Enantiospecific Synthesis of Aza-analogues of the *N*-Acetylglucosamine-*N*-acetylmuramic Acid Repeating Unit in Peptidoglycan

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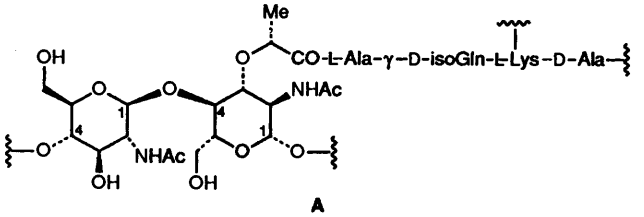
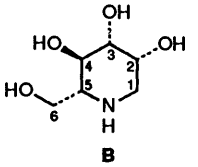
Two substituted piperidyl glycosides, the L-alanyl-D-glutamic acid derivative **22** and the L-alanyl-γ-D-glutamyl-L-lysine derivative **25**, have been prepared as aza-analogues of the repeating disaccharide unit in peptidoglycan and screened as potential antibacterial agents. Starting from diacetoneglucose **1**, the synthesis of an anomeric mixture of intermediate 5-*O*-benzyl-*N*-benzyloxycarbonyl-2,6-dideoxy-2,6-imino-3-*O*-(4-methoxybenzyl)-D-mannofuranosides **11**α/**11**β (α:β ratio ~1:1) is described. Their transformation into the 1,5-dideoxy-1,5-imino-D-mannitol **15** and its corresponding β-glycoside derivative **18**, a common and advanced intermediate *en route* to the aza-analogues **22** and **25**, is also described. Neither product showed any antibacterial activity.

Peptidoglycan, the stress-bearing constituent of the bacterial cell wall, is composed of repeating units of β-1,4-linked *N*-acetylglucosamine-*N*-acetylmuramic acid (NAG-NAM) residues.[1] These glycan strands are mostly cross-linked through relatively short-chain peptides located on the D-lactyl ether moiety on the muramyl ring. The majority of peptides in any bacterial sacculus consists of a sequence of four α-amino acids with alternating configuration L-D-L-D in which the third residue on one glycan strand is attached either directly or through a peptide bridge to the fourth residue on an adjacent strand; thus the cell wall is a macromolecular network which has high tensile strength and rigidity. The nature of the amino acids varies slightly with species and **A**, for example, shows the structure of the repeating unit of peptidoglycan in *Staphylococcus aureus*; a pentaglycine bridge connects adjacent glycan strands.



A



B

The integrity of peptidoglycan is regulated by the action of a number of enzymes which maintain a balance between its synthesis and degradation and control processes such as cell division and cell shaping.[2] For example, both the formation and cleavage of the β-1,4-glycoside bond between NAG-NAM units is catalysed by transglycosylase and glycosidase enzymes, respectively.[3]

Many well-established antibacterial agents, such as β-lactams, vancomycin and moenomycin, upset this balance by inhibiting enzymes involved in the final stages of peptidoglycan assembly.[2] Many synthetic and naturally occurring polyhydroxylated piperidines, aza-analogues of pyranoses in which the ring oxygen atom has been replaced by nitrogen, such as deoxymannojirimycin **B**,† have been shown to be potent and specific inhibitors of glycosidase enzymes,[4] and some have demonstrated antibacterial activity.[5] We wished to investigate whether aza-analogues of NAG-NAM disaccharides would affect the enzymic control of peptidoglycan assembly. This paper describes the enantiospecific synthesis of two aza-analogues of the NAG-NAM unit in peptidoglycan as potential antibacterial agents.

Results and Discussion

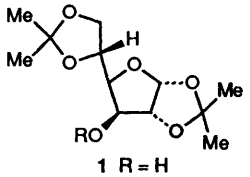
From a synthetic and biological viewpoint, deoxymannojirimycin was chosen as an aza-sugar replacement for the *N*-acetylmuramic acid ring of the NAG-NAM unit. We hoped that a reported synthesis from diacetoneglucose[6] could be modified to meet our needs *via* a change in the protecting-group strategy, by which deoxymannojirimycin could be derivatised at C-3 with a lactyl-bridged peptide and at C-4 by a β-linked *N*-acetylglucosamine ring.

Regarding the transformation of diacetoneglucose **1** into the advanced intermediate aza-sugar **15**, the hydroxy group in compound **1** corresponds to that in target molecule **15**. During the synthesis, the protective group at this position is required to survive catalytic hydrogenation and acidic and strongly basic conditions but be selectively labile in the presence of silyl and benzyl ethers, a benzyl ester and a carbamate function. The *p*-methoxybenzyl[7] (PMB) group, removable with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), was found adequately to meet these requirements.

