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Carbohydrate-based templates for synthetic vaccines and drug delivery

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Abstract—Methyl tetra-*O*-allyl, and tetra-*O*-[2-(tetrahydro-2*H*-pyranyl)oxy.-3-oxapentyl glucosides, and tetra-*O*-(cyanoethyl)galactosyl azide were converted into derivatives containing linkers with terminal carboxylic acid functionalities at the anomeric position and bearing four arms with phthaloyl- or BOC-protected terminal amino groups. These molecules were suitable for use in solid-phase peptide synthesis and for the preparation of dendrimers containing multiple copies of peptides. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Vaccination, the training of the immune system to recognise foreign microorganisms by exposing the host to killed or attenuated bacteria or viruses such that when the host encounters the intact pathogen a strong and rapid immune response is elicited, has proved to be one of the most important and cost-effective public health interventions. Vaccination has led to the world-wide eradication of smallpox, and the control of such diseases as measles, rubella, polio and diptheria.

Many problems remain with conventional vaccines, such as their limited shelf-lives, the need for suitable carriers and adjuvants, and the potential dangers of using live microorganisms. These problems can be addressed by the development of synthetic vaccines. These contain peptide sequences corresponding to the antigenic determinant(s) of the invading microorganisms and, under appropriate conditions, the host can raise an immune response to these synthetic vaccines which offers protection against the disease. The potential advantages of synthetic vaccines are that they would not require refrigerated storage (a great advantage in developing nations) and they are not prepared from pathogenic microbes. As synthetic entities, they would be guaranteed to be of homogeneous composition and, as such, only very small amounts of these molecules would be needed to elicit an immune response. The advantage of preparing such a synthetic vaccine using standard solid-phase peptide synthesis is that carriers and adjuvants could be included in the constructs.[1]

The potential for using dendrimer cores in the preparation of synthetic vaccines has been recognised. Tam's multiple antigenic peptide (MAP) system consists of a core of branching lysine residues, each terminating in a free amino group, to which are attached multiple copies of identical peptide sequences. The advantage of having multiple copies of identical peptides on the one construct is that significantly higher antibody titres can be obtained than for a single peptide sequence when conjugated to a carrier protein.[2]

The incorporation of lipidic molecules as adjuvants into synthetic peptides has been shown to enhance immunogenicity of otherwise poorly immunogenic peptides. For example, Jung and coworkers coupled tripalmitoyl-*S*-glyceryl cysteine (Pam_3Cys) to a peptide epitope and found that the resulting lipopeptide constructs were potent immunogens with self-adjuvanting properties.[3,4] An advanced system which includes features of both Tam's and Jung's constructs is the lipid polylysine core (LCP) system, which uses lipoaminoacids together with a polylysine core.[5] Lipoaminoacids are known to increase peptide immunogenicity,[6] and they also have the potential to be used for the oral delivery of drugs and peptides.[7] In the LCP system the lipoaminoacids are incorporated as a lipidic anchor at the C-terminus of the polylysine system, and can be synthesised by standard solid phase peptide synthesis.

Keywords: synthetic vaccines; carbohydrates; solid-phase synthesis; adjuvants/carriers.
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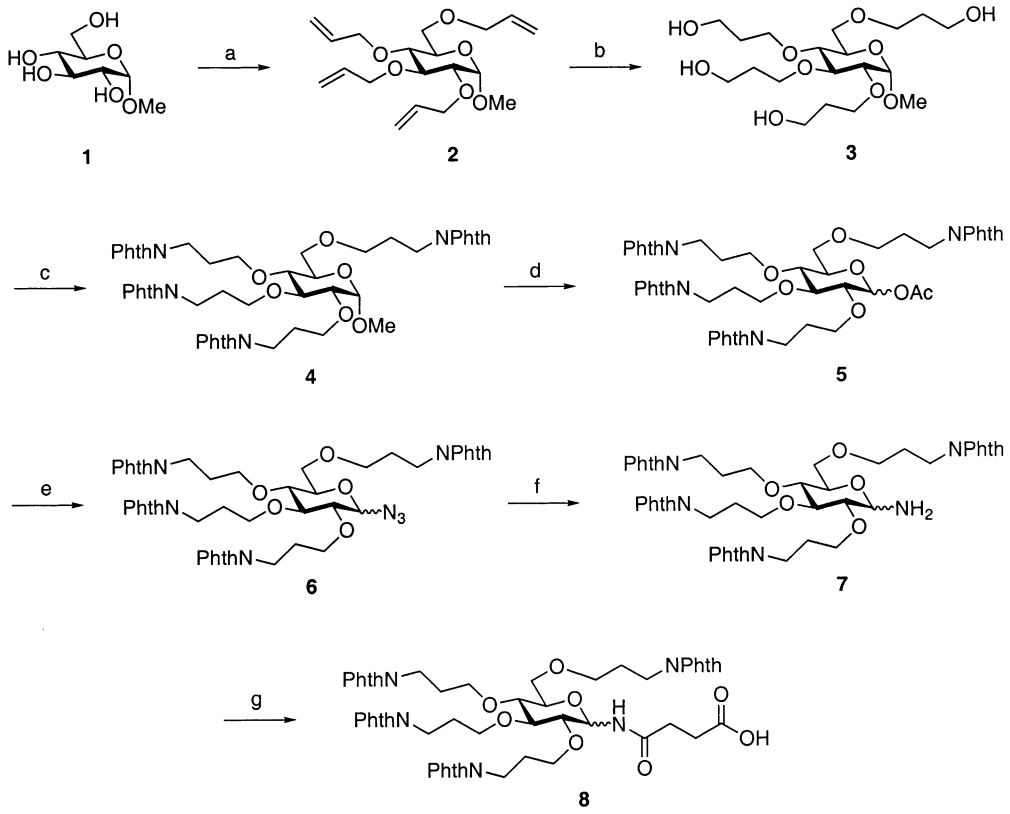


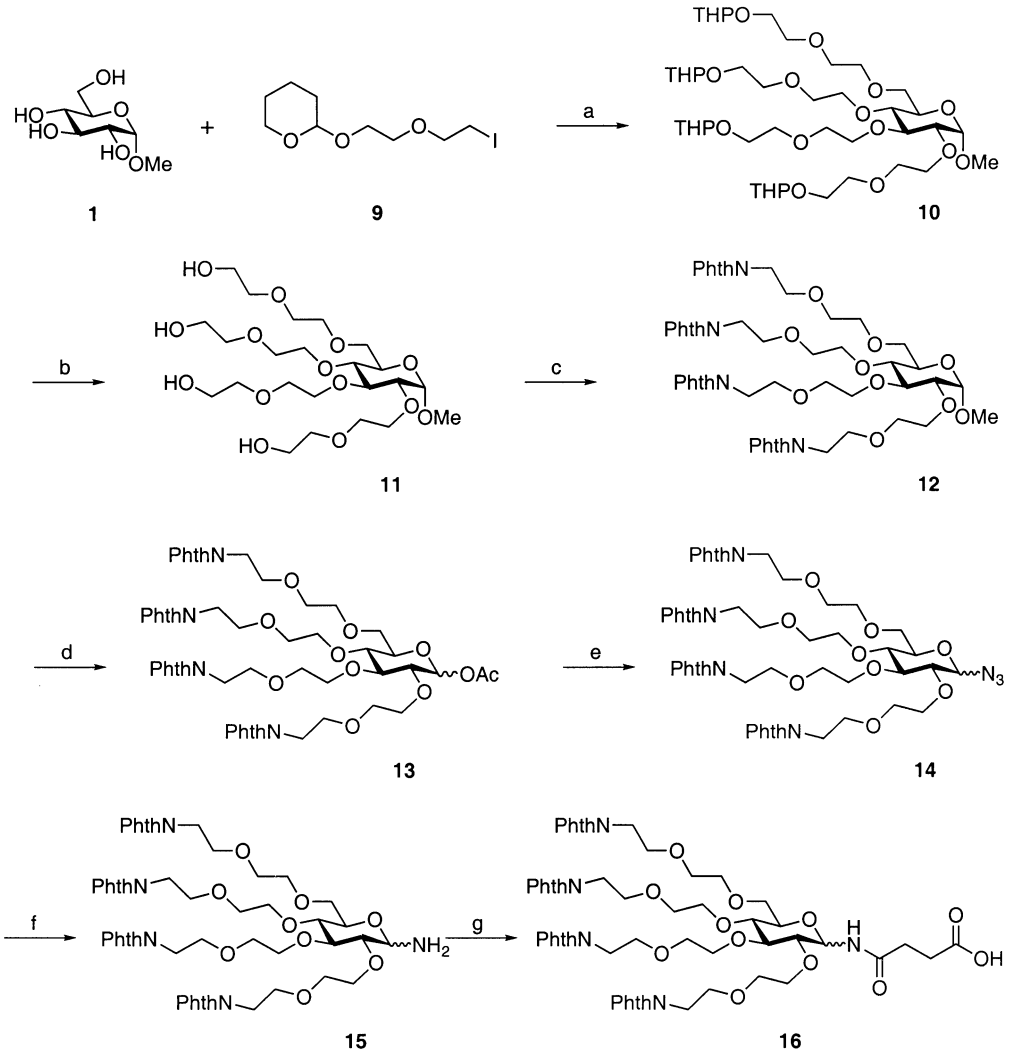
Scheme 1. (a) allyl bromide, NaH, DMF, 0–50°C, 1 h, 67% (b) i. 9-BBN, THF, Δ, 6 h; ii. H_2O_2, NaOH, 0°C–rt, overnight, 70% (c) phthalimide, PPh_3, DEAD, THF, rt, 72 h, 94% (d) Ac_2O, H_2SO_4, −20°C, 3 days, 78% (e) $TMSN_3$, $SnCl_4$, CH_2Cl_2, rt, 1 day, 83% (f) H_2/Pd/C, EtOAc, rt, 2 days, 76% (g) succinic anhydride, CH_2Cl_2, Δ, overnight, 84%.

