 2.9$ Hz, $J = 9.6$ Hz), 4.09 (d, 1H, $J = 11.0$ Hz), 3.66 (s, 3H), 2.33 (t, 2H, $J = 9.5$ Hz), 2.06, 2.00, 1.96, 1.84, 1.83, 1.67 (6 s, 6 × 3H), 1.66−1.56 (m, 4H), 1.42−1.34 (m, 2H), 1.39, 1.17, 1.12, 0.93 (4 d, 4 × 3H, $J = 6.3$ each); ^{13}C NMR ($CDCl_3$) δ 173.9, 170.6, 170.5, 170.3, 169.4, 168.9, 165.9, 165.6, 165.6, 165.5, 165.4, 165.2, 138.3, 138.24, 138.15, 137.9, 137.7, 137.6, 137.38, 137.35, 133.9, 133.4, 133.3, 129.7−126.8, 100.0, 99.5, 99.2, 98.8, 98.2, 97.3, 96.7, 95.6, 81.3, 80.8, 80.0, 79.5, 79.3, 78.8, 78.4, 78.1, 77.92, 77.89, 75.7, 75.6, 75.3, 75.2, 74.9, 74.5, 74.4, 74.3, 74.1, 73.9, 73.42, 73.39, 72.5, 71.3, 71.1, 70.3, 70.1, 69.8, 69.6, 69.5, 68.4, 68.3, 68.2, 68.0, 67.9, 67.83, 67.79, 67.7, 60.7, 51.7, 51.4, 40.4, 33.8, 28.9, 25.5, 24.5, 23.1, 22.9, 21.1, 21.0, 20.7, 20.4, 18.12, 18.06, 17.6, 17.1. TOF-MS-ES$^+$ m/z calcd for $[C_{167}H_{185}ClN_2O_{48}]Na^+$: 3044.2. Found: 3044.2.

*5-(Methoxycarbonyl)pentyl) (2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**37**).* A mixture of compound **36** (2.14 g, 0.7 mmol), thiourea (13.2 mmol), C_5H_5N (1 mL), and DMF (25 mL) was stirred at room temperature for 16 h. The solution was concentrated under reduced pressure. To the residue $CHCl_3$ (50 mL) was added. The solids were removed by filtration and then were washed with $CHCl_3$ thrice. The combined solutions were washed with brine thrice. Concentration of the organic layer afforded a syrup that was purified by column chromatography using hexanes−EtOAc (3:2 → 5:4) as the eluant to afford **37** (1.6 g, 77%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, partial) δ 8.14−8.97 (4 m, 4 × 2H), 7.67−7.55 (m, 4H), 7.54 (m, 8H), 7.38−7.13 (m, 40H), 7.10−703 (m, 10H), 5.75 (dd, 2H, $J = 9.9$ Hz, $J = 12.0$ Hz), 5.57 (dd, 1H, $J = 1.4$ Hz), $J = 3.5$ Hz), 5.40 (m, 1H), 5.37 (dd, 1H, $J = 1.5$ Hz, $J = 3.5$ Hz), 5.32 (m, 1H), 5.29 (br s, 1H), 5.23−5.18 (m, 3H), 5.07 (dd, 1H, $J = 9.1$ Hz, $J = 10.0$ Hz), 4.95 (d, 1H, $J = 3.5$ Hz), 2.33 (t, 2H, $J = 7.4$ Hz), 1.98, 1.96, 1.90, 1.85, 1.76, 1.68 (6 s, 6 × 3H), 1.67−1.57 (m, 4H), 1.42−1.34 (M, 2H), 1.39, 1.16, 1.13, 1.11 (4 d, 4 × 3H, $J \sim 6.3$ Hz each); ^{13}C NMR ($CDCl_3$) δ 173.9, 170.6, 170.5, 170.3, 168.9, 166.1, 165.6, 165.4, 165.3, 138.21, 138.20, 138.1, 138.0, 137.7,137.59, 137.57, 137.3, 133.9, 133.2, 129.77−126.70, 99.2, 99.1, 98.8, 98.4, 98.2, 97.3, 95.8, 95.5, 81.31, 81.28, 81.2, 80.0, 79.5, 78.7, 77.9, 77.8, 76.6, 75.8, 75.59, 75.56, 75.5, 75.22, 75.18, 74.6, 74.5, 74.4, 74.3, 74.1, 74.0, 73.38, 73.35, 73.2, 72.4, 71.2, 71.1, 70.4, 69.9, 69.8, 69.6, 69.4, 69.1, 68.3, 68.2, 68.0, 67.9, 67.79, 67.75, 67.7, 60.8, 60.7, 51.5, 51.4, 33.8, 28.9, 25.5, 24.5, 23.0, 22.8, 21.1, 20.8, 20.6, 20.4, 18.1, 17.9, 17.62, 17.60. TOF-MS-ES$^+$ m/z calcd for $[C_{165}H_{184}N_2O_{47}]Na^+$: 2968.2. Found: 2968.2.

