Aza-analogues of the Repeating Disaccharide Unit of Peptidoglycan. Part 2.[1] Enantiospecific Synthesis of Peptide-derivatised 2-Acetamido-4-*O*-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-3-*O*-carboxymethyl-1,2,5-trideoxy-1,5-imino-D-glucitol

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The L-alanyl-D-glutamic acid derivative **21**, L-alanyl-D-glutamine derivative **23** and L-alanyl-γ-D-glutamyl-L-lysine derivative **25** of 2-acetamido-4-*O*-(2'-acetamido-2'-deoxy-β-D-glucopyranosyl)-3-*O*-carboxymethyl-1,2,5-trideoxy-1,5-imino-D-glucitol **2**(X = OH) have been prepared as aza-analogues of the repeating disaccharide unit in bacterial cell wall peptidoglycan. The synthesis of methyl 5-azido-*N*-benzyloxycarbonyl-2,5,6-trideoxy-2,6-imino-3-*O*-(4-methoxybenzyl)-L-gulofuranoside **8α** and the epimeric azide **9α** from the corresponding D-mannofuranoside precursor **7α** is described starting from a 1:1 anomeric mixture of the 2-*O*-(trifluoromethylsulfonyl)-D-glucofuranoside derivatives **4**. Also reported are the subsequent transformations of the gulofuranoside **8α** into the 2-azido-1,5-imino-D-glucitol **13** and the β-glycoside derivative **19** from which the three target aza-glycopeptides **21**, **23** and **25** are prepared. None of the target compounds showed any antibacterial activity.

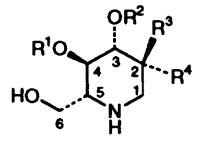
The peptidoglycan layer of bacterial cell walls consists of a matrix of β(1-4)-linked glycan chains of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) units which are cross-linked by short peptides attached between NAM residues[2] (Fig. 1). The sequence of the peptide varies slightly with bacterial species but is generally L-Ala-γ-D-Glu-X-D-Ala, where X is usually either L-lysine or *meso*-diaminopimelic acid (*m* A_2pm). Cross-linking of glycan strands occurs between the terminal amino group in residue X and the D-Ala residue of an adjacent peptide.

Peptidoglycan is unique to bacteria and is essential to the survival of the cell. It is therefore widely considered to be an excellent target for antibacterial action. Enzymes capable of cleaving bonds within peptidoglycan are assumed to maintain its integrity by controlling growth and division of the cell.[3] Naturally, these hydrolases need to be strictly coordinated with the activity of peptidoglycan synthesising enzymes. If this critical balance is disturbed, as it is for example by the action of penicillin, the hydrolases function as autolysins which then cause cell lysis resulting in death.

Aza-analogues of pyranoses, such as deoxymannojirimycin **3**[4] and sometimes their glycoside derivatives,[5,6] have precedent for being potent inhibitors of glycosidases, enzymes which hydrolyse the attachment of one sugar to another. However, some of these compounds exhibit antibacterial activity[7] by an unidentified mechanism of action and this encouraged us to investigate whether aza-analogues of the repeating disaccharide unit of peptidoglycan would induce disruption of its enzyme-controlled assembly. Here we report the enantiospecific synthesis of three aza-analogues of NAG-NAM disaccharides as potential antibacterial agents.

Results and Discussion

In the preceding paper of the series[1] we described the synthesis of aza-analogues of the NAG-NAM disaccharide, in which the aza-sugar replacement for the NAM residue was the 3-*O*-carboxymethyl derivative of deoxymannojirimycin **3**. However,



1 R^1 = β-GlcNAc, R^2 = CH_2COX, R^3 = H, R^4 = OH
2 R^1 = β-GlcNAc, R^2 = CH_2COX, R^3 = NHAc, R^4 = H
} X = peptide, OH
3 R^1 = R^2 = R^3 = H, R^4 = OH (deoxymannojirimycin)

none of these compounds displayed antibacterial activity. As a potentially more promising extension of this work we have now prepared the corresponding 2-*epi*-acetamido derivatives **2** which are closer in structure to the natural disaccharide unit of

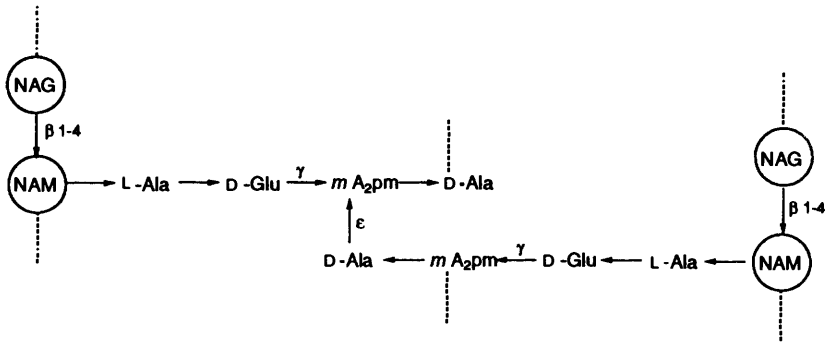
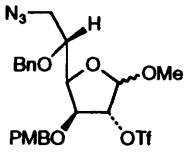


Fig. 1 Representation of the structure of cross-linked disaccharide units of peptidoglycan in *E. coli*



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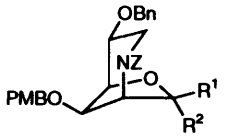
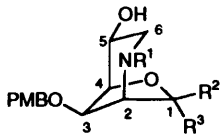
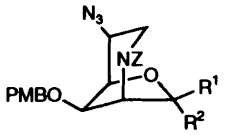


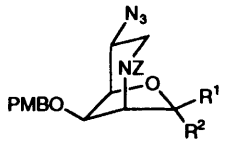
5α R^1 = H, R^2 = OMe
5β R^1 = OMe, R^2 = H



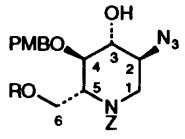
6α R^1 = R^2 = H, R^3 = OMe
6β R^1 = R^3 = H, R^2 = OMe
7α R^1 = Z, R^2 = H, R^3 = OMe
7β R^1 = Z, R^2 = OMe, R^3 = H



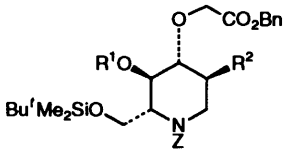
8α R^1 = H, R^2 = OMe
8β R^1 = OMe, R^2 = H



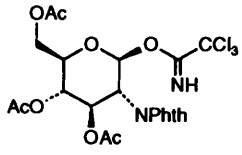
9α R^1 = H, R^2 = OMe
9β R^1 = OMe, R^2 = H



10 R = H
11 R = TBDMS



12 R^1 = PMB, R^2 = N_3
13 R^1 = H, R^2 = N_3
14 R^1 = H, R^2 = NHAc



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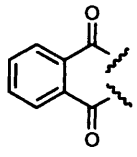
PMB = 4-$MeOC_6H_4CH_2$, Tf = CF_3SO_2, Bn = $PhCH_2$, Z = $PhCH_2OCO$

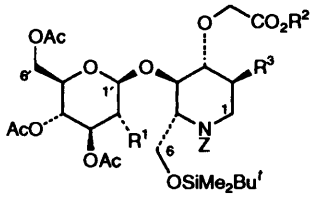
TBDMS = Bu^tMe_2Si Phth =



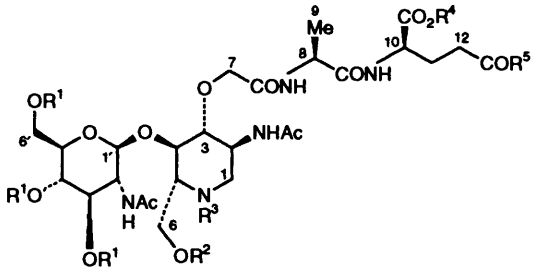
peptidoglycan and which might thereby interfere with its biosynthesis.

