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Design, synthesis and biological evaluation of novel lipoamino acid-based glycolipids for oral drug delivery

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Abstract—A series of lipoamino acid-based glycolipids were synthesised. Suitably derivatised lipoamino acid derivatives were prepared and conjugated to monosaccharides (including glycosyl azides, isothiocyanates, thiols and sulphones) to yield novel *O*-, *N*-, *S*- and *C*-linked glycolipids in good yields. Their potential to improve the oral absorption of piperacillin is reported.

1. Introduction

The successful development of any medicinal compound relies on specific and potent pharmacological activity combined with efficient delivery of the molecule to its target site. Many potential drugs and medicinal peptides fail to reach the marketplace due to poor bioavailability and factors such as molecular size, charge, hydrophilicity, hydrogen bonding potential and enzymatic lability. Their potential therefore rests on the design of effective and stable drug delivery systems. Formulation is one means by which the absorption of a drug can be enhanced, using surfactants, penetration enhancers or through ion pairing.[1,2] Chemical modification is another means, although a compromise between introducing and modifying structural features of a drug moiety that optimises biopharmaceutical properties (e.g., membrane permeability and metabolism) and those that optimise pharmacological activity (e.g., enzyme or receptor binding) must be sought.

This paper describes the design and synthesis of a series of glycolipids, with varying degrees of chemical and biological stability, dictated by the nature of the glycosidic linkage. The molecules have surfactant properties, and can be used as ion-pairing agents. Ion pairs are neutral species, created by the interaction between ions in solution. The resulting ion pair will often have significantly different absorption characteristics, for example allowing dissolution in non-aqueous media.[2] This concept has been extensively studied.[3,4] We demonstrate the potential of such compounds to improve the oral absorption of piperacillin, a beta-lactam antibacterial agent with low oral bioavailability.

Piperacillin (Fig. 1) is a broad spectrum antibacterial agent, but has significant potency against *Pseudomonas aeruginosa*. It is used in the treatment of septicaemias and other serious infections.[5] Due to poor oral bioavailability, it must be administered parenterally as the sodium salt. Methods by which to improve the oral absorption or membrane permeability of piperacillin are therefore of interest to overcome this disadvantage.

2. Compound design

Our objective was the design and synthesis of a series of lipoamino acid-derived glycolipids constructed with glycosidic linkages which would afford variation in lipophilicity and stability in chemical and biological

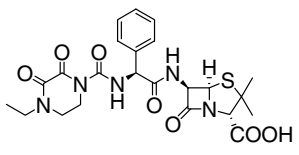

Figure 1. Piperacillin structure.

Keywords: Drug delivery; Glycolipid; Lipoamino acid; Liposaccharide.
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systems. We therefore synthesised novel *O*-, *N*-, *S*- and *C*-linked isosteres of glucose and 2-aminododecanoic acid-based conjugates, affording a series of compounds with diverse physicochemical properties (as exemplified by their calculated log*P* values). This builds on the amide-linked glycolipids we have described previously.[6,7] The synthesis of the sugar donors and lipoamino-acid derivatives is outlined below.

2.1. Synthesis of lipoamino acid derivatives

The lipoamino acids (LAAs) are α-amino acids with long alkyl side chains[8] (e.g., **1**, Scheme 1). They combine the structural properties of lipids (highly hydrophobic side chains) with those of α-amino acids (polar amino and carboxyl functionalities). Their bi-functionality makes LAAs suitable candidates to be chemically conjugated to drugs with a wide range of functional groups and ideal molecules to incorporate into peptide structures.[9] The degree of lipophilicity can be tailored to a particular drug/peptide by varying the length of the alkyl side chain(s) and/or the number of LAAs. Conjugation of one or more LAAs to a molecule will enhance its membrane-like character and may confer a degree of protection from enzymatic degradation.[10] LAA derivatisation has proved to be a successful strategy to enhance the oral absorption of a wide range of therapeutic agents and therapeutic peptides (e.g., morphine,[11] penicillins,[12] enkephalin[13,14]).

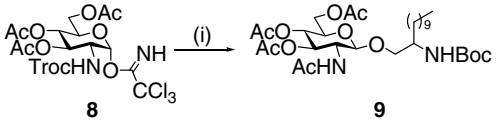
The glycolipids to be produced necessitated synthesis of a range of suitably derivatised LAAs (Scheme 1). A range of LAA derivatives were prepared from the parent Boc-protected derivative **2**. The acid is reduced smoothly, in a modification of the previously published procedure,[15] in quantitative yield to the lipoamino alcohol **3** using borane. Nitrogen was introduced using a Mitsunobu reaction[16] with tetrachlorophthalimide. Subsequent treatment of **4** with ethylenediamine gave amine derivative **5**. Sulphur was introduced through a modified Mitsunobu reaction[17] using 1,1-(azodicarbonyl)dipiperidine and trimethylphosphine (in place of the traditional triphenylphosphine and diethylazodicarboxylate) and thiolacetic acid to yield **6**. These conditions greatly facilitate purification. Thiol **7** was produced following acetate de-protection.

2.2. Synthesis of *O*-glycolipids

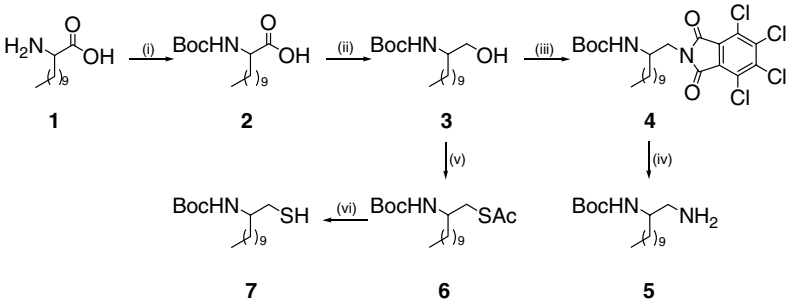
Attempts to synthesise *O*-linked glycosides directly from per-*O*-acetylated sugar donors and lipoamino alcohol **3** were unsuccessful, due to poor reactivity. The trichloroaetimidate procedure of Schmidt[18] was therefore employed, using *N*-acetyl glucosamine in place of simple sugars, to eliminate potential acetate migration at the anomeric position in the early stages of synthesis. Significant oxazoline formation prompted us to adopt the use of trichloroethoxycarbonyl (troc) protected derivative **8**.[19] Reaction with lipoamino alcohol **3** successfully afforded *O*-linked glycolipid **9**, albeit in moderate yield (see Scheme 2).

