Sensitive and specific enzyme immunoassays for antigenic trisaccharide from *Bacillus anthracis* spores

Sandrine G. Y. Dhénin,[a] Vincent Moreau,*[a] Marie-Claire Nevers,[b] Christophe Créminon[b] and Florence Djedaïni-Pilard*[a]

Received 20th July 2009, Accepted 16th September 2009
First published as an Advance Article on the web 19th October 2009
DOI: 10.1039/b914534f

A straightforward synthesis of an anthrose-containing trisaccharide derived from *Bacillus anthracis* was achieved. Antibodies raised against this hapten provide a highly sensitive enzyme immunoassay with a detection limit of 8.5 pmol mL^{-1}. By investigating the specificity of the antibodies obtained using different mono-, di- and trisaccharide synthetic analogues, we demonstrated that the epitope was mainly made up of the methyl group at C-5, the butamido group at C-4 and the hydroxyl at C-3 of the anthrose unit, the other parts of the trisaccharide appearing little involved in the recognition.

Introduction

Bacillus anthracis, a spore-forming Gram positive bacteria, is the causative agent of anthrax. The immunodominant glycoprotein BclA, which covers the surface of the spores, bears a tetrasaccharide **1** containing a unique terminal sugar at the non-reducing end, the so-called anthrose (Fig. 1). Since its structure has been elucidated,[1] and the suggestion that this characteristic antigen may be an interesting target for the development of a vaccine or antibody-based detection system, several studies have reported the synthesis[2] of this sugar or of one of its related fragments[3] and/or their use in developing immunological tools.[3b,4]

It has been clearly demonstrated that the synthetic tetrasaccharide **1** and its related trisaccharide fragment are recognised by antibodies elicited by *B. anthracis* spores.[3b,4b] Moreover, it has been shown that monoclonal antibodies generated from mice immunised with tetrasaccharide **1**-KLH conjugates are able to bind specifically to *B. anthracis* spores.[4a] Recently, we have reported[3c] the synthesis of a derivative of anthrose **2**: when attached to a carrier protein, this very small hapten induced a specific and strong immune response in rabbits.

The main goal of our research is the production of antibodies directed against fragments of tetrasaccharide **1** and their use in the development of an antibody-based detection system. In this competitive context, we wish to design the minimal hapten to be both easily synthesised and allowing the production of a strong specific and sensitive immune response for further detecting anthrax spores. We report here the synthesis of an anthrose-containing trisaccharide **29**-KLH glycoconjugate to produce specific rabbit polyclonal antibodies. A structure-activity relationship study was performed using several mono-, di- and trisaccharide synthetic analogues to check the specificity and sensitivity of the assay and to characterise the optimum synthetic target *via* a competitive enzyme immunoassay (EIA).

[a]*Laboratoire des Glucides, UMR-CNRS 6219, Institut de Chimie de Picardie, Université de Picardie Jules Verne, 33 rue St Leu, F-80039, Amiens, France; Fax: +33 03 22 82 75 62; Tel: +33 03 22 82 75 62. E-mail: florence.pilard@u-picardie.fr, vincent.moreau@u-picardie.fr*
[b]*CEA, iBiTecS, SPI, Laboratoire de Recherche en Immunoanalyse, F-91191, Gif-sur-Yvette, France*

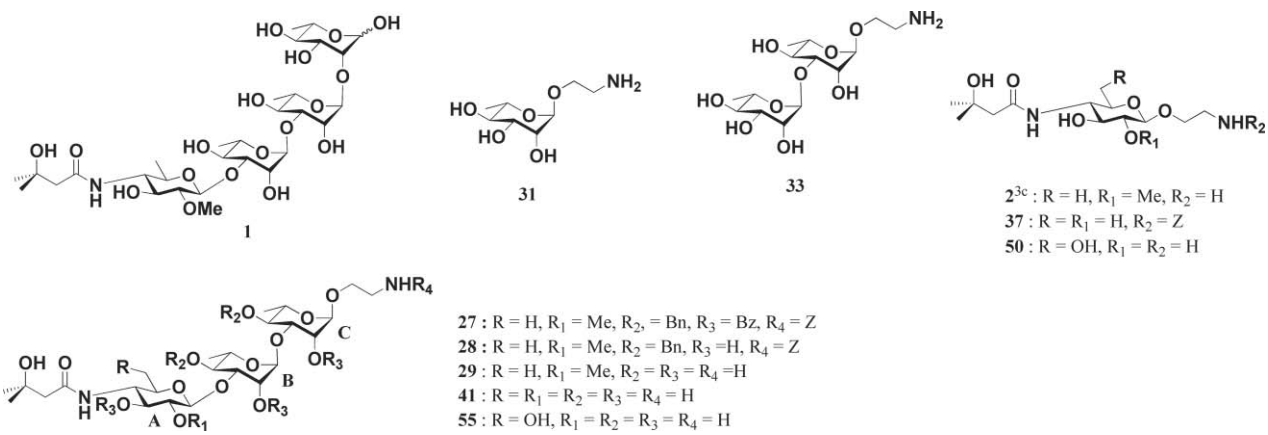


Fig. 1 Structures of the *Bacillus anthracis* antigenic tetrasaccharide **1**, the trisaccharide **29** used for immunization and antibody production after ligation to a protein carrier, and analogues **2**,[3c] **27**, **28**, **37**, **41**, **50** and **55** used for immunoassay experiments (A, B, C letters located below pyranosidic rings refer to each unit and are used to indicate their associated protons and carbons in the NMR data).

Results and discussion

Even if *Bacillus anthracis* is not the only pathogen with potential use for biological warfare, the development of methods allowing rapid and specific detection and diagnosis remains of interest. In this context, the production of specific antibodies to further design immunomethods requires the identification of specific immunogenic target(s). Steinman[4b] and co-workers have demonstrated that the anthrose and anthrose-containing disaccharide are marginally reactive while the anthrose-containing tetrasaccharide is highly reactive against antibodies elicited by the native spores of *Bacillus anthracis*. In this case, the antibody binding reactivity appears to directly correlate with the size of the saccharides. Moreover, Boons and co-workers have reported that the terminal 3-methylbutyryl function is an important antigenic component of the immunological recognition between synthetic trisaccharide and spore-recognising antisera.[3b] In the case of polyclonal antibodies directed against the anthroside unit alone, we showed that the linker used for the synthesis of glycoconjugate is not involved in the recognition, while the C-4 butamido substituent of anthrose is strictly necessary for antibody recognition. Finally, modification at C-3 of anthrose strongly decreases recognition.[3c]

To evaluate the importance of the size of the saccharide hapten, antibodies were raised against anthrose-containing trisaccharide **29** using the protocol previously used to produce antibodies against anthrose derivative **2**.[3c]

Since the introduction of the methoxy group and reduction of primary hydroxyl groups at C-2 and C-6, respectively, are key steps in synthesis, the influence of these functional groups on antibody binding was evaluated by analysing the recognition of the derivatives **37**, **41**, **49** and **55** displayed in Fig. 1.

The specificity of the resulting antibodies for the rhamnose moiety was determined using two rhamnoside analogues **31** and **33** as competitor in enzymatic immunoassays.

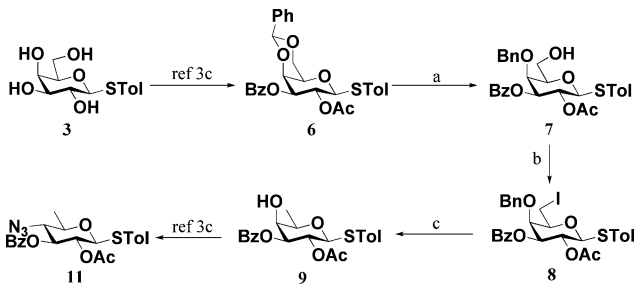
Synthesis of thioglycoside 11

The reported syntheses of the tetrasaccharide **1** and parts thereof differ only slightly except for the *de novo* asymmetric approach starting from 2-acetylfuran described by Guo and O'Doherty.[2d] These former approaches differ mainly in the choice of the starting monosaccharide, the protecting groups and the leaving group used to prepare the intermediate glycoside donor needed to obtain the anthrose moiety. In the most lengthy strategy, D-mannose was used.[2c] Two required the use of rare and expensive D-fucose.[2a,3b] We[3c] and others (Crich[2e] and Kovac[3d]) published almost simultaneously a synthesis starting from D-galactose which compares well with that starting from D-fucose.

Thus, thioglycoside **11** was prepared following our previously described strategy[3c] starting from the known *p*-methylphenyl 1-thio-β-D-galactopyranoside **3**.[5] Reaction of **3** with benzaldehyde dimethyl acetal provided the 4,6-*O*-benzylidene derivative **4**.[6] Selective benzoylation at O-3 was performed with benzoyl cyanide in the presence of triethylamine as a base at −70 °C in high yield (89%) followed by further *O*-acetylation at O-2.

At this stage, our first approach, involving acid-catalysed *O*-debenzylidenation of **6** and regioselective iodination of the resulting diol at the primary hydroxyl, was slightly modified. Iodination in the presence of *N*-iodosaccharine-triphenylphosphine[7] appeared to be more regioselective than the traditional Garegg-Samuelsson procedure[8] but failed to give a high yield on a large scale. We thought that protection at O-4 would allow use of the Garegg-Samuelsson procedure. We thus chose to proceed *via* a reductive ring opening of the 4,6-*O*-benzylidene derivative **6**. As expected, use of BH_3.THF and dibutylboron triflate[9] gave the *p*-methylphenyl 2-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-galactopyranoside **7** in 81% yield. Iodination of compound **7** using Garegg-Samuelsson's conditions (triphenylphosphine/imidazole/iodine in toluene at 60 °C) afforded **8** in good yield (70%) even when carried out on a large scale. Although catalytic hydrogenolysis of **8** induced dehalogenation in high yield, the benzyl group at O-4 appeared totally unreactive under all hydrogenolysis conditions (high pressure and excess of catalyst) attempted. Finally, using the BF_3.OEt_2-NaI reagent system,[10] we were able to release the hydroxyl at O-4 while ensuring the integrity of the tolyl group at the anomeric position. Compound **9** was thus obtained in 77% yield in two steps from **8** without purification of the 4-*O*-benzyl-6-deoxygalactopyranoside intermediate.

Subsequent reaction of **9** with triflic anhydride followed by nucleophilic displacement of the resulting 4-*O*-triflate in **10** with azide[11] gave the expected thioglycoside **11** in 85% yield in 2 steps (Scheme 1).



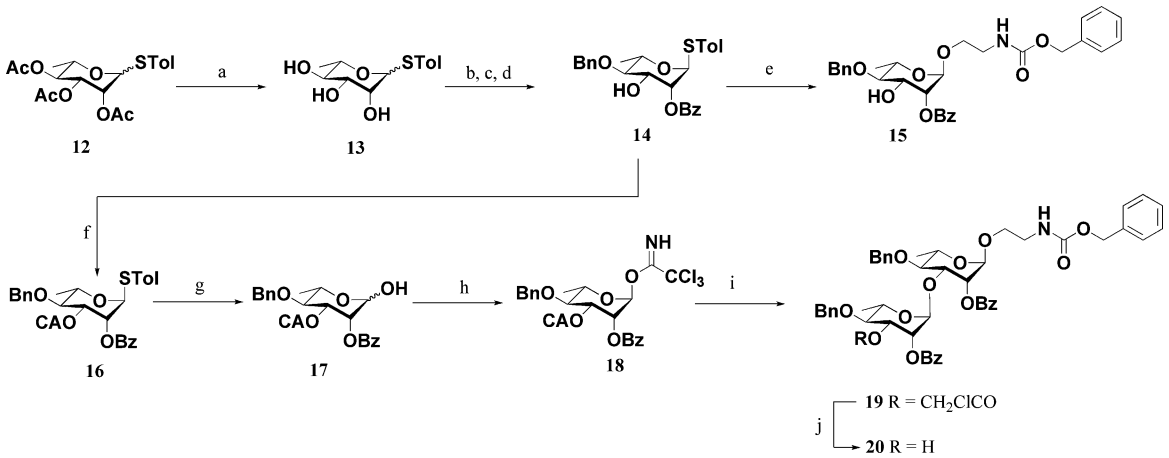
Scheme 1 *Reagents and conditions*: (a) BH_3 (1 M in THF), Bu_2BOTf (1 M in CH_2Cl_2), 0 °C, 5 h, 81%; (b) PPh_3, imidazole, I_2, toluene, 60 °C, 4 h, 70%; (c) DMF, Pd/C, H_2 1 atm., $NaHCO_3$, 5 h then CH_3CN, $BF_3.OEt_2$, NaI, 7 h, 77%.

Synthesis of acceptor 20

Both acceptor **15** and donor **18** were obtained from the key intermediate **14**, namely *p*-methylphenyl 2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside, which was prepared in six steps from L-rhamnose (Scheme 2).

The known fully acetylated *p*-methylphenyl 1-thio-L-rhamnopyranoside[12] **12** was obtained as an α/β mixture in two steps according to the procedure reported by Wong[13] and coworkers in 80% yield. **12** was deacetylated by Zemplèn methanolysis to give **13**[14] which was then reacted with triethyl orthobenzoate in the presence of camphor-10-sulfonic acid as a catalyst. The 2,3-orthoester intermediate thus obtained was benzylated at O-4 in the same pot. The cyclic orthoester was then opened in the presence of aq. 2 M HCl, according to the procedure described by Field,[15] affording the expected *p*-methylphenyl 2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside **14** (76% in 3 steps) and its β analogue (15%).

Glycosylation of benzyl *N*-(2-hydroxyethyl) of NIS/TfOH carbamate with compound **14**, without previous protection of the



Scheme 2 *Reagents and conditions*: (a) MeONa (1 M in MeOH), MeOH, 15 h, 98%; (b) triethyl orthobenzoate, CSA, DMF, 3 h; (c) NaH, BnBr, 15 h; (d) aq. 2 M HCl, 76% in 3 steps. (e) NIS, TfOH, CH_2Cl_2, benzyl *N*-(-2-hydroxyethyl)carbamate, 1 h 30, 58%; (f) chloroacetyl chloride, CH_2Cl_2, pyridine, 3 h, 98%; (g) NBS, acetone, 0 °C to rt, 1 h 20, 91%; (h) CCl_3CN, DBU, 0 °C to rt, 82%; (i) **15**, TMSOTf, CH_2Cl_2, 30 min., 90%; (j) thiourea, $NaHCO_3$, TBAI, THF, 55 °C, 80%.

remaining hydroxyl group, was achieved in the presence to give the acceptor **15** in 58% yield.

Donor **18** was prepared in three steps from **14**. The remaining hydroxy group at O-3 was first chloroacetylated in classic manner in high yield (91%). Cleavage of the thiotoluene group in the presence of NBS followed by subsequent reaction with trichloroacetonitrile under DBU catalysis gave the donor **18**.

The first attempt at glycosylation of acceptor **15** was performed with the thioglycoside donor **16** using standard conditions (NIS/TfOH). The expected disaccharide **19** was obtained in relatively low yield (54%). On the other hand, use of the rhamnosyl trichloroacetimidate donor **18** afforded **19** in high yield (90%, 67% from **16** over 3 steps). Next, the chloroacetyl group was selectively removed by treatment with thiourea giving the dirhamnosyl acceptor **20** (80%) (Scheme 2).

Synthesis of the targeted trisaccharide 29

Glycosylation of the disaccharide **20** with the thioglycoside donor **11** in the presence of NIS and triflic acid as catalyst failed to give the expected trisaccharide. Once again, we chose to use the trichloroacetimidate method,[16] thioglycoside **11** being converted into its trichloroacetimidate analogue **22** in two steps as described above in the case of compound **18**.

Condensation of **20** with donor **22** in the presence of a catalytic amount of TMSOTf afforded the trisaccharide **23**. At this stage, we were not able to remove all the unreacted acceptor and the next step was performed on partially purified trisaccharide **23**. Selective 2-*O*-deacylation of **23** was performed by acid-catalysed methanolysis[17] to afford **24** in 50% yield from **20**. The characteristic methoxy group of anthrose residue was then introduced by reaction of **24** with diazomethane in the presence of BF_3-Et_2O affording the expected derivative **25** in 40% yield.

Next, reduction of the azide in **25** was achieved with sodium borohydride in the presence of nickel chloride to ensure integrity of the carbamate group as previously described by Alpe and Oscarson,[18] affording amine **26**. Treatment of **26** with 3-hydroxy-3-methylbutyric acid in the presence of *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-(4,5-*b*)-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) gave **27** in 85% overall yield (2 steps). Finally, debenzoylation using base-catalysed methanolysis conditions followed by catalytic hydrogenolysis of the benzyl groups and of the carbamate group gave the target spacer arm equipped trisaccharide derivative **29** (Scheme 3).