The target 2-*epi*-acetamido derivatives **2** are prepared by using a modification of the synthesis of aza-glycopeptides **1**.[1] The bicyclic mannofuranosides **5α/5β**, prepared from the diacetoneglucose-derived azides **4**, are intermediates in the synthesis of compounds **1**.[1] Replacement of the benzyloxy group in the bicyclic compounds **5α/5β** by a nitrogen function with the inverted configuration provides convenient access to the target derivatives **2**. However, this transformation requires that the bicyclic intermediates **7α/7β**, in which the C-5 hydroxy groups are unprotected, be prepared from the anomeric mixture of azides **4**. The benzyl groups in the azide mixture **4** were not removed with hydrogen (101 kPa) under palladium catalysis at room temperature, the product under these conditions, after treatment with benzyloxycarbonyl chloride, being the benzyl ethers **5α/5β**. However, *O*-debenzylation was achieved by twice treating a 1:1 mixture of the azides **4** with 10% palladium on carbon under conditions of catalytic hydrogen transfer,[8,9] from which a 1:1 mixture of the amino alcohols **6α/6β** was formed. The corresponding *N*-benzyloxycarbonyl derivatives **7α/7β** of the mixture **6α/6β** were then prepared, purified and characterised. The ^1H NMR spectrum of the bicyclic compounds **7α/7β** has two notable features. First, some signals are duplicated owing to restricted rotation about the NCO_2CH_2Ph amide bond, an observation which is characteristic of such derivatives described here and elsewhere.[1] Secondly, the chemical shifts of the pseudo-axial 6-H protons are typically centred upfield relative to other signals, in the region δ 2.85–3.19 ($J_{6a,5}$ ~9 Hz), when the C-5 substituent is pseudo-equatorial.

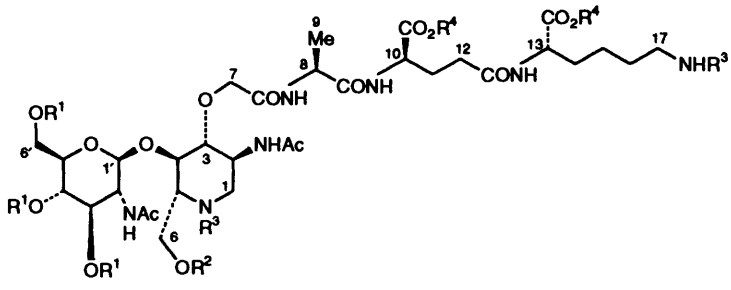
Fleet *et al.* have already described reactions in which the 5-OH group in bicyclic compounds similar to **7α** has been replaced with inversion by azide ion.[10] In their investigation the by-products from these reactions were the 5-azido derivatives in which the configuration had been retained during the displacement reaction. This outcome was attributed to neighbouring group participation of the benzyloxycarbonyl protecting group during the nucleophilic substitution reaction. We examined whether a similar inversion reaction could be carried out on the anomeric mixture **7α/7β**. Thus, the corresponding trifluoromethanesulfonyl ester derivatives of the 1:1 mixture of alcohols **7α/7β** were formed by treatment with trifluoromethanesulfonic anhydride. From the displacement reaction with sodium azide in dimethylformamide (DMF) at 60 °C a four-component mixture was produced. A difficult column chromatographic separation of these components gave the required compounds **8α/8β** in 37% yield and their corresponding epimers **9α/9β** in 39% yield. On the other hand, when the pure anomer **7α** was treated in a similar fashion, the two products formed were much more easily separated by chromatography and identified as the azide **8α** (58%) and the corresponding epimer **9α** (16%). These results imply that the azide-displacement reaction on the trifluoromethanesulfonyl ester derivative of compound **7β** preferentially occurs with unwanted retention of configuration. Therefore to maximise



16 R^1 = NPhth, R^2 = Bn, R^3 = N_3
17 R^1 = NPhth, R^2 = Et, R^3 = NHAc
18 R^1 = NPhth, R^2 = Bn, R^3 = NHAc
19 R^1 = R^3 = NHAc, R^2 = H



20 R^1 = Ac, R^2 = TBDMS, R^3 = Z, R^4 = Bn, R^5 = OBn
21 R^1 = R^2 = R^3 = R^4 = H, R^5 = OH
22 R^1 = Ac, R^2 = TBDMS, R^3 = Z, R^4 = Bn, R^5 = NH_2
23 R^1 = R^2 = R^3 = R^4 = H, R^5 = NH_2



24 R^1 = Ac, R^2 = TBDMS, R^3 = Z, R^4 = Bn
25 R^1 = R^2 = R^3 = R^4 = H

the yield of the bicyclic azide **8α** the separated β-anomeric alcohol **7β** was converted into the α-anomer **7α** in 74% yield, prior to the displacement reaction, by brief treatment with acidic ion-exchange resin in refluxing methanol. The ^{1}H NMR spectrum of the epimeric azide **9α** exhibited a pair of double-doublets (rotamers) at δ 2.97 and 3.02 ($J_{6a,5}$ 10.4 Hz) attributed to the pseudo-axial 6-H proton, consistent with a structure in which the azide substituent is pseudo-equatorial. By comparison, the ^{1}H NMR spectrum of the azide **8α** reveals the corresponding resonance to be downfield (δ > 3.83).

Acetal hydrolysis of the bicyclic azide **8α** occurred upon treatment with 3 mol dm^{-3} hydrochloric acid in tetrahydrofuran (THF) at room temperature and the resulting aldehyde intermediate was reduced with sodium borohydride to afford the dihydroxypiperidine **10** in 68% yield. The primary hydroxy group in compound **10** was then protected as the *tert*-butyldimethylsilyl (TBDMS) ether **11**. The *gluco* configuration of the diol **10** was confirmed by its ^{1}H NMR spectrum in which the 3-H proton appears at δ 3.57 as a triplet (*J* 7.5 Hz) indicating that the 2-, 3- and 4-H protons are all in axial positions.

In our aim to mimic the NAM sugar residue of the disaccharide unit in peptidoglycan we had previously reported difficulties in attempts to prepare 3-*O*-lactyl derivatives of suitably protected deoxymannojirimycin **3**.[1] These difficulties were encountered again during similar attempts to prepare 3-*O*-lactyl derivatives of the silyl ether **11** and we therefore incorporated glycolic acid as a surrogate for lactic acid. Accordingly, the lithium alkoxide species derived from the alcohol **11** was formed with butyllithium in THF and was then treated with the trifluoromethanesulfonyl derivative of glycolic acid benzyl ester[11] to give the highly functionalised piperidine compound **12** in 50% yield. Reaction of compound **12** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) gave rise to the selective removal of the *p*-methoxybenzyl group, affording the secondary alcohol **13** in high yield. The alcohol **13** reacted smoothly with the trichloroacetimidate glycosyl donor **15**[12] under boron trifluoride–diethyl ether (BF_3·Et_2O) catalysis to give the β-glycoside **16**, exclusively, in 84% yield. The ^{1}H NMR spectrum of the product **16** showed a doublet ($J_{1',2'}$ 8.1 Hz) at δ 5.44, assigned to the anomeric proton, which established the diaxial arrangement of the 1'- and 2'-H protons in the β-anomeric product.

Specific reduction of the azide **16** was carried out with sodium hydrogen telluride[10,13] and this was followed by acetylation to give a 45% yield of the acetamido derivative **17** in which transesterification had also occurred; according to the

mechanism of the reduction process, sodium ethoxide is a by-product in the reaction. However, the occurrence of transesterification was of no consequence to the course of the synthesis because in the next step the ester group in the acetamido derivative **17** was hydrolysed with 0.2 mol dm^{-3} potassium hydroxide in methanol. The product from the hydrolysis was then treated with hydrazine monohydrate to remove the phthalimido group and then acetylated with acetic anhydride in pyridine to produce the carboxylic acid **19** in 65% overall yield from the acetamide **17**. The structure of the product **19** was confirmed by its ^1H NMR spectrum which displayed five acetyl resonances (δ 1.79–2.10) and a 5 H aromatic envelope, δ 7.34–7.43.

An alternative, but unsuccessful, strategy towards the synthesis of the acid **19** involved an earlier reduction and acetylation of the azide group in the bicyclic compound **8α** eventually leading to compound **14**. However, under BF_3·Et_2O catalysis, reaction of the imidate **15** with the alcohol **14** gave a very low yield of the product **18** and this route was therefore abandoned.

The carboxylic acid **19** is a stable intermediate which can be coupled to peptides to give intermediate products which yield the target aza-glycopeptides after deprotection in a one-pot reaction sequence. Thus, the acid **19** in the presence of 1-hydroxybenzotriazole (HOBt), *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-methylmorpholine (NMM), was coupled with the hydrochloride salt of L-Ala-D-Glu(OBn)$_2$[1] to give the protected aza-glycopeptide **20** in 78% yield. Hydrogenolysis of compound **20** over palladium black in dil. acetic acid, and then warming of the catalyst-free solution to complete the desilylation step, gave the penultimate product. This product was *O*-deacetylated by treatment with aq. ammonium hydroxide in methanol to afford the aza-glycopeptide **21** in 73% yield from compound **20**. The ^1H–^1H COSY-45 NMR spectrum of the product **21** showed all the expected connectivities in the sugar and aza-sugar rings and in the peptide side chain. The 1D ^1H NMR spectrum of the product **21** featured sharp doublets for 1′-H at δ 4.73 ($J_{1',2'}$ 8.3 Hz) and for L-alanyl Me at δ 1.48 (J 7.2 Hz). In addition, the signals at δ 2.67 (J 12.3 Hz) and δ 3.24 (J 12.3, 4.3 Hz) were assigned to the axial and equatorial 1-H protons, respectively, and are quite distinct from those for the equivalent protons in the spectrum of the corresponding 2-OH analogue **1**,[1] reflecting the difference in C-2 substitution between the two series of compounds.