2.3. Synthesis of *N*-glycolipids

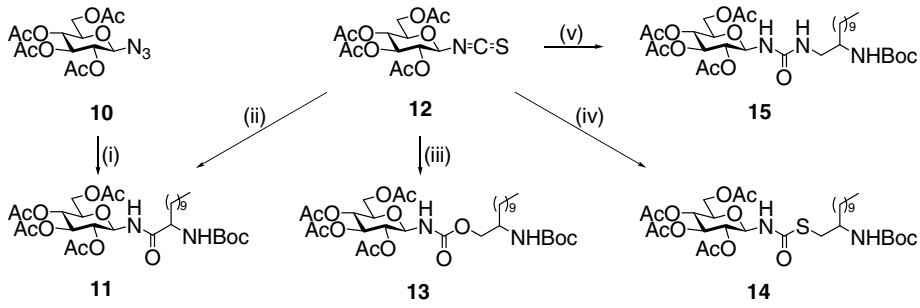
N-linked glycolipids were synthesised via two routes (Scheme 3) in a departure from our previous work.[6,7] The first was through the glycosyl azide, which could be reacted directly in a one-pot modified Staudinger-type procedure with LAA derivative **2** to produce amide-linked glycolipid **13**. This reaction is thought to proceed through an intermediate iminophosphorane, formed by reaction of the azide with tri-*n*-butylphosphine.[20] The second route was via the highly reactive glycosyl isothiocyanate. This afforded greater scope to produce a diverse range of chemical linkages, depending on the nature of the reacting LAA derivative. Isothiocyanates produce amides on reaction with a carboxylic acid, thioureas on reaction with an amine, thiocarbamates on reaction with an alcohol and dithiocarbonates on reaction with a thiol. These types of derivative were all prepared in excellent yields, from glucosyl isothiocyanate **12**[21] and the respective LAA derivative. The glycosyl isocyanates were found to be too reactive and difficult to handle. Reaction of glucosyl isothiocyanate



Scheme 2. Reagents and condition: (i) a—**3** (0.7 equiv), BF_3OEt_2 (3.0 equiv), CH_2Cl_2, 2 h, 0 °C; b—Ac_2O, Zn, 6 h, rt.



Scheme 1. Reagents and conditions: (i) Boc_2O (1.25 equiv), 48 h, rt; (ii) Borane-THF complex (2.0 equiv), 2 h, 0 °C; (iii) DIAD (1.3 equiv), PPh_3 (1.3 equiv), tetrachlorophthalimide (1.4 equiv), THF, 72 h, rt; (iv) ethylenediamine (0.5 equiv), 1 h, rt; (v) ADDP (2.0 equiv), PMe_3 (2.0 equiv), imidazole (2.0 equiv), thiolacetic acid (2.0 equiv), THF, 18 h, rt; (vi) NH_3/MeOH, 1 h, rt.



Scheme 3. Reagents and conditions: (i) PBu_3 (1.5 equiv), **2** (2.0 equiv), CH_2Cl_2, 2 h, rt; (ii) **2** (1.2 equiv), triethylamine (0.2 equiv), toluene, 12 h, reflux; (iii) **3** (1.25 equiv), triethylamine (0.2 equiv), toluene, 12 h, reflux; (iv) **7** (1.2 equiv), triethylamine (0.2 equiv), CH_2Cl_2, 20 min, rt; (v) **5** (1.5 equiv), triethylamine (0.2 equiv), CH_2Cl_2, 1 h, rt.

Table 1. Physicochemical properties of *N*-linked LAA-based glycolipids

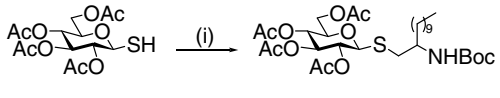
Compound	Linkage	Clog *P*[a]
11	N (amide)	1.33
13	N (thiocarbamate)	2.40
14	N (dithiocarbonate)	2.74
15	N (thiourea)	2.08

[a] Refers to completely de-protected compound.

12 with LAA derivatives **2** and **3** requires more forcing conditions to produce the respective amide **11** and thiocarbamate **13**. However, reaction of isothiocyanate **12** with thiol **7** and amine **5**, to yield dithiocarbonate **14** and thiourea **15**, respectively, proceeds very rapidly at room temperature. The synthesised compounds have differing physicochemical properties (as indicated in Table 1) and are likely to differ in their stability in biological systems.

2.4. Synthesis of *S*-glycolipids

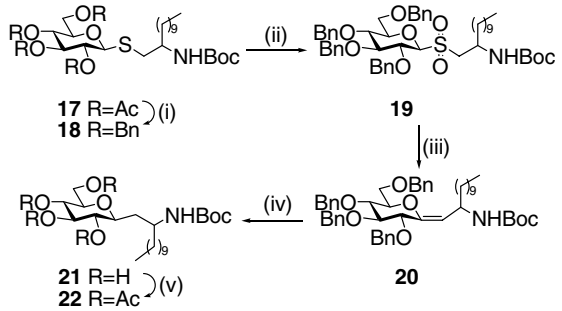
The rationale behind the synthesis of glycolipids linked through a sulphur atom is that, in addition to the increased lipophilicity, there is evidence that such compounds are significantly less chemically and enzymatically labile than their *O*-linked isosteres.[22] In a departure from published methods, such as reaction of per-*O*-acetylated sugars and thiols[23] and reaction of per-*O*-acetylated 1-thiosugars with halogenated agents, we utilised the Mitsunobu reaction.[17] Per-*O*-acetylated 1-thioglucose **16**[24] will participate in a Mitsunobu reaction with lipoamino alcohol **3**, using ADDP and trimethylphosphine under mild conditions, with simple work-up (Scheme 4) to give **17**[17] in excellent yield. The major advantage over the traditional Mitsunobu reagents (DEAD and triphenylphosphine) is that unlike triphenylphosphine oxide, the trimethylphosphine equivalent is water soluble. This is a significant improvement since triphenylphosphine oxide commonly co-elutes with reaction products during chromatography.



Scheme 4. Reagents and conditions: (i) ADDP (2.0 equiv), PMe_3 (2.0 equiv), **7** (2.0 equiv), THF, 18 h, rt.

2.5. Synthesis of *C*-glycolipids

C-glycosides are the most stable glycosides with resistance to both chemical and enzymatic degradation.[25] There are various methods to synthesise such compounds, but most were not applicable to producing the types of compounds required. We have some experience in using free radical-mediated procedures to synthesise *C*-glycosides,[26] but this relies on the use of dehydro-amino acids. We found that the Ramberg-Backlünd rearrangement is a very convenient means by which to synthesise *C*-glycosides from their *S*-linked isosteres. Developed simultaneously by the groups of Franck and Taylor, it proceeds from the sulphone to produce an unsaturated derivative[27] (Scheme 5). *S*-linked glycolipid **17** was re-protected as the per-*O*-benzyl protected derivative (to be compatible with the basic conditions of the reaction). **18** was easily converted to the glycosyl sulphone **19** in excellent yield using *m*-chloroperoxybenzoic acid. Unsaturated derivative **20** was then reduced, with simultaneous de-*O*-benzylation, followed by re-*O*-acetylation to give **22**. This method allows easy access to *C*-linked glycolipids, without the need for synthesis of complex derivatives.



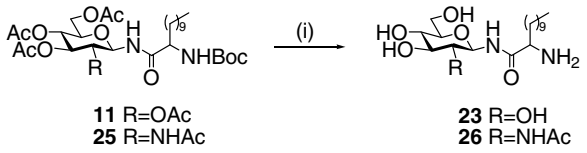
Scheme 5. Reagents and conditions: (i) a—NaOMe (0.1 equiv), methanol, 3 h, rt; b—NaH (8.0 equiv), benzyl bromide (8.0 equiv), DMF, 4 h, 0 °C, rt; (ii) 4-chloroperoxybenzoic acid (1.5 equiv), $CHCl_3$, 3 h, rt; (iii) CBr_2F_2 (12 equiv), KOH/Al_2O_3, CH_2Cl_2/*tert*-butanol 1:2, 4 °C, rt; (iv) 10% Pd/C, H_2, methanol, 12 h, rt; (v) Ac_2O, pyridine, 12 h, rt.

