(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 February 2013 (07.02.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/017254 A1

(51) International Patent Classification:
C07H 3/06 (2006.01) *C07H 15/20* (2006.01)
C07H 3/08 (2006.01) *A61K 31/7032* (2006.01)
C07H 13/08 (2006.01) *A61P 31/04* (2006.01)
C07H 15/04 (2006.01) *A61P 1/12* (2006.01)

(21) International Application Number:
PCT/EP2012/003240

(22) International Filing Date:
30 July 2012 (30.07.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11006355.9 2 August 2011 (02.08.2011) EP
61/514, 095 2 August 2011 (02.08.2011) US

(71) Applicant *(for all designated States except US)*: **MAX-PLANCK-GESELLSCHAFT ZUR FOERDERUNG DER WISSENSCHAFTEN E.V** [DE/DE]; Hofgartenstrasse 8, 80539 München (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SEEBERGER, Peter H.** [DE/DE]; Rudolf-Breitscheid-Strasse 46, 14532 Berlin (DE). **MARTIN, Christopher E.** [DE/DE]; Blissestrasse 27, 10713 Berlin (DE). **BROECKER, Felix** [DE/DE]; Regensburger Str. 15, 10777 Berlin (DE). **ANISH, Chakkumkal** [IN/DE]; Dilgesstrasse 26, 12249 Berlin (DE).

(74) Agent: **KATZAMEYER, Michael**; V. Bezold & Partner, Akademiestraße 7, 80799 München (DE).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) **Title**: OLIGOSACCHARIDES AND OLIGOSACCHARIDE-PROTEIN CONJUGATES DERIVED FROM CLOSTRIDIUM DIFFICILE POLYSACCARIDE PS-I, METHODS OF SYNTHESIS AND USES THEREOF, IN PARTICULAR AS VACCINES AND DIAGNOSTIC TOOLS

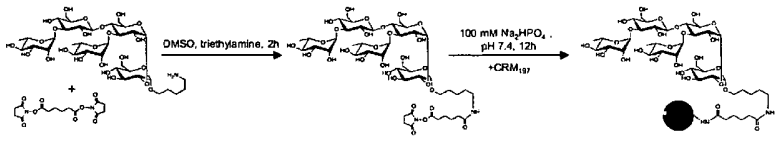


Fig. 3

(57) **Abstract**: The invention relates to a synthetic oligosaccharide representing part of the repeating unit of the Clostridium difficile glycopolymer PS-I and having the sequence of the pentasaccharide a-L-Rhap- (1→3) -β-D-Glcp- (1→4) - [a-L-Rhap- (1→3] -a-D-Glcp- (1→2) -a-D-Glcp or a synthetic fragment or derivative thereof. Preferably, the claimed synthetic oligosaccharide bears at least one linker L for conjugation to a carrier protein or for immobilization on a surface. Further aspects of the invention relate to advantageous methods for synthesizing said synthetic oligosaccharide and oligosaccharide-protein conjugate as well as to uses thereof, in particular as vaccines and diagnostic tools.

WO 2013/017254 A1

Oligosaccharides and Oligosaccharide-protein conjugates derived from *Clostridium difficile* polysaccharide PS-I, methods of synthesis and uses thereof, in particular as vaccines and diagnostic tools

Background

Clostridium difficile is a Gram-positive, spore forming anaerobic bacterium that colonizes the intestinal tract of humans thus leading to *C. difficile* infections (CDI). CDI has become the most commonly diagnosed cause of hospital-acquired diarrhea, particularly in the risk groups including elderly and immunodeficient patients as well as those receiving antibiotic treatment. A steep rise in CDI incidents over the past decade is attributed to the emergence of the hypervirulent, and now predominant strain ribotype 27, causing epidemic outbreaks with increased morbidity, mortality and high relapse rates. The costs to treat patients have greatly increased, particularly in the case of recurring CDI. Preventive methods, such as vaccination of risk groups, may be useful and cost-efficient means to avoid future infections. Although vaccination against *C. difficile* should be economically feasible (B. Y. Lee et al., *Vaccine*, 2010, **28**, 5245) a vaccine has not yet been developed.

Carbohydrates exposed on the cell-surface of pathogens are often immunogenic and constitute potential candidates for vaccine development. When covalently connected to carrier proteins, carbohydrate antigen vaccines can elicit a long lasting T-cell dependent protection (C. Snapper and J. Mond, *J. Immunol.*, 1996, **157**, 2229). Several vaccines containing carbohydrates, isolated from biological sources, are in routine use (G. Ada and D. Isaacs, *Clin. Microbiol. Infect.*, 2003, **9**, 79). Vaccines based on synthetic carbohydrate

antigens against bacteria, viruses, parasites and cancer are currently in preclinical and clinical development (a) R. D. Astronomo and D. R. Burton, *Nature Rev.*, 2010, **9**, 308; b) M.-L. Hecht, P. Stallforth, D. V. Silva, A. Adibekian and P. H. Seeberger, *Curr. Opin. Chem. Biol.*, 2009, **13**, 354).

The chemical structure of two *C. difficile* cell-surface polysaccharides, PS-I and PS-II has been elucidated recently (J. Ganeshapillai et al., *Carbohydr. Res.*, 2008, **343**, 703; WO 2009/033268 A1). Initial focus has been turned towards the PS-II hexasaccharide antigen that is believed to be common to several *C. difficile* strains (a) E. Danieli et al., *Org. Lett.*, 2010, **13**, 378; b) M. Oberli, M.-L. Hecht, P. Bindschädler, A. Adibekian, T. Adam and P. H. Seeberger, *Chem. Biol.*, 2011, **18**, 580). The synthetic PS-II hapten is immunogenic when conjugated to a carrier protein and antibodies found in the stool of *C. difficile* patients bind to the synthetic PS-II hexasaccharide (Oberli et al., ibid.). The pentasaccharide phosphate repeating unit PS-I was reported as [→4)-α-Rha*p*-(1→3)-β-Glc*p*-(1→4)-[α-Rha*p*-(1→3)]-α-Glc*p*-(1→2)-α-Glc*p*-(1→P] and it is suggested to be specific for the strain ribotype 27.

In conclusion, the pathogen *C. difficile* represents a major risk for patients and causes significant costs to health care systems. Unfortunately, however, currently no licensed vaccine against *C. difficile* is available.

Thus, a main object of the present invention is to provide novel and effective means to prevent and/or to treat *C. difficile* associated diseases, in particular related to the hypervirulent strain ribotype 027. A further object is to provide novel and effective means to detect *C. difficile* in a sample and/or a *C. difficile* infection in a subject. A further object is to provide novel and effective means to identify a

certain strain of *C. difficile* in a sample and/or a *C. difficile* infected subject. A further object is to provide novel and effective standards for immunoassays for the detection of *C. difficile*.

The present inventors succeeded in the first total synthesis of a pentasaccharide derived from the repeating unit of the *C. difficile* polysaccharide PS-I, and its conjugation to the diphtheria toxoid Crm_{197}.

Consequently, the above main object of the invention is achieved by providing the synthetic oligosaccharide, in particular pentasaccharide, according to claim 1, the oligosaccharide-protein conjugate according to claim 7 and the composition according to claim 9. Further objects are achieved by providing the antibody of claim 11, the methods of detection and identification according to claims 18-19, and the methods of synthesis according to claims 15-17 and 26-27. Preferred embodiments and other aspects of the invention are the subject of further claims.

Description of the invention

The present invention provides an oligosaccharide, in particular synthetic oligosaccharide, derived from the repeating unit of the *Clostridium difficile* glycopolymer PS-I and an oligosaccharide-protein conjugate comprising said oligosaccharide coupled to a protein carrier.

More specifically, the oligosaccharide is the pentasaccharide having the sequence α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3]-α-D-Glc*p*-(1→2)-α-D-Glc*p* or a (synthetic) fragment or derivative thereof.

The term "derivative" as used herein means generally any structurally related molecule having the same scaffold as the

basic molecule but which is modified by the addition, deletion or substitution of one or more functional groups. For example, the "oligosaccharide derivative" as used herein may be obtained by replacement of one or more of the hydroxyl groups by other functional groups or atoms or by introducing additional substituents such as linker groups.

The term "fragment" as used herein includes tetra-, tri-, di- and monosaccharides which are constituting units of the pentasaccharide having the sequence α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3]-α-D-Glc*p*-(1→2)-α-D-Glc*p* from above or from a derivative thereof, in particular a derivative comprising one or more linker group(s).

Preferably, the oligosaccharide bears at least one linker L for conjugation to a carrier protein or for immobilization on a surface.

The linker or spacer group L may be any moiety that enables to couple the oligosaccharide to a carrier molecule or to the surface of a microarray. A large variety of such linker groups are known in the art and a suitable linker group can be selected in dependence from the respective carrier molecule or surface group. For example, L may be an aliphatic or aromatic residue, e.g. an alkyl(en) group or phenyl(en) group, comprising a reactive functional group, such as an amino group, preferably a primary amino group, (activated) carboxy group, aldehyde, azide, alkenyl or alkinyl group. In specific embodiments L may comprise a polyether or polyester chain. In particular, L is selected from the group comprising primary alkylamines, alkyl or aralkyl residues with a terminal aldehyde, azide, alkine or alkene group or (activated) carboxy group, and alkylaryl and aryl residues, e.g. phenyl residues, comprising a reactive amine, aldehyde or azide group, or (activated) carboxy group.

In a specific embodiment of the invention, L is $(CH_2)_nNH_2$, with n being an integer from 2 to 50, preferably 3 to 20 or 3 to 10, such as 4 to 8.

The carrier may be any carrier molecule known in the art, in particular in the field of vaccine development, e.g. as disclosed in Hecht et al., Curr. Opin. Chem. Biol. *13*, 354-359. (2009). More specifically the carrier is a protein carrier selected from the group comprising diphtheria toxoid CRM_{197}, tetanus toxoid (TT), outer membrane protein (OMP), bovine serum albumin, (BSA), keyhole limpet hemocyanine (KLH), diphtheria toxoid (DT), cholera toxoid (CT), recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA), Clostridium difficile toxin A (TcdA), Clostridium difficile toxin B (TcdB).

The synthetic pentasaccharide derived from the repeating unit of C. difficile PS-I will induce an immunogenic and antigenic response in mice, livestock and human patients.

Consequently, an aspect of the present invention relates to a vaccine against the pathogen *Clostridium difficile* comprising at least one of the group consisting of the synthetic oligosaccharide according to claim 1, the oligosaccharide-protein conjugate according to claim 7, or a conjugate of the oligosaccharide according to claim 1 or derivative thereof with a non-protein carrier molecule.

The oligosaccharide-protein conjugate or the oligosaccharide, in particular the pentasaccharide, of the invention may be advantageously used for preparing a pharmaceutical composition for the treatment or prevention of a disease caused by a pathogenic strain of *Clostridium difficile*.

In a related aspect they may be used in a method for the treatment or prevention of a disease caused by the pathogen *Clostridium difficile*.

In a further related aspect they may be used as diagnostic tools for detecting *Clostridium difficile* or identifying a certain strain of *Clostridium difficile* in a sample and/or a *Clostridium difficile* infection in a subject. Such a method may be, e.g. a diagnostic method for *Clostridium difficile* infection comprising the use of the synthetic oligosaccharide of any one of claims 1-8 or a mixture thereof. They may for example be used as effective standards for immunoassays for the detection of *C. difficile*.

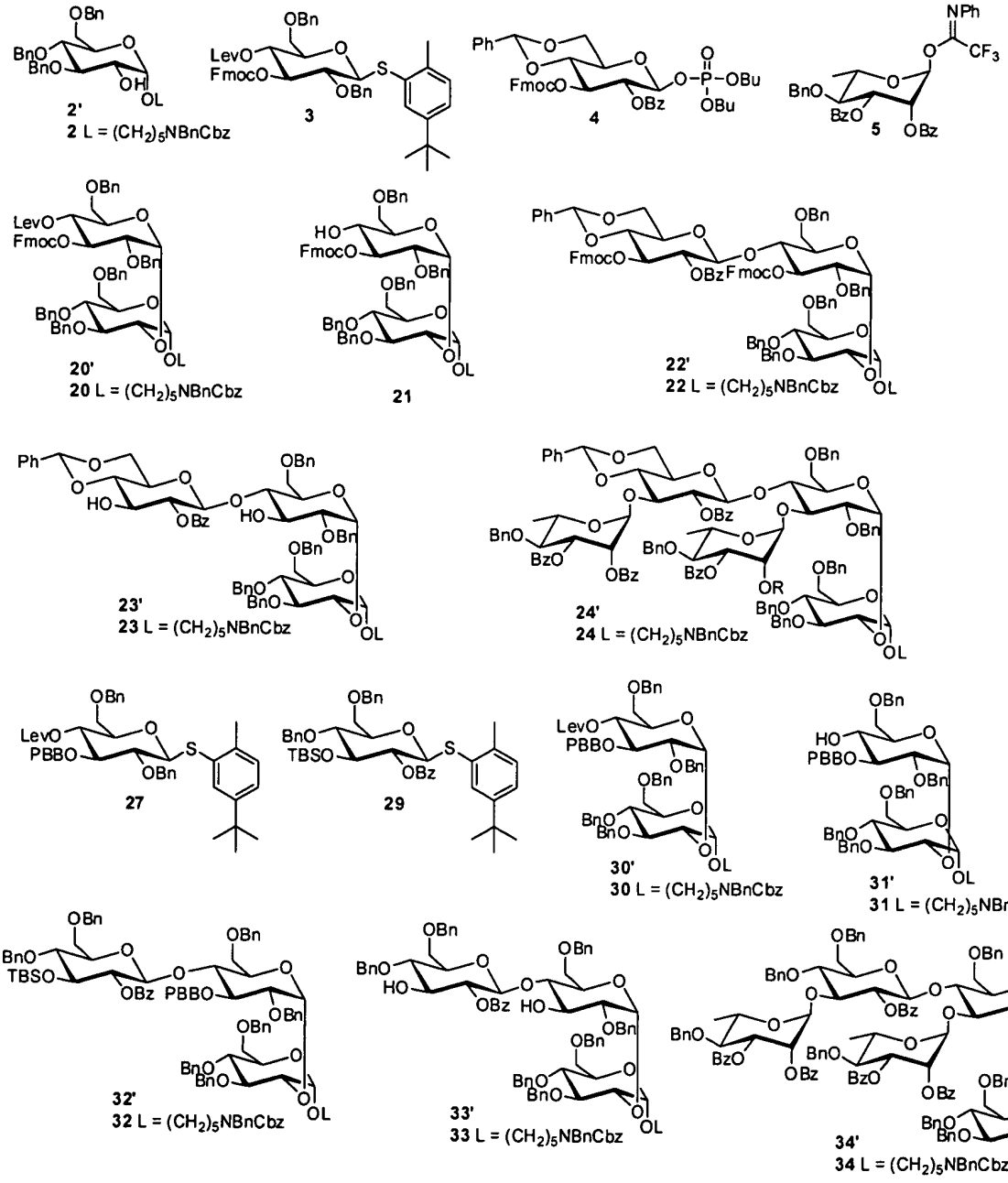
A further aspect of the invention relates to an antibody having specificity for an immunogenic determinant derived from or comprising the repeating unit of the *Clostridium difficile* glycopolymer PS-I. More specifically, the immunogenic determinant comprises or consists of the pentasaccharide of claim 1.

In a specific embodiment, said antibody has been raised against a oligosaccharide-protein conjugate wherein the oligosaccharide is the pentasacharide **1** or a derivative thereof and the protein carrier is diphtheria toxoid CRM_{197}.

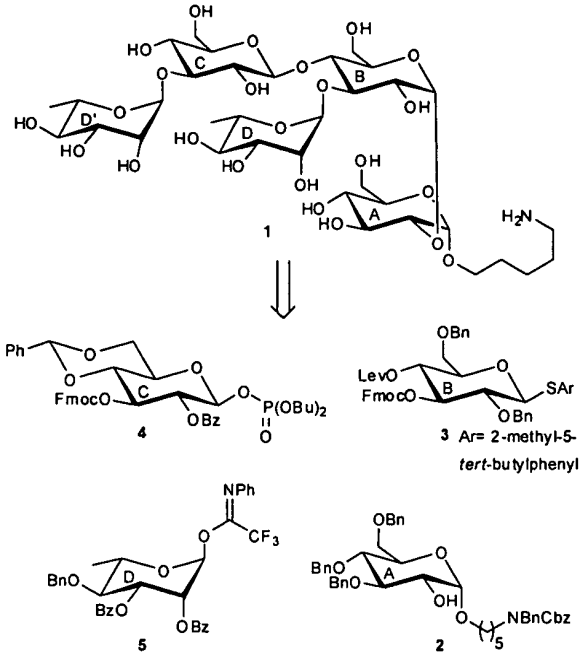
The antibody may be a polyclonal or monoclonal antibody and monoclonal antibodies can be readily prepared by standard methods of the art (e.g. Köhler and Milstein (1975), Nature, 495-497).

The present invention also provides very favourable and efficient methods for synthesizing the pentasaccharide and pentasaccharide-protein conjugates selectively and in high yields.

These methods involve the use of one or more of molecules **2**, **2`**, **3**, **4**, **5**, **20**, **21**, **22**, **23**, **24**, **27**, **29**, **30**, **30`**, **31**, **32**, **32`**, **33**, **33`**, **34`** as shown or defined below as intermediates or building blocks for preparing the pentasaccharide α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p* or of a derivative thereof.



A first preferred method (method A) for synthesizing the pentasaccharide **1** shown in Scheme 1 below



Scheme 1. Retrosynthetic analysis of pentasaccharide **1**.

comprises assembling the monosaccharide building blocks **2** and **3** or **4** shown in Scheme 1 to yield the corresponding disaccharide **21** of Scheme 5, reacting the disaccharide **21** with building block **4** to form the trisaccharide **23** of Scheme 5, subjecting the trisaccharide **23** to a bis-glycosylation reaction with 2 molecules of building block **5** shown in Scheme 1 to yield the fully protected pentasaccharide **24** in Scheme 5 and finally, after deprotection, to yield pentasaccharide **1**.

This method can be generalized for preparing other pentasaccharides having the sequence α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p*-L according to claim 1 wherein the specific amino linker of compound **1** is replaced by any linker L, in particular any linker L as defined in claims 3 or 4. This linker may also be present on a position (sugar moiety) different from the specific position (sugar moiety) indicated above. The generalized method comprises assembling a monosaccharide building block **2`**,

wherein the specific protected amino linker of building block **2** is replaced by a protected or unprotected linker L, in particular a linker L as defined in claim 3 or 4, and building blocks **3** or **4** shown in Scheme 1 to yield the corresponding disaccharide **21`**, reacting the disaccharide **21`** with building block **4** to form the trisaccharide **23`**, subjecting the trisaccharide **23`** to a bis-glycosylation reaction with 2 molecules of building block **5** shown in Scheme 1 to yield the fully protected pentasaccharide **24`** in Scheme 5 and finally, after deprotection, to yield pentasaccharide **1`**, wherein the specific amino linker of pentasaccharide **1** is replaced by a different linker L, in particular a linker L as defined in claim 3 or 4.

The method for preparing the oligosaccharide-protein conjugate of the present invention typically comprises coupling the oligosaccharide of claim 2 bearing a linker or spacer group L, in particular wherein L is $(CH_2)_nNH_2$, with n being an integer from 2 to 50, preferably from 3 to 20, with a protein carrier.

More specifically, said method comprises providing a pentasaccharide having the sequence α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3]-α-D-Glc*p*-(1→2)-α-D-Glc*p*-L bearing a linker *L* = $(CH_2)_nNH_2$, with n being an integer from 2 to 50, preferably from 3 to 20, and reacting the unique terminal amine of the linker L with one of the two NHS-activated esters of Di(N-succinimidyl) adipate to form an amide and subsequent coupling of the activated amide moiety to the protein carrier. The protein carrier may be any carrier disclosed above and in one specific embodiment the protein carrier is CRM_{197}.

In the following, the methods of synthesis according to the invention are outlined in more detail with respect to preferred embodiments but are not limited thereto.

General oligosaccharide synthesis

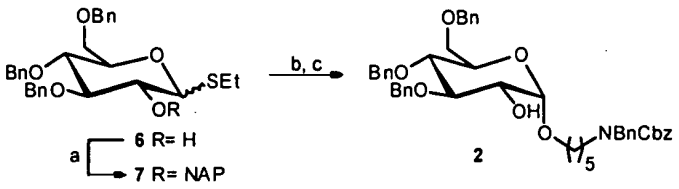
The present inventors developed very effective methods for synthesizing a pentasaccharide having the sequence α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p*-L that comprises the PS-I repeating unit but differs from the natural pentasaccharide by the linker L. In a preferred embodiment, the oligosaccharide was designed to carry a primary amine at the reducing terminus via a linker to facilitate conjugation to a protein carrier and attachment to microarrays or other surfaces. Based on the retrosynthetic analysis (Scheme 1), the pentasaccharide **1** - wherein the linker comprises the $(CH_2)_5NH_2$ group - can be assembled from the monosaccharide building blocks **2** and **3** or **4**, and the monosaccharide building block **5** and these assembling steps are outlined in more detail below.

However, it is to be understood that analogous assembling steps can be performed using an analogous building block **2`** differing from building block **2** only by the presence of a different linker, in particular such as defined in claims 3 or 4, resulting in an analogous pentasaccharide **1`**.

The 1,2-*cis* glycosidic linkages of the glucose residues A and B were installed early in the synthesis by employing the non-participating protecting groups 2-naphthylmethyl (NAP) and benzyl in 2-positions. The temporary protecting groups Lev and Fmoc present in the glucose building blocks B and C were chosen for their compatibility with automated solid phase synthesis (K. R. Love and P. H. Seeberger, *Angew. Chem. Int. Ed.*, 2004, **43**, 602). Both Rha residues D and D' were installed in a single bisglycosylation reaction.

Following placement of the NAP-protection in thioglycoside **6** (S. J. Danishefsky, S. Hu, P. F. Cirillo, M. Eckhardt and P. H. Seeberger, *Chem. Eur. J.*, 1997, **3**, 1617) the terminal

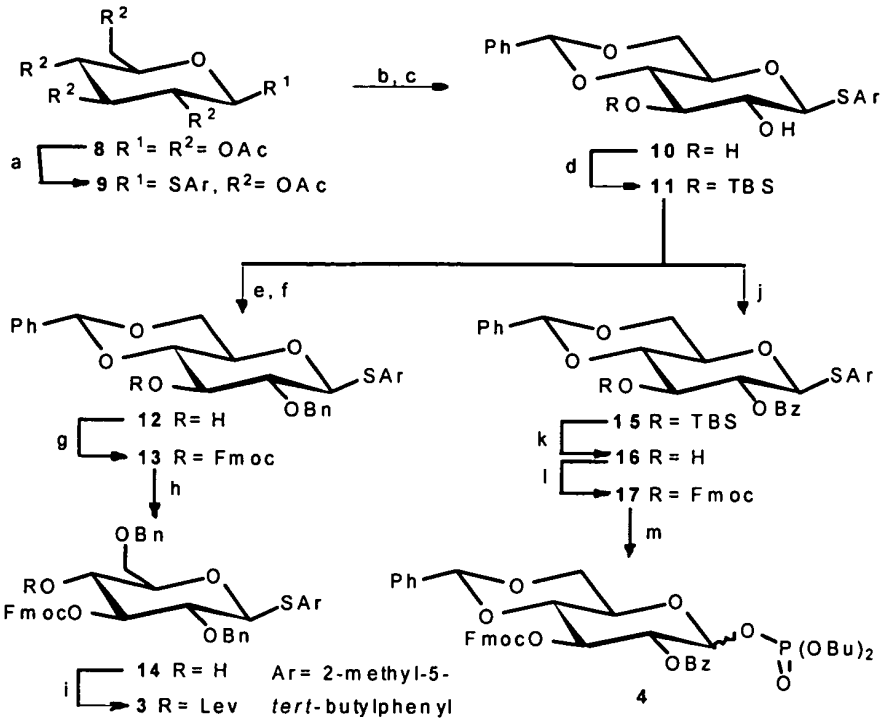
linker carrying a latent amine was introduced by union of thioglucoside **7** and the linker prior to subsequent DDQ-mediated cleavage of the C-2 napthyl ether in order to produce glucose building block **2** (Scheme 2) a) J.-G. Delcros, S. Tomasi, S. Carrington, B. Martin, J. Renault, I. S. Blagbrough and P. Uriac, *J. Med. Chem.*, 2002, **45**, 5098; b) J. Xia, S. A. Abbas, R. D. Locke, C. F. Piskorz, J. L. Alderfer and K. L. Matta, *Tetrahedron Lett.*, 2000, **41**, 169)



Scheme 2. Synthesis of building block **2**.

Reagents and conditions: a) NaH, NAPBr, DMF, 0 °C to rt, 92%; b) $HO(CH_2)_5NBnCbz$, NIS, TfOH, toluene/dioxane, -40°C to -20°C; c) DDQ, DCM, H_2O, 35% over 2 steps.

The synthesis of thioglucoside **11** that served as common precursor for building blocks **3** and **4** commenced from β-d-glucose pentaacetate **8** (Scheme 3). Use of the nontoxic and odorless 2-methyl-5-*tert*-butyl-thiophenol group ensured exclusive formation of β-anomer of thioglucoside **9** (M. Collot, J. Savreux and J.-M. Mallet, *Tetrahedron*, 2008, **64**, 1523). The acetyl groups were removed and the 4- and 6-hydroxyl groups of the resulting tetraol were regioselectively protected as a 4,6-*O*-benzylidene acetal (J. S. S. Rountree and P. V. Murphy, *Org. Lett.*, 2009, **11**, 871) to afford diol **10**. Regioselective placement of a TBS-ether protecting group at the 3-OH gave thioglycoside **11** (K. C. Nicolaou, N. Winssinger, J. Pastor and F. DeRoose, *J. Am. Chem. Soc.*, 1997, **119**, 449).

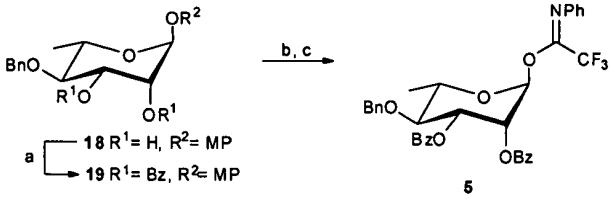


Scheme 3. Synthesis of monosaccharide building blocks **3** and **4**. Reagents and conditions: a) 2-methyl-5-*tert*-butylthiophenol, $BF_3 \cdot OEt_2$, DCM, 85%; b) NaOMe, MeOH, rt; c) benzaldehyde dimethyl acetal, CSA, MeCN, 87% over 2 steps; d) TBS-Cl, imidazole, DMF, 0 °C, 69%; e) NaH, BnBr, DMF, 0 °C to rt; f) 1M TBAF in THF, 0 °C to rt, 93% over 2 steps; g) Fmoc-Cl, pyridine, DCM, 95%; h) TES, TfOH, DCM, 4 Å MS, -78 °C, 73%; i) Lev_2O, pyridine, DCM, 3 days, 79%; j) BzCl, DMAP, pyridine, 70 °C, 88%. k) $TBAF \cdot 3H_2O$, AcOH, DMF, 35 °C, 91%; l) Fmoc-Cl, pyridine, DCM, 96%; m) $HOPO(OBu)_2$, NIS/TfOH, DCM, 4 Å MS 0°C, 81%.