In a similar fashion, the hydrochloride salt of L-Ala-D-Gln(OBn)*, was coupled with the acid **19** to produce compound **22** from which the deprotective reaction sequence yielded the aza-glycopeptide **23** in 56% overall yield from the acid **19**. The tripeptide L-Ala-γ-D-Glu(OBn)-L-Lys(Z)OBn [1] and acid **19** likewise formed the protected intermediate **24** (69%). The tripeptide derivative **24** was similarly deprotected to give the aza-glycopeptide **25** (74%).

None of the target compounds **21**, **23** or **25** exhibited antibacterial activity. Neither were they active in a biological screen[14] designed to identify inhibitors of translocase 1,[15] translocase 2[16a] and transglycosylase,[16b] which are enzymes involved in peptidoglycan biosynthesis.[3]

Experimental

The experimental techniques, materials, solvents and spectroscopic abbreviations and instrumentation employed in this work were as described in Part 1 of the series.[1] Unless otherwise indicated NMR spectra were obtained for solutions in deuteriochloroform. Amberlite CG-120 (100–200 mesh) ion-exchange resin (Na^+-form) was purchased from Fluka AG.

Methyl N-*Benzyloxycarbonyl*-2,6-*dideoxy*-2,6-*imino*-3-O-(4-*methoxybenzyl*)-D-*mannofuranoside* **7α/7β**.—Methyl 6-azido-5-*O*-benzyl-6-deoxy-3-*O*-(4-methoxybenzyl)-2-*O*-(trifluoromethylsulfonyl)-D-glucofuranoside **4**[3] (6.9 g, 12.3 mmol) was prepared as described previously[1] as an orange syrup, identified by ^1H NMR spectroscopy as a 1:1 mixture of anomers. A solution of this syrup (6.9 g) in methanol (100 cm^3) was stirred at room temperature for 20 h with 10% palladium on carbon (2.0 g), anhydrous sodium acetate (1.0 g, 12.2 mmol) and ammonium formate (4.5 g, 71.4 mmol). The reaction mixture was filtered and the residue was washed with water (15 cm^3). The filtrate and washings were combined, and evaporated under reduced pressure to give an oil. The oil was dissolved in dichloromethane (200 cm^3) and the solution was washed with water (200 cm^3). The organic phase was dried, and evaporated under reduced pressure to give an oil. A solution of this oil in methanol (100 cm^3) was re-treated with the same reagents exactly as before and processed in the same way to afford the crude bicyclic amine **6α/6β** (2.0 g) as a syrup [R_f 0.52, 0.37 respectively; EtOAc–EtOH–water (7:2:1)]. This syrup was dissolved in 1,4-dioxane (140 cm^3) and the solution was then diluted with saturated aq. sodium hydrogen carbonate (70 cm^3). Benzyl chloroformate (1.6 cm^3, 11.2 mmol) was added to this mixture at room temperature and the whole was stirred for 3 h, after which time the mixture was concentrated to remove the organic solvent. The remaining aqueous residue was extracted with dichloromethane (2 × 150 cm^3) and the combined extracts were dried, and evaporated at reduced pressure to give a yellow oil. The oil was purified by chromatography [acetone–toluene (1:20→1:4 gradient elution)] to afford separated, pure anomeric components of the title compounds **7α/7β** (1.15 g **7α**; 1.10 g **7β**; combined yield 43%).

α-Anomer **7α**; syrup [R_f 0.23, acetone–toluene (3:17)] (Found: C, 64.2; H, 6.2; N, 3.3. $C_{23}H_{27}NO_7$ requires C, 64.3; H, 6.3; N, 3.3%); $[\alpha]_D$ +21.7 (*c* 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)$/cm^{-1} 3560br (OH), 1690 (C=O) and 1230br; δ(250 MHz) 1.77 (1 H, br s, D_2O-exch, OH), 2.79 and 2.85 (2 × 0.5 H, 2 dd, each $J_{6,6}$13.0, $J_{6a,5}$ 9.7, C*H*HN), 3.39 and 3.40 (2 × 1.5 H, 2 s, anomeric OMe), 3.80 (3 H, s, ArO*Me*), 4.06 (1 H, br becomes br q, J7.2 after D_2O-exch, 5-H), 4.19–4.56 (5 H, m), 4.60 and 4.75 (total 1 H, 2 br s, 2-H), 4.96 (1 H, s, C*H*OMe), 5.10 and 7.19 [total 4 H, 3 d, each J 8.5 (A_2X_2), ArH] and 7.30–7.35 (5 H, m, ArH); *m*/*z* (CI) 447 (MNH_4^+, 3%) 430 (MH^+, 35), 121 (C_8H_9O, 100) and 91 (C_8H_9, 72).

β-*Anomer* **7β**; syrup [R_f 0.14, acetone–toluene (3:17)] (Found: C, 64.3; H, 6.6; N, 3.2%); $[\alpha]_D$ −94.8 (*c* 0.17, $CHCl_3$); $\nu_{max}(CHCl_3)$/cm^{-1} 3575br (OH), 1690 (C=O) and 1230br; δ(250 MHz) 1.59 (1 H, br s, D_2O-exch, OH), 3.17 and 3.19 (total 1 H, 2 dd, each $J_{6,6}$ 12.7, $J_{6a,5}$ 8.8, C*H*HN), 3.47 and 3.49 (total 3 H, 2 s, anomeric OMe), 3.80 (3 H, s, ArO*Me*), 4.01 (1 H, br s becomes sharper after D_2O-exch, 5-H), 3.95 and 4.04 (total 1 H, 2 dd, each $J_{3,4}$ 5.9, $J_{3,2}$ 3.9, 3-H), 4.23–4.60 (4 H, m), 4.66 and 4.87 (total 1 H, 2 t, each $J_{2,3} \sim J_{2,1} \sim$ 3.5, 2-H), 5.04 and 5.10 (total 1 H overlapping, 2 d, $J_{1,2}$ 3.3, anomeric C*H*OMe), 5.07–5.24 (total 2 H, overlapping, m, CO_2CH_2Ph) 6.82–6.86 (2 H, m, ArH), 7.10 and 7.19 [total 2 H, 2 d, each J 8.6 (A_2X_2), ArH] and 7.31–7.35 (5 H, m, ArH); *m*/*z* (CI) 430 (MH^+, 2%), 121 (C_8H_9O, 100) and 91 (C_7H_7, 18).

Conversion of β-*Epimer* **7β** *into* α-*Epimer* **7α**—A solution of compound **7β** (1.08 g) in dry methanol (70 cm^3) was refluxed for 0.5 h with CG120 (H^+) ion-exchange resin (500 mg) which had

* L-Ala-D-Gln(OBn) {hydrochloride salt: $[\alpha]_D$ +32.4 × 10^{-1} deg cm^2 g^{-1} (*c* 0.14, water)} was synthesised in high yield by solution peptide-coupling methodology (DCC–HOBt–NMM) from commercially available constituent amino acids.

previously been washed with methanol (2 × 5 cm^3) and dried. The suspension was cooled to room temperature and the resin was removed by filtration. The filtrate was evaporated under reduced pressure to leave an oil, which was purified by chromatography [acetone–toluene (1:20→3:7 gradient elution)] to give compound **7α** (800 mg, 74%) as an oil. This material was identical in all respects with the authentic compound described above.

*Methyl 5-Azido-*N*-benzyloxycarbonyl-2,5,6-trideoxy-2,6-imino-3-O-(4-methoxybenzyl)-α-*L*-gulofuranoside* **8α**.—Trifluoromethanesulfonic anhydride (1.25 cm^3, 7.4 mmol) was added dropwise to a stirred solution of compound **7α** (2.5 g, 5.8 mmol) in a mixture of dry dichloromethane (37 cm^3) and dry pyridine (0.95 cm^3, 11.8 mmol) at −30 °C under argon. After 1 h at this temperature the mixture was warmed to 0 °C and was then washed with cold water (40 cm^3), dried, and evaporated under reduced pressure to leave a pale yellow oil. The oil was dissolved in DMF (37 cm^3) and the solution was stirred with a mixture of sodium azide (3.75 g, 57.7 mmol) and tetrabutylammonium hydrogen sulfate (1.98 g, 5.8 mmol) at 60 °C for 18 h under argon. The reaction mixture was then evaporated under reduced pressure and the residue was partitioned between dichloromethane (100 cm^3) and water (100 cm^3). The organic layer was separated, dried, and evaporated under reduced pressure to leave an oil, which was purified by chromatography [acetone–toluene (1:200→3:97 gradient elution)] to yield the title compound **8α** (1.54 g, 58%) and the corresponding epimer **9α** (430 mg, 16%).