3. Biological application

In order to demonstrate the utility of such compounds, cationic glycolipid **11** was fully de-protected by treatment with sodium methoxide and trifluoroacetic acid sequentially to yield **23** (Scheme 6). In order to assess the potential of glycolipid **23** to enhance absorption of piperacillin, we used a Caco-2 cell monolayer assay (a model for oral absorption[28]). Compound **23** was admixed with piperacillin by lyophilising from an acetic acid solution of the two components. An LC-MS/MS assay was used to determine piperacillin transport.

As expected, the permeability of piperacillin was very low (Fig. 2), with an apparent permeability (P_{app}) of $3.25 \pm 0.14 \times 10^{-8}$ cm s^{-1}. The permeability observed for the piperacillin-glycolipid admix **24** was determined to be one order of magnitude higher at $2.42 \pm 0.66 \times 10^{-7}$ cm s^{-1}. As a point of reference, drugs with excellent oral bioavailability usually exhibit permeabilities of 10^{-6} cm s^{-1} or greater in the Caco-2 assay,[28] indicating that despite encouraging initial results, there is scope for improvement in the design of the glycolipids.

We sought to confirm that the use of these glycolipids does not affect antimicrobial activity of piperacillin. We achieved this by assessing antibacterial efficacy using a panel of bacterial strains. Minimum inhibitory concentrations (MICs) of piperacillin alone and piperacillin-glycolipid admix **27** were determined using a standard assay (Table 2). **27**, prepared from glycolipid **25** (Scheme 6), was used as an exemplar of these types of molecule. The results indicate that the presence of glycolipid does not affect the antimicrobial activity of piperacillin. Indeed the activity of **27** was marginally greater than that of parent piperacillin. Since the glycolipids alone were inactive, we hypothesise that this increase in activity is likely to have been due to the enhanced permeability of piperacillin (afforded by the presence of the glycolipid) through the agar medium—since piperacillin has extremely poor aqueous solubility.



11 R=OAc
25 R=NHAc

23 R=OH
26 R=NHAc

Scheme 6. Reagents and conditions: (i) a—NaOMe (0.1 equiv), methanol, 3 h, rt; b—TFA/CH_2Cl_2 1:1, 15 min, rt.

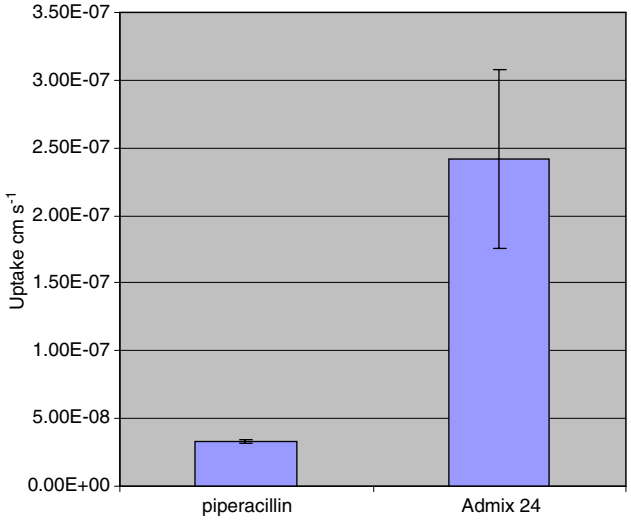


Figure 2. Uptake of piperacillin versus glycolipid-piperacillin admix **24**.

Table 2. MIC values for piperacillin and glycolipid-piperacillin admix **27** (μg/mL)

	Piperacillin	Piperacillin admix **27**
B. subtilis	0.5	0.5
E. coli	4	1
K. aerogenes	8	8
P. aeruginosa	4	4
S. aureus	1	0.5

4. Conclusion

We have designed and synthesised a series of novel amphipathic lipoamino acid-based glycolipids for potential use in improvement of the oral absorption of otherwise poorly bioavailable drugs. *O*-, *N*-, *S*- and *C*-linked glycolipids were prepared. The nature of the glycosidic linkage will determine the lipophilicity and stability of the glycolipids in chemical and biological environments. The exact nature of the lipid component, sugar component and glycosidic linkage can be tailored to meet physicochemical requirements. We have demonstrated the potential use of such compounds with surfactant properties and ion-pairing capability in improving the membrane transport of piperacillin, a poorly orally bioavailable antibacterial.

5. Experimental

5.1. Biology

5.1.1. Caco-2 cell drug uptake experiments and LC-MS/MS analysis. Caco-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine and 1% non-essential amino acids at 95% humidity and 37 °C in an atmosphere of 5% CO_2. The medium was changed every second day. After reaching 80% confluence, the cells were subcultured using 0.2% EDTA and 0.25% trypsin. Six thousand to 8000 cells (passage number 47–50) were seeded onto polycarbonate cell culture inserts (Transwell, mean pore size 0.45, 6.5 mm diameter) and cultivated in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 1% non-essential amino acids, 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were allowed to grow for 21–28 days. The medium was changed every second day. Transport experiments were performed in Hanks' balanced salt solution (HBSS) buffered with 25 mM

Hepes (pH 7.4) in air at 37 °C. The agents under test were dissolved in HBSS Hepes to a final concentration of 0.5 mM. Prior to the study, the monolayers were washed three times in pre-warmed HBSS–Hepes. At the start of the experiments, 100 μL of the drug solutions was added to the donor side of the monolayers. The plates were shaken in a Heidolf Titramax 1000, at 37 °C during the whole experiment. At determined time intervals (30, 90, 120 and 150 min), 0.4 mL samples were taken out from the receiver chambers. The volume in the receiver chamber was maintained constant by replacing the withdrawn volume with the same amount of HBSS–Hepes. Collected samples were kept frozen until LC–MS/MS analysis. The compounds were tested in two independent assays, using three or four wells in each experiment. To ensure integrity of the cells, transepithelial electrical resistance (TEER) of the monolayers was assessed before and after experiments using the Millicell-ERS system (Millipore Corporation, Bedford, MA). The values before starting experiments were typically above 1 $k\Omega\,cm^{-2}$ and no significant decrease was observed after the experiments. Piperacillin concentrations were determined by LC-MS/MS using a gradient HPLC system (Shimadzu LC-10AT system) coupled to a triple quadrupole mass spectrometer (PE Sciex API 3000) operating in MRM mode with positive ion electrospray. The mobile phase was a mixture of solvent A (1% formic acid in water) and solvent B (1% formic acid in 90% acetonitrile/water). A C18 column (Phenomenex luna, 5 micron, 50 × 2.0 mm) was used for RP-HPLC. Flow rate was set to 0.5 mL min^{-1} with a 30–100% B gradient over 4 min, incorporating a splitter (1:10) before the MS unit. The source used for the MS experiments was an ion spray (voltage: 5200 V). Nitrogen was used as nebulizer gas (10 mL min^{-1}), curtain gas (12 mL min^{-1}) and collision gas (4 mL min^{-1}). ESI-MS data were acquired and processed using Analyst 1.4.1 software (Applied Biosystems/MDS Sciex, Canada).