Synthesis of the competitors

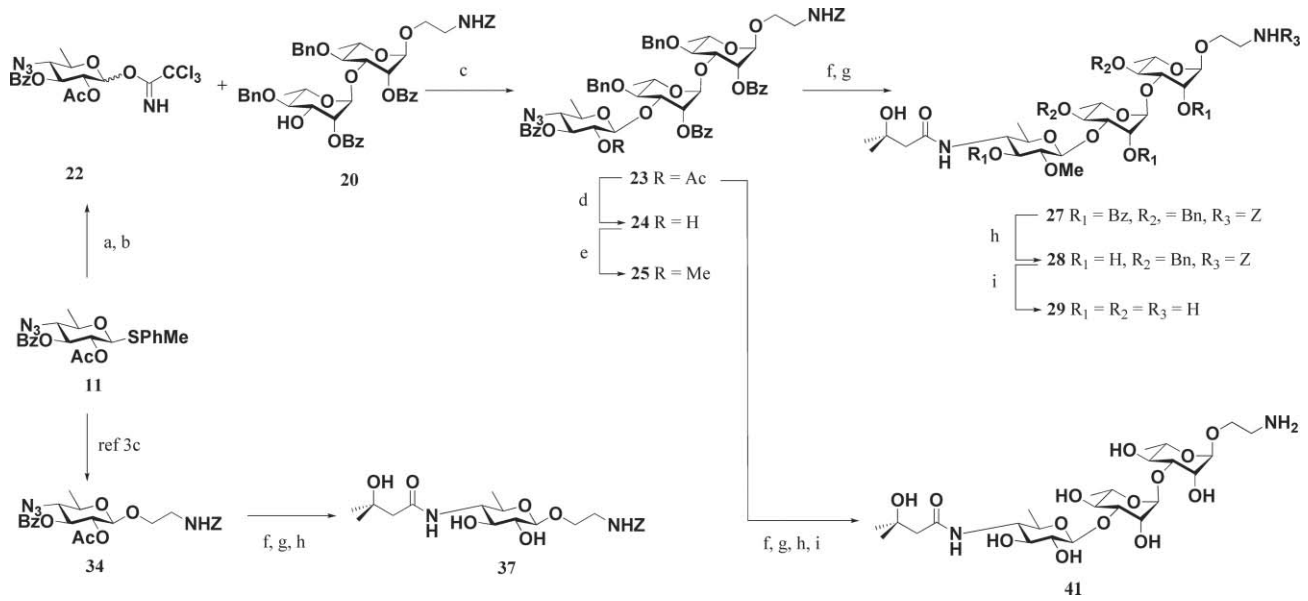
Specificity of the produced antibodies was evaluated using several competitors in EIA: among them, two analogues of the rhamnoside part **31** and **33** as well as two monosaccharides **37** and **50** and two trisaccharides **41** and **55** with a modified anthrose moiety were prepared.

Both rhamnosyl derivatives **31** and **33** were obtained in a deacylation step followed by a catalytic hydrogenolysis step from **15** and **19**, respectively.

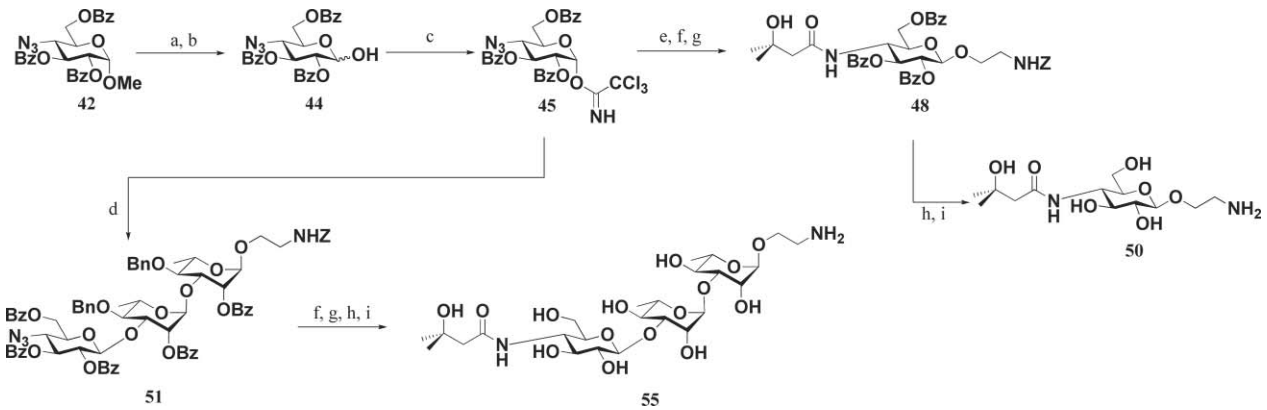
Both competitors **37** and **41**, devoid of the characteristic methoxy group of anthrose, were prepared from the already described *N*-benzyloxyaminoethyl-β-D-glucopyranoside derivative **34**[3c] and the trisaccharide **23**, respectively (Scheme 3). Reduction of the azide, peptide-type coupling of 3-hydroxy-3-methylbutyric acid followed by base-catalysed methanolysis gave the expected 2-hydroxy anthrose analogues **37** and **40**. Treatment of **40** by catalytic hydrogenolysis of the benzyl groups and of the carbamate group afforded the target trisaccharide derivative **41**.

As shown by our work and previous reports, synthesis of the anthropyranoside moiety is quite long and tedious starting from D-galactose as well as from the expensive D-fucose. We thus decided to study the influence of the methyl group at C-5 and the possibility of replacing it by a hydroxymethyl group. Synthesis of competitors **50** and **55** was undertaken (Scheme 4).

Acetolysis of the known methyl 2,3,6-tri-*O*-benzoyl-4-azido-4-deoxy-α-D-glucopyranoside[19] **42** and selective removal of the resulting 1-*O*-acetyl derivative **43** by treatment with hydrazine acetate gave glucopyranose derivative **44**[20] in 67% yield in 2 steps. Subsequent reaction of **44** with trichloroacetonitrile under DBU catalysis gave the donor **45**.



Scheme 3 *Reagents and conditions*: (a) NBS, acetone, 0 °C to rt, 2 h 20 min, 90%; (b) CCl_3CN, DBU, 0 °C, 2 h, 84%; (c) TMSOTf, CH_2Cl_2, 45 min; (d) CH_3COCl, MeOH, CH_2Cl_2, 0 °C to rt, 6 days, 50% in two steps; (e) CH_2N_2, BF_3OEt_2, CH_2Cl_2, 0 °C, 40%; (f) $NaBH_4$, $NiCl_2.6H_2O$, EtOH, CH_2Cl_2; (g) $HO(CH_3)_2CCH_2CO_2H$, HATU, DIPEA, CH_2Cl_2, 18 h, 85% (**27**), 62% (**36**), 53% (**39**) in two steps; (h) MeONa (1 M in MeOH), MeOH, 15 h, 76% (**37**); (i) $Pd(OH)_2$, AcOH, H_2, tBuOH, 5 h to 20 h, 40% (**29**), 50% (**41**) in two steps.



Scheme 4 *Reagents and conditions*: (a) Ac_2O, AcOH, H_2SO_4, 50 °C, 24 h, 74%; (b) hydrazine acetate, DMF, 4 h, 91%; (c) CCl_3CN, DBU, 0 °C, 1 h, 87%; (d) **20**, TMSOTf, CH_2Cl_2, 1 h; (e) TMSOTf, CH_2Cl_2, benzyl *N*-(-2-hydroxyethyl)carbamate, 0 °C, 1 h, 73%; (f) $NaBH_4$, $NiCl_2.6H_2O$, EtOH, CH_2Cl_2; (g) $HO(CH_3)_2CCH_2CO_2H$, HATU, DIPEA, CH_2Cl_2, 18 h and 3 days respectively, 60% (**48**), 47% (**53**) in two steps; (h) MeONa (1 M in MeOH), MeOH, 18 h; (i) $Pd(OH)_2$, AcOH, H_2, tBuOH, 20 h, 72% (**50**), 44% (**55**) in two steps.

Then, condensation of benzyl *N*-(2-hydroxyethyl)carbamate or dirhamnosyl acceptor **20** with **45** was performed in the presence of a catalytic amount of TMSOTf to afford the expected β-linked glycoside **46** and the trisaccharide **51**, respectively. Reduction of the azide, peptide-type coupling of 3-hydroxy-3-methylbutyric acid, base-catalysed methanolysis and finally catalytic hydrogenolysis of the benzyl groups and of the carbamate group afforded the target competitors **50** and **55**.

Synthesis of a trisaccharide-KLH conjugate and investigation of the immune response

The trisaccharide-KLH conjugate was prepared according to our previously described procedure[3c] using glutaraldehyde to ensure coupling between the amino groups of the protein carrier and the trisaccharide **29**. To induce antibody production, rabbits were immunised and given booster injections every two months with **29**-KLH conjugate. Antibody titre was measured by EIA using compound **29** labeled with acetylcholinesterase (AChE) as tracer. The latter was prepared in two steps by first deriving the trisaccharide **29** *via* reaction with S-acetyl thioglycolic acid N-succinimidyl ester before covalently coupling the introduced thiol moiety to AChE through a succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker. This procedure ensures full immunoreactivity of the enzymatic tracer **29**-AChE and avoids the possible involvement of the spacer arm in the recognition by antibodies. A titre above 1/1,000,000 was measured from the first booster injection onwards, showing the strong immunogenicity of the **29**-KLH immunogen (Fig. 2).

Using the **29**-AChE tracer, we determined the optimal dilution of antisera for the assay and plotted standard curves using **29** as reference. Antibodies raising against **29** provided a detection

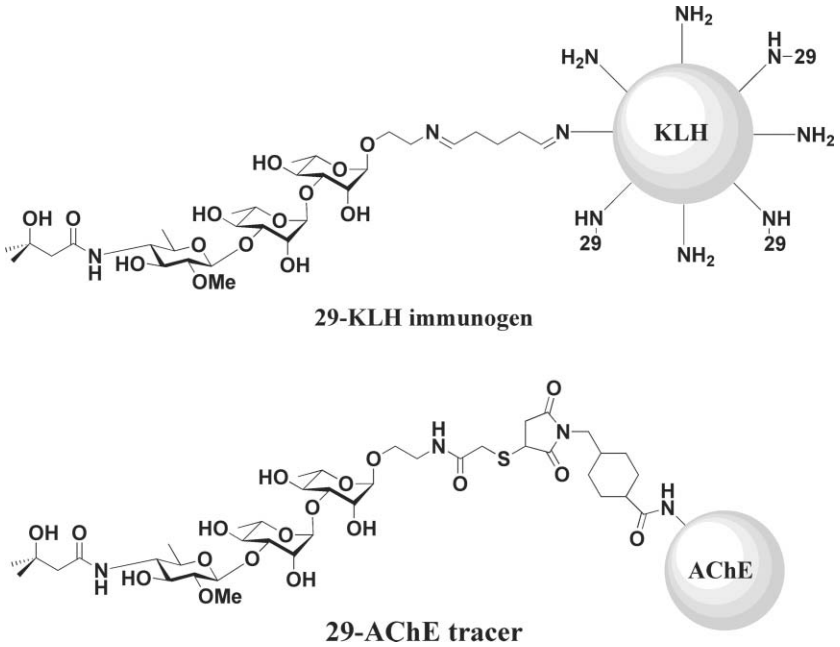


Fig. 2 Structure of **29**-KLH immunogen and of **29**-AChE tracer.

Table 1 Relative cross-reactivity observed with **2**, **27**, **28**, **31**, **33**, **37**, **41**, **50**, and **55** taking **29** as reference and using the glycoconjugate **29**-AchE as tracer

competitor	IC50 (ng mL^{-1})[a]	IC50 (pmol mL^{-1})[a]	CR (%)[b]
29	5.2	8.48	100
31	—	—	—
33	—	—	—
37	37.2	84.45	9.5
41	1.9	3.17	260
50	2600	8065	0.1
55	128	208.25	3.9
27	—	—	—
28	9.8	10.57	80
2	15.9	49.62	16

[a] IC50: concentration of the competitor required to inhibit 50% of the **29**-AchE tracer/antibody binding. [b] Cross reactivity (CR) = IC50(**29**)/IC50(competitor) with IC50 in pmol mL^{-1}.

limit of 8.5 pmol mL^{-1}, similar to the previous assay developed for monosaccharide **2** (8.7 pmol mL^{-1}).[3c] However, the higher immune titre seems to indicate a stronger immunogenic potency for the **29**-KLH immunogen.

Taking compound **29** as reference, different standard curves were plotted using analogues **31**, **33**, **37**, **41**, **50**, **55** and two intermediates of the synthesis (**27** and **28**) as competitor to better analyse the specificity of the antibodies. The previously synthesised hapten **2** was also evaluated (Fig. 1).[3c]

The results are summarised in Table 1. Antigen **29** presented an IC50 value (concentration of the competitor required to inhibit 50% of the tracer/antibody binding) of 5.2 ng mL^{-1}.

As already observed by Steinman,[4b] the rhamnoside derivatives **31** and **33** were not able to bind the antibodies, demonstrating that the rhamnoside part was only negligibly involved in the recognition by the antibodies. By comparing the IC50 obtained with the different monomeric anthrose derivatives, namely **2**, **37** and **50**, **2** exhibited an almost good cross-reactivity, revealing that this part of the immunogen is critical for the antibody recognition. On the other hand, the better recognition of **29** in comparison with **2** could be explained by a size effect. It is also noteworthy that the lack of the C2 methyl only slightly modified the binding (**37** *vs* **2**), while the addition of a hydroxyl group to C6 strongly decreased recognition (**50** *vs* **37**). Considering the different trisaccharides analysed, although the absence of recognition of the fully protected trisaccharide **27** was expected, the partially deprotected derivative **28**, exposing the anthrose residue and the C2 hydroxyl functions of the rhamnose, presented almost the same reactivity as compound **29**.

Next, we evaluated what kind of modification of the anthrose unit itself could be tolerated leading to a potentially more accessible synthesis of hapten. We[3c] and others[3b] have already observed that the C-4 butamido substituent of anthrose is strictly necessary for antibody recognition. Moreover, we know that the latter is dramatically decreased by a modification at C-3.[3c] Data published by Boons[3b] indicate that the methyl ether at C-2 is not critical for the antispore antibody binding. The full cross-reactivity exhibited by the trisaccharide **41** lacking a methoxy group at C-2 (3.2 pmol mL^{-1} *vs* 8.5 pmol mL^{-1} for **29**) corroborates this observation in the case of antibodies elicited by the synthetic **29**-KLH glycoconjugate. On the other hand, the poor recognition of **50** and **55** bearing a hydroxy group at C-6 indicates that the 6-deoxy feature of the anthrose residue has to be conserved to preserve the immunogenic potential of future synthetic analogues.

Our results showed that the presence of specific substituents at positions C-3, C-4 and C-6 of the anthrose unit is dramatic for antibody recognition and for the immune response. On the other hand, the methyl ether at C-2 seems not to be necessary. Finally, while the overall size of the trisaccharide appears to play a role in the recognition phenomenon, the rhamnoside part is poorly immunogenic.

Experimental section

General remarks

All reactions were monitored by TLC on Kieselgel 60 F254 (E. Merck). Detection was achieved by charring with vanillin. Silica gel (E. Merck, 240-400 mesh) was used for chromatography. Solutions were concentrated under reduced pressure. Optical rotation was measured with a JASCO DIP-370 digital polarimeter, using a sodium lamp ($\lambda = 589$ nm) at 20 °C. All NMR experiments were performed at 300.13 and 500.13 MHz using Bruker DMX300 and DRX500 spectrometers equipped with a Z-gradient unit for pulsed-field gradient spectroscopy. Assignments were performed by stepwise identification using COSY, successive RELAY, HSQC and HMBC experiments using standard pulse programs from the Brucker library. Chemical shifts are given relative to external TMS with calibration involving the residual solvent signals. When D_2O was used, TMS was used as internal standard reference in a previous ^{13}C NMR experiment performed in the same experimental conditions. The length of the 90° pulse was approximately 7 μs (^1H NMR) and 10 μs (^{13}C NMR). 1D NMR data spectra were collected using 16 K data points. 2D experiments were run using 1K data points and 512 time increments. The phase-sensitive (TTPI) sequence was used and processing resulted in a 1K*1K (real-real) matrix. A 45° flip angle (3.5 μs) and a total recovery time of 5 s were used to ensure complete relaxation of the protons and quantitative measurements. The digital integration of the transformed spectra was performed after polynomial baseline correction.

Low-resolution ESI mass spectra were obtained on a hybrid quadrupole/time-of-flight (Q-TOF) instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source (Micromass). High-resolution mass spectra were recorded in positive mode on a ZabSpec TOF (Micromass, UK) tandem hydrid mass spectrometer with EBETOF geometry. The compounds were individually dissolved in 1:1 water-MeCN at a concentration of 10 μg cm^{-3} and then infused into the electrospray ion source at a flow rate of 10 mm^3 min^{-1} at 60 °C. The mass spectrometer was operated at 4 kV whilst scanning the magnet at a typical range of 4000-100 Da. The mass spectra were collected as continuum profile data. Accurate mass measurement was achieved using polyethylene glycol as internal reference with a resolving power set to a minimum of 10 000 (10% valley).