Synthesis of building block **3** began with the installation of the non-participating benzyl group at the 2-position of **12** to favor the formation of the α-glycosidic linkage between monosaccharides A and B fragments. Subsequent placement of the 3-*O*-Fmoc-protection furnished compound **13**. Finally, the regioselective opening of the 4,6-*O*-benzylidene acetal with TES-TfOH and protection of the free 4-hydroxyl gave

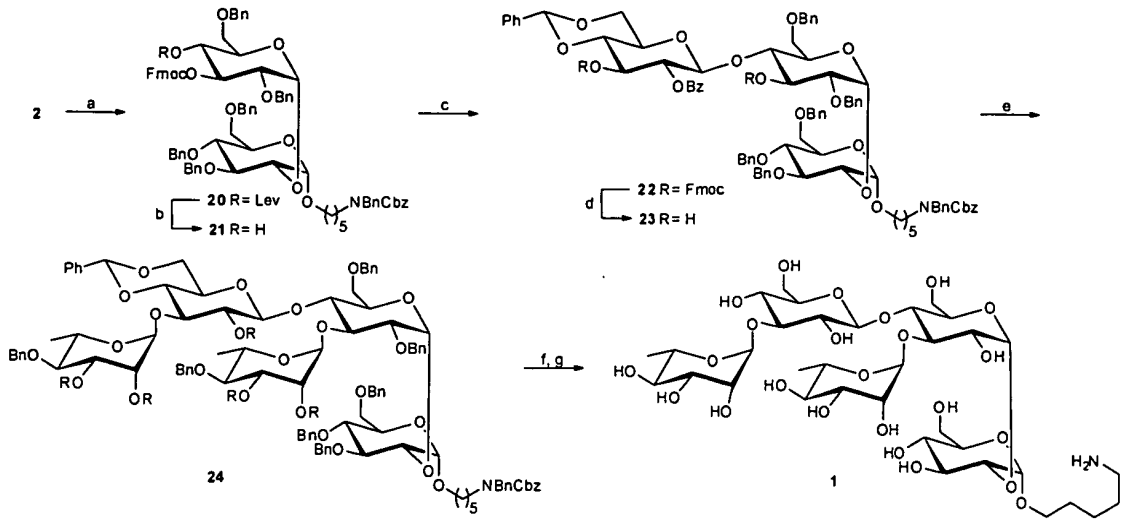
orthogonally protected building block **3**. Preparation of differentially protected glucosyl phosphate **4** from **11** followed a similar route. In anticipation of the formation of a 1,2-*trans* linkage between the B and C saccharide fragments, a participating benzoyl group was installed at the 2-position of **15**. During TBAF-mediated desilylation of **15**, careful control of the TBAF:AcOH ratio was essential to prevent benzoyl-migration from the C2- to C3-positions. Fmoc-protected thioglycoside **17** was further converted to glycosyl phosphate **4**.

Synthesis of the rhamnosyl building block **5** to provide the D fragment commenced with the bis-benzoylation of 4-methoxy-phenyl glycoside **18** (Scheme 3) (D. B. Werz, A. Adibekian and P. H. Seeberger, *Eur. J. Org. Chem.*, 2007, **12**, 1976). CAN-mediated removal of the anomeric *p*-methoxyphenyl group yielded the free lactol that was immediately converted into rhamnosyl *N*-phenyl trifluoroacetimidate **5** (B. Yu and H. Tao, *Tetrahedron Lett.*, 2001, **42**, 2405).



Scheme 4. Synthesis of rhamnosyl building block **5**. Reagents and conditions: a) BzCl, DMAP, pyridine, DCM, 0 °C to rt, 97%; b) CAN, MeCN, H_2O; c) $CF_3C(NPh)Cl$, Cs_2CO_3, DCM, 74% over 2 steps.

The assembly of the pentasaccharide target was achieved in seven linear steps by combining the monosaccharide building blocks in sequence (Scheme 5).



Scheme 5. Synthesis of **1** according to method A. Reagents and conditions: a) **3**, NIS/TfOH, Et_2O, -35 °C to -10°C, 70%; b) $N_2H_4 \cdot H_2O$, AcOH/pyridine, DCM, 94%; c) **4**, TMSOTf, DCM, 4Å MS, -35 °C to -7 °C; d) NEt_3, DCM, rt, 38% over 2 steps; e) **5**, TMSOTf, DCM, 4Å MS, -30 °C to -15 °C, 81%; f) NaOMe, THF/MeOH, 50 °C; g) H_2, 10% Pd/C, MeOH, H_2O,

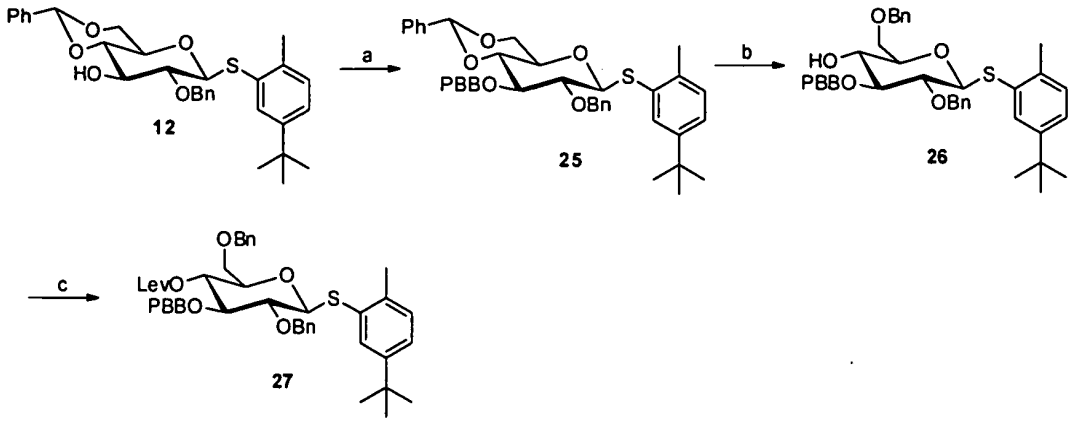
Installation of the α-glycosidic linkage was the result of the union of glycosylating agent **3** and nucleophile **2**. Disaccharide **20** was obtained in good yield and stereoselectivity when NIS and TfOH in Et_2O was employed as promoter system. Selective cleavage of the levulinic ester with hydrazine hydrate in pyridine/AcOH, did not compromise the integrity of the Fmoc-group but cleanly produced disaccharide acceptor **21**. Thioglucoside building block **17**, a very storage-stable monomer unit had been intended for the installation of the next glycosidic linkage to form trisaccharide **22**. Upon a variety of conditions only traces of the desired product **22** were isolated. As a first means to remedy the situation, replacement of the anomeric leaving group was executed. Glycosyl phosphate **4** was activated by TMSOTf to promote the glycosylation of **21** and afforded **22**, although purification was achieved only following Fmoc cleavage to yield **23**. Conversion of diol **23** to fully protected pentasaccharide **24** was achieved

by bis-glycosylation using rhamnosyl-imidate **5** in the presence of TMSOTf. Final deprotection of compound **24** required two transformations: saponification of the benzoate esters and catalytic hydrogenation of the aromatic groups gave pentasaccharide **1**. Careful comparison of the spectroscopic data for synthetic pentasaccharide **1** and NMR spectra of native PS-I revealed excellent agreement.

In summary, the first synthesis of the *C. difficile* cell-surface PS-I pentasaccharide **1** was achieved employing a linear strategy that serves to scout reaction conditions for automated solid phase synthesis and to identify robust and efficient monosaccharide building blocks. Four such building blocks **2-5** were prepared. Glycosyl phosphate **4** proved a significantly better building block than identically protected thioglycoside **3**.

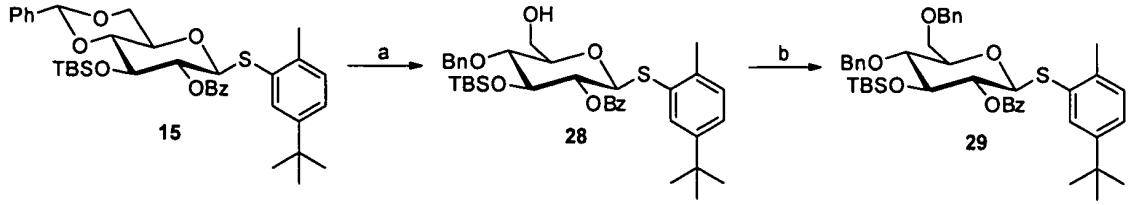
The present inventors also developed an alternative route of synthesis based on a similar strategy as outlined above which is even more efficient and results in greatly improved yields of the PS-I pentasaccharide product.

The innovation of this improved synthesis relies on the use of the protecting group *para*-bromobenzyl (PBB)[Plante et al., J. Am. Chem Soc. 122:7148-7149, 2000; Liu et al., Chem. Commun. 1708-2709 ; 2004]. Building block **27**, modified with PBB at *C*-3 was obtained in three steps from intermediate **12** described above (Scheme 3). PBB-containing **27** was used for the following pentasaccharide synthesis rather than Fmoc-containing **3** used in the method outlined above.



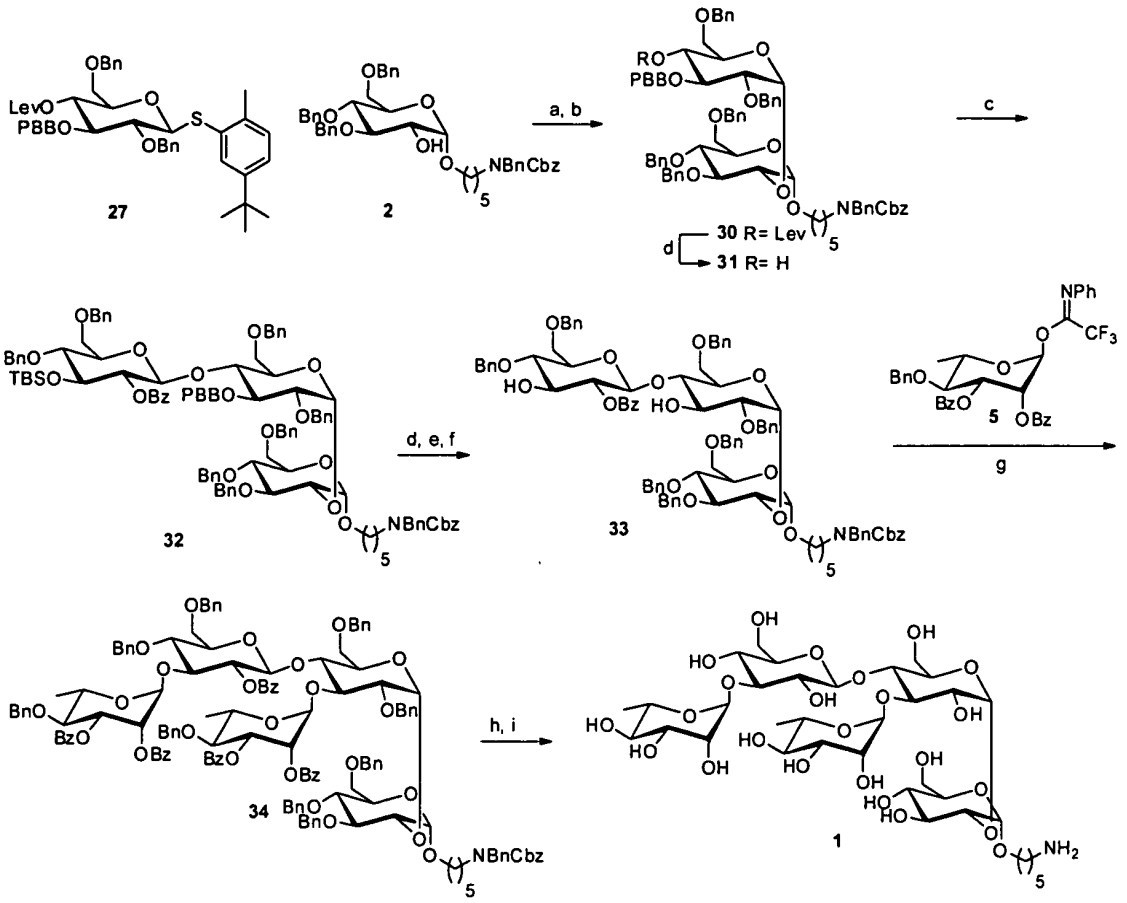
Scheme 6. Synthesis of building block **27**. Reagents and conditions: a) *para*-bromobenzyl (PBB) bromide, NaH, DMF; b) TES, TfOH, 4Å MS, DCM, -78 °C, 58% over 2 steps; c) LevOH, DCC, DMAP, DCM, 87%.

A further improvement of the previous synthesis was achieved by replacing the acid-labile building block **4** with more stable **29**. The 4,6-*O*-benzylideneacetal ring of previously reported intermediate **15** was opened selectively, followed by benzylation to give building block **29**. (Scheme 7)



Scheme 7. Synthesis of building block **29**. Reagents and conditions: a) $BH_3 \cdot THF$, TMSOTf, DCM; b) BnBr, NaH, THF/DMF, 88% over 2 steps.

Assembly of the pentasaccharide took place similarly as described above for method A; changes were made in the deprotection steps d), e) and f) (Scheme 8) due to the modified protective group pattern.



Scheme 8. Synthesis of pentasaccharide **1** (method B). Reagents and conditions: a) NIS/TfOH, Et_2O, -20°C to 0°C, 69%; b) $N_2H_4 \cdot H_2O$, AcOH/Pyridine, DCM, 96%; c) **29**, NIS/TfOH, DCM, -30°C to -10°C, 92%; d) cat. $Pd(OAc)_2$, (3,4-dimethoxyphenyl)boronic acid, TBABr, K_3PO_4, EtOH, 92%; e) DDQ, aq. $NaHCO_3$, H_2O, DCM; f) $TBAF \cdot 3H_2O$, AcOH, DMF, 50°C, 68% over 2 steps; g) TMSOTf, DCM, 4Å MS, -30 °C to -15 °C, 88%; h) NaOMe, THF/MeOH, rt; i) H_2, 10% Pd/C, MeOH, H_2O, AcOH, 59% over 2 steps.

Synthesis of the pentasaccharide **1** according to method B preferably comprises assembling the monosaccharide building blocks **2** and **27** shown in Scheme 8 to yield the corresponding disaccharide **30** of scheme 8, reacting the disaccharide **30** with building block **4** or **29** to form the protected trisaccharide **32** of scheme 8, deprotecting the trisaccharide **32** to obtain trisaccaride **33** and subjecting trisaccharide **33** to a bis-glycosylation reaction with 2 molecules of building block **5**

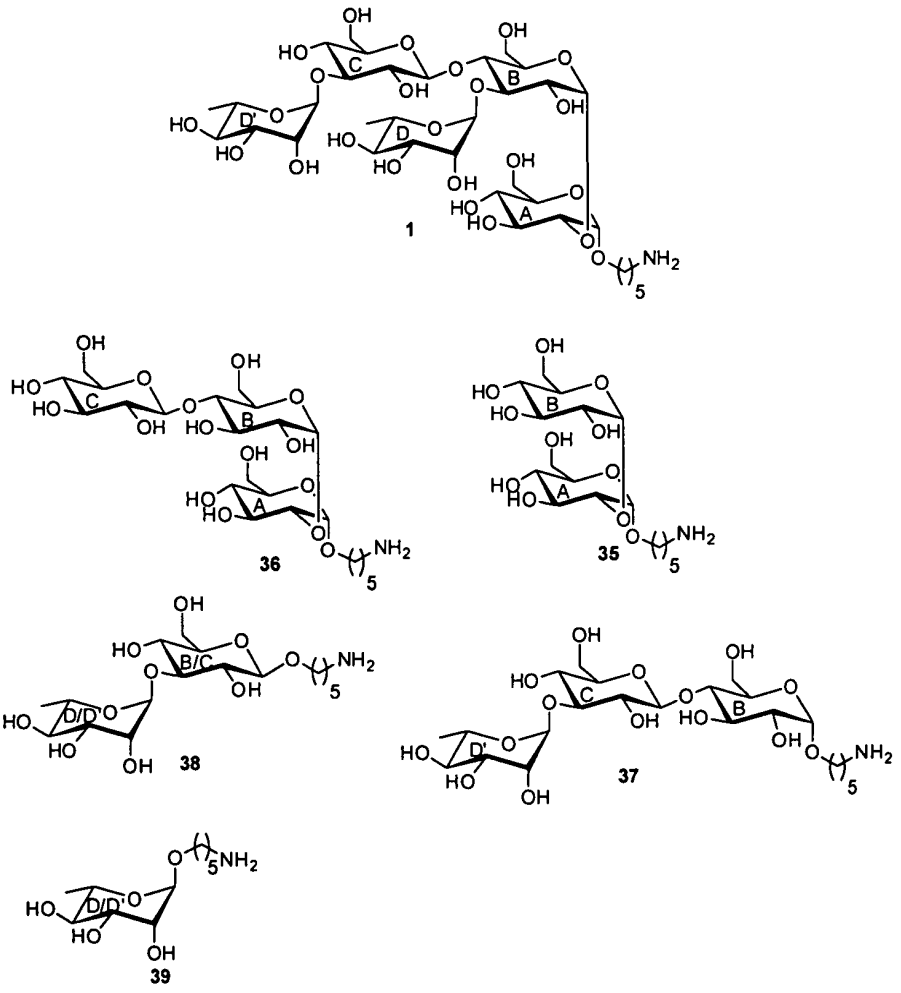
shown in Scheme 8 to yield the fully protected pentasaccharide **34** in Scheme 8 and finally, after deprotection, to yield pentasaccharide **1**.

Formation of the Glc(1→4)Glc linkage (Scheme 8, step c) proceeded in 92% yield, a huge improvement compared to 38% in method A.

This method can be generalized for preparing other pentasaccharides having the sequence α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p*-L according to claim 1 wherein the specific amino linker of compound **1** is replaced by any linker L, in particular any linker L as defined in claims 3 or 4. This linker may also be present on a position (sugar moiety) different from the specific position (sugar moiety) indicated above. The generalized method comprises assembling a monosaccharide building block **2`**, wherein the specific protected amino linker of building block **2** is replaced by a protected or unprotected linker L, in particular a linker L as defined in claim 3 or 4, and building block **27** shown in Scheme 8 to yield the corresponding disaccharide **30`**, reacting the disaccharide **30`** with building block **4** or **29** to form the corresponding protected trisaccharide **32`**, deprotecting the trisaccharide **32`** to obtain trisaccaride **33´**, subjecting the trisaccharide **33`** to a bis-glycosylation reaction with 2 molecules of building block **5** shown in Scheme 1 to yield the fully protected pentasaccharide **34`** and finally, after deprotection, to yield pentasaccharide **1`**, wherein the specific amino linker of pentasaccharide **1** is replaced by a different linker L, in particular as defined in claim 3 or 4.

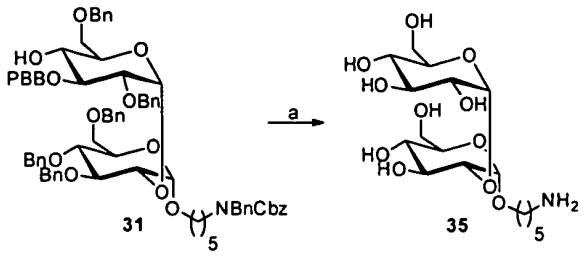
Synthesis of PS-I Substructures

A comprehensive set of PS-I substructures **35-39** (Scheme 9) carrying an amino-linker was synthesized. The pentasaccharide repeating unit **1** is built up from glucose residues A, B and C and terminal rhamnoses D and D'. Disaccharide **35** contains A and B, trisaccharide **36** A, B and C. The sequence BCD' is covered by trisaccharide **37**. Disaccharide **38** covers both the BD and CD' sequence. Rhamnose substructure **39** represents D and D'.

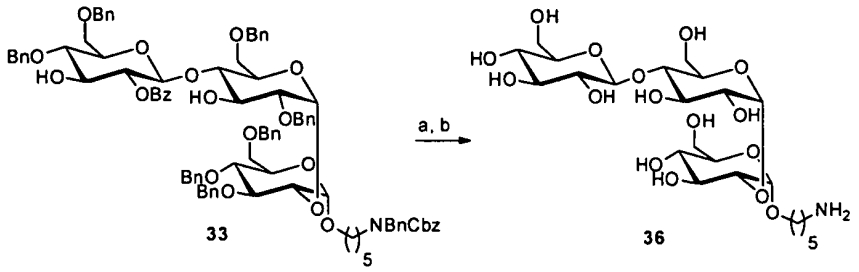


Scheme 9. Pentasaccharide **1** and comprehensive set of substructures **35-39**.

Oligoglucose disaccharide **35** (Scheme 10) and trisaccharide **36** (Scheme 11) were obtained by catalytic hydrogenation of protected disaccharide **31** and trisaccharide **33**.

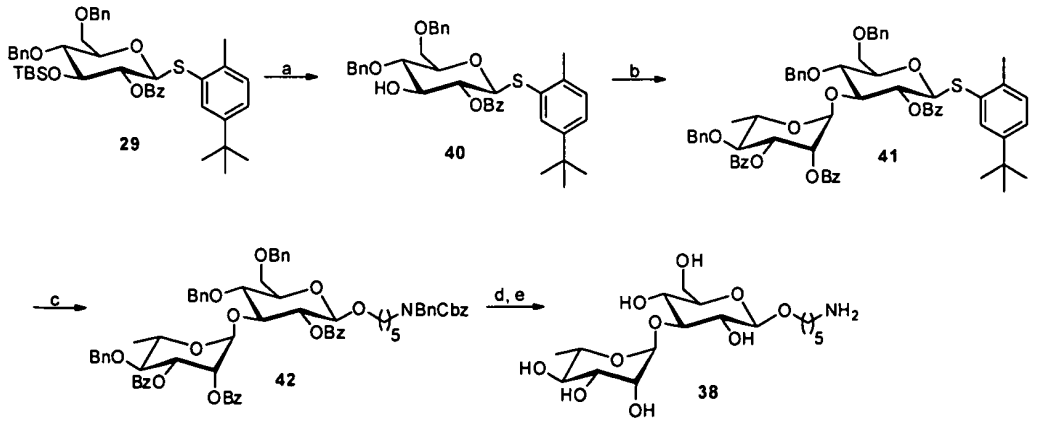


Scheme 10. Synthesis of **35**. Reagents and conditions: a) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 99%.

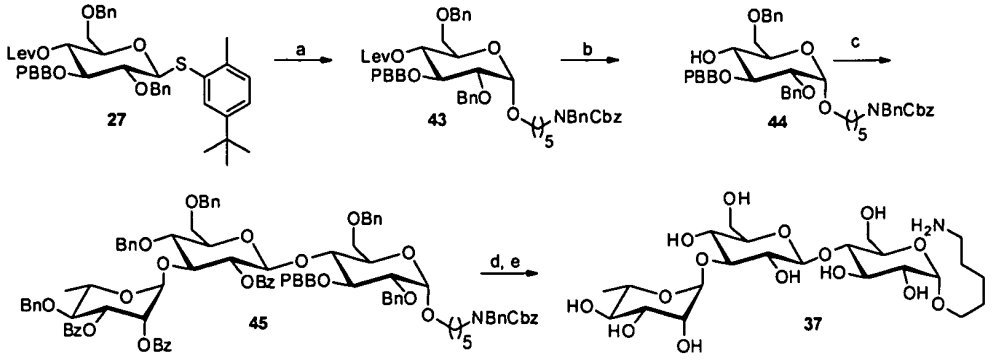


Scheme 11. Synthesis of **36**. Reagents and conditions: a) NaOMe, THF/MeOH; b) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 66% over 2 steps.

Oligosaccharides **38** (Scheme 12) and **37** (Scheme 13) containing a terminal rhamnose residue were synthesized relying on disaccharide **41** which in its turn was obtained by union of **40** and **5**.

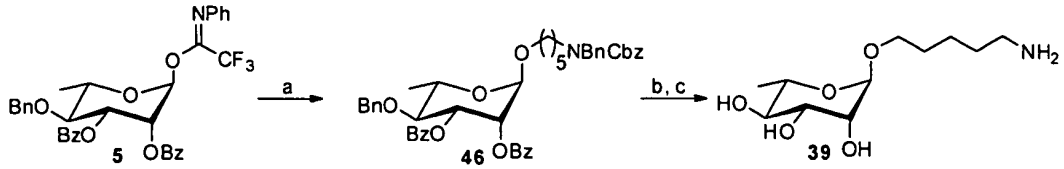


Scheme 12. Synthesis of **38**. Reagents and conditions: a) TBAF·3H_2O, AcOH, DMF, 35°C; b) **5**, TMSOTf, DCM, 4Å MS, -40°C to -20 °C, 79% over 2 steps; c) 5-aminopentanol, NIS/TfOH, DCM, -20°C to 0°C, 91%; d) NaOMe, THF/MeOH; e) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 75% over 2 steps.



Scheme 13. Synthesis of **37**. Reagents and conditions: a) 5-aminopentanol, NIS/TfOH, Et_2O, -10°C to 0°C, 39%; b) $N_2H_4·H_2O$, AcOH/Pyridine, DCM, 81%; c) **41**, NIS/TfOH, DCM, -20°C to 0°C, 95%; d) NaOMe, THF/MeOH; e) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 78% over 2 steps.

Rhamnoside **39** (Scheme 14) bearing an anomeric linker was attained by combining **5** and 5-aminopentanol.