The synthesis of carbohydrate-centred glycoclusters and glycodendrimers has attracted much attention recently[8] and, in principle, protecting group manipulation allows for the attachment of different peptide sequences within the same construct. This paper describes the synthesis of glucose- and galactose-based monosaccharides, incorporating carboxylic acid groups for attachment to solid supports,[9,10] and suitably functionalised with terminal amino groups for the attachment of multiple copies of peptide antigen sequences.

2. Results and discussion

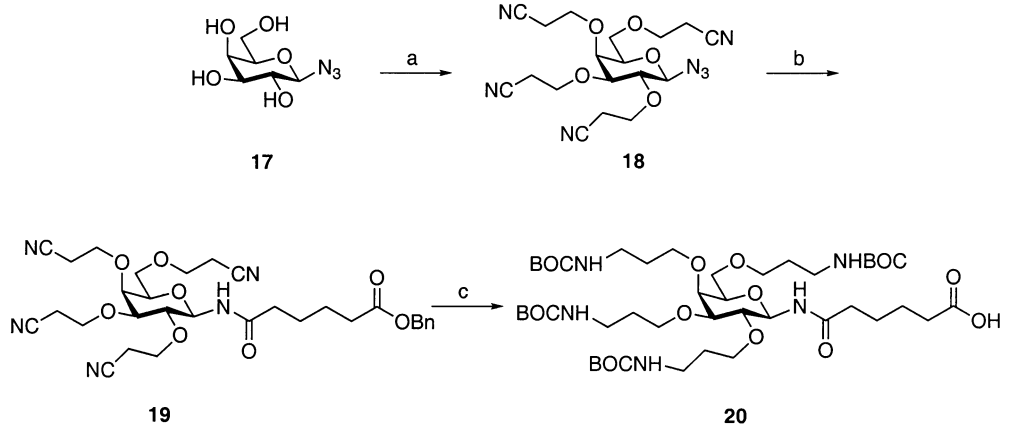
Methyl α-D-glucopyranoside **1** was used as a common starting material for the syntheses of the carbohydrate core molecules **8** and **16** shown in Schemes 1 and 2, respectively. This compound contains suitable protection at the anomeric centre that can be transformed to the required carboxylic functionality at a later stage of synthesis. The synthesis of 'octopus' glycosides has recently been described via per-allylated glycosides. Allylation of glucoside **1** was initially carried out using allyl chloride in aqueous sodium hydroxide in the presence of a phase transfer catalyst, as described by Dubber and Lindhorst.[11] However, the required 2,3,4,6-tetra-*O*-allyl derivative **2** was obtained in very low yield (16%). The yield of the tetraol **2** could be increased significantly by instead employing allyl bromide and sodium hydride in DMF at 50°C. By this method the per-allylated derivative **2** was obtained in 67% yield. Regioselective hydroboration of **2** using 9-borabicyclo-[3.3.1]nonane (9-BBN) reagent gave the tetra-*O*-(3-hydroxypropyl) derivative **3** in good yield (70%). When the tetraol **3** was subjected to Mitsunobu conditions (phthalimide, diethyl azodicarboxylate, triphenylphosphine), the four hydroxyl groups of **3** were converted to their corresponding phthaloyl-protected amine functionalities, giving compound **4** in excellent yield (94%). Acetolysis of the methyl glucoside **4** was accomplished using a mixture of acetic anhydride and sulfuric acid (50:1) at −20°C, which afforded predominantly the α-anomer of the acetate **5** in high yield (78%; α/β≈7:1, determined by integration of the resolved H-1 signals). The acetate **5** was converted the corresponding azido derivative **6** by treatment with azidotrimethylsilane in the presence of tin(IV) chloride as catalyst. The azide **6** was obtained as an anomeric mixture (α/β≈2:1). Catalytic hydrogenation of the azide **6** provided the anomeric amino derivative **7** with the same ratio of anomers as it was found for **6**. Reaction of the amine **7** with succinic anhydride yielded the required carboxylic acid **8**, also as an anomeric mixture, but this time with the ratios of anomers reversed (β/α≈2:1). This fact suggested that the two anomers of compound **7** were in dynamic equilibrium, and that the β-anomer reacted faster with succinic anhydride.

To modify the physicochemical properties of the templates molecules, the introduction of diethyleneglycol ($XCH_2CH_2OCH_2CH_2O$) moieties appeared to be a feasible substitution on the monosaccharide core (Scheme 2). However, many attempts to obtain such tetra-substituted derivatives of the tetraol **1** using electrophiles such as

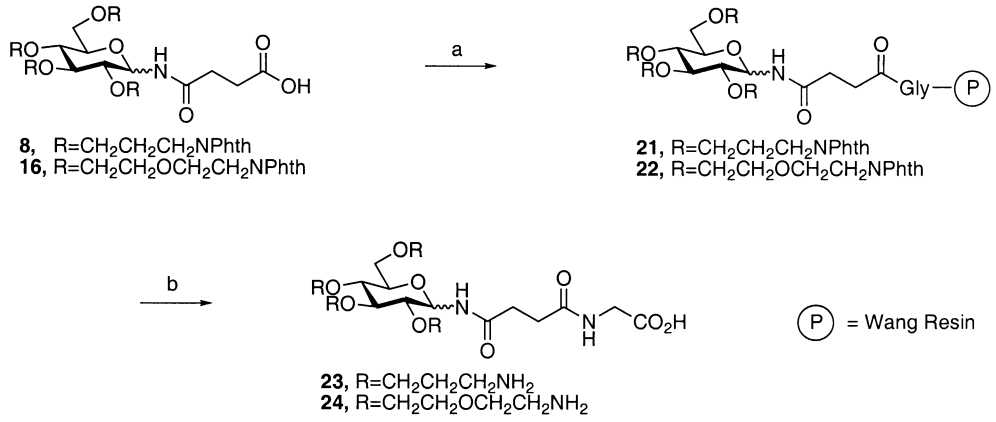


Scheme 2. (a) NaH, DMF, 0–50°C, overnight, 19% (b) *p*-TsOH, $CHCl_3$/MeOH, 55°C, 2 h, 69% (c) phthalimide, PPh_3, DEAD, THF, rt, 72 h, 86% (d) Ac_2O, H_2SO_4, −20°C, 3 days, 78% (e) $TMSN_3$, $SnCl_4$, CH_2Cl_2, rt, 1 day, 88% (f) H_2/Pd/C, EtOAc, rt, 2 days, 76% (g) succinic anhydride, CH_2Cl_2, Δ, overnight, 84%.

