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Preparation of synthetic glycoconjugates as potential vaccines against *Shigella flexneri* serotype 2a disease†‡

Karen Wright, Catherine Guerreiro, Isabelle Laurent, Françoise Baleux and Laurence A. Mulard*

Unité de Chimie Organique, URA CNRS 2128, Institut Pasteur, 28 rue du Dr Roux, 75 724, Paris, Cedex 15, France. E-mail: lmulard@pasteur.fr

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The synthesis of three neoglycopeptides incorporating carbohydrate haptens, differing in length, covalently linked to a non natural universal T helper peptide is disclosed. They were synthesized according to a blockwise strategy based on the condensation of appropriate di-, tri-, and tetrasaccharide trichloroacetimidate donors onto an azidoethyl 2-acetamido-2-deoxy-β-D-glucopyranoside acceptor. Use of thiol–maleimide coupling chemistry allowed site-selective efficient conjugation.

Introduction

Since the discovery of *Shigella dysenteriae* type 1 (Shiga's bacillus) more than a century ago,[2] shigellosis or bacillary dysentery has been known as a serious infectious disease, occurring only in humans.[3] In a recent survey of the literature published between 1966 and 1997,[4] the number of episodes of shigellosis occurring annually throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries. Up to 1.1 million annual deaths were associated with shigellosis during the same period. Occurrence of the disease is seen as a correlate of sanitary conditions, and those are not likely to improve rapidly in areas at risk. The financial status of the populations in which shigellosis exists in its endemic or epidemic forms, as well as the emerging resistance to antimicrobial drugs,[5–9] limit the impact of such drugs. Of the four species of *Shigellae*, *Shigella flexneri* is the major species responsible for the endemic form of the disease, with serotype 2a being the most prevalent. The critical importance of the development of a vaccine against *Shigellae* infections was first outlined in 1987.[10] Due to increasing resistance of all groups of *Shigellae* to antibiotics,[7] it remained a high priority as stated by the World Health Organization ten years later.[11] In the meantime, several experimental vaccines have gone through field evaluation,[12–14] but there are as yet no licensed vaccines for shigellosis.

Shigella's lipopolysaccharide (LPS) is a major surface antigen of the bacterium. The corresponding O-specific polysaccharide domain (O-SP) is both an essential virulence factor and the target of the infected host's protective immune response.[15,16] Indeed, using the pulmonary murine model for shigellosis, it was recently demonstrated that the presence locally, preliminary to infection, of a secretory antibody of isotype A, specific for an epitope located on the O-SP moiety of the LPS of *Shigella flexneri* 5a, prevented any host homologous infection.[17] Based on the former hypothesis that serum IgG anti-LPS antibodies may confer specific protection against shigellosis,[18] several polysaccharide–protein conjugates, targeting either *Shigella sonnei*, *S. dysenteriae* 1 or *S. flexneri* serotype 2a, were evaluated in humans.[14,19] In the case of *S. sonnei*, recent field trials allowed J. B. Robbins and co-workers to demonstrate the efficacy of a vaccine made of the corresponding detoxified LPS covalently linked to the recombinant exoprotein A of *Pseudomonas aeruginosa*.[20] Conversion of polysaccharide T-independent antigens to T-dependent ones through their covalent attachment to a carrier protein has had a tremendous impact in the field of bacterial vaccines. Several such neoglycoconjugate vaccines are currently in use against *Haemophilus influenzae* b,[21] *Neisseria meningitidis*,[22] and *Streptococcus pneumoniae*.[23] These polysaccharide–protein conjugate vaccines are highly complex structures, whose immunogenicity depends on several parameters amongst which are the length and nature of the saccharide component as well as its loading on the protein. It is reasonably admitted that control of these parameters is somewhat difficult when dealing with polysaccharides purified from bacterial cell cultures. As recent progress in carbohydrate synthesis allows access to complex saccharides, it has been suggested that the use of well-defined synthetic oligosaccharides may allow better control, and consequently the optimisation, of these parameters. Indeed, available data on *S. dysenteriae* type 1 indicate that neoglycoconjugates incorporating di-, tri- or tetramers of the O-SP repeating unit were more immunogenic than a detoxified LPS–human serum albumin reference conjugate.[24] Besides, it was reported that short oligosaccharides comprising one repeating unit may be immunogenic in animal models.[25,26] Another critical parameter in the design of neoglycoconjugate vaccines is the carrier protein. As potential applications for these vaccines are expanding, the need for new carrier proteins licensed for human use is growing.[27] It has been suggested,[28] and later on demonstrated,[29,30] that synthetic peptides representing immunodominant T-cell epitopes could act as carriers in polysaccharide and oligosaccharide conjugates. Besides, the use of T-cell epitopes offers several advantages, including potential access to well-defined conjugates with no risk of epitopic suppression, as this latter phenomenon appeared to be a major drawback of protein carriers.[31–34] Polypeptides containing multiple T-cell epitopes have been generated in order to address the extensive polymorphism of HLA molecules.[35] In other strategies, universal T-helper epitopes compatible with human use have been characterized, for example from tetanus toxoid,[36] or engineered such as the pan HLA DR-binding epitope (PADRE).[37] Recently, covalent attachment of the human milk oligosaccharide, lacto-*N*-fucopentose II, to PADRE resulted in a linear glycopeptide of comparable immunogenicity to that of a glycoconjugate employing HSA as the carrier.[38]Along the same lines, a PADRE glycoconjugate was recently shown

† See ref. 1.
‡ Electronic supplementary information (ESI) available: experimental details for compounds **5**, **8**, **15**–**17**, **21**–**24** and **38**–**40**. See http://www.rsc.org/suppdata/ob/b4/b400986j/

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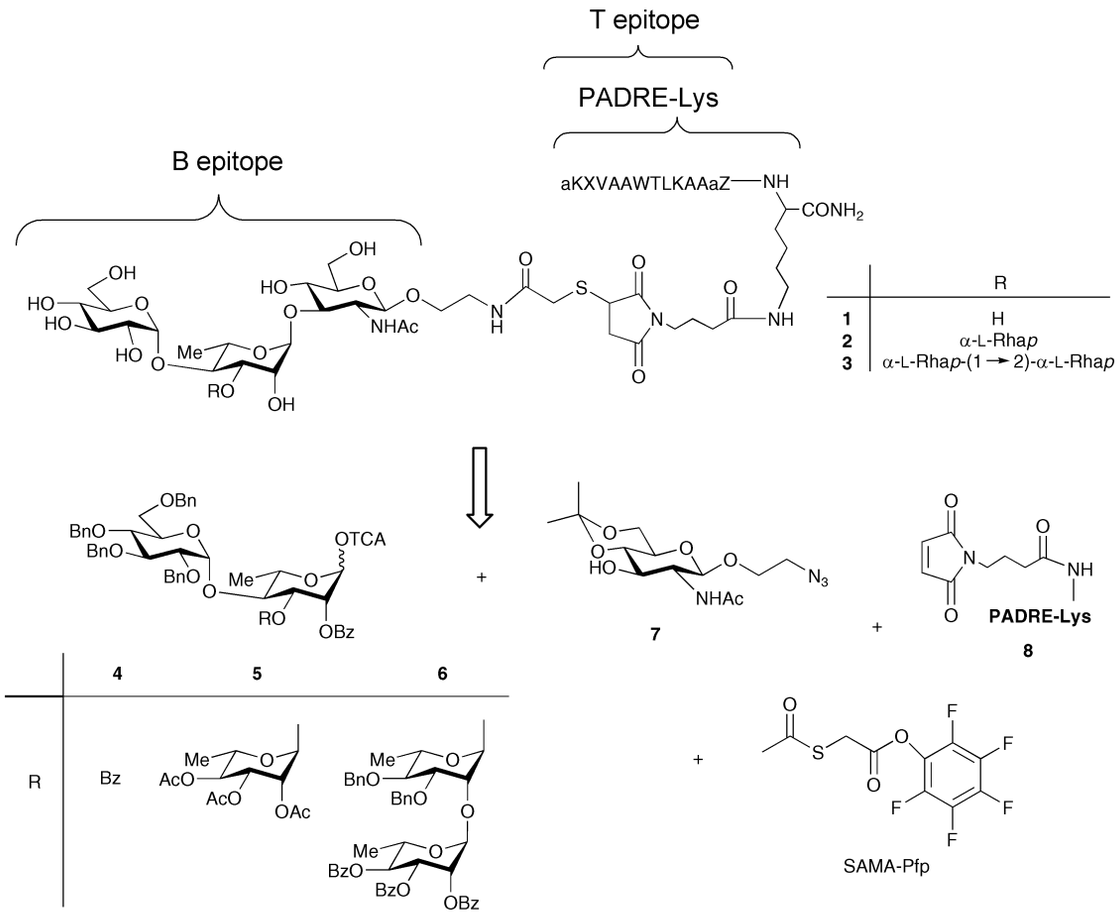


Scheme 1 Retrosynthetic analysis of the target neoglycopeptides **1**, **2** and **3**. (a, X and Z stand for D-Ala, cyclohexyl Ala, and ε-aminohexanoic acid, respectively).

to induce a strong T-cell dependent antibody response specific for the Tn antigen in both outbred and HLA transgenic mice.[39]

Based on these converging data, we focused on the development of well-defined neoglycopeptides as an alternative to polysaccharide–protein conjugate vaccines targeting infections caused by *S. flexneri* 2a. The target neoglycopeptides were constructed by covalently linking a short peptide, serving as a T-helper epitope, to appropriate carbohydrate haptens, serving as B epitopes mimicking the *S. flexneri* 2a O-SP. We have employed a rational approach involving a preliminary study of the interaction between the bacterial O-SP and homologous protective monoclonal antibodies, which helped to define the carbohydrate haptens.

Results and discussion

The O-SP of *S. flexneri* 2a is a heteropolysaccharide defined by the pentasaccharide repeating unit **I**.[40,41] It features a linear tetrasaccharide backbone, which is common to all *S. flexneri* O-antigens, except serotype 6, and comprises an *N*-acetyl glucosamine (**D**) and three rhamnose residues (**A**, **B** and **C**). The serotype specificity is associated with the presence of an α-D-glucopyranose residue linked at C-4 of rhamnose **C**. Besides the known methyl glycoside of the **EC** disaccharide,[42,43] a set of di- to pentasaccharides corresponding to frame-shifted fragments of the repeating unit **I**,[44–47] an octasaccharide,[48] and more recently a decasaccharide,[49] representative of fragments of *S. flexneri* 2a O-SP have been synthesized in this laboratory. Based on the use of these compounds as molecular probes for mapping at the molecular level the binding characteristics of a set of protective monoclonal antibodies against *S. flexneri* 2a infection,[50] fragments **ECD**, **B(E)CD** and **AB(E)CD** were selected as haptens that could act as B epitopes in the conjugates. Three fully synthetic linear neoglycopeptides **1**,**2** and **3**, corresponding to haptens **ECD**, **B(E)CD**, and **AB(E)CD**, respectively, were synthesized according to a strategy built on the concept of chemoselective ligation which allows the selective one-point attachment of the free B and T epitopes in aqueous media. All conjugates involve the peptide PADRE as the universal T-cell epitope.