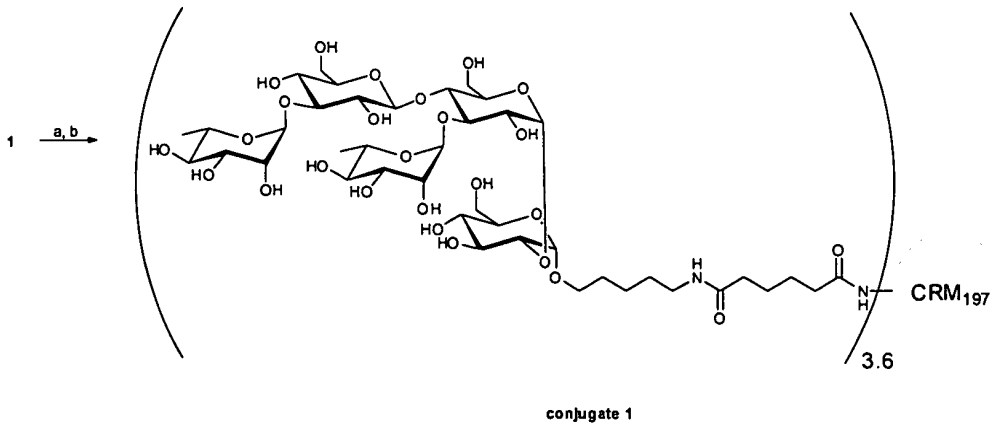
Scheme 14. Synthesis of **39**. Reagents and conditions: a) 5-aminopentanol, TMSOTf, DCM, 4Å MS, -30 °C to -20 °C, 94%; b) NaOMe, THF/MeOH; c) H_2, 10% Pd/C, MeOH, THF, H_2O, AcOH, 94% over 2 steps.

Microarray-chips containing **1** and the substructures **36-39** were prepared. This set of oligosaccharides substructures covalently linked to a surface was used to identify binding

epitopes of anti PS-I pentasaccharide antibodies raised in mice (Figure 6).

The pentasaccharide **1** or **1`** obtained as outlined above or a fragment or derivative thereof can be coupled to a carrier protein by a variety of known methods.

A particular advantageous and preferred method uses the approach shown in scheme 15 below. For this the unique, terminal amine of **1** was first reacted with one of the two NHS-activated esters of Di(N-succinimidyl) adipate to form an amide. The coupling of the activated pentasaccharide to CRM_{197} proceeded in phosphate buffer (any other usual buffer providing the desired pH is also suitable) and in one experiment resulted in a load that averaged 3.6 pentasaccharide units per protein, as determined by MALDI-TOF mass spectrometry. However, other pentasaccharide loads (such as e.g. about 9.6 units per carrier molecule) are also possible by varying the reaction conditions (compare Example 3).



conjugate 1

Scheme 15. Synthesis of **conjugate 1 (1a)**. Reagents and conditions: a) Di(N-succinimidyl) adipate, NEt_3, DMSO; b) CRM_{197}, phosphate buffer (pH 7.5).

Microarray Chips

Oligosaccharides, in particular pentasaccharide 1 and substructures 35 through 39, were immobilized on the surface of NHS-activated glass slides via their terminal primary amine group of the linker moiety. These microarrays were used to detect and quantify oligosaccharide-specific antibodies.

Polyclonal and monoclonal Antibodies

Monoclonal antibodes (mABs) were generated using the standard method by Köhler and Milstein, 1975. These showed specificity for pentasaccharide **1**.

The invention is further illustrating by the following non-limiting Examples and Figures.

FIGURES

Figure 1. Glycoconjugate **1** composed of hapten **1** (pentasaccharide **1**) and protein CRM_{197}

Figure 2. Characterization of glycoconjugate **1a**;
a) SDS-PAGE; b) MALDI-TOF; c) HPLC

Figure 3. Conjugate reaction resulting in glycoconjugate **1b**

Figure 4a. SDS-PAGE analysis of CRM_{197} glycoconjugate **1b**

Figure 4b. MALDI-TOF MS analysis of CRM_{197} glycoconjugate **1b**

Figure 5. Microarray design

Figure 6. Microarray analysis of immune response against glycoconjugate. Dilutions of pooled sera in PBS are indicated under the microarray images.

Figure 7. Antibody titers against the PS-I pentasaccharide (left), CRM_{197} (center), and spacer moiety (right), as determined by microarray analysis

Figure 8. Isotype analysis of the immune response against PS-I pentasaccharide

Figure 9. Microarray design including PS-I pentasaccharide **1** and substructures thereof, **35** through **39**

Figure 10. Immune response against PS-I substructures of mice immunized with PS-I glycoconjugate without adjuvant

Figure 11. Immune response against PS-I substructures of mice immunized with PS-I glycoconjugate and Freund's adjuvant

Figure 12. Immune response against PS-I substructures of mice immunized with PS-I glycoconjugate and Alum adjuvant

Figure 13. Isotype analysis of monoclonal antibodies and their reactivities against PS-I substructures

EXAMPLE 1

Preparation and characterization of a pentasaccharide based on the repeating unit of C. difficile polysaccharide PS-I

The pentasaccharide was designed to provide, by means of a linker group, a primary amine at the reducing terminus to facilitate conjugation to a protein carrier and attachment to microarrays and other surfaces. In the following synthesis, the linker comprises the $(CH_2)_5NH_2$ group and the overall synthesis was performed according to scheme 5 or 8 above as indicated.

General Experimental

Commercial grade reagents and solvents were used without further purification except as indicated below. All batch reactions conducted under an Ar atmosphere. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian 400-MR or Varian 600 spectrometer. The proton signal of residual, non-deuterated solvent (δ 7.26 ppm for $CHCl_3$; δ 4.79 ppm for H_2O, 2.84 ppm for acetone) was used as an internal reference for ^{1}H spectra. For ^{13}C spectra, the chemical shifts are reported relative to the respective solvent (δ 77.16 ppm for $CDCl_3$, δ 29.84 ppm for acetone). For ^{13}C spectra in D_2O, MeOH (δ 49.50 ppm) was added as internal standard. Coupling constants are reported in Hertz (Hz). The following abbreviations are used to indicate the multiplicities: s, singlet; d, doublet; t, triplet; m multiplet. Infrared (IR) spectra were recorded as thin films on a Perkin Elmer Spectrum 100 FTIR spectrophotometer. Optical rotations (OR) were measured with a Schmidt & Haensch UniPol L 1000 at 589 nm and a concentration (c) expressed in g/100 mL. High-resolution mass spectra (HRMS) were recorded with an Agilent 6210 ESI-TOF mass spectrometer at the Freie Universität Berlin, Mass Spectrometry Core Facility. MALDI-TOF spectra were recorded on a Bruker Daltonics Autoflex Speed. Synthetic carbohydrates were measured using a 2,4,6-trihydroxyacetophenone (THAP) matrix, proteins and glycoconjugates were measured using 2,4-dihydroxyacetophenone (DHAP) as matrix.

Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or a 1:1 mixture of H_2SO_4 (2N) and resorcine monomethylether (0.2%) in ethanol. Column chromatography was performed using Kieselgel 60 (230-400 mesh). SEC-HPLC analyses were performed

on a TSKgel-G4000SWXL column connected to an Agilent 1200 HPLC system equipped with a PDA detector. Elution buffer was constituted by 100mM sodium phosphate pH 7.2, 100 mM NaCl flow rate was 0.4 mL/min. SDS PAGE gels were run with 10 % SDS PAGE gel in reducing conditions at 130 V and 50 mA, molecular weight marker (Invitrogen bench marker) was used.

Synthesis of pentasaccharide 1 and intermediates according to schemes 1-5

Ethyl-3,4,6-tri-*O*-benzyl-2-*O*-(2-naphthalenylmethyl)-1-thio-D-glucopyranoside (7)

To a solution of **6** (284 mg, 0.57 mmol) in anhydrous DMF (1 mL), NaH (20.7 mg, 0.86 mmol) followed by NAP-Br (228 mg, 1.03 mmol) were added at 0 °C. The mixture was warmed to room temperature over 1 h, cooled to 0 °C and quenched by the addition of MeOH (0.1 mL). Et_2O was added and the organic layer washed with 0.01 M HCl solution and with saturated aqueous $NaHCO_3$ solution. The phases were separated and the organic layer was dried over $MgSO_4$ and concentrated. Column chromatography (hexanes/ethyl acetate) afforded **7** (335 mg, 0.53 mmol, 92%) in a mixture of α/β-anomers as a white solid. Analytical data is reported for the β-anomer. $[\alpha]_D^{20}$ = +26.1 ° (c = 5.3, $CHCl_3$), IR ν_{max} (film) 3061, 3030, 2864, 1949, 1808, 1603, 1497, 1453, 1360, 1065 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.82-7.69 (4H, m, Ar-H), 7.52-7.09 (18H, m, Ar-*H*), 5.08-5.02 (1H, m, $-CH_2$-Ar), 4.93-4.77 (4H, m, $-CH_2$-Ar), 4.60-4.50 (3H, m, $-CH_2$-Ar), 4.47 (1H, d, *J* 9.7, 1-H), 3.80-3.54 (4H, m), 3.52-3.41 (2H, m), 2.84-2.66 (2H, m, S-CH_2-), 1.31 (3H, t, *J* 7.3, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.7, 138.4, 138.2, 135.6, 133.4, 133.2, 128.56, 128.55, 128.5, 128.2, 128.1, 127.92, 127.87, 127.84, 127.80, 127.77, 127.7, 127.2, 126.5, 126.1, 126.0, 86.8, 85.2 (C-1), 82.0, 79.3, 78.2, 75.9, 75.7, 75.2,

73.6, 69.3, 25.2, 15.3; HRMS (ESI): Calcd for $C_{40}H_{42}O_5S$ $[M+Na]^+$ 657.2651, found 657.2651.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-3,4,6-tri-O-benzyl-ß-D-glucopyranoside (2)

Thioglucoside **7** (335 mg, 0.53 mmol) and $HO(CH_2)_5NBnCbz$ (518 mg, 1.58 mmol) were coevaporated with toluene (3 x 10 ml), dried *in vacuo*, then the compounds were dissolved in a solution of anhydrous toluene:dioxane=2:1 (4.5 ml). The solution was cooled to -40 °C, treated with NIS (131 mg, 0.58 mmol) and TfOH (4.7 µl, 53 µmol) and warmed to -20 °C over 1.5 h. The reaction was quenched with pyridine, diluted with DCM and washed with saturated aqueous $Na_2S_2O_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) gave a mixture of anomers which was dissolved in DCM (10 ml) and water (1 ml) and treated with DDQ (202 mg, 0.89 mmol) at 0 °C for 2 h. The mixture was diluted with DCM and the organic layer washed with saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **2** (140 mg, 0.184 mmol, 35%) as a colorless oil. $[\alpha]_D^{20}$ = +53.3 ° (c = 5.5), IR ν_{max} (film) 3458, 3031, 2927, 1952, 1876, 1808, 1454, 1421, 1360, 1229, 1129, 1067 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d6) δ 7.48-7.10 (25H, m, Ar-H), 5.15 (2H, bs), 4.99 (1H, d, *J* 11.4, $-CH_2$-Bn), 4.84 (1H, d, *J* 11.1, $-CH_2$-Bn). 4.79 (1H, d, *J* 11.4, $-CH_2$-Bn), 4.75 (1H, bs, 1-H), 4.62-4.49 (5H, m, $-CH_2$-Bn), 3.84-3.86 (6H, m), 3.62-3.47 (2H, m), 3.40 (1H, m), 3.31-3.18 (2H, m, linker-CH_2-), 1.67-1.50 (4H, m, linker-CH_2-), 1.43-1.29 (2H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, acetone-d6) δ 140.5, 139.8, 139.7, 139.5, 129.3, 129.1, 129.0, 128.9, 128.60, 128.58, 128.43, 128.41, 128.2, 128.0, 99.9 (C-1), 84.3, 78.7, 75.5, 75.4, 74.2, 73.3, 71.5, 70.2, 68.5, 67.4, 24.1; HRMS (ESI): Calcd for $C_{47}H_{53}NO_8$ $[M+Na]^+$ 782.3669, found 782.3633.

(2-Methyl-5-*tert*-butylphenyl) 2,3,4,6-tetra-*O*-acetyl-1-thio-ß-D-glucopyranoside (9)

1,2,3,4,6-Penta-*O*-acetyl-ß-D-glucopyranose **8** (30 g, 77 mmol) was dissolved in anhydrous DCM (34 mL). 2-Methyl-5-*tert*-butyl thiophenol (17 mL, 92 mmol, 1.2 eq) were added under stirring. $BF_3 \cdot OEt_2$ (13.6 mL, 108 mmol, 1.4 eq) was added dropwise and the resulting yellow solution was stirred over night. After completion the solution was diluted with DCM and extracted with saturated aqueous $NaHCO_3$ and H_2O, and the organic layer was dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was dried in high vacuum. The resulting yellow solid was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **9** (33.4 g, 65.4 mmol, 85%). $[\alpha]_D^{20}$ = -8.0 ° (c = 1.0, $CHCl_3$); IR ($CHCl_3$): 2961, 1747, 1366, 1211, 1034, 912 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.52 (1H, d, *J* 2.0, Ar-H), 7.25-7.10 (2H, m, Ar-H), 5.19 (1H, dd, J_1J_2 9.4, 1-H), 5.10-4.98 (2H, m, 4-H, 2-H), 4.64 (1H, d, *J* 10.6, 1-H), 4.23 (1H, dd, J_1 12.2, J_2 5.0, 6-Ha), 4.10 (1H, dd, J_1 12.2, J_2 1.9, 6-Hb), 3.71-3.63 (1H, m, 5-H), 2.34 (3H, s, CH_3), 2.07-2.03 (6H, m, OAc), 2.00-1.96 (6H, m, OAc), 1.29 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.3, 169.5, 169.4 (C=O OAc), 149.8, 137.51, 131.47, 130.53, 130.2, 125.8, 87.0 (C-1), 75.9 (C-5), 74.2 (C-3), 70.3 (C-3), 68.3(C-4), 62.4 (C-6), 31.4 (*t*Bu), 20.89, 20.88, 20.74, 20.70 (OAc), 20.5 (CH_3); HRMS (ESI): Calcd for $C_{25}H_{34}O_9S$ $[M+Na]^+$ 533.1816, found 533.1832.

(2-Methyl-5-*tert*-butylphenyl)-4,6-*O*-benzylidene-1-thio-ß-D-glucopyranoside (10)

Thioglycoside **9** (1.5 g, 2.94 mmol) was dissolved in of methanol (12 mL). Sodium methoxide (58 mg, 1.07 mmol, 0.37 eq) was added and the reaction was stirred over night. After completion, the solution was neutralized with Amberlite IR 120

(H^+) ion exchange resin, filtered and concentrated *in vacuo*. The remainder was dried in high vacuum to give (2-Methyl-5-*tert*-butylphenyl) 1-thio-ß-D-glucopyranoside **S1** (1.0 g) which was used for the next reaction step without further purification. Tetrol **S1** (1.0 g) was dissolved in anhydrous acetonitrile (11.3 mL) at RT under argon atmosphere and benzaldehyde dimethylacetal (880 µL, 5.84 mmol, 2 eq) and camphorsulfonic acid (7 mg, 0.029 mmol, 0.01 eq) were added. After 2.5 h (TLC: cyclohexane/ethyl acetate, 1:2), the reaction was quenched with triethylamine, and the solvents were evaporated *in vacuo* to give 1.5 g of colorless oil. The crude product was purified by column chromatography on silica gel (cyclohexane/ethyl acetate) to afford **10** (1.09 g, 2.53 mmol, 87%). $[\alpha]_D^{20}$ = -49.4 ° (c = 1.0, CH_2Cl_2); IR (CH_2Cl_2): 3410, 2963, 2870, 1384, 1264, 1082, 1072, 1029, 1003, 972 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (1H, d, *J* 2.0 Hz, Ar-H), 7.51-7.46 (2H, m, Ar-H), 7.39-7.35 (m, 3H, Ar-H), 7.29-7.23 (m, 2H, Ar-H), 7.16 (1H, d, *J* = 8.0, Ar-H), 5.54 (1H, s, benzylidene-H), 4.64 (1H, d, *J* 10.0, 1-H), 4.36 (1H, dd, J_1 10.3, J_2 4.5, 6-Ha), 3.90-3.73 (2H, m, 3-H, 6-Hb), 3.59-3.47 (3H, m, 2-H, 4-H, 5-H), 2.86 (1H, d, *J* 2.2, OH), 2.69 (1H, d, *J* 2.4, OH), 2.42 (3H, s, CH_3), 1.32 (9H, s, *t*-Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 137.1, 137.0, 131.0, 130.3, 130.2, 129.4, 128.5, 126.4, 125.5 (C-aromatic), 102.0 (C-benzylidene), 88.8 (C-1), 80.4 (C-2), 74.8 (C-3), 73.0 (C-4), 70.5 (C-5), 68.7 (C-6), 31.4 (*t*Bu), 20.6 (CH_3); HRMS (ESI): Calcd for $C_{24}H_{30}O_5S$ $[M+Na]^+$ 453.1706, found 453.1714.

(2-Methyl-5-*tert*-butylphenyl)-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-ß-D-glucopyranoside (11)

Compound **10** (658 mg, 1.53 mmol) and imidazole (208 mg, 3.06 mmol, 2 eq) were dissolved in anhydrous DMF (880 µL). TBSCl (346 mg, 2.29 mmol, 1.5 eq) was gradually added with stirring. After 4 h, the solvent was evaporated and the resulting oil was dissolved in DCM. The solution was extracted with 1 M HCl

and saturated aqueous $NaHCO_3$ solution, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The colorless solid was dried in high vacuum and the crude product (820 mg) was purified using flash column chromatography (cyclohexane/ethyl acetate) to afford **11** (573 mg, 1.05 mmol, 69 %). $[\alpha]_D^{20}$ = -49.1 ° (c = 1.0, CH_2Cl_2); IR (CH_2Cl_2): 3559, 2957, 2928, 2858, 1631, 1383, 1259, 1110, 1086, 1067, 1009 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.61 (1H, d, *J* 2.1, Ar-H), 7.51-7.46 (2H, m, Ar-H), 7.39-7.33 (3H, m, Ar-H), 7.26-7.22 (1H, m, Ar-H), 7.15 (1H, d, *J* 8.0, Ar-*H*), 5.52 (1H, s, benzylidene-H), 4.65 (1H, d, *J* 9.8, 1-H), 4.34 (1H, dd, J_1 10.4, J_2 4.4, 6-Ha), 3.84-3.74 (2H, m, 6-Hb, 3-H), 3.54-3.45 (3H, m, 4-H, 5-H, 2-H), 2.42 (3H, s, CH_3), 1.31 (9H, s, *t*Bu), 0.88 (9H, s, *t*Bu), 0.11 (3H, s, CH_3), 0.04 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 137.3, 137.0, 131.4, 130.1, 130.1, 129.1, 128.3, 126.3, 125.3 (C-aromatic), 101.8 (C-benzylidene), 89.0 (C-1), 81.2 (C-4), 76.2 (C-3), 74.0 (C-2), 70.8 (C-5), 68.8 (C-6), 31.4 (*t*Bu), 26.0 (*t*Bu), 20.6 (CH_3), -4.2 (CH_3), -4.6 (CH_3); HRMS (ESI): Calcd for $C_{30}H_{44}O_5SSi$ $[M+Na]^+$ 567.2571, found 567.2584.

(2-Methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-1-thio-ß-D-glucopyranoside (12)

To a solution of **11** (2.00 g, 3.67 mmol) in anhydrous DMF (20 ml), NaH (0.21 g, 8.81 mmol) and BnBr (1.31 ml, 11.01 mmol) were added at 0 °C. The mixture was warmed to room temperature and stirred over night. Then cooled to 0 °C, quenched with MeOH and diluted with Et_2O. The organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded crude (2-methyl-5-*tert*-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-ß-D-glucopyranoside **S2** (2.4 g), which was taken directly to the next step. Crude **S2** (2.4 g) was dissolved in THF (30 ml), cooled to 0 °C and treated with a solution of TBAF (1 M in THF, 7.24 ml, 7.24 mmol). The mixture was warmed to room temperature over night

and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **12** (1.77 g, 3.40 mmol, 93%). $[\alpha]_D^{20}$ = -11.4 ° (c = 3.7, $CHCl_3$), IR ν_{max} (film) 3463, 3033, 2962, 1810, 1670, 1602, 1488, 1455, 1384, 1264, 1215, 1088 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.64-7.61 (1H, m, Ar-H), 7.51-7.20 (11H, m, Ar-H), 7.17-7.12 (1H, m, Ar-H), 5.55 (1H, s, benzylidene-H), 4.99 (1H, d, A of AB, J_{AB} 10.9, $-CH_2$-Bn) , 4.84 (1H, d, B of AB, J_{AB} 10.9, $-CH_2$-Bn), 4.75 (1H, d, *J* 9.8, 1-H), 4.34 (1H, dd, J_1 10.5, J_2 5.0, 6-Ha), 3.97-3.89 (1H, m, 3-H), 3.81 (1H, dd, J_1 J_2 10.3, 6-Hb), 3.60 (1H, dd, J_1 J_2 9.4, 4-H), 3.55-3.42 (2H, m, 2-H, 5-H), 2.52 (1H, d, *J* 2.4, 3-OH), 2.42 (3H, s, CH_3), 1.31 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 138.1, 137.1, 136.3, 132.8, 130.1, 129.4, 129.1, 128.7, 128.5, 128.4, 128.2, 126.4, 125.0, 102.0, 88.2 (C-1), 81.1 (C-2), 80.5 (C-4), 75.7, 75.6 (C-3), 70.1 (C-5), 68.8 (C-6), 34.6, 31.5, 20.5; HRMS (ESI): Calcd for $C_{31}H_{36}O_5S$ $[M+Na]^+$ 543.2181, found 543.2181.

(2-Methyl-5-tert-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (13)

To a solution of **12** (415 mg, 0.80 mmol) and pyridine (129 µl) in DCM (5 ml), Fmoc-Cl (309 mg, 1.20 mmol) was added and the mixture was stirred over night, diluted with DCM and the organic layers were washed with a 0.01 M HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **13** (561 mg, 0.76 mmol, 95%) as a white solid. $[\alpha]_D^{20}$ = -0.3 ° (c = 5.9, $CHCl_3$), IR ν_{max} (film) 3033, 2961, 1955, 1754, 1605, 1451, 1385, 1251, 1077 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.79-7.73 (2H, m, Fmoc-H), 7.65-7.13 (19H, m, Ar-H), 5.55 (1H, s, benzylidene-H), 5.29-5.22 (1H, m), 4.98 (1H, A of AB, J_{AB} 10.7, $-CH_2$-Bn), 4.82 (1H, d, *J* 9.8, H-1), 4.72 (1H, B of AB, *J* 10.7, $-CH_2$-Bn), 4.49-4.42 (1H, m), 4.40-4.28 (2H, m), 4.24-4.18 (1H, m), 3.88-3.67 (3H, m),

3.60-3.52 (1H, m), 2.42 (3H, s, CH_3), 1.31 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.6, 149.8, 143.5, 143.3, 141.4, 137.5, 136.9, 136.6, 130.2, 129.6, 129.2, 128.4, 128.3, 128.2, 128.00, 127.97, 127.30, 127.27, 126.3, 126.2, 125.2, 120.1, 101.6, 88.7 (C-1), 79.5, 79.3, 78.5, 75.7, 70.33, 70.27, 68.8, 46.8, 34.6, 31.4, 20.5; HRMS (ESI): Calcd for $C_{46}H_{46}O_7S$ $[M+Na]^+$ 765.2862, found 765.2886.

(2-Methyl-5-*tert*-butylphenyl)-2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (14)

To a solution of **13** (100 mg, 0.14 mmol) in anhydrous DCM (3 ml) freshly activated molecular sieves (4 Å) were added. The mixture was cooled to -78 °C, TES (64 µl, 0.40 mmol) and TfOH (41 µl, 0.46 mmol) were added. After stirring for 3 hours at -78 °C the reaction was quenched by the addition of pyridine, diluted with DCM and washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **14** (73 mg, 0.10 mmol, 73%). $[\alpha]_D^{20}$ = +10.5 ° (c = 4.9, $CHCl_3$), IR ν_{max} (film) 3486, 3031, 2959, 1951, 1750, 1604, 1451, 1387, 1254, 1054 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (2H, m, Fmoc-H), 7.66-7.56 (3H, m, Ar-H), 7.44-7.09 (16H, m, Ar-H), 4.95 (1H, dd, J_1 J_2 9.2 , 3-H), 4.92 (1H, d, *J* 10.7, -CH_2-Bn), 4.69 (1H, d, *J* 9.8, 1-*H*), 4.68 (1H, d, *J* 10.8, -CH_2-Bn), 4.61 (1H, A of AB, J_{AB} 12.0, -CH_2-Bn), 4.55 (1H, B of AB, J_{AB} 12.0, -CH_2-Bn), 4.50-4.43 (1H, m, Fmoc-CH_2), 4.40-4.31 (1H, m, Fmoc-CH_2), 4.26-4.20 (1H, m, Fmoc-CH), 3.84 (1H, ddd, J_1 J_2 9.5, J_3 3.6, 4-H), 3.81-3.74 (2H, m, 6-H), 3.61 (1H, dd, J_1 J_2 9.5, 2-H), 3.56-4.49 (1H, m, 5-H), 2.97 (1H, d, *J* 3.6, 4-OH), 2.40 (1H, s, CH_3), 1.26 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.7, 149.8, 143.5,143.4, 141.4, 137.7, 137.6, 136.5, 132.8, 130.1, 129.5, 128.6, 128.4, 128.2 128.04, 127.98, 127.9, 127.3, 125.3, 125.2, 125.0, 120.2, 88.1 (C-1), 83.2 (C-3), 78.5 (C-2), 77.8 (C-5), 75.4,

73.9, 71.0 (C-4), 70.4, 70.3 (C-6), 46.9, 34.6, 31.4, 20.5; HRMS (ESI): Calcd for $C_{46}H_{48}O_7S$ $[M+Na]^+$ 767.3018, found 767.3038.

