Synthesis of Two Linear PADRE Conjugates Bearing a Deca- or Pentadecasaccharide B Epitope as Potential Synthetic Vaccines against *Shigella flexneri* Serotype 2a Infection**

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Abstract: The blockwise synthesis of the 2-aminoethyl glycosides of a deca- and a pentadecasaccharide made of two and three repeating units, respectively, of the *Shigella flexneri* serotype 2a specific polysaccharide is reported. The strategy relies on trifluoromethanesulfonic acid mediated glycosylation of a pentasaccharide building block acting as a glycosyl donor and a potential glycoside acceptor. Both targets were made available in amounts large enough for their subsequent conversion into glycoconjugates. Indeed, efficient elongation of the spacer through an acetylthioacetyl moiety and subsequent conjugation onto a Pan HLA DR-binding epitope (PADRE) T-cell-universal peptide resulted in two fully synthetic neoglycopeptides, which will be evaluated as potential vaccines against *S. flexneri* serotype 2a infections.

Keywords: antigens · carbohydrates · glycoconjugates · glycopeptides · vaccines

Introduction

Diarrheal diseases resulting from bacterial or viral infections account for more than 3 million deaths annually. Available field data point to shigellosis, or bacillary dysentery, as the major form of infection leading to this poor prognostic. Indeed, the number of annual episodes of shigellosis was recently estimated to be 164.7 million, with some 1.1 million deaths among the victims.[1] Significantly, 163.2 million of these episodes were reported in developing countries, particularly in areas where sanitary conditions are insufficient. Of the four species of *Shigella*, *Shigella flexneri* is the major one responsible for the endemic form of the disease in developing countries. Furthermore, field data indicate that among the various *S. flexneri* strains known to be pathogenic in humans, serotype 2a is the most prevalent. Infection, which is spread by the feco-oral route, results from colonization of the digestive tract by a number of bacteria as low as a 100; this results in a high transmission rate. More than 69% of the episodes and 61% of all deaths attributable to shigellosis involve children under five years of age, which is of utmost concern. When it is considered that sanitary conditions are not likely to improve rapidly in those areas at risk and that the global impact of shigellosis cannot be adequately controlled with the available means, a safe and effective vaccine against the most common serotypes of *Shigella* would offer great potential to control the disease. Indeed, the development of a vaccine against shigellosis is of high priority, as stated by the World Health Organization in its program against enteric diseases.[2] Several options, resulting in the development of various experimental vaccines, have been undertaken to reach this goal.[3–5] However, there are as yet no licensed vaccines for shigellosis.

As for several other Gram-negative bacteria, *S. flexneri* 2a lipopolysaccharide (LPS) is a major surface antigen. It is both an essential virulence factor and a major target of the infected host's immune response.[6,7] Protection is serotype-specific, a fact which points to the O-specific polysaccharide (O-SP) moiety of the LPS as the target of the protective immune response. In fact, the repeating unit of this polymer of less than 30 kD defines the bacterium serotype. This knowledge has been taken into account and has led to clinical studies of at least three families of LPS-based candidate *Shigella* vaccines, with two of them specifically involving the O-SP moiety in the form of either the detoxified LPS or synthetic fragments thereof. A critical point in the design of such polysaccharide vaccines is that O-SPs are T-cell-inde-

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[**] PADRE = Pan HLA DR-binding epitope. Synthesis of ligands related to the O-specific polysaccharides of *Shigella flexneri* serotype 2a and *Shigella flexneri* serotype 5a, Part 13. For Part 12, see reference [22].

 DOI: 10.1002/chem.200400903

pendent antigens,[8,9] which are not immunogenic by themselves. Nevertheless, benefiting from the successful conversion of bacterial capsular polysaccharides from T-cell-independent antigens to T-cell-dependent ones through their covalent coupling to a protein carrier, it was shown that O-SPs could be turned into potent immunogens. Several polysaccharide–protein conjugates, targeting either *S. sonnei*, *S. dysenteriae* 1, or *S. flexneri* 2a, were shown to be safe and immunogenic in adults[7,10] and also in young children when evaluated in the latter.[11] In addition, a recent outbreak of *S. sonnei* during a randomized, double-blind, field trial allowed Robbins and co-workers to demonstrate that a candidate vaccine made of the corresponding detoxified LPS covalently linked with the nontoxic recombinant exoprotein A of *Pseudomonas aeruginosa* conferred 74% protection in military recruits.[12] Even though encouraging data are available, detoxified-LPS–protein conjugate vaccines remain complex constructs, especially when obtained from randomly activated polysaccharides. Their immunogenicity depends on several parameters, among which are the length and nature of the hapten as well as its loading on the protein. It may be assumed that the use of well-defined synthetic oligosaccharides (OSs) suitable for single-point attachment on to the carrier would allow better control and, consequently, the optimization of the above-mentioned parameters. The fact that low-molecular-weight OSs mimicking bacterial antigenic determinants are immunogenic when conjugated onto a protein carrier was demonstrated in the late 1930s[13,14] and has been exploited on several occasions since then.[15] Several reports indicated that neoglycoproteins incorporating OSs comprising one repeating unit or smaller fragments were immunogenic in mice.[16–18] Furthermore, others demonstrated that conjugates incorporating haptens mimicking a single repeating unit of the natural antigen could induce fully protective antibodies in mice,[19,20] whereas short OSs representing only part of the natural antigen repeating unit appeared to already contain epitopes capable of inducing protection in rabbits.[20] Along this line, we recently reported the synthesis of three fully synthetic glycoconjugates as potential vaccines against *S. flexneri* 2a infection.[21] These incorporated short OS haptens, representative of either part of or the whole repeating unit of the O-SP of *S. flexneri* 2a.

The thymus dependence of an OS–protein conjugate increases with shortening of the OS length. However, it is known that epitope size can vary between species.[22] In particular, longer OSs may be required for an optimal immune response in humans.[23] Indeed, it is anticipated that the better, in terms both of antigenicity and secondary structure, the O-SP mimics used as haptens, the better the immunogenicity of the resulting glycoconjugates. For various reasons, it is theorized that haptens made of at least two contiguous repeating units may be necessary for the corresponding OS conjugates to induce antipolysaccharide antibodies efficiently,[24] as illustrated in the case of *S. dysenteriae* 1[25] or *Streptococcus pneumoniae* 3.[19] Analogous observations resulted from extensive work in the field of *Haemophilus influenzae* b glycoconjugate vaccines.[26] In the latter case, the success of the approach in humans was recently demonstrated.[27] In addition, another important point was addressed, that is, the need for the development of alternatives to the most commonly used protein carriers that are compatible with administration in humans. Relying on synthetic T-helper peptides derived from conventional protein carriers such as tetanus toxoid[28] or selected from the pathogen's own proteins[29] may be an option. To overcome the limitations associated with the extensive polymorphism of human leucocyte antigen (HLA) molecules, recombinant carrier proteins constituted by strings of several human T-helper antigens from various pathogen origins have also been proposed.[30] In other strategies targeting an efficient T-helper contribution in humans, nonnatural T-helper peptides, such as the Pan HLA DR-binding epitope (PADRE), were engineered based on their capacity to bind to a large number of HLA class II molecules.[31] PADRE was found to be efficient when evaluated as a carrier for carbohydrate B antigens, such as the lacto-*N*-fucopentose II and a dodecasaccharide from the O-SP of *Salmonella typhimurium*,[32] as well as more recently when conjugated to various *S. pneumoniae* polysaccharide antigens.[33]

An extension of the latter approach to the design of neoglycopeptides as potential vaccines against *S. flexneri* 2a infection is reported in the following section.

Results and Discussion

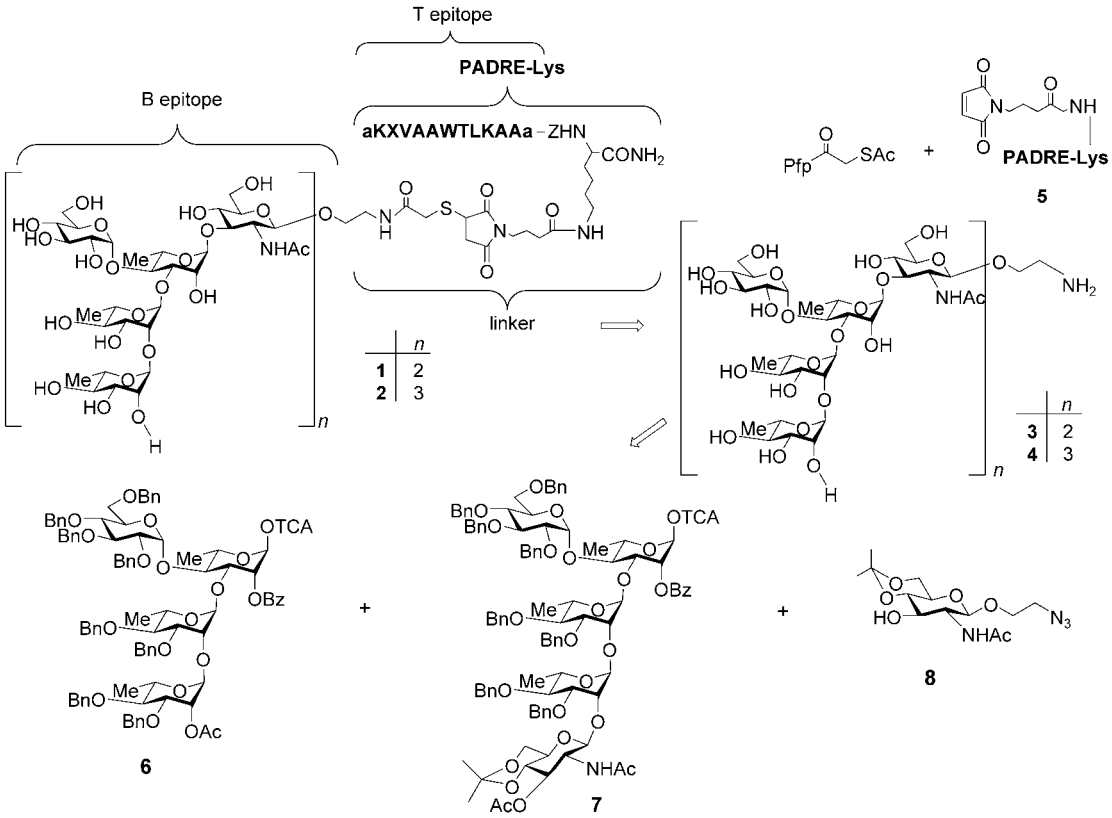
The O-SP of *S. flexneri* 2a is a branched heteropolysaccharide defined by the pentasaccharide repeating unit **I**.[34,35] It

A B E C D
2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-L-Rha*p*-(1→3)-β-D-GlcNAc*p*(1→

I

features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-SPs and comprises an *N*-acetyl glucosamine (D) and three rhamnose residues (A, B, C). The specificity of the serotype is associated with the α-D-glucopyranosyl residue linked to position 4 of rhamnose C.