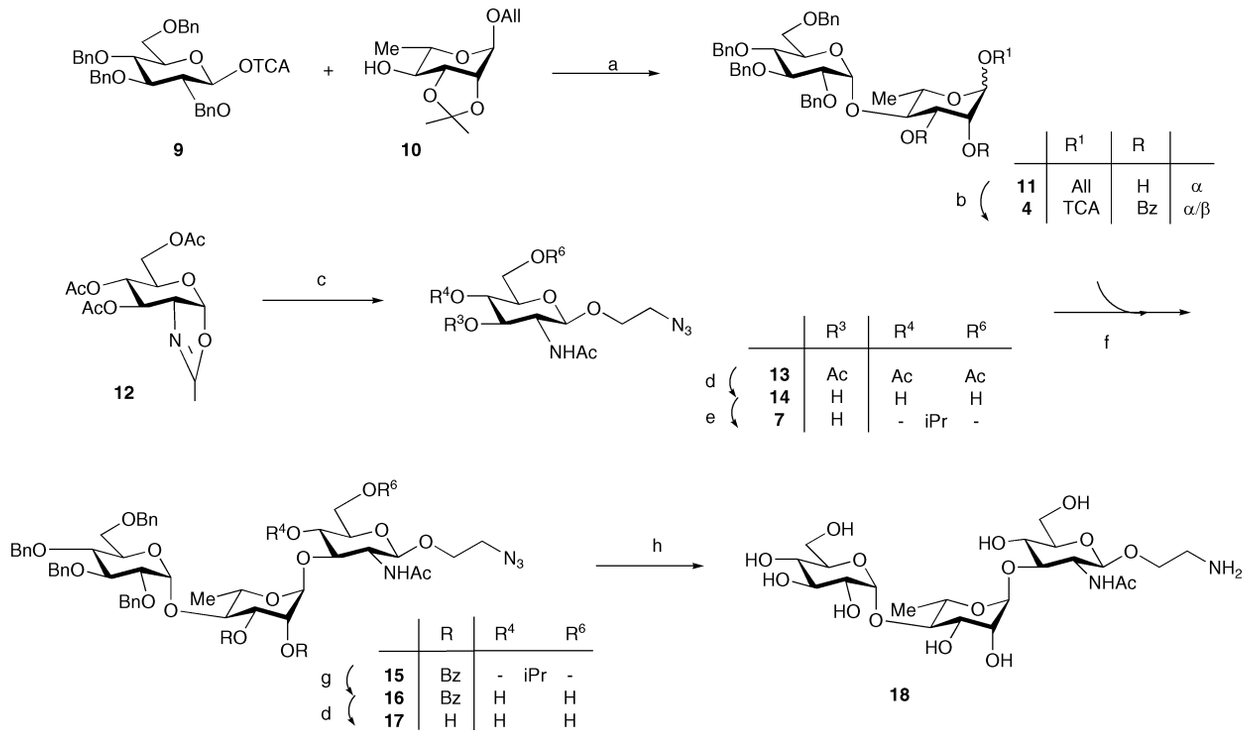
A **B** **E** **C** **D**

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-GlcNA*cp*(1→

I

Retrosynthetic analysis of the saccharidic haptens (Scheme 1)

Analysis of *S. flexneri* 2a O-SP suggests that, due to the 1,2-*cis* glycosidic linkage involved, construction of the **EC** disaccharide is probably the most demanding. Besides, prior work in this laboratory has shown that the **C**–**D** glycosidic linkage was an appropriate disconnection site when dealing with the blockwise synthesis of oligosaccharide fragments of *S. flexneri* 2a O-SP.[45,46,48] These observations supported the design of a synthetic strategy common to all three targets. Basically, it relies on (i) the condensation of an **EC** (**4**),[44] **B(E)C** (**5**)[48] or **AB(E)C** (**6**) donor to a **D** acceptor (**7**), functionalized at the anomeric position with an azidoethyl spacer; and (ii) elongation of the spacer with introduction of a masked thiol group to allow its coupling onto a PADRE peptide derivatized by a maleimido group on a C-terminal lysine (**8**). The carbohydrate synthesis relies on the trichloroacetimidate (TCA) methodology[51] and the use of known building blocks whenever possible. It is worthy of note that the strategy disclosed herein differs from that developed for the preparation of the corresponding methyl glycosides.[45,47]



Scheme 2 *(a)* see ref. 46; *(b)* see ref. 45; *(c)* see ref. 55; *(d)* cat. MeONa, MeOH, rt, 2 h; *(e)* $Me_2C(OMe)_2$, cat. CSA, DMF, rt, 2 h; *(f)* cat. TfOH, 4 Å-MS, CH_2Cl_2, −40 °C⟶rt, 3 h; *(g)* 95% aq. TFA, CH_2Cl_2, 0 °C; 15 min; *(h)* 10% Pd/C, 1 M aq. HCl, EtOH/EtOAc, rt, 24 h.

Synthesis of the aminoethyl ECD building block 18 (Scheme 2)

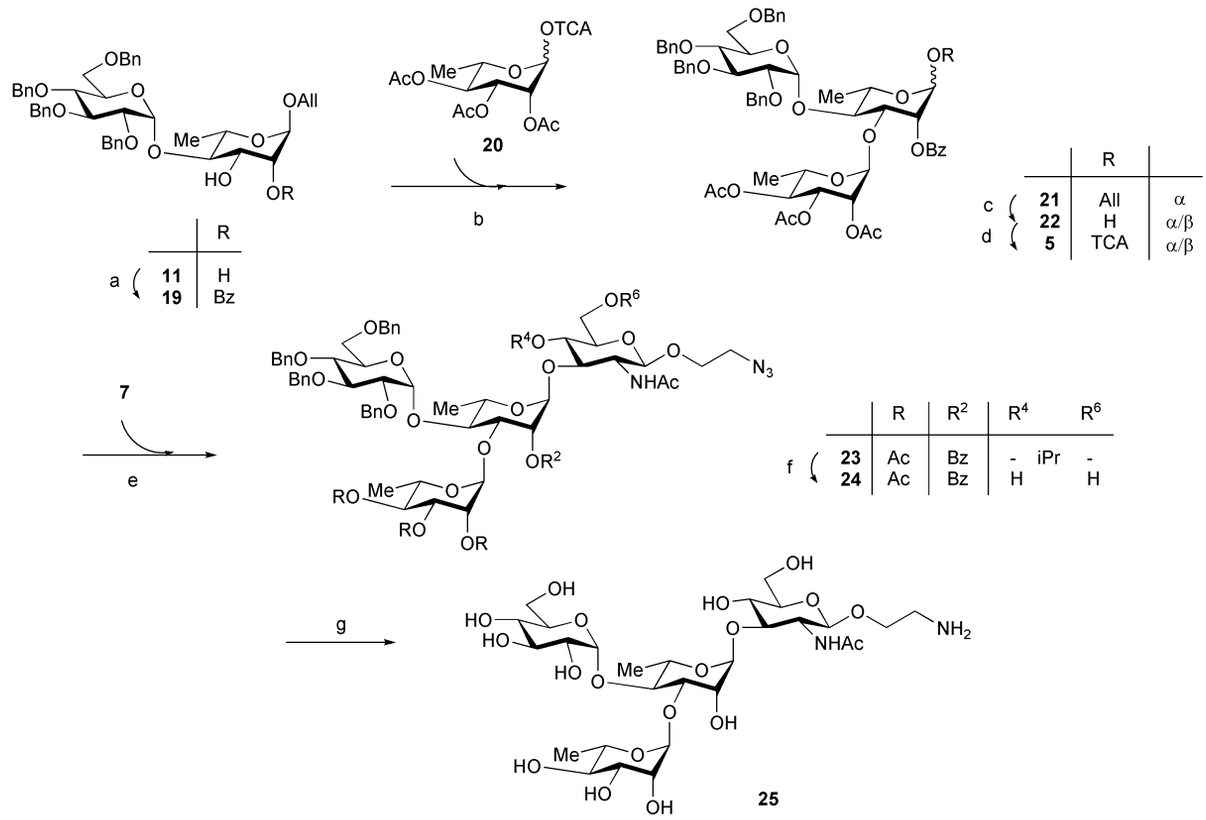
The now easily accessible disaccharide donor **4**,[46] with a benzoyl participating group at C-2_C, was used as the precursor to the **EC** moiety in the construction of **1**. It was prepared, as described,[44] in 5 steps and 45% overall yield from 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate (**9**)[52,53] and allyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside (**10**)[54] through the key intermediate diol **11**[45] (69% from **10**). Introduction of the azidoethyl spacer to a glucosaminyl intermediate was performed according to a known procedure[55] by coupling of azidoethanol[56] onto the oxazoline[57] **12** to give the triacetate **13**.[55] We have shown on several occasions in the *S. flexneri* series, that protection of the 4- and 6-OH groups of precursors to residue **D** with an isopropylidene acetal was appropriate, especially when such precursors are involved in a blockwise synthesis based on the disconnection at the **C–D** linkage.[46,48] Thus, Zemplén deacetylation of **13** gave the triol **14** which was converted to the key acceptor **7** (81% from **13**) upon reaction with 2,2-dimethoxypropane under acid catalysis. When the latter was glycosylated with the donor **4** in the presence of $BF_3.OEt_2$ in CH_2Cl_2, the fully protected trisaccharide **15** was isolated in 58% yield together with the diol **16** (30%), resulting from partial loss of the isopropylidene acetal. When **4** and **7** were glycosylated in the presence of a catalytic amount of TMSOTf, no side reaction was observed, and the condensation product **15** was obtained in 86% yield. Conversion of **15** into **16** (87%) was more conveniently achieved by acidic hydrolysis of the former with 95% aq. TFA. Debenzoylation of **16** gave the tetraol **17** (94%) which was subsequently transformed into the aminoethyl trisaccharide **18** (69%) by hydrogenation in the presence of palladium-on-charcoal (Pd/C) and 1 M aq. HCl to convert the formed amine to its hydrochloride salt as others have pointed out that hydrogenolysis using Pd/C in the presence of a free amine was sluggish and low-yielding.[58–60] In order to prevent any side-reaction at a latter stage of the synthesis, **18** was subsequently submitted to reverse-phase HPLC (RP-HPLC).