(2-Methyl-5-*tert*-butylphenyl)-2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (3)

To a solution of **14** (480 mg, 0.64 mmol) in DCM (8 ml) and pyridine (0.3 ml) Lev_2O (55 mg, 0.26 mmol) was added and stirred for three days. The mixture was diluted with DCM and washed with a 1 M HCl solution and with saturated aqueous $NaHCO_3$ solution. The organic layers were dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **3** (428 mg, 0.51 mmol, 79%). $[\alpha]_D^{20}$ = +19.2 ° (c = 1.0, $CHCl_3$), IR ν_{max} (film) 3065, 2955, 1754, 1719, 1604, 1488, 1452, 1363, 1259, 1152, 1070, 1039 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.80-7.74 (2H, m, Ar-H), 7.68-7.58 (3H, m, Ar-H), 7.44-7.17 (15H, m, Ar-H), 7.15-7.11 (1H, m, Ar-*H*), 5.20 (1H, dd, J_1 J_2 9.7, 4-H), 5.15-5.07 (1H, m, 3-H), 4.95 (1H, A of AB, J_{AB} 10.8, -CH_2-Bn), 4.71 (1H, d, *J* 9.8, 1-H), 4.69 (1H, B of AB, J_{AB} 10.4, -CH_2-Bn), 4.56-4.41 (3H, m), 4.29-4.20 (2H, m), 3.74-3.55 (4H, m, 2-H, 4-H, 6-H), 2.60-2.52 (2H, m, Lev-CH_2), 2.42 (3H, s, Lev-CH_3), 2.41-2.32 (2H, m, Lev-CH_2), 2.02 (3H, s, SPhCH_3), 1.26 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.0, 171.6, 154.8, 149.9, 143.7, 143. 5, 141.4, 141.3, 138.0, 137.6, 136.6, 132.7, 130.1, 129.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 127.4, 127.3, 125.5, 125.4, 125.0, 120.1, 88.2, 80.5, 78.9, 77.3, 75.6, 73.7, 70.6, 69.4, 69.2, 46.7, 37.8, 34.6, 31.4, 29.7, 28.0, 20.5; HRMS (ESI): Calcd for $C_{51}H_{54}O_9S$ $[M+Na]^+$ 865.3386 found 865.3412.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-1-thio-ß-D-glucopyranoside (15)

Thioglycoside **12** (1.00 g, 1.84 mmol) was dissolved under argon in anhydrous pyridine (4 mL). DMAP (67 mg, 0.55 mmol) was

added and the solution was cooled to 0 °C. BzCl (639 µL, 5.51 mmol) was added dropwise and the solution was heated to 70 °C and stirred for 12 h. After completion (TLC: cyclohexane/ethyl acetate, 9:1), the reaction was quenched with methanol. The suspension was diluted with DCM and extracted with 1 M HCl and H_2O. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **15** (1.05 g, 1.62 mmol, 88%). $[\alpha]_D^{20}$ = +22.9 °(c = 1.0, CH_2Cl_2); IR (CH_2Cl_2): 2959, 2929, 2858, 1732, 1384, 1266, 1096, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.08 (2H, dd, *J* 8.3, Ar-H), 7.56 (1H, d, *J* 1.8, Ar-H), 7.52-7.43 (5H, m, Ar-H), 7.37 (3H, dd, J_1 5.2, J_2 2.0, Ar-H), 7.20 (1H, dd, J_1 8.0, J_2 2.1, Ar-H), 7.07 (1H, d, *J* 8.0, Ar-*H*), 5.58 (1H, s, benzylidene-H), 5.35 (1H, dd, J_1 10.3, J_2 8.6, 2-H), 4.84 (1H, d, *J* 10.3, 1-H), 4.38 (1H, dd, J_1 10.5, J_2 5.0, 6-Ha), 4.06 (1H, dd, J_1 J_2 8.9, 3-H), 3.88 (1H, dd, J_1 10.3, J_2 5.0, 6-Hb), 3.69 (1H, dd, J_1 J_2 9.1 Hz, 4-H), 3.60-3.52 (1H, m, 5-H), 2.18 (3H, s, CH_3), 1.28 (9H, s, *t*Bu), 0.70 (9H, s, *t*Bu), -0.05 (3H, s, CH_3), -0.14 (3H, s, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 133.1, 129.9, 129.8, 129.4, 129.1, 128.3, 128.1, 126.2, 125.1 (C-Ar), 101.9 (C-benzylidene), 88.1 (C-1), 81.3 (C-4), 74.3 (C-3), 73.6 (C-2), 70.6 (C-5), 68.7 (C-6), 31.3 (*t*Bu), 25.5 (*t*Bu), 20.2 (CH_3), -4.2 (CH_3), -5.0 (CH_3); HRMS (ESI): Calcd for $C_{37}H_{48}O_6SSi$ $[M+Na]^+$ 671.2833, found 671.2852.

(2-Methyl-5-tert-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-ß-D-glucopyranoside (16)

To a solution of **15** (200 mg, 0.31 mmol) in DMF (1 mL) a solution of TBAF·$3H_2O$ (683 mg, 1.85 mmol) and glacial acetic acid (124 µL, 2.16 mmol) in DMF (1 mL) were added. The mixture was warmed to 35 °C for 9 h, diluted with ether and washed with a 0.01 M HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **16** (150 mg, 0.28 mmol, 91%). $[\alpha]_D^{20}$ = -5.5 ° (c = 0.8, $CHCl_3$); IR ($CHCl_3$): 3455, 2963, 2870,

1729, 1268, 1100, 1071 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11 (2H, d, *J* 7.4, Ar-H), 7.64-7.33 (9H, m, Ar-H), 7.27-7.20 (1H, m, Ar-H), 7.10 (1H, d, *J* 8.0, Ar-H), 5.59 (1H, s, benzylidene-H), 5.25 (1H, dd, $J_1$10.1, J_2 8.7, 2-H), 4.88 (1H, d, *J* 10.1, 1-H), 4.40 (1H, dd, J_1 10.5, J_2 5.0, 6-Ha), 4.09 (1H, dd, J_1 9.0, J_2= 8.7, 3-H), 3.87 (1H, dd, J_1 10.4, J_2 5.0, 6-Hb), 3.71 (1H, dd, J_1 9.0, J_2 9.7, 4-H), 3.57 (1H, td, J_1 9.7, J_2 5.0, 5-H), 2.83 (1H, br, 3-OH), 2.23 (3H, s, CH_3), 1.29 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 166.1 (C=O benzoyl), 149.7, 137.32, 136.9, 133.6, 131.9, 130.4, 130.2, 129.5, 128.6, 128.5, 126.4, 125.6 (aromatics), 102.1 (C-benzylidene), 87.5 (C-1), 80.9 (C-4), 74.0 (C-3), 73.6 (C-2), 70.5 (C-5), 68.7 (C-6), 31.4 (*t*Bu), 20.4 (CH_3); HRMS (ESI): Calcd for $C_{31}H_{34}O_6S$ $[M+Na]^+$ 557.1968, found 557.1975.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenylmethoxycarbonyl-1-thio-ß-D-glucopyranoside (17)

To a solution of **16** (277 mg, 0.52 mmol) and pyridine (130 µl) in DCM (4 ml), Fmoc-Cl (268 mg, 1.04 mmol) was added and the mixture stirred over night, diluted with DCM and the organic layers were washed with a 0.01 M HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **17** (378 mg, 0.50 mmol, 96%). $[\alpha]_D^{20}$ = +50.2 ° (c = 4.5, $CHCl_3$), IR ν_{max} (film) 3066, 2961, 1752, 1732, 1602, 1488, 1450, 1385, 1316, 1268, 1250, 1093 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06-7.99 (2H, m, Ar-H), 7.73-7.67 (2H, m, Ar-H), 7.61-7.07 (19H, m, Ar-H), 5.60 (1H, s, benzylidene-H), 5.51-5.36 (2H, m, 2-H, 3-H), 4.95 (1H, d, *J* 9.9, 1-H), 4.46-4.39 (1H, m, 6-*H*), 4.27-4.16 (2H, m, Fmoc-CH_2), 4.06-4.00 (1H, m, Fmoc-CH), 3.98-3.88 (2H, m, 4-H, 6-H), 3.72-3.63 (1H, m, 5-H) 2.23 (1H, s, CH_3), 1.29 (9H, s, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.3, 154.6, 149.8, 143.4, 143.2, 141.3, 141.2, 137.4, 136.8, 133.6, 131.7, 130.5, 130.2, 130.1, 129.3, 129.2, 128.5, 128.3, 127.9, 127.27, 127.25, 126.3, 125.8,

125.3, 125.1, 120.00, 119.99, 101.8, 88.0 (C-1), 78.3 (4-H), 77.3 (C-3), 71.4 (C-2), 70.8 (C-5), 70.5, 68.7 (C-6), 46.6, 34.6, 31.7, 31.4, 20.4, 14.3; HRMS (ESI): Calcd for $C_{46}H_{44}O_8S$ $[M+Na]^+$ 779.2655, found 779.2649.

Dibutyl-2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-fluorenyl-methoxycarbonyl-D-gluco-pyranosidephosphate (4)

Thioglucoside **17** (690 mg, 0.91 mmol) was coevaporated with toluene three times and dried *in vacuo*, then dissolved in anhydrous DCM (10 ml). Freshly activated molecular sieves (4 Å) and dibutyl hydrogen phosphate (542 µl, 2.73 mmol) were added and the solution cooled to 0 °C. NIS (246 mg, 1.09 mmol), followed by TfOH (10 µl, 0.11 mmol) was added and stirred at 0 °C for one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetate) to afford **4** (583 mg, 0.74 mmol, 81%) in a mixture of α/β-anomers (α/β=1:4). NMR data are reported for the β-anomer. $[\alpha]_D^{20}$ = +8.9 ° (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3067, 2961, 1755, 1733, 1602, 1451, 1268, 1096, 1026 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.06-7.99 (2H, m, Ar-H), 7.72-7.66 (2H, m, Ar-H), 7.55-7.29 (12H, m, Ar-H), 7.18-7.11 (2H, m, Ar-H), 5.60-5.54 (2H, m, benzylidene-H, 1-H), 5.50 (1H, dd, J_1 J_2 9.4, 2-H), 5.36 (1H, dd, J_1 J_2 9.4, 3-H), 4.49-4,41 (1H, m, 6-H), 4.30-4.18 (2H, m, Fmoc-CH_2), 4.10-4.01 (3H, m, Fmoc-H, phosphate-CH_2), 4.00-3.94 (1H, m, 4-H), 3.90-3.86 (1H, m, 6-H), 3.82-3.67 (3H, m, phosphate-CH_2, 5-*H*), 1.67-1.60 (2H, m, phosphate-CH_2), 1.42-1.25 (4H, m, phosphate-CH_2), 1.10-1.01 (2H, m, phosphate-CH_2), 0.92 (3H, t, *J* 7.4, phosphate-CH_3), 0.70 (3H, t, *J* 7.4, phosphate-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.1, 154.5, 143.4, 143.1, 141.3, 136.6, 133.8, 130.1, 129.4, 128.6, 128.4, 127.9, 127.2, 126.3, 125.3, 125.2, 120.0, 101.9, 96.91, 96.86, 78.1, 77.5, 77.2, 76.8, 75.8, 72.6, 70.6,

68.4, 68.1, 67.1, 46.6, 32.2, 32.1, 32.0, 31.9, 18.7, 18.4, 13.7, 13.5; δ_P (160 MHz, $CDCl_3$) -2.95; HRMS (ESI): Calcd for $C_{43}H_{47}O_{12}P$ $[M+Na]^+$ 809.2703, found 809.2690.

4-Methoxyphenyl-2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamno-pyranoside (19)

Rhamnoside **18** (500 mg, 1.39 mmol) was dissolved in a solution of DCM (1 ml) and pyridine (1 ml). DMAP (68 mg, 0.56 mmol) was added and the mixture cooled to 0 °C, then BzCl (780 mg, 5.56 mmol) was added and the reaction warmed to room temperature over night. The reaction was quenched with MeOH, diluted with DCM and the organic layer was washed with a 0.01 M HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **19** (768 g, 1.35 mmol, 97%). $[\alpha]_D^{20}$ = +17.6 ° (c = 3.1, $CHCl_3$), IR ν_{max} (film) 3064, 2934, 1725, 1602, 1506, 1452, 1363, 1273, 1213, 1094, 1027 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.11-8.05 (2H, m, Ar-H), 7.98-7.93 (2H, m, Ar-H), 7.67-7.61 (1H, m, Ar-H), 7.56-7.49 (3H, m, Ar-H), 7.40-7.35 (2H, m, Ar-H), 7.25-7.16 (5H, m, Ar-H), 7.08-7.03 (2H, m, Ar-H), 6.87-6.82 (2H, m, Ar-H), 5.94 (1H, dd, J_1 9.6, J_2 3.4, 3-H), 5.79 (1H, dd, J_1 3.4, J_2 1.9, 2-H), 5.54 (1H, d, J 1.8, 1-H), 4.75 (1H, A of AB, J_{AB} 10.9, $-CH_2$-Bn), 4.68 (1H, B of AB, J_{AB} 10.9, $-CH_2$-Bn), 4.20-4.11 (1H, m, 5-H), 3.88 (1H, dd, J_1 J_2 9.6, 4-H), 3.78 (3H, s, $-CH_3$), 1.41 (3H, d, J 6.2, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.58, 165.55, 155.21, 150.20, 137.7, 133.6, 133.3, 130.0, 129.9, 129.8, 129.71, 128.69, 128.53, 128.48, 128.2, 128.0, 117.9, 114.7, 96.6 (C-1), 79.1 (C-4), 75.3, 72.3 (C-3), 71.2 (C-2), 68.5 (C-5), 55.8, 18.3 (C-6); HRMS (ESI): Calcd for $C_{34}H_{32}O_8$ $[M+Na]^+$ 591.1995, found 591.1985.

2,3-Di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside-*N*-phenyl-trifluoroacetimidate (5)

CAN (2.17 g, 3.96 mmol) was added to a mixture of **19** (750 mg, 1.32 mmol) in MeCN (12 ml) and H_2O (12 ml) and stirred vigorously for 2 h. H_2O and EtOAc were added, the layers separated, the organic layer washed with H_2O and brine, dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded the lactol as an orange solid (548 mg). A solution of the lactol (548 mg) in DCM (10 ml) was cooled to 0 °C, $CF_3C(NPh)Cl$ (438 mg, 2.11 mmol) and Cs_2CO_3 (688 mg, 2.11 mmol) were added and the resulting solution was stirred overnight at room temperature, diluted with DCM, filtered through a plug of celite and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **5** (619 mg, 0.98 mmol, 74%). $[\alpha]_D^{20}$ = +41.2 ° (c = 4.8, $CHCl_3$), IR ν_{max} (film) 3065, 2981, 1727, 1600, 1490, 1452, 1270, 1208, 1164, 1091 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-8.02 (2H, m, Ar-H), 7.96-7.89 (2H, m, Ar-H), 7.66-7.60 (1H, m, Ar-H), 7.57-7.46 (3H, m, Ar-H), 7.40-7.19 (9H, m, Ar-H), 7.40-7.19 (9H, m, Ar-H), 7.14-7.07 (1H, m, Ar-H), 6.91-6.82 (2H, m, Ar-H), 6.35 (1H, bs, 1-H), 5.84 (1H, s, 2-H), 5.77 (1H, dd, J_1 9.4, J_2 3.3, 3-H), 5.35 (1H, dd, J_1 3.7, J_2 1.9, 1-H), 4.76 (1H, A of AB, J_{AB} 10.9, $-CH_2$-Bn), 4.68 (1H, B of AB, J_{AB} 10.9, $-CH_2$-Bn), 4.21-4.08 (1H, m, 5-H), 3.87 (1H, dd, J_1 J_2 9.5, 4-H), 1.48 (3H, d, J 6.1, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.5, 165.3, 143.4, 137.4, 133.7, 133.4, 130.0, 129.8, 129.7, 129.4, 128.9, 128.7, 128.58, 128.57, 128.3, 128.2, 124.6, 119.6, 94.1 (C-1), 78.5 (C-4), 75.5, 72.0 (C-3), 70.7 (C-3), 69.6 (C-2), 18.4 (C-6); HRMS (ESI): Calcd for $C_{35}H_{30}F_3NO_7$ $[M+Na]^+$ 656.1872, found 656.1852.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-_O_-benzyl-3-_O_-fluorenylmethoxycarbonyl-4-_O_-levulinoyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (20)

Glucoside donor **3** (326 mg, 0.34 mmol) and glucoside acceptor **2** (262 mg, 0.35 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in anhydrous Et_2O (3 ml), NIS (93 mg, 0.41 mmol) was added and cooled to -35°C. TfOH (3.7 µl, 41 µmol) was added and the mixture was stirred and warmed up to -10 °C in one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetate) to afford **20** (343 mg, 0.24 mmol, 70%). $[\alpha]_D^{20}$ = +64.4 ° (c = 5.9), IR ν_{max} (film) 3032, 2932, 1755, 1700, 1605, 1497, 1452, 1362, 1259 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.00-6.90 (43H, m, Ar-H), 5.41 (1H, dd, J_1 J_2 9.7), 5.26 (1H, dd, J_1 J_2 9.8), 5.18-5.10 (2H, m), 5.06 (1H, bs, anomeric-H), 5.03-4.96 (2H, m, anomeric-H), 4.88 (1H, app d, J 11.0), 4.82 (1H, app d, J 10.8), 4.68-4.58 (3H, m), 4.52-4.41 (5H, m), 4.39-4.30 (2H, m), 4.26 (1H, app t, J 7.5), 4.14-4.08 (1H, m), 4.07-4.01 (1H, m), 3.82 (1H, dd, J_1 9.9, J_2 3.4), 3.80-3.56 (6H, m), 3.34-3.31 (2H, m), 3.28-3.06 (4H, m), 2.54-2.42 (2H, m), 2.32-2.17 (2H, m), 2.00 (1H, s, Lev-CH_3), 1.65-1.50 (4H, m, linker-CH_2-), 1.30-1.23 (4H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.0, 171.5, 154.9, 143.7, 143.6, 141.40, 141.35, 138.0, 137.8, 128.6, 128.54, 128.53, 128.39, 128.37, 128.2, 128.1, 128.0, 127.94, 127.87, 127.74, 127.66, 127.5, 127.3, 126.3, 125.5, 120.1, 120.0, 95.6 (C-anomeric), 94.0 (C-anomeric), 80.7, 78.2, 77.4, 77.0, 76.8, 76.2, 75.9, 75.4, 73.7, 73.5, 72.4, 70.5, 70.3, 68.8, 68.6, 68.4, 67.3, 46.8, 37.8, 31.4,

29.8, 27.9, 23.7; HRMS (ESI): Calcd for $C_{87}H_{91}NO_{17}$ $[M+Na]^+$ 1444.6179, found 1444.6128.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-2,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (21)

To a solution of **20** (224 mg, 0.16 mmol) in DCM (4.5 ml) hydrazine hydrate (31 µl, 0.63 mmol) dissolved in AcOH (0.4 ml) and pyridine (0.6 ml) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **21** (196 mg, 0.15 mmol, 94%). $[\alpha]_D^{20}$ = +57.7 ° (c = 1.7), IR ν_{max} (film) 3423, 3031, 2926, 1753, 1697, 1605, 1586, 1497, 1452, 1422, 1362, 1255, 1068 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d6) δ 7.92-7.84 (2H, m, Ar-H), 7.78-7.64 (2H, m, Ar-H), 7.56-7.14 (35H, m, Ar-H), 5.44-5.37 (2H, m), 5.20-5.10 (3H, m), 5.07 (1H, d, *J* 10.7), 4.89-4.77 (3H, m), 4.66-4.47 (8H, m), 4.46-4.39 (2H, m), 4.27 (1H, app t, *J* 6.9), 4-20-4.14 (1H, m), 3.99 (1H, app t, *J* 9.3), 3.89-3.80 (2H, m), 3.78-3.59 (7H, m), 3.59-3.52 (1H, m), 3.49-3.42 (1H, m), 3.25-3.15 (2H, m), 2.82-2.79 (1H, m), 1.60-1.44 (4H, m, linker-CH_2-), 1.33-1.25 (2H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, acetone-d6) δ 155.9, 144.7, 144.6, 142.2, 142.1, 139.9, 139.8, 139.74, 139.68, 139.5, 139.4, 129.34, 129.26, 129.1, 129.02, 129.00, 128.9, 128.7, 128.62, 128.55, 128.5, 128.4, 128.20, 128.16, 128.14, 128.05, 128.0, 127.9, 126.1, 126.0, 120.88, 120.86, 96.3, 94.2, 81.8, 80.0, 79.2, 78.0, 76.5, 75.5, 73.8, 73.5, 72.1, 71.9, 71.5, 70.2, 70.08, 70.06, 69.5, 68.6, 67.4, 47.6, 27.5, 24.2; HRMS (ESI): Calcd for $C_{82}H_{85}NO_{15}$ $[M+Na]^+$ 1346.5817, found 1346.5784.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-2-_O_-benzoyl-4,6-_O_-benzylidene-ß-D-glucopyranosyl-(1→4)-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (23)

Phosphate **4** (74 mg, 94 µmol) and **21** (48 mg, 36 µmol) were coevaporated with toluene three times, dried *in vacuo* and then dissolved in anhydrous DCM (1.0 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to -30 °C. TMSOTf (18 µl, 98 µmol) was added and then warmed to -7 °C over 1.5 h. The reaction was quenched with pyridine and concentrated *in vacuo*. Column chromatography on silica gel (toluene/acetone) afforded crude **22**. 20% NEt_3 in DCM (1 ml) was added to crude **22** and stirred for 4 h, the mixture was concentrated *in vacuo* column chromatography on silica gel (toluene/acetone) afforded **23** (20 mg, 14 µmol, 38 %). $[\alpha]_D^{20}$ = +8.1 ° (c = 1.6), IR ν_{max} (film) 3462, 3032, 2924, 1732, 1699, 1603, 1497, 1453, 1364, 1268, 1093 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05-7.92 (2H, m, Ar-H), 7.63-7.06 (43H, m, Ar-H), 5.56 (1H, s, benzylidene-H), 5.24 (1H, app t, *J* 8.5), 5.20-5.11 (2H, m), 5.09-4.98 (2H, m, anomeric-H), 4.88 (1H, app d, *J* 10.7), 4.79-4.66 (4H, m, anomeric-H), 4.62-4.54 (1H, m), 4.49-4.36 (5H, m), 4.19-4.05 (2H, m), 4.03-3.91 (2H, m), 3.89-3.44 (14H, m), 3.39-3.04 (4H, m), 1.57-1.36 (4H, m, linker-CH_2-), 1.32-1.14 (2H, m, linker-CH_2-); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 138.51, 138.46, 138.4, 138.1, 136.8, 133.7, 130.1, 129.6, 129.4, 128.7, 128.6, 128.54, 128.52, 128.47, 128.45, 128.4, 127.98, 127.9, 127.84, 127.78, 127.7, 127.4, 126.4, 102.1, 101.7 (C-anomeric), 95.8 (C-anomeric), 94.5 (C-anomeric), 81.1, 80.8, 80.6, 78.2, 77.9, 77.4, 76.1, 75.2, 74.7, 73.6, 73.4, 72.8, 72.1, 71.6, 70.4, 69.5, 68.7, 68.4, 68.2, 67.8, 67.3, 66.4, 29.8, 29.4, 23.6; HRMS (ESI): Calcd for $C_{87}H_{93}NO_{19}$ $[M+Na]^+$ 1478.6239, found 1478.6136.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)-2-O-benzoyl-4,6-O-benzylidene-3-ß-D-glucopyranosyl-(1→4)-[2,3-Di-O-benzoyl-4-O-benzyl-α-L-rhamnopyranosyl-(1→3)]-2,6-di-O-benzyl-α-D-gluco-pyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (24)

Compounds **5** (26 mg, 41 µmol) and **23** (10 mg, 6.9 µmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (1.0 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to -30 °C. TMSOTf (10 µl of a solution of 7.4 µl TMSOTf in 93 µl DCM, 4.1 µmol) was added and the reaction was stirred at -30 °C for 1.5 h. The reaction was quenched with pyridine and concentrated *in vacuo*. Column chromatography on silica gel (toluene/acetone) afforded **24** (14 mg, 5.5 µmol, 81 %). $[\alpha]_D^{20}$ = + 5.2° (c =0.7), IR ν_{max} (film) 3032, 2933, 1728, 1602, 1585, 1496, 1452, 1363, 1263, 1094, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.20-6.90 (75H, m, Ar-H), 5.79-5.67 (3H, m), 5.46 (1H, s, benylidene-H), 5.33-5.29 (1H, m), 5.28-5.21 (1H, m), 5.17-5.08 (3H, m, anomeric-H), 5.02 (1H, bs, anomeric-H), 4.92-4.78 (4H, m, anomeric-H), 4.74-4.60 (4H, m), 4.59-4.49 (4H, m, anomeric-H), 4.48-4.44 (1H, m), 4.43-4.31 (4H, m), 4.29-4.13 (4H, m, anomeric-H), 4.03-3.88 (3H, m), 3.83-3.45 (13H, m), 3.40-3.02 (7H, m), 1.65 (1H, d, *J* 6.2, Rha-CH_3), 1.53-1.32 (4H, m, linker-CH_2-), 1.24-1.10 (2H, m, linker-CH_2-), 0.90 (1H, d, *J* 6.1, Rha-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 165.48, 165.5, 164.5, 164.2, 138.3, 137.8, 137.6, 133.1, 130.1, 130.0, 129.94, 129.85, 129.7, 129.4, 129.1, 129.0, 128.9, 128.83, 128.76, 128.7, 128.6, 128.51, 128.47, 128.45, 128.42, 128.36, 128.32, 128.29, 128.23, 128.17, 128.04, 128.00, 127.94, 127.88, 127.8, 127.7, 126.5, 126.4, 100.6 (C-anomeric), 100.5 (C-anomeric), 97.9 (C-anomeric), 97.5, 95.8 (C-anomeric), 93.5 (C-anomeric), 80.2, 79.2, 78.1, 77.5, 77.4, 77.2, 76.8, 76.2, 76.1, 76.0, 74.2, 74.0, 73.6, 72.9, 72.1, 71.6, 71.2, 70.9, 68.7, 67.4, 67.2, 50.6, 47.2, 46.2, 29.9, 23.6, 18.4, 17.5;

HRMS (ESI): Calcd for $C_{141}H_{141}NO_{31}$ $[M+Na]^+$ 2366.9385, found 2366.9440.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-ß-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (1)

Fully protected pentasaccharide **24** (10 mg, 4.3 µmol) was dissolved in a solution of NaOMe (0.5 M) in THF/MeOH (1:1, 1 ml) and heated to 50 °C for 12 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH=1:1) afforded the de-benzoylated pentasaccharide (5.6 mg), which was dissolved in a mixture of MeOH (0.9 ml), H_2O (0.1 ml) and AcOH (25 µl). The solution was purged with Argon, 10% Pd/C (10 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **1** (2.3 mg, 2.6 µmol, 61%). NMR data are reported in Table 1, comparison with the data from native PS-I is reported in Table 2. HRMS (MALDI-TOF): Calcd for $C_{35}H_{63}NO_{24}$ $[M+Na]^+$ 904.3632, found 904.3606.