We described recently the synthesis of the ECD, B(E)CD, and AB(E)CD fragments functionalized with an aminoethyl spacer at their reducing end, and we demonstrated that the latter could serve as a suitable anchoring point,[21] as illustrated by the synthesis of the corresponding PADRE neoglycopeptides. Parallel studies into the recognition of synthetic fragments of the *S. flexneri* 2a O-SP by protective monoclonal antibodies outlined the impact of chain elongation on the recognition process.[36] Taking both sets of data into account, we report herein on the synthesis of the 2-aminoethyl glycosides of a decasaccharide (**1**) and a pentadecasaccharide (**2**), which correspond to a dimer $[AB(E)CD]_2$ and a trimer $[AB(E)CD]_3$ of the branched pentasaccharide

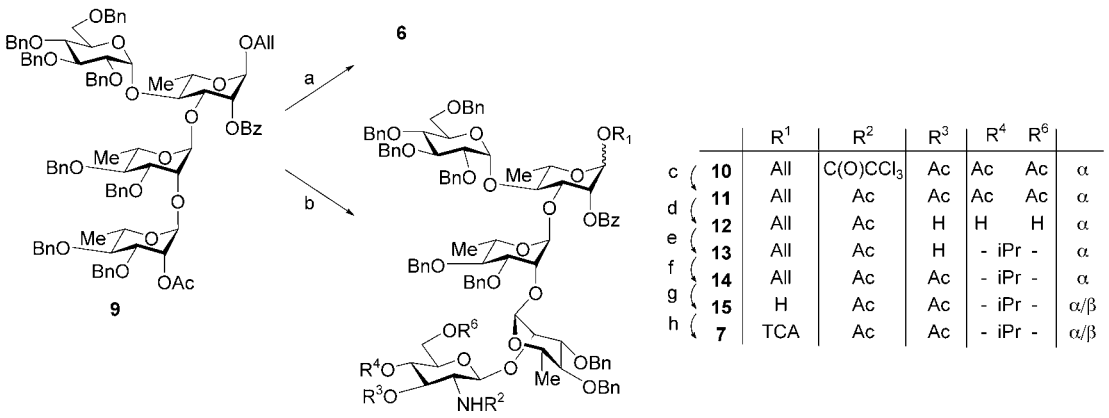


Scheme 1. Retrosynthetic analysis of the aminoethyl glycoside haptens **1** and **2**. a = D-alanine, Ac = acetyl, Bn = benzyl, Bz = benzoyl, Pfp = pentafluorophenyl, TCA = trichloroacetimidate, X = cyclohexylalanine, Z = aminocaproic acid.

I, respectively (Scheme 1). The synthesis is based on a modular approach involving three partners. Basically, it relies on 1) the use of appropriate haptens (**3** and **4**, respectively) functionalized at the anomeric position with an aminoethyl spacer, 2) the incorporation of a thioacetyl acetamido linker as a masked thiol functionality, and 3) the use of a PADRE peptide derivatized by a maleimido group on a C-terminal lysine residue (**5**).

In considering targets **3** and **4**, a disconnection at the D–A linkage would appear most appropriate. However, others have shown that such a disconnection strategy was not suitable even with di- or trisaccharide building blocks;[37,38] thus, this route was avoided. More recently, disconnections at the A–B, B–C, and C–D linkages were evaluated in this laboratory when the methyl glycoside of the frame-shifted decasaccharide D′A′B′(E′)C′DAB(E)C was successfully synthesized.[39] It was demonstrated on that occasion that disconnection at the C–D linkage was indeed appropriate for the construction of large fragments of the *S. flexneri* 2a O-SP. Based on our experience in the field, we designed a blockwise strategy to synthesize targets **3** and **4** that involved an AB(E)C tetrasaccharide donor (**6**), a DAB(E)C potential acceptor acting as a donor (**7**), and the recently disclosed acceptor **8**,[21] bearing a masked aminoethyl spacer, as a precursor to the reducing end D residue (Scheme 1). Although permanent blocking of the OH groups at positions 4_D and 6_D with an isopropylidene acetal may appear somewhat unusual, this choice was a key feature of the strategy. It was based on former observations in the methyl glycoside series, when it was demonstrated that its use could overcome some of the known drawbacks of the corresponding benzylidene acetal,[40,41] including the poor solubility. In order to reduce the number of synthetic steps, it was found appropriate to access the AB(E)C donor and the DAB(E)C building block from a common key AB(E)C tetrasaccharide intermediate **9** (see Scheme 2).[39] Most of all, the design of the pentasaccharide building block **7** was a key element to success. Indeed, a leading concept of the overall strategy was to limit the number of transformations at later stages in the syntheses. With respect to the choice of **7**, the reader's attention is drawn to 1) the permanent blocking of positions 4_D and 6_D with an isopropylidene acetal, 2) the introduction of a participating benzoyl group, which is resistant to Zemplén deacylation, at position 2_A, 3) the temporary protection of position 3_D as an orthogonal acetate, 4) the early introduction of the required acetamido functionality at position 2_D, and 5) the activation of the anomeric position as a trichloroacetimidate (TCA). Indeed, the syntheses disclosed herein are based on the use of the trichloroacetimidate chemistry,[42] and known building blocks were used whenever possible.

Synthesis of the tetrasaccharide building block 6 (Scheme 2): The fully protected tetrasaccharide **9** could be obtained in high yield when the condensation was run on a

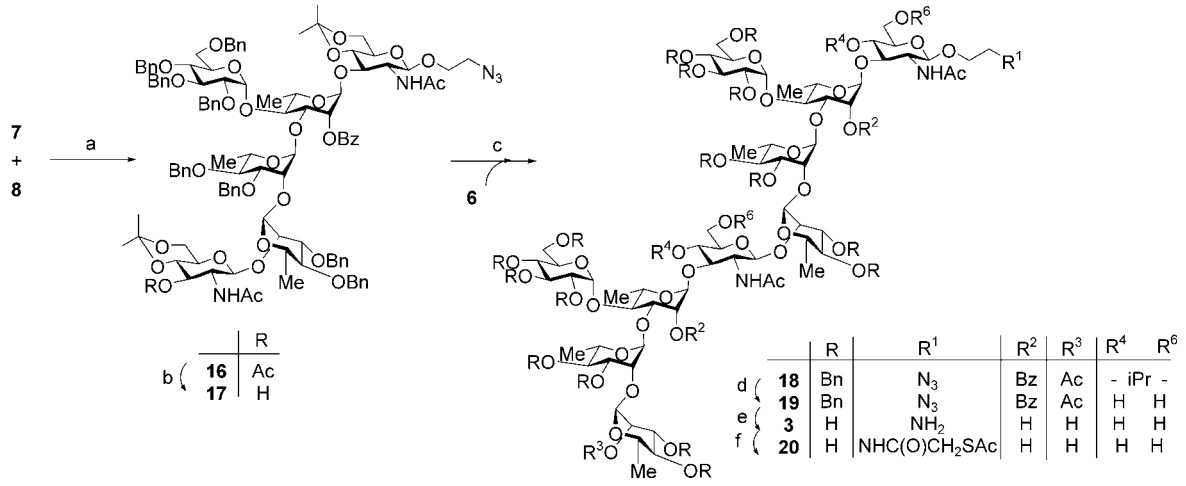


Scheme 2. Synthesis of building blocks **6** and **7**. a) 1. $[Ir(cod)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$ (cat.), THF, room temperature, 2 h; 2. I_2, THF/water, room temperature, 1 h; 3. CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1 h; b) see ref. [21]; c) Bu_3SnH, AIBN (cat.), toluene, 100 °C, 1 h; d) MeONa (cat.), MeOH, room temperature, 25 min; e) $Me_2C(OMe)_2$, PTSA (cat.), DMF, room temperature, overnight; f) Ac_2O, pyridine, room temperature, 2.5 h; g) 1. $[Ir(cod)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$ (cat.), THF, room temperature, 2 h; 2. HgO, $HgBr_2$, acetone/water, room temperature, 1 h; h) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1 h. AIBN = 2,2′-azobisisobutyronitrile, All = allyl, cod = cycloocta-1,5-diene, DBU = 1,8-diazabicyclo [5.4.0]undec-7-ene, DMF = *N*,*N*-dimethylformamide, PTSA = *p*-toluenesulfonic acid, THF = tetrahydrofuran.

15 g scale. Preparation of **6** was conveniently achieved with 67% yield from this crucial intermediate according to a conventional protocol, namely, selective removal of the anomeric allyl group and subsequent activation upon treatment of the resulting hemiacetal with trichloroacetonitrile in the presence of catalytic DBU.

Synthesis of the pentasaccharide building block 7 (Scheme 2): We recently described the synthesis of the DAB(E)C building block **10** bearing a trichloroacetamido function at position 2_D by starting from the tetrasaccharide **9**. Compound **10**, now conveniently prepared on a 5–10 g scale, was used successfully as the donor in the synthesis of the D′A′B′(E′)C′DAB(E)C decasaccharide, once it had been converted into the corresponding trichloroacetimidate.[39] However, for the present study, we reasoned that conversion of the trichloroacetamide moiety into the required acetamide at an early stage in the synthesis was preferable. Reductive free-radical dechlorination of **10** by using Bu_3SnH in the presence of catalytic AIBN allowed the conversion of the *N*-trichloroacetyl moiety into an *N*-acetyl group, to give the known compound **11** (68%), previously obtained according to an alternative and somewhat lower yielding strategy.[39] Controlled de-*O*-acetylation of **11** by using a catalytic amount of methanolic sodium methoxide gave the triol **12**, which was next converted into the corresponding alcohol **13** upon reaction with 2,2-dimethoxypropane (81% from **11**). Conventional acetylation at position 3_D then gave the fully protected intermediate **14** (94%), with the good overall yield of this three-step conversion (**11**→**14**, 76%) outlining its usefulness. The latter compound was transformed into the hemiacetal **15** by following a two-step process, involving iridium-complex-promoted isomerization of the allyl moiety into the corresponding propen-1-yl group[43] and hydrolysis of the latter upon treatment with mercuric chloride, since it was originally demonstrated that labile isopropylidene groups were stable to such neutral conditions.[44] Subsequent treatment of **15** with trichloroacetonitrile in the presence of catalytic DBU cleanly gave the key building block **7** (82% from **14**).

Synthesis of the aminoethyl decasaccharide 3 (Scheme 3): Previous glycosidation attempts in the series indicated that, when run at low temperature or at room temperature, reactions with the D acceptor **8** occasionally resulted in a somewhat poor yield of the condensation product. This was tentatively explained by the still rather low solubility of **8**. When using 1,2-DCE as the solvent, the condensation could be performed at higher temperature, which proved rewarding. Indeed, optimized coupling conditions of **7** and **8**, used in slight excess, relied on the concomitant use of a catalytic amount of triflic acid in the presence of 4 Å molecular sieves as the promoter and 1,2-DCE as the solvent, while the condensation was performed at 75 °C, according to a known protocol[45] that had recently been adapted to the use of acceptor **8** in the *S. flexneri* series.[21] The fully protected hexasaccharide **16** was isolated in a satisfactory 76% yield. The resistance of the two isopropylidene acetals to the harsh acidic conditions of the glycosidation reaction is noteworthy. The fact that the hemiacetal **15**, resulting from the hydrolysis of the excess donor, can be recovered is a great advantage if one considers scaling up the process (not described). Resistance of isolated benzoyl groups to Zemplén transesterification has been reported.[46–48] It was also observed previously in the series, upon attempted removal of a benzoyl group located at position 2_C.[39] Thus, as anticipated, selective deacetylation of the OH group at position 3 of the nonreducing residue gave the D′AB(E)CD acceptor **17** in a yield of 97%; this result confirmed the orthogonality of the various protecting groups in use at this stage. Condensation of the latter and **6** was performed in 1,2-DCE with triflic acid as the promoter. One may note that although the condensa-