$N_3CH_2CH_2OCH_2CH_2OTos$, $ClCH_2CH_2OCH_2CH_2OTHP$, $ClCH_2CH_2OCH_2CH_2Cl$ and $DdeNHCH_2CH_2OCH_2CH_2O$-Tos, either in the presence of phase transfer catalysts, or using strong base (sodium hydride) at room temperature, failed. This may have been due either to insufficient reactivity of these electrophiles, or the tendency for these halides to undergo elimination rather than substitution, leading to terminal enol ethers.[12] Nevertheless, by employing



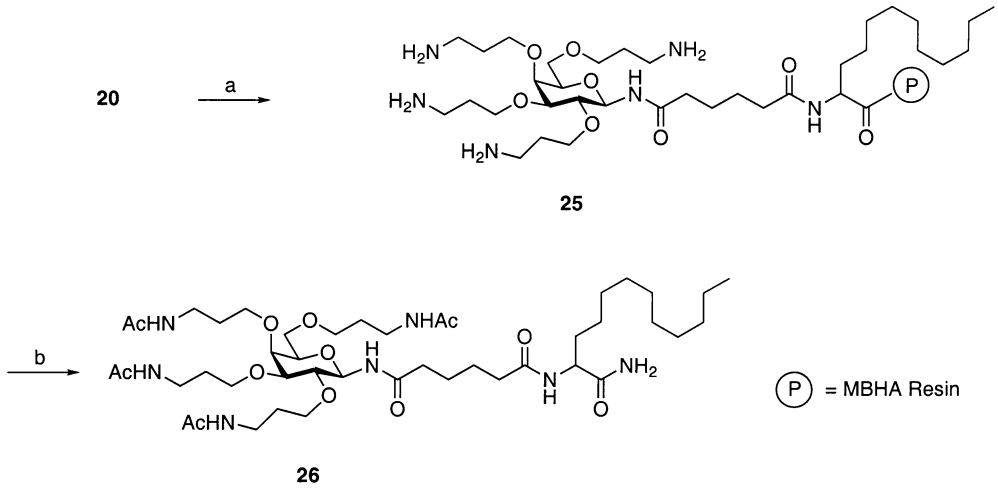
Scheme 3. (a) acrylonitrile, DBU, MeCN, rt, overnight, 64% (b) i. H_2/Pd/C, THF, rt, 2 h; ii. adipic acid monobenzyl ester, HBTU, DIPEA, THF, rt, overnight, 55% (c) i. $NaBH_4$, $CoCl_2{\cdot}6H_2O$, BOC_2O, MeOH, 0°C–rt, 4 h; ii. LiOH, MeOH/H_2O, rt, overnight, 46%.



Scheme 4. (a) Gly-Wang Resin, HBTU, HOBt, DIPEA, DMF, rt, 2×1 h (b) i. $NH_2NH_2{\cdot}H_2O$, EtOH, 60°C, 24 h; ii. TFA/TIPS/H_2O, 2 h.

two equivalents of $ICH_2CH_2OCH_2CH_2OTHP$ (**9**) per hydroxyl group of the tetraol **1**, and excess sodium hydride in DMF at 50°C, the tetra-alkylated derivative **10** was obtained, albeit in low yield (19%). Insufficiently alkylated side products were also isolated which, by mass spectrometry, were shown to be mixtures of the trialkylated derivatives (17%), and the dialkylated derivatives (21%) of the starting material **1**. Removal of the tetrahydropyranyl protecting groups of the polyether **10** was accomplished using 4-toluenesulfonic acid in chloroform–ethanol at 55°C to give the tetrahydroxy intermediate **11** in moderate yield (69%). The tetraol **11** was converted to the tetraphthalimide **12**, using the same Mitsunobu conditions employed previously to convert compound **3** to compound **4**. Replacement of the methoxyl group at the anomeric position of compound **12** with an acetoxyl group, using sulfuric acid in acetic anhydride, provided the acetate **13**, mainly as the α-anomer (α/β≈7:1, with overall yield 78%), from which the azido derivative **14** was prepared as anomeric mixture (α/β≈2:1) by treating compound **13** with azidotrimethylsilane in the presence of tin(IV) chloride. Hydrogenation of the azido group of compound **14** provided the amino derivative **15**, which was refluxed with succinic anhydride in dichloromethane to yield the protected tetraamine **16**, containing a carboxylic acid functional group for attachment to a solid support.

To reduce the number of steps required to prepare these template molecules, the synthetic route outlined in Scheme 3 was devised. The crystalline β-galactosyl azide[13] **17** was cyanoethylated using acrylonitrile and 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) in acetonitrile, giving the tetranitrile **18** in 64% yield. It was essential to use a non-aqueous solvent, as attempts to employ the conditions described by Bazin and coworkers for cyanoethylating carbohydrates (acrylonitrile in aqueous sodium hydroxide)[14] gave low yields, and large amounts of the side-product bis(cyanoethyl) ether, which proved to be inseparable from the tetranitrile **18**. Selective reduction of the azido group in **18** was achieved using catalytic hydrogenation. The intermediate anomeric amine was not isolated, but was coupled directly with adipic acid, monobenzyl ester, using the peptide coupling agent HBTU, to give the ester **19**, as the pure β-anomer, and in 55% yield for the two steps. The four nitrile fuctional groups of **19** were reduced to the corresponding amines using sodium borohydride and cobalt(II) chloride in methanol.[15] By performing this reduction in the presence of di-*tert*-butyl dicarbonate (BOC_2O), the transient amino groups were converted to their corresponding BOC-protected derivatives in situ. Final treatment of the reaction mixture with aqueous lithium hydroxide saponified the ester group to give the free acid **20**, in 46% yield for the two steps.



Scheme 5. (a) i. 2-aminododecanoyl-MBHA resin, HBTU, DIPEA, DMF, rt, 30 min; ii. TFA, rt, 2×1 min (b) i. AcOH, HBTU, DIPEA, DMF, rt, 30 min; ii. HF-cleavage.

The phthalimido-protected compounds **8** and **16** are suitable substrates for solid-phase peptide synthesis using either BOC- or FMOC-chemistries. They were coupled to preloaded glycine-Wang resin by employing HBTU/HOBt/DIPEA coupling reagents in DMF (Scheme 4). The phthaloyl protecting groups on the resin-bound sugar derivatives **21** and **22** were removed by treatment with hydrazine in ethanol at 60°C, yielding the corresponding resin-bound tetraamines. The success of the protecting group removal was confirmed by a quantitative ninhydrin analysis on the resin-bound products, and by mass spectrometry of the products **23** and **24**, obtained by cleavage from the Wang resin with trifluoroacetic acid/triisopropylsilane/water.

The synthesis of the lipoaminoacid-containing galactoside **26**, is shown in Scheme 5. 2-(Butoxycarbonylamino)dodecanoic acid was attached to MBHA resin then, after trifluoroacetic acid deprotection, the BOC-protected tetraamine **20** was coupled to the solid phase. After a second deprotection and subsequent N-acetylation, the construct was cleaved from the resin using standard anhydrous hydrofluoric acid/scavenger conditions, giving the galactose-based core molecule **26**, whose identity was confirmed by high-resolution mass spectrometry and ^{1}H NMR spectroscopy.

3. Conclusion

Two glucose-based templates and one galactose-based template have been prepared, each bearing a carboxylic acid for attachment to solid supports, and four protected amines attached to spacers of differing hydrophobicity, suitable for chain extension via BOC- or FMOC-based solid phase peptide synthesis. The stability of these constructs towards the conditions of resin cleavage using either trifluoroacetic acid or hydrofluoric acid has been demonstrated, as has their utility for preparing lipoamino-acid-containing dendrimers on solid supports.

4. Experimental

4.1. General methods

Optical rotations were determined with AA-10R polarimeter (1 dm cells, Na-D-line: 589 nm, Optical Activity Ltd). Mass spectra were run on a Perkin–Elmer API 3000 LC/MS/MS electrospray instrument, or with a VG Analytical ZAB-SE instrument using fast atom bombardment (FAB) techniques (20 kV Cs+ ion bombardment) with 2 μL of appropriate matrix (3-nitrobenzyl alcohol or thioglycerol) added. ^{1}H NMR spectra were recorded on either a Bruker AM 500 instrument or a Varian Gemini 300 machine. ^{13}C NMR spectra were run on either a Bruker AM250 instrument or a Varian Gemini 300 machine. All reactions were performed under inert gas atmospheres.

4.1.1. Methyl 2,3,4,6-tetra-*O*-allyl-α-D-glucopyranoside (2). Sodium hydride (60% dispersion in oil, 4.00 g, 100 mmol) was added portionwise to a well stirred solution of **1** (3.88 g, 20.0 mmol) at 0°C in 100 mL dry DMF. After 30 min allyl bromide (10.4 mL, 120 mmol) was added dropwise and the temperature was raised to 50°C. When the reaction was complete (about 1 h) the reaction flask was placed in an ice bath and the excess sodium hydride was quenched by the slow addition of methanol (20 mL). The mixture was evaporated to dryness, then the residue was dissolved in CH_2Cl_2 (200 mL) and washed with water (3×80 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated, then the residual oil was purified by flash chromatography (hexane/EtOAc 8:2), yielding the tetra-ether **2** as a colourless oil (4.77 g, 67%). R_f 0.19 (hexane/EtOAc 8:2). $[\alpha]^{24}_D$=+96.0 (*c* 1.0, $CHCl_3$). ν_{max}(thin film) 3080, 2980, 2910, 2846, 1647, 1460, 1426, 1384, 1349, 1194, 1153, 1083, 1050, 980, 924, 770, 720, 670, 560 cm^{-1}. MS (FAB): 377 $(M+Na)^+$, 355 $(M+H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.37 (s, 3H, CH_3), 3.38 (dd, 1H, $J_{2,3}$=9.0 Hz, H-2), 3.45 (dd, 1H, $J_{3,4}$=9.2 Hz, H-4), 3.62 (3H, m, H-5, H-6, H-6′) 3.68 (t, 1H, $J_{3,4}$=9.2 Hz, H-3), 3.97–4.34 (m, 8H, 4×OC*H*$_2$CHCH$_2$), 4.74 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.10–5.28 (m, 8H, J_{gem}=1.2 Hz, 4×OCH$_2$CHC*H*$_2$), 5.87–5.93 (m, 4H, J_{vic}=5.6 Hz, 4×OCH$_2$C*H*CH$_2$). ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 55.0 (OCH_3), 66.7, 70.0, 72.5, 73.8, 74.2 (allyl-C-1), 77.5, 79.5, 81.5, 98.3 (C-1), 116.2, 116.5, 117.0, 117.4 (allyl-C-3), 134.7, 134.9, 135.0 (allyl-C-2). Anal. Calcd for $C_{19}H_{30}O_6$: C, 64.38; H, 8.53. Found: C, 64.21; H, 8.22.