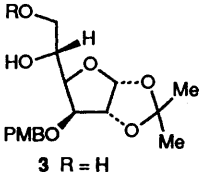
The *p*-methoxybenzyl derivative **2** was prepared as a crude oil by treatment of diacetoneglucose **1** with *p*-methoxybenzyl chloride and sodium hydride.[8] Selective hydrolysis of the 5,6-isopropylidene group in compound **2** with a 0.05 mol dm^{-3} solution of hydrochloric acid in methanol afforded the diol **3**, which was tosylated at the primary hydroxy group to give compound **4** as a pure oil after column chromatography in 64% overall yield from diacetoneglucose. Reaction of the tosyl derivative **4** with excess of sodium azide gave the crude azido alcohol **5**, from which the benzyl ether **6** was prepared as a pure oil in 74% yield from compound **4**. Treatment of the benzyl

† Systematic name 1,5-dideoxy-1,5-imino-D-mannitol.

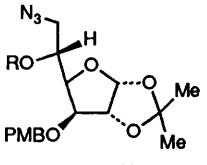
ether **6** with 0.5 mol dm^{-3} hydrochloric acid in methanol afforded a mixture of the required alcohols **7α/7β** (α:β ratio ~2:3 by ^1H NMR spectroscopy) in 79% yield. In addition, a mixture of diols **8α/8β** (17% yield) was formed, arising from the slight instability of the PMB ether group to acid. The ^1H NMR spectra of the separated anomers of the mixture **7α/7β** are comparable to the ^1H NMR spectra recorded for the α- and β-anomer of the dibenzyl analogue (**7α/7β** where $R^1 = CH_2Ph$) reported by Fleet *et al.*[6] The anomeric configurations of the two methyl glycosides were assigned by inspection of their ^1H NMR spectra: the anomeric proton in isomer **7α** appears at δ 5.02 as a doublet (J_{12} 4.5 Hz) whereas the corresponding proton in isomer **7β** appears as a singlet at δ 4.80. These assignments were confirmed by nuclear Overhauser enhancement (NOE) difference experiments on both anomers: irradiation of the 1-H atom in the ^1H NMR spectrum of isomer **7α** gave rise only to enhancements of the 2-H atom (3.2%) and the anomeric OMe signal (0.8%); on the other hand, irradiation of the 1-H atom in the spectrum of isomer **7β** gave rise to enhancements of the 2-H atom (1.1%), the anomeric OMe signal (0.8%) and the 4-H atom (1.9%).



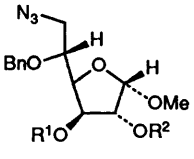
1 R = H
2 R = PMB



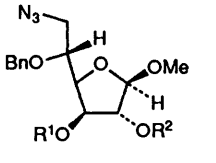
3 R = H
4 R = Ts



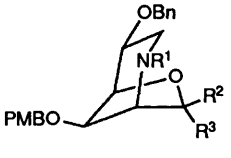
5 R = H
6 R = Bn



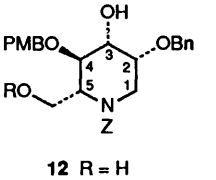
7α R^1 = PMB, R^2 = H
8α $R^1 = R^2$ = H
9α R^1 = PMB, R^2 = Tf



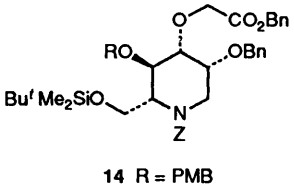
7β R^1 = PMB, R^2 = H
8β $R^1 = R^2$ = H
9β R^1 = PMB, R^2 = Tf



10α $R^1 = R^2$ = H, R^3 = OMe
10β $R^1 = R^3$ = H, R^2 = OMe
11α R^1 = Z, R^2 = H, R^3 = OMe
11β R^1 = Z, R^2 = OMe, R^3 = H



12 R = H
13 R = TBDMS



14 R = PMB
15 R = H

PMB = 4-$MeOC_6H_4CH_2$, Ts = 4-$MeC_6H_4SO_2$, Bn = $PhCH_2$, Tf = CF_3SO_2, Z = $PhCH_2OCO$, TBDMS = Bu^tMe_2Si

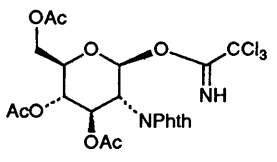
Treatment of the mixture **7α/7β** with trifluoromethanesulfonic anhydride at −50 °C gave a corresponding mixture of triflates **9α/9β** (α:β ~1:1), from which the piperidine ring system was formed *via* reduction of the azide group to amine under catalytic hydrogenation conditions, followed by cyclisation through intramolecular displacement of the triflate group. The mixture of secondary bicyclic amines **10α/10β** so formed was isolated as the *N*-benzyloxycarbonyl derivatives **11α/11β** (α:β ~1:1) in 59% yield from substrates **7α/7β**. The ^1H NMR spectra of carbamates **11α/11β** and all subsequent compounds which contain the NCO_2CH_2Ph moiety were complicated by doubling of some signals due to the existence of rotamers about the amide bond in these molecules; evidently at 27 °C the speed of rotation about this bond is slow on the NMR time-scale. In addition, it is noteworthy that in this part of the synthetic sequence involving the formation of anomeric mixtures there is no benefit to be gained in their separation before they are progressed. Acetal hydrolysis of the bicyclic mixture **11α/11β** ensued upon brief treatment with trifluoroacetic acid (TFA)–water–tetrahydrofuran (THF) (3:3:1), which was followed by sodium borohydride reduction of the aldehyde function, affording the dihydroxypiperidine derivative **12** as an oil in 83% yield.