5.1.2. MIC determination. The minimum inhibitory concentrations (MICs) of piperacillin and piperacillin-glycolipid admix **27** were determined. Five species of bacteria were used in these experiments, namely *Bacillus subtilis*, *Escherichia coli*, *Klebsiella aerogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. One microlitre samples of culture and 1 μL samples from 10^2- and 10^4-fold dilutions (in the broth in which they had been grown) were inoculated onto a series of plates containing solid nutrient media with decreasing concentrations of the test compounds. Inoculation was performed using a Denley Multipoint Inoculator (Denley Products, Billinghurst, UK). The plates were subsequently incubated at 37 °C for 24 h under aerobic conditions. The MICs were determined as the lowest concentration of antibacterial that inhibited visible growth of an organism.

5.2. Chemistry

5.2.1. General information. All moisture sensitive reactions were carried out under a nitrogen atmosphere using oven-dried glassware. Solvents were dried over standard drying agents and freshly distilled prior to use. Reactions were carried out at room temperature unless otherwise specified. Purification was achieved by column chromatography through Sorbsil C60-H40/60, using the mobile phases indicated for thin layer chromatography unless otherwise specified. All commercial reagents were used without further purification.

NMR spectra were recorded at room temperature for $CDCl_3$ solutions (unless otherwise indicated). ^1H NMR spectra were recorded using a Bruker AM 500 instrument operating at a field of 500 MHz. Chemical shifts are reported in ppm downfield from internal TMS. Assignments were made using (1D) ^1H spectra and (2D) COSY spectra. Mass spectra were obtained using a VG Analytical ZAB-SE instrument using Fast Atom Bombardment (FAB) techniques—20 kV Cs^+ ion bombardment, with 2 μL of appropriate matrix, either 3-nitrobenzyl alcohol (MNOBA) or thioglycerol with NaI (MeOH) solution added where necessary to produce natriated species when no protonated molecular ions were observed. Alternatively, spectra were obtained using a Finnigan MassLab Navigator quadropole mass spectrometer, using electrospray ionisation (N_2 flow, 300 L/h; temperature, 180 °C; cone voltage, 49 V) where indicated.

Analytical RP-HPLC was carried out on a Vydac C_4 Protein column (25.0 cm × 4.6 mm). Separation was achieved using a linear gradient at a flow-rate of 1.2 mL/min effected by a Waters 600 S controller and 616 pump running Solvent A: 0.1% TFA; Solvent B: 0.1% TFA in 90% MeCN; 0% B to 70% B over 20 min, then 70% B to 0% B over 5 min. Separation was monitored with a Waters 486 absorbance detector at 214 nm.

The calculated log *P* (C log *P*) values quoted in this paper were generated with 'HyperChem Pro' release 5.1 Molecular Modelling system, using 'ChemPlus QSAR properties' extension version 1.6. These values refer to fully de-protected, non-ionised compounds.

5.2.2. (3) *tert*-Butyl *N*-[1-(*R*/*S*)-(hydroxymethyl)undecyl]carbamate. 2-(*R*/*S*)-[(*tert*-Butoxycarbonyl) amino] dodecanoic acid **2b** (0.93 g, 2.92 mmol) in abs. THF (3 mL) was added slowly dropwise to BH_3-THF complex (1.0 M, 5.8 mL, 5.80 mmol) at 0 °C. After stirring for 2 h, the reaction mixture was quenched with 10% acetic acid in methanol (v/v) and evaporated. The residue was taken up in CH_2Cl_2 (10 mL) and washed with 1 M $KHSO_{4(aq)}$ (1 × 20 mL) and brine (2 × 20 mL). The solution was then dried over $MgSO_4$, filtered and evaporated. Purification by column chromatography gave **3**. R_F = 0.50 hexane:ethyl acetate 4:1 (v/v); yield 87%; ^1H NMR δ 3.65–3.48 (m, 3H, αCH, CH_2), 1.43 (s, 9H, 3 × Boc CH_3), 1.24 (m, 18H, 9CH_2), 0.86 (t, 3H, CH_3); FAB MS $C_{17}H_{35}NO_3$ (301.46) *m*/*z* (%) 302 $[M+H]^+$ (15), 324 $[M+Na]^+$ (5), 434 $[M+Cs]^+$ (10), 202 $[M\text{-}Boc+H]^+$ (95).

5.2.3. (4) *tert*-Butyl *N*-{1-(*R*/*S*)-[4,5,6,7-tetrachlorophthalimidomethyl]undecyl}carbamate. Diisopropyl azidodicarboxylate (873 mg, 4.32 mmol) dissolved in abs. THF (1 mL) was added to a stirred solution of

triphenylphosphine (1.13 g, 4.32 mmol), tetrachlorophthalimide (1.33 g, 4.65 mmol) and *tert*-butyl *N*-[1-(*R*/*S*)-(hydroxymethyl)undecyl]carbamate **3** (1.00 g, 3.32 mmol) in abs. THF (150 mL). The reaction mixture was then stirred at room temperature for 72 h. The solvent was then evaporated and the residue was purified by column chromatography in hexane:ethyl acetate 5:1 (v/v) to give **4**. $R_F = 0.23$ hexane:ethyl acetate 1:1 (v/v); yield 75%; ^{1}H NMR δ 4.44 (d, 1H, NH), 3.94 (m, 1H, αCH), 3.65 (m, 2H, CH_2), 1.44–1.25 (m, 27H, 3 × Boc CH_3, 9CH_2), 0.88 (t, 3H, CH_3); FAB MS $C_{25}H_{34}Cl_4N_2O_4$ (568.36) *m*/*z* (%) 591 $[M+Na]^+$ (5), 470 $[M\text{-}Boc+H]^+$ (100).

5.2.4. (5) 2-(*R*/*S*)-(2-Aminododecyl)-1-(4,5,6,7-tetrachlorophthalimide). *tert*-Butyl *N*-{1-(*R*/s)-[4,5,6,7-tetrachlorophthalimidomethyl]undecyl}carbamate **4** (342 mg, 0.602 mmol) was dissolved in CH_2Cl_2:TFA 1:1 (4 mL) and stirred at room temperature for 10 min. After evaporation, the residue was taken up in CH_2Cl_2 (20 mL) and washed with $NaHCO_{3(sat,aq)}$ (2 × 20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to give **5**. $R_F = 0.41$ chloroform:methanol 10:2 (v/v); yield 88%; ^{1}H NMR ($CDCl_3$:CD_3OD 1:1) δ 3.80–3.46 (m, 3H, αCH, CH_2), 1.25 (m, 18H, 9CH_2), 0.86 (t, 3H, CH_3); FAB MS $C_{20}H_{26}Cl_4N_2O_2$ (468.24) *m*/*z* (%) 469 $[M+H]^+$ (50).