*5-(Methoxycarbonyl)pentyl) (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**38**).* A stirred solution of compounds **33** (1.0 g, 1.2 mmol) and **37** (0.66 g, 0.23 mmol) in CH_2Cl_2 (10 mL) was treated with TMSOTf (5 μL) under external cooling with ice−water. After 5 min the cooling bath was removed, and then the solution was allowed to reach room temperature over a period of 1 h. To the solution was added Et_3N (approximately 0.1 mL) followed by concentration under reduced pressure and column chromatography of the resulting syrup to afford **38** (0.73 g, 88%): ^{1}H NMR ($CDCl_3$, partial) δ 8.12−8.06 (m, 2H), 8.03−7.94 (m, 8H), 7.67−7.32 (m, 16H), 7.31−7.12 (m, 34H), 7.17−7.00 (m, 16H), 6.98−6.92 (m, 4H), 5.74 (t, 1H, $J = 9.1$ Hz), 5.54 (m, 1H), 5.39 (br s, 1H), 3.66 (s, 3H), 2.33 (t, 2H, $J = 7.5$ Hz), 1.98, 1.95, 1.91, 1.88, 1.85, 1.82, 1.77, 1.75, 1.67, 1.45 (10 s, 10 × 3H), 1.68−1.56 (m, 4H), 1.44−1.40 (m, 2H), 1.38, 1.22, 1.00 (3 d, 3 × 3H, $J = 6.3$ each), 1.25 (d, 6H, $J = 6.3$ Hz). ^{13}C NMR ($CDCl_3$) δ 174.1, 171.2, 170.7, 170.63, 170.57, 170.5, 170.4, 170.0, 169.1, 169.0, 168.9, 165.7, 165.5, 165.45, 165.41, 165.1, 138.45, 138.36, 138.24, 138.22, 137.82, 137.79, 137.74, 137.73, 137.68, 137.49, 137.45, 134.0, 133.9, 133.3, 129.9−126.8, 99.5, 99.27, 99.25, 99.0, 98.7, 98.4, 97.5, 96.2, 95.8, 93.8, 93.6, 93.2, 81.3, 81.2, 80.24, 80.20, 79.7, 79.6, 78.9, 78.0, 77.9, 75.7, 75.5, 75.3, 74.63, 74.56, 74.5, 74.2, 73.5, 73.4, 73.0, 72.7, 72.52, 72.45, 71.6, 71.3, 71.2, 70.1, 70.0, 69.7, 69.5, 69.3, 69.1, 68.9, 68.7, 68.5, 68.3, 68.1, 68.0, 67.9, 67.8, 67.7, 67.6, 67.4, 67.2, 60.9, 60.8, 60.7, 51.5, 51.4, 51.2, 51.0, 33.9, 29.0, 25.7, 24.6, 23.1, 23.0, 22.5, 22.4, 21.27, 21.26, 20.73, 20.70, 20.68, 20.59, 20.56, 20.5, 20.4, 18.3, 18.2, 18.1, 18.0, 17.7, 17.5. TOF-MS-ES$^+$ m/z calcd for $[C_{199}H_{223}N_3O_{60}]Na^+$: 3637.2. Found: 3637.4.