Compound **8α**; syrup [R_f 0.1, acetone–toluene (1:50)]; $[\alpha]_D$ + 9.6 (*c* 0.41, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 2105 (N_3), 1695 (C=O) and 1230br; δ(250 MHz; resolution enhanced) 3.31 and 3.36 (total 3 H, 2 s, anomeric OMe), 3.61 and 3.70 (total 1 H, 2 dt, each pattern *J* 2.5, *J* ~ 6.3, 5-H), 3.79 and 3.80 (total 3 H, 2 s, ArO*Me*), 3.83–4.65 (7 H, m), 4.78 and 4.85 (total 1 H, 2 s, anomeric C*H*OMe), 5.07–5.21 (2 H, m, CO_2CH_2Ph) and 6.82–7.36 (9 H, m, ArH); *m/z* FAB (3-NOBA) 477 (MNa^+), 455 (MH^+), 121 (C_8H_9O) and 91 (C_7H_7).

Compound **9α**; syrup [R_f 0.11, acetone–toluene (1:50)]; $[\alpha]_D$ + 43.7 (*c* 0.82, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 2105 (N_3), 1690 (C=O) and 1230br; δ(250 MHz) 2.97 and 3.02 (total 1 H, 2 dd, each pattern $J_{6,6}$ 12.8, $J_{6a,5}$ 10.4, C*H*HN), 3.39 and 3.41 (total 3 H, 2 s, anomeric OMe), 3.73–3.84 (1 H, overlapping, m, 5-H), 3.80 (3 H, overlapping s, ArO*Me*), 4.18–4.87 (6 H, m), 4.99 (1 H, s, anomeric C*H*OMe), 5.06–5.17 (2 H, m, CO_2CH_2Ph) and 6.82–7.36 (9 H, m, ArH); *m/z* FAB (3-NOBA) 477 (MNa^+), 121 (C_8H_9O) and 91 (C_7H_7).

*2-Azido-*N*-benzyloxycarbonyl-1,2,5-trideoxy-1,5-imino-4-O-(4-methoxybenzyl)-*D*-glucitol* **10**.—Compound **8α** (1.15 g, 2.5 mmol) was dissolved in a mixture of THF (18.5 cm^3) and conc. hydrochloric acid (relative density 1.18; 6.5 cm^3) and the resulting solution was stirred at room temperature for 15 min before being poured slowly into a mixture of dichloromethane (100 cm^3), water (100 cm^3) and sodium hydrogen carbonate (10 g), and this mixture was stirred vigorously for 15 min. The layers were separated and the aqueous fraction was extracted with dichloromethane (50 cm^3). The combined organic phases were dried, and evaporated under reduced pressure to give an oil, which was kept at room temperature for 1 h *in vacuo*. The residue was dissolved in ethanol (24 cm^3) and to the stirred solution, cooled to 0 °C, was slowly added a solution of sodium borohydride (186 mg, 4.9 mmol) in water (5 cm^3). The temperature of the solution was maintained at 0 °C for 15 min and was then allowed to reach ambient during another 15 min. Excess of ammonium chloride was then added to the stirred mixture; after 15 min the mixture was concentrated to give an oil, which was dissolved in dichloromethane (100 cm^3) and the solution was washed with water (100 cm^3). The aqueous fraction was re-extracted with dichloromethane (50 cm^3) and the combined organic phases were dried, and evaporated under reduced pressure to afford an oil. Purification of the oil by chromatography [acetone–toluene (1:20→1:1 gradient elution)] afforded the title compound **10** (760 mg, 68%) as a foam [R_f 0.23; acetone–toluene (1:4)]; $[\alpha]_D$ −33.0 (*c* 0.18, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3600br and 3420br (OH), 2115 (N_3), 1685 (C=O) and 1230; δ(250 MHz) 1.85 (~ 2 H, br s, D_2O-exch, OH), 3.05–3.14 (1 H, br m, C*H*HN), 3.38–3.46 (2 H, m, CH*H*N and C*H*CH_2OH), 3.57 (1 H, t, $J_{3,2}$ ~ $J_{3,4}$ ~ 7.5, C*H*OH), 3.66 (1 H, t, $J_{4,3}$ ~ $J_{4,5}$ ~ 7.4, C*H*OPMB), 3.81 (3 H, s, OMe), 3.96–4.08 (1 H, overlapping, m, CHN_3), 3.94 and 4.09 (2 H, overlapping, 2 dd, $J_{6,6}$ 12.7, $J_{6,5}$ 4.4, $J_{6,5}$ 2.4, CH_2OH), 4.67 and 4.80 [total 2 H, 2 d, each *J* 11.3 (AB), ArCH_2], 5.12 and 5.19 [total 2 H, 2 d, each *J* 12.3 (AB), CO_2CH_2Ph], 6.90 [2 H, d, *J* 8.6 (A_2X_2), ArH] and 7.26–7.38 (7 H, m, ArH); ^1H–^1H correlations: δ 3.05 to 3.14 (1-H)–3.38 to 3.46 (1-H) and 3.96–4.08 (2-H); 3.38 to 3.46 (2-H)–3.66 (4-H), 3.94 and 4.09 (6-H); 3.38 to 3.46 (1-H)–3.05 to 3.14 (1-H) and 3.96 to 4.08 (2-H); 3.57 (3-H)–3.66 (4-H) and 3.96 to 4.08 (weak, 2-H); 3.66 (4-H)–3.57 (3-H) and 3.38 to 3.46 (5-H); 3.94 (6-H)–4.09 (6-H) and 3.38 to 3.46 (5-H); 3.96 to 4.08 (2-H)–3.57 (weak, 3-H), 3.38 to 3.46 (1-H) and 3.05 to 3.14 (1-H); 4.09 (6-H)–3.94 (6-H) and 3.38 to 3.46 (5-H); 4.67 (ArCH_2)–4.80 (ArCH_2, *gem*); 5.12 (ArCH_2)–5.19 (ArCH_2, *gem*); 6.90 (PMB ArH)–7.30 (PMB ArH, *gem*); and 7.30–6.90 (PMB ArH, *gem*); *m/z* FAB (3-NOBA) 465 (MNa^+) and 121 (C_8H_9O).

*2-Azido-*N*-benzyloxycarbonyl-6-O-(tert-butyldimethylsilyl)-1,2,5-trideoxy-1,5-imino-4-O-(4-methoxybenzyl)-*D*-glucitol* **11**.—A solution of TBDMSCl (1.06 g, 7.0 mmol) in DMF (16 cm^3) was added during 5 min to a stirred, ice-cooled solution of the diol **10** (1.81 g, 4.1 mmol), imidazole (1.11 g, 16.3 mmol) and 4-(dimethylamino)pyridine (20 mg) in DMF (32 cm^3) under argon. The reaction mixture was warmed to room temperature and was stirred for 24 h to give an oil, which was dissolved in dichloromethane (100 cm^3) and the solution was washed with water (2 × 100 cm^3). The dried organic extract was concentrated to a syrup and purified by chromatography [acetone–toluene (1:24→1:3 gradient elution)] to yield the title compound **11** (1.85 g, 82%) as an oil [R_f 0.24, acetone–toluene (1:24)]; $[\alpha]_D$ −30.8 (*c* 0.5, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3350br (OH), 2110 (N_3), 1690 (C=O) and 1250br; δ(250 MHz) 0.01 and 0.03 (~ 6 H, masked by $SiMe_4$, 2 s, $SiMe_2$), 0.87 (9 H, s, SiBu^t), 1.60 (1 H, br s, OH), 3.49–3.72 (4 H, m), 3.80 (3 H, s, OMe), 3.78–4.17 (4 H, excluding OMe, m), 4.60 (2 H, s, PMB CH_2), 5.16 (2 H, s, CO_2CH_2Ph), 6.87 and 7.26 [4 H, 2 d, each *J* 8.6 (A_2X_2), ArH] and 7.34 (5 H, s, ArH); *m/z* FAB (3-NOBA) 579 (MNa^+) and 121 (C_8H_9O).