5.2.5. (6) *S*-{2-(*R*/*S*)-[(tert-Butoxycarbonyl)amino]dodecyl} ethanethioate. Trimethylphosphine (1.0 M, 0.664 mmol) was added dropwise to a stirred solution of 1,1′-(azodicarbonyl)dipiperidine [ADDP] (168 mg, 0.664 mmol) and imidazole (46.0 mg, 0.664 mmol) in abs. THF (10 mL) at 0 °C. After stirring for 30 min, *tert*-butyl *N*-[1-(*R*/s)-(hydroxymethyl)undecyl]carbamate **3** (100 mg, 0.332 mmol) and thiolacetic acid (51.0 mg, 0.664 mmol) in abs. THF (2 mL) were added to the solution. The reaction was stirred at room temperature overnight. The solvent was then evaporated. The product was dissolved in hexane, and the insoluble organic precipitate filtered off. Following evaporation of the solvent, the residue was taken up in CH_2Cl_2 (50 mL) and was washed with $NaHCO_{3(sat,aq)}$ (2 × 50 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 5:1 (v/v) to give **6**. $R_F = 0.47$ hexane:ethyl acetate 4:1 (v/v); yield 76%; ^{1}H NMR δ 4.46 (d, 1H, NH), 3.74 (m, 1H, αCH), 3.12, 2.99 (2m, 2H, CH_2), 2.36 (s, 3H, SAc), 1.43 (s, 9H, 3 × Boc CH_3), 1.27 (m, 18H, 9CH_2), 0.87 (t, 3H, CH_3); ^{13}C NMR δ 195.5, 155.5, 79.3, 50.5, 34.5–26.4, 25.9, 22.7, 14.1; FAB MS $C_{19}H_{37}NO_3S$ (359.57) *m*/*z* (%) 360 $[M+H]^+$ (7), 382 $[M+Na]^+$ (20), 260 $[M\text{-}Boc+H]^+$ (100).

5.2.6. (7) *S*-{2-(*R*/*S*)-[(tert-Butoxycarbonyl)amino]dodecyl}ethanethiol. *S*-{2-(*R*/*S*)-[(*tert*-Butoxycarbonyl)amino] dodecyl}ethanethioate **6** (1.50 g, 4.18 mmol) was dissolved in methanolic ammonia and stirred at room temperature for 1 h. Following evaporation, the residue was taken up in CH_2Cl_2 (50 mL) and was washed with brine (1× 50 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated to give a white solid **7**. $R_F = 0.53$ hexane:ethyl acetate 4:1 (v/v); yield 88%; ^{1}H NMR δ 4.59 (d, 1H, NH), 3.71 (m, 1H, αCH), 2.66 (m, 2H, CH_2), 1.44 (s, 9H, 3 × Boc CH_3), 1.25 (m, 18H, 9CH_2), 0.88 (t, 3H, CH_3); ^{13}C NMR δ 155.4, 79.9, 51.4, 32.9, 31.9, 29.5, 29.3, 28.4, 25.9, 22.6, 14.0; FAB MS $C_{17}H_{35}NO_2S$ (317.53) *m*/*z* (%) 340 $[M+Na]^+$ (15), 450 $[M+Cs]^+$ (3), 533 $[2M\text{-}2H\text{-}Boc+H]^+$ (10), 633 $[2M\text{-}2H+H]^+$ (2), 656 $[2M\text{-}2H+Na]^+$ (10); IR Raman shift: 2582 cm^{-1} (0.26) S–H stretch.

5.2.7. (9) *tert*-Butyl 1-[(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy)-methyl]-(*R*/*S*)-undecylcarbamate. *O*-[3,4,6-tri-*O*-Acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-gluco-pyranosyl] tri-chloroacetimidate **8**[19] (125 mg, 0.20 mmol), *tert*-butyl *N*-[1-(*R*/*S*)-(hydroxymethyl)-undecyl]carbamate **3** (45.0 mg, 0.150 mmol) and molecular sieves (200 mg) were stirred in abs. CH_2Cl_2 (5 mL) for 15 min. Boron trifluoride etherate (64.0 mg, 0.451 mmol) in abs. CH_2Cl_2 (3 mL) was added dropwise at 0 °C over 20 min. The mixture was stirred for 2 h at room temperature. The reaction mixture was then diluted with CH_2Cl_2 (10 mL) and filtered through a Celite pad. The solution was washed with $NaHCO_{3(sat,aq)}$ (1 × 10 mL) and water (1 × 10 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography using hexane:ethyl acetate 6:4 (v/v). $R_F = 0.35$ hexane:ethyl acetate 1:1 (v/v); yield 35%; ^{1}H NMR δ 5.28–5.21 (m, 2H, H-3, H-4), 4.79, 4.63 (2m, 2H, $Cl_3CC\underline{H}_2$), 4.56 (d, 1H, H-1, $J_{1,2} = 8.2$ Hz), 4.25, 4.14 (2m, 2H, H-6, H-6′), 3.82 (m, 1H, H-2), 3.70–3.55 (m, 4H, H-5, αCH, CH_2), 2.16, 2.08, 2.02 (3s, 9H, 3Ac), 1.44 (s, 9H, 3 × Boc CH_3), 1.28–1.23 (m, 18H, 9CH_2), 0.87 (t, 3H, CH_3); FAB MS $C_{32}H_{53}Cl_3N_2O_{12}$ (764.13) *m*/*z* (%) 787 $[M+Na]^+$ (100), 462 $[M\text{-}lipid]^+$ (75), 663 $[M\text{-}Boc+H]^+$ (70). This intermediate, *tert*-butyl 1-{[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl- amino)-β-D-glucopyranosyloxy]methyl}-(*R*/s)-undecylcarbamate (27.0 mg, 0.0353 mmol) was dissolved in acetic anhydride (1 mL) into which activated zinc powder (4.6 mg, 0.0706 mmol) had been added. The reaction was stirred for 6 h, after which it was filtered and evaporated (and co-evaporated with benzene and toluene). The residue was purified by column chromatography to give **9**. $R_F = 0.17$ hexane:ethyl acetate 1:1 (v/v); yield 49%; ^{1}H NMR δ 5.24–5.16 (m, 2H, H-3, H-4), 4.51 (d, 1H, H-1, $J_{1,2} = 8.5$ Hz), 4.27, 4.11 (2m, 2H, H-6, H-6′), 3.72 (m, 1H, H-2), 3.71–3.57 (m, 4H, H-5, αCH, CH_2), 2.16, 2.08, 2.02, 1.96 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 × Boc CH_3), 1.29–1.24 (m, 18H, 9CH_2), 0.87 (t, 3H, CH_3); FAB MS $C_{31}H_{54}N_2O_{11}$ (630.77) *m*/*z* (%) 653 $[M+Na]^+$ (60), 531 $[M\text{-}Boc+H]^+$ (90).