Selective protection of the primary hydroxy function in the dihydroxypiperidine **12** was required next. For this purpose, the *tert*-butyldimethylsilyl[7] (TBDMS) group was chosen as one which would survive forthcoming contact with strong base, Lewis acid and DDQ. The dihydroxy compound **12** was therefore treated with TBDMS chloride and imidazole to produce the silyl ether **13** in excellent yield. With the structure of *N*-acetylmuramic acid in mind two attempts were made to introduce a lactic acid residue at the C-3 position of compound **13**, using slightly modified literature procedures,[9] but without success. Hence, no reaction occurred when the alcohol **13** was treated initially with potassium hydride in THF to prepare the potassium alkoxide species, and then treated with (2*S*)-2-chloropropionic acid at 60 °C for 24 h. In addition, when (2*S*)-2-(trifluoromethylsulfonyloxy)propionic acid benzyl ester was used in place of (2*S*)-2-chloropropionic acid and added at −20 °C to the lithium alkoxide species, prepared from compound **13** by using butyllithium as base, a reaction did occur in which substrate **13** was consumed to give only a mixture of unwanted products.

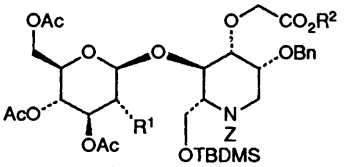
The glycolyl residue has successfully been used as a lactyl-residue surrogate in the synthesis of muramyl dipeptide analogues as potent immunostimulants[10–12] and we therefore turned our efforts towards introducing the glycolyl unit at position C-3 of compound **13**. The lithium alkoxide species, derived from compound **13**, was treated with the trifluoromethanesulfonate derivative of glycolic acid benzyl ester[13] to give the highly functionalised piperidine derivative **14** in 56% yield. Reaction between the benzyl ester **14** and DDQ selectively removed the *p*-methoxybenzyl group, affording the secondary alcohol **15** in high yield as an oil after chromatography.

At this point in the synthesis glycosylation at the C-4 hydroxy group of compound **15** could, in principle, be undertaken either before, or after, coupling the peptide side-chain to the glycolic acid residue. We investigated both options and found only the former to be productive; peptide derivatives of the alcohol **15** failed to react with the trichloroacetamidate glycosyl donor **16**[14] under boron trifluoride–diethyl ether (BF_3·Et_2O) catalysis. On the other hand, when the alcohol **15** was mixed with the imidate **16** and a catalytic amount of BF_3·Et_2O the β-glycoside **17** was formed in 50% yield with 42% recovery of the alcohol **15**. No unwanted α-glycoside formation was detected in this reaction. The ^1H NMR spectrum of the β-glycoside **17** featured a pair of doublets ($J_{1'2'}$ 8.4 Hz for each rotamer) centred at δ 5.46 which were attributed to the anomeric proton in the product and which established the β-configuration of the glycoside linkage in which the 1′-H and 2′-H protons are *trans*-diaxially disposed.

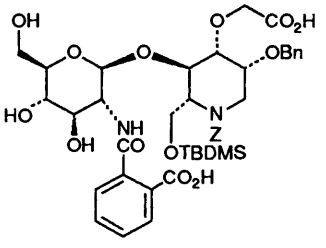
Hydrolysis of the benzyl ester **17** in 0.5 mol dm^{-3} aq. potassium hydroxide at room temperature gave a nearly quantitative yield of the carboxylic acid **19**, in which base-induced phthalimido-ring opening had also occurred.[15] The IR spectrum of the product exhibited peaks at ν_{max} 1700 and



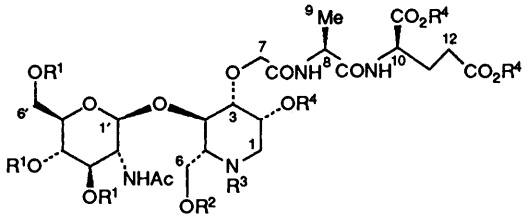
16



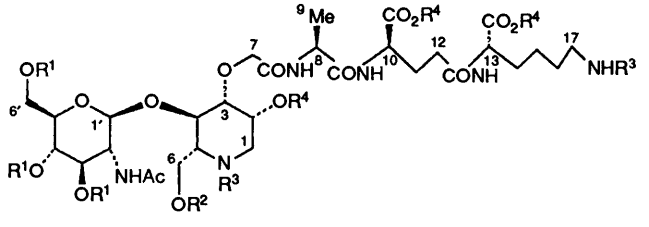
17 R^1 = NPhth, R^2 = Bn
18 R^1 = NHAc, R^2 = H