Synthesis of the aminoethyl B(E)CD building block 25 (Scheme 3)

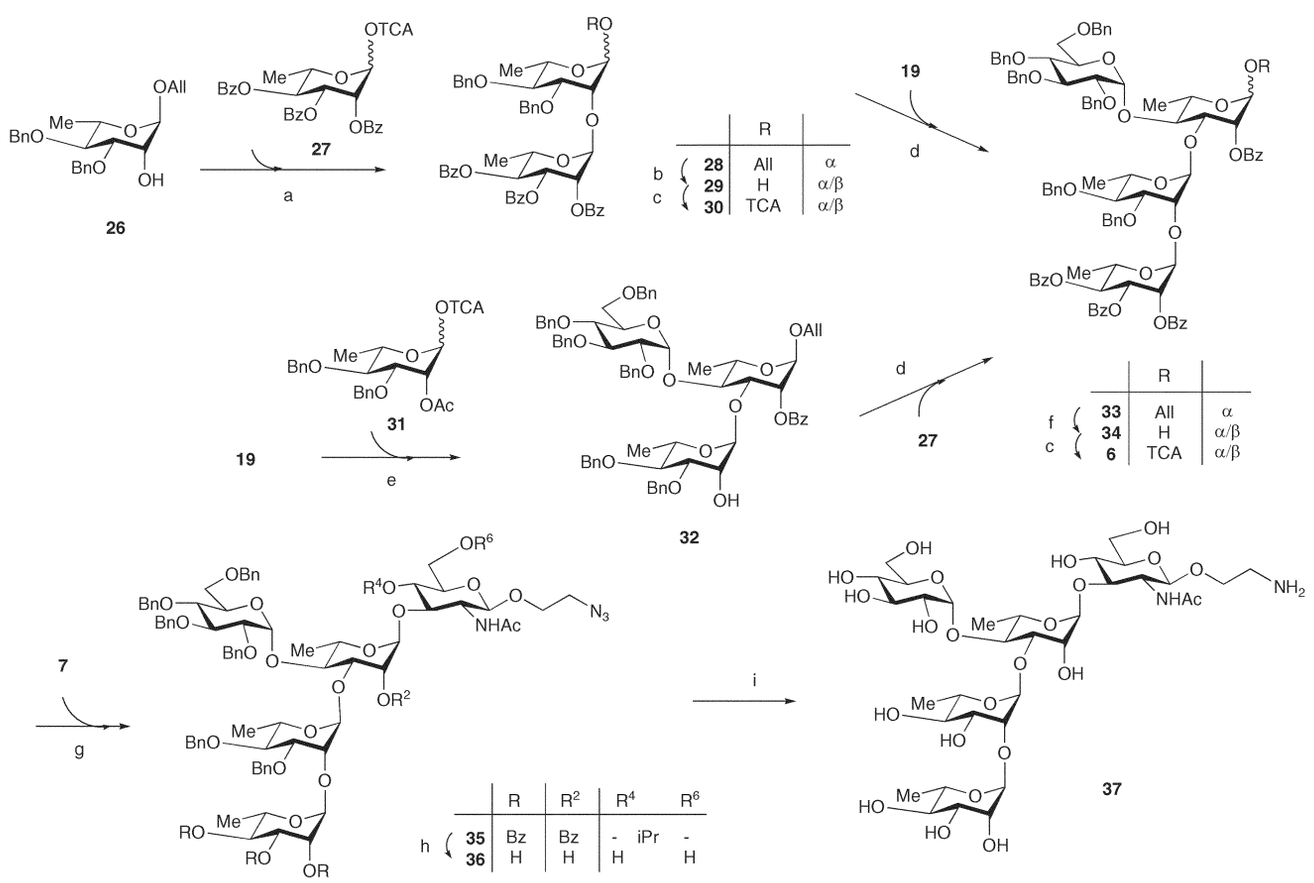
The known rhamnopyranosyl trichloroacetimidate **20**,[61] acetylated at its 2-, 3-, and 4-OH groups thus acting as a chain terminator, was chosen as the precursor to residue **B**. Benzoylation of diol **11** to give **19**[46] was performed by regioselective opening of the cyclic orthoester intermediate as described.[46] Glycosylation of the latter by donor **20**, with activation by a catalytic amount of TMSOTf, proceeded smoothly in Et_2O to yield the fully protected trisaccharide **21** (89%), which was de-*O*-allylated into the hemiacetal **22** (80%) following a two step process involving (i) iridium(I)-catalysed isomerisation of the allyl glycoside to the prop-1-enyl glycoside[62] and (ii) subsequent hydrolysis.[54,63] The selected trichloroacetimidate leaving group was introduced by treatment of **22** with trichloroacetonitrile in the presence of a catalytic amount of DBU, which resulted in the formation of **5** (99%). Condensation of the latter with acceptor **7** was performed in CH_2Cl_2 in the presence of a catalytic amount of trifluoromethanesulfonic acid (TfOH) to give the required tetrasaccharide **23** (76%). Acidic hydrolysis of the latter using 95% aq. TFA gave the intermediate diol **24** in 95% yield. Deacylation of **24** followed by debenzylation and concomitant conversion of the azide into the corresponding amine with hydrogen in the presence of Pd/C under acidic conditions gave the key aminoethyl tetrasaccharide **25** in a yield of 77% after gel filtration. Again, compound **25** was purified by RP-HLPC before elongation of the spacer for conjugation.

Synthesis of the aminoethyl AB(E)CD building block 37 (Scheme 4)

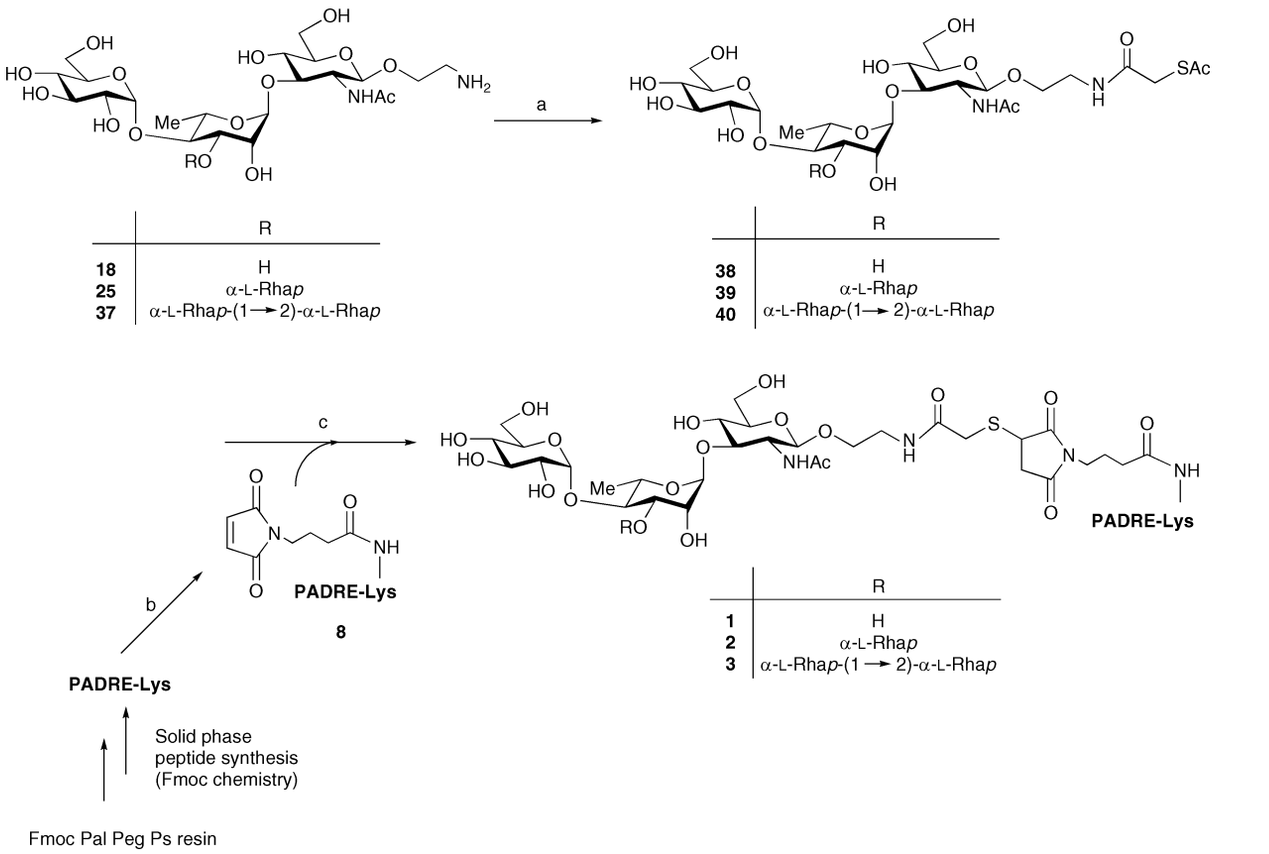
The synthesis of **37** is based on the condensation of acceptor **7** and donor **6**, which resulted from the selective deallylation and anomeric activation of the key intermediate tetrasaccharide **33**. The latter was obtained by two routes following either a block strategy (route 1) based on the condensation of an **AB** disaccharide donor (**30**) and the **EC** disaccharide acceptor **19**, or a linear strategy (route 2) involving the stepwise elongation of **19**. The construction of the donor **30** was based on the use of the known allyl rhamnopyranoside **26**,[64] having permanent protecting groups at C-3 and C-4, as the precursor to residue **B**, and the trichloroacetimidate chain terminator **27**,[65] acting as a precursor to residue **A**. Condensation of the two entities in the presence of a catalytic amount of TMSOTf resulted in the fully



Scheme 3 *(a)* see ref. 46; *(b)* cat. TMSOTf, Et_2O, −50 °C⟶rt, 2 h; *(c) i.* cat. $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$, THF, rt, 20 h, *ii.* HgO, $HgCl_2$, acetone/water, rt, 2 h; *(d)* CCl_3CN, DBU, CH_2Cl_2, rt, 30 min; *(e)* cat. TfOH, 4 Å-MS, 1,2-DCE, 65 °C, 1 h; *(f)* 50% aq. TFA, CH_2Cl_2, 0 °C, 1 h; *(g)* i. cat. MeONa, MeOH, rt, 18 h; ii. 10% Pd/C, 1 M aq. HCl, EtOH/EtOAc, rt, 72 h.



Scheme 4 *(a)* cat. TMSOTf, Et_2O, −70 °C⟶rt, 8 h; *(b)* i. cat. $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$, THF, rt, 16 h, ii. HgO, $HgBr_2$, acetone/water, rt, 1 h; *(c)* CCl_3CN, DBU, CH_2Cl_2, rt, 2 h; *(d)* cat. TMSOTf, Et_2O, −60 °C⟶−30 °C, 2 h; *(e)* see ref. 49; *(f)* i. cat. $[Ir(COD)\{PCH_3(C_6H_5)_2\}_2]^+PF_6^-$, THF, rt, 16 h, ii. HgO, $HgCl_2$, acetone/water, rt, 1 h; *(g)* cat. TMSOTf, 4 Å-MS, CH_2Cl_2, rt, 3 h; *(h)* i. 50% aq. TFA, CH_2Cl_2, 0 °C; 2 h; ii. cat. MeONa, MeOH, 55 °C, 2 h; *(i)* 10% Pd/C, 1 M aq. HCl, EtOH/EtOAc, rt, 2 h.



Scheme 5 *(a)* SAMA-Pfp, 0.1 M phosphate buffer (pH 7.4), rt, 45 min; *(b) i.* maleimide butyric acid, DCC, CH_2Cl_2, 10 min; *ii.* TFA : TIS : H_2O (95 : 2.5 : 2.5); *(c)* $HONH_2$.HCl, H_2O, CH_3CN, 0.5 M phosphate buffer (pH 6.0), rt, 1 h.

protected **28** (96%), which was selectively de-*O*-allylated into **29** (84%) according to the protocol described above for the preparation of **22**. Subsequent treatment of **29** with trichloroacetonitrile and a catalytic amount of DBU gave the required **30** (96%). Glycosylation of **19** with the latter under TMSOTf promotion afforded the fully protected tetrasaccharide **33** in 55% yield. No β-anomer was detected. The stereochemical outcome of this glycosylation step involving a rhamnosyl donor glycosylated at C-2, thus lacking any participating group at this position is not without precedent. Related examples involving rhamnopyranosyl donors may be found in the synthesis of oligosaccharides representative of the capsular polysaccharide of the β-hemolytic *Streptococcus* Group A,[66] or of the O-Ag of *Serratia marcescens* O18[67] as well as in our own work on *S. flexneri* serotype 2a.[49] Route 1 was considered initially in order to prevent extensive consumption of the **EC** disaccharide **11**. Given the relatively low yield of coupling of **19** and **30**, route 2 was considered as well. Of all precursors to **34**, only that to residue **B**, namely the donor and potential acceptor **31**,[68] differed from those used in route 1. Conventional glycosylation of disaccharide **19** and **31** and subsequent selective deacetylation using methanolic HBF_4, gave the acceptor **32** in 70% yield from **19**.[49] The trisaccharide **32** was glycosylated with trichloroacetimidate **27** in an analogous fashion to glycosylation of **19** with **30**, yielding **33** (92%). Anomeric de-*O*-allylation of this key intermediate, as described above for the preparation of **22**, gave the corresponding hemiacetal **34** (90%) which was converted into the required trichloroacetimidate **6** (88%) upon treatment with trichloroacetonitrile and DBU. Condensation of donor **6** with the glucosaminyl acceptor **7** was performed under promotion by TfOH or TMSOTf, which resulted in the fully protected pentasaccharide **35** in 62% and 80% yield, respectively. Following the process described for the preparation of **25**, compound **35** was submitted to acid hydrolysis (97%) and subsequent deacylation to give the partially deblocked **36** (87%), which was next converted to the aminoethyl pentasaccharide **37** upon treatment with hydrogen in the presence of Pd/C. Final RP-HPLC purification resulted in the isolation of **37** in 53% yield.