Elemental analyses were performed at the Service de Microanalyse of the Université de Champagne-Ardennes in Reims, France. The samples were previously dried under reduced pressure for one week.

Acetylcholinesterase (AChE, EC 3.1.1.7) from the electric organs of the electric eel *Electrophorus electricus*, was purified by affinity chromatography as reported.[21] The modified Ellman's reagent was a solution of acetylthiocholine iodide (enzyme substrate) and 5,5-dithiobis-2-nitrobenzoic acid (chromogen) in phosphate buffer (pH 7.4). All reagents used for the EIA were diluted in the following buffer (EIA buffer): 0.1 M potassium phosphate (pH 7.4) containing 0.15 M NaCl, 0.1% bovine serum albumin (BSA) and 0.01% sodium azide. The washing buffer was 10 mM phosphate (pH 7.4) containing 0.05% Tween 20. Solid phase EIA was performed in 96-well microtitre plates (immunoplate Maxisorb with certificate, Nunc, Roskilde Denmark) with as specialised microtitration equipment a washer (ELX 405, Bio-Tek Instruments) using automatic plate washer and automatic plate-reader (Multiskan, Thermo Life Sciences).

p-Methylphenyl 2-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-1-thio-β-D-galactopyranoside (7)

p-Methylphenyl 2-*O*-acetyl-3-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**6**)[3c] (5.34 g, 10.3 mmol) was dissolved in a solution of BH_3 (1 M in THF, 30 mL) and cooled at 0 °C. A solution of dibutylboron triflate (1 M in CH_2Cl_2, 10 mL) was added and the reaction mixture stirred for 5 h. Methanol (around 1 mL with care until the gaseous emission ceased) was added. The solvents were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography (7.5:2.5 cyclohexane-EtOAc) to give **7** (4.37 g, 81%), $[\alpha]_D$ +34 (c 0.72, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.04-7.13 (m, 14H, Ar), 5.65 (t, 1H, $J_{1,2} = J_{2,3}$ 10 Hz, H-2), 5.24 (dd, 1H, $J_{3,4}$ 3 Hz, H-3), 4.76 (d, 1 H, H-1), 4.75 (d, 1H, *J* 11.5 Hz, OCH_2Ar), 4.50 (d, 1 H, OCH_2Ar), 4.14 (d, 1H, H-4), 3.95-3.86 (m, 1H, H-6a), 3.73 (t, 1H, $J_{5,6}$ 5.5 Hz, H-5), 3.66-3.57 (m, 1H, H-6b), 2.37 (s, 3H, $SC_6H_4CH_3$), 2.04 (s, 3H, CH_3CO); δ_C (75 MHz, $CDCl_3$) 169.4 (CH_3CO), 165.8 (OCOAr), 137.9-127.5 (Ar), 86.5 (C-1), 78.8 (C-5), 75.9 (C-3), 74.5 (OCH_2Ar), 73.6 (C-4), 67.8 (C-2), 61.8 (C-6), 21.1 ($SC_6H_4CH_3$), 20.8 (CH_3CO); HRMS (ES^+) Calcd. for $C_{29}H_{30}NaO_7S$ (MNa^+) 545.1610. Found 545.1614.

p-Methylphenyl 2-*O*-acetyl-3-*O*-benzoyl-4-*O*-benzyl-6-deoxy-6-iodo-1-thio-β-D-galactopyranoside (8)

To compound **7** (4.37 g, 8.37 mmol) in toluene (200 mL) was added successively at room temperature triphenylphosphine (3.3 g, 12.6 mmol), imidazole (870 mg, 12.8 mmol) and iodine (3.17 mg, 12.5 mmol). The reaction mixture was stirred at 60 °C for 4 h. The reaction mixture was cooled, saturated aq. $NaHCO_3$ (70 mL) was added and the mixture was stirred for 5 min. Iodine was added in portions until the red color persists. Excess iodine was removed by the addition of saturated aq $Na_2S_2O_3$ (15 mL). The mixture was diluted with ethyl acetate. Then the organic layer was separated and extracted with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (9:1 cyclohexane-EtOAc) to give **8** (3.71 g, 70%), $[\alpha]_D$ +49 (c 1.6, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.09-7.22 (m, 14H, Ar), 5.60 (t, 1H, $J_{1,2} = J_{2,3}$ 10.0 Hz, H-2), 5.27 (dd, H, $J_{3,4}$ 3 Hz, H-3), 4.85 (d, 1H, *J* 11.5 Hz, OCH_2Ar), 4.77 (d, 1H, H-1), 4.60 (d, 1H, OCH_2Ar), 4.41 (d, 1H, H-4), 3.86 (t, 1H, $J_{5,6}$ 7 Hz, H-5), 3.42-3.29 (m, 2H, H-6a, H-6b), 2.45, (s, 3H, $SC_6H_4CH_3$), 2.05 (s, 3H, CH_3CO); δ_C (75 MHz, $CDCl_3$) 169.4 (CH_3CO), 165.7 (OCOAr), 138.1-127.7 (Ar), 86.6 (C-1), 79.0 (C-5), 75.8 (C-3), 75.2 (OCH_2Ar), 74.50 (C-4), 67.3 (C-2), 21.1 ($SC_6H_4CH_3$), 20.7 (CH_3CO), 1.6 (C-6); HRMS (ES^+) Calcd. for $C_{29}H_{29}INaO_6S$ (MNa^+) 655.0627. Found 655.064.

p-Methylphenyl 2-*O*-acetyl-3-*O*-benzoyl-1-thio-β-D-fucopyranoside (9)

To compound **8** (1.6 g, 2.53 mmol) in dry DMF (80 mL) was added 20% palladium-on-charcoal catalyst (650 mg), and $NaHCO_3$ (688 mg, 8.19 mmol). The reaction mixture was stirred for 5 h

in a hydrogen atmosphere. The catalyst was filtered on celite 521 and the filtrate was concentrated. The residue was dissolved in ethyl acetate. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The residue was dissolved in CH_3CN (10 mL) and NaI (3.79 g, 25.3 mmol) and $BF_3.OEt_2$ (3.2 mL, 25.3 mmol) were added. The reaction mixture was stirred at rt for 7 h, then diluted with CH_2Cl_2. The organic solution was washed with aq. $Na_2S_2O_3$ and brine, dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography (4:1 cyclohexane-EtOAc) afford **9** (810 mg, 77%) as a white powder, $[\alpha]_D$ +25 (*c* 0.15, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.03 (d, 2H, *J* 8 Hz, $SC_6H_4CH_3$), 7.60-7.41 (m, 5H, OCOAr), 7.16 (d, 2H, $SC_6H_4CH_3$), 5.44 (t, 1H, $J_{1,2} = J_{2,3}$ 10 Hz, H-2), 5.15 (dd, 1H, $J_{3,4}$ 3 Hz, H-3), 4.70 (d, 1H, H-1), 4.06 (broad s, 1H, H-4), 3.84 (q, 1H, $J_{5,6}$ 6.5 Hz, H-5), 2.36 (s, 3H, $SC_6H_4CH_3$), 2.02 (s, 3H, CH_3CO), 1.39 (d, 3H, H-6); δ_C (75 MHz, $CDCl_3$) 169.5 (CH_3CO), 165.8 (OCOAr), 139.0-128.0 (Ar), 86.3 (C-1), 75.9 (C-3), 74.6 (C-5), 70.0 (C-4), 67.3 (C-2), 21.1 ($SC_6H_4CH_3$), 20.8 (CH_3CO), 16.4 (C-6); HRMS (ES^+) Calcd. for $C_{22}H_{24}O_6Na^{32}S$ (MNa^+) 439.1191. Found 439.1211. Found: C, 63.08; H, 5.92. $C_{22}H_{24}O_6S$ requires C, 63.44; H, 5.81%.

p-Methylphenyl 1-thio-L-rhamnopyranoside (13)

To a suspension of *p*-methylphenyl 2,3,4-tri-*O*-acetyl-1-thio-L-rhamnopyranoside **12**[12] (20.82 g, 52.58 mmol) in methanol (300 mL) was added dropwise at 0 °C a solution of sodium methoxide (26 mL, 1 M in MeOH). The reaction mixture was stirred at room temperature for 15 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed to afford derivative **13** (13.90 g, 98%) as a white powder, δ_H (300 MHz, $CDCl_3$) 7.31 (d, 2H, *J* 8.1 Hz, $SC_6H_4CH_3$), 7.03 (d, 2H, $SC_6H_4CH_3$), 5.47 (broad s, 1H, $J_{1\alpha,2\alpha} <$ 0.5 Hz, H-1α), 4.78 (broad s, 1H, $J_{1\beta,2\beta} <$ 0.5 Hz, H-1β), 4.28-4.27 (m, 2H, H-2α, H-2β), 4.26-4.21 (m, 1H, H-5α), 3.87 (dd, 2H, $J_{2\alpha,3\alpha} = J_{2\beta,3\beta}$ 2.6 Hz, $J_{3\alpha,4\alpha} = J_{3\beta,4\beta}$ 9.4 Hz, H-3α, H-3β), 3.66-3.57 (m, 2H, H-4α, H-4β), 3.32-3.28 (m, 1H, H-5β), 2.30 (s, 3H, $SC_6H_4CH_3$), 1.41 (d, 3H, $J_{5\beta,6\beta}$ 6.0 Hz, H-6β), 1.35 (d, 3H, $J_{5\alpha,6\alpha}$ 6.1 Hz, H-6α); δ_C (75 MHz, $CDCl_3$) 137.3-129.7 ($SC_6H_4CH_3$), 88.2 (C-1α), 87.4 (C-1β), 76.6 (C-5β), 74.8 (C-4β), 73.1 (C-4α), 72.5 (C-2α, C-2β), 72.1 (C-3α, C-3β), 69.3 (C-5α), 21.0 ($SC_6H_4CH_3$), 17.9 (C-6β), 17.5 (C-6α); LRMS (ES+) Calcd. for $C_{13}H_{18}NaO_4S$ ($M Na)^+$ 293. Found 293.

p-Methylphenyl 2-*O*-benzoyl-4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (14)

To a suspension of derivative **13** (3 g, 11.1 mmol) in dry DMF (100 mL) were added triethyl orthobenzoate (4 mL, 17.8 mmol) and camphor-10-sulfonic acid (516 mg, 2.22 mmol). The reaction mixture was stirred for 3 h at room temperature. The solution was neutralised with triethylamine (1.5 mL) and cooled to 0 °C. NaH (800 mg, 33.33 mmol) was added followed by dropwise addition of benzyl bromide (2.4 mL, 20 mmol). The reaction mixture was stirred for 15 h at room temperature, then methanol (30 mL) was slowly added. The solution was evaporated and the residue was dissolved with ethyl acetate. The solution was successively washed with water and twice with 2 M HCl. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a syrup from which was separated by flash chromatography (4:1 cyclohexane-EtOAc) the desired compound **14** (3.92 g, 76%) and its β-analogue (0.59 g, 15%). Data for **14**: $[\alpha]_D$ −23 (c 1.15, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.23 (d, 2H, *J* 8.2 Hz, $SC_6H_4CH_3$), 7.67-7.38 (m, 10 H, Ar), 7.22 (d, 2H, $SC_6H_4CH_3$), 5.83 (dd, 1H, $J_{1,2}$ 1.3 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.71 (d, 1H, H-1), 5.11 (d, 1H, *J* 11.2 Hz, $OCH_2C_6H_5$), 4.91 (d, 1H, $OCH_2C_6H_5$), 4.51 (dd, 1H, $J_{4,5}$ 9.3 Hz, $J_{5,6}$ 6.2 Hz, H-5), 4.43-4.39 (m, 1H, H-3), 3.79 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 3.49 (d, 1H, *J* 4.7 Hz, OH), 2.42 (s, 3H, $SC_6H_4CH_3$), 1.58 (d, 3H, H-6); δ_C (75 MHz, $CDCl_3$) 165.9 (OCOAr), 137.9-127.5 (Ar), 85.9 (C-1), 81.1 (C-4), 74.7 (C-2, OCH_2Ar), 70.6 (C-3), 68.4 (C-5), 20.7 ($SC_6H_4CH_3$), 17.7 (C-6); HRMS (ES^+) Calcd. for $C_{27}H_{28}NaO_5{}^{32}S$ (MNa^+) 487.1555. Found 487.1548. Found: C, 69.22; H, 6.03. $C_{27}H_{28}O_5S$ requires C, 69.80; H, 6.07%.

N-Benzyloxycarbonylaminoethyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (15)

A solution of **14** (2 g, 4.31 mmol) and benzyl *N*-(2-hydroxyethyl)carbamate[22] (6.72 g, 34.48 mmol) in dry CH_2Cl_2 (130 mL) containing molecular sieves 4 Å (1 g) was cooled to 0 °C. *N*-Iodosuccinimide (1.94 g, 8.62 mmol) and trifluoromethanesulfonic acid (765 μL, 8.62 mmol) were added. The reaction mixture was stirred at room temperature for 1 h 30 min, then quenched by addition of Et_3N. The molecular sieves were removed by filtration. The organic layer was washed with saturated aq $Na_2S_2O_3$, water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **15** (1.34 g, 58%) as an oil, $[\alpha]_D$ −11 (c 0.18, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.11-7.33 (m, 10H, Ar), 5.39 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.4 Hz, H-2), 5.28 (t, 1H, *J* 4.9 Hz, NH), 5.15 (broad s, 2H, $NHCOOCH_2Ar$), 4.93-4.89 (m, 2H, OCH_2Ar, H-1), 4.78 (d, 1H, *J* 11.1 Hz, OCH_2Ar), 4.25 (dd, 1H, $J_{3,4}$ 9.4 Hz, H-3), 3.87-3.72 (m, 2H, CH_2CH_2NH, H-5), 3.57-3.49 (m, 2H, CH_2CH_2NH, H-4), 3.46-3.42 (m, 2H, CH_2CH_2NH), 1.42 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR: δ_C (75 MHz, $CDCl_3$) 166.2 (OCOAr), 156.3 (NHCO), 138.0-127.9 (Ar), 97.5 (C-1), 81.4 (C-4), 75.2 (OCH_2Ar), 73.1 (C-2), 70.3 (C-3), 67.7 (C-5), 66.9 (CH_2CH_2NH), 66.8 ($NHCOOCH_2Ar$), 40.8 (CH_2CH_2NH), 18.1 (C-6); HRMS (ES^+) Calcd. for $C_{30}H_{33}NNaO_8$ (MNa^+) 558.2104. Found 558.2107. Found: C, 67.02; H, 6.21; N, 2.51. $C_{30}H_{33}NO_8$ requires C, 67.28; H, 6.21; N 2.62%.

p-Methylphenyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside (16)

To a solution of **14** (3.66 g, 7.89 mmol) in CH_2Cl_2 (65 mL) containing pyridine (3.3 mL) was added dropwise at 0 °C a solution of chloroacetyl chloride (2.5 mL, 31.56 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was stirred at room temperature for 3 h, then stopped by careful addition at 0 °C of methanol (20 mL). The solution was evaporated and the residue was dissolved in CH_2Cl_2. The solution was successively washed with aq. $KHSO_4$, saturated aq. $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **16** (4.17 g, 98%) as a brown oil, $[\alpha]_D$ −53 (c 0.33, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.13 (d, 2H, *J* 8.2 Hz, $SC_6H_4CH_3$), 7.71-7.30 (m, 10H, Ar), 7.20 (d, 2H, $SC_6H_4CH_3$), 5.86 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$

3.2 Hz, H-2), 5.56 (dd, 1H, $J_{3,4}$ 9.7 Hz, H-3), 5.53 (d, 1H, H-1), 4.83 (d, 1H, J 11.3 Hz, OCH_2Ar), 4.78 (d, 1H, OCH_2Ar), 4.54-4.44 (m, 1H, H-5), 4.00 (d, 1H, J 14.9 Hz, $COCH_2Cl$), 3.92 (d, 1H, $COCH_2Cl$), 3.80 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 2.39 (s, 3H, $SC_6H_4CH_3$), 1.50 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); δ_C (75 MHz, $CDCl_3$) 166.3, 165.4 (OCOAr, $COCH_2Cl$), 138.1-127.8 (Ar), 85.9 (C-1), 78.7 (C-4), 75.2 (OCH_2Ar), 74.1 (C-3), 71.8 (C-2), 69.1 (C-5), 40.5 ($COCH_2Cl$), 21.0 ($SC_6H_4CH_3$), 17.9 (C-6); HRMS (ES^+) Calcd. for $C_{29}H_{29}{}^{35}ClNaO_6{}^{32}S$ (MNa^+) 563.1271. Found 563.1285. Found: C, 63.84; H, 5.31. $C_{29}H_{29}ClO_6S$ requires C, 64.38; H, 5.40%.