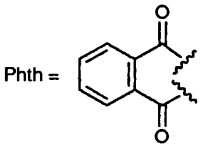
19



20 R^1 = Ac, R^2 = TBDMS, R^3 = Z, R^4 = Bn
21 R^1 = Ac, R^2 = R^3 = R^4 = H
22 R^1 = R^2 = R^3 = R^4 = H



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



1670sh cm^{-1} assigned to the C=O stretching frequencies of the amide and carboxy groups in acid **19**. The spectrum showed no absorptions corresponding to the phthalimido group, which in the IR spectrum of the precursor **17** appeared at ν_{max} 1780 and 1720 cm^{-1}.[16] Corroborating evidence for the open ring structure of acid **19** came from its negative-ion FAB mass spectrum which displayed a base peak at m/z 867 corresponding to M − H]$^-$. When the carboxylic acid **19** was treated with hydrazine hydrate followed by acetic anhydride in pyridine, the acetamide derivative **18** was isolated in 54% yield. The ^{1}H NMR spectrum of this product showed, amongst other signals, a 10 H aromatic envelope (δ 7.26–7.39), a D_2O-exchangeable doublet at δ 5.50 (J 9.1), assigned to the amide proton, and four acetyl singlets δ 1.83–2.10.

Coupling of the carboxylic acid **18** with protected peptides gave products which, after purification, could be deprotected in a one-pot reaction sequence to yield the required piperidyl glycosides. Thus, reaction of the hydrochloride salt of L-ala-D-glu(OBn)$_2$* with compound **18** in the presence of 1-hydroxybenzotriazole (HOBt), *N*,*N*′-dicyclohexylcarbodi-imide (DCC) and *N*-methylmorpholine afforded the dipeptide **20** in 64% yield. Hydrogenolysis of the dipeptide **20** over palladium black in dilute acetic acid was followed by warming of the filtered solution at 60 °C for 3 h to ensure removal of the silyl group. The product was identified as the glycopeptide **21** whose ^{1}H NMR spectrum showed no doubling of signals previously observed in the spectra of *N*-benzyloxycarbonyl derivatives. The synthesis was completed by *O*-deacetylation of the glycopeptide **21** upon treatment with aq. ammonium hydroxide in methanol at room temperature affording the polyhydroxylated piperidyl glycoside **22** in 79% yield from dipeptide **20**. The ^{1}H COSY-45 NMR spectrum of compound **22** displayed the expected ^{1}H–^{1}H correlations consistent with the structure of the product. The 1D ^{1}H NMR spectrum of product **22** was well resolved, featuring sharp doublets for 1′-H at δ 4.69 ($J_{1'2'}$ 7.9) and for L-alanyl Me at δ 1.42 (J 7.2), indicating that neither anomerisation nor racemisation had occurred in the product.

In the same manner, the tripeptide L-ala-γ-D-glu(OBn)-L-lys-(Z)OBn* was coupled with the acid **18** to produce compound **23**. Hydrogenolysis of compound **23** in acetic acid gave the tetra-acetyl derivative **24** from which the tripeptide **25** was formed in moderate overall yield from intermediate acid **18**.

Neither the dipeptide **22** nor the tripeptide **25** showed any antibacterial activity (results not shown). Currently, we are examining the synthesis of aza-analogues of the NAG-NAM unit in which the stereochemistry and substitution in the piperidine ring is closer to that in *N*-acetylmuramic acid.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 K on a Brüker AM 250 (250 MHz) or AM 400 (400 MHz) spectrometer. Unless otherwise indicated deuteriochloroform was employed as solvent; chemical shifts are reported in ppm from tetramethylsilane (TMS) as internal standard and all J-values are in Hz. The ^{1}H nuclear Overhauser enhancement (NOE) difference experiments were conducted using a modification[18] of the method of Hunter and Sanders.[17] Mass spectra were recorded on a VG ZAB IF or VG Trio-2 spectrometer in electron impact (EI), chemical ionisation (CI) or fast-atom bombardment (FAB) mode, as specified. In the CI mode ammonia was used as the reagent gas; in the FAB mode 3-nitrobenzyl alcohol–sodium acetate (3-NOBA) or thioglycerol (THIOG) were used as the matrix. Accurate mass measurements were taken in the FAB mode with glycerol as matrix. Optical rotations were measured at 293 K using an Optical activity AA-1000 single-wavelength polarimeter. $[\alpha]_D$-Values are reported in units of 10^{-1} deg cm^2 g^{-1}. Microanalyses were carried out on a CEC 440 or Carbo Erba 1106 elemental analyser. IR spectra were recorded either on a Perkin-Elmer 983 dispersive or Philips PU 9706 spectrometer. The homogeneity of products and the progress of reactions were determined by TLC using Merck silica gel 60 F254 precoated (0.2 mm thickness) aluminium-backed sheets. TLC Sheets were visualised under UV light at 254 nm or by gently baking after spraying with a 2% solution of dodecamolybdophosphoric acid in ethanol or alternatively by spraying with 0.5% aq. potassium permanganate followed by fixing with a 0.1% solution of Bromophenol Blue in ethanol. Column