Synthesis of the target neoglycopeptides 1-3 (Scheme 5)

In all cases, chemoselective ligation of the B and T epitopes was achieved through coupling of the carbohydrate haptens pre-functionalized with a thiol function and a maleimide group introduced at the C terminus of the T helper peptide. Such a strategy was chosen in order to exploit the high reactivity and specificity of thiol groups towards the maleimide functionality,[69] which allows specific and high-yielding modification of the former in the presence of other nucleophiles.[70] It was used previously under various forms in the coupling of carbohydrate haptens to either proteins[71,72] or peptides.[30,72] To our knowledge, in all the reported cases the maleimide functionality was introduced onto the carbohydrate hapten. On the contrary, our strategy relies on the introduction of this activating group to the T helper peptide. The immunogenicity of various maleimide-derived coupling reagents was evaluated in a model system.[73] Based on the reported data,[73] 4-(*N*-maleimido)-*n*-butanoyl was selected as the linker, and incorporated by covalent linkage to the side chain amino group of a lysine residue added to the C-terminus of the PADRE sequence (PADRE-Lys). It is worth mentioning that the strategy described herein differs somewhat from that described by others when demonstrating the usefulness of PADRE in the construction of immunogenic neoglycopeptides.[38]

The lysine-modified PADRE (PADRE-Lys) was assembled using standard Fmoc chemistry solid-phase peptide synthesis.[74] Standard side chain protecting groups were used, except for that of the C-terminal lysine side chain which was protected by the 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) group.[75] Indeed, this orthogonal protecting group strategy allows specific introduction of the maleimide group to

the C-terminal lysine, upon selective cleavage of the ivDde group by hydrazine. The thiol functionality was introduced onto the carbohydrate haptens as a masked thiol function (acetylthioester), which is easily generated *in situ* during the conjugation process. Thus, reaction of **18**, **25** and **37**, with *S*-acetylthioglycolic acid pentafluorophenyl ester (SAMA-Pfp) resulted in the site-selective elongation of their aminoethyl spacer with a thioacetyl acetamido linker. Derivatization could be monitored by RP-HPLC with detection at 215 nm. Under these conditions, the required thioacetyl-armed intermediates, **38**, **39** and **40** were isolated in 53%, 74%, and 75% yield, respectively. Their structure was confirmed based on MS and NMR analysis. Conjugation of the carbohydrate haptens to the maleimido activated PADRE-Lys (**8**) was performed in phosphate buffer at pH 6.0 in the presence of hydroxylamine[76] and monitored by RP-HPLC. Lastly, RP-HPLC purification gave the target neoglycopeptides **1**, **2** and **3** as single products, whose identity was assessed by MS analysis, in yields of 58%, 48% and 46%, respectively.

Conclusion

The synthesis of three fully synthetic glycopeptides incorporating tri-, tetra-, and pentasaccharide haptens representative of fragments of the O-SP of *S. flexneri* serotype 2a covalently linked to the PADRE-sequence, which acts as a universal T cell epitope compatible with human use is reported. The carbohydrate haptens were selected based on a preliminary study of the recognition of synthetic oligosaccharides with homologous protective antibodies. They were synthesized following a common block strategy, in a form allowing their coupling by chemical ligation onto a maleimido-activated PADRE. Evaluation of the immunogenicity of the conjugates in mice is ongoing.

Experimental

General methods

Optical rotations were measured for $CHCl_3$ solutions at 25 °C, except where indicated otherwise, with a Perkin-Elmer automatic polarimeter, Model 241 MC. TLC on precoated slides of Silica Gel 60 F_{254} (Merck) was performed with solvent mixtures of appropriately adjusted polarity consisting of *A*, CH_2Cl_2/methanol; *B*, cyclohexane/ethyl acetate, *C*, cyclohexane/acetone and *D*, toluene/ethyl acetate. Detection was effected when applicable, with UV light, and/or by charring with orcinol (35 mM) in 4 N aq. H_2SO_4. Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 60–43 μm). RP-HPLC (215 nm) used a Kromasil 5 μm C18 100 Å 4.6 × 250 mm analytical column (1 mL min^{-1}). NMR spectra were recorded at 20 °C for solutions in $CDCl_3$ unless stated otherwise, on a Bruker Advance 400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C). External references: for solutions in $CDCl_3$, TMS (0.00 ppm for both ^1H and ^{13}C); for solutions in D_2O, dioxane (67.4 ppm for ^{13}C) and trimethylsilyl-3-propionic acid sodium salt (0.00 ppm for ^1H). Proton signal assignments were made by first-order analysis of the spectra as well as analysis of two-dimensional ^1H-^1H correlation maps (COSY) and selective TOCSY experiments. In addition to s, d, t, and q, multiplicity is given as pt for pseudo t, and br s for broad s. Of the two magnetically non-equivalent 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 ^{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 identified by a subscript in listing of signal assignments. Low-resolution mass spectra were obtained by either chemical ionisation (CI-MS) using NH_3 as the ionising gas, by electrospray mass spectrometry (ES-MS), or by fast atom bombardment mass spectrometry (FAB-MS) in the positive-ion mode using dithioerythritol/dithio-L-threitol (4 : 1, magic bullet) as the matrix, in the presence of NaI, and xenon as the gas. High-resolution mass spectra were obtained by Matrix Assisted Laser Desorption Ionisation mass spectrometry (MALDI-MS). Anhydrous dichloromethane (CH_2Cl_2), 1,2-dichloroethane (1,2-DCE) and Et_2O, sold on molecular sieves were used as such. 4 Å powder molecular sieves were kept at 100 °C and activated before use by heating at 250 °C under vacuum. Solid phase peptide synthesis was performed using standard Fmoc chemistry protocols on a Pioneer peptide synthesiser (AppliedBiosystem). 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. All others reagents and amino acids were purchased from Applied Biosystem.

2-Azidoethyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (7)

1 M methanolic sodium methoxide (1 mL) was added to a solution of **13**[55] (3.6 g, 8.65 mmol) in MeOH (20 mL) and the mixture was stirred at rt for 2 h. The mixture was neutralised with Amberlite IR-120 (H^+) resin and filtered. The filtrate was concentrated to give crude **14** (2.6 g). Camphorsulfonic acid (200 mg, 0.9 mmol) was added to a solution of triol **14** (2.3 g) in a mixture of DMF (4 mL) and 2,2-dimethoxypropane (4 mL). After 3 h at rt, low boiling point solvents were evaporated under reduced pressure and more 2,2-dimethoxypropane (2 mL, 15.8 mmol) was added. The mixture was stirred for 2 h at rt, Et_3N was added, and the mixture was concentrated. The crude product was purified by column chromatography (solvent A, 19 : 1) to give **7** as a white solid (1.97 g, 78% from **13**), $[\alpha]_D$ −91.1 (*c* 1.0); ^1H NMR: δ 6.15 (d, 1H, $J_{NH,2}$ = 5.9 Hz, NH), 4.70 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.05 (m, 1H, OCH_2), 3.97–3.89 (m, 2H, H-6a, 3), 3.79 (pt, 1H, $J_{5,6b} = J_{6a,6b}$ = 10.5 Hz, H-6b), 3.70 (m, 1H, OCH_2), 3.62–3.46 (m, 3H, H-2, 4, OCH_2), 3.35–3.26 (m, 3H, H-5, CH_2N_3), 2.05 (s, 3H, CH_3CO), 1.52 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$); ^{13}C NMR: δ 172.4 (C=O), 100.9 (C-1), 100.0 ($C(CH_3)_2$), 74.3 (C-4), 71.8 (C-3), 68.6 (OCH_2), 67.3 (C-5), 62.0 (C-6), 58.7 (C-2), 50.7 (CH_2N_3), 29.0 ($C(CH_3)_2$), 23.6 (CH_3CO), 19.1 ($C(CH_3)_2$). CI-MS for $C_{13}H_{22}N_4O_6$ (M, 330) *m/z* 331 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{22}N_4O_6 \cdot 0.5H_2O$: C, 46.01; H, 6.83; N, 16.51%. Found: C, 46.37; H, 6.69; N, 16.46%.

2-Aminoethyl α-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (18)

The trisaccharide **17** (368 mg, 0.38 mmol) was dissolved in a mixture of EtOH (10 mL) and EtOAc (1 mL). A 1 M solution of aq. HCl (0.77 mL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (400 mg) for 24 h. The mixture was diluted with water and filtered. The filtrate was concentrated, then freeze-dried. The residue was dissolved in a solution of $NaHCO_3$ (75 mg) in water (1 mL) and purified by passing first through a column of C_{18} silica gel (eluting with water), then through a column of Sephadex G_{10} (eluting with water) to give, after lyophilization, **18** (151 mg, 69%). Starting from 75 mg of the latter, further RP-HPLC purification gave 46 mg of RP-HPLC pure **18**. HPLC (215 nm): Rt 4.09 min (Kromasil 5 μm C18 100 Å 4.6 × 250 mm analytical column, using a 0–20% linear gradient over 20 min of CH_3CN in 0.01 M aq. TFA at 1 mL min^{-1} flow rate). ^1H NMR (D_2O): δ 4.97 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1_E), 4.78 (d, 1H, $J_{1,2}$ = 1.2 Hz, H-1_C), 4.54 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_D), 4.02 (m, 1H, H-5_C), 4.00–3.90 (m, 3H, H-5_E, $6a_D$, CH_2O), 3.88–3.67 (m, 6H, H-2_C, 2_D, 3_C, $6a_E$, $6b_E$, $6b_D$, CH_2O), 3.61 (dd, 1H, *J* = 9.8, *J* = 9.1 Hz, H-3_E), 3.60–3.42 (m, 5H, H-2_E, 4_C, 4_D, 4_E, 5_D), 3.54 (m, 1H, H-3_D), 3.03 (m, 2H, CH_2NH_2), 2.00 (s, 3H, CH_3CO), 1.25 (d, 3H, $J_{5,6}$ = 6.3 Hz,

H-6$_C$); ^{13}C NMR (D_2O): δ 175.2 (C=O), 101.6 (C-1$_C$), 100.7 (C-1$_D$), 100.0 (C-1$_E$), 82.1 (C-3$_D$), 81.4 (C-4$_C$), 76.3 (C-2$_E$), 73.1 (C-3$_E$), 72.2 (C-5$_E$), 71.9 (C-4$_D$), 71.3 (C-2$_C$), 69.7 (C-4$_E$), 69.3 (C-3$_C$), 68.8 (C-5$_D$), 68.5 (C-5$_C$), 66.0 (CH_2O), 60.9 (C-6$_D$), 60.5 (C-6$_E$), 55.5 (C-2$_D$), 39.8 (CH_2NH_2), 22.5 (CH_3CO), 17.1 (C-6$_C$). ES-MS for $C_{22}H_{40}N_2O_{15}$ (M, 572) *m/z* 573 [M + H]$^+$. HRMS (MALDI) Calcd for $C_{22}H_{40}N_2O_{15}Na$: 595.2326. Found: 595.2341.