2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-L-rhamnopyranose (17)

N-Bromosuccinimide (1.73 g, 9.72 mmol) was added at 0 °C to a solution of thioglycoside **16** (3.50 g, 6.48 mmol) in acetone (180 mL). The reaction mixture was stirred at 0 °C for 20 min., then at room temperature for 1 h. Ammonium chloride (500 mg) was added. After 10 min of stirring, the mixture was diluted with ethyl acetate (100 mL) and washed with water (80 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **17** (2.57 g, 91%) as a colourless oil, δ_C (75 MHz, $CDCl_3$) 166.4, 165.7 ($OCOC_6H_5$, $OCOCH_2Cl$), 137.7-127.8 ($OCOC_6H_5$, $OCH_2C_6H_5$), 92.4 (C-1β), 92.0 (C-1α), 78.5 (C-4α, C-2β), 77.8 (C-4β), 75.7, 75.3 ($OCH_2C_6H_5$), 73.6 (C-3α), 71.8 (C-5β), 71.2 (C-3β), 70.8 (C-2α), 67.7 (C-5α, C-5β), 40.6, 40.5 ($OCOCH_2Cl$), 18.0 (C-6α, C-6β); HRMS (ES^+) Calcd. for $C_{22}H_{23}{}^{35}ClNaO_7$ (MNa^+) 457.1030. Found 457.1022.

2-*O*-Benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl trichloroacetimidate (18)

To a solution of **17** (2.57 g, 5.91 mmol) in dry CH_2Cl_2 (15 mL) were added at 0 °C trichloroacetonitrile (12.4 mL, 0.12 mol) and DBU (222 µL, 1.5 mmol). After 1 h of stirring at room temperature, solvent was removed. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc, Et_3N 2%) to afford the donor **18** (2.81 g, 82%), $[\alpha]_D$ +9 (c 0.33, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.78 (s, 1H, NH), 8.15-7.34 (m, 10H, Ar), 6.38 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 5.78 (dd, 1H, $J_{2,3}$ 3.3 Hz, H-2), 5.57 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 4.79 (d, 1H, J 11.2 Hz, OCH_2Ar), 4.73 (d, 1H, OCH_2Ar), 4.22-4.15 (m, 1H, H-5), 3.99 (d, 1H, J 15.0 Hz, $COCH_2Cl$), 3.90 (d, 1H, $COCH_2Cl$), 3.78 (t, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 1.50 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); δ_C (75 MHz, $CDCl_3$) 166.3, 165.4 (OCOAr, $OCOCH_2Cl$), 160.1 ($OCNHCCl_3$), 137.4-128.0 (Ar), 94.8 (C-1), 77.8 (C-4), 75.4 (OCH_2Ar), 73.6 (C-3), 70.7 (C-5), 68.7 (C-2), 40.5 ($COCH_2Cl$), 18.0 (C-6); HRMS (ES^+) Calcd. for $C_{24}H_{23}{}^{35}Cl_4NaO_7$ (MNa^+) 600.0126. Found 600.0126.

N-Benzyloxycarbonylaminoethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (19)

TMSOTf (223 µL, 1.23 mmol) was added under argon at –40 °C to a solution of donor **18** (1.71 g, 2.95 mmol) and acceptor **15** (1.31 g, 2.46 mmol) in dry CH_2Cl_2 (70 mL) containing molecular sieves 4 Å (500 mg). After 30 min of stirring, the solution was neutralised with Et_3N. The molecular sieves were removed by filtration. The reaction mixture was washed with water (30 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **19** (2.11 g, 90%) as a white powder, $[\alpha]_D$ +30 (c 0.33, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.18-7.16 (m, 25H, Ar), 5.65 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.2 Hz, H-2B), 5.47-5.43 (m, 2H, H-3B, H-2C), 5.22 (d, 1H, H-1B), 5.16 (broad s, 3H, $NHCOOCH_2Ar$, NH), 5.03 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.95 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1C), 4.78 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.64 (d, 1H, J 11.6 Hz, OCH_2Ar), 4.59 (d, 1H, J 11.6 Hz, OCH_2Ar), 4.30 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 9.2 Hz, H-3C), 4.00-3.77 (m, 5H, H-5C, H-5B, CH_2CH_2NH, $COCH_2Cl$), 3.70 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4C), 3.64-3.55 (m, 2H, H-4B, CH_2CH_2NH), 3.50-3.43 (m, 2H, CH_2CH_2NH), 1.39 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6C), 1.26 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6B); δ_C (75 MHz, $CDCl_3$) 166.3, 166.0 (OCOAr), 165.4 ($COCH_2Cl$), 156.3 ($NHCOOCH_2Ar$), 137.7-127.4 (Ar), 99.6 (C-1B), 97.1 (C-1C), 79.8 (C-4C), 78.9 (C-3C), 78.0 (C-4B), 75.8, 74.3 (OCH_2Ar), 73.5, 72.5 (C-3B, C-2C), 70.4 (C-2B), 68.6 (C-5C), 68.0 (C-5B), 67.1 (CH_2CH_2NH), 66.8 ($NHCOOCH_2Ar$), 40.8 (CH_2CH_2NH), 40.6 ($COCH_2Cl$), 18.1 (C-6C), 17.8 (C-6B); HRMS (ES^+) Calcd. for $C_{52}H_{54}{}^{35}ClNNaO_{14}$ (MNa^+) 974.3131. Found 974.3132. Found: C, 65.11; H, 5.66; N, 1.52. $C_{52}H_{54}ClNO_{14}$ requires C, 65.57; H, 5.71; N 1.47%.

N-Benzyloxycarbonylaminoethyl 2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-L-rhamnopyranoside (20)

To a solution of derivative **19** (720 mg, 0.76 mmol) in THF (35 mL) were added thiourea (115 mg, 1.51 mmol), $NaHCO_3$ (144 mg, 1.51 mmol) and a catalytic amount of TBAI (14 mg, 38 µmol). The solution was warmed to 55 °C and stirred for 12 h. The reaction mixture was then cooled to room temperature, diluted with CH_2Cl_2 (40 mL) and washed with water. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **20** (530 mg, 80%) as a white powder, $[\alpha]_D$ +31 (c 0.17, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.14-7.26 (m, 25H, Ar), 5.45 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.4 Hz, H-2B), 5.43 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.3 Hz, H-2C), 5.24 (d, 1H, H-1B), 5.19-5.17 (m, 1H, NH), 5.15 (broad s, 2H, $NHCOOCH_2Ar$), 4.98 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.92 (d, 1H, H-1C), 4.76-4.72 (m, 2H, OCH_2Ar), 4.68 (d, 1H, J 11.4 Hz, OCH_2Ar), 4.29 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-3C), 4.17-4.12 (m, 1H, H-3B), 3.92-3.84 (m, 1H, H-5B), 3.83-3.78 (m, 2H, H-5C, CH_2CH_2NH), 3.67 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4C), 3.60-3.54 (m, 1H, CH_2CH_2NH), 3.50-3.44 (m, 3H, H-4B, CH_2CH_2NH), 2.19 (d, 1H, OH), 1.37 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6C), 1.30 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6B); δ_C (75 MHz, $CDCl_3$) 165.9, 165.8 (OCOAr), 156.3 ($NHCOOCH_2Ar$), 138.2-127.7 (Ar), 99.5 (C-1B), 97.2 (C-1C), 81.1 (C-4B), 80.2 (C-4C), 77.6 (C-3C), 75.7, 74.0 (OCH_2Ar), 73.1 (C-2B), 72.6 (C-2C), 69.8 (C-3B), 68.3 (C-5B), 68.0 (C-5C), 67.1 (CH_2CH_2NH), 66.7 ($NHCOOCH_2Ar$), 40.8 (CH_2CH_2NH), 18.0 (C-6C), 17.9 (C-6B); HRMS (ES^+) Calcd. for $C_{50}H_{53}NNaO_{13}$ (MNa^+) 898.3415. Found 898.3429. Found: C, 68.22; H, 5.90; N, 1.57. $C_{50}H_{53}NO_{13}$ requires C, 68.58; H, 6.10; N, 1.60%.

2-*O*-Acetyl-4-azido-3-*O*-benzoyl-4,6-dideoxy-D-glucopyranose (21)

N-Bromosuccinimide (640 mg, 3.6 mmol) was added at 0 °C to a solution of thioglycoside **11** (1.06 g, 2.4 mmol) in acetone (85 mL).

The reaction mixture was stirred at 0 °C for 20 min, then at room temperature for 2 h. Ammonium chloride (500 mg) was added. After 10 min of stirring, the mixture was diluted with ethyl acetate (50 mL) and washed with water. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (1:1 cyclohexane-EtOAc) to give **21** (723 mg, 90%) as a colourless oil, δ_H (300 MHz, $CDCl_3$) 8.13-7.38 (m, 5H, Ar), 5.78 (t, 1H, $J_{2,3} = J_{3,4}$ 10.0 Hz, H-3α), 5.47-5.41 (m, 2H, H-1α, H-4β), 5.10-5.04 (m, 2H, H-2α, H-2β), 4.87-4.85 (m, 2H, H-1β, H-3β), 4.16-4.06 (m, 1H, H-5α), 3.58-3.43 (m, 1H, H-5β), 3.38 (t, 1H, $J_{4,5}$ 10.0 Hz, H-4α), 1.96 (s, 3H, CH_3CO), 1.42 (d, 3H, $J_{5,6}$ 5.8 Hz, H-6β), 1.36 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6α); δ_C (75 MHz, $CDCl_3$) 170.5 (CH_3CO), 165.6 (OCOAr), 133.5-128.3 (Ar), 94.6 (C-1β), 89.9 (C-1α), 73.4 (C-4β), 73.1 (C-2β, C-3β), 71.5 (C-2α), 70.7 (C-3α, C-5β), 66.1 (C-4α), 65.6 (C-5α), 20.3 (CH_3CO), 17.9 (C-6α, C-6β); HRMS (ES^+) Calcd. for $C_{15}H_{17}N_3NaO_6$ (MNa^+) 358.1015. Found 358.1021. Found: C, 53.15; H, 5.11; N, 12.49. $C_{15}H_{17}N_3O_6$ requires C, 53.73; H, 5.11; N, 12.53%.

2-*O*-Acetyl-4-azido-3-*O*-benzoyl-4,6-dideoxy-D-glucopyranosyl trichloroacetimidate (22)

To a solution of **21** (723 mg, 2.16 mmol) in dry CH_2Cl_2 (5 mL) were added at 0 °C trichloroacetonitrile (4.5 mL, 45.3 mmol) and DBU (81 µL, 0.54 mmol). After 2 h of stirring at 0 °C, solvent was removed. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc, Et_3N 2%) to afford the donor **22** (865 mg, 84%), δ_H (300 MHz, $CDCl_3$) 8.74 (broad s, 1H, NH-β), 8.67 (broad s, 1H, NH-α), 8.09-7.40 (m, 5H, Ar), 6.50 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1α), 5.90 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1β), 5.80 (t, 1H, $J_{2,3} = J_{3,4}$ 10.1 Hz, H-3α), 5.48 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3β), 5.38 (dd, 1H, H-2β), 5.25 (dd, 1H, H-2α), 4.07-3.97 (m, 1H, H-5α), 3.72-3.63 (m, 1H, H-5β), 3.52-3.42 (m, 2H, H-4α, H-4β), 1.92 (s, 3H, CH_3CO), 1.46 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6β), 1.42 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6α); δ_C (75 MHz, $CDCl_3$) 169.9 (CH_3CO), 165.3 (OCOAr), 160.8 ($OCNHCCl_3$), 133.5-128.5 (Ar), 95.3 (C-1β), 93.1 (C-1α), 73.5 (C-3β), 71.8 (C-5β), 70.4 (C-2β, C-3α), 70.0 (C-2α), 68.9 (C-5α), 65.7 (C-4α), 65.5 (C-4β), 20.3 (CH_3CO), 18.2 (C-6α, C-6β); LRMS (ES^+) Calcd. for $C_{17}H_{17}Cl_3NNaO_6$ (MNa^+) 501. Found 501.

N-Benzyloxycarbonylaminoethyl 2-*O*-acetyl-4-azido-3-*O*-benzoyl-4,6-dideoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranoside (23)

TMSOTf (73 µL, 0.4 mmol) was added under argon at −70 °C to a solution of donor **22** (383 mg, 0.8 mmol) and acceptor **20** (465 mg, 0.53 mmol) in dry CH_2Cl_2 (20 mL) containing molecular sieves 4 Å (500 mg). After 45 min of stirring, the solution was neutralised with Et_3N. The molecular sieves were removed by filtration. The reaction mixture was washed with water (15 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (9:1 cyclohexane-EtOAc) to give a mixture (920 mg) of the expected trisaccharide **23** and some unreacted acceptor **20** which was used in the next step without further purification. A sample was purified by high performance liquid chromatography (Prevail C_{18} 5 µm (4.6 × 250 mm), H_2O-MeOH 6:4 to 0:1 in 30 min.) to afford pure **23** as a white powder, $[\alpha]_D$ +3 (c 0.1, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 8.25-7.31 (m, 30H, Ar), 5.56-5.55 (m, 1H, H-2C), 5.54-5.53 (m, 1H, H-2B), 5.34 (d, 1H, $J_{1,2} < 1$ Hz, H-1B), 5.27 (t, 2H, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3A, NH), 5.22 (broad s, 2H, $NHCOOCH_2Ar$), 5.15-5.12 (m, 2H, H-2A, OCH_2Ar), 4.98 (d, 1H, $J_{1,2} < 1$ Hz, H-1C), 4.86 (d, 1H, J 11.4 Hz, OCH_2Ar), 4.83 (d, 1H, J 10.9 Hz, OCH_2Ar), 4.64 (d, 1H, J 11.4 Hz, OCH_2Ar), 4.56 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1A), 4.40 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.3 Hz, H-3C), 4.22 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.3 Hz, H-3B), 3.95-3.88 (m, 3H, H-5B, H-5C, CH_2CH_2NH), 3.76 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4C), 3.65-3.60 (m, 2H, H-4B, CH_2CH_2NH), 3.57-3.48 (m, 2H, CH_2CH_2NH), 3.26 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4A), 3.09-3.07 (m, 1H, H-5A), 1.69 (s, 3H, CH_3CO), 1.46 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6C), 1.24 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6B), 1.14 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6A); δ_C (125 MHz, $CDCl_3$) 169.3 (CH_3CO), 165.8, 165.6 (OCOAr), 156.3 ($NHCOOCH_2Ar$), 138.2-127.3 (Ar), 100.5 (C-1A), 98.6 (C-1B), 97.3 (C-1C), 80.1 (C-4C), 79.0 (C-4B), 78.7 (C-3B), 77.3 (C-3C), 75.4, 74.3 (OCH_2Ar), 73.8 (C-3A), 72.3 (C-2B, C-2C), 71.7 (C-2A), 70.5 (C-5A), 68.6 (C-5B), 68.0 (C-5C), 67.2 (CH_2CH_2NH), 66.7 ($NHCOOCH_2Ar$), 65.5 (C-4A), 40.8 (CH_2CH_2NH), 20.2 (CH_3CO), 18.0 (C-6C), 17.8 (C-6A, C-6B); HRMS (ES^+) Calcd. for $C_{65}H_{68}N_4NaO_{18}$ (MNa^+) 1215.4426. Found 1215.4399. Found: C, 64.43; H, 5.74; N, 4.51. $C_{65}H_{68}N_4O_{18}$ requires C, 65.43; H, 5.74; N 4.7%.

N-Benzyloxycarbonylaminoethyl 4-azido-3-*O*-benzoyl-4,6-dideoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranoside (24)

A freshly prepared solution of acetyl chloride (1.9 mL, 26.3 mmol) in methanol (48 mL) was added dropwise at 0 °C to a solution of crude compound **23** (920 mg) in CH_2Cl_2 (36 mL). The reaction mixture was stirred at room temperature for 6 days, then poured into saturated aq. $NaHCO_3$. The organic layer was washed with water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **24** (307 mg, 50% from **20**) as a white powder, $[\alpha]_D$ +17 (c 0.05, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 8.15-7.03 (m, 30H, Ar), 5.52-5.50 (m, 1H, H-2B), 5.48-5.46 (m, 1H, H-2C), 5.24 (d, 1H, $J_{1,2} < 1$ Hz, H-1B), 5.17-5.14 (m, 3H, $NHCOOCH_2Ar$, NH), 5.09 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3A), 5.05 (d, 1H, J 10.9 Hz, OCH_2Ar), 4.90 (d, 1H, $J_{1,2} < 1$ Hz, H-1C), 4.85 (d, 1H, J 11.0 Hz, OCH_2Ar), 4.73 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.64 (d, 1H, J 11.0 Hz, OCH_2Ar), 4.34-4.30 (m, 2H, H-1A, H-3C), 4.21 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.4 Hz, H-3B), 3.92-3.88 (m, 1H, H-5B), 3.83-3.79 (m, 2H, H-5C, CH_2CH_2NH), 3.67 (t, 1H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4C), 3.63-3.58 (m, 2H, H-4B, CH_2CH_2NH), 3.57-3.42 (m, 3H, H-2A, CH_2CH_2NH), 3.15 (t, 1H, $J_{3,4} = J_{4,5}$ 9.6 Hz, H-4A), 3.03-2.88 (m, 1H, H-5A), 2.68 (broad s, 1H, OH), 1.37 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6C), 1.22 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6B), 1.07 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6A); δ_C (125 MHz, $CDCl_3$) 166.8, 166.2, 166.1 (OCOAr), 156.8 ($NHCOOCH_2Ar$), 138.5-128.1 (Ar), 103.6 (C-1A), 99.3 (C-1B), 97.8 (C-1C), 80.5 (C-4C), 80.2 (C-4B), 78.2 (C-3B), 78.1 (C-3C), 76.4 (C-3A), 75.9, 75.1 (OCH_2Ar), 73.6 (C-2A), 73.0 (C-2B), 72.8 (C-2C), 70.9 (C-5A), 69.2 (C-5B), 68.5 (C-5C), 67.7 (CH_2CH_2NH), 67.2 ($NHCOOCH_2Ar$), 66.1 (C-4A), 41.3 (CH_2CH_2NH), 18.5, 18.4 (C-6A, C-6B, C-6C); HRMS (ES^+) Calcd. for $C_{63}H_{66}N_4NaO_{17}$ (MNa^+) 1173.4321. Found 1173.4292. Found: C, 65.47; H, 5.75; N, 4.82. $C_{63}H_{66}N_4O_{17}$ requires C, 65.73; H, 5.78; N, 4.87%.