*5-(Methoxycarbonyl)pentyl) (2-O-acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**39**).* To a stirred solution of **28** (1.00 g, 0.82 mmol) and **37** (0.66 g, 0.23 mmol) in CH_2Cl_2 (15 mL) was added TMSOTf (5 μL) under cooling in ice−water. After 15 min the cooling bath was removed, and then the solution was allowed to reach room temperature in 1 h. The solution was treated with Et_3N (~0.1 mL) followed by removal of the volatiles. Column chromatographic purification of the residue using hexanes−EtOAc (3:2 → 5:4) as the eluant afforded **39** (0.95 g, 91%) as an amorphous substance, the purity of which was about 95% (NMR): ^{1}H NMR ($CDCl_3$, partial) δ 8.11−8.08 (m), 8.02−7.95 (m, 7.64−7.54 (m), 7.50−7.39 (m), 7.32−6.89 (m), 5.83 (d, 1H, $J = 10$ Hz), 5.73 (t, 2 × 1H, $J = 9.3$ Hz), 5.55, 5.54 (2 d, 2 × 1H, $J = 3.5$ Hz each), 5.42−5.38 (2 m, 2 × 1H), 3.66 (s, 3H), 2.33 (t, 2H, $J = 7.5$ Hz), 1.96, 1.95, 1.93, 1.88, 1.85, 1.82, 1.75, 1.71, 1.67, 1.63 (10 s, 10 × 3H), 1.38, 1.19, 0.99 (3 d, 3 × 3 H, $J = 6.3$ Hz), 1.14 (d, 6H, $J = 6.3$ Hz); ^{13}C NMR ($CDCl_3$) δ 174.0, 171.1, 170.60, 170.57, 170.5, 170.3, 170.1, 169.2, 168.9, 165.7, 165.5, 165.4, 165.0, 138.4, 138.3, 138.20, 138.16, 137.8, 137.7, 137.6, 137.44, 137.40, 134.0, 133.7, 133.29, 133.26, 129.8−125.3, 99.4, 99.3, 99.20, 98.9,98.7, 98.4, 97.4, 96.20, 95.7, 95.5, 94.2, 81.3, 81.1, 80.20, 80.16, 79.9, 79.6,

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79.0, 78.0, 77.9, 77.5, 76.6, 75.73, 75.70, 75.6, 75.3, 74.6, 74.5, 74.4, 74.3, 7.35, 73.4, 73.2, 73.0, 72.8, 72.5, 72.4, 71.20, 71.16, 70.5, 70.3, 70.1, 70.0, 69.5, 69.2, 68.9, 68.6, 68.4, 68.1, 68.04, 67.99, 67.9, 67.7, 60.78, 60.76, 53.4, 51.5, 51.4, 50.9, 33.9, 31.6, 29.0, 25.6, 24.6, 23.0, 22.9, 22.7, 22.6, 21.4, 21.24, 21.21, 20.8, 20.70, 20.68, 20.53, 20.51, 20.2, 18.2, 18.1, 17.9, 17.7, 17.4, 14.2, 14.1, 11.4. TOF-MS-ES$^+$ m/z calcd for $[C_{226}H_{251}N_3O_{65}]Na^+$: 4069.5. Found: 4069.6.