2-Aminoethyl α-L-rhamnopyranosyl-(1⟶3)-[α-D-glucopyranosyl-(1⟶4)]-α-L-rhamnopyranosyl-(1⟶3)-2-acetamido-2-deoxy-β-D-glucopyranoside (25)

An ice cold solution of 95% aq. TFA (2.4 mL) in CH_2Cl_2 (21.6 mL) was added to the tetrasaccharide **23** (1.93 g, 1.40 mmol). The mixture was kept at 0 °C for 5 min, then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. The residue was dissolved in MeOH (65 mL), and a 1 M solution of sodium methoxide in MeOH (3 mL) was added. The mixture was left to stand at rt for 18 h, then neutralised with Amberlite IR-120 (H^+) resin, and filtered. The filtrate was concentrated, and the residue was purified by column chromatography (solvent B, 9 : 1) to give **24** (1.38 g, 89%) as a colourless foam. The tetrasaccharide **24** (1.38 g, 1.25 mmol) was dissolved in a mixture of EtOH (35 mL) and EtOAc (3.5 mL). A 1 M solution of aq. HCl (2.5 mL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (1.5 g) for 72 h, then diluted with water and filtered. The filtrate was concentrated, then freeze-dried. The residue was dissolved in a solution of 5% aq. $NaHCO_3$ and purified by passing first through a column of C_{18} silica (eluting with water), then through a column of Sephadex G$_{10}$ (eluting with water) to give, after lyophilization, **25** (693 mg, 77%). Further HPLC purification of 373 mg of the latter gave 351 mg of RP-HPLC pure **25**. HPLC (215 nm): Rt 4.78 min (Kromasil 5 μm C18 100 Å 4.6 × 250 mm analytical column, using a 0–20% linear gradient over 20 min of CH_3CN in 0.01 M aq. TFA at 1 mL min^{-1} flow rate). ^{1}H NMR (D_2O): δ 5.10 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1$_E$), 4.89 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1$_B$), 4.73 (d, 1H, $J_{1,2}$ = 1.0 Hz, H-1$_C$), 4.50 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1$_D$), 4.08 (dq, 1H, $J_{4,5}$ = 9.3 Hz, H-5$_C$), 3.96 (m, 2H, H-2$_B$, CH_2O), 3.88–3.64 (m, 12H, H-2$_C$, 2$_D$, 3$_B$, 3$_C$, 4$_C$, 5$_B$, 5$_E$, 6a$_D$, 6b$_D$, 6a$_E$, 6b$_E$, CH_2O), 3.59 (pt, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, H-3$_E$), 3.52 (dd, 1H, $J_{2,3}$ = 8.6, $J_{3,4}$ = 9.7 Hz, H-3$_D$), 3.48–3.33 (m, 5H, H-2$_E$, 4$_B$, 4$_D$, 4$_E$, 5$_D$), 3.15 (m, 2H, CH_2NH_2), 1.98 (s, 3H, CH_3CO), 1.27 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6$_C$), 1.20 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6$_B$); ^{13}C NMR (D_2O): δ 174.8 (C=O), 103.2 (br s, C-1$_B$), 101.4 (C-1$_C$), 100.9 (C-1$_D$), 98.6 (C-1$_E$), 81.9 (C-3$_D$), 79.0 (br s, C-3$_C$), 76.6 (br s, C-4$_C$), 76.3 (C-2$_E$), 72.9 (C-3$_E$), 72.3 (2C, C-5$_E$, 4$_B$), 71.8 (C-4$_D$), 71.1 (br s, C-2$_C$), 70.5 (C-2$_B$, 3$_B$), 69.7, 69.5 (2C, C-5$_B$, 4$_E$), 69.2, 68.8 (C-5$_D$, 5$_C$), 67.9 (CH_2O), 61.0 (C-6$_D$), 60.8 (C-6$_E$), 55.5 (C-2$_D$), 40.0 (CH_2NH_2), 22.6 (CH_3CO), 18.0 (C-6$_C$), 17.0 (C-6$_B$). FAB-MS for $C_{28}H_{50}N_2O_{19}$ (M, 718.3) *m/z* 741 [M + Na]$^+$. HRMS (MALDI) Calcd for $C_{28}H_{50}N_2O_{19}Na$: 741.2905. Found: 741.2939.

Allyl (2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (28)

TMSOTf (11 μL, 59 μmol) was added to a solution of the rhamnoside **26** (2.26 g, 5.88 mmol) and the trichloroacetimidate **27** (4.23 g, 6.82 mmol) in anhydrous Et_2O (60 mL) at −70 °C. The reaction mixture was stirred for 8 h while the cooling bath was slowly coming back to rt. Et_3N (100 μL) was added, and the mixture was stirred at rt for 15 min. Solvents were evaporated, and the crude material was purified by column chromatography (solvent B, 49 : 1⟶9 : 1), to give **28** as a white foam (4.78 g, 96%). [*a*]$_D$ +81.7 (*c* 1.0); ^{1}H NMR: δ 8.17–7.12 (m, 25H, Ph), 5.97–5.85 (m, 3H, H-2$_A$, 3$_A$, CH=), 5.67 (pt, 1H, $J_{3,4}$ = 9.6 Hz, H-4$_A$), 5.34–5.19 (m, 3H, H-1$_A$, CH_2=), 5.01 (d, 1H, *J* = 9.0 Hz, CH_2Ph), 4.92 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1$_B$), 4.82–4.74 (m, 2H, CH_2Ph), 4.71 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 4.31 (dq, 1H, $J_{4,5}$ = 9.7 Hz, H-5$_A$), 4.21 (m, 1H, OCH_2), 4.10 (dd, 1H, H-2$_B$), 4.02 (m, 1H, OCH_2), 3.97 (dd, 1H, $J_{2,3}$ = 3.0, $J_{3,4}$ = 9.2 Hz, H-3$_B$), 3.82 (dq, 1H, $J_{4,5}$ = 9.4 Hz, H-5$_B$), 3.71 (dd, 1H, H-4$_B$), 1.43 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6$_B$), 1.37 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_A$); ^{13}C NMR: δ 166.3, 165.9, 165.7 (C=O), 139.0–127.9 (CH=, Ph), 117.8 (CH_2=), 99.9 (C-1$_A$), 98.3 (C-1$_B$), 80.6 (C-4$_B$), 80.2 (C-3$_B$), 76.5 (C-2$_B$), 76.0, 72.9 (2C, CH_2Ph), 72.3 (C-4$_A$), 71.0 (C-2$_A$*), 70.4 (C-3$_A$*), 68.7 (C-5$_B$), 68.1 (OCH_2), 67.5 (C-5$_A$), 18.4 (C-6$_B$), 18.1 (C-6$_A$). FAB-MS for $C_{50}H_{50}O_{12}$ (M, 842.3) *m/z* 865.1 [M + Na]$^+$. Anal. Calcd. for $C_{50}H_{50}O_{12}$: C, 71.24; H, 5.98%. Found: C, 71.21; H, 5.99%.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α/β-L-rhamnopyranose (29)

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (25 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, until the colour had changed to yellow. The solution was then degassed again in an argon stream. A solution of **28** (4.71 g, 5.59 mmol) in THF (40 mL) was degassed and added. The mixture was stirred at rt overnight, then concentrated. The residue was taken up in acetone (350 mL) and water (82 mL). Mercuric bromide (3.23 g, 8.96 mmol) and mercuric oxide (2.64 g, 12.2 mmol) were added to the mixture, which was protected from light. The suspension was stirred at rt for 1 h, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 3 : 1) to give **29** (3.87 g, 84%) as a colourless foam. ^{1}H NMR (α anomer): δ 8.15–7.12 (m, 25H, Ph), 5.94–5.88 (m, 3H, H-2$_A$, 3$_A$, CH=), 5.70 (pt, 1H, $J_{3,4}$ = 9.7 Hz, H-4$_A$), 5.31 (dd, 1H, $J_{1,OH}$ = 3.0 Hz, H-1$_B$), 5.28 (br s, 1H, H-1$_A$), 4.98 (d, 1H, *J* = 11.0 Hz, CH_2Ph), 4.82–4.68 (m, 3H, CH_2Ph), 4.31 (dq, 1H, $J_{4,5}$ = 9.8 Hz, H-5$_A$), 4.13 (dd, 1H, $J_{1,2}$ = 2.1 Hz, H-2$_B$), 4.06–3.99 (m, 2H, H-3$_B$, 5$_B$), 3.72 (pt, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz, H-4$_B$), 2.79 (br s, 1H, OH-1$_B$), 1.41 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_B$), 1.37 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6$_A$); ^{13}C NMR (α anomer): δ 166.2, 165.9, 165.7 (C=O), 138.9–127.9 (Ph), 99.7 (C-1$_A$), 94.2 (C-1$_B$), 80.5 (C-4$_B$), 79.6 (C-3$_B$), 77.6 (C-2$_B$), 76.5, 72.5 (2C, CH_2Ph), 72.3 (C-4$_A$), 71.0 (C-2$_A$*), 70.4 (C-3$_A$*), 68.8 (C-5$_B$), 67.6 (C-5$_A$), 18.5 (C-6$_B$*), 18.1 (C-6$_A$*). FAB-MS for $C_{47}H_{46}O_{12}$ (M, 802.3) *m/z* 825.1 [M + Na]$^+$. Anal. Calcd. for $C_{47}H_{46}O_{12}$·0.5 H_2O: C, 69.53; H, 5.84%. Found: C, 69.55; H, 5.76%.

(2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl)-(1⟶2)-3,4-di-*O*-benzyl-α/β-L-rhamnopyranosyl trichloroacetimidate (30)

The hemiacetal **29** (3.77 g, 4.71 mmol) was dissolved in CH_2Cl_2 (15 mL) and the solution was cooled to 0 °C. Trichloroacetonitrile (2.5 mL) was added, then DBU (200 μL). The mixture was stirred at rt for 2 h. Toluene was added, and co-evaporated twice from the residue. The crude material was purified by flash chromatography (solvent B, 4 : 1 + 0.1% Et_3N) to give **30** as a white foam (4.29 g, 96%). Some hydrolyzed material **29** (121 mg, 3%) was eluted next. The trichloroacetimidate **30**, isolated as an α/β mixture had ^{1}H NMR (α anomer): δ 8.62 (s, 1H, NH), 8.20–7.18 (m, 25H, Ph), 6.31 (s, 1H, H-1$_B$), 5.94 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2$_A$), 5.89 (dd, 1H, $J_{2,3}$ = 3.4, $J_{3,4}$ = 9.9 Hz, H-3$_A$), 5.71 (pt, 1H, H-4$_A$), 5.27 (br s, 1H, H-1$_A$), 5.02 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.84 (d, 1H, *J* = 11.9 Hz, CH_2Ph), 4.79 (d, 1H, CH_2Ph), 4.72 (d, 1H, CH_2Ph), 4.36 (dq, 1H, $J_{4,5}$ = 9.8 Hz, H-5$_A$), 4.13 (dd, 1H, H-2$_B$), 4.03–3.97 (m, 2H, H-3$_B$, 5$_B$), 3.80 (pt, 1H, $J_{3,4}$ = 9.5 Hz, H-4$_B$), 1.45 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6$_B$), 1.40 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6$_A$); ^{13}C NMR (α anomer): δ 166.2, 165.9, 165.7 (3C, C=O), 160.8 (C=NH), 138.6–128.2 (Ph), 99.9 (C-1$_A$), 97.2 (C-1$_B$), 91.4 (CCl_3), 79.9 (C-4$_B$), 79.1 (C-3$_B$), 76.2 (CH_2Ph), 74.9 (C-2$_B$), 73.3 (CH_2Ph), 72.1 (C-4$_B$), 71.7 (C-5$_B$), 71.0 (C-2$_A$), 70.2 (C-3$_A$), 67.8 (C-5$_A$), 18.4 (C-6$_B$), 18.0 (C-6$_A$). Anal. Calcd. for

$C_{49}H_{46}Cl_3NO_{12}$: C, 62.13; H, 4.89; N, 1.48%. Found C, 61.81; H, 4.86, N, 1.36%.