_N_-Benzyloxycarbonylaminoethyl 4-azido-3-_O_-benzoyl-4,6-dideoxy-2-_O_-methyl-β-D-glucopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-α-D-rhamnopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-α-D-rhamnopyranoside (25)

A freshly prepared solution of diazomethane[23] was added dropwise at 0 °C to a solution of **24** (80 mg, 0.7 mmol) in CH_2Cl_2 (3 mL) in the presence of $BF_3.OEt_2$ (3 μL, 0.21 mmol) until the yellow colour persisted. The reaction mixture was stirred at 0 °C until TLC showed complete consumption of starting material and was then allowed to reach room temperature. The white precipitate was filtered and the filtrate washed with saturated aq. $NaHCO_3$ (6 mL) and water (6 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **25** (32 mg, 40%) as a colourless oil, $[\alpha]_D$ +33 (c 0.06, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.16-7.23 (m, 30H, Ar), 5.56 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.2 Hz, H-2B), 5.51 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.2 Hz, H-2C), 5.27 (d, 1H, H-1B), 5.23-5.16 (m, 4H, H-3A, NHCOOCH_2Ar, NH), 5.06 (d, 1H, J 10.7 Hz, OCH_2Ar), 4.92 (d, 1H, H-1C), 4.88 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.73 (d, 1H, J 10.7 Hz, OCH_2Ar), 4.63 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.57 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1A), 4.33 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.3 Hz, H-3C), 4.27 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.4 Hz, H-3B), 3.94-3.77 (m, 3H, H-5B, H-5C, C$H_2$$CH_2$NH), 3.69 (t, 1H, $J_{3,4} = J_{4,5}$ 9.4 Hz, H-4C), 3.66-3.58 (m, 2H, H-4B, C$H_2$$CH_2$NH), 3.50-3.38 (m, 2H, CH_2CH_2NH), 3.35 (s, 3H, OCH_3), 3.20-3.11 (m, 2H, H-2A, H-4A), 3.08-2.99 (m, 1H, H-5A), 1.37 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6C), 1.25 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6B), 1.02 (d, 3H, $J_{5,6}$ 5.9 Hz, H-6A); δ_C (75 MHz, $CDCl_3$) 165.8, 165.6, 165.5 (OCOAr), 156.3 (NHCOOCH_2Ar), 137.7-127.7 (Ar), 102.7 (C-1A), 99.1 (C-1B), 97.2 (C-1C), 81.9 (C-2A), 80.2 (C-4B), 79.9 (C-4C), 77.9 (C-3C), 76.0 (C-3B), 75.6 (OCH_2Ar), 74.7 (C-3A), 74.3 (OCH_2Ar), 73.0 (C-2B), 72.4 (C-2C), 70.2 (C-5A), 68.6 (C-5B), 68.0 (C-5C), 67.2 ($C$$H_2$$CH_2$NH), 66.5 (NHCOO$C$$H_2$Ar), 65.9 (C-4A), 60.6 ($OCH_3$), 40.8 ($CH_2$$C$$H_2$NH), 18.0 (C6-C), 17.9 (C-6A, C-6B); HRMS (ES^+) Calcd. for $C_{64}H_{68}N_4NaO_{17}$ (MNa^+) 1187.4477. Found 1187.4490. Found: C, 65.32; H, 5.81; N, 4.73. $C_{64}H_{68}N_4O_{17}$ requires C, 65.97; H, 5.88; N, 4.81%.

_N_-Benzyloxycarbonylaminoethyl 4-(3-hydroxy-3-methylbutamido)-3-_O_-benzoyl-4,6-dideoxy-2-_O_-methyl-β-D-glucopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-α-D-rhamnopyranosyl-(1→3)-2-_O_-benzoyl-4-_O_-benzyl-α-D-rhamnopyranoside (27)

To a solution of **25** (53 mg, 45 μmol) in a mixture of dry CH_2Cl_2 (1.5 mL) and EtOH (5 mL) was added $NaBH_4$ (4 mg, 90 μmol) and a catalytic amount of $NiCl_2.6H_2O$. The reaction mixture was stirred at room temperature for 1 h and concentrated. The residue was dissolved in CH_2Cl_2 (5 mL). The organic layer was washed with water (3 mL) and brine (3 mL), dried over Na_2SO_4, filtered and concentrated. Amine **26** (51 mg) was used in the next step without purification. To a solution of compound **26** (51 mg) in dry CH_2Cl_2 (5 mL) was added dropwise in the following order: 3-hydroxy-3-methylbutanoic acid (8.5 μL, 67 μmol), HATU (26 mg, 68 μmol) then DIPEA (12 μL, 68 μmol). The reaction mixture was stirred at room temperature under argon for 18 h. The residue was diluted with CH_2Cl_2 (5 mL). The organic layer was washed with saturated aq. $NaHCO_3$ (5 mL), water (5 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (3:2 cyclohexane-EtOAc) to give **27** (48 mg, 85%) as a colourless oil, $[\alpha]_D$ = +52 (c 0.05, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.14-7.27 (m, 30H, Ar), 5.79 (d, 1H, $J_{NH,H4}$ 9.4 Hz, NHCO), 5.54 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.1 Hz, H-2B), 5.45 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.0 Hz, H-2C), 5.27 (d, 1H, H-1B), 5.18-5.15 (m, 3H, NHCOOCH_2Ar, NHCOOCH_2Ar), 5.07 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.97 (t, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3A), 4.91-4.87 (m, 2H, H-1C, OCH_2Ar), 4.72 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.62 (d, 1H, J 10.8 Hz, OCH_2Ar), 4.53 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1A), 4.32 (dd, 1H, $J_{2,3}$ 3.0 Hz, $J_{3,4}$ 9.2 Hz, H-3C), 4.27 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 9.4 Hz, H-3B), 4.00-3.77 (m, 4H, H-4A, H-5C, H-5B, C$H_2$$CH_2$NH), 3.73-3.60 (m, 2H, H-4B, H-4C), 3.58-3.52 (m, 1H, C$H_2$$CH_2$NH), 3.47-3.42 (m, 2H, CH_2CH_2NH), 3.37 (s, 3H, OCH_3), 3.25 (dd, 1H, H-2A), 3.02-2.94 (m, 1H, H-5A), 2.21 (d, 1H, J 14.7 Hz, NHCOCH_2C$(CH_3)_2$OH), 2.13 (d, 1H, NHCOCH_2C$(CH_3)_2$OH), 1.71 (broad s, 1H, OH), 1.35 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6C), 1.23 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6B), 1.10 (s, 3H, NHCOCH_2C(CH_3)$_2$OH), 0.98 (s, 3H, NHCOCH_2C(CH_3)$_2$OH), 0.91 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6A); δ_C (75 MHz, $CDCl_3$) 172.3 (NHCO), 167.2, 165.8, 165.6 (OCOAr), 156.3 (NHCOOCH_2Ar), 138.2-127.6 (Ar), 102.8 (C-1A), 99.2 (C-1B), 97.2 (C-1C), 81.5 (C-2A), 80.3 (C-4B), 79.8 (C-4C), 78.1 (C-3C), 76.4 (C-3B), 75.5 (OCH_2Ar), 75.0 (C-3A), 74.3 (OCH_2Ar), 72.9 (C-2B), 72.5 (C-2C), 71.3 (C-5A), 69.2 (NHCOC$H_2$$C$$(CH_3)_2$OH), 68.6 (C-5B), 68.0 (C-5C), 67.2 ($C$$H_2$$CH_2$NH), 66.8 (NHCOO$C$$H_2$$C_6H_5$), 60.7 ($OCH_3$), 54.6 (C-4A), 47.9 (NHCO$C$$H_2C(CH_3)_2$OH), 40.8 ($CH_2$$C$$H_2$NH), 29.1, 28.9 (NHCOCH$_2$C($CH_3$)$_2$OH), 18.0 (C-6C), 17.9 (C-6B), 17.5 (C-6A); HRMS (ES^+) Calcd. for $C_{69}H_{78}N_2NaO_{19}$ (MNa^+) 1261.5096. Found 1261.5105.

_N_-Benzyloxycarbonylaminoethyl 4-(3-hydroxy-3-methylbutamido)-4,6-dideoxy-2-_O_-methyl-β-D-glucopyranosyl-(1→3)-4-_O_-benzyl-α-D-rhamnopyranosyl-(1→3)-4-O-benzyl-α-D-rhamnopyranoside (28)

A solution of sodium methoxide (1 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **27** (39 mg, 31 μmol) in MeOH (15 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed. The residue was then purified by flash chromatography (2:3 cyclohexane-EtOAc) to give **28** (23 mg, 80%) as a colourless oil, $[\alpha]_D$ +13 (c 0.07, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 7.76-7.32 (m, 15H, Ar), 6.05 (d, 1H, $J_{NH,H4}$ 8.6 Hz, NHCO), 5.17 (d, 1H, $J_{1,2}$ < 1 Hz, H-1B), 5.15-5.13 (m, 3H, NHCOOCH_2Ar, NHCOOCH_2Ar), 4.92 (d, 1H, J 10.6 Hz, OCH_2Ar), 4.80 (d, 1H, J 10.7 Hz, OCH_2Ar), 4.77 (d, 1H, $J_{1,2}$ < 1 Hz, H-1C), 4.66-4.59 (m, 2H, OCH_2Ar), 4.53 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1A), 4.17-4.13 (m, 1H, H-2B), 4.07 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 8.7 Hz, H-3B), 4.02-4.00 (m, 1H, H-2C), 3.98-3.90 (m, 2H, H-3C, H-5C), 3.77-3.65 (m, 3H, H-4A, H-5B, C$H_2$$CH_2$NH), 3.62 (s, 3H, OCH_3), 3.60-3.33 (m, 8H, H-4B, H-3A, H-3C, H-4C, C$H_2$$CH_2$NH, H-5A, CH_2CH_2NH), 3.11 (t, 1H, $J_{1,2} = J_{2,3}$ 7.8 Hz, H-2A), 3.00-2.98 (m, 1H, OH-B), 2.39 (s, 2H, NHCOCH_2C$(CH_3)_2$OH), 1.38 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6C), 1.34-1.27 (m, 9H, H-6B, NHCOCH$_2$C(CH$_3$)$_2$OH), 1.16 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6A); δ_C (75 MHz, $CDCl_3$) 172.9 (NHCO), 156.3 (NHCOOCH_2Ar), 138.0-127.8 (Ar), 103.0 (C-1A), 101.3 (C-1B), 99.4 (C-1C), 83.8 (C-2A), 80.6 (C-3B), 80.0 (C-3C), 79.8 (C-4B, C-4C), 75.4, 74.9 (OCH$_2$Ar), 74.6 (C-3A), 70.7 (C-2B, C-2C,

C-5A), 69.9 (NHCOCH$_2$*C*(CH$_3$)$_2$OH), 68.3 (C-5C), 67.7 (C-5B), 66.9 (*C*H$_2$CH$_2$NH), 66.8 (NHCOO*C*H$_2$Ar), 60.9 (OCH$_3$), 56.8 (C-4A), 48.6 (NHCO*C*H$_2$C(CH$_3$)$_2$OH), 40.8 (CH$_2$*C*H$_2$NH), 29.8, 29.2 (NHCOCH$_2$C(*C*H$_3$)$_2$OH), 18.0 (C-6A), 17.8 (C-6B, C-6C); HRMS (ES$^+$) Calcd. for $C_{48}H_{66}N_2NaO_{16}$ (MNa$^+$) 949.4310. Found 949.4296.

2-Aminoethyl 4-(3-hydroxy-3-methylbutamido)-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranosyl-(1→3)-α-D-rhamnopyranosyl-(1→3)-α-D-rhamnopyranoside (29)

To a solution of **28** (23 mg, 25 μmol) in tBuOH (2.3 mL) was added AcOH (1.9 mL) and a catalytic amount of Pd(OH)$_2$. The reaction mixture was stirred at room temperature under hydrogen. The reaction was monitored by mass spectrometry. After 5 h, the reaction was complete. The catalyst was filtered over celite and the filtrate was concentrated. The residue was dissolved in water and washed with CHCl$_3$. The organic layer was extracted twice with water. The aq. layers were combined and concentrated. The residue was purified on a resin cation exchanger (Bio-Rad, AG MP-50 Resin, analytical grade 100-200 mesh). A glass column (400 mm × 15 mm) was packed with the resin (10 mL) which was washed with water and then acidified to pH 1 with a solution of HCl (1 N). An acidified aq. solution of the residue (1 mL, pH 1) was added on the top of the column. The resin was then washed with water (2 × 10 mL) and **29** was eluted with a solution of ammonia 10% (3 × 10 mL). After lyophilisation, compound **29** (6 mg, 40%) was obtained as a white solid, [α]$_D$ + 45 (c 0.05, H$_2$O); δ$_H$ (500 MHz, D$_2$O) 4.97 (d, 1H, $J_{1,2}$ < 1 Hz, H-1B), 4.74 (d, 1H, $J_{1,2}$ < 1 Hz, H-1C), 4.68 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1A), 4.23-4.22 (m, 1H, H-2B), 4.00-3.99 (m, 1H, H-2C), 3.95-3.92 (m, 2H, H-3B, CH$_2$C*H*$_2$NH), 3.84-3.78 (m, 2H, H-3C, H-5C), 3.70-3.63 (m, 2H, H-5C, CH$_2$C*H*$_2$NH), 3.58 (s, 3H, OCH$_3$), 3.56-3.47 (m, 5H, H-4B, H-3A, H-4A, H-5A, H-4C), 3.08 (d, 1H, $J_{1,2} = J_{2,3}$ 8.3 Hz, H-2A), 3.05-2.98 (m, 2H, C*H*$_2$CH$_2$NH), 2.41 (s, 2H, NHCOC*H*$_2$C(CH$_3$)$_2$OH), 1.25-1.23 (m, 12H, H-6B, H-6C, NHCOCH$_2$C(C*H*$_3$)$_2$OH), 1.17 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6A); δ$_C$ (75 MHz, D$_2$O) 174.1 (NHCO), 103.6 (C-1A), 102.3 (C-1B), 99.8 (C-1C), 83.3 (C-2A), 79.5 (C-3B), 78.5 (C-3C), 72.8 (C-3A), 71.1 (C-4B, C-4C), 70.8 (C-5A), 70.2 (NHCOCH$_2$*C*(CH$_3$)$_2$OH), 69.7 (C-2B, C-2C), 69.3 (C-5C), 69.0 (C-5B), 63.4 (CH$_2$*C*H$_2$NH), 60.0 (OCH$_3$), 56.6 (C-4A), 48.9 (NHCO*C*H$_2$C(CH$_3$)$_2$OH), 39.1 (*C*H$_2$CH$_2$NH), 28.3, 28.1 (NHCOCH$_2$C(*C*H$_3$)$_2$OH), 17.0 (C-6A), 16.6 (C-6B, C-6C); HRMS (ES$^+$) Calcd. for $C_{26}H_{48}N_2NaO_{14}$ (MNa$^+$) 635.3003. Found 635.3011.