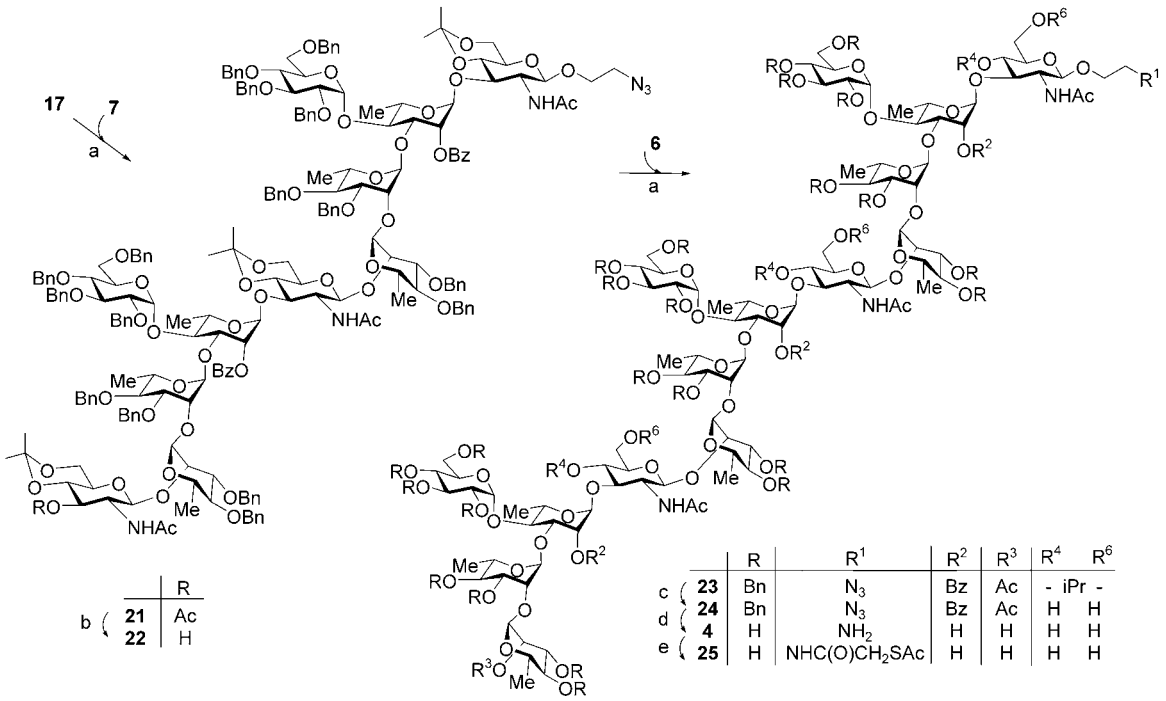


Scheme 3. Synthesis of the decasaccharide hapten **20** bearing a masked-thiol-type spacer. a) TfOH (cat.), MS, 1,2-DCE, 75 °C, 2.5 h; b) MeONa (cat.), MeOH, room temperature, 3 h; c) TfOH (cat.), MS, 1,2-DCE, −35 °C→10 °C, 2.5 h; d) 50% aqueous TFA, CH_2Cl_2, 0 °C; 3 h; e) 1. MeONa (cat.), MeOH, 55 °C, overnight; 2. 10% Pd/C, 1 N aqueous HCl, EtOH/EtOAc, room temperature, 72 h; f) SAMA-Pfp, 0.1 M phosphate buffer (pH 7.4), room temperature, 1 h. 1,2-DCE = 1,2-dichloroethane, MS = 4 Å molecular sieves, SAMA-Pfp = *S*-acetylthioglycolic acid pentafluorophenyl ester, Tf = triflate = trifluoromethanesulfonyl, TFA = trifluoroacetic acid.

tion involves the construction of the C–D linkage, thus somewhat resembling the preparation of hexasaccharide **16**, heating was not required and the glycosylation went smoothly at 10 °C to give the fully protected decasaccharide **18** (82%). Acidic hydrolysis of the acetals gave the tetraol **19** (75%). Transesterification of the acyl groups was best performed by overnight heating of **19** in methanolic sodium methoxide. Final hydrogenolysis of the benzyl groups and concomitant conversion of the azido group into the corresponding amine gave the target **3** (71% from **19**). As observed previously,[21,49] the latter transformation was best performed under acidic conditions.

Synthesis of the aminoethyl pentadecasaccharide 4 (Scheme 4): The rather convenient access to building block **7** allowed larger sequences to be targeted. Thus, having the hexasaccharide acceptor **17** in hand, we repeated the two-step glycosylation/deacetylation process involving **7**. Analogously to the condensation step leading to the fully protected decasaccharide, condensation of **17** and the pentasaccharide donor **7** in the presence of triflic acid was run at a temperature below 5 °C. Under such conditions, the fully protected undecasaccharide **21** was isolated in an excellent yield of 90%, a result that once more outlines the compatibility of the rather labile isopropylidene groups with the glycosylation conditions in use. Zemplén transesterification at the nonreducing OH group at position 3_D of **21**, resulting in the required acceptor **22** (91%), proved to be efficient. Condensation of this key intermediate with the tetrasaccharide trichloroacetimidate donor **6** was performed according to the same protocol, by using triflic acid as the promoter. The fully protected pentadecasaccharide **23** was isolated in a satisfactory yield of 82%. Conversion of **23** into the target **4** was performed by running the stepwise sequence described for the preparation of **3**. Acidic hydrolysis of the isopropylidene groups afforded the hexaol **24** (83%). Again, the resistance of the isolated benzoyl groups at position 2_C to methanolic transesterification could be overcome by running the transesterification step at high temperature. Lastly, conventional hydrogenolysis of the benzyl groups and concomitant reduction of the azide moiety allowed the smooth conversion of the de-*O*-acylated intermediate into the pentadecasaccharide hapten **4** (65% from **24**). Interestingly, although the number of synthetic steps involved may be somewhat challenging, the steps are generally high yielding and large amounts of **4** are reachable.

Synthesis of the target conjugates 1 and 2: Chemoselective ligation of the carbohydrate B and peptide T epitopes was achieved through coupling of the carbohydrate haptens prefunctionalized with a thiol function and a maleimido group correctly introduced at the C terminus of the T-helper peptide, which allows specific and high-yielding modification of the former in the presence of other nucleophiles.[50] Based on reported data on the immunogenicity of various maleimide-derived coupling agents,[51] 4-(*N*-maleimido)-*n*-butanoyl was selected as the linker. It was covalently linked to the side-chain amino group of a lysine residue added to the C terminus of the PADRE sequence (PADRE-Lys) according to an in-house process,[21] which differs from that described previously by others.[32] Treatment of **3** and **4** with SAMA-Pfp resulted in the site-selective elongation of their aminoethyl spacers with a thioacetyl acetamido linker to yield **20** and **25** in 61% and 63% yield, respectively (Schemes 3 and 4).[21] Derivatization could be monitored by reversed-phase (RP) HPLC with detection at 215 nm, and structure confirmation was based on MS and NMR spectroscopy analysis. Conjugation of the carbohydrate haptens to the maleimido-activated PADRE-Lys (**5**) was performed in phosphate buffer at pH 6.0 in the presence of hydroxylamine[52] and monitored by RP HPLC. Lastly, RP HPLC purification gave the target neoglycopeptides **1** and **2** as single



Scheme 4. Synthesis of the pentadecasaccharide hapten **25** bearing a masked-thiol-type of spacer. a) TfOH (cat.), MS, 1,2-DCE, −30 °C→5 °C, 2.5 h; b) MeONa (cat.), MeOH, room temperature, 3 h; c) 50% aqueous TFA, CH_2Cl_2, 0 °C, 3 h; d) 1. MeONa (cat.), MeOH, 55 °C, overnight; 2. 10% Pd/C, 1 N aqueous HCl, EtOH/EtOAc, room temperature, 48 h; e) SAMA-Pfp, 0.1 M phosphate buffer (pH 7.4), room temperature, 2 h.

products, whose identity was assessed by MS analysis, in yields of 44 and 67%, respectively.

Conclusion

The synthesis of the O-SP of *S. flexneri* Y by way of polycondensation of a tritylated cyanoethylidene tetrasaccharide has been reported by others.[53] However, this is to our knowledge is the first report of the total synthesis of fully defined oligomeric repeating-unit glycosides mimicking the branched bacterial O-SPs in the *S. flexneri* series. The strategy disclosed herein gives access to extended fragments of the O-SP of *S. flexneri* serotype 2a in a spacer-armed form suitable for immunological studies. Indeed, by using solution-phase methods, large enough amounts were made for the synthesis of fully synthetic oligosaccharide conjugates as potential vaccines targeting *S. flexneri* serotype 2a infection. The preparation of such conjugates in the form of two linear PADRE–oligosaccharide conjugates is exemplified. It is noteworthy that the synthesis of the pentadecasaccharide **2** differs from that of the decasaccharide **1** by only two additional steps.

Experimental Section

General methods: Optical rotations were measured at 25 °C with a Perkin–Elmer model 241 MC automatic polarimeter. TLC on precoated slides of silica gel 60 F_{254} (Merck) was performed with solvent mixtures of appropriately adjusted polarity. Detection was effected, when applicable, with UV light and/or by charring with orcinol (35 mM) in 4 N aqueous H_2SO_4 or EtOH/H_2SO_4 (95/5). Preparative chromatography was performed by elution from columns of silica gel 60 (particle size: 60–43 μm). RP HPLC (215 nm) was performed with a Kromasil 5 μm C18 100 Å 4.6×250 mm analytical column (flow rate: 1 mL min^{-1}). NMR spectra were recorded at 20 °C on a Bruker Advance 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). The external references were TMS (0.00 ppm for both ^{1}H and ^{13}C) for solutions in $CDCl_3$, and dioxane (67.4 ppm for ^{13}C) and trimethylsilyl-3-propionic acid sodium salt (0.00 ppm for ^{1}H) for solutions in D_2O. Proton signal assignments were made by first-order analysis of the spectra as well as analysis of two-dimensional ^{1}H–^{1}H correlation maps (COSY). Of the two magnetically nonequivalent geminal protons at C-6, the one resonating at lower field is denoted H-6a and the one at higher field is denoted H-6b. Interchangeable assignments in the ^{1}H and ^{13}C NMR spectra are marked with an asterisk. Sugar residues in oligosaccharides are serially lettered according to the lettering of the repeating unit of the O-SP and are identified by a subscript in the listing of signal assignments. Low-resolution mass spectra were obtained by fast-atom-bombardment (FAB) MS in the positive-ion mode with dithioerythridol/dithio-L-threitol (4:1, MB) as the matrix, in the presence of NaI, and with xenon as the gas. Anhydrous dichloromethane (DCM) and 1,2-dichloroethane (1,2-DCE), sold on molecular sieves, were used as received. 4 Å powdered molecular sieves were kept at 100 °C and activated before use by heating at 250 °C under vacuum. Solid-phase peptide synthesis was performed by using standard 9-fluorenylmethoxycarbonyl (Fmoc) chemistry protocols on a Pioneer peptide synthesizer (Applied Biosystem). Fmoc-Lys(iv-Dde)-OH, Fmoc-Cha-OH, Fmoc-D-Ala-OH, Fmoc-ε-Ahx-OH and Boc-D-Ala-OH were purchased from NovaBiochem (iv-Dde = 1-(4,4-dimethyl-2,6-dioxocyclohexlidene)-3-methylbutyl, Cha (X) = cyclohexylalanine, ε-Ahx (Z) = aminocaproic acid). All others reagents and amino acids were purchased from Applied Biosystem.