4.1.2. Methyl 2,3,4,6-tetra-*O*-(3-hydroxypropyl)-α-D-glucopyranoside (3). 9-BBN (0.5 M solution in THF, 70 mL, 35 mmol) was added to a stirred solution of **2** (1.03 g, 2.9 mmol) in dry THF (25 mL), then the reaction was heated under reflux for 6 h. The excess 9-BBN was destroyed by the dropwise addition of water (3.0 mL) at 0°C. The hydroboration mixture was oxidised by the dropwise addition of 3 M NaOH (aq., 36 mL) and 30% H_2O_2 (36 mL) at 0°C, followed by stirring overnight at room temperature. The aqueous phase was saturated with K_2CO_3 then the THF phase was separated. The aqueous phase was extracted with THF (2×50 mL), then the combined THF layers were dried over $MgSO_4$, filtered, and concentrated. The oily residue was purified by column chromatography (9:1→8:2 $CHCl_3$/MeOH) to yield the tetraol **3** as a colourless oil (0.86 g, 70%). R_f 0.26 ($CHCl_3$/MeOH 8:2). ν_{max}($CHCl_3$) 3630, 2960, 2800, 1650, 1460, 1430, 1380, 1350, 1050, 980, 940, 770, 720, 670, 560 cm^{-1}. MS(FAB): 449 $(M+Na)^+$, 427 $(M+H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.77–1.82 (m, 8H, 4×OCH$_2$C*H*$_2$CH$_2$OH), 3.24 (dd, 1H, $J_{4,5}$=9.2 Hz, H-4), 3.28 (dd, 1H, H-2), 3.38 (s, 3H, OCH_3), 3.48 (1H, t, $J_{3,4}$=9.5 Hz, H-3), 3.52–3.74 (m, 20H, 4×OCH$_2$CH$_2$-C*H*$_2$O*H*), 3.80 (m, 1H, H-6), 3.82–3.87 (m, 2H, H-5, H-6′), 4.80 (1H, d, $J_{1,2}$=3.5 Hz, H-1). Anal. Calcd for $C_{19}H_{38}O_{10}$: C, 53.51; H, 9.00. Found: C, 53.60; H, 8.72.

4.1.3. Methyl 2,3,4,6-tetra-*O*-3-phthalimidopropyl-α-D-glucopyranoside (4). A solution of diethyl azodicarboxylate (DEAD) (0.93 mL, 5.9 mmol) in dry THF (5 mL) was added dropwise to a solution of the tetraol **3** (0.48 g, 1.13 mmol), phthalimide (0.93 g, 6.30 mmol), and triphenylphosphine (1.57 g, 6.0 mmol) in dry THF (40 mL) and the reaction was stirred at room temperature for 72 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (50 mL) and was washed with brine, then dried

($MgSO_4$), filtered and concentrated. Purification of the residue by flash chromatography (EtOAc/hexane 8:2) afforded the tetraphthalimide **4** (1.00 g, 94%) as a colourless oil. R_f 0.28 (EtOAc/hexane 7:3). $[\alpha]^{24}_D$=+28.5 (*c* 1.0, $CHCl_3$). ν_{max}($CHCl_3$) 3020, 1780, 1716, 1420, 1380, 1216, 1060, 930, 760, 669, 540 cm^{-1}. MS (FAB): 966 $(M+H+Na)^+$, 943 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 1.91–1.98 (m, 8H, 4×$OCH_2CH_2CH_2N$), 3.06–3.11 (m, 2H, H-4, H-2), 3.29 (s, 3H, OCH_3), 3.43 (t, 1H, $J_{3,4}$=9.5 Hz, H-3), 3.46–3.63 (m, 8H, 4×$OCH_2CH_2CH_2N$), 3.65–3.92 (m, 11H, 4×$OCH_2CH_2CH_2N$, H-5, H-6, H-6′), 4.70 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 7.45–7.80 (16H, m, 4×ArH). ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 28.8, 29.3, 29.4, 29.6 ($OCH_2CH_2CH_2N$), 35.3, 35.7, 35.8 ($OCH_2CH_2CH_2N$), 54.9 (OCH_3), 68.7, 69.2, 69.8, 70.0, 70.6, 71.0, 76.5 ($OCH_2CH_2CH_2N$, C-5, C-6), 78.24 (C-4), 80.8 (C-2), 81.9 (C-3), 97.7 (C-1), 123.0, 123.1, 131.9, 132.0, 132.2, 132.4, 133.7, 133.8 (ArC), 168.2 (CON). Anal. Calcd for $C_{51}H_{50}O_{14}N_4$: C, 64.96; H, 5.35. Found: C, 64.68; H, 5.42.

4.1.4. 1-*O*-Acetyl-2,3,4,6-tetra-*O*-3-phthalimidopropyl-D-glucopyranose (5). A solution of the tetraphthalimide **4** (1.00 g, 1.06 mmol) in acetic anhydride (10 mL) was stirred at −20°C for 10 min. To this stirred solution was added precooled (0°C) Ac_2O/H_2SO_4 (50:1, 5 mL), dropwise during 5 min, then the reaction mixture was left at −20°C for 3 days. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and was washed successively with saturated $NaHCO_3$ (50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated, then co-distilled with several portions of toluene. The residue was purified by flash chromatography (EtOAc/hexane 7:3) to yield the acetate **5** as a colourless oil (0.80 g, 78%). R_f 0.19 (EtOAc/hexane 7:3). ν_{max}($CHCl_3$) 3020, 1780, 1750, 1716, 1380, 1240, 1080, 950, 760, 670, 550 cm^{-1}. MS (FAB): 1104 $(M+Cs)^+$, 994 $(M+Na)^+$. ^1H NMR (500 MHz, $CDCl_3$) (α-anomer only): δ 1.91–1.95 (m, 8H, 4×$OCH_2CH_2CH_2N$), 2.10 (s, 3H, OAc), 3.14–3.19 (2H, m, H-4, H-2), 3.40 (m, 1H, H-3), 3.45 (m, 1H, H-6), 3.51–3.79 (m, 17H, H-6′, 4×$OCH_2CH_2CH_2N$, 3.82–3.90 (m, 1H, H-5), 6.12 (1H, d, $J_{1,2}$=3.5 Hz, H-1), 7.45–7.80 (m, 16H, 4×ArH). Integration of the signals of the H-1 protons indicated that the α/β ratio was 7:1. ^{13}C NMR (62.9 Hz, $CDCl_3$) (α-anomer only): δ 21.0 (Ac–C-1) 28.8, 29.2, 29.4, 29.5 ($OCH_2CH_2CH_2N$), 35.3, 35.6 ($OCH_2CH_2CH_2N$), 68.5, 69.3, 69.3, 70.9, 71.1, 72.8, 76.5 ($OCH_2CH_2CH_2N$, C-5, C-6), 77.5 (C-4), 79.7 (C-2), 81.6 (C-3), 89.6 (C-1), 123.0, 123.1, 131.9, 132.0, 132.2, 132.3, 133.7, 133.7 (ArC), 168.2 (CON). Anal. Calcd for $C_{52}H_{50}O_{15}N_4$: C, 64.32; H, 5.19. Found: C, 64.41; H, 5.22.