N-Benzyloxycarbonylaminoethyl 4-*O*-benzyl-α-L-rhamnopyranoside (30)

A solution of sodium methoxide (3.7 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **15** (1 g, 1.87 mmol) in MeOH (50 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H$^+$) was added to neutralise MeONa. The resin was filtered and the solvent removed. The residue was then purified by flash chromatography (3:2 cyclohexane-EtOAc) to give **30** (572 mg, 71%) as a white powder, [α]$_D$ −29 (c 0.16, CHCl$_3$); δ$_H$ (300 MHz, CDCl$_3$, 300 MHz) 7.39-7.30 (m, 10H, Ar), 5.33-5.31 (m, 1H, NH), 5.13 (broad s, 2H, NHCOOC*H*$_2$Ar), 4.83 (d, 1H, *J* 11.2 Hz, OCH$_2$Ar), 4.77 (broad s, 1H, H-1), 4.71 (d, 1H, OCH$_2$Ar), 3.93-3.92 (m, 2H, H-2, H-3), 3.76-3.67 (m, 2H, H-5, OH), 3.50-3.34 (m, 5H, H-4, C*H*$_2$CH$_2$NH, CH$_2$C*H*$_2$NH), 3.09 (broad s, 1H, OH), 1.35 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); δ$_C$ (75 MHz, CDCl$_3$) 156.4 (NH*C*OOCH$_2$Ar), 138.2-127.8 (Ar), 99.4 (C-1), 81.3 (C-4), 74.9 (OCH$_2$Ar), 71.3, 70.9 (C-2, C-3), 67.4 (C-5), 66.7 (NHCOO*C*H$_2$Ar), 66.5 (*C*H$_2$CH$_2$NH), 40.6 (CH$_2$*C*H$_2$NH), 17.9 (C-6); HRMS (ES$^+$) Calcd. for $C_{23}H_{29}NNaO_{11}$ (MNa$^+$) 454.1842. Found 454.1847. Found: C, 64.40; H, 6.80; N, 3.33. $C_{23}H_{29}NO_7$ requires C, 64.02; H, 6.77; N, 3.25%.

2-Aminoethyl α-L-rhamnopyranoside (31)

AcOH (30 mL) and a catalytic amount of Pd(OH)$_2$ were added to a solution of **30** (495 mg, 1.14 mmol) in tBuOH (40 mL). The reaction mixture was stirred at room temperature under hydrogen. The reaction was monitored by mass spectrometry. After 12 h, the reaction was complete. The catalyst was filtered over celite and the filtrate was concentrated. The residue was dissolved in water (30 mL) and washed with CHCl$_3$ (15 mL). The organic layer was extracted with water (5 × 10 mL). The aq. layers were combined and concentrated. The residue was purified by high performance liquid chromatography (Prevail C$_{18}$ 5 μm (4.6 × 250 mm), H$_2$O-MeOH 1:0 to 0:1 in 30 min.) to afford **31** (159 mg, 67%) as a white powder, [α]$_D$ −8 (c 0.16, H$_2$O); δ$_H$ (300 MHz, D$_2$O) 4.74 (broad s, 1H, H-1), 3.91-3.88 (m, 1H, H-2), 3.72 (dd, 2H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.7 Hz, H-3, CH$_2$C*H*$_2$NH), 3.51-3.44 (m, 1H, CH$_2$C*H*$_2$NH), 3.38 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 3.17 (t, 1H, *J* 5.5 Hz, NH$_2$), 2.84 (broad s, 2H, C*H*$_2$CH$_2$NH), 1.23 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); δ$_C$ (75 MHz, D$_2$O) 99.9 (C-1), 72.0 (C-4), 70.2 (C-2), 70.0 (C-3), 68.6 (C-5), 68.0 (CH$_2$*C*H$_2$NH), 39.8 (*C*H$_2$CH$_2$NH), 16.6 (C-6); HRMS (ES$^+$) Calcd. for $C_8H_{18}NO_5$ (MH$^+$) 208.1185. Found 208.1186. Found: C, 42.03; H, 8.24; N, 5.85. $C_8H_{17}NO_5$ requires C, 42.66; H, 8.50; N, 5.85%.

N-Benzyloxycarbonylaminoethyl 4-*O*-benzyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzyl-α-L-rhamnopyranoside (32)

A solution of sodium methoxide (1.5 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **19** (320 mg, 0.37 mmol) in MeOH (10 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H$^+$) was added to neutralise MeONa. The resin was filtered and the solvent removed. The residue was then purified by flash chromatography (3:2 cyclohexane-EtOAc) to give **32** (200 mg, 82%) as a white powder, [α]$_D$ −52 (c 0.18, CHCl$_3$); δ$_H$ (300 MHz, CDCl$_3$) 7.40-7.30 (m, 10H, Ar), 5.23-5.21 (m, 1H, NH), 5.14 (broad s, 2H, NHCOOC*H*$_2$Ar), 5.11 (d, 1H, *J* < 1 Hz, H-1B), 4.81 (d, 1H, *J* 11.2 Hz, OCH$_2$Ar), 4.75-4.60 (m, 4H, H-1C, OCH$_2$Ar), 3.99-3.86 (m, 4H, H-2B, H-3B, H-2C, H-5C), 3.75-3.69 (m, 3H, H-5B, H-3C, *CH*$_2$CH$_2$NH), 3.51-3.38 (m, 5H, H-4B, H-4C, *CH*$_2$CH$_2$NH, CH$_2$*CH*$_2$NH), 2.82, 2.76, 2.68 (3 broad s, 3H, OH), 1.38 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6B or H-6C), 1.31 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6B or H-6C); δ$_C$ (75 MHz, CDCl$_3$)156.3 (NHCOOCH$_2$Ar), 138.1-127.8 (Ar), 101.2 (C-1B), 99.3 (C-1C), 81.3, 80.2 (C-4B, C-4C), 79.2 (C-2B or C-2C), 75.4, 75.1 (OCH$_2$Ar), 71.3, 70.7 (C-2B or C-2C, C-3B, C-5C), 68.2, 67.8 (C-5B, C-3C), 66.7 (*CH*$_2$CH$_2$NH, NHCOO*C*H$_2$Ar), 40.6 (CH$_2$*C*H$_2$NH), 18.0, 17.7 (C-6B, C-6C); HRMS (ES$^+$) Calcd. for $C_{36}H_{45}NNaO_{11}$ (MNa$^+$) 690.2890. Found

690.2889. Found: C, 64.29; H, 6.72; N, 2.16. $C_{36}H_{45}NO_{11}$ requires C, 64.75; H, 6.79; N, 2.10%.

2-Aminoethyl α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranoside (33)

AcOH (11 mL) and a catalytic amount of $Pd(OH)_2$ were added to a solution of **32** (145 mg, 0.22 mmol) in tBuOH (16 mL). The reaction mixture was stirred at room temperature under hydrogen. The reaction was monitored by mass spectrometry. After 12 h, the reaction was complete. The catalyst was filtered over celite and the filtrate was concentrated. The residue was dissolved in water (15 mL) and washed with $CHCl_3$ (7 mL). The organic layer was extracted with water (5 × 5 mL). The aq. layers were combined and concentrated. The residue was purified on a resin cation exchanger (Bio-Rad, AG MP-50 Resin, analytical grade 100-200 mesh). After lyophilisation, compound **33** (50 mg, 65%) was obtained as a white solid, $[\alpha]_D$ −66 (c 0.06, H_2O); δ_H (300 MHz, D_2O) 5.04 (d, 1H, $J_{1,2}$ < 1 Hz, H1-B), 4.79 (broad s, 1H, H-1C), 4.09-4.04 (m, 2H, H-2B, H-2C), 3.87-3.73 (m, 5H, H-3B, H-5B, H-3C, H-5C, CH_2CH_2NH), 3.57-3.44 (m, 3H, H-B, H-4C, CH_2CH_2NH), 3.25 (t, 2H, *J* 5.3 Hz, NH_2), 3.02-3.00 (m, 2H, CH_2CH_2NH), 1.31-1.25 (m, 6H, H-6B, H-6C); δ_C (75 MHz, D_2O) 102.5 (C-1B), 99.8 (C-1C), 78.4 (C-3B), 72.0, 71.3 (C-4B, C-4C), 70.1, 69.9 (C-2B, C-2C, C-3C), 69.1, 68.8 (C-5B, C-5C), 66.9 (CH_2CH_2NH), 39.6 (CH_2CH_2NH), 16.6 (C-6B, C-6C); HRMS (ES^+) Calcd. for $C_{14}H_{28}NO_9$ (MH^+) 354.1764. Found 354.1769. Found: C, 36.79; H, 8.44; N, 3.74. $C_{14}H_{27}NO_9$ requires C, 36.44; H, 8.52; N, 3.04%.

N-Benzyloxycarbonylaminoethyl 2-*O*-acetyl-4-(3-hydroxy-3-methylbutamido)-3-*O*-benzoyl-4,6-dideoxy-β-D-glucopyranoside (36)

$NaBH_4$ (32 mg, 0.84 mmol) and a catalytic amount of $NiCl_2.6H_2O$ were added to a solution of **34**[3c] (215 mg, 0.42 mmol) in a mixture of dry CH_2Cl_2 (10 mL) and EtOH (40 mL). The reaction mixture was stirred at room temperature for 1 h and concentrated. The residue was dissolved in CH_2Cl_2 (30 mL). The organic layer was washed with water (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated. Amine **35** (203 mg, 0.42 mmol) was used in the next step without purification. To a solution of compound **35** (203 mg) in dry CH_2Cl_2 (30 mL) was added dropwise in the following order: 3-hydroxy-3-methylbutanoic acid (79 µL, 0.62 mmol), HATU (240 mg, 0.63 mmol) then DIPEA (108 µL, 0.63 mmol). The reaction mixture was stirred at room temperature under argon for 18 h. The residue was diluted with CH_2Cl_2 (10 mL). The organic layer was washed with saturated aq $NaHCO_3$ (10 mL), water (10 mL), dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (1:1 cyclohexane-EtOAc) to give **36** (152 mg, 62%) as a white powder, $[\alpha]_D$ = +18 (c 1.8, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 7.91-7.10 (m, 10H, Ar), 6.26 (d, 1H, $J_{NH,H4}$ 10.1 Hz, NHCO), 5.55-5.53 (m, 1H, $NHCOOCH_2Ar$), 5.35 (t, 1H, $J_{2,3} = J_{3,4}$ 10.1 Hz, H-3), 5.04 (m, 3H, H-2, $NHCOOCH_2Ar$), 4.49 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.05 (q, 1H, $J_{3,4} = J_{4,5} = J_{NH,H4}$ 10.1 Hz, H-4), 3.83-3.78 (m, 1H, CH_2CH_2NH), 3.68-3.61 (m, 2H, H-5, CH_2CH_2NH), 3.33-3.31 (m, 1H, CH_2CH_2NH), 2.22 (d, 1H, *J* 14.6 Hz, $NHCOCH_2C(CH_3)_2OH$), 2.13 (d, 1H, $NHCOCH_2C(CH_3)_2OH$), 1.81 (s, 3H, CH_3CO), 1.22 (d, 3H, $J_{5,6}$ 5.8 Hz, H-6), 1.03, 0.92 (2 s, 6H, $NHCOCH_2C(CH_3)_2OH$); δ_C (75 MHz, $CDCl_3$) 173.0 (NHCO), 169.9 (CH_3CO), 166.4 (OCOAr), 156.6 ($NHCOOCH_2Ar$), 136.4-121.1 (Ar), 100.6 (C-1), 72.8 (C-3), 71.8 (C-2), 70.8 (C-5), 69.1 (CH_2CH_2NH), 68.8 ($NHCOCH_2C(CH_3)_2OH$), 66.3 ($NHCOOCH_2Ar$), 54.4 (C-4), 47.3 ($NHCOCH_2C(CH_3)_2OH$), 40.5 (CH_2CH_2NH), 28.8, 28.6 ($NHCOCH_2C(CH_3)_2OH$), 20.2 (CH_3CO), 17.0 (C-6); LRMS (ES^+) Calcd. for $C_{30}H_{38}N_2NaO_{10}$ (MNa^+) 609. Found 609.

N-Benzyloxycarbonylaminoethyl 4-(3-hydroxy-3-methylbutamido)-4,6-dideoxy-β-D-glucopyranoside (37)

A solution of sodium methoxide (320 µL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **36** (52 mg, 88 µmol) in MeOH (8 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed to give **37** (30 mg, 76%) as a white powder, $[\alpha]_D$ = −5 (c 0.04, $CHCl_3$); δ_H (300 MHz, MeOD) 7.39-7.32 (m, 5H, Ar), 5.12 (broad s, H, $NHCOOCH_2Ar$), 4.27 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 3.92-3.86 (m, 1H, CH_2CH_2NH), 3.69-3.58 (m, 2H, H-4, CH_2CH_2NH), 3.49-3.39 (m, 4H, H-3, H-5, CH_2CH_2NH), 3.26 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 2.41 (d, 2H, *J* 2.8 Hz, $NHCOCH_2C(CH_3)_2OH$), 1.32, 1.31 (2 s, 6H, $NHCOCH_2C(CH_3)_2OH$), 1.24 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6); δ_C (75 MHz, MeOD) 175.4.0 (NHCO), 156.6 ($NHCOOCH_2Ar$), 131.6-120.9 (Ar), 105.2 (C-1), 76.7 (C-2), 76.2, 73.2 (C-3, C-5), 71.5 ($NHCOCH_2C(CH_3)_2OH$), 70.8 (CH_2CH_2NH), 68.4 ($NHCOOCH_2Ar$), 59 (C-4), 49.9 ($NHCOCH_2C(CH_3)_2OH$), 42.8 (CH_2CH_2NH), 30.5, 30.4 ($NHCOCH_2C(CH_3)_2OH$), 19.3 (C-6); HRMS (ES^+) Calcd. for $C_{21}H_{32}N_2NaO_8$ (MNa^+) 463.2056. Found 463.2036.

N-Benzyloxycarbonylaminoethyl 4-(3-hydroxy-3-methylbutamido)-2-*O*-acetyl-3-*O*-benzoyl-4,6-dideoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranoside (39)

$NaBH_4$ (15 mg, 0.4 mmol) and a catalytic amount of $NiCl_2.6H_2O$ were added to a solution of **23** (146 mg, 0.12 mmol) in a mixture of dry CH_2Cl_2 (2 mL) and EtOH (9 mL). The reaction mixture was stirred at room temperature for 3 h 30 min and concentrated. The residue was dissolved in CH_2Cl_2 and washed with water and brine, then dried over Na_2SO_4, filtered and concentrated. Amine **38** (131 mg) was used in the next step without purification. To a solution of compound **38** (131 mg) in dry CH_2Cl_2 (2.5 mL) was added in the following order: 3-hydroxy-3-methylbutanoic acid (30 µL, 236 µmol), HATU (90 mg, 236 µmol) then DIPEA (40 µL, 236 µmol). The reaction mixture was stirred at room temperature under argon for 18 h. The residue was diluted with CH_2Cl_2 and the organic solution was washed twice with saturated aq $NaHCO_3$, then with water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (3:2 cyclohexane-EtOAc) to give **39** (82 mg, 53%) as a colourless oil, $[\alpha]_D$ = +10 (c 0.77, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.11-7.17 (m, 30H, Ar), 6.01 (d, 1H, $J_{NH,H4}$ 9 Hz, NHCO), 5.43-5.38 (m, 2H, H-2B, H-2C), 5.22 (broad s, 2H, H-1B, $NHCOOCH_2Ar$), 5.12-5.01 (m, 5H, H-2A, H-3A, OCH_2Ar, $NHCOOCH_2Ar$), 4.85 (broad s, 1H, H-1C), 4.75 (d, 1H, *J* 12 Hz, OCH_2Ar), 4.75 (d, 1H, *J* 12 Hz, OCH_2Ar), 4.69 (d, 1H, *J* 12 Hz, OCH_2Ar), 4.51 (d, 1H, *J* 12 Hz, OCH_2Ar), 4.43 (d, 1H, $J_{1,2}$ 9 Hz, H-1A), 4.28 (dd, 1H, $J_{2,3}$ 3 Hz,

$J_{3,4}$ 9 Hz, H-3B or H-3C), 4.10 (dd, 1H, $J_{2,3}$ 3 Hz, $J_{3,4}$ 9 Hz, H-3B or H-3C), 3.92-3.30 (m, 9H, H-4A, H-4B, H-4C, H-5B, H-5C, CH_2CH_2NH), 3.03-2.94 (m, 1H, H-5A), 2.22 (d, 1H, J 15 Hz, $NHCOCH_2C(CH_3)_2OH$), 2.14 (d, 1H, $NHCOCH_2C(CH_3)_2OH$), 1.53 (s, 3H, CH_3CO), 1.32 (d, 3H, $J_{5,6}$ 6 Hz, H-6B or H6-C), 1.16 (d, 3H, $J_{5,6}$ 6 Hz, H-6B or H-6C), 1.10 (s, 3H, $NHCOCH_2C(CH_3)_2OH$), 1.01 (s, 3H, $NHCOCH_2C(CH_3)_2OH$), 0.90 (d, 3H, $J_{5,6}$ 6 Hz, H-6A); δ_C (75 MHz, $CDCl_3$) 172.1 (NHCO), 169.1 (CH_3CO), 166.9, 165.8, 165.7 ($OCOC_6H_5$), 156.3 ($NHCOOCH_2Ar$), 138.0-127.2 (Ar), 100.5 (C-1A), 98.7 (C-1B), 97.2 (C-1C), 79.9, 79.0 (C-4B, C-4C), 78.7, 77.7 (C-3B, C-3C), 75.3 (OCH_2Ar), 74.2 (OCH_2Ar), 73.3 (C-3A), 72.3, 72.1 (C-2B, C-2C), 71.5 (C-2A), 71.3 (C-5A), 69.2 ($NHCOCH_2C(CH_3)_2OH$), 68.5, 67.9 (C-5B, C-5C), 67.1 (CH_2CH_2NH), 66.7 ($NHCOOCH_2Ar$), 54.5 (C-4A), 47.9 ($NHCOCH_2C(CH_3)_2OH$), 40.8 (CH_2CH_2NH), 29.1, 29.0 ($NHCOCH_2C(CH_3)_2OH$), 20.2 (CH_3CO), 18.0 (C-6B or C-6C), 17.7 (C-6B or C-6C), 17.3 (C-6A); HRMS (ES^+) Calcd. for $C_{70}H_{78}N_2NaO_{20}$ (MNa^+) 1289.5046. Found 1289.5012.