* The two peptides, L-ala-D-glu(OBn)$_2$ {$[\alpha]_D$ +18.3 (*c* 0.51, $CHCl_3$)} and L-ala-γ-D-glu(OBn)-L-Lys(Z)OBn {$[\alpha]_D$ +5.7 (*c* 1.0, $CHCl_3$)}, were synthesized in high yields by solution peptide-coupling methodology (DCC–HOBT–*N*-methylmorpholine). Their constituent, protected amino acids are either commercially available or easily prepared.

chromatography. was performed at elevated pressure (~4 psi, 27 570 Pa) using a Medcalf air-pump, over silica gel (Merck silica 60, <230 mesh) with the specified eluent. Extracts were dried over anhydrous sodium sulfate. THF was distilled from calcium hydride and used directly. Other commercial, high-grade solvents were dried over activated molecular sieve (type 3 Å).

1,2-O-*Isopropylidene*-3-O-(4-*methoxybenzyl*)-α-D-*glucofuranose* **3**.—To a stirred solution of 1,2;5,6-di-*O*-isopropylidene-3-*O*-(4-methoxybenzyl)-α-D-glucofuranose **2**[8] (76 g, 0.2 mol) in methanol (450 cm^3) was added 0.6 mol dm^{-3} hydrochloric acid (45 cm^3). After 18 h the solution was neutralised by the addition of conc. ammonium hydroxide and was then concentrated to an oil. The oil was dissolved in ethyl acetate (500 cm^3) and the solution was washed with water (2 × 250 cm^3), dried, and evaporated under reduced pressure to give the title compound **3** (65 g) as a crude yellow oil [R_f 0.18, EtOAc–hexane (1:1)]. The oil was used in the next stage without purification.

A small quantity of the oil (150 mg) was purified by chromatography [EtOAc–hexane (3:7 → 1:1 gradient elution)] to give *compound* **3** (81 mg) as a pure syrup (Found: C, 59.8; H, 7.2. $C_{17}H_{24}O_7$ requires C, 60.0; H, 7.1%); $[\alpha]_D$ −74.9 (*c* 0.26, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3560br (OH), 1610 and 1230br; δ(250 MHz) 1.69 (2 H, br s, 2 × OH), 1.33 and 1.49 (2 × 3 H, 2 s, CMe_2), 3.67 (1 H, dd, J_{65} 5.6, J_{66} 11.4, C*H*HOH), 3.81 (3 H, s, OMe), 3.80 (1 H, dd, J_{65} 3.5, CH*H*OH), 3.97–4.04 (1 H, m, C*H*OH), 4.07–4.14 (2 H, m), 4.63 (1 H, d, J_{21} 3.8, 2-H), 4.45 and 4.68 (2 × 1 H, 2 d, *J* 11.5, ArCH_2), 5.94 (1 H, d, J_{12} 3.8, 1-H) and 6.90 and 7.28 (2 × 2 H, 2 d, *J* 8.6, ArH); *m/z* FAB (3-NOBA) 363 (MNa^+) and 121 (C_8H_9O).

1,2-O-*Isopropylidene*-3-O-(4-*methoxybenzyl*)-6-O-(p-*tolylsulfonyl*)-α-D-*glucofuranose* **4**.—A solution of toluene-4-sulfonyl chloride (36.6 g, 192 mmol) in dry pyridine (90 cm^3) was added during 30 min to a stirred solution of the crude diol **3** (65 g) in pyridine (330 cm^3) at −20 °C. The temperature was maintained for 19 h during which time pyridine hydrochloride crystallised out. The suspension was filtered, washed with a little dichloromethane, and the filtrate was evaporated under reduced pressure to give a yellow oil. The oil was dissolved in ethyl acetate (500 cm^3) and the solution was washed with water (2 × 250 cm^3), dried, and concentrated to give an oil. The oil was purified by chromatography [EtOAc–hexane (1:9 → 3:2 gradient elution)] to give the *title compound* **4** (61 g, 64% from compound **1**) as a pale yellow syrup [R_f 0.33, acetone–toluene (1:9)] (Found: C, 58.6; H, 5.55; S, 6.4. $C_{24}H_{30}O_9S$ requires C, 58.3; H, 6.1; S, 6.5%); $[\alpha]_D$ −28.7 (*c* 0.28, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3560br (OH) 1370, 1230 and 1175; δ(250 MHz) 1.31 and 1.47 (2 × 3 H, 2 s, CMe_2), 1.65 (1 H, br s, D_2O-exch., OH), 2.44 (3 H, s, Ar*Me*), 4.17 (5 H, m), 3.81 (3 H, s, OMe), 4.47 and 4.63 (2 × 1 H, 2 d, *J* 11.3, ArCH_2), 4.58 (1 H, d, J_{21} 3.7, 2-H), 5.87 (1 H, d, 1-H), 6.89 and 7.25 (2 × 2 H, 2 d, *J* 8.6, ArH) and 7.33 and 7.78 (2 × 2 H, 2 d, *J* 8.20, ArH); *m/z* FAB (3-NOBA) 518 (MNa^+) and 121 (C_8H_9O).