*2-Azido-*N*-benzyloxycarbonyl-3-O-(benzyloxycarbonylmethyl)-6-O-(tert-butyldimethylsilyl)-1,2,5-trideoxy-1,5-imino-4-O-(4-methoxybenzyl)-*D*-glucitol* **12**.—A 1.5 mol dm^{-3} solution of butyllithium in hexane (1.2 cm^3, 1.8 mmol) was added during 10 min to a stirred solution of compound **11** (1.0 g, 1.8 mmol) in dry THF (10 cm^3) at −55 °C under argon. After 10 min at this temperature the mixture was treated with a solution of benzyl *O*-(trifluoromethylsulfonyl)glycolate[11] (805 mg, 2.7 mmol) in dry THF (4 cm^3) during 5 min. The solution was maintained at −55°C for 0.5 h after which the cooling bath was removed and the reaction mixture was allowed to warm to room temperature during 2 h. Sodium hydrogen carbonate (800 mg) was then added to the reaction mixture followed, after 5 min, by water (5 cm^3). The mixture was concentrated, diluted with water, and extracted with dichloromethane (2 × 20 cm^3). The combined extracts were dried, and evaporated under reduced pressure to give an oil,

which was purified by chromatography [acetone–toluene; (1:99→1:24 gradient elution)] to afford the title compound **12** (634 mg, 50%) as a syrup [R_f 0.43, acetone–toluene (1:24)]; $[\alpha]_D$ + 1.7 (*c* 1.5, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 2115 (N_3), 1755 (ester C=O), 1690 (amide C=O), 1250br and 1120br; δ(400 MHz) 0.0 and 0.2 (~6 H masked by $SiMe_4$, 2 s, $SiMe_2$), 0.88 (9 H, s, SiBu^t), 3.48 (1 H, dd, $J_{1,2}$ 4.0, $J_{1,1}$ 14.4, C*H*HN), 3.58 (1 H, t, *J* 5.3), 3.81 (3 H, s, OMe), 3.71–3.84 (3 H excluding OMe, m), 3.93 (1 H, t, *J* 5.3), 4.05 (1 H, br d), 4.18–4.21 (1 H, m, C*H*CH_2O), 4.20 and 4.30 [2 H, 2 d, each *J* 16.3 (AB), OCH_2CO], 4.62 (2 H, br s, PMB CH_2), 5.19 and 5.22 (total 4 H, 2 s, 2 × CO_2CH_2Ph), 6.86 and 7.26 [4 H, 2 d, each *J* 8.5 (A_2X_2), ArH] and 7.34–7.41 (10 H, m, ArH); *m/z* FAB (3-NOBA) 727 (MNa^+) and 121 (C_8H_9O).

2-*Azido*-N-*benzyloxycarbonyl*-3-O-(*benzyloxycarbonylmethyl*)-6-O-(tert-*butyldimethylsilyl*)-1,2,5-*trideoxy*-1,5-*imino*-D-*glucitol* **13**.—DDQ (521 mg, 2.3 mmol) was added in portions to a rapidly stirred mixture of compound **12** (1.08 g, 1.5 mmol) in a mixture of dichloromethane (14 cm^3) and water (0.9 cm^3) at room temperature. After 1.3 h, the mixture was filtered through Celite with a little more solvent. The filtrate was washed with saturated aq. sodium hydrogen carbonate (2 × 15 cm^3), dried, and evaporated under reduced pressure to give an oil, which was purified by chromatography [acetone–toluene (1:99→1:24 gradient elution)] to give the title compound **13** (723 mg, 81%) as a pale orange oil [R_f 0.28, acetone–toluene (1:24)]; $[\alpha]_D$ +1.3 (*c* 0.39, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3440br (OH), 2115 (N_3), 1740 (ester C=O), 1690 (amide C=O), 1250br and 1125br; δ(250 MHz) −0.01 and 0.15 (~6 H masked by $SiMe_4$, 2 s, $SiMe_2$), 0.86 (9 H, s, SiBu^t), 3.37 (1 H, dd, $J_{3,4}$ 5.4, $J_{3,2}$ 8.1, C*H*OCH_2), 3.49 (1 H, dd, $J_{1,1}$ 14.8, $J_{1,2}$ 4.4, C*H*HN), 3.74–3.79 (1 H, m, CHN_3), 3.79 (1 H, br s, D_2O-exch, OH), 3.85–3.94 (3 H, m, $CHCH_2OSi$), 4.0–4.06 (1 H, m, C*H*OH), 4.09 (1 H, br d, $J_{1,1}$ 14.8, CH*H*N), 4.30 and 4.42 [2 H, 2 d, each *J* 16.9 (AB), OCH_2CO_2], 5.12 and 5.19 [2 H, 2 d, each *J* 12.3 (AB), CO_2CH_2], 5.21 (2 H, s, CO_2CH_2) and 7.35–7.38 (10 H, m, ArH); ^1H–^1H correlations δ 3.37 (3-H)–3.74 to 3.79 (2-H), 4.0 to 4.06 (4-H); 3.49 (1-H)–3.74 to 3.79 (2-H), 4.09 (1-H); 3.74 to 3.79 (2-H)–3.49 (1-H), 4.09 (1-H), 3.37 (3-H); 3.85 to 3.94 [5-H, 6-H, 6-H (*gem*)]–3.85 to 3.94; 4.0 to 4.06 (4-H)–3.37 (3-H), 3.94 (5-H); 4.09 (1-H)–3.49 (1-H), 3.74 to 3.79 (2-H); 4.30 (7-H)–4.42 (7-H); 5.12 (ArC*H*$_2$)–5.19 (ArC*H*$_2$); *m/z* FAB (3-NOBA) 607 (MNa^+) and 585 (MH^+).

2-*Azido*-N-*benzyloxycarbonyl*-3-O-(*benzyloxycarbonylmethyl*)-6-O-(tert-*butyldimethylsilyl*)-1,2,5-*trideoxy*-1,5-*imino*-4-O-(3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-D-*glucitol* **16**.—Boron trifluoride–diethyl ether (46 mm^3, 0.37 mmol) was added to a stirred solution of the alcohol **13** (723 mg, 1.2 mmol) and the imidate **15** [12] (1.1 g, 1.9 mmol) in dry dichloromethane (7 cm^3) at −20 °C under argon. After 2 h at −20 °C the mixture was treated with sodium hydrogen carbonate (100 mg) followed, after 5 min, by water (3 cm^3) and then the mixture was warmed to room temperature. The layers were separated and the aqueous fraction was extracted with dichloromethane (2 × 10 cm^3). The combined organic phases were dried, and evaporated under reduced pressure to afford an oil, which was partially purified by chromatography [EtOAc–hexane (1:9→3:7 gradient elution)] to give an oil (1.1 g). The oil was stirred with tetrachloromethane (5 cm^3) and the precipitated trichloroacetamide by-product was removed by filtration. The filtrate was evaporated under reduced pressure to produce the title compound **16** (1.0 g, 84%) as a syrup [R_f 0.49, EtOAc–hexane (2:3)]; $[\alpha]_D$ +10.8 (*c* 0.37, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 2115 (N_3), 1775sh (Phth C=O, sym), 1745 (ester C=O), 1720 (Phth C=O, asym), 1695sh (amide C=O) and 1230br; δ(400 MHz) 0.0 and 0.07 (~6 H masked by $SiMe_4$, 2 s, $SiMe_2$), 0.88 (9 H, s, SiBu^t), 1.83 and 2.02 (9 H, 2 s, 3 × Ac), 3.20 (1 H, d, $J_{1,1}$ 14.3, C*H*HN), 3.49 (1 H, br t, *J* ~7.5), 3.61 (1 H, br s), 3.69–3.88 (4 H, m), 4.1–4.6 (8 H, m), 5.04–5.24 (4 H, m, 2 × CO_2CH_2Ph), 5.44 (1 H, d, $J_{1,2'}$ 8.1, 1′-H), 5.73–5.9 (1 H, br m, 3′-H), 7.15–7.36 (10 H, m, 2 × Ph) and 7.63–7.88 (4 H, m, Phth); *m/z* FAB (3-NOBA) 1024 (MNa^+), 1002 (MH^+), 298 ($C_{16}H_{12}NO_5$) and 256 ($C_{14}H_{10}NO_4$).