Allyl (2,3,4-tri-*O*-benzoyl-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 (33)

(a) The acceptor **19** (465 mg, 0.56 mmol) was dissolved in Et_2O (3 mL). The solution was cooled to −60 °C and TMSOTf (65 μL, 0.36 mmol) was added. The donor **30** (690 mg, 0.73 mmol) was dissolved Et_2O (6 mL) and added to the acceptor solution in two portions with an interval of 30 min. The mixture was stirred at −60 °C to −30 °C over 2 h. Et_3N (100 μL) was added. The mixture was concentrated and the residue was purified by column chromatography (solvent B, 7 : 1) to give **33** (501 mg, 55%).

(b) A solution of the donor **27** (1.41 g, 2.25 mmol) and the acceptor **32**[49] (1.07 g, 1.79 mmol) in anhydrous Et_2O (88 mL) was cooled to −60 °C. TMSOTf (63 μL) was added, and the mixture was stirred at −60 °C to −20 °C over 2.5 h. Et_3N was added (100 μL). The mixture was concentrated and the residue was purified by column chromatography (solvent D, 49 : 1) to give **33** (2.66 g, 92%); $[\alpha]_D$ +74.1 (*c* 0.5); ^{1}H NMR: δ 8.11–7.06 (m, 50H, Ph), 6.00–5.87 (m, 3H, H-2_A, 3_A, CH=), 5.72 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4_A), 5.49 (dd, 1H, $J_{1,2}$ = 2.0, $J_{2,3}$ = 3.0 Hz, H-2_C), 5.35–5.22 (m, 3H, H-1_A, CH_2=), 5.12 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1_B), 5.08 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 4.96 (br s, 1H, H-1_C), 4.98–4.62 (m, 7H, CH_2Ph), 4.57 (br s, 1H, H-2_B), 4.54–4.31 (m, 6H, H-5_A, CH_2Ph), 4.21–4.16 (m, 2H, H-3_C, OCH_2), 4.09–3.99 (m, 3H, H-3_E, 5_E, OCH_2), 3.84 (m, 2H, H-4_C, 5_C), 3.77–3.54 (m, 6H, H-3_B, 4_B, 4_E, 5_B, $6a_E$, $6b_E$), 3.49 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2_E), 1.42 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A), 1.37 (d, 3H, $J_{5,6}$ = 5.5 Hz, H-6_C), 1.11 (d, 3H, $J_{5,6}$ = 5.9 Hz, H-6_B); ^{13}C NMR: δ 166.0, 165.9, 165.4, 165.1 (C=O), 138.7–127.1 (CH=, Ph), 117.8 (CH_2=), 101.3 (br s, C-1_B), 99.6 (C-1_A), 97.9 (C-1_E), 96.1 (C-1_C), 81.9 (C-3_E), 81.0 (C-2_E), 80.1 (br s, C-3_C), 79.8 (C-4_B), 78.9 (C-3_B), 77.9 (br s, C-4_C), 77.4 (C-4_E), 75.9 (C-2_B), 75.6, 75.0, 74.9, 73.9, 72.9 (CH_2Ph), 72.4 (C-2_C), 71.9 (C-4_A), 71.2 (C-5_E), 70.9 (CH_2Ph), 70.7 (C-2_A), 70.0 (C-3_A), 69.2 (C-5_B), 68.5 (OCH_2), 68.1 (C-6_E), 67.6 (C-5_C), 67.2 (C-5_A), 18.8 (C-6_A), 18.1 (C-6_C), 17.8 (C-6_B). FAB-MS for $C_{97}H_{98}O_{22}$ (1614) *m/z* 1637 $[M + Na]^+$. Anal. Calcd. for $C_{97}H_{98}O_{22}\cdot H_2O$: C, 71.31; H, 6.17%. Found: C, 71.35; H, 6.21%.

(2,3,4-Tri-*O*-benzoyl-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/β-L-rhamnopyranose (34)

1,5-Cyclooctadiene-bis(methyldiphenylphosphine)iridium hexafluorophosphate (12.5 mg) was dissolved in THF (5 mL) and the resulting red solution was degassed in an argon stream. Hydrogen was then bubbled through the solution, causing the colour to change to yellow. The solution was then degassed again in an argon stream. A solution of **33** (1.14 g, 0.70 mmol) in THF (15 mL) was degassed and added. The mixture was stirred at rt overnight. The mixture was concentrated. The residue was taken up in acetone (7 mL) and water (0.7 mL). Mercuric chloride (285 mg, 1.05 mmol) and mercuric oxide (303 mg, 1.4 mmol) were added to the mixture, which was protected from light. The mixture was stirred at rt for 1 h, then concentrated. The residue was taken up in CH_2Cl_2 and washed three times with sat. aq. KI, then with brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (solvent B, 7 : 3) to give **34** (992 mg, 90%) as a colourless foam. ^{1}H NMR (α anomer): δ 8.16–7.05 (m, 50H, Ph), 5.97–5.88 (m, 2H, H-2_A, 3_A), 5.74 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4_A), 5.56 (br s, 1H, H-2_C), 5.35 (br s, 1H, H-1_A), 5.29 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1_C), 5.18 (br s, 1H, H-1_B), 5.01 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1_E), 4.99–4.78 (m, 6H, CH_2Ph), 4.68 (d, 1H, CH_2Ph), 4.61 (br s, 1H, H-2_B), 4.58–4.47 (m, 5H, H-5_A, CH_2Ph), 4.38 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.24 (br d, 1H, $J_{3,4}$ = 8.5 Hz, H-3_C), 4.09–3.99 (m, 3H, H-3_E, 5_C, 5_E), 3.86 (pt, 1H, $J_{3,4} = J_{4,5}$ = 8.9 Hz, H-4_C), 3.80–3.60 (m, 6H, H-3_B, 4_E, 4_B, 5_B, $6a_E$, $6b_E$), 3.54 (dd, 1H, H-2_E), 3.17 (d, 1H, OH), 1.46 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_A), 1.42 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_C), 1.16 (d, 3H, $J_{5,6}$ = 5.7 Hz, H-6_B); ^{13}C NMR (α anomer): δ 166.0, 165.6, 165.2 (C=O), 138.9–127.2 (Ph), 101.1 (br s, C-1_B), 99.7 (C-1_A), 98.1 (C-1_E), 91.6 (C-1_C), 81.9 (C-3_E), 81.1 (C-2_E), 79.9 (C-4_B), 79.4 (br s, C-3_C), 78.9 (C-3_B), 78.3 (br s, C-4_C), 77.6 (C-4_E), 76.1 (C-2_B), 75.8, 75.3, 75.1, 74.0, 73.1 (5C, CH_2Ph), 72.7 (C-2_C), 72.1 (C-4_A), 71.4 (C-5_E), 71.1 (CH_2Ph), 70.8 (C-2_A), 70.2 (C-3_A), 69.4 (C-5_B), 68.3 (C-6_E), 67.7 (C-5_C), 67.3 (C-5_A), 19.0 (C-6_A), 18.2 (C-6_C), 17.9 (C-6_B). FAB-MS for $C_{94}H_{94}O_{22}$ (M, 1574) *m/z* 1597 $[M + Na]^+$. Anal. Calcd. for $C_{94}H_{94}O_{22}$: C, 71.65; H, 6.01%. Found: C, 71.48; H, 6.17%.

(2,3,4-Tri-*O*-benzoyl-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/β-L-rhamnopyranosyl trichloroacetimidate (6)

The hemiacetal **34** (412 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (5 mL) and the solution was cooled to 0 °C. Trichloroacetonitrile (0.26 mL) was added, then DBU (4 μL). The mixture was stirred at 0 °C for 1 h. The mixture was concentrated and toluene was co-evaporated from the residue. The residue was purified by flash chromatography (solvent B, 4 : 1 + 0.1% Et_3N) to give **6** (393 mg, 88%). ^{1}H NMR (α anomer): δ 8.75 (s, 1H, NH), 8.10–7.03 (m, 50H, Ph), 6.42 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1_C), 5.90 (m, 2H, H-2_A, 3_A), 5.74 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4_A), 5.59 (br s, 1H, H-2_C), 5.32 (br s, 1H, H-1_A), 5.15 (br s, 1H, H-1_B), 5.09 (d, 1H, $J_{1,2}$ = 2.7 Hz, H-1_E), 5.04–4.79 (m, 6H, CH_2Ph), 4.70–4.42 (m, 7H, H-2_B, 5_A, CH_2Ph), 4.32 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.24 (m, 1H, H-3_C), 4.09–3.96 (m, 3H, H-3_E, 5_B, 5_E), 3.91 (m, 1H, H-4_C), 3.74–3.57 (m, 6H, H-3_B, 4_B, 4_E, 5_C, $6a_E$, $6b_E$), 3.52 (dd, 1H, $J_{1,2}$ = 3.4, $J_{2,3}$ = 9.8 Hz, H-2_E), 1.42 (d, 3H, $J_{5,6}$ = 6.0 Hz, H-6_C 1.39 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_A), 1.10 (d, 3H, $J_{5,6}$ = 4.3 Hz, H-6_B); ^{13}C NMR (α anomer): δ 166.3, 165.9, 165.8, 165.5 (C=O), 160.5 (C=NH), 138.7–127.2 (Ph), 101.2 (C-1_B), 99.7 (C-1_A), 98.3 (C-1_E), 94.3 (C-1_C), 90.9 (CCl_3), 82.2 (C-3_E), 81.3 (br s, C-2_E), 80.0 (2C, C-3_C, 4_B), 79.0 (C-3_B), 77.9 (C-4_E), 77.5 (br s, C-4_C), 76.4 (br s, C-2_B), 76.0, 75.5, 75.4, 74.4, 73.3 (5C, CH_2Ph), 72.2 (C-4_A), 71.7 (C-5_E), 71.3 (CH_2Ph), 71.1 (C-2_A*), 71.0 (C-2_C*), 70.7 (br s, C-5_C*), 70.4 (C-3_A), 69.8 (br s, C-5_B*), 68.4 (C-6_E), 67.6 (C-5_A), 19.1 (br s, C-6_C), 18.2 (C-6_A), 18.1 (C-6_B). Anal. Calcd. for $C_{96}H_{94}Cl_3NO_{22}\cdot H_2O$: C, 66.34; H, 5.57; N, 0.81%. Found: C, 66.26; H, 5.72; N, 0.94%.

2-Azidoethyl (2,3,4-tri-*O*-benzoyl-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 (35)

(a) The tetrasaccharide donor **6** (500 mg, 0.29 mmol) and the acceptor **7** (140 mg, 0.42 mmol) were dissolved in 1,2-DCE (5 mL) and 4 Å-MS (400 mg) were added. The mixture was stirred at rt for 2 h. The mixture was cooled to 0 °C and TfOH (7 μL, 72 μmol) was added. The mixture was stirred at 0 °C to rt over 1 h 30 min. The mixture was then heated at 65 °C for 1 h 30 min. The mixture was allowed to cool, Et_3N (0.5 mL) was added, and the mixture was stirred at rt for 20 min. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 4 : 3) to give **35** (340 mg, 62%).