4.1.5. 2,3,4,6-Tetra-*O*-3-phthalimidopropyl-D-glucopyranosyl azide (6). A solution of the acetate **5** (0.44 g, 0.45 mmol) in dry CH_2Cl_2 (20 mL) was stirred with azidotrimethylsilane (0.15 mL, 1.13 mmol) and tin(IV) chloride (0.026 mL, 0.23 mmol) for 1 day. The solution was diluted with CH_2Cl_2 (20 mL) and washed with 1 M KF solution (10 mL) then with water (10 mL). The organic extract was dried ($MgSO_4$), filtered, and concentrated to afford a white foam (0.36 g, 83%). R_f 0.30 (EtOAc/hexane 7:3). $[\alpha]^{24}_D$=+51.8 (*c* 1.0, $CHCl_3$). ν_{max}($CHCl_3$) 3030, 2120, 1780, 1713, 1360, 1240, 1080, 950, 760, 670, 550 cm^{-1}. MS(FAB): 977 $(M+Na)^+$, 955 $(M+1)^+$. ^1H NMR (500 MHz, $CDCl_3$) (α-anomer only): δ 1.89–1.97 (m, 8H, 4×$OCH_2CH_2CH_2N$), 3.06–3.15 (m, 2H, H-2, H-4), 3.29 (t, 1H, $J_{2,3}$=9.0 Hz, H-3), 3.44–3.87 (m, 19H, H-5, H-6, H-6′, 4×$OCH_2CH_2CH_2N$), 5.36 (1H, d, $J_{1,2}$=3.5 Hz, H-1), 7.45–7.80 (m, 16H, 4×ArH). Integration of the signals of the H-1 protons indicated that the α/β ratio was 2:1. Anal. Calcd for $C_{50}H_{47}O_{13}N_7$: C, 63.47; H, 4.97. Found: C, 63.41; H, 4.88.

4.1.6. 2,3,4,6-Tetra-*O*-3-phthalimidopropyl-D-glucopyranosylamine (7). The azido sugar **6** (0.38 g, 0.4 mmol) dissolved in EtOAc (10 mL) was hydrogenated over 10% Pd/C (90 mg) catalyst for 2 days at room temperature. The catalyst was filtered off and washed with EtOAc (40 mL) and the filtrate was evaporated. The residue was purified by flash chromatography (EtOAc/Et_2O 9:1, containing 0.5% triethylamine) to give the amine **7** as a colourless foam (280 mg, 76%). ν_{max}($CHCl_3$) 3520, 3400, 3020, 1780, 1716, 1380, 1240, 1080, 950, 760, 670, 550 cm^{-1}. MS (FAB): 951 $(M+Na)^+$, 928 $(M)^+$. ^1H NMR (500 MHz, $CDCl_3$) (α-anomer only): δ 1.84–1.99 (m, 8H, 4×$OCH_2CH_2CH_2N$), 3.01–3.11 (m, 3H, H-4, H-2, H-3), 3.44–3.92 (m, 19H, H-5, H-6, H-6′, 4×$OCH_2CH_2CH_2N$), 4.95 (t, 1H, H-1), 7.45–7.80 (m, 16H, 4×ArH). Integration of the signals of the H-1 protons indicated that the α/β ratio was 2:1. ^{13}C NMR (62.9 Hz, $CDCl_3$) (α-anomer only): δ 28.7, 28.9, 29.4, 29.6 ($OCH_2CH_2CH_2N$), 35.4, 35.7 ($OCH_2CH_2CH_2N$), 69.3, 70.0, 70.2, 70.4, 70.8, 71.0, 75.6 ($OCH_2CH_2CH_2N$, C-5, C-6), 78.6 (C-4), 84.1 (C-2), 85.9 (C-3), 89.3 (C-1), 123.1, 131.88, 132.4, 133.5, 133.6, 133.7 (ArC), 166.2 (CON). Anal. Calcd for $C_{50}H_{49}O_{13}N_5$: C, 64.72; H, 5.32. Found: C, 64.41; H, 5.12.

4.1.7. 4-(2,3,4,6-Tetra-*O*-(3-phthalimidopropyl)-D-glucopyranosylamino)-4-oxobutanoic acid (8). The amino sugar **7** (140 mg, 0.15 mmol) was refluxed with succinic anhydride (17 mg, 1.7 mmol) in dry CH_2Cl_2 (5 mL) overnight. The solvent was evaporated and the residue was purified by flash chromatography ($CHCl_3$/MeOH 93:7) to give the acid **8** as a colourless foam (130 mg, 84%). R_f 0.33 ($CHCl_3$/MeOH 9:1). $[\alpha]^{24}_D$=+24.0 (*c* 1.0, $CHCl_3$). ν_{max}(KBr) 3460, 3410, 2928, 2874, 1770, 1713, 1650, 1530, 1397, 1371, 1188, 1130, 1098, 1040, 940, 880, 720, 531 cm^{-1}. MS(FAB): 1051 $(M+Na)^+$, 1029 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$) (β-anomer only): δ 1.79–1.96 (m, 8H, 4×$OCH_2CH_2CH_2N$), 2.59 (m, 2H, $HNOCCH_2$), 2.73 (m, 2H, $HOOCCH_2$), 2.96 (m, 1H, H-2), 3.19 (m, 2H, H-5, H-3), 3.32 (m, 1H, H-6), 3.41 (m, 1H, H-6′), 3.46–3.83 (m, 17H, H-4, 4×$OCH_2CH_2CH_2N$), 4.86 (t, 1H, $J_{1,2}$=8.5 Hz, H-1), 7.47 (d, 1H, NH), 7.62–7.80 (m, 16H, 4×ArH). Integration of the signals of the H-1 protons indicated that the α/β ratio was 1:2. ^{13}C NMR (62.9 Hz, $CDCl_3$) (α-anomer only): δ 28.8, 29.0, 29.4, 29.6 ($OCH_2CH_2CH_2N$), 30.7 ($NCOCH_2$) 34.9, 35.6 ($OCH_2CH_2CH_2N$), 69.0, 69.2, 69.3, 69.6, 70.3, 70.5, 70.8, 76.3 ($OCH_2CH_2CH_2N$, C-5, C-6), 79.0 (C-4), 78.2, 80.9 (C-2), 81.5 (C-3), 86.3 (C-1), 123.1, 123.2, 123.3, 123.4, 132.0, 132.1, 132.2, 133.8, 133.9, 134.0, 134.1 (ArC), 166.2, 166.37, 166.9 (CON), 173.2 (NHCO). Anal. Calcd for $C_{54}H_{53}O_{16}N_5$: C, 63.09; H, 5.20; N, 6.81. Found: C, 63.21; H, 5.31, N, 6.64.

4.1.8. 2-[2-(2-Iodoethoxy)ethoxy]tetrahydro-2*H*-pyran (9). 2-Chloroethoxyethanol (6.0 g, 48 mmol) was added to

ice-cold 3,4-dihydro-2*H*-pyran (15 mL) and the mixture was stirred for 5 h at room temperature. The excess 3,4-dihydro-2*H*-pyran was distilled off under high vacuum. The pale yellow oily residue (9.8 g; R_f 0.41 hexane/EtOAc 1:1) was added without further purification to a solution of dry sodium iodide (8.8 g, 58.8 mmol) in dry acetone (60 mL), then the mixture was heated under reflux for 24 h. The precipitated salt was filtered off and the solvent was evaporated. The residue was taken up in CH_2Cl_2 (60 mL) and the precipitate was again filtered off. The filtrate was washed with 40 mL of water, then dried ($MgSO_4$) and concentrated to give the iodide **9** as an oil (13.5 g, 82%) which was used in the next step without purification. R_f 0.30 (hexane/EtOAc 8:2). ν_{max}(thin film) 3510, 2940, 2870, 1464, 1454, 1440, 1384, 1353, 1262, 1201, 1078, 1035, 1021, 990, 870, 814, 736, 617, 430 cm^{-1}. MS(FAB): 215(M−THP)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.51–1.83 (m, 6H), 3.37(t, 2H), 3.50 (m, 1H), 3.61 (m, 1H), 3.69 (m, 5H), 3.87 (m, 1H), 4.62 (m, 1H, OCHO). ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 19.4, 25.6, 30.5, 50.7, 62.2, 66.7, 70.0, 70.6, 74.5, 99.0.