2-*Acetamido*-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-1,2,5-*trideoxy*-3-O-(*ethoxycarbonylmethyl*)-1,5-*imino*-4-O-(3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-D-*glucitol* **17**.—A suspension of tellurium powder (200 mesh; 344 mg, 2.7 mmol) and sodium borohydride (252 mg, 6.7 mmol) in dry ethanol was heated under reflux for 2.5 h under argon. The deep-red solution was then slowly cooled to room temperature and to it was added a solution of compound **16** (1.0 g, 1.0 mmol) in dry diethyl ether (29 cm^3) during 5 min. The resulting black mixture was stirred for 15 min at room temperature after which time the reaction flask was opened to the atmosphere and stirred for a further 0.5 h. The reaction mixture was filtered through Celite and the colourless filtrate was evaporated under reduced pressure to leave an oily solid. The oily solid was dissolved in dry pyridine (36 cm^3) and to the solution was added acetic anhydride (20 cm^3). The solution was stirred at room temperature for 18 h and was then evaporated under reduced pressure to leave an oil, which was dissolved in dichloromethane (100 cm^3) and the solution was washed with water (100 cm^3). The aqueous layer was extracted with dichloromethane (50 cm^3) and the combined organic phases were dried, and evaporated under reduced pressure to leave an oil, which was purified by chromatography [acetone–toluene (1:50→3:7 gradient elution)] to give the title compound **17** (447 mg, 45%) as an oil [R_f 0.39, acetone–toluene (3:7)]; $[\alpha]_D$ −6.0 (*c* 0.23, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420 (NH), 1775sh (Phth C=O, sym), 1750 (ester C=O), 1720 (Phth C=O, asym), 1690sh, 1670sh (amide C=O) and 1230br; δ(250 MHz) −0.01 and 0.03 (~6 H, masked by $SiMe_4$, 2 s, $SiMe_2$), 0.83, 0.85 and 0.88 (9 H, 3 s, SiBu^t), 1.27 (3 H, t, *J* 7.2, *Me*CH_2), 1.85, 2.04, 2.06 and 2.12 (total 12 H, 4 s, 4 × Ac), 3.15 (1 H, d, $J_{1,1}$ 12.8, C*H*HN), 3.47 (1 H, dd, *J* 9.1 and 6.5), 3.62–4.42 (~16 H, m), 5.17 (1 H, t, $J_{4',3'} \sim J_{4',5'}$ ~ 10.0, 4′-H), 5.33 (1 H, d, $J_{1',2'}$ 8.5, 1′-H), 6.0 (1 H, t, $J_{3',2'} \sim J_{3',4'}$ ~ 9.9, 3′-H), 6.34 (1 H, d, *J* 8.5, D_2O-exch, N*H*Ac), 7.05–7.35 (5 H, m, Ph) and 7.61–8.0 (4 H, m, Phth); *m/z* FAB (3-NOBA) 978 (MNa^+), 956 (MH^+), 298 ($C_{16}H_{12}NO_5$) and 256 ($C_{14}H_{10}NO_4$).

2-*Acetamido*-4-O-(2′-*acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-3-O-*carboxymethyl*-1,2,5-*trideoxy*-1,5-*imino*-D-*glucitol* **19**.—Aq. potassium hydroxide (1 mol dm^{-3}; 2.4 cm^3, 2.4 mmol) was added dropwise to a stirred solution of compound **17** (380 mg, 0.4 mmol) in methanol (9.8 cm^3) at room temperature. After 3 h the solution was stirred for 10 min with ion-exchange resin [CG120 (H^+-form), 2 g]. The resin was removed by filtration and the filtrate was evaporated under reduced pressure to give a foam (310 mg). A solution of this material (310 mg) and hydrazine monohydrate (190 mm^3, 3.9 mmol) in ethanol (21 cm^3) was heated under reflux for 20 h. The solution was evaporated under reduced pressure to give an oil, which was reconcentrated with toluene (2 × 20 cm^3). The residue was kept at room temperature for 3 h *in vacuo* and was then stirred at room temperature for 20 h with acetic anhydride (7 cm^3) and pyridine (9.5 cm^3). The solution was evaporated under reduced pressure to afford an oil, which was dissolved in dichloromethane (20 cm^3) and the solution was stirred for 5 min with CG120 resin (H^+-form) (0.5 g). The resin was filtered off and the filtrate was washed with water (20 cm^3), then was dried, and evaporated under reduced pressure to leave an oil. The oil

was purified by chromatography [EtOAc; EtOAc–EtOH (9:1); EtOAc–EtOH–water (36:3:1; 16:3:1; 12:5:3)] to give the title compound **19** as a glass (218 mg, 65%) [R_f 0.12, EtOAc–EtOH–water (36:3:1)]; $[\alpha]_D$ −19.9 (*c* 0.17, MeOH); ν_{max}(KBr)/cm^{-1} 3405br, 1750 (ester C=O), 1700br, 1640br and 1240; δ(250 MHz; CD_3OD) 0.06 and 0.1 (~6 H masked by $SiMe_4$, 2 s, $SiMe_2$), 0.91 and 0.94 (9 H, 2 s, SiBu^t), 1.79, 1.88, 2.02, 2.05, and 2.09 and 2.10 (total 15 H, 5 s, 5 × Ac), 3.45 (1 H, d, $J_{1,1}$ 14.2, C*H*HN), 3.68–4.68 (~16 H, m), 4.95–5.37 (~5 H, partly obscured by HOD, m) and 7.34–7.43 (5 H, m, ArH); *m/z* FAB (THIOG) 862 (MNa^+), 840 (MH^+), 330 ($C_{14}H_{20}NO_8$), 210 ($C_{10}H_{12}NO_4$) and 168 ($C_8H_{10}NO_3$).

N-{[2-*Acetamido*-4-O-(2′-*acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-1,2,3,5-*tetradeoxy*-1,5-*imino*-D-*glucitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-D-*glutamic Acid Dibenzyl Ester* **20**.—HOBt hydrate (9.7 mg, 71 μmol) was added to a stirred solution of L-alanyl-D-glutamic acid dibenzyl ester hydrochloride [1] (34 mg, 77 μmol) and compound **19** (50 mg, 60 μmol) in DMF (0.5 cm^3) at room temperature. This was followed by the successive additions of NMM (7.2 mm^3, 65 μmol) and a solution of DCC (14.7 mg, 71 μmol) in THF (0.5 cm^3). The solution was stirred for 22 h and was then evaporated under reduced pressure to give an oil, which was triturated with tetrachloromethane (1 cm^3). The suspended *N,N*′-dicyclohexylurea was filtered off and the filtrate was diluted with dichloromethane (5 cm^3) and washed with water (5 cm^3). After being dried, the organic phase was evaporated under reduced pressure to afford an oil, and this was purified by chromatography [EtOAc; EtOAc–EtOH–water (36:3:1)] to give the title compound **20** (57 mg, 78%) as a foam [R_f 0.71, EtOAc–EtOH–water (36:3:1)]; $[\alpha]_D$ −24.2 (*c* 0.19, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3400br (NH), 3350sh br (NH), 1738 (ester C=O), 1685sh, 1665 and 1230; δ(400 MHz) − 0.02 and 0.04 (6 H, 2 s, $SiMe_2$), 0.87 (9 H, s, SiBu^t), 1.40 (3 H, d, *J* 7.0, Ala Me), 1.80, 2.04, 2.05, 2.10 and 2.11 (total ~15 H overlapping, 5 s, 5 × Ac), 2.02–2.46 (4 H overlapping, m, Glu CH_2CH_2), 3.27 (1 H, d, $J_{1,1}$ 12.4, equatorial C*H*HN), 3.56–3.61 (2 H, m), 3.66 (1 H, dd, *J* 7.1 and 9.6), 3.82 (1 H, t, *J* 9.7), 4.02–4.15 (5 H, m), 4.26–4.42 (5 H, m), 4.49 (1 H, t, *J* 7.3 becomes q after D_2O-exch), 4.62 (1 H, dd, *J* 7.8 and 4.9), 4.97–5.17 (8 H, m, 3′- and 4′-H and 3 × CO_2CH_2Ph), 5.78 (1 H, d, *J* 8.9, D_2O-exch, NH), 6.83 (1 H, d, *J* 7.5, D_2O-exch, NH), 6.93 (1 H, d, *J* 9.0, D_2O-exch, NH), 7.03 (1 H, d, *J* 7.5, D_2O-exch, NH) and 7.30–7.36 (15 H, m, 3 × Ph); *m/z* FAB (3-NOBA) 1242 (MNa^+), 1220 (MH^+), 330 ($C_{14}H_{20}NO_8$) and 210 ($C_{10}H_{12}NO_4$).