2-Aminoethyl 4-(3-hydroxy-3-methylbutamido)-6-deoxy-β-D-glucopyranosyl-(1→3)-α-D-rhamnopyranosyl-(1→3)-α-D-rhamnopyranoside (41)

A solution of sodium methoxide (0.6 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **39** (75 mg, 59 µmol) in MeOH (2 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed. AcOH (3 mL) and a catalytic amount of $Pd(OH)_2$ were added to a solution of crude **40** in tBuOH (6 mL). The reaction mixture was stirred at room temperature under hydrogen. The reaction was monitored by mass spectrometry. After 20 h, the reaction was complete. The catalyst was filtered over celite and the filtrate was concentrated. The residue was purified on a resin cation exchanger (Bio-Rad, AG MP-50 Resin, analytical grade 100-200 mesh). After lyophilisation, compound **41** (18 mg, 50%) was obtained as a white solid, $[\alpha]_D$ −58 (c 0.45, H_2O); δ_C (75 MHz, D_2O) 174.0 (NHCO), 103.5 (C-1A), 102.2 (C-1B), 99.8 (C-1C), 79.5, 78.4, 74.0, 73.4, 71.9, 71.2, 71.0, 69.8, 68.9 (C-2A, C-3A, C-5A, C-2B, C-3B, C-4B, C-5B, C-2C, C-3C, C-4C, C-5C, $NHCOCH_2C(CH_3)_2OH$), 65.9 (CH_2CH_2NH), 56.5 (C-4A), 48.9 ($NHCOCH_2C(CH_3)_2OH$), 39.5 (CH_2CH_2NH), 28.3, 28.1 ($NHCOCH_2C(CH_3)_2OH$), 17.08, 16.6, 16.5 (C-6A, C-6B, C-6C); HRMS (ES^+) Calcd. for $C_{25}H_{47}N_2O_{14}$ (MH^+) 599.3027. Found 599.3030.

1-*O*-Acetyl-4-azido-2,3,6-tri-O-benzoyl-4-deoxy-D-glucopyranose (43)

A solution of the known methyl glucoside derivative **42**[19] (4,8 g, 9 mmol) in a mixture Ac_2O (38 mL), AcOH (11 mL) and H_2SO_4 (280 µL) was stirred at 50 °C for 24 hours. The reaction mixture was concentrated and the residue dissolved in ethyl acetate (50 mL). The organic layer was washed with saturated aq. $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (4:1 cyclohexane-EtOAc) to give **43** (3.74 g, 74%) as an α/β (9:1) mixture, δ_H (300 MHz, $CDCl_3$) 8.10-7.35 (m, 15H, Ar), 6.54 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1α), 5.98 (t, 2H, $J_{2,3} = J_{3,4}$ 10 Hz, H-3α, H-1β), 5.73 (t, 1H, $J_{2,3} = J_{3,4}$ 9 Hz, H-3β), 5.52 (dd, 1H, $J_{1,2}$ 8 Hz, H-2β), 5.41 (dd, 1H, H-2α), 4.72-4.60 (m, 4H, 2H-6α, 2H-6β), 4.17-4.11 (m, 2H, H-5α, H-4β), 4.01-3.95 (m, 2H, H-4α, H-5β), 2.16 (s, 3H, CH_3CO); δ_C (75 MHz, $CDCl_3$) 168.58 (CH_3CO), 166.01, 165.56, 165.33 (OCOAr), 133.56-128.47 (Ar), 91.84 (C-1β), 89.24 (C-1α), 73.60 (C-3β), 73.43 (C-5β), 70.80 (C-3α), 70.68 (C-2β), 70.60 (C-5α), 70.11 (C-2α), 62.89 (C-6β), 62.76 (C-6α), 60.41 (C-4α, C-4β), 20,78 (CH_3CO); HRMS (ES^+) Calcd. for $C_{29}H_{25}N_3NaO_9$ (MNa^+) 582.1489. Found 582.1473. Found: C, 62.44; H, 4.69; N, 7.42. $C_{29}H_{25}N_3O_9$ requires C, 62.25; H, 4.50; N 7.51%.

4-Azido-2,3,6-tri-*O*-benzoyl-4-deoxy-D-glucopyranose (44)

Hydrazine acetate (450 mg, 4.9 mmol) was added to a solution of derivative **43** (2.57 g, 4.6 mmol) in DMF (15 mL). The reaction mixture was stirred for 4 hours and then diluted in ethyl acetate (50 mL). The organic layer was washed with brine (2 × 50 mL) and with water (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to afford crude **44** (2.17 g, 91%) as an α/β (7.5:2.5) mixture which was used in the next step without further purification, δ_H (300 MHz, $CDCl_3$) 8.09-7.24 (m, 15H, Ar), 6.06 (t, 1H, $J_{2,3} = J_{3,4}$ 10 Hz, H-3α), 5.74 (t, 1H, $J_{2,3} = J_{3,4}$ 9.8 Hz, H-3β), 5.68 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1α), 5.29 (dd, 1H, $J_{1,2}$ 8 Hz, H-2β), 5.22 (dd, 1H, H-2α), 4.97 (d, 1H, H-1β), 4.75-4.55 (m, 4H, 2H-6α, 2H-6β), 4.34-4.28 (m, 1H, H-5α), 3.90 (t, 1H, $J_{4,5}$ 10 Hz, H-4β), 3.82-3.78 (m, 1H, H-5α); δ_C (75 MHz, $CDCl_3$) 166.34, 165.94, 165.65 (OCOAr), 133.43-128.43 (Ar), 95.91 (C-1β), 90.49 (C-1α), 74.11 (C-2β), 72.88 (C-3β, C-5β), 72.08 (C-2α), 70.71 (C-3α), 69.09 (C-5α), 63.22 (C-6α, C-6β), 60.95 (C-4α, C-4β); LRMS (ES^+) Calcd. for $C_{27}H_{23}N_3NaO_8$ (MNa^+) 540. Found 540.

4-Azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-glucopyranosyl trichloroacetimidate (45)

Trichloroacetonitrile (5 mL) and DBU (80 µL) were added to a solution of compound **44** (1.08 g, 2 mmol) in dry CH_2Cl_2 (5 mL). After 1 h of stirring at 0 °C, solvent was removed. The residue was purified by flash chromatography (7.5:2.5 cyclohexane-EtOAc, Et_3N 2%) to afford the donor **45** (1.2 g, 87%), δ_H (300 MHz, $CDCl_3$) 8.60 (s, 1H, NH), 8.09-7.34 (m, 15H, Ar), 6.73 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 6.09 (t, 1H, $J_{2,3} = J_{3,4}$ 10 Hz, H-3), 5.48 (dd, 1H, H-2), 4.68-4.66 (m, 2H, H-6a, H-6b), 4.29-4.24 (m, 1H, H-5), 4.01 (t, 1H, $J_{4,5}$ 10 Hz, H-4); δ_C (75 MHz, $CDCl_3$) 165.99, 165.40 (OCOAr), 160.42 ($OCNHCCl_3$), 133.59-128.43 (Ar), 93.14 (C-1), 70.95 (C-5), 70.74 (C-3), 70.47 (C-2), 62.77 (C-6), 60.26 (C-4); LRMS (ES^+) Calcd. for $C_{29}H_{23}Cl_3N_3NaO_8$ (MNa^+) 683. Found 683.

N-Benzyloxycarbonylaminoethyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy -β-D-glucopyranoside (46)

TMSOTf (130 µL, 0.71 mmol) was added at 0 °C to a solution of **45** (1.2 g, 1.8 mmol) and benzyl *N*-(2-hydroxyethyl)carbamate[21] (1.1 g, 5.64 mmol) in dry CH_2Cl_2 (12 mL). The reaction mixture was stirred at 0 °C for 1 h, then quenched by addition of Et_3N. The reaction mixture was concentrated. The residue was purified by flash chromatography (7.5:2.5 cyclohexane-EtOAc) to give **46** (920 mg, 73%), $[\alpha]_D$ +66 (c 3.7, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.12-7.29 (m, 20H, Ar), 5.68 (t, 1H, $J_{2,3} = J_{3,4}$ 9.8 Hz,

H-3), 5.41 (dd, 1H, $J_{1,2}$ 7.9 Hz, H-2), 5.20 (broad s, 1H, NH), 5.05 (d, 1 H, J 12 Hz, NHCOOCH_2Ar), 4.96 (d, 1 H, NHCOOCH_2Ar), 4.80 (dd, 1H, $J_{5,6}$ 2.3 Hz, $J_{6,6}$ 12.2 Hz H-6a), 4.74 (d, 1H, H-1), 4.62 (dd, 1H, $J_{5,6}$ 4.5 Hz, H-6b), 3.98-3.90 (m, 3H, H-4, CH_2CH$_2$NH), 3.85-3.70 (m, 1H, H-5), 3.40-3.35 (m, 2H, CH$_2$CH_2NH); δ_C (75 MHz, $CDCl_3$) 166.06, 165.54, 165.29 (OCOAr), 156.26 (NH*C*OOCH$_2$Ar), 133.49-127.98 (Ar), 101.34 (C-1), 73.46 (C-3), 72.66 (C-5), 71.78 (C-2), 69.55 (*C*H$_2$CH$_2$NH), 66.51 (NHCOO*C*H$_2$Ar), 63.07 (C-6), 60.60.74 (C-4), 40.80 (CH$_2$*C*H$_2$NH); HRMS (ES$^+$) Calcd. for $C_{37}H_{34}N_4NaO_{10}$ (MNa$^+$) 717.2173. Found 717.2175.

N-Benzyloxycarbonylaminoethyl 4-(3-hydroxy-3-methylbutamido)-2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-glucopyranoside (48)

$NaBH_4$ (95 mg, 2.5 mmol) and a catalytic amount of $NiCl_2.6H_2O$ were added to a solution of **46** (880 mg, 1.26 mmol) in a mixture of dry CH_2Cl_2 (18 mL) and EtOH (90 mL). The reaction mixture was stirred at room temperature for 1 h and concentrated. The residue was dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated. Amine **47** (709 mg) was used in the next step without purification. To a solution of compound **47** (709 mg 1.06 mmol) in dry CH_2Cl_2 (25 mL) was added dropwise in the following order: 3-hydroxy-3-methylbutanoic acid (293 µL, 2.3 mmol), HATU (582 mg, 1.53 mmol) then DIPEA (253 µL, 1.46 mmol). The reaction mixture was stirred at room temperature under argon for 18 h. The residue was diluted with CH_2Cl_2. The organic layer was washed twice with saturated aq. $NaHCO_3$, water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (2:3 cyclohexane-EtOAc) to give **48** (575 mg, 60%), $[\alpha]_D$ = +57 (c 0.705, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.12-7.26 (m, 20H, Ar), 6.61 (d, 1H, $J_{NH,H4}$ 9.2 Hz, NHCO), 5.69 (t, 1H, $J_{2,3}$ = $J_{3,4}$ 10.1 Hz, H-3), 5.47 (dd, 1H, $J_{1,2}$ 7.9 Hz, H-2), 5.25-5.21 (m, 1H, NHCOOCH_2Ar), 5.04 (d, 1H, J 12.3 Hz, NHCOOCH_2Ar), 4.94 (d, 1H, NHCOOCH_2Ar), 4.77 (d, 1H, H-1), 4.74 (dd, 1H, $J_{5,6}$ 2 Hz, $J_{6,6}$ 12.3 Hz, H-6a), 4.51-4.41 (m, 2H, H-4, H-6b), 4.03 (ddd, 1H, $J_{5,6b}$ 6.3 Hz, H-5), 3.94-3.88 (m, 1H, CH_2CH$_2$NH), 3.74-3.66 (m, 1H, CH_2CH$_2$NH), 3.41-3.34 (m, 2H, CH$_2$CH_2NH), 2.28 (d, 2H, J 1.8 Hz, NHCOCH_2C(CH$_3$)$_2$OH), 1.55 (s, 1H, OH), 1.13, 1.09 (2 s, 6H, NHCOCH$_2$C(CH_3)$_2$OH); δ_C (75 MHz, $CDCl_3$) 172.4 (NHCO), 166.8, 166.4, 165.2 (OCOAr), 156.3 (NHCO), 133.6-127.9 (Ar), 101.1 (C-1), 73.69 (C-5), 72.6 (C-3), 71.9 (C-2), 69.4 (*C*H$_2$CH$_2$NH), 66.5 (NHCOO*C*H$_2$Ar), 63.7 (C-6), 50.7 (C-4), 48.3 (NHCO*C*H$_2$C(CH$_3$)$_2$OH), 40.8 (CH$_2$*C*H$_2$NH), 29.2 (NHCOCH$_2$C(*C*H$_3$)$_2$OH); HRMS (ES$^+$) Calcd. for $C_{42}H_{44}N_2NaO_{12}$ (MNa$^+$) 791.2792. Found 791.2781. Found: C, 65.29; H, 5.72; N, 3.96. $C_{42}H_{44}N_2O_{12}$ requires C, 65.61; H, 5.77; N 3.6%.

2-Aminoethyl 4-(3-hydroxy-3-methylbutamido)-4-deoxy-β-D-glucopyranoside (50)

A solution of sodium methoxide (1 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **48** (138 mg, 0.18 mmol) in MeOH (9 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H$^+$) was added to neutralise MeONa. The resin was filtered and the solvent removed. AcOH (10 mL) and a catalytic amount of $Pd(OH)_2$ were added to a solution of crude **49** in tBuOH (12 mL). The reaction mixture was stirred at room temperature under hydrogen. The reaction was monitored by mass spectrometry. After 20 h, the reaction was complete. The catalyst was filtered over celite and the filtrate was concentrated. The residue was purified on a resin cation exchanger as described for **29**. After lyophilisation, compound **50** was obtained as a white powder (42 mg, 72%), $[\alpha]_D$ −15 (c 0.5, H_2O); δ_C (75 MHz, D_2O) 174.3 (NHCO), 102 (C-1), 74.9, 73.5, 73.3 (C-2, C-3, C-5), 71.9 (NHCOCH$_2$*C*(CH$_3$)$_2$OH), 65.8 (CH$_2$*C*H$_2$NH), 60.8 (C-6), 51.4 (C-4), 48.8 (NHCO*C*H$_2$C(CH$_3$)$_2$OH), 39.5 (*C*H$_2$CH$_2$NH), 28.3, 28.1 (NHCOCH$_2$C(*C*H$_3$)$_2$OH); HRMS (ES$^+$) Calcd. for $C_{13}H_{27}N_2O_7$ (MH$^+$) 323.1818. Found 323.1817.

N-Benzyloxycarbonylaminoethyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranoside (51)

TMSOTf (73 µL, 0.4 mmol) was added under argon at −50 °C to a solution of donor **45** (479 mg, 0.72 mmol) and acceptor **20** (500 mg, 0.57 mmol) in dry CH_2Cl_2 (25 mL) containing molecular sieves 4 Å (600 mg). After 60 min of stirring, the reaction mixture was allowed to reach room temperature for overnight additional stirring. The solution was neutralised with Et_3N and the molecular sieves were removed by filtration. The residue was purified by flash chromatography (7:3 cyclohexane-EtOAc) to give a mixture (838 mg) of the expected trisaccharide **51** and some unreacted acceptor **20** which was used in the next step without further purification. A pure fraction was used for the following characterisation of **51**, $[\alpha]_D$ +38 (c 1.2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 8.16-6.91 (m, 40H, Ar), 5.48-5.44 (m, 3H, H-3A, H-2B, H-2C), 5.38 (dd, 1H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.8 Hz, H-2A), 5.29 (t, 1H, J 5.6 Hz, NHCOO), 5.20 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1B), 5.14 (d, 1H, J 12.3 Hz, NHCOOCH_2Ar), 5.11 (d, 1H, NHCOOCH_2Ar), 5.02 (d, 1H, J 11 Hz, OCH$_2$Ar), 4.89 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1C), 4.73 (d, 1H, OCH$_2$Ar), 4.62 (d, 1H, H-1A), 4.54 (d, 1H, J 11.6 Hz, OCH$_2$Ar), 4.36-4.30 (m, 3H, H-6Aa, H-3C, OCH$_2$Ar), 4.23 (dd, 1H, $J_{5,6}$ 1.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6Ab), 4.18 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 9.3 Hz, H-3B), 3.86-3.78 (m, 3H, H-5B, H-5C, OCH_2CH$_2$NHZ), 3.76 (t, 1H, $J_{3,4}$ = $J_{4,5}$ 9.3 Hz, H-4A), 3.66 (t, 1H, $J_{3,4}$ = $J_{4,5}$ 9.4 Hz, H-4C), 3.56 (m, 1H, OCH_2CH$_2$NH), 3.49-3.37 (m, 3H, H-4B, CH$_2$CH_2NH), 3.05 (m, 1H, H-5A), 1.39 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6C), 1.01 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6B); δ_C (125 MHz, $CDCl_3$) 165.8, 165.7, 165,6, 165.5, 165,2 (OCOAr), 156.3 (NHCOO), 137.9-127.2 (Ar), 101.2 (C-1A), 98.1 (C-1B), 97.3 (C-1C), 80.1 (C-4C), 79.1 (C-3B), 79.0 (C-4B), 76.9 (C-3C), 75.3, 74.4 (OCH$_2$Ar), 73.5, 72.2, 72.0 (C-3A, C-5A, C-2B, C-2C), 71.7 (C-2A), 68.5 (C-5C), 67.9 (C-5B), 67.1 (*C*H$_2$CH$_2$NH), 66.7 (NHCOO*C*H$_2$Ar), 62.3 (C-6A), 64.0 (C-4A), 40.8 (CH$_2$*C*H$_2$NH), 17.9 (C-6C), 17.7 (C-6B); HRMS (ES$^+$) Calcd. for $C_{77}H_{74}N_4NaO_{20}$ (MNa$^+$) 1397.4794. Found 1397.4733.