5.2.8. (11) 2,3,4,6-Tetra-*O*-acetyl-*N*-{1-(*R*/*S*)-[(tert-butoxycarbonyl)amino]dodecyl}-β-D-glucopyranosylamide. *Method A*: Tributyl-*n*-phosphine (4.88 g, 24.2 mmol) was dissolved in abs. CH_2Cl_2 (50 mL) and added dropwise to a stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide **24** (6.00 g, 16.1 mmol) and 2-(*R*/s)-[(*tert*-butoxycarbonyl)amino]dodecanoic acid **2** (10.3 g, 32.3 mmol) in abs. CH_2Cl_2 (100 mL) over 20 min. After stirring for 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with $NaHCO_{3(sat,aq)}$ (2 × 100 mL). The organic

phase was dried over $MgSO_4$, filtered and evaporated. The product was purified by column chromatography in chloroform:methanol 10:0.2 (v/v) to give **11** (yield 82%). *Method B*: Triethylamine (66.0 mg, 0.653 mmol) was added to a stirred solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate **12**[21] (1.28 g, 3.29 mmol) and 2-(*R*/s)-[(*tert*-butoxycarbonyl)amino]dodecanoic acid **2** (1.24 g, 3.95 mmol) in abs. toluene (15 mL). The reaction was refluxed for 12 h. After evaporation, the residue was taken up in CH_2Cl_2 (20 mL) and washed with $NaHCO_{3(sat,aq)}$ (2 × 20 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The product was purified by column chromatography in chloroform:ethyl acetate 10:3 (v/v) to give **11** (yield 68%). R_F = 0.87 chloroform:methanol 10:2.5 (v/v); ^{1}H NMR δ 5.31–5.22 (m, 2H, H-1, H-3), 5.06 (m, 1H, H-4), 4.93 (m, 1H, H-2), 4.79 (br s, 1H, NH), 4.28 (m, 1H, H-6), 4.13–4.05 (m, 2H, H-6′, αCH), 3.80 (m, 1H, H-5), 2.06, 2.03, 2.01, 2.00 (4s, 12H, 4Ac), 1.44 (s, 9H, 3 × Boc CH_3), 1.28–1.23 (m, 18H, 9CH_2), 0.87 (t, 3H, CH_3); FAB MS $C_{31}H_{52}N_2O_{12}$ (644.75) *m*/*z* (%) 667 $[M+Na]^+$ (10), 777 $[M+Cs]^+$ (100), 545 $[M\text{-}Boc+H]^+$ (15).

5.2.9. (13) 2,3,4,6-Tetra-*O*-acetyl-*N*-[({2-(R/s)-[(*tert*-butoxycarbonyl)amino]dodecyl}oxy)-carbonothioyl]-β-D-glucopyranosylamine. 2,3,4,6-tetra-*O*-Acetyl-β-D-glucopyranosyl isothiocyanate **12** (1.00 g, 2.57 mmol), *tert*-butyl *N*-[1-(*R*/*S*)-(hydroxymethyl)undecyl]carbamate **3** (967 mg, 3.21 mmol) and triethylamine (130 mg, 1.29 mmol) were dissolved in abs. toluene (10 mL) and stirred under reflux for 12 h. Following evaporation, the residue was purified by column chromatography in hexane:ethyl acetate 2:1 to give **13**. R_F = 0.69 chloroform:methanol 10:2 (v/v); yield 77%; ^{1}H NMR δ 7.02 (d, 1H, NH), 5.54, 5.32, 5.05, 4.96 (4m, 4H, H-1, H-2, H-3, H-4), 4.37 (m, 1H, αCH), 4.28 (m, 1H, H-6), 4.09 (m, 3H, CH_2, H-6′), 3.81 (d, 1H, H-5), 2.05, 2.01, 2.00, 1.99 (4s, 12H, 4Ac), 1.41 (s, 9H, 3 × Boc CH_3), 1.28–1.21 (m, 18H, 9CH_2), 0.85 (t, 3H, CH_3); ^{13}C NMR δ 170.6, 170.4, 169.9, 169.4, 155.3, 83.2, 81.9, 73.7, 72.7, 70.5, 69.8, 68.3, 67.6, 65.8, 61.6, 61.2, 60.2, 52.9, 49.6, 31.8–13.9; FAB MS $C_{34}H_{54}N_2O_{12}S$ (690.84) *m*/*z* (%) 713 $[M+Na]^+$ (25), 823 $[M+Cs]^+$ (100), 591 $[M\text{-}Boc+H]^+$ (40).

5.2.10. (14) 2,3,4,6-Tetra-*O*-acetyl-*N*-[({2-(*R*/*S*)-[(*tert*-butoxycarbonyl)amino]dodecyl}thio)-carbonothioyl]-β-D-glucopyranosylamine. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate **12** (100 mg, 0.257 mmol), *S*-{2-(*R*/s)-[(*tert*-butoxycarbonyl)amino]dodecyl}ethanethiol **7** (98.0 mg, 0.308 mmol) and triethylamine (5.20 mg, 0.0514 mmol) were dissolved in abs. CH_2Cl_2 and stirred at room temperature for 20 min. Following evaporation, the residue was purified by column chromatography in hexane:ethyl acetate 3:1 to give **14**. R_F = 0.61 hexane:ethyl acetate 1:1 (v/v); yield 71%; ^{1}H NMR δ 5.34, 5.16, 5.08 (3m, 4H, H-1, H-2, H-3, H-4), 4.29, 4.09 (2m, 2H, H-6, H-6′), 3.84 (d, 1H, H-5), 3.71, 3.44 (2m, 3H, αCH, CH_2), 2.06, 2.04, 2.01, 2.00 (4s, 12H, 4Ac), 1.46 (s, 9H, 3 × Boc CH_3), 1.30–1.25 (m, 18H, 9CH_2), 0.87 (t, 3H, CH_3); FAB MS $C_{32}H_{54}N_2O_{11}S_2$ (706.91) *m*/*z* (%) 707 $[M+H]^+$ (5), 729 $[M+Na]^+$ (10), 839 $[M+Cs]^+$ (15), 607 $[M\text{-}Boc+H]^+$ (80), 331 (25).

5.2.11. (15) 2,3,4,6-Tetra-*O*-acetyl-*N*-[({2-(*R*/*S*)-[(*tert*-butoxycarbonyl)amino]dodecyl}amino)-carbonothioyl]-β-D-glucopyranosylamine. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate **12** (25.0 mg, 0.0617 mmol), *tert*-butyl *N*-[1-(*R*/*S*)-(aminomethyl)undecyl]carbamate **5** (27.8 mg, 0.0927 mmol) and triethylamine (12.5 mg, 0.0124 mmol) were dissolved in abs. CH_2Cl_2 (5 mL) and stirred at room temperature for 1 h. Following evaporation, the residue was purified by column chromatography to give **15**. R_F = 0.39 chloroform:methanol 10:0.2 (v/v); yield 94%; ^{1}H NMR δ 5.11–4.99 (m, 3H, H-1, H-3, H-4), 4.23, 4.10 (2m, 2H, H-6, H-6′), 3.87–3.61 (m, 3H, H-2, H-5, αCH), 2.09, 2.01, 2.00, 1.96 (4s, 12H, 4Ac), 1.43 (s, 9H, 3 × Boc CH_3), 1.24 (m, 18H, 9CH_2), 0.88 (t, 3H, CH_3); FAB MS $C_{32}H_{55}N_3O_{11}S$ (689.86) *m*/*z* (%) 690 $[M+H]^+$ (10), 712 $[M+Na]^+$ (30), 590 $[M\text{-}Boc+H]^+$ (100).