4.1.9. Methyl 2,3,4,6-tetra-*O*-[(5-(tetrahydro-2*H*-pyranyl)oxy)-3-oxapentyl]-α-D-glucopyranoside (10). Sodium hydride (60% dispersion in oil, 8.0 g, 200 mmol) was added to a stirred solution of methyl α-D-glucoside **1** (4.85 g, 25 mmol) in anhydrous DMF (125 mL) at 0°C. After stirring for 30 min, the iodide **9** (60.0 g, 200 mmol) was added dropwise at 0°C. The cooling bath was removed and the flask was immersed in an oil bath at 50°C. After stirring overnight the reaction mixture was evaporated, then the residue was dissolved in CH_2Cl_2 (300 mL) and washed with water (2×100 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated, then the oily residue was purified by flash chromatography (0→5% MeOH in $CHCl_3$) to afford the polyether **10** as a yellow oil (4.2 g, 19%). R_f 0.37 ($CHCl_3$/MeOH 95:5). ν_{max}($CHCl_3$) 3490, 3040, 2870, 1460, 1450, 1384, 1350, 1202, 1078, 1020, 990, 930, 884, 760, 680 cm^{-1}. MS(FAB): 906 (M+Na)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.54 (m, 16H, CH_2 of THP), 1.68 (m, 4H, CH_2 of THP), 1.79 (m, 4H, CH_2 of THP), 3.27 (m, 1H, H-2), 3.34 (s, 3H, CH_3), 3.42–3.85 (m, 4H), 3.51–3.76 (m, 29H), 3.78–3.85 (m, 9H), 3.90–4.07 (m, 3H), 4.59 (4H, s, CH of THP), 4.76 (1H, d, J=3.5 Hz, H-1). Anal. Calcd for $C_{43}H_{78}O_{18}$: C, 58.49; H, 8.90. Found: C, 58.11; H, 8.99.

4.1.10. Methyl 2,3,4,6-tetra-*O*-(5-hydroxy-3-oxapentyl)-α-D-glucopyranoside (11). 4-Toluenesulfonic acid monohydrate (0.23 g, 1.2 mmol) was added to a solution of the polyether **10** (4.2 g, 4.8 mmol) in $CHCl_3$ (30 mL) and MeOH (30 mL). The solution was stirred at 55°C for 2 h, then the catalyst was neutralised by the addition of triethylamine (0.2 mL) and the solvent was evaporated. The residue was purified by flash chromatography ($CHCl_3$/MeOH 9:1) to give the tetraol **11** as a colourless oil (1.8 g, 69%). R_f 0.19 ($CHCl_3$/MeOH 85:15). ν_{max}($CHCl_3$) 3620, 2960, 1460, 1450, 1380, 1360, 1222, 1080, 1020, 990, 940, 872, 750, 680, 550 cm^{-1}. MS(FAB): 679 (M+Cs)$^+$, 585 (M+K)$^+$, 569 (M+Na)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.17 (t, 1H, $J_{2,3}$=9.5 Hz, H-2), 3.26 (dd, 1H, $J_{5,6}$=6.5 Hz, H-5), 3.31 (s, 3H, OCH_3), 3.42–3.3.61 (m, 29H), 3.60 (m, 2H), 3.66 (m, 2H), 3.70 (m, 1H), 3.87 (m, 2H), 4.79 (d, 1H, J=3.4 Hz, H-1). Anal. Calcd for $C_{23}H_{46}O_{14}$: C, 50.54; H, 8.48. Found: C, 50.41; H, 8.21.

4.1.11. Methyl 2,3,4,6-tetra-*O*-(5-phthalimido-3-oxapentyl)-α-D-glucopyranoside (12). A solution of diethyl azodicarboxylate (DEAD) (2.69 mL, 17.1 mmol) in dry THF (5 mL) was added dropwise to a stirred solution of the tetraol **11** (1.8 g, 3.3 mmol), phthalimide (2.72 g, 18.5 mmol), and triphenylphosphine (4.58 g, 17.5 mmol) in dry THF (15 mL), then the reaction mixture was stirred at room temperature for 72 h. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (100 mL) and washed with brine and dried ($MgSO_4$), filtered, and concentrated. Purification of the residue by flash chromatography (EtOAc/Et_2O 9:1) afforded the product **12** as a colourless oil (3.0 g, 86%). $[\alpha]^{24}_D$=+60.4 (*c* 1.0, $CHCl_3$). ν_{max}($CHCl_3$) 3020, 1775, 1716, 1460, 1430, 1396, 1216, 1100, 1042, 756, 720, 670, 550 cm^{-1}. MS(FAB): 1085 (M+Na)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.16 (m, 2H, H-2, H-5), 3.27 (s, 3H, OCH_3), 3.42–3.48 (m, 2H, H-6,6′), 3.51–3.63 (m, 14H), 3.62–3.77 (m, 10H), 3.80–3.89 (m, 10H), 4.65 (1H, d, J=3.5 Hz, H-1), 7.44–7.83 (16H, m, 4×ArH). ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 37.3, 37.4 (CH_2N), 54.7 (OCH_3), 67.5, 67.6, 67.8, 69.5, 69.9, 70.0, 70.2, 70.4, 70.5, 70.8, 72.0, 72.2, 76.5, 77.8, 80.7, 82.1, 98.0, 123.2, 131.9, 132.0, 132.1, 132.2, 133.8, 168.1. Anal. Calcd for $C_{55}H_{58}O_{18}N_4$: C, 62.14; H, 5.50. Found: C, 62.31; H, 5.32.

4.1.12. 1-*O*-Acetyl-2,3,4,6-tetra-*O*-(5-phthalimido-3-oxapentyl)-D-glucopyranose (13). A solution of the phthalimide **12** (3.5 g, 3.3 mmol) in acetic anhydride (15 mL) was stirred at −20°C for 10 min. To this solution was added precooled (0°C) Ac_2O/H_2SO_4 (50:1, 7.5 mL) dropwise during 5 min, then the reaction mixture was left at −20°C for 3 days. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and was washed successively with cold saturated $NaHCO_3$ (100 mL) and water (2×100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated, then co-distilled with several portions of toluene. The residue was purified by flash chromatography (EtOAc/Et_2O 85:15) to give the acetate **13** as a colourless oil (2.8 g, 78%). R_f 0.34 (EtOAc/Et_2O 9:1). $[\alpha]^{24}_D$=+33.0 (*c* 1.0, $CHCl_3$). ν_{max}($CHCl_3$) 3020, 1775, 1750, 1716, 1400, 1220, 1100, 1050, 930, 760, 670, 540 cm^{-1}. MS(FAB): 1224 (M+Cs)$^+$, 1114 (M+Na)$^+$. ^{1}H NMR (500 MHz, $CDCl_3$) (α-anomer only): δ 2.02 (s, 3H, CH_3 of OAc), 3.20–3.28 (m, 2H, H-2, H-5), 3.52–3.75 (m, 24H, CH_2 and sugar protons), 3.72–3.91 (m, 12H, CH_2 and sugar protons), 6.14 (1H, d, $J_{1,2}$=3.5 Hz, H-1), 7.63–7.82 (16H, m, 4×ArH). Integration of the signals of the H-1 protons indicated that the α/β ratio was 7:1. ^{13}C NMR (62.9 Hz, $CDCl_3$) (α-anomer only): δ 21.0, 37.3, 37.4, 67.6, 67.7, 67.8, 67.9, 69.2, 69.9, 70.2, 70.4, 70.6, 70.7, 70.8, 72.3, 72.7, 76.5, 78.8, 81.7, 90.0, 123.2, 132.2, 133.9, 168.6. Anal. Calcd for $C_{56}H_{58}O_{19}N_4$: C, 61.65; H, 5.36. Found: C, 61.27; H, 5.23.

4.1.13. 2,3,4,6-Tetra-*O*-(5-phthalimido-3-oxapentyl)-D-glucopyranosyl azide (14). A solution of the acetate **13** (1.3 g, 1.2 mmol) in dry CH_2Cl_2 (30 mL) was stirred with azidotrimethylsilane (0.4 mL, 3.0 mmol) and tin(IV) chloride (0.07 mL, 0.6 mmol) for 24 h at room temperature. The solution was diluted with CH_2Cl_2 (30 mL) and washed

with 1 M KF solution (30 mL) then with water (50 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated to afford the azide **14** as a colourless foam (1.13 g, 88%). R_f 0.41 (EtOAc/Et_2O 9:1). $[\alpha]^{24}_D$=+18.6 (*c* 1.0, $CHCl_3$). ν_{max}($CHCl_3$) 3020, 2120, 1775, 1716, 1380, 1220, 1090, 940, 760, 670, 540 cm^{-1}. MS(FAB): 1207 $(M+Cs)^+$, 1097 $(M+Na)^+$. ^1H NMR (500 MHz, $CDCl_3$) (α-anomer only): δ 3.25 (2H, m, H-2, H-5), 3.47–3.69 (24H, m, CH_2 and sugar protons), 3.70–3.88 (12H, m, CH_2 and sugar protons), 5.94 (1H, d, *J*=3.5 Hz, H-1), 7.54–7.80 (16H, m, 4×ArH). Integration of the signals of the H-1 protons indicated that the α/β ratio was 2:1. ^{13}C NMR (62.9 Hz, $CDCl_3$) (α-anomer only): δ 37.4, 67.9, 68.0, 69.2, 69.5, 70.0, 70.3, 70.5, 70.7, 71.00, 71.4, 72.0, 72.3, 72.5, 76.5, 77.5, 80.8, 81.9, 85.0, 87.8, 91.0, 123.2, 132.2, 133.7, 166.1. Anal. Calcd for $C_{54}H_{55}O_{17}N_7$: C, 60.39; H, 5.16. Found: C, 60.52; H, 5.01.