(2-*O*-Acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (6): (1,5-Cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexa-

fluorophosphate (17 mg) was dissolved in THF (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the color to change to yellow. The solution was then degassed again in an argon stream. A solution of **9**[39] (550 mg, 0.37 mmol) in THF (3 mL) was degassed and added. The mixture was stirred at room temperature for 2 h. A solution of I_2 (188 mg, 0.74 mmol) in a mixture of THF (3.5 mL) and water (1 mL) was added. The mixture was stirred at room temperature for 1 h and the volatiles were evaporated. The residue was taken up in DCM and the organic phase was washed successively with a solution of 5% aqueous $NaHSO_3$, 5% aqueous $NaHCO_3$, water, and brine. The organic phase was dried by being passed through a phase separator and was concentrated. The residue was eluted from a column of silica gel with cyclohexane/EtOAc (9:1) to give the corresponding hemiacetal (489 mg, 91%). Trichloroacetonitrile (360 μL) and DBU (6 μL) were added to a solution of the residue (479 mg) in anhydrous DCM (3.1 mL) at 0°C. After 1 h, toluene was added and the mixture was concentrated. The residue was eluted from a column of silica gel with cyclohexane/EtOAc (90:10→85:15) and 0.2% Et_3N to give **6** as a white foam (389 mg, 74%); $[\alpha]_D = +22°$ ($c = 1$, $CHCl_3$); ^1H NMR: $\delta = 8.72$ (s, 1H, C=NH), 8.00–7.00 (m, 45H, Ph), 6.39 (d, 1H, $J_{1,2} = 2.5$ Hz, H-1_C), 5.60 (dd, 1H, $J_{2,3} = 3.0$ Hz, H-2_C), 5.58 (dd, 1H, $J_{1,2} = 1.7$, $J_{2,3} = 3.0$ Hz, H-2_A), 5.12 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1_E), 5.08 (m, 2H, H-1_A, 1_B), 5.00–4.00 (m, 16H, CH_2Ph), 4.20 (dd, 1H, H-3_C), 4.05 (dd, 1H, H-3_E), 4.00–3.35 (m, 14H, H-2_E, 4_E, 5_E, $6a_E$, $6b_E$, 4_C, 5_C, 2_B, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A), 2.05 (s, 3H, OAc), 1.42, 1.36, 1.00 (3d, 9H, H-6_A, 6_B, 6_C) ppm; ^{13}C NMR: $\delta = 170.3$, 165.8 (2C, C=O), 138–127 (Ph), 99.9 (2C, C-1_A, 1_B), 98.5 (C-1_E), 94.7 (C-1_C), 82.1, 81.2, 80.4, 80.0, 79.1, 78.1, 78.0, 75.2, 71.7, 71.2, 70.7, 69.5, 69.4, 68.7 (16C, C-2_A, 3_A, 4_A, 5_A, 2_B, 3_B, 4_B, 5_B, 2_C, 3_C, 4_C, 5_C, 2_E, 3_E, 4_E, 5_E), 76.0, 75.7, 75.5, 75.1, 74.3, 73.3, 72.2, 71.2 (8C, PhCH_2), 68.5 (C-6_E), 21.4 (OAc), 19.2, 18.5, 18.1 (C-6_A, 6_B, 6_C) ppm; elemental analysis calcd (%) for $C_{91}H_{96}Cl_3NO_{20}$: C 67.05, H 5.94, N 0.86; found: C 66.44, H 6.21, N 0.93.

Allyl (2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (11): A mixture of **10**[39] (3.14 g, 1.6 mmol), Bu_3SnH (2.5 mL, 9.3 mmol), and AIBN (240 mg) in dry toluene (40 mL) was stirred for 30 min at room temperature under a stream of dry argon and was then heated for 1 h at 100°C, cooled, and concentrated. The residue was eluted from a column of silica gel with petroleum ether/EtOAc (3:2) to give **11** as a white foam (2.0 g, 68%); $[\alpha]_D = +3°$ ($c = 1$, $CHCl_3$); ^1H NMR: $\delta = 8.00$–7.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.58 (d, 1H, $J_{2,NH} = 8.0$ Hz, N-H$_D$), 5.35 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 2.3$ Hz, H-2_C), 5.19 (m, 2H, All), 5.10 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1_A), 4.92 (dd, 1H, $J_{2,3} = 10.5$, $J_{3,4} = 10.5$ Hz, H-3_D), 4.92 (d, 1H, $J_{1,2} = 3.3$ Hz, H-1_E), 4.90 (d, 1H, $J_{1,2} = 1.7$ Hz, H-1_B), 4.89 (d, 1H, H-1_C), 4.88 (dd, 1H, $J_{4,5} = 10.0$ Hz, H-4_D), 4.62 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1_D), 4.90–4.35 (m, 16H, CH_2Ph), 4.40 (m, 1H, H-2_B), 4.10–4.00 (m, 2H, All), 4.08 (dd, 1H, $J_{2,3} = 2.4$ Hz, H-2_A), 4.02 (dd, 1H, H-3_C), 3.91 (m, 1H, H-2_D), 3.90–3.70 (m, 11H, H-4_C, 5_C, 3_A, 5_A, $6a_D$, $6b_D$, 3_E, 4_E, 5_E, $6a_E$, $6b_E$), 3.61 (dd, 1H, $J_{3,4} = 9.5$ Hz, H-3_B), 3.55 (m, 1H, H-5_B), 3.41–3.40 (m, 3H, H-4_A, 5_D, 2_E), 3.47 (m, 1H, $J_{4,5} = 9.5$, $J_{5,6} = 6.1$ Hz, H-5_B), 3.35–3.33 (m, 3H, H-4_A, 5_D, 2_E), 3.25 (dd, 1H, H-4_B), 1.95, 1.70 (3s, 9H, OAc), 1.65 (s, 3H, NHAc), 1.32 (d, 3H, $J_{5,6} = 6.1$ Hz, H-6_A), 1.30 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6_C), 0.97 (d, 3H, $J_{5,6} = 6.0$ Hz, H-6_B) ppm; ^{13}C NMR: $\delta = 171.1$, 170.8, 170.2, 169.6, 166.2 (5C, C=O), 138.2–118.5 (Ph, All), 103.1 (C-1_D), 101.4 (C-1_B), 101.2 (C-1_A), 98.5 (C-1_E), 96.4 (C-1_C), 82.2 (C-3_E), 81.7 (C-2_E), 81.7 (C-4_A), 80.4 (C-4_B), 80.2 (C-3_C), 79.0 (C-3_A), 78.6 (C-3_B), 78.1 (C-2_A), 77.8 (C-4_C), 77.6 (C-4_E), 76.0, 75.8, 75.4, 74.7, 74.3, 74.2, 73.3, 70.5 (8C, CH_2Ph), 74.9 (C-2_B), 72.7 (C-2_C), 72.6 (C-3_D), 71.9 (2C, C-5_E, 5_D), 69.1 (C-5_B), 68.9 (2C, All, C-5_A), 68.3 (C-6_E), 67.8 (C-5_C), 62.3 (C-6_D), 54.6 (C-2_D), 23.5 (NHAc), 21.1, 21.0, 20.8 (3C, OAc), 19.0 (C-6_C), 18.4 (C-6_A), 18.2 (C-6_B) ppm; FAB MS for $C_{104}H_{117}NO_{27}$ $[M]^+$ (1913.1): m/z: 1936.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{104}H_{117}NO_{27}$: C 68.90, H 6.50, N 0.77; found: C 68.64, H 6.66, N 1.05.

Allyl (2-acetamido-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (13): The pentasaccharide **11** (2.65 g, 1.47 mmol) was dissolved in MeOH (20 mL). MeONa was added until a value of pH 10 was reached. The mixture was stirred for 25 min then treated with IR 120 (H^+) resin until a neutral pH value was reached. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with DCM/MeOH (9:1) to give the expected triol **12** which was then treated overnight at room temperature with 2,2-dimethoxypropane (11 mL, 0.1 mol) and PTSA (20 mg, 0.17 mmol) in DMF (20 mL). Et_3N was added and the solution was evaporated. The residue was eluted from a column of silica gel with cyclohexane/EtOAc (1:1) and 0.2% Et_3N to give **13** as a white foam (2.05 g, 81% from **11**); $[\alpha]_D = +3°$ ($c = 1$, $CHCl_3$); ^1H NMR: $\delta = 6.98$–8.00 (m, 45H, Ph), 6.17 (brs, 1H, NH$_D$), 5.82 (m, 1H, All), 5.30 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 3.0$ Hz, H-2_C), 5.11–5.25 (m, 2H, All), 5.06 (brs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1_E), 4.88 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1_B), 4.84 (brs, 1H, H-1_C), 4.35 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20–4.80 (m, 16H, CH_2Ph), 4.05 (dd, 1H, H-2_A), 3.36 (dd, 1H, H-2_E), 2.90–4.10 (m, 22H, All, H-2_D, 3_A, 3_B, 3_C, 3_D, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.5 (s, 3H, NHAc), 1.2–0.9 (m, 15H, C(CH_3)$_2$, H-6_A, 6_B, 6_C) ppm; ^{13}C NMR: $\delta = 172.7$, 164.9 (2C, C=O), 137.7–116.7 (Ph, All), 102.3 (C-1_D), 100.2 (C-1_B), 100.0 (C-1_A), 98.9 (*C*(CH$_3$)$_2$), 97.2 (C-1_E), 95.1 (C-1_C), 82.1, 82.0, 81.8, 81.6, 80.6, 80.3, 79.0, 78.8, 78.3, 77.8, 77.6, 75.7, 75.6, 75.0, 74.3, 72.8, 71.8, 71.6, 70.8, 70.3, 69.0, 68.5, 67.8, 67.4, 61.9, 60.8, 60.5, 29.4 (C(*C*H$_3$)$_2$), 22.7 (NHAc), 19.0 (C(*C*H$_3$)$_2$), 18.9, 18.4, 18.2 (3C, C-6_A, 6_B, 6_C) ppm; FAB MS for $C_{101}H_{115}NO_{24}$ $[M]^+$ (1726.9): m/z: 1749.7 $[M+Na]^+$; elemental analysis calcd (%) for $C_{101}H_{115}NO_{24}\cdot H_2O$: C 69.52, H 6.76, N 0.80; found: C 69.59, H 6.71, N 0.57.

Allyl (2-acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranoside (14): A mixture of **13** (2.05 g, 1.19 mmol) in pyridine (60 mL) was cooled to 0°C. Acetic anhydride (20 mL) was added and the solution was stirred for 2.5 h. The solution was concentrated and coevaporated with toluene. The residue was eluted from a column of silica gel with cyclohexane/EtOAc (2:1) and 0.2% Et_3N to give **14** as a white foam (1.99 g, 94%); $[\alpha]_D = +1°$ ($c = 1$, $CHCl_3$); ^1H NMR: $\delta = 6.95$–8.00 (m, 45H, Ph), 5.82 (m, 1H, All), 5.46 (d, 1H, $J_{2,NH} = 8.0$ Hz, NH$_D$), 5.29 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 3.0$ Hz, H-2_C), 5.11–5.25 (m, 2H, All), 5.00 (brs, 1H, H-1_A), 4.90 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1_E), 4.85 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1_B), 4.83 (brs, 1H, H-1_C), 4.70 (dd, 1H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3_D), 4.44 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20–4.80 (m, 16H, CH_2Ph), 4.02 (dd, 1H, H-2_A), 3.37 (dd, 1H, H-2_E), 2.90–4.10 (m, 21H, All, H-2_D, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.92 (s, 3H, OAc), 1.57 (s, 3H, NHAc), 1.27–0.90 (m, 15H, C(CH$_3$)$_2$, H-6_A, 6_B, 6_C) ppm; ^{13}C NMR: $\delta = 171.3$, 170.3, 166.2 (3C, C=O), 138.7–117.9 (Ph, All), 103.9 (C-1_D), 101.5 (C-1_B), 101.4 (C-1_A), 99.9 (*C*(CH$_3$)$_2$), 98.5 (C-1_E), 96.3 (C-1_C), 82.1, 81.7, 81.6, 80.3, 80.1, 78.8, 78.1, 77.8, 76.0, 75.8, 75.3, 75.1, 74.7, 74.2, 73.6, 73.3, 72.7, 71.9, 71.4, 70.8, 69.0, 68.8, 68.7, 68.4, 68.1, 67.8, 62.1, 55.0 (C-2_D), 30.0 (C(*C*H$_3$)$_2$), 23.5 (NHAc), 21.6 (OAc), 19.2 (C(*C*H$_3$)$_2$), 19.0, 18.3, 18.2 (3C, C-6_A, 6_B, 6_C) ppm; FAB MS for $C_{103}H_{117}NO_{25}$ $[M]^+$ (1769.0): m/z: 1791.9 $[M+Na]^+$; elemental analysis calcd (%) for $C_{103}H_{117}NO_{25}$: C 69.93, H 6.67, N 0.79; found: C 69.77, H 6.84, N 0.72.