*5-(Methoxycarbonyl)pentyl (2-O-benzoyl-4-O-benzyl-3-O-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4-O-benzyl-α-L-rhamnopyranoside (**40**).* To a stirred solution of imidate **8** (1.6 g, 0.98 mmol) and octasaccharide **37** (1.5 g, 0.52 mmol) in CH_2Cl_2 (15 mL) was added TMSOTf (7.5 μL) under cooling in ice−water. The solution was allowed to reach room temperature. After 1 h the solution was treated with Et_3N (0.1 mL) followed by concentration. Repeated column chromatography of the residue using hexanes−EtOAc (3:2 → 6:5) as the eluant afforded **40** (1.45 g, 64%) as an amorphous material: ^{1}H NMR ($CDCl_3$, partial) δ 8.15−7.97 (m, 12H), 7.7−7.57 (m, 7H), 7.55−7.43 (m, 14H), 7.35−7.21 (m, 46H), 7.21−6.96 (m, 26H), 3.69 (s, 3H), 5.83−5.74 (m, 3H), 5.63−5.55 (m, 3H), 5.43 (br s, 1H), 5.39 (dd, 1H, J = 3.5 Hz, J = 9.2 Hz), 5.34 (br s, 1H), 5.29 (br s, 3H), 5.25−5.19 (m, 3H), 5.16−5.09 (m, 3H), 4.97 (d, 1H, J = 3.2 Hz), 2.36 (t, 4H, J = 7.5 Hz), 2.09, 2.01, 1.98, 1.90, 1.88, 1.86, 1.82, 1.77, 1.70 (9 s, 9 × 3H), 1.70−1.60 (m, 4H), 1.43−1.37 (m, 2H), 1.41, 1.19, 1.18, 1.12, 1.09, 0.95 (6 d, 6 × 3H, J = 6.3 each); ^{13}C NMR ($CDCl_3$) δ 174.0, 170.64, 170.62, 170.58, 170.5, 170.4, 170.3, 169.4, 168.9, 166.0, 165.6, 165.52, 165.46, 165.44, 165.42, 165.3, 165.2, 138.36, 138.35, 138.3, 138.2, 138.23, 138.16, 138.0, 137.80, 137.75, 137.70, 137.69, 137.67, 137.5, 137.43, 137.41, 137.39, 137.38, 133.93, 133.87, 133.4, 133.3, 133.2, 129.8 − 126.8, 100.1, 99.6, 99.2, 98.9, 98.3, 97.4, 96.8, 95.9, 95.7, 81.28, 81.25, 80.8, 80.2, 80.1, 79.6, 79.40, 78.9, 78.8, 78.4, 78.1, 77.9, 75.72,75.67, 75.65, 75.4, 75.2, 74.9, 74.6, 74.5, 74.4, 74.2, 74.1, 73.9, 73.45, 73.43, 73.38, 72.5, 72.4, 71.3, 71.2, 71.1, 70.4, 70.2, 69.9, 69.6, 69.5, 69.4, 68.44, 68.39, 68.2, 68.1, 68.0, 67.9, 67.8, 67.72, 67.68, 60.8, 60.65, 51.7, 51.5, 51.3, 25.6, 24.6, 23.2, 23.0, 22.9, 21.2, 21.1, 21.0, 20.7, 20.5, 18.2, 18.1, 18.0, 17.6, 17.5, 17.1. TOF-MS-ES$^+$ m/z calcd for $[C_{246}H_{270}ClN_3O_{70}]Na^+$: 4443.7. Found: 4444.0.

*5-Methoxycarbonylpentyl α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**41**).* For ^{1}H and ^{13}C NMR data, see Tables 1, 3, 5, and 7.

*5-Methoxycarbonylpentyl α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→3)-α-L-rhamnopyranoside (**42**).* For ^{1}H and ^{13}C NMR data, see Tables 1, 3, 5, and 7.

Growth of Bacteria, Isolation of LPS and O-SP. *E. coli* O148 strain 201 and rabbit typing antiserum were obtained from the Centers for Disease Control and Prevention (Atlanta, GA) and cultured in tryptic soy broth for 20 h at 37 °C with stirring and aeration. The pH was maintained at ∼7.5 by the addition of ammonium hydroxide. The identity of the bacteria was confirmed by the Clinical Microbiology Laboratory at the NIH, Bethesda, MD. LPS was extracted by the hot phenol method.[42] LPS was purified by ultracentrifugation in a Sorvall Discovery SE centrifuge twice at 35 000 rpm for 5 h, at 4 °C. The contents of proteins and nucleic acids in the final LPS preparation were less than 1.5%. To isolate O-SP, LPS (100 mg) was treated with 10 mL of 1% acetic acid at 100 °C for 1.5 h. Lipid A was removed by ultracentrifugation as above, and the soluble products were separated by gel chromatography on a BioGel P-10 (1 × 100 cm) column equilibrated with 0.05 M pyridinium acetate buffer at pH 5.5. The chromatography was monitored with a differential refractometer. O-SP was eluted in the void volume fraction with a yield of 28%.