4.1.14. 2,3,4,6-Tetra-*O*-(5-phthalimido-3-oxapentyl)-D-glucopyranosylamine (15). The azido sugar **14** (110 mg, 0.102 mmol) dissolved in EtOAc (10 mL) was hydrogenated using 10% Pd/C catalyst (60 mg) for 2 days at room temperature. The catalyst was filtered off and was washed with EtOAc (40 mL), then the filtrate was evaporated. The residue was purified by flash chromatography ($CHCl_3$/MeOH 97:3) to give the amine **15** as a colourless foam (80 mg, 76%). R_f 0.20 ($CHCl_3$/MeOH 95:5). ν_{max}($CHCl_3$) 3500, 3410, 3020, 1775, 1720, 1380, 1220, 1090, 950, 770, 670, 540 cm^{-1}. MS(FAB): 1071 $(M+Na)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 3.12–3.21 (m, 2H, H-2, H-5), 3.47–3.93 (m, 38H), 5.21 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 7.68–7.81 (m, 16H, 4×ArH). ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 37.4, 67.6, 67.8, 69.7, 70.0, 70.2 70.6, 71.7, 71.9, 72.1, 72.2, 75.5, 75.7, 76.5, 77.0, 77.9, 79.6, 81.9, 82.3, 83.9, 85.7, 86.0, 86.4, 89.1, 91.0, 123.2, 132.2, 133.9, 168.1. Anal. Calcd for $C_{54}H_{57}O_{17}N_5$: C, 61.68; H, 5.48. Found: C, 60.31; H, 5.29.

4.1.15. 4-[2,3,4,6-Tetra-*O*-(5-phthalimido-3-oxapentyl)-D-glucopyranosylamino]-4-oxobutanoic acid (16). The amino sugar **15** (45 mg, 0.04 mmol) was refluxed with succinic anhydride (5.4 mg, 0.05 mmol) in dry CH_2Cl_2 (5 mL) overnight. The solvent was evaporated and the residue was purified by chromatography ($CHCl_3$/MeOH 95:5) to give the acid **16** as a colourless foam (47 mg, 84%). R_f 0.12 ($CHCl_3$/MeOH 95:5). ν_{max}(KBr) 3470, 3400, 2980, 1778, 1716, 1660, 1530, 1460, 1380, 1240, 1080, 1040, 966, 825, 740, 670, 540, 440 cm^{-1}. MS (FAB): 1171 $(M+Na)^+$, 1149 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 2.59 (m, 2H, HNOCCH_2), 2.73 (m, 2H, HOOCCH_2), 3.10–3.18 (m, 2H, H-2, H-5), 3.44–3.90 (m, 37H), 5.20 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 7.67–7.80 (m, 16H, 4×ArH). ^{13}C NMR (62.9 Hz, $CDCl_3$): δ 30.8, 31.2, 37.4, 67.6, 67.8, 69.7, 70.0, 70.2 70.6, 71.7, 71.9, 72.1, 72.2, 75.5, 75.7, 76.5, 77.0, 77.9, 79.6, 81.9, 82.3, 83.9, 85.7, 86.0, 86.4, 89.1, 91.0, 123.2, 132.2, 133.9, 168.1. Anal. Calcd for $C_{58}H_{61}O_{20}N_5$: C, 60.68; H, 5.35. Found: C, 60.41; H, 5.22.

4.1.16. 2,3,4,6-Tetra-*O*-(cyanoethyl)-β-D-galactopyranosyl azide (18). Acrylonitrile (6.4 mL, 5.2 g, 97 mmol) was added to a stirred suspension of the galactosyl azide[13] **17** (2.00 g, 9.75 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.60 mL, 0.60 g, 3.9 mmol) in acetonitrile (50 mL). After stirring vigorously for 3 h a clear, pale yellow solution had formed and after a further 1 h more acrylonitrile (6.4 mL) and DBU (0.60 mL) were added. After stirring overnight the solution was evaporated and the residue was purified by flash chromatography (50–75% EtOAc/hexane) to give the tetranitrile **18** as a colourless oil, 2.61 g (64%). R_f 0.69 (EtOAc). $[\alpha]^{24}_D$=−19.1 (*c* 1.1, $CHCl_3$). ν_{max}(thin film) 2251, 2118, 1117 cm^{-1}. ES-MS: 418 $(MH)^+$, 435 $(M+NH_4)^+$, 440 $(M+Na)^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 2.59–2.66 (m, 8H, 4×CH_2CN), 3.38 (dd, 1H, H-3, *J*=2.6, 9.6 Hz), 3.45 (dd, 1H, H-2, *J*=8.0, 9.6 Hz), 3.61–4.26 (m, 12H, 5×CH_2O and 2×CHO), 4.58 (d, 1H, H-1, *J*=8.0 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.9, 19.2, 19.2, 19.4, 65.9, 66.5, 67.3, 67.8, 68.2, 74.5, 74.7, 78.9, 82.4, 90.0, 117.9, 118.0, 118.1, 118.4. HRMS (TOF) calcd for $C_{18}H_{24}N_7O_5$ $(M+H)^+$ 418.1839, found 418.1865.

4.1.17. 6-(2,3,4,6-Tetra-*O*-(2-cyanoethyl)-β-D-galactopyranosylamino)-6-oxohexanoic acid, benzyl ester (19). A solution of the tetranitrile azide **18** (1.22 g, 2.92 mmol) in THF (75 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (100 mg) for 2 h, at which time TLC indicated the complete consumption of starting material. The atmosphere was replaced with argon, and adipic acid, mono benzyl ester (0.76 g, 3.2 mmol), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexaflurophosphate (HBTU) (1.16 g, 3.07 mmol) and *N*,*N*-diisopropylethylamine (610 μL, 450 mg, 3.5 mmol) were added. After stirring overnight the mixture was filtered through Celite® and the filter pad was washed well with methanol. The filtrate was evaporated and the residue was dissolved in EtOAc (200 mL) and washed with 5% HCl (3×100 mL), saturated $NaHCO_3$ (2×100 mL) and brine (50 mL) then dried ($MgSO_4$) and evaporated to a pale yellow syrup. This was purified by flash chromatography (EtOAc) to give the α-anomer benzyl ester **19** as a colourless oil (0.98 g, 55%). R_f 0.39 (EtOAc). ν_{max}(thin film) 2251, 1732, 1670, 1541, 1115 cm^{-1}. ES-MS: 610 $(M+H)^+$, 627 $(M+NH_4)^+$, 632 $(M+Na)^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 1.62–1.68 (m, 4H, CH_2CH_2), 2.23–2.28 (m, 2H, CH_2CON), 2.36 (t, 2H, CH_2CO_2, *J*=6.9 Hz), 2.51–2.65 (m, 8H, 4×CH_2CN), 3.47 (dd, 1H, $H6_A$, *J*=2.4, 9.4 Hz), 3.57, (br d, 1H, $H6_B$, *J*=9.0 Hz), 3.65–3.98 (m, 12H, 4×CH_2O and 4×CHO) 5.04 (t, 1H, H-1, *J*=9.2 Hz), 5.09 (s, 2H, CH_2OPh), 6.84 (d, 1H, NH, *J*=9.3 Hz), 7.33 (br s, 5H, 5×Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.8, 19.2, 19.3, 19.4, 24.2, 24.5, 33.7, 35.9, 65.7, 65.8, 66.2, 67.5, 68.3, 73.4, 74.5, 78.3, 79.0, 83.5, 118.1, 118.2, 118.6, 118.7, 128.0, 128.2, 128.5, 135.9, 173.3, 173.7. HRMS (TOF) calcd for $C_{31}H_{40}N_5O_8$ $(M+H)^+$ 610.2875, found 610.2877.