(2-Acetamido-3-*O*-acetyl-4,6-*O*-isopropylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-2-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (7): 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (50 mg, 58 μmol) was dissolved in THF (10 mL), and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the color to change to yellow. The solution was then degassed again in an argon stream. A solution of **14** (1.8 g, 1.02 mmol) in THF (20 mL) was degassed and added. The mixture was stirred at room temperature overnight, then concentrated to dryness. The residue was dissolved in acetone (9 mL), then water (2 mL), mercuric chloride (236 mg), and mercuric oxide (200 mg) were added successively. The mixture was protected from light and stirred at room temperature for 2 h, then the acetone was evaporated. The resulting suspension was taken up in DCM, washed twice with 50% aqueous KI, water, and saturated aqueous NaCl, dried, and concentrated. The residue was eluted

from a column of silica gel with cyclohexane/EtOAc (3:2) and 0.2% Et_3N to give the corresponding hemiacetal **15**. Trichloroacetonitrile (2.4 mL) and DBU (72 μL) were added to a solution of the residue in anhydrous DCM (24 mL) at 0°C. After 1 h, the mixture was concentrated. The residue was eluted from a column of silica gel with cyclohexane/EtOAc (3:2) and 0.2% Et_3N to give **7** as a colorless oil (1.58 g, 82% from **14**); $[\alpha]_D = +2°$ ($c = 1$, $CHCl_3$); ^{1}H NMR: $\delta = 8.62$ (s, 1H, NH), 6.95–8.00 (m, 45H, Ph), 6.24 (d, 1H, $J_{1,2} = 2.6$ Hz, H-1_C), 5.48 (dd, 1H, $J_{2,3} = 3.0$ Hz, H-2_C), 5.41 (d, 1H, $J_{2,NH} = 8.4$ Hz, NH_D), 4.99 (brs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1_E), 4.88 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1_B), 4.69 (dd, 1H, $J_{2,3} = J_{3,4} = 10.0$ Hz, H-3_D), 4.44 (d, 1H, H-1_D), 4.34 (dd, 1H, H-2_B), 4.20–4.80 (m, 16H, CH_2Ph), 4.02 (dd, 1H, H-2_A), 3.38 (dd, 1H, H-2_E), 2.90–4.10 (m, 19H, H-2_D, 3_A, 3_B, 3_C, 3_E, 4_A, 4_B, 4_C, 4_D, 4_E, 5_A, 5_B, 5_C, 5_D, 5_E, $6a_D$, $6b_D$, $6a_E$, $6b_E$), 1.95 (s, 3H, OAc), 1.55 (s, 3H, NHAc), 1.30–0.85 (m, 15H, C(CH_3)$_2$, H-6_A, 6_B, 6_C) ppm; ^{13}C NMR: $\delta = 172.4$, 171.4, 166.9 (3C, C=O), 140.2–128.9 (Ph), 104.2 (C-1_D), 101.4 (2C, C-1_A, 1_B), 101.1 (*C*(CH_3)$_2$), 98.0 (C-1_E), 94.8 (C-1_C), 92.4 (CCl_3), 82.1, 81.5, 80.2, 80.1, 78.6, 78.1, 77.8, 77.6, 76.0, 75.8, 75.5, 75.0, 74.3, 74.2, 73.5 (C-3_D), 73.4, 71.9, 71.4, 71.0, 70.5, 69.2, 68.8, 68.3, 68.1, 62.1, 54.9 (C-2_D), 29.3 (C(*C*H_3)$_2$), 23.4 (NHAc), 21.4 (OAc), 19.2 (C(*C*H_3)$_2$), 19.0, 18.2, 18.1 (3C, C-6_A, 6_B, 6_C) ppm; FAB MS for $C_{102}H_{113}Cl_3N_2O_{25}$ $[M]^+$ (1873.3): *m/z*: 1896.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{102}H_{113}Cl_3N_2O_{25}$: C 65.40, H 6.08, N 1.50; found: C 65.26, H 6.02, N 1.31.

2-Azidoethyl (2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (16): A mixture of donor **7** (745 mg, 0.4 mmol) and acceptor **8**[21] (170 mg, 0.51 mmol), 4 Å molecular sieves, and dry 1,2-DCE (12 mL) was stirred for 1 h then cooled to 0°C. Triflic acid (25 μL) was added. The stirred mixture was allowed to reach room temperature over 10 min then stirred again for 2.5 h at 75°C. After cooling to room temperature, Et_3N (100 μL) was added and the mixture was filtered. After evaporation, the residue was eluted from a column of silica gel with cyclohexane/EtOAc (1:2) and 0.2% Et_3N to give **16** as a white foam (615 mg, 76%); $[\alpha]_D = +0°$ ($c = 1$, $CHCl_3$); ^{1}H NMR: $\delta = 6.95$–7.90 (m, 45H, Ph), 6.02 (d, 1H, $J_{2,NH} = 7.1$ Hz, NH_D), 5.46 (d, 1H, $J_{2,NH} = 8.6$ Hz, $NH_{D'}$), 5.20 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 3.0$ Hz, H-2_C), 5.03 (d, 1H, $J_{1,2} = 8.1$ Hz, H-1_D), 5.02 (brs, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2} = 3.1$ Hz, H-1_E), 4.85 (d, 1H, $J_{1,2} = 1.6$ Hz, H-1_B), 4.82 (brs, 1H, H-1_C), 4.70 (dd, 1H, H-$3_{D'}$), 4.44 (d, 1H, H-$1_{D'}$), 4.30 (dd, 1H, H-2_B), 4.20–4.80 (m, 16H, CH_2Ph), 3.99 (dd, 1H, H-2_A), 3.37 (dd, 1H, H-2_E), 2.90–3.95 (m, 29H, H-2_D, $2_{D'}$, 3_A, 3_B, 3_C, 3_D, 3_E, 4_A, 4_B, 4_C, 4_D, $4_{D'}$, 4_E, 5_A, 5_B, 5_C, 5_D, $5_{D'}$, 5_E, $6a_D$, $6b_D$, $6a_{D'}$, $6b_{D'}$, $6a_E$, $6b_E$, $OCH_2CH_2N_3$), 2.00 (s, 3H, NHAc), 1.92 (s, 3H, OAc), 1.57 (s, 3H, NHAc), 1.27–0.90 (m, 21H, 2×C(CH_3)$_2$, H-6_A, 6_B, 6_C) ppm; ^{13}C NMR: $\delta = 172.1$, 171.5, 170.3, 166.2 (4C, C=O), 139.0–127.7 (Ph), 103.9 (C-$1_{D'}$), 101.7 (C-1_B), 101.2 (C-1_A), 100.0 (C-1_D), 99.9, 99.8 (2C, *C*(CH_3)$_2$), 98.3 (C-1_E), 97.8 (C-1_C), 82.0, 81.7, 81.5, 80.8, 80.2, 80.1, 78.9, 78.6, 78.0, 77.9, 76.0, 75.9, 75.8, 75.3, 74.8, 74.6, 74.2, 74.0, 73.6, 73.5, 73.4, 73.0, 71.9, 71.4, 70.8, 69.1, 69.0, 68.8, 68.6, 68.0, 67.7, 67.6, 62.6, 62.1, 60.8, 59.7 (C-2_D), 55.0 (C-$2_{D'}$), 51.1 (CH_2N_3), 29.5 (C(*C*H_3)$_2$), 29.3 (C(*C*H_3)$_2$), 23.9 (NHAc), 23.5 (NHAc), 21.3 (OAc), 19.7 (C(*C*H_3)$_2$), 19.2 (C(*C*H_3)$_2$), 18.8, 18.4, 18.2 (3C, C-6_A, 6_B, 6_C) ppm; FAB MS for $C_{113}H_{133}N_5O_{30}$ $[M]^+$ (2041.3): *m/z*: 2064.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{113}H_{133}N_5O_{30}$: C 66.49, H 6.57, N 3.43; found: C 65.93, H 6.57, N 2.61.