6-*Azido*-6-*deoxy*-1,2-O-*isopropylidene*-3-O-(4-*methoxybenzyl*)-α-D-*glucofuranose* **5**.—A suspension of sodium azide (32 g, 492 mmol) was stirred with a solution of the tosyl derivative **4** (60.5 g, 122 mmol) in *N*,*N*-dimethylformamide (DMF) (500 cm^3) at 55 °C for 16 h under argon. The cooled reaction mixture was filtered and the filtrate was concentrated to give an oil. The oil was dissolved in ethyl acetate (500 cm^3) and the solution was washed with water (2 × 250 cm^3), dried, and evaporated under reduced pressure to give the title compound **5** as a crude syrup (46.5 g), which was used in the next stage without purification.

A small quantity of the crude syrup (180 mg) was purified by chromatography [EtOAc–hexane (1:9 → 1:1 gradient elution)] to yield pure *compound* **5** (130 mg) as a syrup [R_f 0.24, EtOAc–hexane (1:4)] (Found: C, 56.0; H, 6.8; N, 11.4. $C_{17}H_{23}N_3O_6$ requires C, 55.9; H, 6.3; N, 11.5%); $[\alpha]_D$ −68.8 (*c* 0.81, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3560br (OH) and 2110 (N_3); δ(250 MHz) 1.33 and 1.49 (2 × 3 H, 2 s, CMe_2), 1.7 (1 H, br s, D_2O-exch., OH), 3.38 (1 H, dd, J_{66} 11.8, J_{65} 5.3, C*H*HN_3), 3.53 (1 H, br d, CH*H*N_3), 3.81 (3 H, s, OMe), 4.07 (3 H, s, 3-, 4- and 5-H), 4.44 and 4.68 (2 × 1 H, 2 d, *J* 11.6, ArCH_2), 4.63 (1 H, d, J_{21} 3.8, 2-H), 5.93 (1 H, d, 1-H) and 6.91 and 7.28 (2 × 2 H, 2 d, *J* 8.6, ArH); *m/z* FAB (3-NOBA) 388 (MNa^+) and 121 (C_8H_9O).

6-*Azido*-5-O-*benzyl*-6-*deoxy*-1,2-O-*isopropylidene*-3-O-(4-*methoxybenzyl*)-α-D-*glucofuranose* **6**.—Hexane-washed sodium hydride (5.4 g; 60% dispersion in oil, 135 mmol) was added in portions to a stirred solution of the crude azide **5** (44.9 g, 123 mmol) in dry THF (315 cm^3) in apparatus closed with an argon bubbler. When evolution of hydrogen had ceased (*ca.* 1.5 h), tetrabutylammonium iodide (1.25 g, 3.4 mmol) and benzyl bromide (16.1 cm^3, 135 mmol) were added and the solution was stirred for a further 20 h. Excess of sodium hydride was destroyed by the addition of methanol 15 cm^3) and after 30 min the reaction mixture was filtered. The filtrate was evaporated under reduced pressure to give an oil, which was purified by chromatography [EtOAc–hexane (1:19 → 1:4 gradient elution)] to afford the *title compound* **6** (41.1 g, 74% from compound **4**) as a syrup [R_f 0.45, EtOAc–hexane (1:4)] (Found: C, 63.5; H, 6.4; N, 8.9. $C_{24}H_{29}N_3O_6$ requires C, 63.3; H, 6.4; N, 9.2%); $[\alpha]_D$ −74.4 (*c* 0.40, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 2110 (N_3), 1610 and 1230br; δ(250 MHz) 1.32 and 1.49 (2 × 3 H, 2 s, CMe_2), 3.42 (1 H, dd, J_{66} 12.9, J_{65} 5.0, C*H*HN_3), 3.66 (1 H, dd, J_{65} 2.6, CH*H*N_3), 3.77 (3 H, s, OMe), 4.00 (1 H, ddd, J_{54} 9.0, J_{56} 5.2 and 2.7, C*H*OBn), 4.10 (1 H, d, J_{34} 3.1, C*H*OPMB), 4.26 (1 H, dd, 4-H), 4.41, 4.45, 4.60 and 4.68 (4 × 1 H, 4 d, each *J* 11.1, 2 × ArCH_2), 4.61 (1 H, d, J_{21} 3.7, 2-H), 5.88 (1 H, d, 1-H), 6.83 and 7.20 (2 × 2 H, 2 d, *J* 8.6, ArH) and 7.26–7.35 (5 H, m, Ph); *m/z* FAB (3-NOBA) 478 (MNa^+) and 121 (C_8H_9O).