(b) The tetrasaccharide donor **6** (250 mg, 145 μmol) and the acceptor **7** (67 mg, 204 μmol) were dissolved in CH_2Cl_2 (1.5 mL) and 4 Å-MS (200 mg) were added. The mixture was stirred at −40 °C for 30 min and TMSOTf (5 μL) was added. The mixture was stirred at rt over 3 h, triethylamine was added, and

the mixture was stirred at rt for 15 min. The mixture was diluted with CH_2Cl_2 and filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (solvent B, 9 : 1⟶1 : 1) to give **35** (219 mg, 80%). $[\alpha]_D$ +64.0 (*c* 1); ^{1}H NMR: δ 8.14–7.10 (m, 50H, Ph), 6.31 (d, 1H, $J_{NH,2}$ = 7.6 Hz, NH), 6.01 (dd, 1H, $J_{1,2}$ = 1.6 Hz, H-2_A), 5.98 (dd, 1H, $J_{2,3}$ = 3.3 Hz, H-3_A), 5.79 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, H-4_A), 5.47 (dd, 1H, $J_{1,2}$ = 1.6, $J_{2,3}$ = 2.0 Hz, H-2_C), 5.41 (br s, 1H, H-1_A), 5.24 (m, 1H, H-1_B), 5.21 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1_E), 5.10 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1_D), 5.05 (br s, 1H, H-1_C), 5.04–4.81 (m, 4H, CH_2Ph), 4.73–4.68 (m, 2H, CH_2Ph), 4.65 (br s, 1H, H-2_B), 4.62–4.42 (m, 8H, CH_2Ph), 4.55 (m, 1H, H-5_A), 4.39 (pt, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3_D), 4.23–4.12 (m, 3H, H-3_C, 5_C, 5_E), 4.08 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-3_E), 4.02–3.97 (m, 2H, H-$6a_D$, CH_2O), 3.91 (pt, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4_C), 3.85–3.66 (m, 8H, H-3_B, 4_B, 4_E, 5_B, $6b_D$, $6a_E$, $6b_E$, CH_2O), 3.63 (pt, 1H, $J_{4,5}$ = 9.3 Hz, H-4_D), 3.59 (dd, 1H, $J_{2,3}$ = 9.7 Hz, H-2_E), 3.47 (m, 1H, H-5_D), 3.41–3.33 (m, 2H, H-2_D, CH_2N_3), 3.16 (m, 1H, CH_2N_3), 2.21 (s, 3H, CH_3CO), 1.53–1.47 (m, 9H, H-6_A, $(CH_3)_2C$), 1.39 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6_C), 1.19 (d, 3H, $J_{5,6}$ = 5.Hz, H-6_B); ^{13}C NMR: δ 172.3, 166.4, 166.3, 165.9, 165.5 (C=O), 139.3–127.5 (Ph), 101.8 (br s, C-1_B), 100.3 (C-1_D), 100.0 (C-1_A), 99.9 ($C(CH_3)_2$), 98.0 (2C, C-1_C, 1_E), 82.1 (C-3_E), 81.5 (C-2_E), 80.4 (br s, C-3_C), 80.2 (C-4_E*), 79.4 (C-4_B*), 78.0 (C-3_B), 77.9 (br s, C-4_C), 76.6 (C-3_D), 76.4 (C-2_B), 76.0, 75.4, 75.3, 74.2, 73.5 (5C, CH_2Ph), 73.4 (C-4_D*), 73.2 (C-2_C), 72.3 (C-4_A), 71.8 (C-2_A), 71.5 (CH_2Ph), 71.2 (C-5_E), 70.5 (C-3_A), 69.7 (C-5_B), 69.1 (OCH_2), 68.8 (C-6_E), 67.9 (C-5_C), 67.7 (C-5_A), 67.6 (C-5_D), 62.7 (C-6_D), 59.2 (C-2_D), 51.0 (CH_2N_3), 29.5 (C(CH_3)$_2$), 24.0 (CH_3CO), 19.2 (C(CH_3)$_2$), 19.1 (C-6_A), 18.5 (C-6_C), 18.2 (C-6_B). FAB-MS for $C_{107}H_{114}N_4O_{27}$ (M, 1886) *m/z* 1909 [M + Na]$^+$. Anal. Calcd. for $C_{107}H_{114}N_4O_{27}$: C, 68.07, H, 6.09; N, 2.97%. Found: C, 68.18, H, 6.07; N, 2.79%.

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-glucopyranoside (37)

An ice cold solution of 50% aq. TFA (2.1 mL) in CH_2Cl_2 (8 mL) was added to the pentasaccharide **35** (283 mg, 0.15 mmol). The mixture was kept at 0 °C for 2 h, then diluted with toluene and concentrated. Toluene was co-evaporated from the residue. Chromatography of the residue (solvent B, 7 : 3⟶1 : 1) gave the intermediate diol (265 mg, 96%). The latter (265 mg) was dissolved in MeOH (6 mL), and a 1% solution of methanolic sodium methoxide (4.0 mL) was added. The mixture was stirred at 55 °C for 2 h, then neutralised with Dowex X8 (H^+) resin, and filtered. The filtrate was concentrated. The mixture was purified by column chromatography (solvent A, 100 : 0 1⟶95 : 5) to give **36** (195 mg, 87%) as a colourless foam, whose structure was confirmed from mass spectrometry analysis (FAB-MS for $C_{76}H_{94}N_4O_{23}$ (M, 1430) *m/z* 1453 [M + Na]$^+$). Pentasaccharide **36** (171 mg, 0.11 mmol) was dissolved in EtOH (18 mL). A 1 M solution of aq. HCl (210 µL) was added. The mixture was stirred under hydrogen in the presence of 10% Pd/C (96 mg) for 2 h. The mixture was diluted with EtOH and water, then filtered through a pad of Celite. The filtrate was concentrated and preliminarily purified by passing through a column of C_{18} silica (eluting with water). The residue was purified by RP-HPLC to give, after lyophilization, **37** (50 mg, 53%). HPLC (215 nm): Rt 5.87 min (Kromasil 5 µm C18 100 Å 4.6 × 250 mm analytical column, using a 0–20% linear gradient over 20 min of CH_3CN in 0.01 M aq. TFA at 1 mL min^{-1} flow rate). ^{1}H NMR (D_2O): δ 5.15 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1_E), 5.00 (br s, 1H, H-1_A), 4.92 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1_B), 4.76 (br s, 1H, H-1_C), 4.53 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1_D), 4.10 (m, 1H, H-5_C), 4.03 (m, 2H, H-2_A, 2_B), 4.01 (m, 3H, H-4_A, 4_B, CH_2O), 3.88–3.38 (m, 7H, H-2_C, 2_D, 3_A, $6a_D$, $6b_D$, $6a_E$, CH_2O), 3.69–3.76 (m, 7H, H-3_B, 3_C, 3_E, 4_C, 5_A, 5_B, $6b_E$), 3.52 (pt, 1H, H-3_D), 3.33–3.54 (m, 5H, H-2_E, 4_D, 4_E, 5_D, 5_E), 3.09 (m, 2H, CH_2NH_2), 1.98 (s, 3H, CH_3CO), 1.28 (d, 3H, H-6_C), 1.22 (m, 6H, H-6_A, 6_B); ^{13}C NMR (D_2O): δ 175.3 (C=O), 102.9 (C-1_A), 101.7 (br s, C-1_B), 101.4 (C-1_C), 100.9 (C-1_D), 97.9 (br s, C-1_E), 81.8 (C-3_D), 79.8 (C-2_B), 79.4 (br s, C-3_C), 76.3 (C-2_E), 75.1 (br s, C-4_C), 72.9, 72.4, 72.4, 72.2, 71.7, 71.2 (br s), 70.5 (2C), 70.4, 70.1, 70.0, 69.7, 69.6, 69.4, 68.7, 66.7 (CH_2O), 61.0 (2C, C-6_D, 6_E), 55.5 (C-2_D), 39.9 (CH_2NH_2), 22.6 (CH_3C=O), 18.2 (C-6_C), 17.2 (C-6_A), 17.0 (C-6_B). HRMS (MALDI) Calcd for $C_{34}H_{60}N_2O_{23}$ + H: 865.3665. Found: 865.3499.

General procedure for the preparation of targets 1, 2 and 3

The oligosaccharide bearing a masked thiol group (5.8 µmol) was dissolved in water (500 µL) and added to a solution of **8** (13 mg, 7.4 µmol) in a mixture of water (1 mL), CH_3CN (200 µL) and 0.5 M phosphate buffer (pH 5.7, 1.2 mL). 117 µL of a solution of hydroxylamine hydrochloride (139 mg mL^{-1}) in 0.5 M phosphate buffer (pH 5.7) was added, and the mixture was stirred for 1 h. RP-HPLC purification gave the pure neoglycopeptide

PADRE-lys-(thiomethyl)carbonylaminoethyl α-D-glucopyranosyl-(1⟶4)-α-L-rhamnopyranosyl-(1⟶3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

62% from **38**. HPLC (230 nm): Rt 10.40 min (100% pure, Kromasil 5 µm C18 100 Å 4.6 × 250 mm analytical column, using a 0–20% linear gradient over 20 min of CH_3CN in 0.01 M aq. TFA at 1 mL min^{-1} flow rate). ES-MS Calcd for $C_{109}H_{181}N_{23}O_{35}S$: 2405.85. Found: 2405.52.

PADRE-lys-(thiomethyl)carbonylaminoethyl α-L-rhamnopyranosyl-(1⟶3)-[α-D-glucopyranosyl)-(1⟶4)]-α-L-rhamnopyranosyl-(1⟶3)-2-acetamido-2-deoxy-β-D-glucopyranoside (2)

48% from **39**. HPLC (230 nm): Rt 11.60 min (100% pure, Kromasil 5 µm C18 100 Å 4.6 × 250 mm analytical column, using a 20–50% linear gradient over 20 min of CH_3CN in 0.01 M aq. TFA at 1 mL min^{-1} flow rate). ES-MS Calcd for $C_{125}H_{191}N_{23}O_{39}S$: 2552.99. Found: 2551.90.

PADRE-lys-(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-glucopyranoside (3)

46% from **40**. HPLC (230 nm): Rt 10.33 min (100% pure, Kromasil 5 µm C18 100 Å 4.6 × 250 mm analytical column, using a 20–50% linear gradient over 20 min of CH_3CN in 0.01 M aq. TFA at 1 mL min^{-1} flow rate). ES-MS Calcd for $C_{121}H_{201}N_{23}O_{43}S$: 2698.14. Found: 2698.09.

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References

1 Part 12 of the series: Synthesis of ligands related to the O-specific polysaccharides of *Shigella flexneri* serotype 2a and *Shigella flexneri* serotype 5a. For part 11, see ref. 47.
2 R. Shields and W. Burnett, *Zentralbl. Bakteriol.*, 1898, **24**, 817–828.
3 T. G. Keusch and M. L. Bennish, *Shigellosis*, Plenum Medical Book Company, New York, 1991, pp. 593–620.
4 K. L. Kotloff, J. P. Winickoff, B. Ivanoff, J. D. Clemens, D. L. Swerdlow, P. J. Sansonetti, G. K. Adak and M. M. Levine, *Bull. WHO*, 1999, **77**, 651–666.