2-Azidoethyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (17): The hexasaccharide **16** (615 mg, 0.30 mmol) was dissolved in MeOH (8 mL). MeONa was added until a value of pH 9 was reached. The mixture was stirred for 3 h then treated with IR 120 (H^+) resin until a neutral pH value was reached. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with DCM/MeOH (25:1) and 0.2% Et_3N to give **17** as a white foam (590 mg, 97%); $[\alpha]_D = +1°$ ($c = 1$, $CHCl_3$); ^{1}H NMR: $\delta = 8.00$–7.00 (m, 45H, Ph), 6.10 (d, 1H, $NH_{D'}$), 6.05 (d, 1H, $J_{2,NH} = 7.4$ Hz, NH_D), 5.20 (dd, 1H, $J_{1,2} = 1.7$, $J_{2,3} = 3.0$ Hz, H-2_C), 5.10 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1_A), 4.99 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1_D), 4.96 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1_E), 4.90 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1_B), 4.86 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1_C), 4.52 (d, 1H, $J_{1,2} = 7.5$ Hz, H-$1_{D'}$), 4.37 (dd, 1H, H-2_B), 4.22 (dd, 1H, H-3_D), 4.02 (dd, 1H, H-2_A), 4.80–4.00 (m, 16H, CH_2Ph), 4.00–2.95 (m, 30H, H-2_D, 4_D, 5_D, $6a_D$, $6b_D$, 2_E, 3_E, 4_E, 5_E, $6a_E$, $6b_E$, 3_C, 4_C, 5_C, 3_B, 4_B, 5_B, 3_A, 4_A, 5_A, $2_{D'}$, $3_{D'}$, $4_{D'}$, $5_{D'}$, $6a_{D'}$, $6b_{D'}$, $OCH_2CH_2N_3$), 2.00–0.92 (6s and 3d, 27H, NHAc, C(CH_3)$_2$, H-6_A, 6_B, 6_C) ppm; ^{13}C NMR (partial): $\delta = 173.9$, 172.1, 166.3 (3C, C=O), 140.0–125.0 (Ph), 103.6 (C-$1_{D'}$), 101.7 (C-1_B), 101.2 (C-1_A), 100.2 (*C*(CH_3)$_2$), 100.2 (C-1_D), 99.9 (*C*(CH_3)$_2$), 98.2 (C-1_E), 97.8 (C-1_C), 51.1 (CH_2N_3), 29.4, 29.3, 23.9, 22.8, 19.6, 19.2, 18.9, 18.4, 18.2 (C-6_A, 6_B, 6_C, NHAc, C(*C*H_3)$_2$) ppm; FAB MS for $C_{111}H_{131}N_5O_{29}$ $[M]^+$ (1999.2): *m/z*: 2021.8 $[M+Na]^+$; elemental analysis calcd (%) for $C_{111}H_{131}N_5O_{29}$: C 66.68, H 6.60, N 3.50; found: C 66.63, H 6.78, N 3.32.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-L-glucopyranoside (18): A mixture of alcohol **17** (110 mg, 55 μmol), trichloroacetimidate **6** (179 mg, 110 μmol) and 4 Å molecular sieves in anhydrous 1,2-DCE (2.5 mL) was stirred for 1 h under dry argon. After cooling at −35°C, triflic acid (5 μL, 50 μmol) was added dropwise and the mixture was stirred for 2.5 h while it was allowed to reach 10°C. Et_3N (25 μL) was added, and the mixture was filtered and concentrated. The residue was eluted from a column of silica gel with toluene/EtOAc (4:1→3:1) and 0.2% Et_3N to give **18** as a white foam (158 mg, 82%); $[\alpha]_D = +18°$ ($c = 1$, $CHCl_3$); ^{1}H NMR: $\delta = 8.00$–6.90 (90H, m, Ph), 5.90 (d, 1H, $J_{2,NH} = 7.0$ Hz, NH_D), 5.58 (d, 1H, $J_{2,NH} = 7.5$ Hz, $NH_{D'}$), 5.45, 5.22 (m, 2H, $J_{1,2} = 1.0$, $J_{2,3} = 2.0$ Hz, H-2_C, $2_{C'}$), 5.12 (dd, 1H, H-$2_{A'}$), 5.11 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1_D), 5.05 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1_A), 5.01 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1_E), 4.96 (d, 1H, $J_{1,2} = 1.0$ Hz, H-1_C), 4.94 (m, 2H, H-1_E, 1_B), 4.86 (d, 1H, H-1_B), 4.82 (d, 1H, H-1_C), 4.72 (d, 1H, H-$1_{D'}$), 4.70 (d, 1H, H-$1_{A'}$), 4.90–4.20 (m, 36H, 16×OCH_2Ph, H-2_B, $2_{B'}$, 3_D, $3_{D'}$), 4.00–2.90 (m, 45H, H-2_D, 4_D, 5_D, $6a_D$, $6b_D$, 3_C, 4_C, 5_C, 2_E, 3_E, 4_E, 5_E, $6a_E$, $6b_E$, 3_B, 4_B, 5_B, 2_A, 3_A, 4_A, 5_A, $2_{D'}$, $4_{D'}$, $5_{D'}$, $6a_{D'}$, $6b_{D'}$, $3_{C'}$, $4_{C'}$, $5_{C'}$, $2_{E'}$, $3_{E'}$, $4_{E'}$, $5_{E'}$, $6a_{E'}$, $6b_{E'}$, $3_{B'}$, $4_{B'}$, $5_{B'}$, $3_{A'}$, $4_{A'}$, $5_{A'}$, $OCH_2CH_2N_3$), 2.00 (s, 3H, NHAc), 1.88 (s, 3H, OAc), 1.86 (s, 3H, NHAc), 1.40–0.82 (m, 30H, H-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$, C(CH_3)$_2$) ppm; ^{13}C NMR (partial): $\delta = 172.1$, 171.4, 170.2, 166.2, 165.9 (5C, C=O), 102.7 (C-$1_{D'}$), 101.6, 101.2 (2C, C-1_B, $1_{B'}$), 101.1 (C-1_A), 99.8 (C-1_D), 99.7 (C-1_C), 98.2 (2C, C-1_E, $1_{A'}$), 97.2 (2C, C-1_C, 1_E), 63.3, 62.6 (2C, C-6_E, $6_{E'}$), 60.0, 57.8 (2C, C-2_D, $2_{D'}$), 51.0 (CH_2N_3), 29.5, 29.4 (2C, C(*C*H_3)$_2$), 24.0 (2C, NHAc), 21.3 (OAc), 19.6, 19.5 (2C, C(*C*H_3)$_2$), 19.1, 18.9, 18.8, 18.5, 18.2, 18.1 (6C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; FAB MS for $C_{200}H_{225}N_5O_{48}$ $[M]^+$ (3446.9): *m/z*: 3489.5 $[M+Na]^+$.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (19): A solution of TFA (2 mL) and water (2 mL) was added dropwise to a solution of **18** (630 mg, 181 μmol) in DCM (12 mL) at 0°C. The mixture was stirred for 3 h at this temperature then concentrated by coevaporation with water and then with toluene. The residue was eluted from a column of silica gel with toluene/EtOAc (1:1) to give **19** as a white foam (460 mg, 75%); $[\alpha]_D = +9°$ ($c = 1$, $CHCl_3$); FAB MS for $C_{194}H_{217}N_5O_{48}$ $[M]^+$ (3386.8): *m/z*: 3409.2 $[M+Na]^+$; elemental analysis calcd (%) for $C_{194}H_{217}N_5O_{48}\cdot H_2O$: C 68.43, H 6.45, N 2.06; found: C 68.40, H 7.02, N 1.61.

2-Aminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-

(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (3): A mixture of **19** (130 mg, 38 μmol) in MeOH (4 mL) was treated with MeONa until a value of pH 9 was reached. The mixture was stirred for 1 h at room temperature then heated at 55 °C overnight. After the mixture was cooled to room temperature, IR 120 (H^+) resin was added until a neutral pH value was reached, and the solution was filtered and concentrated. The residue was eluted from a column of silica gel with DCM/MeOH (25:1→20:1) to give an amorphous residue. A solution of this residue in EtOH (1.5 mL), EtOAc (150 μL), 1 M HCl (66 μL, 2 equiv) was hydrogenated in the presence of Pd/C (100 mg) for 72 h at room temperature. The mixture was filtered and concentrated into a residue which was eluted from a column of C-18 resin with water then lyophilized to afford amorphous **3** as a white foam (41 mg, 71 %); $[\alpha]_D = -7°$ ($c = 1$, water); ^{1}H NMR (D_2O; partial): $\delta = 4.90$ (m, 2H, $J_{1,2} = 3.5$ Hz, H-1_E, H-$1_{E'}$), 4.82, 4.76, 4.72, 4.67, 4.52, 4.51 (6×brs, 6H, H-1_A, 1_B, 1_C, $1_{A'}$, $1_{B'}$, $1_{C'}$), 4.41 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1_D*), 4.29 (d, 1H, $J_{1,2} = 8.6$ Hz, H-$1_{D'}$*), 1.77 (s, 6H, NHAc), 1.15–0.96 (m, 18H, H-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; ^{13}C NMR (D_2O; partial): $\delta = 174.8$, 174.7 (2C, C=O), 102.6 (C-$1_{D'}$*), 102.9, 101.8, 101.6, 101.4, 101.3 (6C, C-1_A, 1_B, 1_C, $1_{A'}$, $1_{B'}$, $1_{C'}$), 100.8 (C-$1_{D'}$*), 97.9 (2C, C-1_E, $1_{E'}$), 56.0, 56.4 (2C, 2 C-6_D, $6_{D'}$), 22.7, 22.6 (2C, NHAc), 18.2, 17.2, 17.0, 16.9 (6C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; HRMS (MALDI) calcd (%) for $C_{66}H_{113}N_5O_{45}Na$ $[M+Na]^+$: 1690.6544; found: 1690.6537.

2-Azidoethyl (2-acetamido-3-*O*-acetyl-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (21): A mixture of donor **7** (835 mg, 0.44 mmol), acceptor **17** (590 mg, 0.3 mmol), 4 Å molecular sieves, and dry 1,2-DCE (12 mL), was stirred for 1 h then cooled to −30 °C. Triflic acid (35 μL) was added. The stirred mixture was allowed to reach 5 °C over 2.5 h. Et_3N (150 μL) was added, and the mixture was filtered. After evaporation, the residue was eluted from a column of silica gel with cyclohexane/EtOAc (1:2) and 0.2 % Et_3N to give **21** as a white foam (990 mg, 90 %); $[\alpha]_D = +10°$ ($c = 1$, $CHCl_3$); ^{1}H NMR ($CDCl_3$; partial): $\delta = 6.95$–7.90 (m, 90H, Ph), 5.98 (d, 1H, $J_{2,NH} = 6.9$ Hz, NH_D), 5.60 (d, 1H, $J_{2,NH} = 7.5$ Hz, NH_D), 5.45 (d, 1H, $J_{2,NH} = 8.5$ Hz, NH_D), 5.22 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 3.0$ Hz, H-2_C), 5.13 (dd, 1H, $J_{1,2} = 1.0$, $J_{2,3} = 3.0$ Hz, H-2_C), 5.08 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1_D), 5.07 (brs, 1H, H-1_A), 5.04 (brs, 1H, H-1_A), 4.97 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1_E), 4.94 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1_E), 4.90 (brs, 1H, H-1_B), 4.86 (brs, 1H, H-1_B), 4.82 (brs, 1H, H-1_C), 4.73 (d, 1H, H-1_D), 4.70 (brs, 1H, H-1_C), 4.43 (d, 1H, H-1_D), 4.20–4.80 (m, 16H, CH_2Ph), 2.00, 1.85, 1.58 (3×s, 9H, NHAc), 1.95 (s, 3H, OAc), 1.37–0.85 (m, 36H, 3×$C(CH_3)_2$, H-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; ^{13}C NMR (partial): $\delta = 171.7$, 170.8, 169.8, 165.8, 165.4 (6C, C=O), 139.0–127.7 (Ph), 103.9 (C-1_D), 102.8 (C-1_D), 101.5 (2C, C-1_B), 101.3 (C-1_A), 101.1 (C-1_A), 100.0 (C-1_D), 99.5, 99.3 (3C, $C(CH_3)_2$), 98.3 (C-1_E), 98.1 (2C, C-1_C, 1_E), 97.8 (C-1_C), 82.0, 81.7, 81.6, 81.4, 80.3, 80.2, 80.1, 79.5, 79.2, 78.9, 78.7, 78.4, 78.1, 77.9, 77.8, 77.6, 76.0, 75.8, 75.3, 75.2, 74.7, 74.4, 74.1, 74.0, 73.6, 73.5, 73.4, 73.3, 73.0, 72.7, 71.9, 71.4, 70.9, 70.8, 69.1, 69.0, 68.9, 68.7, 68.6, 68.5, 68.1, 67.8, 67.7, 67.5, 62.6, 62.3, 62.1, 60.8, 59.9, 57.9, 55.0 (3C, C-2_D, $2_{D'}$, $2_{D''}$), 51.1 (CH_2N_3), 29.5, 29.4, 29.3 (3C, $C(CH_3)_2$), 24.0, 23.9, 23.5 (3C, NHAc), 21.3 (OAc), 19.7, 19.6, 19.2 (3C, $C(CH_3)_2$), 18.9, 18.8, 18.6, 18.5, 18.2, 18.1 (6C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; FAB MS for $C_{211}H_{242}N_6O_{53}$ $[M]^+$ (3710.2): m/z: 3733.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{211}H_{242}N_6O_{53}$: C 68.31, H 6.57, N 2.27; found: C 68.17, H 6.74, N 2.12.