N-Benzyloxycarbonylaminoethyl 4-(3-hydroxy-3-methylbutamido)-2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranoside (53)

$NaBH_4$ (50 mg, 1.3 mmol) and a catalytic amount of $NiCl_2.6H_2O$ were added to a solution of **51** (885 mg, 0.64 mmol) in a mixture of

dry CH_2Cl_2 (10 mL) and EtOH (45 mL). The reaction mixture was stirred at room temperature for 2 h, then additional $NaBH_4$ (50 mg, 1.3 mmol) and a catalytic amount of $NiCl_2.6H_2O$ were added. The reaction mixture was stirred overnight and concentrated. The residue was dissolved in CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated. Amine **52** (642 mg) was used in the next step without purification. To a solution of compound **52** (642 mg) in dry CH_2Cl_2 (10 mL) were added dropwise in the following order: 3-hydroxy-3-methylbutanoic acid (132 µL, 1.05 mmol), HATU (399 mg, 1.05 mmol) then DIPEA (180 µL, 1.05 mmol). The reaction mixture was stirred at room temperature under argon for 3 days. The residue was diluted with CH_2Cl_2. The organic layer was washed with saturated aq. $NaHCO_3$, water, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (3:2 cyclohexane-EtOAc) to give **53** (442 mg, 47%), $[\alpha]_D = +21$ (c 0.675, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 8.16-6.91 (m, 40 H, Ar), 6.15 (d, 1H, $J_{NH,H4}$ 9 Hz, NHCO), 5.57-5.06 (m, 9H, H-2A, H-3A, H-1B, H-2B, H-2C, NHCOOCH_2Ar, OCH_2Ar, NHCOO), 4.91 (broad s, 1H, H-1C), 4.74-4.70 (m, 2H, H-1A, OCH_2Ar), 4.57 (d, 1H, J 12 Hz, OCH_2Ar), 4.39-4.16 (m, 5H, H-4A, H-6Aa, H-3B, H-3C, OCH_2Ar), 3.86-3.35 (m, 10H, H-6Ab, H-4B, H-5B, H-4C, H-5C, CH_2CH_2NH), 2.18 (broad s, 2H, NHCOCH_2C$(CH_3)_2$OH), 1.44 (s, 3H, NHCOCH$_2$C(CH_3)$_2$OH), 1.36 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6B or H-6C), 1.08 (s, 3H, NHCOCH$_2$C(CH_3)$_2$OH), 1.00 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6B or H-6C); δ_C (75 MHz, $CDCl_3$) 172.0 (NHCO), 166.6, 166.2, 165.7, 165.6, 165.0 (OCOAr), 156.3 (NH*C*OOCH$_2$Ar), 138.1-127.2 (Ar), 101.2 (C-1A), 98.4 (C-1B), 97.2 (C-1C), 80.1, 79.0 (C-4B, C-4C), 78.9, 77.4 (C-3C, C-3B), 75.2, 74.2 (OCH_2Ar), 72.7, 72.6, 72.3, 72.1, 71.9 (C-2A, C-3A, C-5A, C-2B, C-2C), 69.3 (NHCOCH$_2$*C*(CH$_3$)$_2$OH), 68.4, 67.8 (C-5B, C-5C), 67.1 (*C*H$_2$CH$_2$NH), 66.7 (NHCOO*C*H$_2$Ar), 62.4 (C-6A), 50.2 (C-4A), 48.1 (NHCO*C*H$_2$C(CH$_3$)$_2$OH), 40.7 (CH$_2$*C*H$_2$NH), 29.2, 29.1 (NHCOCH$_2$C(*C*H$_3$)$_2$OH), 18.0, 17.7 (C-6B, C-6C); HRMS (ES$^+$) Calcd. for $C_{82}H_{84}N_2NaO_{22}$ (MNa^+) 1471.5413. Found 1471.5450. Found: C, 67.82; H, 6.41; N, 1.97. $C_{82}H_{84}N_2O_{22}$ requires C, 67.94; H, 5.84; N 1.93%.

2-Aminoethyl 4-(3-hydroxy-3-methylbutamido)-4-deoxy-β-D-glucopyranosyl-(1→3)-α-D-rhamnopyranosyl-(1→3)-α-D-rhamnopyranoside (55)

A solution of sodium methoxide (1.6 mL, 1 M in MeOH) was added dropwise at 0 °C to a solution of **53** (227 mg, 0.15 mmol) in MeOH (5 mL). The reaction mixture was stirred at room temperature for 18 h. An acidic resin (Amberlite IR 120 H^+) was added to neutralise MeONa. The resin was filtered and the solvent removed. AcOH (8 mL) and a catalytic amount of $Pd(OH)_2$ were added to a solution of crude **54** in tBuOH (15 mL). The reaction mixture was stirred at room temperature under hydrogen. The reaction was monitored by mass spectrometry. After 20 h, the reaction was complete. The catalyst was filtered over celite and the filtrate was concentrated. The residue was purified on a resin cation exchanger (Bio-Rad, AG MP-50 Resin, analytical grade 100-200 mesh). After lyophilisation, compound **55** (44 mg, 44%) was obtained as a white solid, $[\alpha]_D$ –48 (c 0.78, H_2O); δ_C (75 MHz, D_2O) 174.2 (NHCO), 103.6 (C-1A), 102.2 (C-1B), 99.8 (C-1C), 80.0, 78.4, 74.8, 73.8, 73.3, 71.3, 71.0, 70.1, 69.8, 69.0, (C-2A, C-3A, C-5A, C-2B, C-3B, C-4B, C-5B, C-2C, C-3C, C-4C, C-5C, NHCOCH$_2$*C*(CH$_3$)$_2$OH), 66.8 (CH$_2$*C*H$_2$NH), 60.9 (C-6A), 51.4 (C-4A), 48.8 (NHCO*C*H$_2$C(CH$_3$)$_2$OH), 39.6 (*C*H$_2$CH$_2$NH), 28.3, 28.1 (NHCOCH$_2$C(*C*H$_3$)$_2$OH), 16.6 (C-6B, C-6C); HRMS (ES$^+$) Calcd. for $C_{25}H_{47}N_2O_{15}$ (MH^+) 615.2977. Found 615.2979.

Antiserum production

For the production of antibodies, the trisaccharide derivative **29** was covalently linked to KLH (keyhole limpet haemocyanin) using glutaraldehyde, which cross-links primary amino groups of the carrier protein to the spacer arm of the monosaccharide analogue. Derivative **29** (3.54 mg, 5.8 µmol) dissolved in 50% MeOH was reacted with KLH (20 mg, 0.29 µmol) and glutaraldehyde (25 µL, 25% in water) in phosphate buffer (0.1 M, 5 mL, pH 7.4). The reaction mixture was stirred overnight at 4 °C in the dark before aliquoting and freezing at –20 °C.

Rabbits (Blanc de Bousact, Evic, France) are housed under normal conditions in accredited animal husbandry, receiving tap water and food given *ad libitum*. All experiments are performed according to the European Community rules of animal care and are supervised by authorized researchers (permissions delivered by the French Veterinary Authorities). Rabbits were immunised and given booster injections every two months with immunogen (1 mg in complete Freund's adjuvant) in multiple subcutaneous injections according to the procedure described by Vaitukaitis.[24] Rabbits were bled from the central ear artery before the first immunisation (serum S_0), then on a weekly basis after each booster (serums S_1, S_2,...). The sera were kept at 4 °C in the presence of sodium azide (0.01% final concentration).

Preparation of the enzymatic tracer

The tracer was obtained by covalently coupling derivative **29** to AChE using the procedure previously described for haptens[25] and proteins.[26] In the first step, a thiol group was introduced into the hapten **29** by reaction of its primary amino group with *N*-succinimidyl-*S*-acetyl thioacetate (SATA). Briefly, a solution of SATA (1.15 mg, 5 µmol) and derivative **29** (0.31 mg, 0.5 µmol) in a borate buffer (130 µL, 0.1 M, pH 8.5) was stirred for 1 h at 20 °C. The thiolated derivative was purified using a Sep-Pak-C18 cartridge (Waters, Milford, USA) before deprotecting the thiol function in the presence of hydroxylamine. The enzyme conjugate was obtained by mixing the thiolated derivative **29** (0.1 nmol) with AChE-SMCC (0.1 nmol) prepared as previously described[24] for 18 h at 4 °C. The enzyme conjugate was purified by molecular sieve chromatography on a Bio-Gel A 1.5 M column (90 × 1.5 cm Bio-Rad) eluted with EIA buffer and was stored at –20 °C until use.

Competitive EIA procedure

Competitive EIA was performed in 96-well microtitre plates coated with mouse monoclonal anti-rabbit immunoglobulin antibodies in order to ensure separation between the free and bound moieties of the enzymatic tracer during the immunological reaction. The assay was performed in a total volume of 150 µL, each reagent (enzymatic tracer, diluted rabbit antisera and standard (derivatives **2**, **27**, **28**, **29**, **31**, **33**, **37**, **41**, **50**, **55**)) being added in a 50 µL volume. The optimal working dilution of antiserum was previously determined by serial dilution. After 18 h

of immunological reaction at 4 °C, the plates were washed with the washing buffer and AChE substrate (200 μL, Ellman's reagent) was added to each well. After 1 or 2 h of gentle shaking in the dark at room temperature, the absorbance of each well was measured at 414 nm. The results were expressed in terms of $(B/B_0) \times 100$ as function of concentration (logarithmic scale) where B and B_0 represent bound enzymatic activity in the presence and absence of the competitor (antigen or analogues), respectively. A linear log-logit transformation was used to fit the calibration curve. The sensitivity of the assay was characterised by the concentration of standard inducing a 50% lowering of the binding observed in the absence of competitor (B/B_0 50%). Nonspecific binding represented less than 0.1% of the total enzyme activity. All experiments were done in duplicate, and in quadruplicate for B_0.

Acknowledgements

The authors gratefully acknowledge Dr. David Lesur for mass spectrometry. This work was supported by the Conseil Régional de Picardie. S. Dhénin is grateful to the Ministère de l'Enseignement et de la Recherche for a doctoral fellowship.

Notes and references

1 J. M. Daubenspeck, H. Zeng, P. Chen, S. Dong, C. T. Steichen, N. R Krishna, D. G. Pritchard and C. L. Turnbough, Jr., *J. Biol. Chem.*, 2004, **279**, 30945–30953.
2 (*a*) D. B. Werz and P. H. Seeberger, *Angew. Chem., Int. Ed.*, 2005, **44**, 6315–6318; (*b*) R. Saksena, R. Adamo and P. Kovac, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 615–617; (*c*) R. Adamo, R. Saksena and P. Kovac, *Carbohydr. Res.*, 2005, **340**, 2579–2582; (*d*) H. Guo and G. A. O'Doherty, *Angew. Chem., Int. Ed.*, 2007, **46**, 5206–5208; (*e*) D. Crich and O. Vinogradova, *J. Org. Chem.*, 2007, **72**, 6513–6520.
3 (*a*) R. Saksena, R. Adamo and P. Kovac, *Carbohydr. Res.*, 2005, **340**, 1591–1600; (*b*) A. S. Metha, E. Saile, W. Zhong, T. Buskas, R. Carlson, E. Kannenberg, Y. Reed, C. P. Quin and G.-J. Boons, *Chem.–Eur. J.*, 2006, **12**, 9136–9149; (*c*) S. G. Y. Dhénin, V. Moreau, N. Morel, M-C. Nevers, H. Volland, C. Créminon and F. Djedaïni-Pilard, *Carbohydr. Res.*, 2008, **343**, 2101–2110; (*d*) S. Hou and P. Kovac, *Eur. J. Org. Chem.*, 2008, 1947–1952.
4 (*a*) M. Tamborrini, D. B. Werz, J. Frey, G. Pluschke and P. H. Seeberger, *Angew. Chem., Int. Ed.*, 2006, **45**, 6581–6582; (*b*) D. Wang, G. T. Carroll, N. J. Turro, J. T. Koberstein, P. Kovac, R. Saksena, R. Adamo, L. A. Herzenberg, L. A. Herzenberg and L. Steinman, *Proteomics*, 2007, **7**, 180–184.
5 C.-T. Tai, S. S. Kulkarni and S.-C. Hung, *J. Org. Chem.*, 2003, **68**, 8719–8722.
6 Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov and C.-H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 734–753.
7 H. Firouzabadi, N. Iranpoor and F. Ebrahimzadeh, *Tetrahedron Lett.*, 2006, **47**, 1771–1775.
8 P. J. Garegg and B. Samuelsson, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2866–2869.
9 L. Jiang and T.-H. Chan, *Tetrahedron Lett.*, 1998, **39**, 355–358.
10 (*a*) Y. D. Vankar and C. T. Rao, *J. Chem. Res., Synop.*, 1985, **7**, 232–233; (*b*) A. Brar and Y. D. Vankar, *Tetrahedron Lett.*, 2006, **47**, 5207–5210.
11 B. Elchert, J. Li, J. Wang, Y. Hui, R. Rai, R. Ptak, P. Ward, J. Y. Takemoto, M. Bensaci and C.-W. T. Chang, *J. Org. Chem.*, 2004, **69**, 1513–1523.
12 B. Mukhopadhyay, K. P. R. Kartha, D. A. Russell and R. A. Field, *J. Org. Chem.*, 2004, **69**, 7758–7760.
13 O. Plettenburg, V. Bodmer-Narkevitch and C.-H. Wong, *J. Org. Chem.*, 2002, **67**, 4559–4564.
14 I. Cumpstey, A. J. Fairbanks and A. J. Redgrave, *Tetrahedron*, 2004, **60**, 9061–9074.
15 B. Mukhopadhyay and R. A. Field, *Carbohydr. Res.*, 2003, **338**, 2149–2152.
16 R. R. Schmidt and W. Kinzi, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21–123.
17 N. E. Byramova, M. V. Ovchinnikov, L. Backinowsky and N. K. Kochetkov, *Carbohydr. Res.*, 1983, **124**, C8–C11.
18 M. Alpe and S. Oscarson, *Carbohydr. Res.*, 2002, **337**, 1715–1722.
19 S. K. Sinha and K. Brew, *Carbohydr. Res.*, 1980, **81**, 239–247.
20 B. Leon, S. Liemann and W. Klaffke, *J. Carbohydr. Chem.*, 1993, **12**, 597–610.
21 J. Massoulie and S. Bon, *Eur. J. Biochem.*, 1976, **68**, 531–539.
22 H. Harada, O. Asano, T. Kawata, T. Inoue, T. Horizoe, N. Yasuda, K. Nagata, M. Murakami, J. Nagaoka, S. Kobayashi, I. Tanaka and S. Abe, *Bioorg. Med. Chem.*, 2001, **9**, 2709–2726.
23 J. O. Deferrari, E. G. Gros and I. M. E. Thiel, *Methods Carbohydr. Chem.*, 1972, **6**, 365–367.
24 J. L. Vaitukaitis, *Methods Enzymol.*, 1981, **73**, 46–61.
25 L. L. Mclaughlin, Y. Wei, P. T. Stockmann, K. M. Leahy, P. Needleman, J. Grassi and P. Pradelles, *Biochem. Biophys. Res. Commun.*, 1987, **144**, 469–476.
26 J. Grassi, Y. Frobert, P. Pradelles, F. Chercuitte, D. Gruaz, J.-M. Dayer and P. E. Poubelle, *J. Immunol. Methods*, 1989, **123**, 193–210.