5 M. U. Khan, *Int. J. Epidemiol.*, 1985, **14**, 607–613.
6 M. A. Salam and M. L. Bennish, *Rev. Infect. Dis.*, 1991, **13**, S332–S341.
7 S. Ashkenazi, M. May-Zahav, J. Sulkes and Z. Samra, *Antimicrob. Agents Chemother.*, 1995, **39**, 819–823.
8 E. R. Iversen, H. Colding, L. Petersen, R. Ngetich and G. D. Shanks, *Trans. R. Soc. Trop. Med. Hyg.*, 1998, **92**, 30–31.
9 B. A. Iwalokun, G. O. Gbenle, S. I. Smith, A. Ogunledun, K. A. Akinsinde and E. A. Omonigbehin, *J. Health Popul. Nutr.*, 2001, **19**, 183–190.
10 World, Health and Organization, *Bull. W.H.O.*, 1987, **65**, 17–25.
11 WHO, *Wkly. Epidemiol. Rec.*, 1997, **72**, 73–79.
12 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 and T. L. Hale, *Infect. Immun.*, 1999, **67**, 3437–3443.
13 L. F. Fries, A. D. Montemarano, C. P. Mallett, D. N. Taylor, T. L. Hale and G. H. Lowell, *Infect. Immun.*, 2001, **69**, 4545–4553.
14 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 and R. Schneerson, *Infect. Immun.*, 2001, **69**, 1351–1357.
15 D. Cohen, M. S. Green, C. Block, T. Rouach and I. Ofek, *J. Infect. Dis.*, 1988, **157**, 1068–1071.
16 D. Cohen, M. S. Green, C. Block, R. Slepon and I. Ofek, *J. Clin. Microbiol.*, 1991, **29**, 386–389.
17 A. Phalipon, M. Kauffmann, P. Michetti, J.-M. Cavaillon, M. Huerre, P. Sansonetti and J.-P. Krahenbuhl, *J. Exp. Med.*, 1995, **182**, 769–778.
18 J. B. Robbins, C. Chu and R. Schneerson, *Clin. Infect. Dis.*, 1992, **15**, 346–361.
19 D. N. Taylor, A. C. Trofa, J. Sadoff, C. Chu, D. Bryla, J. Shiloach, D. Cohen, S. Ashkenazi, Y. Lerman, W. Egan, R. Schneerson and J. B. Robbins, *Infect. Immun.*, 1993, **61**, 3678–3687.
20 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 and J. B. Robbins, *The Lancet*, 1997, **349**, 155–159.
21 R. W. Ellis and D. M. Granoff, *Development and clinical use of Haemophilus b conjugate vaccines*, M. Dekker, New York, 1994.
22 P. Richmond, R. Borrow, E. Miller, S. Clark, F. Sadler, A. Fox, N. Begg, R. Morris and K. Cartwright, *J. Infect. Dis.*, 1999, **179**, 1569–1572.
23 M. B. Rennels, K. M. Edwards, H. L. Keyserling, K. S. Reisinger, D. A. Hogerman, D. V. Madore, I. Chang, P. R. Paradiso, F. J. Malinoski and A. Kimura, *Pediatrics*, 1998, **101**, 604–611.
24 V. Pozsgay, C. Chu, L. Panell, J. Wolfe, J. B. Robbins and R. Schneerson, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 5194–5197.
25 B. Benaissa-Trouw, D. J. Lefeber, J. P. Kamerling, J. F. G. Vliegenthart, K. Kraaijeveld and H. Snippe, *Infect. Immun.*, 2001, **69**, 4698–4701.
26 F. Mawas, J. Niggemann, C. Jones, M. J. Corbet, J. P. Kamerling and J. F. G. Vliegenthart, *Infect. Immun.*, 2002, **70**, 5107–5114.
27 J. B. Robbins, R. Schneerson, S. C. Szu and V. Pozsgay in *Polysaccharide-protein conjugate vaccines*, ed. S. Plotkin and B. Fantini, Elsevier, Paris, 1996, pp. 135–143.
28 G. J. P. H. Boons, P. Hoogerhout, J. T. Poolman, G. A. van der Marel and J. H. van Boom, *Bioorg. Med. Chem.*, 1991, **1**, 303–308.
29 E. Lett, S. Gangloff, M. Zimmermann, D. Wachsmann and J.-P. Klein, *Infect. Immun.*, 1994, **62**, 785–792.
30 A. Kandil, N. Chan, M. Klein and P. Chong, *Glycoconjugate J.*, 1997, **14**, 13–17.
31 T. Barington, M. Skettrup, L. Juul and C. Heilmann, *Infect. Immun.*, 1993, **61**, 432–438.
32 T. A. Barington, K. Gyhrs, K. Kristensen and C. Heilman, *Infect. Immun.*, 1994, **62**, 9–14.
33 C. C. A. M. Peeters, A.-M. Tenbergen-Meekes, J. T. Poolman, M. Beurret, B. J. M. Zegers and G. T. Rijkers, *Infect. Immun.*, 1991, **59**, 3504–3510.
34 M.-P. Schutze, C. Leclerc, M. Jolivet, F. Audibert and L. Chedid, *J. Immunol.*, 1985, **135**, 2319–2322.
35 P. R. Paradiso, K. Dermody and S. Pillai, *Vaccine Res.*, 1993, **2**, 239–248.
36 D. Valmori, A. Pessi, E. Bianchi and G. P. Corradin, *J. Immunol.*, 1992, **149**, 717–721.
37 J. Alexander, J. Sidney, S. Southwood, J. Ruppert, C. Oseroff, A. Maewal, K. Snoke, H. M. Serra, R. T. Kubo, A. Sette and H. M. Grey, *Immunity*, 1994, **1**, 751–761.
38 J. Alexander, A.-F. del Guercio, A. Maewal, L. Qiao, J. Fikes, R. W. Chesnut, J. Paulson, D. R. Bundle, S. DeFrees and A. Sette, *J. Immunol.*, 2000, **164**, 1625–1633.
39 S. Vichier-Guerre, R. Lo-Man, L. BenMohamed, E. Dériaud, S. Kovats, C. Leclerc and S. Bay, *J. Peptide Res.*, 2003, **62**, 117–124.
40 D. A. R. Simmons, *Bacteriol. Rev.*, 1971, **35**, 117–148.
41 A. A. Lindberg, A. Karnell and A. Weintraub, *Rev. Infect. Dis.*, 1991, **13**, S279–S284.
42 J. M. Berry and G. G. S. Dutton, *Can. J. Chem.*, 1974, **54**, 681–683.
43 G. M. Lipkind, A. S. Shashkov, A. V. Nikolaev, S. S. Mamyan and N. K. Kochetkov, *Bioorg. Khim.*, 1987, **13**, 1081–1092.
44 C. Costachel, P. J. Sansonetti and L. A. Mulard, *J. Carbohydr. Chem.*, 2000, **19**, 1131–1150.
45 L. A. Mulard, C. Costachel and P. J. Sansonetti, *J. Carbohydr. Chem.*, 2000, **19**, 849–877.
46 F. Segat and L. A. Mulard, *Tetrahedron: Asymmetry*, 2002, **13**, 2211–2222.
47 L. A. Mulard and C. Guerreiro, *Tetrahedron*, 2004, **60**, 2475–2488.
48 F. Bélot, C. Costachel, K. Wright, A. Phalipon and L. A. Mulard, *Tetrahedron. Lett.*, 2002, **43**, 8215–8218.
49 F. Bélot, K. Wright, C. Costachel, A. Phalipon and L. A. Mulard, *J. Org. Chem.*, 2004, **69**, 1060–1074.
50 A. Phalipon, C. Costachel, A. Thuizat, F. Nato, B. Vulliez-Le Normand, V. Marcel-Peyre, G. Bentley, P. J. Sansonetti and L. A. Mulard, *in preparation*.
51 R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21–123.
52 R. R. Schmidt and J. Michel, *Tetrahedron Lett.*, 1984, **25**, 821–824.
53 R. R. Schmidt, J. Michel and M. Roos, *Liebigs Ann. Chem.*, 1984, 1343–1357.
54 R. Gigg, S. Payne and R. Conant, *J. Carbohydr. Chem.*, 1983, **2**, 207–223.
55 T. Eklind, R. Gustafsson, A.-K. Tidén, T. Norberg and P.-M. Åberg, *J. Carbohydr. Chem.*, 1996, **15**, 1161–1174.
56 A. Chernyak, G. Sharma, L. Konokov, P. Krishna, A. Levinsky, N. Kochetkov and A. R. Rao, *Carbohydr. Res.*, 1992, **223**, 303–309.
57 R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, 1975, **97**, 4063–4069.
58 W. Stahl, U. Sprengard, G. Kretschmar and H. Kunz, *Angew. Chem., Int. Ed.*, 1994, **33**, 2096–2098.
59 N. M. Spikjer, C. A. Keuning and M. Hooglugt, *Tetrahedron*, 1996, **52**, 5945–5960.
60 Q. Li, H. Li, Q.-H. Lou, B. Su, M.-S. Cai and Z.-J. Li, *Carbohydr. Res.*, 2002, **337**, 1929–1934.
61 I. Kitagawa, N. I. Baek, K. Ohashi, M. Sakagami, M. Yoshikawa and H. Shibuya, *Chem. Pharm. Bull.*, 1989, **37**, 1131–1133.
62 J. J. Oltvoort, C. A. A. van Boeckel, J. H. der Koning and J. H. van Boom, *Synthesis*, 1981, 305–308.
63 R. Gigg and C. D. Warren, *J. Chem. Soc. C*, 1968, 1903–1911.
64 P. Westerduin, P. E. der Haan, M. J. Dees and J. H. van Boom, *Carbohydr. Res.*, 1988, **180**, 195–205.
65 T. Ziegler, F. Bien and C. Jurish, *Tetrahedron: Asymmetry*, 1998, **9**, 765–780.
66 K. B. Reimer, S. L. Harris, V. Varma and B. M. Pinto, *Carbohydr. Res.*, 1992, **228**, 399–414.
67 J. Zhang, J. M. Mao, H. M. Chen and M. S. Cai, *Tetrahedron: Asymmetry*, 1994, **5**, 2283–2290.
68 J. C. Castro-Palomino, M. H. Rensoli and V. Verez Bencomo, *J. Carbohydr. Chem.*, 1996, **15**, 137–146.
69 D. H. Marrian, *J. Chem. Soc. C*, 1949, 1515–1516.
70 G. T. Hermanson, *Bioconjugate techniques*, Academic Press, New York, 1996, p. 148.
71 G. Ragupathi, R. R. Koganty, D. Qiu, K. O. Llyod and P. O. Livingston, *Glycoconjugate J.*, 1998, **15**, 217–221.
72 I. Shin, H. Jung and M. Lee, *Tetrahedron Lett.*, 2001, **42**, 1325–1328.
73 J. M. Peeters, T. G. Hazendonk, E. C. Beuvery and G. I. Tesser, *J. Immunol. Methods*, 1989, **120**, 133–143.
74 W. C. Chan and P. D. White, *Fmoc solid phase peptide synthesis*, Oxford University Press, New York, 2000.
75 S. R. Chhabra, B. Hothi, D. J. Evans, P. D. White, B. W. Bycroft and W. C. Chan, *Tetrahedron Lett.*, 1998, **39**, 1603–1606.
76 H. F. Brugghe, H. A. M. Timmermans, L. M. A. van Unen, G. J. T. Hove, G. W. der. Werken, J. T. Poolman and P. Hoogerhout, *Int. J. Peptide Protein Res.*, 1994, **43**, 166–172.