Conjugation of Oligosaccharides. To 15 mg of BSA or rDT (H21G)[27] in 2.2 mL of Buffer A (PBS, 0.1% glycerol, 5 mM EDTA, pH 7.2), 4 mg of *N*-succinimidyl 3-(bromoacetamido) propionate in 40 μL of DMSO was added at pH 7.2, at rt with mixing. After 1.5 h, the solution was applied to a Sephadex G-50 column (1 × 50 cm) in PBS. The void volume fraction containing bromoacetylated protein (Pr−Br) was concentrated using an Amicon Ultra-15 centrifuge filter device to 2.6 mL. 13 mg of the protein was recovered, and 0.1 mL of the solution was removed for analysis. To 12 mg of Pr−Br in 2.4 mL of Buffer A, 10 mg *of O*-(3-thiopropyl)hydroxylamine were added in 300 μL of 1 M KCl, reacted at pH 7.2, rt with mixing for 3 h. Next, the solution was passed through Sephadex G-50 (1 × 50 cm), and the void volume fraction containing aminooxylated protein (Pr-ONH_2) was concentrated to 2.6 mL as above, and 0.2 mL removed for analysis. Pr-ONH_2 (10 mg) was treated with the oligosaccharide (10 mg) in 3 mL of Buffer A at pH 7.2 at rt with mixing for 12 h. The solution was then passed through Sephadex G-50 in PBS, and the void volume fraction was collected and analyzed for sugar and protein contents and molecular mass.

Analytical Methods. Protein concentration was measured by the BCA protein assay according to the manufacturer's protocol. Sugar was quantitated by the anthrone assay.[43] SDS-PAGE and Western blot were performed using standard protocols. LPS (2 μg) was loaded into 14% Tris-Glycine gels and transferred into polyvinylidene (PDVF) membranes according to the manufacturer's instructions (Bio-Rad, Hercules, CA). After the transfer, the membranes were blocked with 1% BSA in PBS and incubated for 3 h with anti-*SD* or anti-*E. coli* O148 sera raised by whole bacteria or by the synthetic oligosaccharide/protein conjugates diluted 1:100 in blocking buffer. After washing 3 × 15 min with PBS + 0.05% Tween, the membranes were incubated for 1 h with phosphatase labeled goat antimouse IgG (KPL, Gaithersburg, MD) diluted 1:500 in blocking buffer, washed again as above, and visualized using BCIP/NBT phosphate substrate (KPL, Gaithersburg, MD). For dot-blot analyses, 2 μg of conjugates were pipetted onto stripes of a nitrocellulose membrane and developed the same way as the Western Blot. MALDI-TOF mass spectra of the derivatized proteins and of the conjugates were obtained with a MALDI-TOF instrument operated in the linear mode. Samples for analysis were desalted, and a 1 μL aliquot was mixed with 20 μL of a saturated sinapinic acid matrix solution made in 30% aq CH_3CN containing 0.1% trifluoroacetic acid. One microliter of the solution so obtained was applied to and dried on the sample stage.

Immunization. All animal experiments were approved by the National Institute of Child Health and Human Development Animal Care and Use Committee. Five to six-week-old female NIH general purpose mice were immunized subcutaneously 3 times at 2 week intervals, with 2.5 μg of oligosaccharide as a conjugate in 0.1 mL of phosphate buffered saline (PBS). Groups of 10 mice were exsanguinated 7 days after the third injection.[44] Controls received PBS.

Serology. IgG antibodies were measured by ELISA using BSA or human serum albumin as appropriate for blocking. Antibody levels were calculated relative to a pool of highest antibody level sera obtained from mice immunized 3 times with *S. dysenteriae* type 1 conjugates[14] and assigned a value of 100 ELISA units (EU). Results were computed with an ELISA immunoassay data processing program provided by the Biostatistics and Information Management Branch, CDC.[45] Competitive inhibition ELISA was done by incubating sera from mice injected with either *S. dysenteriae* type 1 or *E. coli* O148 synthetic 12-mers conjugated to rDT, diluted in PBS to give an A_{405} of 1.0, with 0.04, 0.2, 5, or 80 μg/well of either O-SP, incubated for 1 h at 37 °C, followed by incubation at 4 °C for 12 h. The assay was then continued as described above. Sera with inhibitor were compared to the same serum dilution without an inhibitor. Percent inhibition was defined as (1-A_{405} adsorbed serum/A_{405} nonadsorbed serum) × 100%.

Statistics. ELISA values are expressed as the geometric mean (GM). Unpaired *t*-tests were used to compare GMs of different groups.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **1**−**8**, **10**, **13**, **14**, **18**−**30**, **32**, and **34**−**42**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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