Methyl 6-*Azido*-5-O-*benzyl*-6-*deoxy*-3-O-(4-*methoxybenzyl*)-D-*glucofuranoside* **7α/7β**.—The isopropylidene derivative **6** (40.8 g, 89.6 mmol) was dissolved in dry methanolic hydrogen chloride (0.5 mol dm^{-3}; 380 cm^3) and stirred at room temperature for 15 h. The reaction mixture was diluted with dichloromethane (500 cm^3) and washed successively with saturated aq. sodium hydrogen carbonate (500 cm^3) and then water (500 cm^3). The combined aqueous washings was extracted with dichloromethane (200 cm^3) and the pooled organic extracts were dried and evaporated under reduced pressure to give an oil. The oil was purified by chromatography (acetone–toluene; gradient elution) to give the title compounds **7α/7β** (30.4 g, 79%) as a syrup, identified by ^1H NMR spectroscopy as a mixture of anomers (α:β ratio ~2:3). The mixture was used in the next stage without separation. Further elution from the column yielded methyl 6-azido-5-*O*-benzyl-6-deoxy-D-glucofuranoside **8α/8β** (4.7 g, 17%) as a syrup [α:β ratio ~2:3; R_f 0.24, 0.21 respectively, acetone–toluene (1:4)].

A small quantity of the anomeric mixture **7α/7β** was separated by chromatography [acetone–toluene (1:19 → 1:4 gradient elution)] to yield the pure components.

α-*Anomer* **7α**; syrup [R_f 0.59, acetone–toluene (1:4)] (Found: C, 61.7; H, 6.4; N, 9.3. $C_{22}H_{27}N_3O_6$ requires C, 61.5; H, 6.3; N, 9.8%); $[\alpha]_D$ +0.6 (*c* 1.84, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3520br (OH), 2110 (N_3), 1610, 1510 and 1230br; δ(250 MHz) 2.87 (1 H, br s, D_2O-exch., OH), 3.40 (1 H, dd, J_{66} 13.1, J_{65} 5.2, C*H*HN), 3.48 (3 H, s, anomeric OMe), 3.61 (1 H, dd, J_{65} 2.7, CH*H*N), 3.78 (3 H, s, ArO*Me*), 3.95 (1 H, ddd, J_{54} 8.0, 5-H),

4.03 (1 H, dd, J_{34} 4.5, J_{32} 1.9, 3-H), 4.23 (1 H, br s, C*H*OH), 4.27 (1 H, dd, 4-H), 4.44, 4.50, 4.64 and 4.65 [4 × 1 H, 4 d, each *J* 11.3 (AB), 2 × ArCH_2], 5.02 (1 H, d, J_{12} 4.5, C*H*OMe) [in an NOE experiment irradiation of the signal at δ 5.02 gave rise to enhancements at δ 4.23 (3.2%) and δ 3.48 (0.8%)], 6.83 and 7.20 [2 × 2 H, 2 d, *J* 8.63 (A_2X_2), ArH] and 7.26–7.35 (5 H, m, Ph); *m/z* FAB (3-NOBA) 452 (MNa^+) and 121 (C_8H_9O).

β-*Anomer* **7**β; syrup [R_f 0.46, acetone–toluene (1:4)] (Found: C, 61.6; H, 6.5; N, 9.5%); $[\alpha]_D$ −82.9 (*c* 1.67, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3520br (OH), 2110 (N_3), 1610, 1510 and 1230br; δ(250 MHz) 1.61 (1 H, br s, D_2O-exch., OH), 3.40 (3 H, s, anomeric OMe), 3.42 (1 H, dd, J_{66} 13.2, J_{65} 4.6, C*H*HN), 3.68 (1 H, dd, J_{65} 2.5, CH*H*N), 3.78 (3 H, s, ArO*Me*), 3.96 (1 H, dd, J_{34} 5.0, J_{32} 1.2, 3-H), 4.03 (1 H, ddd, J_{54} 8.5, 5-H), 4.19 (1 H, br s, C*H*OH), 4.37 (1 H, dd, 4-H), 4.48 and 4.57 [2 × 1 H, 2 d, *J* 11.19 (AB), ArCH_2], 4.51 and 4.68 [2 × 1 H, 2 d, *J* 11.19 (AB), ArCH_2], 4.80 (1 H, s, C*H*OMe) [in an NOE experiment irradiation of the signal at δ 4.80 gave rise to enhancements at δ 4.37 (1.9%), δ 4.19 (1.1%) and δ 3.40 (0.8%)], 6.82 and 7.21 [2 × 2 H, 2 d, *J* 8.60 (A_2X_2) ArH] and 7.27–7.37 (5 H, m, Ph); *m/z* FAB (3-NOBA) 452 (MNa^+) and 121 (C_8H_9O).