Table 1: ^{1}H NMR δ (600 MHz, D_2O) and ^{13}C NMR δ (150 MHz, D_2O) of pentasaccharide **1**.[a]

	α-Glc **(A)**	*α-Glc* **(B)**	*β-Glc* **(C)**	*α-Rha* **(D)**	*α-Rha* **(D')**	*Linker*
H-1	5.18	5.09	4.53	5.24	5.14	
C-1	96.1	96.8	102.4	101.8	102.0	
H-2	3.70	3.73	3.38	4.06	4.06	
C-2	72.7	73.4	75.3	71.4	71.2	
H-3	3.70	4.03	3.61	3.88	3.81	
C-3	76.1	77.0	83.2	71.1	71.2	
H-4	3.48	3.86	3.46	3.47	3.47	
C-4	70.5	73.8	69.1	73.0	73.0	
H-5	3.82	4.05	3.45	4.43	4.03	
C-5	72.5	72.3	77.2	69.5	69.8	
H-6	3.88/3.	3.92	3.80/3.	1.27	1.27	
a/b	78		96			
C-6	61.6	60.3	62.2	17.5	17.5	
H-1'						3.79/3.
a/b						59
C-1'						68.7
H-2'						1.70
C-2'						29.0
H-3'						1.49
C-3'						23.5
H-4'						1.70
C-4'						27.7
H-5'						3.01
C-5'						40.4

[a] ^{1}H and ^{13}C NMR resonances were assigned based on HSQC, HMBC, COSY and TOCSY experiments.

Table 2: Comparison of ^{1}H and ^{13}C NMR δ between **1** and the native PS-I repeating unit.[a]

	α-Glc **(A)**	*α-Glc* **(B)**	*β-Glc* **(C)**	*α-Rha* **(D)**	*α-Rha* **(D')**
H-1	5.18	5.09	4.53	5.24	5.14
	5.75	*5.13*	*4.53*	*5.23*	*5.17*
C-1	96.1	96.8	102.4	101.8	102.0
	93.5	*98.0*	*102.4*	*101.9*	*101.4*
H-2	3.70	3.73	3.38	4.06	4.06
	3.68	*3.70*	*3.38*	*4.07*	*4.09*
C-2	72.7	73.4	75.3	71.4	71.2
	77.3	*73.6*	*75.2*	*71.1*	*71.2*
H-3	3.70	4.03	3.61	3.88	3.81
	3.89	*4.01*	*3.62*	*3.85*	*3.97*
C-3	76.1	77.0	83.2	71.1	71.2
	72.1	*77.5*	*83.0*	*71.0*	*70.9*
H-4	3.48	3.86	3.46	3.47	3.47
	3.53	*3.86*	*3.46*	*3.46*	*4.07*
C-4	70.5	73.8	69.1	73.0	73.0
	70.1	*73.6*	*69.1*	*73.0*	*78.9*
H-5	3.82	4.05	3.45	4.43	4.03
	3.91	*4.06*	*3.45*	*4.44*	*4.12*
C-5	72.5	72.3	77.2	69.5	69.8
	73.8	*72.4*	*77.1*	*69.4*	*68.6*
H-6 a/b	3.88/3.78	3.92	3.80/3.96	1.27	1.27
	n.d.	*n.d.*	*3.80/3.95*	*1.27*	*1.33*
C-6	61.6	60.3	62.2	17.5	17.5
	n.d.	*n.d.*	*62.2*	*17.5*	*17.8*

[a] data of native PS-I reported in italic taken from: J. Ganeshapillai *et al.*, *Carbohydr. Res.*, 2008, 343, 703.

Synthesis of pentasaccharide 1 and intermediates according to schemes 6-8

(2-Methyl-5-tert-butylphenyl) 4,6-*O*-benzylidene-2-*O*-benzyl-3-*O*-(4-bromo)benzyl-1-thio-β-D-glucopyranoside (25)

To a solution of **12** (200 mg, 0.38 mmol) in anhydrous DMF (2 ml), NaH (22 mg, 0.92 mmol) was added followed by *para*-bromobenzyl (PBB) bromide (288 mg, 1.15 mmol) at 0 °C. The mixture was warmed to room temperature over 2 h, cooled to 0 °C and quenched by the addition of MeOH. Et_2O was added and the organic layer washed with 0.1 M HCl solution and with saturated aqueous $NaHCO_3$ solution. The phases were separated and the organic layer was dried over $MgSO_4$ and concentrated. Column chromatography (cyclohexane/ethyl acetate) afforded **25** (276 mg) along with aromatic impurities and was taken to the next step without further purification.

(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-(4-bromo) benzyl-1-thio-β-D-glucopyranoside (26)

To a solution of **25** (140 mg, 0.20 mmol) in anhydrous DCM (4 ml) freshly activated molecular sieves (4 Å) were added. The mixture was cooled to -78 °C, TES (97 µl, 0.61 mmol) and TfOH (61 µl, 0.69 mmol) were added. After stirring for 3 hours at -78 °C, the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, diluted with DCM and washed with a saturated aqueous $NaHCO_3$ solution. The organic phase was then dried over $MgSO_4$, filtered and concentrated. Column chromatography on silica gel (cyclohexane/ethyl acetate) afforded **26** (81 mg, 0.12 mmol, 58%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.65 -7.60 (m, 1H, ArH), 7.54 -7.10 (m, 16H, ArH), 4.98 (d, 1H, *J*=10.3 Hz, benzyl), 3.65-3.44 (m, 6H, benzyl, 1-H), 3.79-3.70 (m, 3H, 6-H, 4-H), 3.56-3.43 (m, 3H, 2-H, 3-H, 5-H), 2.76 (d, 1H, *J*= 2.2 Hz, 4-OH), 2.40 (s, 3H, CH_3), 1.26 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.7, 138.1, 137.72, 137.67, 136.1,

133.3, 131.7, 130.0, 129.6, 128.9, 128.6, 128.5, 128.3, 128.0, 128.0, 124.7, 121.8, 88.2 (C-1), 86.2 (C-2), 80.7 (C-3), 77.5 (C-5), 75.7, 74.7, 73.9, 72.7 (C-4), 70.7 (C-6), 31.4, 20.5; HRMS (ESI): Calcd for $C_{38}H_{43}BrO5SNa^+$ $[M+Na]^+$ 713.1907, found 713.1951.

(2-Methyl-5-*tert*-butylphenyl) 2,6-di-*O*-benzyl-3-*O*-(4-bromo) benzyl-4-*O*-levulinoyl-1-thio-β-D-glucopyranoside (27)

To a solution of **26** (1.55 g, 2.24 mmol) in DCM (20 ml) at 0 °C, DMAP (274 mg, 2.24 mmol), LevOH (1.30 ml, 11.20 mmol) and DCC (2.31 g, 11.20 mmol) were added. The solution was warmed to room temperature and stirred for 16 h. The reaction was diluted with DCM and the organic layers were washed with a 0.1 M HCl solution and saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **27** (1.54 g, 1.95 mmol, 87%). $[\alpha]_D^{20}$ = + 6.4° (c =3.4, $CHCl_3$), IR ν_{max} (film) 2963, 1744, 1718, 1488, 1361, 1261, 1068, 1038, 1012 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.70-7.05 (m, 17H, Ar-H), 5.11-5.04 (m, 1H, 4-H), 4.97 (app. d, 1H, *J*=10.4 Hz, benzyl-H), 4.77-4.60 (m, 4H, benzyl-H, 1-H), 4.48 (s, 1H, PBB-H), 3.70-3.54 (m, 5H, 2-H, 3-H, 5-H, 6-H), 2.64-2.55 (m, 2H, Lev-CH_2), 2.40 (s, 3H, S-CH_3), 2.35-2.29 (m, 2H, Lev-CH_2), 2.12 (s, 3H, Lev-CH_3), 1.25 (s, 9H, *t*Bu); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.2 (Lev-carbonyl), 171.7, 149.8, 138.1, 138.0, 137.5, 136.2, 133.2, 131.6, 130.0, 129.6, 128.9, 128.5, 128.4, 128.3, 128.1, 128.0, 127.69, 124.71, 121.6, 88.3 (C-1), 84.1, 81.1, 77.4, 75.8, 74.5, 73.7, 71.3, 69.7, 37.8, 34.6, 31.4, 29.9, 28.0, 20.5; HRMS (MALDI-TOF): Calcd for $C_{43}H_{49}BrO_7SNa^+$ $[M+Na]^+$ 811.2275, found 811.2026.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-ß-D-glucopyranoside (28)

To a solution of **15** (800 mg, 1.23 mmol) in anhydrous DCM (12 ml) freshly BH_3·THF (1 M in THF, 7.4 ml, 7.4 mmol) and TMSOTf (0.11 ml, 0.62 mmol) were added drop wise at 0°C. The reaction was warmed to room temperature over 2 hours, cooled to 0°C again and quenched by the drop wise addition of saturated aqueous $NaHCO_3$ solution. The Emulsion was diluted with DCM and washed with a saturated aqueous NaHCO3 solution. The organic phase was then dried over MgSO4, filtered and concentrated. Crude **28** was taken to the next step.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-ß-D-glucopyranoside (29)

To a solution of crude **28** (approx. 1.23 mmol) in THF/DMF (9:1, 10 ml) at 0°C, BnBr (0.18 ml, 1.50 mmol) and NaH (36 mg, 1.50 mmol) were added. The solution was warmed to room temperature over 2h, then cooled to 0°C again and further BnBr (0.18 ml, 1.50 mmol) was added. The reaction was warmed to room temperature over 30 min, cooled to 0°C and quenched by the addition of water. After dilution with Et_2O the phases were separated and the aqueous layer extracted with Et_2O. The organic phase was then dried over MgSO4, filtered and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **29** (797 mg, 1.08 mmol, 88%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.14-7.00 (m, 18H, Ar-H), 5.31 (dd, 1H, J_1=10.1 Hz, J_2=8.9 Hz, 2-H), 4.83 (app. d, 1H, J=11.3 Hz, benzyl-H_a), 4.72 (d, 1H, J=10.2 Hz, 1-H), 4.63 (app. d, 1H, J=11.0 Hz, benzyl-H_b), 4.58 (app. d, 2H, J=3.1 Hz, benzyl-H), 3.95 (app. t, 1H, J=8.7 Hz, 3-H), 3.78-3.51 (m, 4H, 4-H, 5-H, 6-H), 2.15 (s, 3H, S-CH_3), 1.25 (s, 9H, S- *t*Bu), 0.79 (s, 9H, TBS- *t*Bu), 0.00 (s, 3H, TBS-CH_3), -0.16 (s, 3H, TBS-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 149.7, 138.2, 136.5, 133.2, 130.5, 130.1, 129.8, 129.2, 128.5, 128.4, 128.0, 127.72,

127.68, 127.6, 124.7, 88.0 (C-1), 79.5 (C-5), 78.9 (C-4), 77.0 (C-3), 75.1, 73.6 (C-2), 73.5, 69.0 (C-6), 31.4, 25.8, 20.3, -3.9, -4.1; HRMS (ESI): Calcd for $C_{44}H_{56}O_6SSiNa^+$ $[M+Na]^+$ 763.3459, found: 763.3500

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-O-benzyl-3-O-(4-bromo)benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-Tri-O-benzyl-α-D-gluco-pyranoside (30)

Thioglucoside **27** (323 mg, 0.41 mmol) and glucoside **2** (222 mg, 0.29 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in Ether (4 ml), freshly activated and acid washed molecular sieves (4 Å) and NIS (105 mg, 0.47 mmol) were added and cooled to -40°C. TfOH (4.2 µl, 0.05 mmol) was added and the mixture was stirred and warmed up to -10 °C in one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford **30** (276 mg, 0.20 mmol, 69%). $[\alpha]_D^{20}$ = + 54.1° (c =4.8, $CHCl_3$), IR ν_{max} (film) 3031, 2923, 2864, 1744, 1698, 1497, 1454, 1420, 1360, 1209 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.60-7.02 (m, 39H, Ar-H), 5.24-5.10 (m, 3-H), 5.09-4.93 (m, 3H, 2 × anomeric-H), 4.89-4.37 (m, 13H), 4.13-4.00 (m, 2H), 3.99-3.56 (m, 8H), 3.50-3.08 (m, 5H), 2.63-2.47 (m, 2H), 2.25-2.18 (m, 2H), 2.13 (s, 3H, Lev-CH_3), 1.71-1.38 (m, 4H, linker-H), 1.36-1.14 (m, 2H, linker-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3 (Lev-carbonyl), 171.4, 138.7, 138.3, 138.1, 138.1, 137.8, 131.4, 129.6, 128.7, 128.5, 128.4, 128.3, 128.1, 128.03, 127.98, 127.93, 127.88, 127.8, 127.60, 127.57, 127.4, 121.4, 95.5 (C-anomeric), 93.5 (C-anomeric), 80.9, 79.3, 78.8, 78.1, 75.7, 75.3, 74.1, 73.7, 73.5, 72.3, 70.5, 70.2, 68.6, 68.3, 68.1,

67.3, 37.8, 30.0, 29.5, 27.9, 23.7; HRMS (MALDI-TOF): Calcd for $C_{79}H_{86}BrNO_{15}Na^+$ $[M+Na]^+$ 1390.5073, found 1390.5105.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-O-benzyl-3-O-(4-bromo)benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-Tri-O-benzyl-α-D-gluco-pyranoside (31)

To a solution of **30** (300 mg, 0.22 mmol) in DCM (5.0 ml) hydrazine hydrate (32 µl, 0.66 mmol) dissolved in AcOH (0.4 ml) and pyridine (0.6 ml) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **31** (117 mg, 0.09 mmol, 96%). $[\alpha]_D^{20}$ = + 56.5° (c =2.7, $CHCl_3$), IR ν_{max} (film) 3453, 2963, 1695, 1454, 1420, 1360, 1259, 1013 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.90-7.00 (39H, m, Ar-H), 5.25-5.13 (m, 2H), 5.10 (bs, 1H, anomeric-H), 5.05 (bs, 1H, anomeric-H), 4.98-4.43 (m, 14H), 4.10-3.53 (m, 13H), 3.45-3.10 (m, 3H), 1.65-1.40 (m, 4H, linker-H), 1.34-1.15 (m, 2H, linker-H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 138.7, 138.2, 138.1, 131.6, 129.7, 128.6, 128.49, 128.45, 128.1, 128.0, 127.97, 127.91, 127.85, 127.74, 127.71, 127.3, 121.6, 95.6 (C-anomeric), 93.9 (C-anomeric), 81.4, 81.0, 78.9, 78.1, 77.4, 77.2, 77.0, 75.8, 75.2, 74.4, 73.6, 73. 6, 72.1, 71.1, 70.5, 69.3, 68.6, 68.3, 67.3, 50.3, 47.2, 46.2, 43.3, 29.5, 27. 7, 23.6; HRMS (MALDI-TOF): Calcd for $C_{74}H_{80}BrNO_{13}Na^+$ $[M+Na]^+$ 1292.4705, found 1292.4701.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-O-benzoyl-4,6-di-O-benzyl-3-O-*tert*-butyldimethylsilyl-α-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-3-O-(4-bromo)benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (32)

Thioglucoside **29** (233 mg, 0.31 mmol) and disaccharide **31** (266 mg, 0.21 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (7 ml), freshly activated and acid washed molecular sieves (4 Å) and

NIS (80 mg, 0.36 mmol) were added and cooled to -30°C. TfOH (3.2 µl, 0.04 mmol) was added and the mixture was stirred and warmed up to -17 °C in one hour. The reaction was quenched by the addition of pyridine, diluted with DCM and washed with aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solutions. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford **32** (354 mg, 0.19 mmol, 92%). $[\alpha]_D^{20}$ = + 52.5° (c =2.6, $CHCl_3$), IR ν_{max} (film) 3031, 2928, 2859, 1733, 1699, 1603, 1497, 1454, 1421, 1362, 1314, 1265, 1070 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.91-7.05 (m, 54H, Ar-H), 5.21-5.11 (m, 3H), 5.04 (bs, 1H, anomeric-H), 5.01-4.95 (m, 2H, anomeric-H), 4.81 (app. d, 1H, *J*=11.3 Hz), 4.74-4.35 (m, 17H, anomeric-H), 4.23 (app. d, 1H, *J*=12.3 Hz), 3.98 (app. t, 1H, *J*=9.4 Hz), 3.93-3,87 (m, 1H), 3.82 (app. t, 1H, *J*=9.3 Hz), 3.74-3.66 (m, 4H), 3.64-3.45 (m, 10H), 3.42-3.36 (m, 1H), 3.34-3.06 (m, 4H), 1.56-1.35 (m, 4H), 1.25-1.09 (m, 2H), 0.79 (s, 9H, *t*Bu), 0.02 (s, 3H), -0.19 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 164.8, 138.7, 138.7, 138.4, 138.4, 138.4, 138.1, 133.1, 131.1, 130.1, 130.0, 129.6, 128.7, 128.6, 128.49, 128.48, 128.43, 128.41, 128.40, 128.37, 128.35, 128.2, 128.0, 127.93, 127.86, 127.8, 127.7, 127.64, 127.58, 127.5, 127.4, 120.8, 100.3 (C-anomeric), 96.1 (C-anomeric), 59,0 (C-anomeric) 80.5, 80.0, 79.1, 78.6, 77.7, 76.1, 75.5, 75.4, 75.3, 75.2, 74.7, 74.4, 73.8, 73.6, 73.5, 72.3, 70.6, 70.3, 69.1, 68.7, 67.6, 67.2, 50.6, 47.2, 46.3, 29.4, 28.1, 25.8, 23.6, 17.9, -3.86, -3.89; HRMS (MALDI-TOF): Calcd for $C_{107}H_{120}BrNO_{19}SiNa^+$ $[M+Na]^+$ 1852.7299 found 1852.7375.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2-O-benzoyl-4,6-di-O-benzyl-ß-D-glucopyranosyl-(1→4)-2,6-di-O-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-glucopyranoside (33)

A solution of **32** (100 mg, 0.06 mmol), (3,4-dimethoxyphenyl)boronic acid (20 mg, 0.11 mmol), TBABr (1.8 mg, 5.5 µmol), K_3PO_4 (35 mg, 0.16 mmol) in EtOH (4 ml) was subjected to three freeze-pump-saw cycles. To this solution $Pd(OAc)_2$ (1.2 mg, 5.5 µmol) was added and stirred for 2 hours. The mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ solution. The aqueous phase was back extracted with EtOAc. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford the Suzuki coupling product (95 mg, 0.05 mmol, 92%) which was dissolved in DCM/H_2O/saturated aqueous $NaHCO_3$ (100:9:1, 11 ml). To this emulsion DDQ (34 mg, 0.15 mmol) was added, stirred vigorously for 16 hours, diluted with DCM and washed with saturated aqueous $NaHCO_3$ solutions. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was dissolved in DMF (2.5 ml), and treated with a solution of TBAF·$3H_2O$ (137 mg, 0.43 mmol) and AcOH (29 µl, 0.51 mmol) in DMF (2.5 ml) at 50°C for three days. After dilution with Et_2O the phases were separated and the organic phase washed with a 0.1 M HCl solution, saturated aqueous $NaHCO_3$ solution and brine. The organic phase was then dried over MgSO4, filtered and concentrated. The crude product was purified by column chromatography on silica gel (toluene/acetone) to afford **33** (52 mg, 0.03 mmol, 68%). $[\alpha]_D^{20}$ = + 38.9° (c =1.5, $CHCl_3$), IR ν_{max} (film) 3462, 3031, 2924, 2867, 1729, 1699, 1497, 1454, 1422, 1362, 1315, 1268, 1095, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.07-7.00 (m, 50H), 5.25-5.05 (m, 3H), 5.03-4.94 (m, 2H, 2 × anomeric-H), 4.90 (app. d, *J*=10.6, 1H), 4.82-4.35 (m, 16H), 4.27 (app. d, *J*=12.1, 1H),

4.16 (app. dd, *J*=9.2, 8.8, 1H), 4.06 (app. d, *J*=12.2, 1H), 3.99 (app. t, *J*=9.3, 1H), 3.93-3.42 (m, 15H), 3.28 (s, 4H), 1.73-1.36 (m, 4H, linker-H), 1.34-1.08 (m, 2H, linker-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 166.2, 139.0, 138.5, 138.4, 137.9, 137.7, 133.6, 130.1, 129.4, 128.72, 128.65, 128.62, 128.59, 128.57, 128.52, 128.46, 128.45, 128.40, 128.36, 128.30, 128.24, 128.18, 127.97, 127.95, 127.93, 127.88, 127.8, 127.61, 127.57, 127.37, 101.2 (C-anomeric), 95.9 (C-anomeric), 94.8 (C-anomeric), 81.6, 78.4, 78.2, 78.0, 77.5, 77.4, 77.2, 76.8, 76.6, 76.3, 75.0, 74.7, 73.9, 73.6, 73.2, 72.7, 72.2, 70.4, 69.3, 69.1, 68.7, 67.3, 50.4, 47.3, 29.5, 28.1, 23.6; HRMS (MALDI-TOF): Calcd for $C_{94}H_{101}NO_{19}Na^+$ $[M+Na]^+$ 1570.6860, found 1570.6362.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)-2-_O_-benzoyl-4,6-_O_-benzyl-ß-D-glucopyranosyl-(1→4)-[2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)]-2,6-di-_O_-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-_O_-benzyl-α-D-glucopyranoside (34)

Rhamnosyl-imidate **5** (72 mg, 140 µmol) and trisaccharide **33** (42 mg, 27 µmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (3.0 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to -40 °C. TMSOTf (25 µl of a solution of 100 µl TMSOTf in 900 µl DCM, 14 µmol) was added and the reaction was warmed to -20 °C over 1.5 h. The reaction was quenched with TEA and concentrated. Size exclusion chromatography on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) afforded **34** (58 mg, 24 µmol, 88 %). $[\alpha]_D^{20}$ = + 49.7° (c =2.2, $CHCl_3$), IR ν_{max} (film) 3031, 2927, 2863, 1729, 1700, 1602, 1497, 1453, 1273, 1264, 1095, 1069 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.08- 6.98 (m, 80, Ar-H), 5.89 (app. dd, *J*=9.4, 3.5, 1H), 5.85 (app. dd, *J*=3.5, 1.7, 1H), 5.66 (app. dd, *J*=9.4, 3.5, 1H), 5.49-5.42 (m, 1H), 5.39 (app. dd, *J*=3.5, 1.8, 1H), 5.32- 5.25 (m, 1H), 5.16- 5.12 (m, 2H), 5.085.05 (m,

1H), 5.04-4.99 (m, 1H), 4.97 (d, J=1.6, 1H), 4.954.25 (m, 20H), 4.24-3.43 (m, 20H), 3.39- 3.00 (m, 5H), 1.67 (d, J=6.2, 3H), 1.60-1.32 (m, 4H), 1.32-1.06 (m, 2H), 0.94 (d, J=6.1, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 165.43, 165.21, 164.49, 164.12, 139.19, 138.54, 138.37, 138.16, 137.83, 137.74, 133.13, 133.01, 132.95, 132.69, 132.68, 130.38, 130.14, 130.11, 129.99, 129.94, 129.89, 129.78, 129.75, 129.74, 129.45, 128.95, 128.70, 128.67, 128.65, 128.62, 128.55, 128.47, 128.45, 128.41, 128.39, 128.36, 128.26, 128.23, 128.19, 128.15, 128.02, 127.95, 127.93, 127.91, 127.75, 127.66, 127.36, 127.22, 99.53, 97.97, 97.73, 95.81, 93.74, 80.83, 80.40, 80.26, 79.27, 78.40, 78.25, 77.52, 76.58, 76.15, 75.91, 75.74, 75.16, 74.68, 74.20, 74.01, 73.68, 73.58, 73.27, 72.87, 72.19, 71.93, 71.20, 71.16, 70.59, 70.33, 68.69, 68.09, 67.99, 67.33, 67.22, 50.61, 47.18, 46.26, 29.44, 23.56, 18.58, 17.75; HRMS (MALDI-TOF): Calcd for $C_{148}H_{149}NO_{31}Na^+$ $[M+Na]^+$ 2459.0006, found 2459.0636.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-ß-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→3)]-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (1)

To a solution of fully protected pentasaccharide **34** (23 mg, 9.4 µmol) in THF (1.5 ml) NaOMe (0.5 M, in MeOH, 1 ml) was added and stirred for 12 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. Column chromatography on silica gel (DCM/acetone/MeOH) afforded the de-benzoylated pentasaccharide (16 mg), which was dissolved in a mixture of THF (1 ml) MeOH (1 ml), H_2O (0.7 ml) and AcOH (0.1 ml). The solution was purged with Ar, 10% Pd/C (30 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Size exclusion chromatography on Sephadex LH-20 (MeOH) afforded **1** (5.0 mg, 5.7 µmol, 60%). NMR data is consistent with previously reported.[3]

EXAMPLE 2

Preparation of PS-1 Substructures

5-Amino-pentanyl D-glucopyranosyl-(1→2)-α-D-glucopyranoside (35)

A solution of protected disaccharide **33** (40 mg, 31 µmol) in a mixture of MeOH (5.0 ml),THF (2.5 ml) H_2O (2.0 ml) and AcOH (0.5 ml) was purged with Ar. After that 10% Pd/C (70 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. The crude product was purified by reversed phase solid phase extraction (RP SPE) (Waters Sep-Pak®, C18) to afford **35** (13.3 mg, 31 µmol, 99%). ^{1}H-NMR (600 MHz, D_2O) δ 5.23 (d, *J*=3.4, 1H, anomeric), 5.16 (d, *J*=3.6, 1H, anomeric), 4.02-3.80 (m, 8H), 3.75 (app. dd, *J*=9.9, 3.5, 2H) 3.68-3.61 (m, 2H), 3.53 (app. td, J=9.6, 4.7, 2H), 3.09 (app. t, J=7.5, 2H), 1.81-1.71 (m, 4H, linker), 1.59-1.49 (m, 2H, linker); ^{13}C-NMR (150 MHz, D_2O) δ 98.6 (anomeric), 97.9 (anomeric), 77.7, 75.4, 74.5, 74.4, 74.2, 74.0, 72.3, 72.1, 70.4, 63.3, 63.1, 42.1, 30.6, 29.2, 25.1; HRMS (MALDI-TOF): Calcd for $C_{17}H_{33}NO_{11}H^+$ $[M+H]^+$ 428.2126, found 428.2147.

5-Amino-pentanyl ß-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (36)

To a solution of protected trisaccharide **33** (60 mg, 31 µmol) in THF (2 ml) NaOMe (0.5 M in MeOH, 0.5 ml) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (5.0 ml) MeOH (2.5 ml), H_2O (2.0 ml) and AcOH (0.5 ml). The solution was purged with Ar, then 10% Pd/C (30 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **36** (13.3 mg, 31

µmol, 66%). ^{1}H-NMR (600 MHz, D_2O) δ 5.22 (d, *J*=3.3, 1H, anomeric α-Glc), 5.15 (d, *J*=3.6, 1H, anomeric α-Glc), 4.60 (d, *J*=7.9, 1H, anomeric β-Glc), 4.14-4.08 (m, 1H), 4.03-3.91 (m, 5H), 3.90-3.79 (m, 4H), 3.78-3.72 (m, 3H), 3.71-3.62 (m, 2H), 3.62-3.47 (m, 4H), 3.40 (t, *J*=8.7, 1H), 3.09 (t, *J*=7.5, 2H), 1.83-1.72 (m, 4H, linker), 1.59-1.49 (m, 2H, linker). ^{13}C-NMR (150 MHz, D_2O) δ 100.7 (anomeric β-Glc), 94.0 (anomeric α-Glc), 93.4 (anomeric α-Glc), 76.8, 74.2, 73.7, 73.5, 71.3, 69.8, 69.6, 69.5, 69.2, 68.7, 67.7, 67.6, 65.9, 58.8, 57.9, 37.5, 26.1, 24.6, 20.6; HRMS (MALDI-TOF): Calcd for $C_{23}H_{43}NO_{16}Na^+$ $[M+Na]^+$ 612.2474, found 612.2424.