5.2.12. (18) *tert*-Butyl 1-{[(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)thio]methyl}-(*R*/*S*)-undecylcarbamate. *tert*-Butyl 1-{[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thio]methyl}-(*R*/*S*)-undecylcarbamate **17** (4.00 g, 6.182 mmol) was dissolved in abs. methanol (40 mL). Sodium methoxide was added (0.5 M, 0.618 mmol) and the reaction was stirred for 3 h. The reaction was neutralised with Amberlite H^+ ion exchange resin. The solution was then filtered and the resin washed with methanol. The solution was then evaporated to a residue. R_F = 0.43 chloroform:methanol 10:2 (v/v); yield 91%; ^{1}H NMR δ 4.31 (t, 1H, H-1), 3.80 (m, 1H, H-6), 3.60 (m, 2H, H-3, H-6′), 3.28–3.13 (m, 4H), 2.83–2.59 (m, 2H, CH_2), 1.42 (s, 9H, 3 × Boc CH_3), 1.28 (m, 18H, 9CH_2), 0.87 (t, 3H, CH_3); FAB MS $C_{23}H_{45}NO_7S$ (479.67) *m*/*z* (%) 502 $[M+Na]^+$ (100), 380 $[M\text{-}Boc+H]^+$ (15). The *tert*-butyl 1-{[(β-D-glucopyranosyl)thio] methyl}-(R/*S*)-undecylcarbamate residue (2.70 g, 5.64 mmol) was dissolved in abs. DMF (20 mL) and added slowly dropwise to a stirred suspension of NaH (1.08 g, 45.1 mmol) in abs. DMF (40 mL) at 0 °C. After stirring the mixture for 2 h, benzyl bromide (7.71 g, 45.1 mmol) was added dropwise to the reaction mixture at 0 °C. The solution was stirred for a further 90 min. Methanol was cautiously added to quench the reaction. The solution was evaporated, taken up in ether (100 mL) and washed with water (3× 100 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 9:1 (v/v) to give **18**. R_F = 0.56 hexane:ethyl acetate 2:1 (v/v); yield 64%; ^{1}H NMR δ 7.37–7.17 (m, 20H, arom.H), 4.92–4.52 (m, 8H, 4CH_2Ar), 4.41 (d, 1H, H-1), 3.73–3.65 (m, 5H, αCH, H-2, H-3, H-4, H-6), 3.48 (m, 2H, H-5, H-6′), 2.90, 2.75 (2m, 2H, CH_2), 1.45, 1.42 (2s, 9H, 3 × Boc CH_3), 1.28–1.20 (m, 18H, 9CH_2), 0.89 (t, 3H, CH_3);^{13}C NMR δ 157.5, 140.1, 130.3, 130.1, 129.9, 129.8, 129.7, 129.6, 129.5, 88.9, 88.3–71.0, 52.3, 39.0–24.6, 15.9; FAB MS $C_{51}H_{69}NO_7S$ (840.16) *m*/*z* (%) 841 $[M+H]^+$ (5), 863 $[M+Na]^+$ (7), 972 $[M+Cs]^+$ (100).

5.2.13. (19) 2,3,4,6-Tetra-*O*-benzyl-1-({2-(*R*/*S*)-[(*tert*-butoxycarbonyl)amino]dodecyl}-sulphonyl)-1,5-anhydro-D-gluco-hexitol. *tert*-Butyl 1-{[(2,3,4,6-tetra-*O*-benzyl-

β-D-glucopyranosyl)thio]methyl}-(*R*/*S*)-undecylcarbamate **18** (2.03 g, 2.42 mmol) was dissolved in $CHCl_3$ (50 mL). 4-chloroperoxybenzoic acid (728 mg, 3.63 mmol) was added. The reaction was stirred at room temperature for 3 h. The solution was then diluted with $CHCl_3$ (25 mL) and washed with 1M $K_2HPO_{4(aq)}$ (2 × 25 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography in hexane:ethyl acetate 4:1 (v/v) to give **19**. R_F = 0.51 hexane:ethyl acetate 2:1 (v/v); yield 76%; ^{1}H NMR δ 7.38–7.25 (m, 20H, arom.H), 4.97–4.52 (m, 8H, $4CH_2Ar$), 4.27 (d, 1H, H-1), 4.13–3.66 (m, 7H), 3.47, 3.30 (2m, 2H, CH_2), 1.43 (s, 9H, 3 × Boc CH_3), 1.25 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_3); ^{13}C NMR δ 155.7, 155.3, 138.3, 137.8, 137.7, 137.4, 128.8, 128.5, 128.3, 127.9, 127.8, 127.7, 90.8, 89.8, 86.4, 86.1, 79.6–68.8, 55.8, 54.9, 46.9, 46.8, 35.7–22.7, 14.1; FAB MS $C_{51}H_{69}NO_9S$ (872.16) *m*/*z* (%) 894 $[M+Na]^+$ (60), 1004 $[M+Cs]^+$ (100), 772 $[M\text{-}Boc+H]^+$ (15).

5.2.14. (20) 2,3,4,6-Tetra-*O*-acetyl-1-{2-[(*tert*-butoxycarbonyl)amino]dodecylidene}-1,5-anhydro-D-*gluco*-hexitol. CBr_2F_2 (1.27 g, 6.06 mmol) dissolved in CH_2Cl_2 (2 mL) was added to a stirred mixture of 2,3,4,6-tetra-*O*-benzyl-1-({2-(*R*/*S*)-[(*tert*-butoxycarbonyl)amino]dodecyl}-sulphonyl)-1,5-anhydro-D-*gluco*-hexitol **19** (440 mg, 0.505 mmol) and KOH/Al_2O_3 catalyst (see below) in CH_2Cl_2:*tert*-butanol 1:2 (v/v) (10.5 mL) at 4 °C. The mixture was stirred for 15 min, then for 3 h at room temperature. The reaction was diluted with CH_2Cl_2 (50 mL) and passed through a celite pad. The solvents were evaporated and the residue purified by column chromatography in hexane:ethyl acetate 9:1 to give **20**. Preparation of catalyst: Potassium hydroxide pellets (10 g) were dissolved in methanol and shaken with neutral alumina (70–230 mesh) for 4 h. The solvent was then evaporated at 60 °C until the powder was free-flowing. R_F = 0.51 hexane:ethyl acetate 2:1 (v/v); yield 67%; ^{1}H NMR δ 7.36–7.15 (m, 20H, arom.H), 4.79–4.53 (m, 10H), 3.92–3.54 (m, 6H), 1.43, 1.41 (2s, 9H, 3 × Boc CH_3), 1.25 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_3); FAB MS $C_{51}H_{67}NO_7$ (806.08) *m*/*z* (%) 829 $[M+Na]^+$ (70), 845 $[M+K]^+$ (25), 705 $[M\text{-}Boc+H]^+$ (40).

5.2.15. (21) 1-{2-(*R*/*S*)-[(*tert*-Butoxycarbonyl)amino]dodecyl}-1,5-anhydro-D-*gluco*-hexitol (109). 2,3,4,6-Tetra-*O*-acetyl-1-{2-[(*tert*-butoxycarbonyl)amino]dodecylidene]-1,5-anhydro-D-*gluco*-hexitol **20** (270 mg, 0.335 mmol) was dissolved in methanol (7 mL). THF (2 mL) was added to fully dissolve the material. Palladium catalyst (10% on carbon, 50.0 mg) was added in one portion to the solution, which was stirred for 12 h under a hydrogen atmosphere. The catalyst was subsequently filtered off, and the solvent evaporated to give **21**, which was used for the next reaction step unpurified. R_F = 0.09 ethyl acetate; yield 80%; ESI MS $C_{23}H_{45}NO_7$ (447.61) *m*/*z* (%) 448 $[M+H]^+$ (100), 470 $[M+Na]^+$ (10).