4.1.18. 6-(2,3,4,6-Tetra-*O*-(3-*tert*-butoxycarbonylaminopropyl)-β-D-galactopyranosylamino)-6-oxohexanoic acid (20). Sodium borohydride (2.26 g, 59.7 mmol) was added cautiously and portionwise to a stirred, chilled (0°C) solution of the tetranitrile **19** (910 mg, 1.49 mmol), $CoCl_2 \cdot 6H_2O$ (2.84 g, 11.9 mmol) and di-*tert*-butyl dicarbonate (1.63 g, 7.48 mmol) in MeOH (40 mL). The ice-bath was removed and stirring was continued for 4 h. Water (10 mL) was added, then the pH was raised to 13 by the addition of $LiOH \cdot H_2O$. After stirring overnight the MeOH was evaporated and the residue was diluted with water (100 mL) and

filtered through a pad of Celite®. The filtrate was washed with Et_2O (20 mL), then $CHCl_3$ (200 mL) was added and the pH of the aqueous phase was lowered to 2 with 5% HCl. The layers were separated and the organic phase was evaporated. The residue was dissolved in a little 10% MeOH in $CHCl_3$ and filtered through a plug of silica. Evaporation of the filtrate gave the product **20** as a colourless foam, 640 mg (46%). R_f 0.54 (5% MeOH in EtOAc). $[\alpha]^{24}_D$=+7.0 (*c* 1.0, $CHCl_3$). ν_{max}(thin film) 3348, 1693, 1521 cm^{-1}. ES-MS: 959 $(M+Na)^+$, 937 $(MH)^+$, 837 $(MH-BOC)^+$. ^1H NMR (300 MHz) δ 1.40 (br s, 36H, 4×CMe_3), 1.50–1.80 (m, 12H, 6×CH_2), 2.25–2.35 (m, 4H, 2×CH_2CO), 3.00–3.75 (m, 22H, 4×CH_2N, 5×CH_2O and 4×CHO), 4.67 (br s, 1H, H-1), 4.90–5.10 (m, 5H, 5×NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1, 24.2, 24.5, 28.3, 29.5, 29.6, 29.7, 30.1, 33.5, 33.6, 35.7, 35.8, 37.4, 37.8, 37.9, 38.5, 64.7, 68.2, 68.4, 69.2, 69.9, 70.2, 70.4, 73.7, 73.8, 78.8, 78.9, 79.2, 156.0, 156.1, 156.4, 156.6, 173.9, 176.6. HRMS (TOF) calcd for $C_{44}H_{82}N_5O_{16}$ $(M+H)^+$ 936.5757, found 936.5742.

4.2. Protocol for loading compounds 8 and 16 onto Wang resin

FMOC-Gly-Wang resin (100 mg, 0.069 mmol) was swollen in DMF in a solid phase peptide synthesis reaction vessel for 3 h. The FMOC protecting group was removed with 20% piperidine in DMF (10 mL) by shaking the resin twice (2×10 min). The resin was thoroughly washed with DMF, then 1.5 molar eqvivalents (0.10 mmol) of either **8** or **16** was added together with 2.0 equiv. of HBTU (52 mg, 0.138 mmol), hydroxybenzotriazole (21 mg, 0.138 mmol) and *N*,*N*-diisopropylethylamine (48 μL, 0.28 mmol) in the minimum amount of DMF. The vessel was shaken for 1 h, then the process was repeated with the same amount of either compound **8** or **16**, and the coupling reagents, resulting in a negative ninhydrin test. The resin was then washed with DMF several times, giving either compound **21** or **22**, respectively.

4.2.1. Protocol for phthaloyl deprotection of 21 and 22, and for cleaving 23 and 24 from the solid support. Resin-bound glucosides **21** and **22** were treated with hydrazine hydrate (67 μL, 1.38 mmol) in ethanol (5 mL) at 60°C for 1 day. To check the efficiency of the deprotection, a sample of the resin was thoroughly washed with DMF, then with CH_2Cl_2/MeOH, and finally with CH_2Cl_2. The resin was dried and the glucosides **23** and **24** were cleaved from the resin by stirring the resin with TFA/water/triisopropylsilane (95:2.5:2.5) for 2 h. The resin was filtered off and washed with TFA. In both cases the solvent was evaporated and the residue was lyophilised from water/acetonitrile, then purified by flash chromatography ($CHCl_3$/MeOH 9:1). Analytical samples were obtained by trituration with diethyl ether. MS(FAB); **23**: 587 $(M+Na)^+$, 565 $(M+H)^+$; **24**:707 $(M+Na)^+$, 685 $(M+H)^+$. Anal. Calcd for $C_{24}H_{48}O_9N_6$ (**23**): C, 51.05; H, 8.56; N, 14.88. Found: C, 50.78; H, 8.22, N; 14.50. Calcd for $C_{28}H_{56}O_{13}N_6$ (**24**): C, 49.11; H, 8.24; N, 12.27. Found: C, 49.41; H, 8.07; N, 12.36.

4.3. Protocol for loading compound 20 onto MBHA resin

MBHA resin (431 mg, 0.289 mmol) was swollen in DMF in a solid phase peptide synthesis reaction vessel for 30 min. The resin was washed with 10% *N*,*N*-diisopropylamine in DMF by shaking the resin for 2×1 min. The resin was thoroughly washed with DMF, then the resin was shaken for 10 min with 2-(*tert*-butoxycarbonylamino)dodecanoic acid (364 mg, 1.16 mmol, 4.0 equiv.), HBTU (2.4 mL of a 0.5 M solution in DMF) and DIPEA (255 μL, 189 mg, 1.46 mmol), resulting in a negative ninhydrin test. After rinsing the resin well with DMF, the BOC-group was removed by treatment with neat TFA (2×1 min), then the resin was again washed well with DMF. Compound **20** (352 mg, 0.577 mmol) was coupled to the resin using the same protocol. The four BOC groups were removed by treatment with neat TFA (2×1 min), and the resin was washed well with DMF, then MeOH and finally CH_2Cl_2, then dried under vacuum to give 570 mg of loaded resin (**25**).

4.3.1. Synthesis of compound 26. The loaded resin **25** (285 mg) was swelled in DMF for 30 min, then coupled with acetic acid using the protocol described above. The resin was washed successively with DMF, MeOH and CH_2Cl_2, dried under vacuum, then cleaved using anhydrous hydrofluoric acid with 10% cresol as scavenger at 0°C for 1 h. The product was precipitated with diethyl ether, then lyophilised from water/acetonitrile to give the tetraacetamide **26** as a white powder (37 mg, 28%). ES-MS: 901 $(M+H)^+$, 802, 451 $(M+2H)^{2+}$. ^1H NMR (300 MHz, $(CD_3)_2SO/CDCl_3$) δ 0.67 (t, 3H, CH_3, *J*=6.8 Hz), 1.04 (br s, 16H, 7×CH_2), 1.43–1.67 (br m, 14H, 7×CH_2), 1.75 (br s, 12H, 4×Ac), 2.04 (br s, 4H, 2×CH_2CO), 2.75–4.12 (m, 22H, H_α, 4×CH_2N, 3×OCH and 5×OCH_2), 6.12 (br s, 1H, H-1), 6.95–7.74 (m, 8H, 6×NH and NH_2). HRMS (TOF) calcd for $C_{44}H_{82}N_7O_{12}$ $(M+H)^+$ 900.6022, found 900.6014.

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References

1. Olive, C.; Toth, I.; Jackson, D. *Mini Rev. Med. Chem.* **2001**, 23 (in press).
2. Tam, J. P. *Proc. Natl Acad. Sci. USA* **1988**, *85*, 5409–5413.
3. Wiesmuller, K. H.; Bessler, W.; Jung, G. *Hoppe Seylers Z. Physiol. Chem.* **1983**, *364*, 593–606.
4. Deres, K.; Schild, H.; Wiesmuller, K. H.; Jung, G.; Rammensee, H. G. *Nature* **1989**, *342*, 561–564.
5. Toth, I.; Danton, M.; Flinn, N.; Gibbons, W. A. *Tetrahedron Lett.* **1993**, *34*, 3924–3925.
6. Zong, G.; Toth, I.; Reid, R.; Brunham, R. C. *J. Immunol.* **1993**, *151*, 3728–3736.
7. Toth, I. *J. Drug Target.* **1994**, *2*, 217–239.
8. Roy, T. *Top. Curr. Chem.* **1997**, *187*, 241–274.
9. Falconer, R. A.; Jablonkai, I.; Toth, I. *Tetrahedron Lett.* **1999**, *40*, 8663–8666.
10. Malkinson, J. P.; Falconer, R. A.; Toth, I. *J. Org. Chem.* **2000**, *65*, 5249–5252.

11. Dubber, M.; Lindhorst, T. K. *Carbohydr. Res.* **1998**, *310*, 35–41.
12. Boekman, R. K.; Estep, K. G.; Nelson, S. G. *Tetrahedron Lett.* **1991**, *32*, 4095–4098.
13. Broeder, W.; Horst, K. *Bioorg. Med. Chem.* **1997**, *5*, 1–20.
14. Bazin, H.; Bouchu, A.; Descotes, G. *J. Carbohydr. Chem.* **1995**, *14*, 1187–1207.
15. Satoh, T.; Suzuki, S.; Suzuki, Y.; Miyaji, Y.; Imai, Z. *Tetrahedron Lett.* **1969**, *10*, 4555–4558.