(2-Methyl-5-*tert*-butylphenyl) 2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-ß-D-glucopyranoside (40)

A solution of TBAF·$3H_2O$ (1.10 g, 3.48 mmol) and acetic acid (266 µl, 4.64 mmol) in DMF (4 ml) was added to a solution of **29** (430 mg, 0.58 mmol) in DMF (4 ml). The mixture was stirred for 3 days at 35°C. After dilution with Et_2O the phases were separated and the organic phase washed with a 0.1 M HCl solution, saturated aqueous $NaHCO_3$ solution and brine. The organic phase was then dried over MgSO4, filtered and concentrated. The product **40** was taken directly to the next step.

(2-Methyl-5-*tert*-butylphenyl) 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-ß-D-glucopyranoside (41)

Rhamnosyl-imidate **5** (373 mg, 0.59 mmol) and glucoside **40** (approx. 0.58 mmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (3.0 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to -40 °C. TMSOTf (10 µl, 53 µmol) was added and the reaction was warmed to -20 °C over 1.5 h. The reaction was quenched with TEA and concentrated. Column chromatography

on silica gel (hexanes/ethyl acetate) afforded **41** (490 mg, 0.46 mmol, 79 %). $[\alpha]_D^{20}$ = + 70.7° (c =1.9, $CHCl_3$), IR ν_{max} (film) 2963, 1728, 1602, 1451, 1259, 1090, 1067, 1025 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.02-7.03 (m, 33H), 5.72 (dd, *J*=9.4, 3.5, 1H), 5.53-5.42 (m, 2H), 5.22 (d, *J*=1.9, 1H), 4.88 (d, *J*=10.6, 1H), 4.77-4.47 (m, 6H), 4.24-4.13 (m, 2H), 3.92-3.80 (m, 3H), 3.68-3.59 (m, 2H), 2.18 (s, 3H), 1.25 (s, 9H), 1.08 (d, *J*=6.2, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 149.8, 138.1, 138.0, 133.1, 130.3, 130.0, 129.9, 129.8, 129.7, 129.7, 128.64, 128.57, 128.51, 128.46, 128.42, 128.39, 128.37, 128.32, 128.28, 128.24, 128.20, 128.1, 128.00, 127.97, 127.9, 127.83, 127.80, 127.75, 125.7, 124.4, 97.6, 86.6, 79.3, 77.5, 77.2, 76.8, 75.7, 75.6, 75.0, 74.4, 73.8, 72.0, 71.3, 68.3, 67.9, 31.5, 19.5, 18.0; HRMS (MALDI-TOF): Calcd for $C_{65}H_{66}O_{12}SNa^+$ $[M+Na]^+$ 1093.4167, found 1093.4159.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranosyl-(1→3)2-_O_-benzoyl-4,6-di-_O_-benzyl-1-thio-ß-D-glucopyranoside (42)

Disaccharide **41** (50 mg, 47 µmol) and 5-aminopentanol (31 mg, 93 µmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (3 ml) and NIS (13 mg, 56 µmol) was added and cooled to -20 °C. TfOH (0.5 µl, 6 µmol) was added and the mixture was stirred and warmed up to 0 °C in two hours. The reaction was quenched by the addition of aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetates) to afford **42** (52 mg, 43 µmol, 91%). $[\alpha]_D^{20}$ = + 50.3° (c =2.6, $CHCl_3$), IR ν_{max} (film) 3032, 2936, 1730, 1698, 1452, 1265, 1069 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.23-6.80 (m, 40H, aromatic), 5.73 (dd, *J*=9.4, 3.5, 1H), 5.46 (dd, *J*=3.4, 1.9, 1H), 5.35 (dd, *J*=9.2, 7.9, 1H), 5.24 (d, *J*=1.9, 1H, anomeric Rha), 5.14

(bs, 2H), 4.89 (app. d, *J*=10.6, 1H), 4.72-4.59 (m, 4H), 4.56-4.35 (m, 4H, anomeric Glc), 4.22-4.12 (m, 2H), 3.91-3.76 (m, 4H), 3.68-3.58 (m, 2H), 3.42-3.33 (m, 1H), 3.05-2.88 (m, 2H), 1.50-1.29 (m, 4H, linker), 1.24-0.98 (m, 5H, linker, Rha CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.7, 164.8, 138.2, 138.0, 137.6, 133.1, 132.8, 30.0, 129.92, 129.88, 129.8, 129.7, 128.6, 128.5, 128.42, 128.38, 128.36, 128.31, 128.30, 128.2, 128.0, 127.94, 127.93, 127.8, 127.7, 101.1 (anomeric Glc), 97.6 (anomeric Rha), 79.3, 77.8, 76.9, 75.64, 75.59, 74.9, 74.6, 73.8, 71.9, 71.3, 68.9, 68.3, 67.2, 29.2, 23.2, 18.0 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{74}H_{75}NO_{15}Na^+$ $[M+Na]^+$ 1240.5029, found 1240.4792.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-ß-D-glucopyranoside (38)

To a solution of protected disaccharide **42** (50 mg, 41 µmol) in THF (2 ml) NaOMe (0.5 M in MeOH, 0.5 ml) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (5.0 ml) MeOH (2.5 ml), H_2O (2.0 ml) and AcOH (0.5 ml). The solution was purged with Ar, then 10% Pd/C (100 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **38** (15.7 mg, 27 µmol, 78%). ^{1}H-NMR (600 MHz, D_2O) δ 5.20 (s, 1H, anomeric Rha), 4.53 (d, *J*=8.1, 1H, anomeric Glc), 4.15-4.04 (m, 2H), 4.02-3.96 (m, 2H), 3.85 (app. dd, *J*=9.7, 3.3, 1H), 3.81-3.73 (m, 2H), 3.66 (app. t, *J*=8.7, 1H), 3.56-3.49 (m, 3H), 3.44 (t, *J*=8.7, 1H), 3.08 (app. t, *J*=7.5, 2H), 1.75 (tt, *J*=14.6, 7.2, 4H, linker), 1.57-1.49 (m, 2H, linker), 1.32 (d, *J*=6.3, 3H, Rha CH_3); ^{13}C-NMR (150 MHz, D_2O) δ 100.0 (anomeric Glc), 99.1 (anomeric Rha), 80.3, 73.9, 71.8, 70.0, 68.4, 68.2, 68.1, 66.9, 66.2, 58.8, 37.4, 26.2, 24.4, 20.1, 14.5 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{17}H_{33}NO_{10}Na^+$ $[M+Na]^+$ 434.1997, found 434.1975.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-O-benzyl-3-O-(4-bromo)benzyl-4-O-levulinoyl-1-thio-β-D-glucopyranoside (43)

Thioglucoside **27** (300 mg, 0.38 mmol) and 5-aminopentanol (200 mg, 0.61 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in Ether (4 ml) and Dioxane (4 ml), NIS (103 mg, 0.46 mmol) was added and cooled to -10 °C. TfOH (4 µl, 46 µmol) was added and the mixture was stirred and warmed up to 0 °C in three hours. The reaction was quenched by the addition of aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetates) to afford **43** (140 mg, 0.15 mmol, 39%). $[\alpha]_D^{20}$ = + 22.0° (c =3.4, $CHCl_3$), IR ν_{max} (film) 2920, 1743, 1697, 1454, 1420, 1360, 1208, 1153, 1069, 1038 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.696.92 (m, 24H, ar), 5.22-5.15 (m, 2H), 5.09- 5.03 (m, 1H), 4.81 (app. d, *J*=11.9, 1H), 4.76-4.68 (m, 2H, anomeric), 4.63-4.56 (m, 2H), 4.54-4.46 (m, 4H), 3.89 (app. t, *J*=9.4, 1H), 3.84-3.78 (m, 1H), 3.62-3.45 (m, 4H), 3.38-3.18 (m, 3H), 2.66-2.53 (m, 2H), 2.43-2.29 (m, 2H), 2.13 (s, 3H, Lev CH_3), 1.66-1.48 (m, 4H, linker), 1.38-1.27 (m, 2H, linker); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 206.3 (Lev carbonyl), 171.6, 138.2, 138.1, 138.0, 131.4, 129.6, 129.4, 128.7, 128.5, 128.3, 128.1, 128.03, 127.99, 127.9, 127.6, 127.4, 121.3, 96.9 (anomeric), 79.8, 79.6, 74.3, 73.7, 73.2, 70.9, 69.0, 68.9, 68.3, 67.3, 37.8, 29.9 (Lev CH_3), 29.2, 28.0, 23.6; HRMS (MALDI-TOF): Calcd for $C_{52}H_{58}BrNO_{10}Na^+$ $[M+Na]^+$ 958.3134, found 958.3112.

N-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,6-di-O-benzyl-3-O-(4-bromo)benzyl-1-thio-β-D-glucopyranoside (44)

To a solution of **43** (140 mg, 0.15 mmol) in DCM (5.0 ml) hydrazine hydrate (26 µl, 0.54 mmol) dissolved in AcOH (0.4

ml) and pyridine (0.6 ml) was added and the solution stirred for 1 h. The reaction was then quenched by the addition of acetone and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **44** (102 mg, 0.12 mmol, 81%). $[\alpha]_D^{20}$ = + 24.3° (c =4.2, $CHCl_3$), IR ν_{max} (film) 3454, 3031, 2920, 1696, 1454, 1422, 1229, 1055 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.48-7.04 (m, 24H, Ar), 5.16-5.09 (m, 2H), 4.86 (app. d, *J*=11.7, 1H), 4.70-4.43 (m, 8H), 3.75-3.55 (m, 6H), 3.45 (app. dd, *J*=9.5, 3.6, 1H), 3.32-3.14 (m, 3H), 1.59-1.44 (m, 4H, linker), 1.33-1.23 (m, 2H, linker); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 138.3, 138.1, 138.0, 131.6, 129.5, 128.6, 128.52, 128.47, 128.02, 127.98, 127.9, 127.8, 127.7, 127.4, 121.6, 96.9 (anomeric), 81.7, 79.8, 74.6, 73.7, 72.9, 71.4, 70.1, 69.8, 68.1, 67.3, 50.4, 47.3, 29.2, 27.7, 23.7; HRMS (MALDI-TOF): Calcd for $C_{47}H_{52}BrNO_8Na^+$ $[M+Na]^+$ 860.2769, found 860.2508.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzyl-ß-D-glucopyranosyl-(1→4)-2,6-di-*O*-benzyl-3-*O*-(4-bromo)benzyl-α-D-glucopyranoside (45)**

Disaccharide **41** (144 mg, 0.13 mmol) and glucoside **44** (102 mg, 0.12 mmol) were coevaporated with toluene three times and dried *in vacuo*. The mixture was dissolved in DCM (4 ml) and NIS (36 mg, 0.16 mmol) was added and cooled to -20 °C. TfOH (1.4 µl, 16 µmol) was added and the mixture was stirred and warmed up to 0 °C in two hours. The reaction was quenched by the addition of aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexanes/ethyl acetates) to afford **45** (200 mg, 0.12 mmol, 95%). $[\alpha]_D^{20}$ = + 36.9° (c =5.2, $CHCl_3$), IR ν_{max} (film) 3031, 2866, 1730, 1698, 1602, 1452, 1262, 1092 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.31-6.72 (m, 54H,

Ar), 5.73 (app. dd, *J*=9.4, 3.4, 1H), 5.44 (app. dd, *J*=3.4, 1.9, 1H), 5.37 (app. dd, *J*=9.3, 8.1, 1H), 5.21 (bs, 2H), 5.17-5.09 (m, 2H), 4.88 (app. d, *J*=10.9, 1H), 4.76-4.39 (m, 13H), 4.31 (app. d, *J*=12.2, 1H), 4.19 (app. dd, *J*=9.5, 6.1, 1H), 4.03-3.63 (m, 9H), 3.49-3.42 (m, 3H), 3.37-3.15 (m, 4H), 1.59-1.40 (m, 4H), 1.28-1.11 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.2, 164.6, 164.5, 138.9, 138.5, 138.2, 138.0, 137.89, 137.87, 137.6, 133.1, 133.0, 132.9, 131.1, 129.9, 129.8, 129.7, 129.63, 129.59, 129.4, 129.2, 128.7, 128.6, 128.5, 128.43, 128.35, 128.3, 128.24, 128.19, 128.14, 128.08, 128.0, 127.90, 127.89, 127.74, 127.67, 127.61, 127.55, 127.3, 120.7, 100.3 (anomeric), 97.7 (anomeric), 96.9 (anomeric), 80.3, 79.1, 78.0, 77.4, 76.7, 75.6, 75.2, 74.9, 74.8, 74.5, 73.6, 73.5, 73.1, 71.9, 71.1, 69.7, 68.8, 68.3, 68.0, 67.7, 67.2, 29.0, 23.3, 17.9 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{101}H_{102}BrNO_{20}Na^+$ $[M+Na]^+$ 1750.6071, found 1759.5921.

5-Amino-pentanyl α-L-rhamnopyranosyl-(1→3)-ß-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (37)

To a solution of protected trisaccharide **45** (61 mg, 35 µmol) in THF (2 ml) NaOMe (0.5 M in MeOH, 0.5 ml) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The crude product was dissolved in a mixture of THF (5.0 ml) MeOH (2.5 ml), H_2O (2.0 ml) and AcOH (0.5 ml). The solution was purged with Ar, then 10% Pd/C (100 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **37** (12.5 mg, 30 µmol, 75%). ^{1}H-NMR (600 MHz, D_2O) δ 5.21 (s, 1H, anomeric Rha), 4.99 (d, *J*=2.9, 1H, anomeric α-Glc), 4.61 (d, *J*=8.0, 1H, anomeric β-Glc), 4.15-4.05 (m, 2H), 4.02-3.97 (m, 2H), 3.93-3.79 (m, 6H), 3.73-3.66 (m, 3H), 3.64-3.49 (m, 5H), 3.09 (t, *J*=7.1, 2H), 1.81-1.71 (m, 4H, linker), 1.59-1.50 (m, 2H,

linker), 1.33 (d, *J*=6.0, 3H, Rha CH_3); ^{13}C-NMR (150 MHz, D_2O) δ 102.9 (anomeric Rha), 101.7 (anomeric β-Glc), 98.4 (anomeric α-Glc), 82.7, 79.7, 76.5, 74.5, 72.6, 72.4, 71.6, 71.1, 71.0, 70.8, 69.4, 68.6, 68.5, 61.2, 60.6, 40.0, 28.6, 27.1, 23.0, 17.1. (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{23}H_{43}BrNO_{15}Na^+$ $[M+Na]^+$ 596.2525, found 596.2540.

_N_-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl 2,3-di-_O_-benzoyl-4-_O_-benzyl-α-L-rhamnopyranoside (46)

Rhamnoside-imidate (127 mg, 0.20 mmol) and 5-aminopentanol (160 mg, 0.49 mmol) were coevaporated with toluene three times, dried *in vacuo* and dissolved in anhydrous DCM (3 ml). Freshly activated molecular sieves (4 Å) were added and the mixture cooled to -30 °C. TMSOTf (3.6 µl, 20 µmol) was added and the reaction was warmed to -20 °C over 1 h. The reaction was quenched with TEA and concentrated. Column chromatography on silica gel (hexanes/ethyl acetate) afforded **46** (145 mg, 0.19 mmol, 94 %). $[\alpha]_D^{20}$ = + 54.1° (c =2.6, $CHCl_3$), IR ν_{max} (film) 2963, 1727, 1260, 1018 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.28-7.00 (m, 25H, Ar), 5.73 (app. dd, *J*=9.6, 3.4, 1H), 5.59 (bs, 1H), 5.19 (app d, *J*=11.3, 2H), 4.87 (bs, 1H, anomeric), 4.68 (app. dd, *J*=28.1, 10.9, 2H), 4.53 (bs, 2H), 3.96 (bs, 1H), 3.79 (app. t, *J*=9.5, 1H), 3.75-3.61 (m, 1H), 3.48-3.21 (m, 3H), 1.65-1.51 (m, 4H), 1.45-1.27 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 165.6, 165.5, 138.1, 137.8, 133.4, 133.2, 130.0, 129.9, 129.7, 128.7, 128.6, 128.47, 128.46, 128.2, 127.99, 127.95, 127.4, 97.5 (anomeric), 79.3, 75.3, 72.6, 71.5, 68.0, 67.8, 67.3, 29.3, 23.6, 18.3; HRMS (MALDI-TOF): Calcd for $C_{47}H_{49}NO_9Na^+$ $[M+Na]^+$ 794.3300, found 794.3264.

5-Amino-pentanyl α-L-rhamnopyranoside (39)

To a solution of protected rhamnoside **46** (145 mg, 0.19 mmol) in THF (4 ml) NaOMe (0.5 M in MeOH, 0.5 ml) was added and stirred for 4 h. The mixture was neutralized with Amberlite IR 120 (H^+) ion exchange resin, filtered and concentrated. The

crude product was dissolved in a mixture of THF (10 ml) MeOH (5 ml), H_2O (4 ml) and AcOH (1 ml). The solution was purged with Ar, then 10% Pd/C (300 mg) was added and the solution purged with H_2 for 30 min, then stirred under an H_2 atmosphere for 12 h, filtered and concentrated. Purification by RP SPE (Waters Sep-Pak®, C18) afforded **39** (44 mg, 0.18 mmol, 94%). ^{1}H-NMR (600 MHz, D_2O) δ 4.85 (s, 1H, anomeric Rha), 4.01-3.96 (m, 1H), 3.81-3.70 (m, 3H), 3.62-3.57 (m, 1H), 3.50 (app. t, *J*=9.6, 1H), 3.11-3.03 (m, 2H), 1.78-1.67 (m, 4H, linker), 1.56-1.46 (m, 2H), 1.34 (d, *J*=6.3, 3H, Rha CH_3). ^{13}C-NMR (150 MHz, D_2O) δ 98.3 (anomeric), 70.6, 70.0, 68.8, 67.1, 66.1, 38.0, 26.6, 25.1, 21.0, 15.2 (Rha CH_3); HRMS (MALDI-TOF): Calcd for $C_{11}H_{23}NO_5Na^+$ $[M+Na]^+$ 272.1468, found 272.1433.

EXAMPLE 3

Preparation and characterization of an pentasaccharide-protein Conjugate

Polysaccharide vaccines provoke exclusively a T-cell independent immune response and do not induce an immunoglobulin class switch. The synthetic repeating unit **1** of the *Clostridium difficile* glycopolymer PS-I was conjugated to the protein carrier Crm_{197}. The detoxified diphtheria toxoid Crm_{197} was chosen as a carrier since it is an approved constituent of licensed vaccines (Barocchi et al. (2007), Vaccine 25, 2963-73).

Conjugations

A) To a solution of Di(N-succinimidyl) adipate (5.8 mg, 17 µmol) in DMSO (250 µl) and NEt_3 (20 µl) pentasaccharide **1** (500 µg, 0.57 µmol) dissolved in DMSO (250 µl) was added dropwise. The solution was stirred for 2 h, diluted with phosphate buffer (1.0 ml, 100 µM, pH 7.5) and extracted with $CHCl_3$. CRM_{197} (rDNA) (250 µl, 250 µg, Pfenex Inc (USA)) was added to the

aqueous layer and stirred for 5 h. Conjugate **1a** was desalted and concentrated. An average load of 3.6 pentasaccharide units per protein was determined by MALDI-TOF MS, SEC-HPLC and SDS PAGE confirmed modification of the protein (Fig. 2). SEC-HPLC t_R= 22.49 min, MS (MALDI-TOF) found 61853 Da.

B) First, the primary amine group of the linker moiety of PS-I pentasaccharide **1** was reacted with one of the ester groups of the spacer molecule di(N-succinimidyl) adipate in water-free DMSO (12.7 mg in 120 µl) in the presence of 10 µl triethylamine at room temperature over 2 hours, with the spacer used in 10-fold molar excess to avoid dimer formation. After addition of 400 µL 0.1 M Na-phosphate buffer, pH 7.4, unreacted spacer molecules were removed by chloroform extraction. The remaining ester group of the spacer moiety was then reacted with the ε-amino gropus of lysine residues on the CRM_{197} protein (Pfenex) in 0.1 M Na-phosphate buffer, pH 7.4, at room temperature over 12 hours (Figure 3). For one reaction, 3 mg of PS-I pentasaccharide and 1 mg of CRM_{197} (solubilized in 1 mL 0.1 M Na-phosphate buffer, pH 7.4) was used. The resulting conjugate was purified by ultrafiltration (10 kDa, Amicon, Millipore) with deionized water. The protein concentration was determined by bicinchoninic acid (BCA) assay (Pierce).

Successful conjugation was confirmed by SDS-PAGE as shown in Figure 4a. Marker M is PageRuler Plus Prestained Protein Ladder (Thermo Scientific). Conjugate samples are shifted towards higher masses compared with unconjugated CRM_{197}.

The oligosaccharide/CRM_{197} ratio was determined by MALDI-TOF MS. The mass analysis of CRM_{197} yielded a m/z ion at 58.2 kDa. The mass analysis of the conjugate yielded a major m/z ion at 67.7 kDa and further peaks ~1000 Da apart, corresponding to conjugates of different valencies (Figure 4b). An average of

9.6 PS-I pentasaccharide **1** molecules were loaded on one CRM_{197} protein, resulting in conjugate **1b**.

Knowing the protein concentration of the conjugate, as determined by bicinchoninic acid (BCA) assay, and the average sugar loading, the carbohydrate content was calculated to 300±46 µg/mL (mean±SD) and verified by colorimetric anthrone assay (302±76 µg/mL), an approved method for the carbohydrate determination of the licensed pneumococcal conjugate vaccine Prevenar (Pfizer).

SDS-PAGE

Pentasaccharide **1**-CRM_{197} conjugate and unconjugated CRM_{197} were dissolved in Lämmli buffer (0.125 M Tris, 20% (v/v) glycerol, 4% (w/v) SDS, 5% (v/v) beta-mercaptoethanol, bromophenol, pH 6.8) and boiled at 95°C for 5 minutes. Samples were run in 10% polyacrylamide gels and stained with 0.025% (w/v) Coomassie Brilliant blue R-250 in an aqueous solution containing 40% (v/v) methanol and 7% (v/v) acetic acid.

MALDI-TOF mass spectrometry

Conjugation was confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) using an Autoflex™ Speed instrument (Bruker Daltonics, Bremen, Germany). The mass spectrometer was operated in positive linear mode. Spectra were acquired over an m/z range from 50,000 to 85,000 Da and data was analyzed with the FlexAnalysis software provided with the instrument. 2',4'-dihydroxyacetonephenone (DHAP) was used as matrix, samples were spotted using the dried droplet technique.

Anthrone assay

Anthrone assays were performed in 96-well format in a modified assay according to Leyva et al., *Biologicals* 36:134-141, 2008.

Briefly, 75 µL of anthrone reagent (0.1% (w/v) in concentrated sulfuric acid) was added to each well of a 96-well microtiter plate containing 25 µL of standard solutions, sample dilutions and blank. Plates were first placed at 4°C for 10 minutes, then incubated at 100°C for 20 minutes, and cooled down at room temperature for 20 minutes. Absorbance at 579 nm was determined in a microplate reader. Colorimetric response was compared to a standard curve based on glucose and rhamnose in a 3:2 molar ratio.

EXAMPLE 4

Immunization and monoclonal antibodies

To test the immunogenicity of the PS-I pentasaccharide hapten, three groups of six female C57BL/6 mice each were immunized subcutaneously (s.c.) with conjugate (one group without adjuvant, one group with Freund's adjuvant, one group with Alum adjuvant). Each mouse received an amount of conjugate corresponding to 3 µg PS-I pentasaccharide **1** antigen. Initial immunizations (priming) was followed by an immunization after two weeks (boosting). Sera were collected in one-week intervals. IgG antibody responses were evaluated by microarray. PS-I pentasaccharide **1** in three different concentrations (1, 0.5 and 0.1 mM), CRM_{197} (1, 0.5 and 0.1 µM) and bovine serum albumin (BSA)-spacer-GlcNAc conjugate (1, 0.5 and 0.1 µM) were spotted in triplicate onto the surface of the microarray slides (N-hydroxysuccinimid ester-activated glass slides (CodeLink)) as shown in Figure 5. BSA-spacer-GlcNAc was used to assess immunogenicity against the spacer moiety of the conjugate. As negative controls, phosphate-buffer saline (PBS), as well as two unrelated oligosaccharides (both at a concentration of 1 mM) were also included. Microarrays were designed such that high-throughput analysis of 64 samples per array was possible.

PS-I pentasaccharide-specific IgG antibody responses were identified in pooled sera of three groups (each n=6) of immunized mice after priming, and more pronounced after boosting (week 3), as determined by microarray analysis (Figure 6).

IgG antibody responses were quantified by determination of the fluorescence intensity values using the sera of individual mice. While the conjugate already showed immunogenicity without adjuvant (Figure 7, left diagram, white bars), IgG titers against PS-I pentasaccharide were markedly increased when Freund's adjuvant was used (light grey bars), and, more pronounced, with Alum adjuvant (dark grey bars). IgG antibody titers against the carrier protein CRM_{197} were lower in mice immunized without adjuvant than in mice immunized with Freund's and Alum adjuvant (Figure 7, central diagram). There was no IgG response against the spacer moiety in mice immunized without adjuvant, but in mice immunized with Freund's and Alum adjuvants (Figure 7, right diagram).

As an IgG-specific detection antibody, Anti-Mouse IgG (whole molecule)-FITC (Sigma) was used in the tests of Figures 6 and 7. Slides were analyzed on a GenePix Pro 4300A microarray scanner and data was analyzed using the GenePix Pro 7 software (both Molecular Devices). Individual mice sera at week 0 ('prebleed'), week 2 ('primed') and week 3 ('boosted') were analyzed by microarray (Figure 6). Total fluorescence intensity values were determined with the GenePix Pro 7 software and background fluorescence (PBS) was subtracted for each value. Data shown is mean ± S.E.M. (standard error of the mean) for n=6 values. "Unrel. OS" in Figure 5 means unrelated oligosaccharide.