2-Azidoethyl (2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (22): The undecasaccharide **21** (990 mg, 0.27 mmol) was dissolved in MeOH (30 mL). MeONa was added until a value of pH 9 was reached. The mixture was stirred for 3 h then treated with IR 120 (H^+) resin until a neutral pH value was reached. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with toluene/EtOAc (1:1) and 0.2 % Et_3N to give **22** as a white foam (900 mg, 91 %); $[\alpha]_D = +15°$ ($c = 1$, $CHCl_3$); ^{1}H NMR (partial): $\delta = 6.95$–8.00 (m, 90H, Ph), 6.19 (brs, 1H, NH_D*), 5.96 (d, 1H, $J_{2,NH} = 6.8$ Hz, NH_D*), 5.57 (d, 1H, $J_{2,NH} = 6.8$ Hz, $NH_{D''}$*), 5.22 (dd, 1H, H-2_C*), 5.13 (dd, 1H, H-$2_{C'}$*), 5.10 (d, 1H, H-1_D), 5.07 (brs, 1H, H-1_A*), 5.04 (brs, 1H, H-$1_{A'}$*), 4.96 (d, 1H, H-1_E*), 4.94 (d, 1H, H-$1_{E'}$*), 4.85 (brs, 1H, H-1_B*), 4.84 (brs, 1H, H-$1_{B'}$*), 4.82 (brs, 1H, H-1_C*), 4.70 (d, 1H, H-$1_{C'}$*), 4.67 (d, 1H, H-1_D*), 4.44 (d, 1H, H-$1_{D''}$*), 4.20–4.80 (m, 16H, CH_2Ph), 2.00, 1.85, 1.58 (3×s, 9H, NHAc), 1.37–0.80 (m, 36H, $C(CH_3)_2$, H-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; ^{13}C NMR (partial): $\delta = 172.8$, 170.9, 170.3, 165.1, 164.7 (5C, C=O), 139.0–127.7 (Ph), 103.5, 103.1 (2C, C-1_D, $1_{D'}$), 101.5 (2C, C-1_B, $1_{B'}$), 101.2, 101.1 (2C, C-1_A, $1_{A'}$), 99.9 (C-$1_{D''}$), 99.0, 98.8, 98.7 (3C, $C(CH_3)_2$), 98.3 (C-1_E*), 98.1 (2C, C-1_C*, $1_{E'}$*), 97.8 (C-$1_{C'}$*), 82.1, 82.0, 81.9, 81.7, 81.6, 81.5, 80.6, 80.3, 80.2, 80.1, 79.7, 79.1, 78.9, 78.5, 77.9, 77.6, 75.7, 74.9, 74.6, 74.3, 73.3, 73.0, 72.7, 71.9, 71.8, 69.1, 68.9, 68.7, 68.5, 68.0, 67.8, 67.7, 67.6, 67.5, 62.6, 62.3, 61.9, 60.5, 59.9, 57.4, 55.0 (3C, C-2_D, $2_{D'}$, $2_{D''}$), 51.0 (CH_2N_3), 29.5, 29.3 (3C, $C(CH_3)_2$), 24.0, 23.9, 22.7 (3C, NHAc), 19.7, 19.6, 19.3 (3C, $C(CH_3)_2$), 19.0, 18.9, 18.6, 18.5, 18.2, 18.1 (6C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$) ppm; FAB MS for $C_{209}H_{240}N_6O_{52}$ $[M]^+$ (3668.1): m/z: 3690.8 $[M+Na]^+$; elemental analysis calcd (%) for $C_{211}H_{242}N_6O_{53}$: C 68.43, H 6.59, N 2.29; found: C 68.28, H 6.72, N 2.11.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (23): A mixture of donor **6** (377 mg, 0.230 mmol), acceptor **22** (427 mg, 0.115 mmol), 4 Å molecular sieves, and dry 1,2-DCE (10 mL) was stirred for 1 h then cooled to −30 °C. Triflic acid (20 μL) was added. The stirred mixture was allowed to reach 5 °C over 2.5 h. Et_3N (150 μL) was added, and the mixture was filtered. After evaporation, the residue was eluted from a column of silica gel with toluene/EtOAc (3:1) and 0.2 % Et_3N to give **23** as a foam (490 mg, 82 %); $[\alpha]_D = +20°$ ($c = 1$, $CHCl_3$); ^{1}H NMR (partial): $\delta = 6.90$–8.00 (m, 135H, Ph), 5.95 (d, 1H, $J_{2,NH} = 6.6$ Hz, NH_D*), 5.60 (d, 1H, $J_{2,NH} = 8.0$ Hz, $NH_{D'}$*), 5.59 (d, 1H, $J_{2,NH} = 7.5$ Hz, $NH_{D''}$*), 5.44 (dd, 1H, H-2_C), 5.22 (dd, 1H, H-2_C), 5.10 (dd, 1H, H-2_C), 2.20 (s, 3H, OAc), 2.00, 1.85, 1.84 (3×s, 9H, AcNH), 1.40–0.80 (m, 45H, 3×$C(CH_3)_2$, H-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$, $6_{A''}$, $6_{B''}$, $6_{C''}$) ppm; ^{13}C NMR (partial): $\delta = 173.2$, 172.6, 172.5, 171.3, 167.4, 167.0, 166.9 (C=O), 140.2–126.8 (Ph), 102.8, 102.7, 101.5, 101.3, 101.1, 99.9, 99.8, 98.1, 97.8, 82.0, 81.7, 81.5, 81.4, 80.2, 80.1, 79.6, 79.4, 78.9, 78.6, 78.0, 77.9, 77.6, 75.5, 73.4, 73.3, 73.0, 72.8, 71.9, 71.6, 69.4, 69.1, 69.0, 68.6, 67.8, 67.7, 67.6, 67.5, 62.6, 62.3, 60.0, 57.9, 57.7, 51.0 (CH_2N_3), 30.5 (3C, $C(CH_3)_2$), 25.0, 22.4 (3C, NHAc), 22.9 (OAc), 20.7, 20.6, 20.2 (3C, $C(CH_3)_2$), 20.0, 19.9, 19.8, 19.7, 19.6, 19.3, 19.2, 19.1 (9C, C-6_A, 6_B, 6_C, $6_{A'}$, $6_{B'}$, $6_{C'}$, $6_{A''}$, $6_{B''}$, $6_{C''}$) ppm; FAB MS for $C_{298}H_{334}N_6O_{71}$ $[M]^+$ (5135.8): m/z: 5159.3 $[M+Na]^+$; elemental analysis calcd (%) for $C_{298}H_{334}N_6O_{71}$: C 69.69, H 6.55, N 1.64; found: C 69.74, H 6.72, N 1.49.

2-Azidoethyl (2-*O*-acetyl-3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→4)]-(2-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-2-acetamido-2-

deoxy-β-D-glucopyranoside (24): A solution of 50% aqueous TFA (3.0 mL) was added dropwise to a solution of the pentadecasaccharide **23** (480 mg, 93 μmol) in DCM (14 mL) at 0°C. The mixture was stirred for 3 h then concentrated by coevaporation first with water and then with toluene. The residue was eluted from a column of silica gel with toluene/EtOAc (1:1) to give **24** as a white foam (390 mg, 83%); $[\alpha]_D = +12°$ (c = 1, $CHCl_3$); FAB MS for $C_{289}H_{322}N_6O_{71}$ $[M]^+$ (5015.6): m/z: 5037.2 $[M+Na]^+$.

2-Aminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (4): A solution of the partially deprotected pentadecasaccharide **24** (390 mg, 77 μmol) in MeOH (10 mL) was treated by MeONa until a value of pH 10 was reached. The mixture was stirred overnight at 55°C. After the mixture was cooled at room temperature, IR 120 (H^+) resin was added until a neutral pH value was reached. The solution was filtered and concentrated. The residue was eluted from a column of silica gel with DCM/MeOH (20:1) to give the benzylated residue (252 mg). A solution of this residue in EtOH (3 mL), EtOAc (250 μL), and 1 M HCl (106 μL) was hydrogenated in the presence of Pd/C (300 mg) for 48 h at room temperature. The mixture was filtered and concentrated. The residue was eluted from a column of C-18 with water and freeze-dried to afford amorphous **4** (127 mg, 65%); $[\alpha]_D = -5°$ (c = 1, water); ^{1}H NMR (D_2O; partial): δ = 5.13 (m, 3H, H-1$_E$, 1$_{E'}$, 1$_{E''}$), 5.07, 4.99, 4.95, 4.90, 4.75 (m, 9H, H-1$_A$, 1$_B$, 1$_C$, 1$_{A'}$, 1$_{B'}$, 1$_{C'}$, 1$_{A''}$, 1$_{B''}$, 1$_{C''}$), 4.63, 4.51 (2d, 3H, $J_{1,2}$ = 8.5 Hz, H-1$_D$, 1$_{D'}$, 1$_{D''}$), 2.00 (s, 9H, NHAc), 1.30–1.18 (m, 27H, H-6$_A$, 6$_B$, 6$_C$, 6$_{A'}$, 6$_{B'}$, 6$_{C'}$, 6$_{A''}$, 6$_{B''}$, 6$_{C''}$) ppm; ^{13}C NMR (D_2O; partial): δ = 174.8, 174.7 (3C, C=O), 102.9, 102.6, 101.7, 101.3, 100.8, 97.9, 81.8, 81.7, 79.6, 79.0, 76.3, 76.2, 73.0, 72.7, 72.4, 72.1, 71.6, 70.5, 70.1, 70.0, 69.7, 69.6, 69.4, 68.7, 68.6, 66.0, 61.0, 56.0, 55.4, 39.8, 22.7, 22.6 (NHAc), 18.2, 17.2, 17.0, 16.9 (9C, C-6$_A$, 6$_B$, 6$_C$, 6$_{A'}$, 6$_{B'}$, 6$_{C'}$, 6$_{A''}$, 6$_{B''}$, 6$_{C''}$) ppm; MALDI MS for $C_{98}H_{166}N_4O_{67}Na$ $[M]^+$ (2493.96): m/z: 2494.96.

(*S*-Acetylthiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (20): A solution of SAMA-Pfp (2.8 mg, 9.5 μmol) in CH_3CN (60 μL) was added to the aminoethyl decasaccharide **3** (6.4 mg, 3.84 μmol) in 0.1 M phosphate buffer (pH 7.4, 500 μL). The mixture was stirred at room temperature for 1 h and purified by RP HPLC to give **20** (4.2 mg, 61%); HPLC (230 nm): R_t = 14.17 min (99.9% pure; Kromasil 5 μm C18 100 Å 4.6×250 mm analytical column; 0–20% linear gradient of CH_3CN in 0.01 M aqueous TFA over 20 min at a flow rate of 1 mL min^{-1}); ES MS for $C_{70}H_{117}N_3O_{47}S$ $[M]^+$ (1784.76): m/z: 1784.70.

(*S*-Acetylthiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (25): A solution of SAMA-Pfp (2.8 mg, 9.6 μmol) in CH_3CN (50 μL) was added to pentadecasaccharide **4** (9.4 mg, 3.8 μmol) in 0.1 M phosphate buffer (pH 7.4, 500 μL). The mixture was stirred at room temperature for 2 h and purified by RP HPLC to give **25** (6.3 mg, 63%); HPLC (230 nm): R_t = 13.97 min (99.0% pure; Kromasil 5 μm C18 100 Å 4.6×250 mm analytical column; 0–20% linear gradient of CH_3CN in 0.01 M aqueous TFA over 20 min at a flow rate of 1 mL min^{-1}); ES MS for $C_{102}H_{170}N_4O_{69}S$ $[M]^+$ (2588.53): m/z: 2588.67.

PADRE (thiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1): Compound **20** (6.0 mg, 3.36 μmol) was dissolved in water (300 μL) and added to a solution of PADRE-Mal (7.1 mg, 4.0 μmol) in a mixture of water (630 μL), CH_3CN (120 μL), and 0.1 M phosphate buffer (pH 5.6, 750 μL). A solution (68 μL) of hydroxylamine hydrochloride (139 mg mL^{-1}) in 0.1 M phosphate buffer (pH 5.6) was added, and the mixture was stirred for 2 h. RP HPLC purification gave the pure target **1** (5.2 mg, 44%); HPLC (230 nm): R_t = 10.03 min (100% pure; Kromasil 5 μm C18 100 Å 4.6×250 mm analytical column; 20–50% linear gradient of CH_3CN in 0.01 M aqueous TFA over 20 min at a flow rate of 1 mL min^{-1}); ES MS for $C_{153}H_{254}N_{24}O_{65}S$ $[M]^+$ (3501.91): m/z: 3501.15.