N-{[2-*Acetamido*-4-O-(2′-*acetamido*-2′-*deoxy*-β-D-*glucopyranosyl*)-1,2,3,5-*tetradeoxy*-1,5-*imino*-D-*glucitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-D-*glutamic Acid* **21**.—A solution of compound **20** (54 mg, 44 μmol) in glacial acetic acid (3 cm^3)–water (1.5 cm^3) with suspended palladium black (54 mg) was shaken at room temperature for 22 h with hydrogen at atmospheric pressure. The catalyst was removed by filtration and was washed with the same solvent mixture (1 cm^3). The combined washings and filtrate were heated at 60 °C for 3 h, cooled, and evaporated under reduced pressure and the residue was concentrated from toluene (2 × 10 cm^3) to give an oil (43 mg) [R_f 0.64, EtOAc–EtOH–water (1:1:1)]. The oil was dissolved in methanol (3 cm^3) and conc. aq. ammonium hydroxide (relative density 0.88; 0.3 cm^3) was added. The solution was covered and stirred at room temperature for 20 h. After this time the solution was evaporated under reduced pressure to afford an oily residue, which was purified by chromatography [graded elution: EtOAc–EtOH–water (7:2:1; 12:5:3; 11:5:4; 9:7:4; 1:1:1)]. The product was dissolved in water (2 cm^3) and the solution was freeze-dried to yield the *title compound* **21** (21.4 mg, 73% from compound **20**) as an amorphous solid [R_f 0.40, EtOAc–EtOH–water (1:1:1)] (Found: *m/z*, 666.2830. $C_{26}H_{44}N_5O_{15}$ requires MH, 666.2834); $[\alpha]_D$ −26.4 (*c* 0.05, water); ν_{max}(KBr)/cm^{-1} 3392br, 1652 and 1559; δ(400 MHz; D_2O) 1.48 (3 H, d, *J* 7.2, Ala Me), 1.89–1.98 (1 H, m, Glu C*H*HCH$_2$CO); 2.03 (3 H, s, sugar Ac), 2.11 (3 H, s, piperidine Ac), 2.09–2.17 (1 H, m, Glu CH*H*CH$_2$CO), 2.25 (2 H, br t, *J* 6.7, Glu CH$_2$C*H*$_2$CO), 2.67 (1 H, br t, $J_{1,1}$ ~ $J_{1,2}$ ~ 12.3, axial C*H*HN), 2.89 (1 H, br s, piperidine C*H*CH$_2$OH), 3.24 (1 H, dd, $J_{1,1}$ 12.3, $J_{1,2}$ 4.3, equatorial CH*H*N), 3.38–3.41 (1 H, m, 5′-H), 3.46 (1 H, dd, $J_{4',5'}$ 9.7, $J_{4',3'}$ 8.7, 4′-H), 3.58 (1 H, dd, $J_{3',2'}$ 10.3, $J_{3',4'}$ 8.7), 3.65 (1 H, t, $J_{3,2}$ ~ $J_{3,4}$ ~ 9.9, piperidine C*H*OCH$_2$), 3.67–3.75 (2 H, m, 6′-H and piperidine C*H*HOH), 3.76 (1 H, dd, $J_{2',3'}$ 10.3, $J_{2',1'}$ 8.3, 2′-H), 3.82–3.90 (3 H, m, 6′-H and piperidine CH*H*OH and C*H*OCH), 4.04 (1 H, dt, $J_{2,1'}$ 12.3 and 4.3, C*H*NHAc), 4.21 (1 H, dd, *J* 8.9 and 4.4, Glu C*H*CO$_2$H), 4.29 [1 H, d, *J* 15.3 (AB), OC*H*HCO], 4.51 (1 H, q, *J* 7.1, Ala CH), 4.60 [1 H, d, *J* 15.3 (AB), OCH*H*CO] and 4.73 (1 H, d, $J_{1',2'}$ 8.3, 1′-H); ^{1}H–^{1}H correlations: 9-H–8-H; 11-H (δ 1.89–1.98)–11-H (*gem*), 12-H, 10-H; 11-H (δ 2.09–2.17)–11-H (*gem*), 12-H, 10-H; 12-H–11-H, 11-H; 1-H (δ 2.67)–1-H (*gem*), 2-H; 5-H–6-H, 6-H, 4-H (very weak); 1-H (δ 3.24)–1-H (*gem*), 2-H; 5′-H–4′-H, 6′-H, 6′-H; 4′-H–5′-H, 3′-H; 3′-H–4′-H, 2′-H; 3-H–4-H (δ 3.86, t), 2-H; 6-H (δ ~ 3.7)–6-H (*gem*), 5-H; 6′-H (δ ~ 3.74)–6′-H (*gem*), 5′-H; 2′-H–3′-H, 1′-H; 4-H (δ 3.86)–5-H (very weak), 3-H; 6′-H (δ ~ 3.88)–6′-H (*gem*), 5′-H; 6-H (δ ~ 3.9)–6-H (*gem*), 5-H; 2-H–3-H, 1-H, 1-H; 10-H–11-H, 11-H; 7-H (δ 4.29)–7 H (*gem*); 8-H–9-H; 7-H (δ 4.60)–7-H (*gem*); 1′-H–2′-H; *m/z* FAB (THIOG) 688 (MNa^+) and 666 (MH^+).

N-{[2-*Acetamido*-4-O-(2′-*acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-1,2,3,5-*tetradeoxy*-1,5-*imino*-D-*glucitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-D-*glutamine Benzyl Ester* **22**.—In a similar manner to that described for the preparation of compound **20** the acid **19** (50 mg) and L-alanyl-D-glutamine benzyl ester hydrochloride* (26.8 mg) afforded, after chromatography [graded elution: EtOAc–EtOH–water (95:4:1; 36:3:1; 7:2:1)], the title compound **22** (51 mg, 75%) as a glass; {R_f 0.44, EtOAc–EtOH–water (36:3:1)]; $[\alpha]_D$ −21.9 (*c* 0.11, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3420br, 3360br, 1740 (ester C=O), 1670br (amide C=O) and 1230; δ(250 MHz) 0.0 and 0.05 (~6 H masked by $SiMe_4$, 2 s, $SiMe_2$), 0.87 (9 H, s, SiBu^t), 1.43 (3 H, d, *J* 7.0, Ala Me), 1.72, 1.79, 2.03, 2.05, 2.09 and 2.10 (total 15 H, 6 s, 5 × Ac), 2.0–2.29 (~4 H overlapping, m, Gln CH_2CH_2), 3.78 (1 H, d, $J_{1,1}$ 12.5, axial C*H*HN), 3.54–3.68 (3 H, m), 3.84 (1 H, t, *J* 9.7), 4.01–4.58 (~12 H, m), 4.96–5.21 (6 H, m, 3′-, 4′-H and 2 × CO_2CH_2Ph), 5.74 (1 H, d, *J* 9.0, D_2O-exch, NH), 5.70 and 6.0 (total 2 H, 2 br s, D_2O-exch, NH_2), 6.99 (1 H, d, *J* 7.4, D_2O-exch, NH), 7.02 (1 H, d, *J* 7.4, D_2O-exch, NH) and 7.29–7.40 (11 H reduces to 10 H after D_2O-exch, m, NH and 2 × Ph); *m/z* FAB (THIOG) 1129 (MH^+), 330 ($C_{14}H_{20}NO_8$), 210 ($C_{10}H_{12}NO_3$) and 168 ($C_8H_{10}NO_3$).

N-{[2-*Acetamido*-4-O-(2′-*acetamido*-2′-*deoxy*-β-D-*glucopyranosyl*)-1,2,3,5-*tetradeoxy*-1,5-*imino*-D-*glucitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-D-*glutamine* **23**.—In a similar manner to that described for the preparation of compound **21**, the dipeptide **22** (48 mg) was hydrogenolysed, desilylated and *O*-deacetylated to give, after chromatography [graded elution: EtOAc–EtOH–water (12:5:2; 11:5:4; 9:7:4; 1:1:1)], the *title compound* **23** (22 mg, 74% from compound **22**) as a freeze-dried, amorphous solid [R_f 0.25, EtOAc–EtOH–water (9:7:4)] (Found: *m/z*, 665.3016. $C_{26}H_{45}N_6O_{14}$ requires MH,

* See footnote on p. 1790.