To get an insight into the subclasses of IgG antibodies raised against PS-I pentasaccharide, microarray analysis with pooled

sera using subclass-specific detection antibodies against IgG1, IgG2a and IgG3 was performed.

Figure 8 shows the isotype analysis of the IgG immune response by microarray. Pooled sera at a 1:100 dilution were analyzed with isotype-specific detection antibodies (anti-IgG1, Invitrogen A21125; anti-IgG2a, Invitrogen A21241; anti-IgG3, Invitrogen A21151). Data shown is mean, n=6, S.E.M., normalized to background fluorescence intensity, of mice after boosting (week 3).

As evident from Figure 8, while antibodies against PS-I are almost exclusively of the IgG1 subtype in mice immunized with conjugate without adjuvant (left panel) or Alum adjuvant (right panel), mice immunized with Freund's adjuvant show a relatively high proportion of antibodies of the IgG2a and IgG3 subclasses in addition to IgG1. IgG3 and IgG2a are mainly induced by T-cell independent antigens such as polysaccharides, while IgG1 is mainly T-cell dependent and directed against protein antigens.

To assess whether antibodies raised with PS-I pentasaccharide antigen **1** recognize substructures of the antigen as well, which allows to define the minimal epitope, microarray slides with substructures **35-39** in addition to **1** were prepared (Figure 9). CRM_{197}, BSA-spacer-GlcNAc were included as well as two unrelated oligosaccharides and PBS as negative controls. This array was used to assess immune responses in pooled sera of the three groups of mice immunized with conjugate.

Figure 10 shows the deletion sequence analysis of the immune response of mice immunized with glycoconjugate without adjuvant. Pooled sera of mice (n=6) were analyzed on deletion sequence microarray as in Figure 9, using Alexa Fluor 635 goat

anti-mouse IgG (Invitrogen) as detection antibody. Unrel. OS, unrelated oligosaccharide.

Figure 11 shows the deletion sequence analysis of the immune response of mice immunized with glycoconjugate and Freund's adjuvant.

Figure 11 shows the deletion sequence analysis of the immune response of mice immunized with glycoconjugate and Alum adjuvant.

As shown in Figures 10 and 11, sera of mice immunized without adjuvant or with Freund's adjuvant contain antibodies against substructure with rhamnose, while the IgG responses against disaccharide **38** is generally higher than those against trisaccharide **37**, albeit **37** is closer to the original PS-I pentasaccharide antigen **1** used for immunization. The IgG antibody response in mice immunized with Alum adjuvant shows a more specific reactivity against the PS-I pentasaccharide with lower titers against deletion sequences **38** and **37** (Figure 12). No antibody response against oligoglucose disaccharide **35** nor trisaccharide **36** was detected in any of the groups. Disaccharide **38** may be the minimal epitope of the PS-I pentasaccharide.

Monoclonal antibodies were generated with the traditional hybridoma technique [Köhler and Milstein, 1975]. Three monoclonal antibodies (mAbs), 2C5, 10A1 and 10D6, were selected for evaluation with deletion sequence microarray and isotype-specific detection antibodies. All three mABs showed identical patterns on the microarray, exclusively bound to PS-I pentasaccharide **1** but none of the substructures, and were of the IgG1 subtype (Figure 13).

Figure 13 shows different monoclonal antibodies against PS-I. One mouse of the Alum group was subjected to a second boosting immunization (s.c.) at week 5 and three final boostings (intraperitoneal, i.p.) at three consecutive days in week 7. One day after final boosting, the mouse was sacrificed, the spleen was removed and subjected to monoclonal antibody development. After three rounds of subcloning, supernatants of three monoclonal antibodies (mAB)-producing clones, 2C5, 10A1 and 10D6, were subjected to isotype analysis as in Figure 8, using hybridoma supernatants in a 1:3125 dilution.

Immunizations

Six to eight-weeks old female C57BL/6 mice were immunized s.c. with conjugate corresponding to 3 μg PS-I pentasaccharide **1** with Freund's (priming immunizations with Freund's Complete Adjuvant, boosting immunizations with Freund's Incomplete Adjuvant, both Sigma) or Aluminium Hydroxide Gel Adjuvant (Brenntag Biosector, Frederikssund, Denmark), or without adjuvant. Mice received boosting injections after 2 weeks. For all immunizations, antigen was diluted in sterile PBS to a total injection volume of 100 μL per mouse. Blood was collected in one-week intervals via the tail vein and erythrocytes separated from serum by centrifugation. Serum serum antibody responses were analyzed by microarray. One mouse of the Alum group received a second boosting injection s.c. at week 5 after first immunization, and, prior to being sacrificed, three final boosting injections via the intraperitoneal (i.p.) route, on three consecutive days at week 7.

Preparation of microarrays

Oligosaccharides bearing an amine linker, or proteins, were dissolved in sodium phosphate buffer (50 mM, pH 8.5) and printed robotically using a piezoelectric spotting device

(S11, Scienion, Berlin, Germany) onto NHS-activated glass slides (CodeLink). Slides were incubated in a humid chamber to complete reaction for 24 hours and stored in an anhydrous environment. Prior to the experiment, remaining succinimidyl groups were quenched by incubating slides in 100 mM ethanolamine in sodium phosphate buffer (pH 9, 50 mM) for 1 hour at 50°C. Slides were rinsed three times with deionized water and dried by centrifugation.

Microarray binding assays

The quenched array slides were blocked for 1 hour with 1% (w/v) BSA in PBS, then washed three times with PBS and dried by centrifugation. A FlexWell 64 (Grace Bio-Labs, Bend, OR, USA) grid was applied to the slides. Resulting 64 wells were used for 64 individual experiments. Slides were incubated with sera dilutions or hybridoma supernatants (all dilutions were prepared with PBS) for 1 hour at room temperature in a humid chamber, washed three times with PBS-Tween-20 (0.1% v/v) and dried by centrifugation. Then, slides were incubated with fluorescence-labeled detection antibody diluted in 1% BSA in PBS (w/v) for 1 hour at room temperature in a humid chamber. Slides were washed three times with PBS-Tween-20 (0.1% v/v) and rinsed once with deionized water and dried by centrifugation. Slides were scanned with a GenePix 4300A scanner (Molecular Devices) using the GenePix Pro 7 software. Detection antibodies used were Anti-Mouse IgG (whole molecule)-FITC (Sigma), Alexa Fluor 635 Goat Anti-Mouse IgG (H+L) (Life Technologies) and Alexa Fluor 594 Goat Anti-Mouse IgG1 (γ1) (Life Technologies) in 1:400 dilutions, as well as Alexa Fluor 647 Goat Anti-Mouse IgG2a (γ2a) and Alexa Fluor 488 Goat Anti-Mouse IgG3 (γ3) (Life Technologies) in 1:200 dilutions.

Monoclonal antibodies

Monoclonal antibodes (mABs) were generated using the standard method by Köhler and Milstein, 1975. Briefly, spleenocytes of one mouse were fused with 10^8 mouse myeloma cells in the presence of 50% PEG 1500. Fused cells were selected with complete growth medium (IMDM supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 24 µM beta-mercaptoethanol, 100 µM hypoxanthine, 16 µM thymidine, non-essential amino acids, 100 U/mL penicillin, 100 µg/mL streptomycin, 50 µg/mL gentamycin, 10% hybridoma cloning supplement (BM Condimed H1, Roche)) with 0.4 µM aminopterin. Cells were maintained at 37°C at 5% CO_2. Hybridoma cells were subjected to three consecutive subcloning steps by limited dilution. Clones producing antibodies against PS-I pentasaccharide were identified by microarray analysis.

CLAIMS

1. A synthetic oligosaccharide representing part of the repeating unit of the *Clostridium difficile* glycopolymer PS-I and having the sequence of the pentasaccharide α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p* or a fragment or derivative thereof.

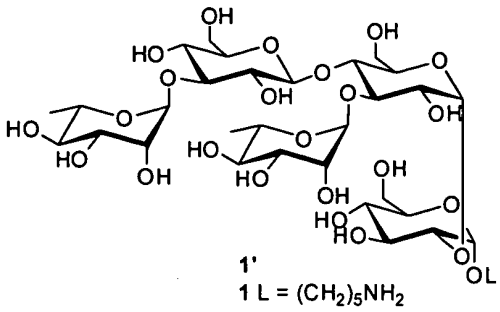
2. The synthetic oligosaccharide according to claim 1 bearing at least one linker L for conjugation to a carrier protein or for immobilization on a surface.

3. The synthetic oligosaccharide according to claim 2 wherein the linker L is selected from the group comprising an aliphatic or aromatic residue, e.g. an alkyl(en) group or phenyl(en) group, comprising a reactive functional group, such as an amino group, preferably a primary amino group, (activated) carboxy group, aldehyde, azide, alkenyl or alkinyl group, in particular primary alkylamines.

4. The synthetic oligosaccharide according to claim 3 wherein the linker L is $(CH_2)_nNH_2$, with n being an integer from 2 to 50, preferably 3 to 20 or 3 to 10.

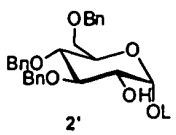
5. The synthetic oligosaccharide according to any one of claims 1-4 in which one or more of the hydroxyl groups is/are derivatized and/or substituted by other functional groups or atoms.

6. The synthetic oligosaccharide according to any one of claims 1-5 which is **1** or **1`** containing L particular as defined in claim 3 or 4

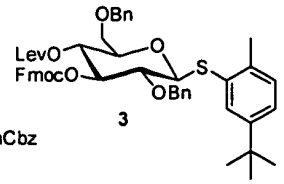


1'
1 L = $(CH_2)_5NH_2$

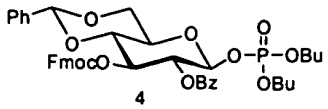
or a fragment **2**, **3**, **4**, **5**, **20**, **21**, **22**, **23**, **24**, **27**, **29**, **30**, **31**, **32**, **33**, **34** **35**, **36**, **37**, **38**, **39** of **1**; or a fragment **2'**, **20'**, **21'**, **22'**, **23'**, **24'**, **30'**, **31'**, **32'**, **33'**, **34'** **35'**, **36'**, **37'**, **38'**, **39'** of **1`** containing L particular as defined in claim 3 or 4



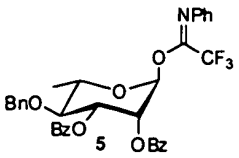
2'
2 L = $(CH_2)_5NBnCbz$



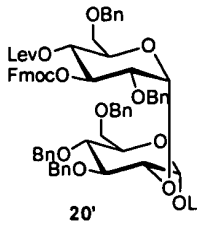
3



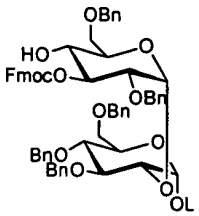
4



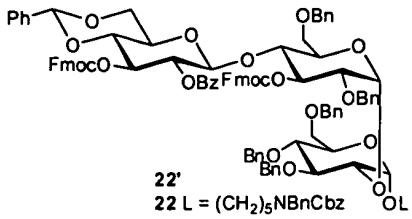
5



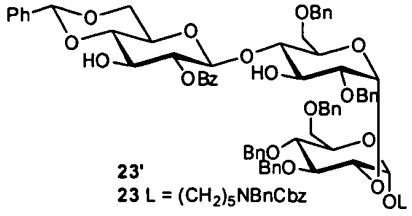
20'
20 L = $(CH_2)_5NBnCbz$



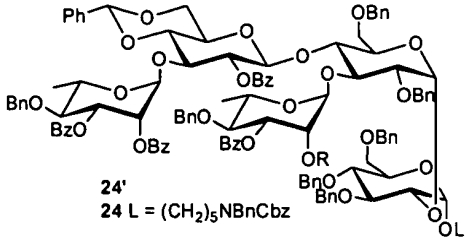
21



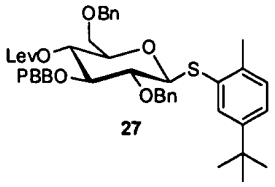
22'
22 L = $(CH_2)_5NBnCbz$



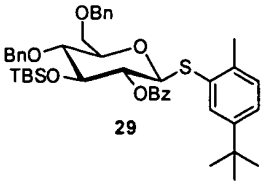
23'
23 L = $(CH_2)_5NBnCbz$



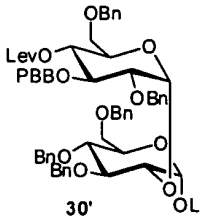
24'
24 L = $(CH_2)_5NBnCbz$



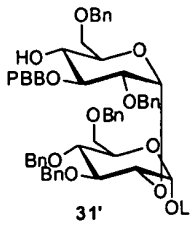
27



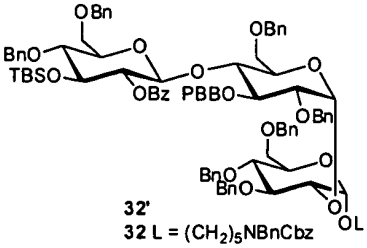
29



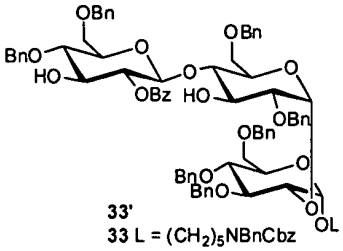
30'
30 L = $(CH_2)_5NBnCbz$



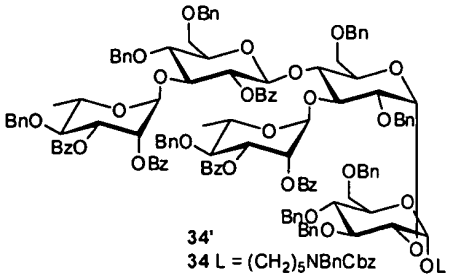
31'
31 L = $(CH_2)_5NBnCbz$



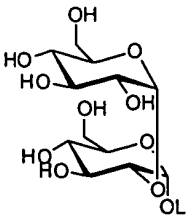
32'
32 L = $(CH_2)_5NBnCbz$



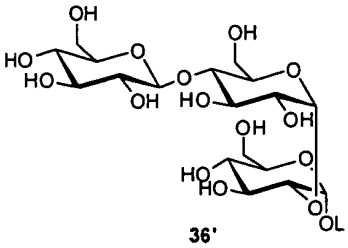
33'
33 L = $(CH_2)_5NBnCbz$



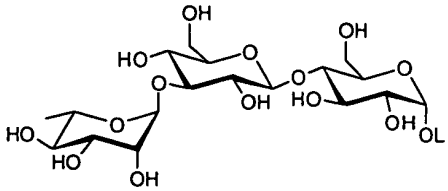
34'
34 L = $(CH_2)_5NBnCbz$



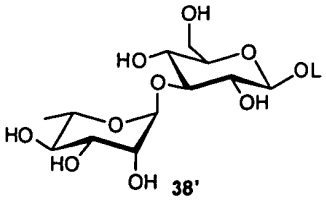
35'
35 L = $(CH_2)_5NH_2$



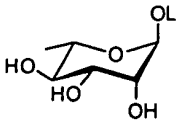
36'
36 L = $(CH_2)_5NH_2$



37'
37 L = $(CH_2)_5NH_2$



38'
38 L = $(CH_2)_5NH_2$



39'
39 L = (CH2)5NH2

or derivative thereof.

7. The synthetic oligosaccharide according to any one claims 1-6 conjugated to a carrier protein.

8. The synthetic oligosaccharide according to claim 7, wherein the carrier protein is diphtheria toxoid CRM_{197}, tetanus toxoid (TT), outer membrane protein (OMP), bovine serum albumin, (BSA), keyhole limpet hemocyanine (KLH), diphtheria toxoid (DT), cholera toxoid (CT), recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA), Clostridium difficile toxin A (TcdA), Clostridium difficile toxin B (TcdB).

9. A composition, in particular a vaccine composition, comprising a synthetic oligosaccharide according to any one of claims 1-8 in a pharmaceutically acceptable formulation.

10. The composition according to claim 9 comprising a synthetic oligosaccharide of any one of claims 1-8 in a pharmaceutically acceptable formulation with an immuno-stimulatory component, such as an adjuvant.

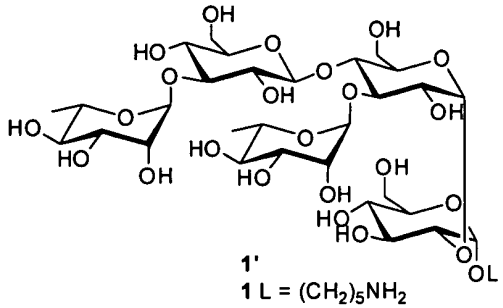
11. An antibody having specificity for an immunogenic determinant which comprises or consists of the pentasaccharide of claim 1 or of a truncated derivative thereof.

12. The antibody according to claim 11 which has been raised against the oligosaccharide-protein conjugate according to claim 7.

13. The antibody according to claim 11 or 12 which is a polyclonal or monoclonal antibody.

14. The monoclonal antibody of claim 13 which is the antibody 2C5, 10A1 or 10D6.

15. A method for preparing the pentasaccharide of claim 1 having the following formula **1**



1'
1 L = $(CH_2)_5NH_2$

which comprises

a) assembling the monosaccharide building blocks **2** and **3** or **4** shown in claim 6 to yield the corresponding disaccharide **21** shown in claim 6, reacting the disaccharide **21** with building block **4** to form the trisaccharide **23** of claim 6, subjecting the trisaccharide **23** to a bis-glycosylation reaction with 2 molecules of building block **5** of claim 6 to yield the fully protected pentasaccharide **24** of claim 6 and finally, after deprotection, to yield pentasaccharide **1**, or

b) assembling the monosaccharide building blocks **2** and **27** shown in claim 6 to yield the corresponding disaccharide **30** of claim 6, reacting the disaccharide **30** with building block **4** or **29** to form the protected trisaccharide **32** of claim 6, deprotecting the trisaccharide **32** to obtain trisaccaride **33** and subjecting trisaccharide **33** to a bis-glycosylation reaction with 2 molecules of building block **5** of claim 6 to yield the fully protected pentasaccharide **34** of claim 6 and finally, after deprotection, to yield pentasaccharide **1**.

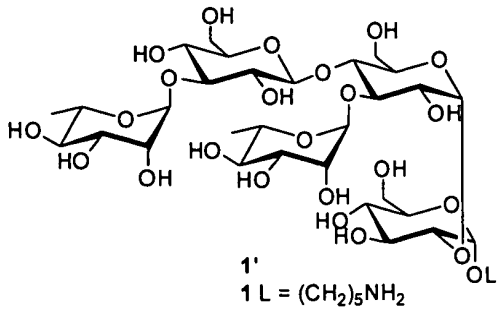
16. A method for preparing the pentasaccharide α-L-Rha*p*-(1→3)-β-D-Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p* or a derivative thereof which comprises

a) assembling a monosaccharide building block **2`**, wherein the specific protected amino linker of building block **2** shown in claim 6 is replaced by a different protected or unprotected linker L, in particular as defined in claim 3 or 4, and building blocks **3** or **4** shown in claim 6 to yield the corresponding disaccharide **21`**, reacting the disaccharide **21`** with building block **4** to form the trisaccharide **23`**, subjecting the trisaccharide **23`** to a bis-glycosylation reaction with 2 molecules of building block **5 of** claim 6 to yield the corresponding fully protected pentasaccharide **24`** and finally, after deprotection, to yield pentasaccharide **1`**, wherein the specific amino linker of pentasaccharide **1** is replaced by a different linker L, in particular as defined in claim 3 or 4, or

b) assembling a monosaccharide building block **2`**, wherein the specific protected amino linker of building block **2** shown in claim 6 is replaced by a different protected or unprotected linker L, in particular as defined in claim 3 or 4, and building block **27** of claim 6 to yield the corresponding disaccharide **30`**, reacting the disaccharide **30`** with building block **4** or **29** of claim 6 to form the corresponding protected trisaccharide **32`**, deprotecting the trisaccharide **32`** to obtain trisaccaride **33´**, subjecting the trisaccharide **33`** to a bis-glycosylation reaction with 2 molecules of building block **5** of claim 6 to yield the corresponding fully protected pentasaccharide **34`** and finally, after deprotection, to yield pentasaccharide **1`**, wherein the specific amino linker of pentasaccharide **1** is replaced by a different linker L, in particular as defined in claim 3 or 4.

17. Use of one or more of molecules **2**, **2`**, **3**, **4**, **5**, **20**, **21**, **22**, **23**, **24**, **27**, **29**, **30**, **30`**, **31**, **32**, **32`**, **33**, **33`**, **34`** as shown in claim 6 or defined in claim 18 as intermediates in a method for preparing the pentasaccharide α-L-Rha*p*-(1→3)-β-D-

Glc*p*-(1→4)-[α-L-Rha*p*-(1→3)]-α-D-Glc*p*-(1→2)-α-D-Glc*p* of claim 1 having the following formula **1**



1'
1 L = $(CH_2)_5NH_2$

or the pentasaccharide **1`**, wherein the specific amino linker of pentasaccharide **1** is replaced by a different linker L, in particular as defined in claim 3 or 4.

18. An *in vitro* method of detecting *Clostridium difficile* comprising the use of the synthetic oligosaccharide of any one of claims 1-8 or a mixture thereof, in particular immobilized on a microarray surface or any other surface.

19. A method of identifying a certain strain of *Clostridium difficile* comprising the use of the synthetic oligosaccharide of any one of claims 1-8 or a mixture thereof.

20. The use of the synthetic oligosaccharide of any one of claims 1-8 or a mixture thereof as an analytical standard for immunoassays.

21. Use of the synthetic oligosaccharide according to any one of claims 1-8 or of the antibody according to any one of claims 11-14 for preparing a pharmaceutical composition for the treatment or prevention of a disease caused by the pathogen *Clostridium difficile.*

22. Use of the synthetic oligosaccharide according to any one of claims 1-8 or of the antibody according to any one of

claims 11-14 for the treatment or prevention of a disease caused by the pathogen *Clostridium difficile*.

23. A method of inducing immune response against *Clostridium difficile* in a subject comprising administering the synthetic oligosaccharide according to any one of claims 1-9 or a mixture thereof.

24. A method of treating or preventing *Clostridium difficile infection* in a subject comprising administering the synthetic oligosaccharide according to any one of claims 1-8 or a mixture thereof or the composition according to claim 9 or 10.

25. A diagnostic method for *Clostridium difficile* infection comprising the use of the synthetic oligosaccharide of any one of claims 1-8 or a mixture thereof or of the composition according to claim 9 or 10.

26. A method for preparing the oligosaccharide according to claim 7 which comprises reacting a unique terminal amine of the linker L with one of the two NHS-activated esters of Di(N-succinimidyl) adipate to form an amide and subsequent coupling of the activated amide moiety to the protein carrier.

27. The method according to claim 26 wherein the protein carrier is diphtheria toxoid CRM_{197}, tetanus toxoid (TT), outer membrane protein (OMP), bovine serum albumin, (BSA), keyhole limpet hemocyanine (KLH), diphtheria toxoid (DT), cholera toxoid (CT), recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA), Clostridium difficile toxin A (TcdA), Clostridium difficile toxin B (TcdB).