5.2.16. (22) 2,3,4,6-tetra-*O*-Acetyl-1-{2-(*R*/*S*)-[(*tert*-butoxycarbonyl)amino]dodecyl}- 1,5-anhydro-D-*gluco*-hexitol. 1-{2-(*R*/*S*)-[(*tert*-Butoxycarbonyl)amino]dodecyl}-1,5-anhydro-D-*gluco*-hexitol **21** (120 mg, 26.9 mmol) was dissolved in acetic anhydride (2 mL) and pyridine (3 mL). The reaction mixture was stirred for 12 h. The product was concentrated, taken up in CH_2Cl_2 (10 mL) and washed with 1 M $KHSO_{4(aq)}$ (1 × 10 mL) and water (1 × 10 mL). The organic phase was dried over $MgSO_4$, filtered and evaporated. The product was purified by column chromatography in hexane:ethyl acetate 1:2 to give **22** (yield 79%) as a mixture of diastereomers **22a** and **22b** in a ratio of 4:3. R_F = 0.66 ethyl acetate; **22a**: ^{1}H NMR δ 5.16 (t, 1H, H-3), 5.04 (m, 1H, H-4), 4.84 (t, 1H, H-4), 4.47 (d, 1H, NH), 4.22 (dd, 1H, H-6), 4.10 (dd, 1H, H-6′), 3.81 (m, 1H, αCH), 3.60 (m, 1H, H-5), 3.53 (t, 1H, H-1β), 2.13, 2.04, 2.03, 1.99 (4s, 12H, 4Ac), 1.60 (m, 2H, CH_2), 1.45 (s, 9H, 3 × Boc CH_3), 1.26 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_3); **22b**: ^{1}H NMR δ 5.15 (t, 1H, H-3), 5.02 (m, 1H, H-4), 4.85 (t, 1H, H-4), 4.47 (d, 1H, NH), 4.21 (dd, 1H, H-6), 4.13 (dd, 1H, H-6′), 3.70 (m, 1H, αCH), 3.62 (m, 1H, H-5), 3.53 (t, 1H, H-1β), 2.13, 2.04, 2.03, 1.99 (4s, 12H, 4Ac), 1.60 (m, 2H, CH_2), 1.45 (s, 9H, 3 × Boc CH_3), 1.26 (m, 18H, $9CH_2$), 0.88 (t, 3H, CH_3); ^{13}C NMR δ 170.5, 170.3, 169.7, 169.6, 155.5, 79.0, 76.1, 75.8, 75.7, 75.2, 74.5, 72.1, 68.9, 68.8, 62.4, 48.9, 47.5, 36.8–20.5, 14.0; FAB MS $C_{31}H_{53}NO_{11}$ (615.75) *m*/*z* (%) 638 $[M+Na]^+$ (8), 654 $[M+K]^+$ (15), 516 $[M\text{-}Boc+H]^+$ (100).

5.2.17. (23) *N*-(1-Amino-(*R*/*S*)-dodecoyl)-β-D-glucopyranosylamine. De-*O*-acetate protection of **23**-procedure as for **18**. The residue (1.34 g, 2.82 mmol) was dissolved in CH_2Cl_2:TFA 1:1 (v/v) (6 mL) and stirred at room temperature for 15 min. The solvent was evaporated and co-evaporated with benzene and toluene to give **23**. R_F = 0.05 chloroform:methanol 10:2 (v/v); yield 81%; ^{1}H NMR δ 4.88–3.30 (m, 8H), 1.28–1.16 (m, 18H, $9CH_2$), 0.78 (t, 3H, CH_3); FAB MS $C_{18}H_{36}N_2O_6$ (376.49) *m*/*z* (%) 377 $[M+H]^+$ (10), 399 $[M+Na]^+$ (30).

5.2.18. (24) Piperacillin/*N*-(1-amino-(*R*/*S*)-dodecoyl)-β-D-glucopyranosylamine ionic complex. Piperacillin (2.00 g, 3.86 mmol) and **23** (1.45 g, 3.86 mmol) were dissolved in 95% acetic acid. Once fully dissolved, the solution was filtered and lyophilised to give **24** as a white solid (3.34 g, 97%). RP-HPLC: R_t = 12.83 min. ESI MS [M (complex **24**) = 893.4; M^1 (glycolipid **23**) = 376.3] *m*/*z* (%) 894.3 $[M+H]^+$, 1411.7 $[(M\text{-piperacillin})+H]^+$, 1270.4 $[(M\text{-}M^1)+H]^+$, 377.8 $[M^1+H]^+$.

5.2.19. (25) 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*N*-{1-(*R*/*S*)-[(*tert*-butoxycarbonyl)amino]-dodecyl}-β-D-glucopyranosylamide. Procedure as for **11**, using 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[21] in place of the glucose derivative. R_F = 0.64 chloroform:methanol 10:0.7 (v/v); yield 76%; ^{1}H NMR δ 5.09–4.98 (m, 2H, H-3, H-4), 4.41 (d, 1H, H-1, $J_{1,2}$ = 9.6 Hz), 4.20–4.08 (m, 3H, αCH, H-6, H-6′), 3.68 (m, 2H, H-2, H-5), 2.07, 1.99, 1.96 (3s, 12H, 4Ac), 1.44 (s, 9H, 3 × Boc CH_3), 1.26 (m, 18H, $9CH_2$), 0.87 (t, 3H, CH_3); FAB MS $C_{31}H_{53}N_3O_{11}$ (643.77) *m*/*z* (%) 644 $[M+H]^+$ (40), 544 $[M\text{-}Boc+H]^+$ (100).

5.2.20. (26) 2-Acetamido-2-deoxy-*N*-(1-amino-(*R*/*S*)-dodecoyl)-β-D-glucopyranosylamine. De-*O*-acetate protection of **25**-procedure as for **18**. The residue (1.34 g,

2.82 mmol) was dissolved in CH_2Cl_2:TFA 1:1 (v/v) (6 mL) and stirred at room temperature for 15 min. The solvent was evaporated and co-evaporated with benzene and toluene to give **26**. $R_F = 0.05$ chloroform:methanol 10:2 (v/v); yield 81%; ^{1}H NMR δ 7.35 (m, 1H, NH), 4.91 (m, 1H, H-1), 3.94–3.31 (m, 8H), 1.28–1.20 (m, 18H, $9CH_2$), 0.82 (t, 3H, CH_3); FAB MS $C_{20}H_{39}N_3O_6$ (417.54) *m/z* (%) 418 $[M+H]^+$ (3), 440 $[M+Na]^+$ (5).

5.2.21. (27) Piperacillin/2-acetamido-2-deoxy-*N*-(1-amino-(*R*/*S*)-dodecoyl)-β-D-gluco-pyranosylamine ionic complex. Piperacillin (2.00 g, 3.87 mmol) and **26** (1.61 g, 3.87 mmol) were dissolved in 95% acetic acid. Once fully dissolved, the solution was filtered and lyophilised to give **27** as a white solid (3.50 g, 97%). RP-HPLC: $R_t = 12.46$ min. ESI MS [M (complex **27**) = 934; M^1 (glycolipid **26**) = 417] *m/z* (%) 935 $[M+H]^+$ (100), 418 $[M^1+H]^+$ (45).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.07.048.

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