665.2994); $[\alpha]_D$ − 22.7 (*c* 0.12, water); ν_{max}(KBr)/cm^{-1} 3340br, 1654 and 1558; δ(250 MHz; D_2O; resolution enhanced) 1.38 (3 H, d, *J* 7.1, Ala Me), 1.93 and 2.01 (total 6 H overlapping, 2 s, 2 × NH*Ac*), 1.83–2.15 (2 H, overlapping, m, Gln C*H*$_2$CH$_2$CO), 2.24 (2 H, t, *J* 7.2, Gln CH_2CO), 2.56 (1 H, br t, $J_{1,1}$ ~ $J_{1a,2}$ ~ 12.4, piperidine axial C*H*HN), 2.79 (1 H, br s, piperidine C*H*CH$_2$OH), 3.16 (1 H, dd, $J_{1,1}$ 12.4, $J_{1e,2}$ 4.8, piperidine equatorial CH*H*N), 3.28–3.81 (10 H, m), 3.93 (1 H, dt, $J_{1,2}$ ~ $J_{2,3}$ ~ 10.4, piperidine C*H*NHAc), 4.15 (1 H, dd, *J* 8.6 and 4.6, Gln C*H*CO$_2$H), 4.20 [1 H, d, *J* 15.3 (AB), OC*H*HCO], 4.38 (1 H, q, *J* 7.2, Ala C*H*Me), 4.52 [1 H, d, *J* 15.3 (AB), OC*H*HCO] and 4.61 (1 H, d, $J_{1',2'}$ 8.5, 1'-H); *m/z* FAB (THIOG) 687 (MNa^+) and 665 (MH^+).

N-{[2-*Acetamido*-4-O-(2'-*acetamido*-3',4',6'-*tri*-O-*acetyl*-2'-*deoxy*-β-D-*glucopyranosyl*)-N-*benzyloxycarbonyl*-6-O-(*tert*-*butyldimethylsilyl*)-1,2,3,5-*tetradeoxy*-1,5-*imino*-D-*glucitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-γ-D-*glutamyl*-N$^{\varepsilon}$-*benzyloxycarbonyl*-L-*lysine Dibenzyl Ester* **24**.—In a similar manner to that described for the preparation of compound **20** the acid **19** (50 mg) and L-alanyl-γ-D-glutamyl-ε-(*N*-benzyloxycarbonyl)-L-lysine dibenzyl ester hydrochloride[1] (54 mg) afforded, after chromatography [acetone–toluene (1:9→3:2 gradient elution)], the title compound **24** (61 mg, 69%) as a syrup [R_f 0.47, acetone–toluene (3:2)]; $[\alpha]_D$ −14.6 (*c* 0.21, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3420br (NH), 3340br (NH), 1740 (ester C=O), 1690sh and 1660 (amide C=O) and 1230; δ(250 MHz) 0.0 (~6 H masked by $SiMe_4$, br s, $SiMe_2$), 0.87 (9 H, s, SiBu^t), 1.39 (3 H overlapping, d, *J* 6.6, Ala Me), 1.30–1.80 (6 H overlapping, m, Lys C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$N), 1.79, 2.03, 2.04, 2.09 and 2.10 (15 H overlapping, 5 s, 5 × Ac), 1.9–2.3 (4 H overlapping, m, Glu CH_2CH_2CO), 3.12 (2 H, br, q, *J* 5.9 becomes t, *J* 6.3, after D_2O-exch, Lys CH_2N), 3.25 (1 H, br d, $J_{1,1}$ 13.5, piperidine C*H*HN), 3.54–3.68 (3 H, m), 3.82 (1 H, t, *J* 9.6), 3.98–4.55 (12 H, m), 4.95–5.22 (11 H, m, 1'-, 3'-, 4'-H and 4 × CO_2CH_2Ph), 5.71 (1 H, d, *J* 8.9, D_2O-exch, NH), 6.92 (1 H, d, *J* ~ 6, D_2O-exch, NH), 6.99 (2 H, d, *J* 7.5, D_2O-exch, 2 × NH) and 7.16–7.33 (~22 H reduces to ~20 H after D_2O-exch, m, 2 × NH and 4 × Ph); *m/z* FAB (3-NOBA) 1504 (MNa^+), 1482 (MH^+) and 91 (C_7H_7).

N-{[2-*Acetamido*-4-O-(2'-*acetamido*-2'-*deoxy*-β-D-*glucopyranosyl*)-1,2,3,5-*tetradeoxy*-1,5-*imino*-D-*glucitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-γ-D-*glutamyl*-L-*lysine* **25**.—In a similar manner to that described for the preparation of compound **21**, the tripeptide **24** (58 mg) was hydrogenolysed over palladium black (58 mg), desilylated at 60 °C (3 h), and *O*-deacetylated by treatment with aq. ammonium hydroxide in methanol to afford, after chromatography [graded elution: EtOAc–EtOH–water (11:5:4; 9:7:4; 1:1:1)], the *title compound* **25** (23 mg, 74% from compound **24**) as a freeze-dried, amorphous solid [R_f 0.21, EtOAc–EtOH–water (1:1:1)] (Found: *m/z*, 794.3828. $C_{32}H_{55}N_7O_{16}$ requires MH, 794.3784); $[\alpha]_D$ −23.4 (*c* 0.11, water); ν_{max}(KBr)/cm^{-1} 3400br, 1646 (amide C=O) and 1559br; δ(400 MHz; D_2O) 1.41 (3 H overlapping, d, *J* 7.2, Ala Me), 1.36–1.44 (2 H overlapping, m, $CH_2CH_2[CH_2]_2N$), 1.61–1.73 (3 H, m, C*H*HCH$_2$C*H*$_2$CH$_2$N), 1.76–1.85 (1 H, m, CH*H*[CH$_2$]$_3$N), 1.98 (3 H, s, sugar NH*Ac*), 2.05 (3 H, s, piperidine NH*Ac*), 1.90–2.15 (2 H, m, Glu C*H*$_2$CH$_2$CO), 2.31 (2 H, t, *J* 7.8, Glu CH_2CO), 2.73 (1 H, br t, $J_{1,1}$ ~ $J_{1,2}$ ~ 12, piperidine C*H*HN), 2.98 (3 H, t, *J* 7.4, Lys CH_2N and piperidine C*H*CH$_2$OH), 3.29 (1 H, dd, $J_{1,1}$ 12.5, $J_{1,2}$ 4.4, piperidine CH*H*N), 3.34–3.37 (1 H, m, 5'-H), 3.41 (1 H, dd, $J_{4',3'}$ ~ $J_{4',5'}$ ~ 8.7, 4'-H), 3.52 (1 H, dd, $J_{3',2'}$ ~ $J_{3',4'}$ ~ 8.6, 3'-H), 3.61–3.70 (3 H, m, 6'-H and piperidine CHC*H*HOH and C*H*OCH$_2$), 3.72 (1 H, dd, $J_{2',1'}$ ~ $J_{2',3'}$ ~ 9, 2'-H), 3.80–3.92 (3 H, m, 6'-H and piperidine C*H*OCH and CHCH*H*OH), 4.07 (1 H, dt, $J_{2,3}$ ~ $J_{2,1}$ ~ 11.1, $J_{2,1}$ 4.5, piperidine C*H*NHAc), 4.15 (1 H, dd, *J* 5.0 and 8.3, Lys C*H*CO$_2$H), 4.19 (1 H, dd, *J* 5.1 and 7.5, Glu C*H*CO$_2$H), 4.25 [1 H, d, *J* 15.4 (AB), OCH*H*CO], 4.45 (1 H, q, *J* 7.2, Ala CH), 4.55 [1 H, d, *J* 15.4 (AB), OC*H*HCO] and 4.66 (1 H, d, $J_{1',2'}$ 8.3, 1'-H); ^{1}H–^{1}H correlations: 9-H–8-H; 15-H–14-H, 16-H; 16-H (δ ~ 1.7)–15-H, 17-H; 14-H (δ ~ 1.7)–15-H, 13-H, 14-H (*gem*); 14-H (δ ~ 1.8)–15-H, 13-H, 14-H (*gem*); 11-H (δ ~ 2.0)–12-H, 10-H, 11-H (*gem*); 11-H (δ ~ 2.1)–12-H, 10-H, 11-H (*gem*); 12-H–11-H, 11-H; 1-H (δ 2.73)–2-H, 1-H (*gem*); 17-H–16-H; 5-H–4-H, 6-H (weak), 6-H (weak); 1-H (δ 3.29)–1-H (*gem*), 2-H (very weak); 5'-H–4'-H, 6'-H (δ 3.7), 6'-H (δ 3.82); 4'-H–3'-H, 5'-H; 3'-H–2'-H, 4'-H; 3-H (δ 3.66)–4-H (δ 3.90), 2-H; 6-H (δ 3.69)–6-H (δ ~ 3.86), 5-H (weak); 6'-H (δ 3.7)–5'-H, 6'-H (δ 3.82); 2-H–3-H, 1-H (δ 2.73), 1-H (δ 3.29, very weak); 13-H–14-H, 14-H; 10-H–11-H, 11-H; 7-H (δ 4.25)–7-H (δ 4.55, *gem*); 8-H–9-H; 7-H (δ 4.55)–7-H (*gem*); 1'-H–2'-H; *m/z* FAB (THIOG) 794 (MH^+).

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