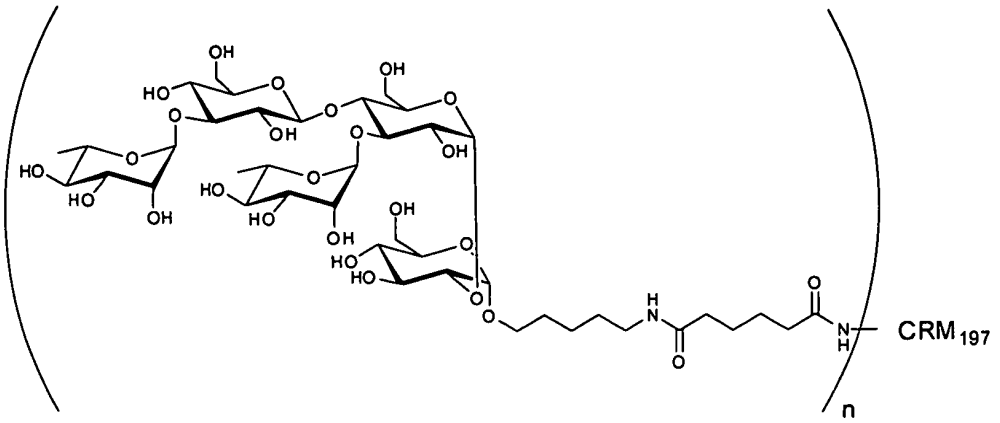


Fig. 1

Conjugate 1: MS (MALDI-TOF)

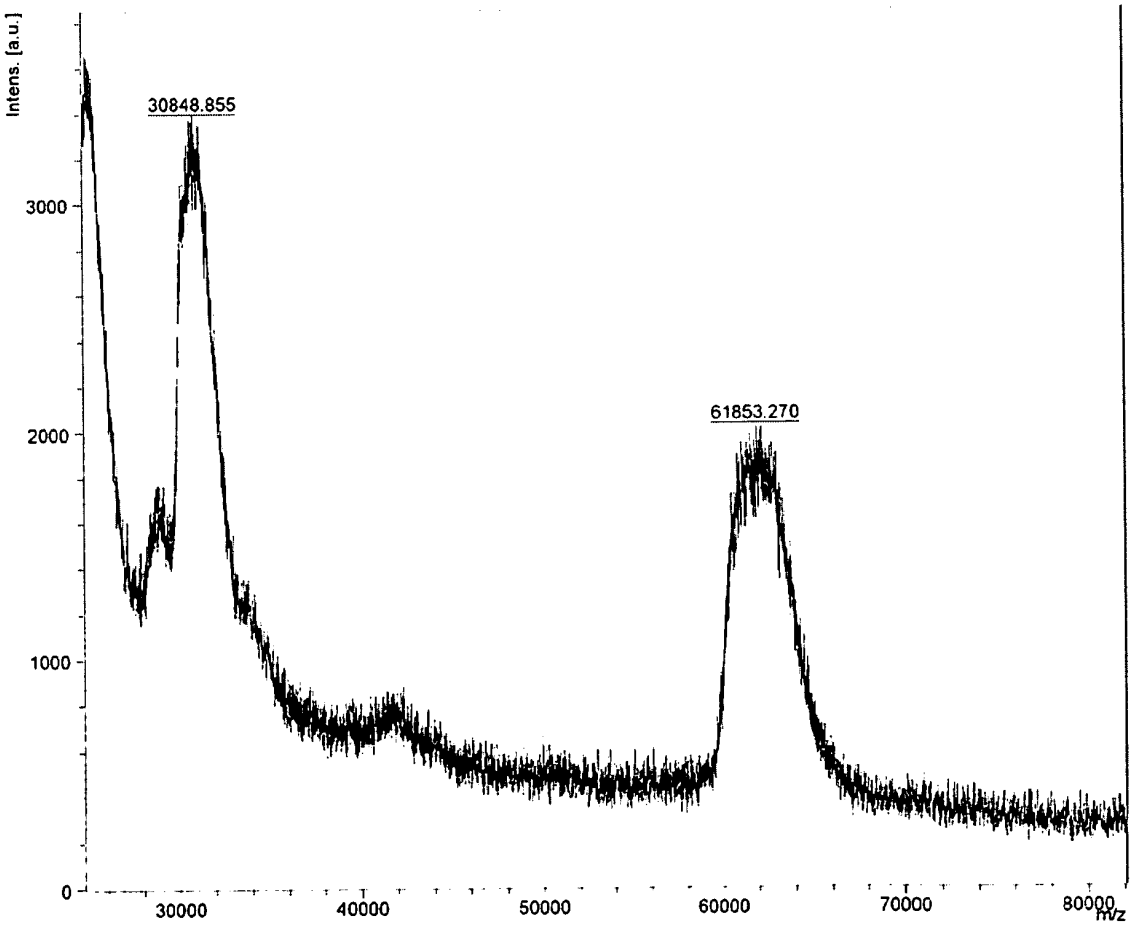


Fig. 2a

Conjugate 1: HPLC (blue t_R= 22.49 min, overlaid with unconjugated CRM_{197} standard red t_R= 22.86 min)

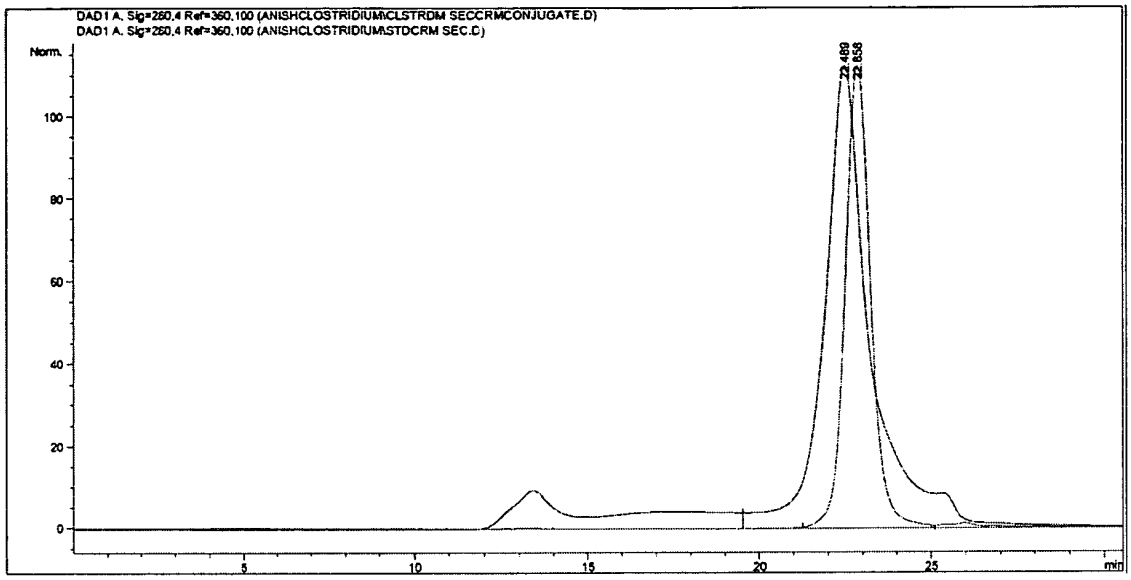


Fig. 2b

Conjugate 1: SDS-PAGE (Lanes: 1: molecular weight marker (Invitrogen bench marker); 2: unconjugated CRM_{197} standard; 3, 4, 5: conjugate 1)

1 2 3 4 5

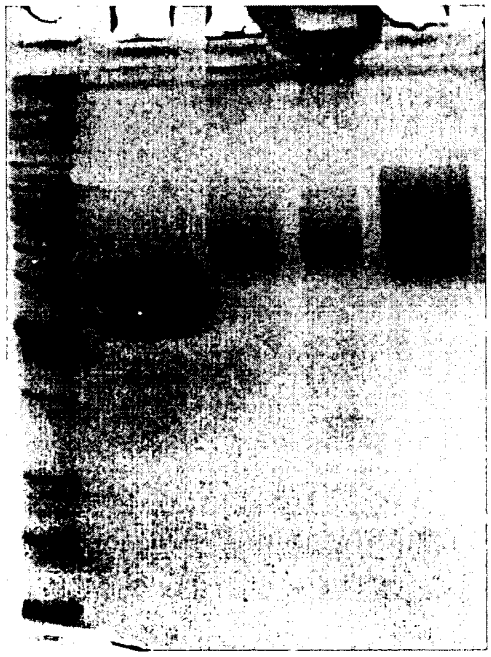


Fig. 2c

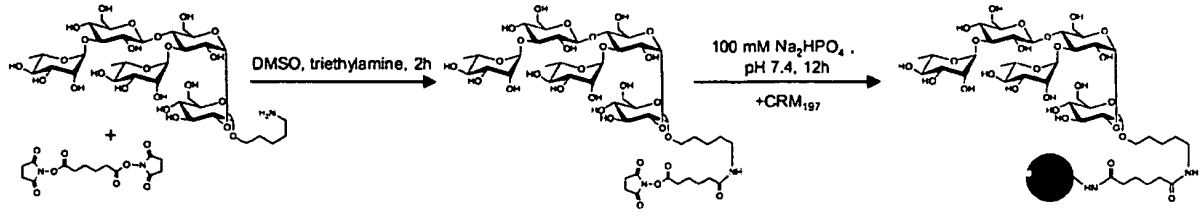


Fig. 3

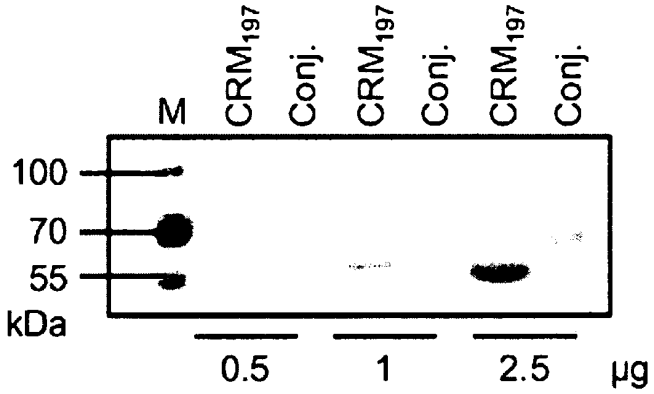


Fig. 4a

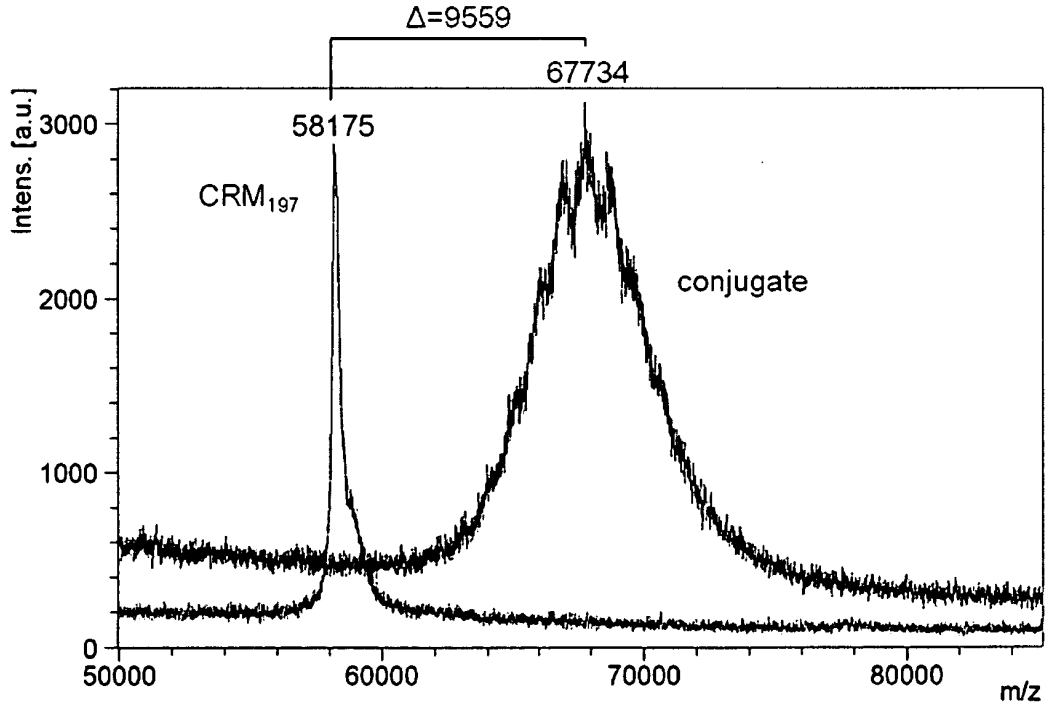


Fig. 4b

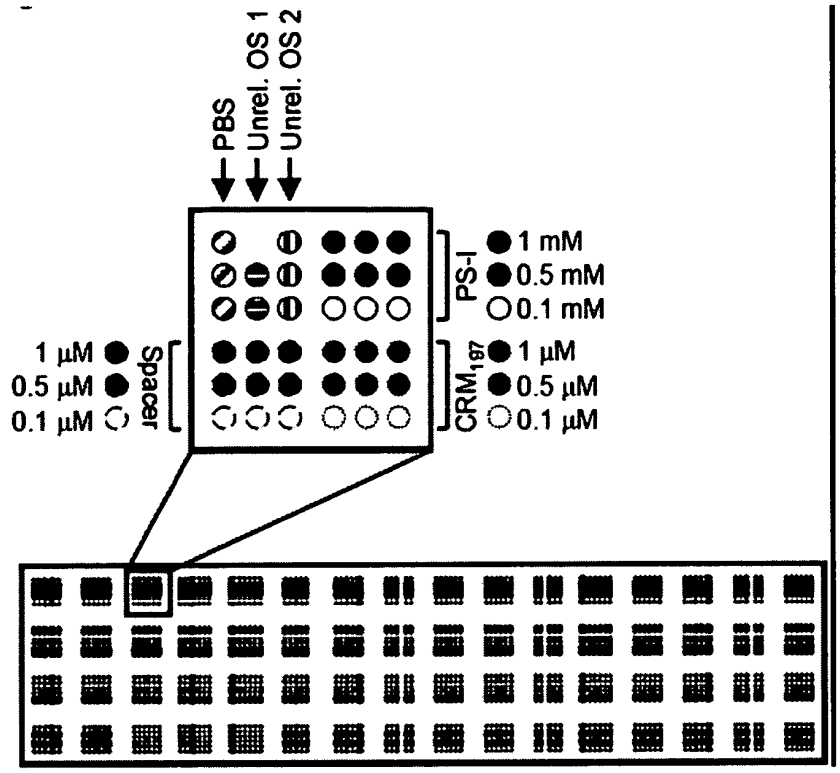

PBS
Unrel. OS 1
Unrel. OS 2
PS-I
1 mM
0.5 mM
0.1 mM
CRM197
1 µM
0.5 µM
0.1 µM
Spacer
1 µM
0.5 µM
0.1 µM

Fig. 5

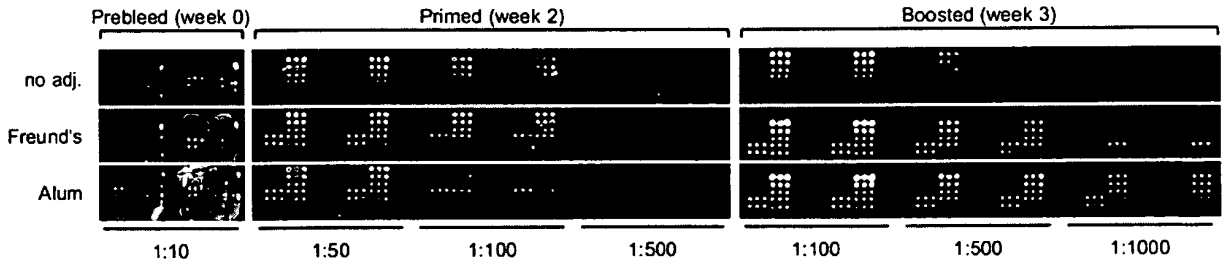

Prebleed (week 0)
Primed (week 2)
Boosted (week 3)
no adj.
Freund's
Alum
1:10
1:50
1:100
1:500
1:100
1:500
1:1000

Fig. 6

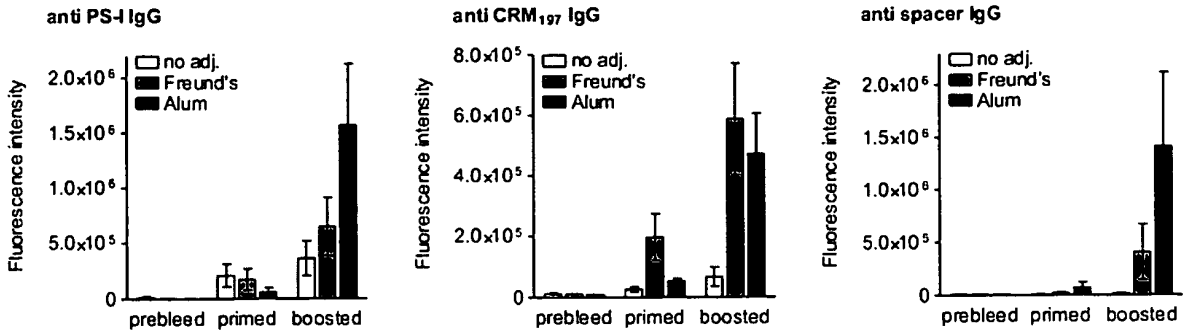

anti PS-I IgG
anti CRM197 IgG
anti spacer IgG
Fluorescence intensity
no adj.
Freund's
Alum
prebleed
primed
boosted

Fig. 7

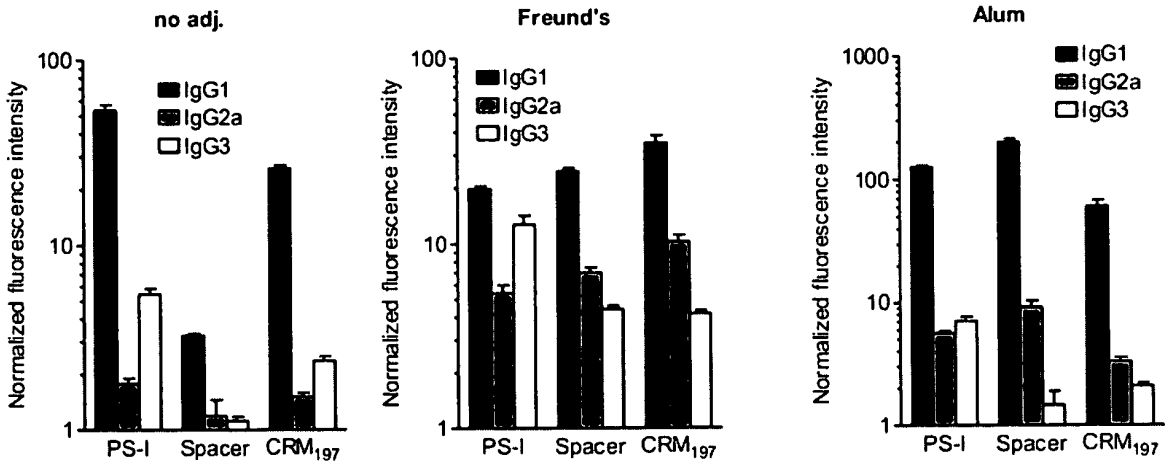

no adj.
Freund's
Alum
Normalized fluorescence intensity
IgG1
IgG2a
IgG3
PS-I
Spacer
CRM197

Fig. 8

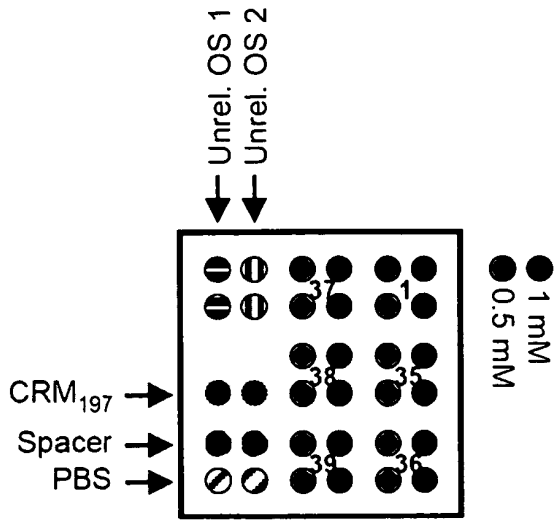
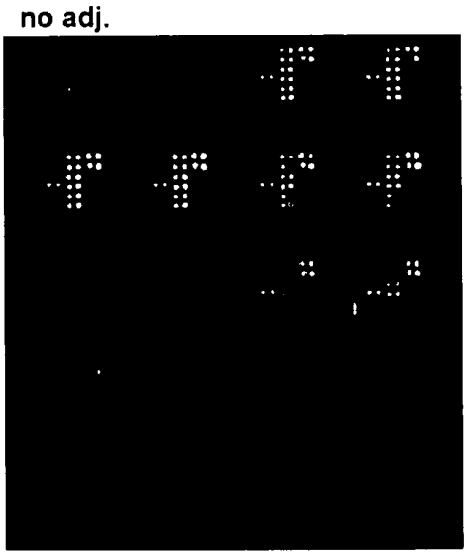


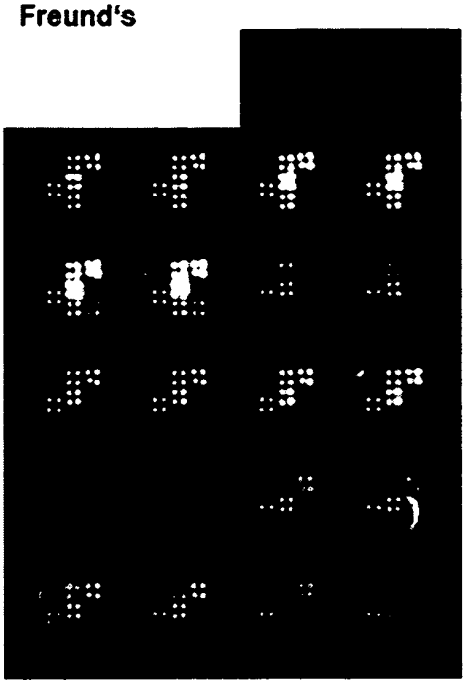
Fig. 9



prebleed 1:50	prebleed 1:50	primed 1:50	primed 1:50
boosted (week 3) 1:50	boosted (week 3) 1:50	boosted (week 5) 1:50	boosted (week 5) 1:50
primed 1:250	primed 1:250	boosted (week 3) 1:250	boosted (week 3) 1:250
boosted (week 5) 1:250	boosted (week 5) 1:250	primed 1:1000	primed 1:1000
boosted (week 3) 1:1000	boosted (week 3) 1:1000	boosted (week 5) 1:1000	boosted (week 5) 1:1000

Fig. 10

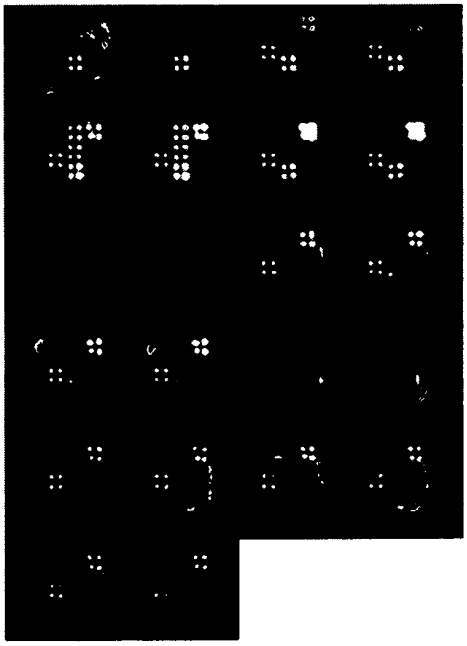
Freund's



		prebleed 1:50	prebleed 1:50
primed 1:50	primed 1:50	boosted (week 3) 1:50	boosted (week 3) 1:50
boosted (week 5) 1:50	boosted (week 5) 1:50	primed 1:250	primed 1:250
boosted (week 3) 1:250	boosted (week 3) 1:250	boosted (week 5) 1:250	boosted (week 5) 1:250
primed 1:1000	primed 1:1000	boosted (week 3) 1:1000	boosted (week 3) 1:1000
boosted (week 5) 1:1000	boosted (week 5) 1:1000	boosted (week 5) 1:2000	boosted (week 5) 1:2000

Fig. 11

Alum



prebleed 1:50	prebleed 1:50	primed 1:50	primed 1:50
boosted (week 3) 1:50	boosted (week 3) 1:50	boosted (week 5) 1:50	boosted (week 5) 1:50
primed 1:250	primed 1:250	boosted (week 3) 1:250	boosted (week 3) 1:250
boosted (week 5) 1:250	boosted (week 5) 1:250	primed 1:1000	primed 1:1000
boosted (week 3) 1:1000	boosted (week 3) 1:1000	boosted (week 5) 1:1000	boosted (week 5) 1:1000
boosted (week 5) 1:2000	boosted (week 5) 1:2000		

Fig. 12

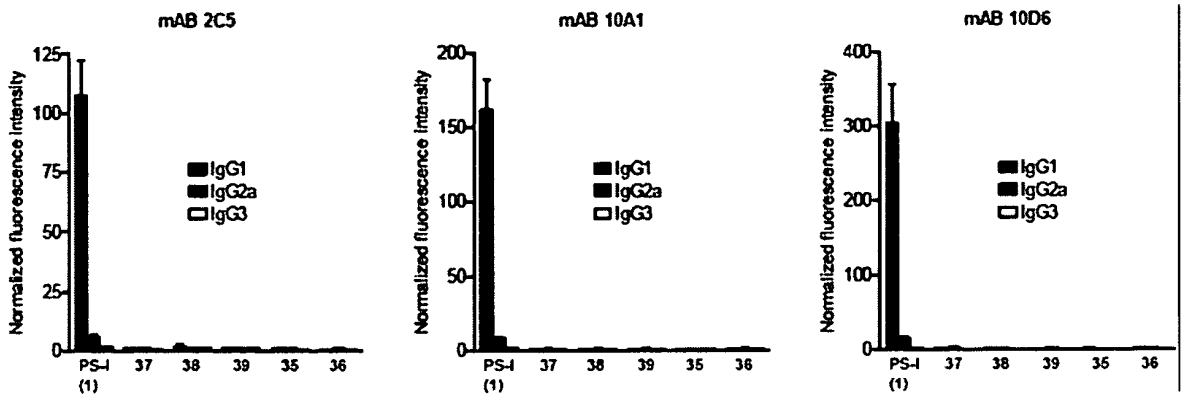

mAB 2C5
mAB 10A1
mAB 10D6
Normalized fluorescence intensity
IgG1
IgG2a
IgG3
PS-I (1)
37
38
39
35
36

Fig. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/003240

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07H3/06 C07H3/08 C07H13/08 C07H15/04 C07H15/20
A61K31/7032 A61P31/04 A61P1/12
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/033268 A1 (UNIV GUELPH [CA]; MONTEIRO MARIO ARTUR [CA]; GANESHAPILLAI JEYABARATHY) 19 March 2009 (2009-03-19)	1,2,5, 7-13, 21-24
Y	claims 3,14-17, 22-30 paragraph [00147]	4,6,14, 18-20, 25-27
A		15-17
	----- -/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 September 2012	11/09/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mezzato, Stefano

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GANESHAPILLAI ET AL: "Clostridium difficile cell-surface polysaccharides composed of pentaglycosyl and hexaglycosyl phosphate repeating units", CARBOHYDRATE RESEARCH, PERGAMON, GB, vol. 343, no. 4, 12 January 2008 (2008-01-12), pages 703-710, XP022497595, ISSN: 0008-6215, DOI: 10.1016/J.CARRES.2008.01.002 cited in the application page 708; compounds PS-I	1,2,5
Y	MATTHIAS A. OBERLI ET AL: "A Possible Oligosaccharide-Conjugate Vaccine Candidate for Clostridium difficile Is Antigenic and Immunogenic", CHEMISTRY & BIOLOGY, vol. 18, no. 5, 1 May 2011 (2011-05-01), pages 580-588, XP055007928, ISSN: 1074-5521, DOI: 10.1016/j.chembiol.2011.03.009 cited in the application page 584 - page 585; figures 3-4	4,6,14, 18-20, 25-27
X	ZHANG J ET AL: "Linking Carbohydrates to Proteins Using N-(2,2-Dimethoxyethyl)-6-hydroxy Hexanamide", TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 54, no. 39, 24 September 1998 (1998-09-24), pages 11783-11792, XP004133357, ISSN: 0040-4020, DOI: 10.1016/S0040-4020(98)83039-1 page 11785 - page 11786; compounds 12-16	1-3,5
X	OPHÉLIE MILHOMME ET AL: "Access to Antigens Related to Anthrose Using Pivotal Cyclic Sulfite/Sulfate Intermediates", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 76, no. 15, 16 June 2011 (2011-06-16) , pages 5985-5998, XP55036957, ISSN: 0022-3263, DOI: 10.1021/jo200340q page 5989; compound 37	1-3,5

-/--

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/003240

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUBOIS E P ET AL: "Chemical approaches to bacterial vaccines. Synthesis of mycobacterial oligosaccharide-protein conjugates for use as serodiagnostics and immunogens", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, ELSEVIER SCIENCE, GB, vol. 6, no. 12, 18 June 1996 (1996-06-18), pages 1387-1392, XP004134846, ISSN: 0960-894X, DOI: 10.1016/0960-894X(96)00235-1 page 1390; compounds 18-20 page 1388; compounds 2-5 -----	1-3,5

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2012/003240

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-27(partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-27(partially)

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of claims 1-27 may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, the search was performed taking into consideration the non-compliance in determining the extent of the search of claims 1-27. The search of claims 1-27 was restricted to: - compounds of claim 6, wherein the linker L is (CH2)nNH2, with n being an integer from 3 to 10.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2012/003240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009033268 A1	19-03-2009	AU 2008299536 A1	19-03-2009
		CA 2698157 A1	19-03-2009
		CN 101896197 A	24-11-2010
		EP 2195018 A1	16-06-2010
		JP 2010539256 A	16-12-2010
		NZ 583750 A	25-05-2012
		US 2010330125 A1	30-12-2010
		WO 2009033268 A1	19-03-2009