PADRE (thiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2): Compound **25** (10.3 mg, 3.98 μmol) was dissolved in water (350 μL) and added to a solution of PADRE-Mal (9.0 mg, 5.0 μmol) in a mixture of water (740 μL), CH_3CN (140 μL), and 0.5 M phosphate buffer (pH 5.6, 890 μL). A solution (80 μL) of hydroxylamine hydrochloride (139 mg mL^{-1}) in 0.5 M phosphate buffer (pH 5.7) was added, and the mixture was stirred for 3 h. RP HPLC purification gave the pure conjugate **2** (11.5 mg, 67%); HPLC (230 nm): R_t = 9.07 min (100% pure; Kromasil 5 μm C18 100 Å 4.6×250 mm analytical column; 20–50% linear gradient of CH_3CN in 0.01 M aqueous TFA over 20 min at a flow rate of 1 mL min^{-1}); ES MS for $C_{185}H_{307}N_{25}O_{87}S$ $[M]^+$ (4305.69): m/z: 4305.45.

Acknowledgements

The authors are grateful to J. Ughetto-Monfrin (Unité de Chimie Organique, Institut Pasteur) for recording the NMR spectra. The authors thank the Bourses Roux Foundation for the postdoctoral fellowship awarded to F.B. and the Institut Pasteur for its financial support (grant no.: PTR 99).

[1] K. L. Kotloff, J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak, M. M. Levine, *Bull. W. H. O.* **1999**, *77*, 651–666.
[2] World Health Organization, *Weekly Epidemiol. Rec.* **1997**, *72*, 73–79.
[3] T. S. Coster, C. W. Hoge, L. L. van der Verg, A. B. Hartman, E. V. Oaks, M. M. Venkatesan, D. Cohen, G. Robin, A. Fontaine-Thompson, P. J. Sansonetti, T. L. Hale, *Infect. Immun.* **1999**, *67*, 3437–3443.
[4] L. F. Fries, A. D. Montemarano, C. P. Mallett, D. N. Taylor, T. L. Hale, G. H. Lowell, *Infect. Immun.* **2001**, *69*, 4545–4553.
[5] J. H. Passwell, E. Harlev, S. Ashkenazi, C. Chu, D. Miron, R. Ramon, N. Farzan, J. Shiloach, D. A. Bryla, F. Majadly, R. Roberson, J. B. Robbins, R. Schneerson, *Infect. Immun.* **2001**, *69*, 1351–1357.
[6] D. Cohen, M. S. Green, C. Block, T. Rouach, I. Ofek, *J. Infect. Dis.* **1988**, *157*, 1068–1071.
[7] D. Cohen, M. S. Green, C. Block, R. Slepon, I. Ofek, *J. Clin. Microbiol.* **1991**, *29*, 386–389.
[8] C. V. Harding, J. Kihlberg, M. Elofsson, G. Magnusson, E. R. Unanue, *J. Immunol.* **1993**, *151*, 2419–2425.
[9] G. Y. Ishioka, A. G. Lamont, D. Thomson, N. Bulbow, F. C. A. Gaeta, A. Sette, H. M. Grey, *J. Immunol.* **1992**, *148*, 2446–2451.
[10] D. N. Taylor, A. C. Trofa, J. Sadoff, C. Chu, D. Bryla, J. Shiloach, D. Cohen, S. Ashkenazi, Y. Lerman, W. Egan, R. Schneerson, J. B. Robbins, *Infect. Immun.* **1993**, *61*, 3678–3687.
[11] S. Ashkenazi, J. H. Passwell, E. Harlev, D. Miron, R. Dagan, N. Farzan, R. Ramon, F. Majadly, D. A. Bryla, A. B. Karpas, J. B. Robbins, R. Schneerson, *J. Infect. Dis.* **1999**, *179*, 1565–1568.

[12] D. Cohen, S. Ashkenazi, M. S. Green, M. Gdalevich, G. Robin, R. Slepon, M. Yavzori, N. Orr, C. Block, I. Ashkenazi, J. Shemer, D. N. Taylor, T. L. Hale, J. C. Sadoff, D. Pavliovka, R. Schneerson, J. B. Robbins, *The Lancet* **1997**, *349*, 155–159.
[13] W. F. Goebel, *J. Exp. Med.* **1940**, *72*, 33–48.
[14] W. F. Goebel, *J. Exp. Med.* **1939**, *69*, 353–364.
[15] V. Pozsgay in *Oligosaccharide–protein conjugates as vaccine candidates against bacteria, Vol. 56* (Ed.: D. Horton), Academic Press, San Diego, **2000**, pp. 153–199.
[16] E. Alonso de Velasco, A. F. M. Verheul, G. H. Veeneman, L. J. F. Gomes, J. H. van Boom, J. Verhoef, H. Snippe, *Vaccine* **1993**, *11*, 1429–1436.
[17] W. T. M. Jansen, A. F. M. Verheul, G. H. Veeneman, J. H. van Boom, H. Snippe, *Vaccine* **2002**, *20*, 19–21.
[18] F. Mawas, J. Niggemann, C. Jones, M. J. Corbet, J. P. Kamerling, J. F. G. Vliegenthart, *Infect. Immun.* **2002**, *70*, 5107–5114.
[19] B. Benaissa-Trouw, D. J. Lefeber, J. P. Kamerling, J. F. G. Vliegenthart, K. Kraaijeveld, H. Snippe, *Infect. Immun.* **2001**, *69*, 4698–4701.
[20] W. T. M. Jansen, S. Hogenboom, M. J. L. Thijssen, J. P. Kamerling, J. F. G. Vliegenthart, J. Verhoef, H. Snippe, A. F. M. Verheul, *Infect. Immun.* **2001**, *69*, 787–793.
[21] K. Wright, C. Guerreiro, I. Laurent, F. Baleux, L. A. Mulard, *Org. Biomol. Chem.* **2004**, *2*, 1518–1527.
[22] E. Alonso de Velasco, A. F. Verheul, A. M. van Steijn, H. A. Dekker, R. G. Feldman, I. M. Fernandez, J. P. Kamerling, J. F. Vliegenthart, J. Verhoef, H. Snippe, *Infect. Immun.* **1994**, *62*, 799–808.
[23] W. T. M. Jansen, H. Snippe, *Indian J. Med. Res.* **2004**, *119 (Suppl)*, 7–12.
[24] C. C. A. M. Peeters, P. R. Lagerman, O. der Weers, L. A. Oomen, P. Hoogerhout, M. Beurret, J. T. Poolman in *Methods Mol. Med. Vol. 87: Vaccine Protocols*, 2nd ed. (Eds.: A. Robinson, M. J. Hudson, M. P. Cranage), Humana Press, Totowa, **2003**, pp.153–173.
[25] V. Pozsgay, C. Chu, L. Panell, J. Wolfe, J. B. Robbins, R. Schneerson, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5194–5197.
[26] C. C. A. M. Peeters, D. Evenberg, P. Hoogerhout, H. Käyhty, L. Saarinen, C. A. A. van Boeckel, G. A. van der Marel, J. H. van Boom, J. T. Poolman, *Infect. Immun.* **1992**, *60*, 1826–1833.
[27] V. Verez-Bencomo, V. Fernández-Santana, E. Hardy, M. E. Toledo, M. C. Rodríguez, L. Heynngnezz, A. Rodriguez, A. Baly, L. Herrera, M. Izquierdo, A. Villar, Y. Valdés, K. Cosme, M. L. Deler, M. Montane, E. Garcia, A. Ramos, A. Aguilar, E. Medina, G. Toraño, I. Sosa, I. Hernandez, R. Martínez, A. Muzachio, A. Carmenates, L. Costa, F. Cardoso, C. Campa, M. Diaz, R. Roy, *Science* **2004**, *305*, 522–525.
[28] D. Valmori, A. Pessi, E. Bianchi, G. P. Corradin, *J. Immunol.* **1992**, *149*, 717–721.
[29] A. Kandil, N. Chan, M. Klein, P. Chong, *Glycoconjugate J.* **1997**, *14*, 13–17.
[30] F. Falugi, R. Petracca, M. Mariani, E. Luzzi, S. Mancianti, V. Carinci, M. L. Melli, O. Finco, A. Wack, A. D. Tommasco, M. T. d. Magistris, P. Costantino, G. d. Giudice, S. Abrignani, R. Rappuoli, G. Grandi, *Eur. J. Immunol.* **2001**, *31*, 3816–3824.
[31] J. Alexander, J. Sidney, S. Southwood, J. Ruppert, C. Oseroff, A. Maewal, K. Snoke, H. M. Serra, R. T. Kubo, A. Sette, H. M. Grey, *Immunity* **1994**, *1*, 751–761.
[32] J. Alexander, A.-F. d. Guercio, A. Maewal, L. Qiao, J. Fikes, R. W. Chesnut, J. Paulson, D. R. Bundle, S. DeFrees, A. Sette, *J. Immunol.* **2000**, *164*, 1625–1633.
[33] J. Alexander, M.-F. der Guercio, B. Frame, A. Maewal, A. Sette, M. H. Nahm, M. J. Newman, *Vaccine* **2004**, *22*, 2362–2367.
[34] D. A. R. Simmons, *Bacteriol. Rev.* **1971**, *35*, 117–148.
[35] A. A. Lindberg, A. Karnell, A. Weintraub, *Rev. Infect. Dis.* **1991**, *13*, S279–S284.
[36] A. Phalipon, C. Costachel, A. Thuizat, F. Nato, B. V.-L. Normand, V. Marcel-Peyre, P. Sansonetti, L. A. Mulard, *J. Immunol. Chem.* **2004**, submitted.
[37] B. M. Pinto, K. B. Reimer, D. G. Morissette, D. R. Bundle, *J. Org. Chem.* **1989**, *54*, 2650–2656.
[38] B. M. Pinto, K. B. Reimer, D. G. Morissette, D. R. Bundle, *Carbohydr. Res.* **1990**, *196*, 156–166.
[39] F. Bélot, K. Wright, C. Costachel, A. Phalipon, L. A. Mulard, *J. Org. Chem.* **2004**, *69*, 1060–1074.
[40] J. Banoub, D. R. Bundle, *Can. J. Chem.* **1979**, *57*, 2091–2097.
[41] L. A. Mulard, C. Costachel, P. J. Sansonetti, *J. Carbohydr. Chem.* **2000**, *19*, 849–877.
[42] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–123.
[43] J. J. Oltvoort, C. A. A. van Boeckel, J. H. der Koning, J. van Boom, *Synthesis* **1981**, 305–308.
[44] R. Gigg, C. D. Warren, *J. Chem. Soc. C* **1968**, 1903–1911.
[45] F. Bélot, D. Rabuka, M. Fukuda, O. Hindsgaul, *Tetrahedron Lett.* **2002**, *43*, 7743–7747.
[46] A. Lipták, Z. Szurmai, P. Nanasi, A. Neszmelyi, *Carbohydr. Res.* **1982**, *99*, 13–21.
[47] Z. Szurmai, J. Kerékgyarto, J. Harangi, A. Lipták, *Carbohydr. Res.* **1987**, *164*, 313–325.
[48] Z. Szurmai, A. Lipták, G. Snatzke, *Carbohydr. Res.* **1990**, *200*, 201–208.
[49] Q. Li, H. Li, Q.-H. Lou, B. Su, M.-S. Cai, Z.-J. Li, *Carbohydr. Res.* **2002**, *337*, 1929–1934.
[50] G. T. Hermanson, *Bioconjugate techniques*, Academic Press, New York, **1996**, p. 148.
[51] J. M. Peeters, T. G. Hazendonk, E. C. Beuvery, G. I. Tesser, *J. Immunol. Methods* **1989**, *120*, 133–143.
[52] H. F. Brugghe, H. A. M. Timmermans, L. M. A. van Unen, G. J. T. Hove, G. W. der Werken, J. T. Poolman, P. Hoogerhout, *Int. J. Pept. Protein Res.* **1994**, *43*, 166–172.
[53] N. K. Kochetkov, N. E. Byramova, Y. E. Tsvetkov, L. V. Backinovsky, *Tetrahedron* **1985**, *41*, 3363–3375.

Received: September 3, 2004
Published online: January 24, 2005