2:3 Anomeric mixture **8**α/**8**β; syrup [R_f 0.24, 0.21 respectively, acetone–toluene (1:4)]; $\nu_{max}(CHCl_3)/cm^{-1}$ 3500br (OH), 2110 (N_3) and 1035; δ(250 MHz) 1.97, 2.59 and 2.77 (3 H, 3 br, s, D_2O-exch., 3 × OH), 2.93 (1 H, br d, *J* 10.0, D_2O-exch., OH), 3.37 (3 H, s, **8**β OMe), 3.38–3.52 (2 H, overlapping m, **8**α/**8**β C*H*HN), 3.50 (3 H, overlapping, s, **8**α Me), 3.59 (1 H, dd, J_{66} 13.2, J_{65} 3.5, **8**α CH*H*N), 3.66 (1 H, dd, J_{65} 2.9, **8**β CH*H*N), 3.90–3.99 (2 H, m, **8**α/**8**β C*H*CH$_2$N), 4.06 (1 H, br s, **8**α 2-H) 4.11–4.25 (4 H, m, **8**α 4-H, **8**β 2-H, **8**α/**8**β 3-H), 4.30 (1 H, dd, *J* 3.8 and 8.9, **8**β 4-H), 4.65 and 4.78 [2 × 1 H, 2 d, each *J* 11.4 (AB), **8**α PhCH_2], 4.74 (2 H, s, **8**β PhCH_2), 4.84 (1 H, s **8**β 1-H), 5.02 (1 H, d, J_{12} 4.3, **8**α 1-H) and 7.16–7.41 (10 H, m, **8**α/**8**β 2 × Ph); *m/z* (CI) 327 (MNH_4^+, 100%) and 310 (MH^+, 7).

Methyl 6-*Azido*-5-O-*benzyl*-6-*deoxy*-3-O-(4-*methoxybenzyl*)-2-O-(*trifluoromethylsulfonyl*)-D-*glucofuranoside* **9**α/**9**β.—Trifluoromethanesulfonic anhydride (1.72 cm^3, 10.20 mmol) was added dropwise to a stirred solution of the 2:3 anomeric mixture **7**α/**7**β (4.0 g, 9.3 mmol) in a mixture of dry dichloromethane (40 cm^3) and dry pyridine (1.6 cm^3, 19.5 mmol) at −50 °C under argon. After 0.5 h at this temperature, the mixture was warmed to room temperature during 1 h and was then washed with cold water (2 × 40 cm^3), dried, and evaporated under reduced pressure to give the crude title compounds **9**α/**9**β (5.1 g) as an orange syrup, identified by ^{1}H NMR spectroscopy as a mixture (~1:1) of anomers. The syrup was used in the next stage without purification. Attempts to separate the anomers of the 1:1 mixture by chromatography [Et_2O–hexane (1:9 → 3:7 gradient elution)] gave rise only to an enrichment of each anomer which allowed assignments to be made in the ^{1}H NMR spectrum of the 1:1 mixture.

1:1 Anomeric mixture **9**α/**9**β [R_f 0.44, 0.38 respectively, Et_2O–hexane (3:7)]; $\nu_{max}(CHCl_3)/cm^{-1}$ 2110 (N_3), 1610, 1510, 1415 and 1230br; δ(250 MHz) 3.34–3.42 (1 H, m, C*H*HN, **9**α/**9**β), 3.42 (1.5 H, s, anomeric OMe, **9**β), 3.46 (1.5 H, s, anomeric OMe, **9**α), 3.61 (0.5 H, dd, J_{66} 13.2, J_{65} 2.8, CH*H*N, **9**α), 3.69 (0.5 H, dd, J_{66} 13.3, J_{65} 2.6, CH*H*N, **9**β), 3.77 (1.5 H, s, ArO*Me*), **9**β), 3.78 (1.5 H, s, ArO*Me*, **9**α), 3.92 (0.5 H, ddd, J_{54} 7.5, J_{56} 4.7 and 2.92, 5-H, **9**α), 4.02 (0.5 H, ddd, J_{54} 6.7, J_{56} 3.9 and 2.80, 5-H, **9**β), 4.34–4.69 (6 H, m), 5.06 (0.5 H, s, C*H*OMe, **9**β), 5.11 (0.5 H, d, J_{12} 4.5, C*H*OMe, **9**α), 6.82 and 6.83 (2 × 1 H, 2 d, each *J* 8.7, ArH, **9**α/**9**β) and 7.14–7.37 (7 H, m, ArH, **9**α/**9**β); *m/z* FAB (3-NOBA) 584 (MNa^+) and 121 (C_8H_9O).

Enriched β-*anomer* **9**β (Found: C, 49.0; H, 4.6; N, 6.9; S, 5.7. $C_{23}H_{26}F_3N_3O_8S$ requires C, 49.2; H, 4.7; N, 7.5; S, 5.7%; *m/z* FAB (3-NOBA) 584 (MNa^+) and 121 (C_8H_9O).

Methyl 5-O-*Benzyl*-2,6-*dideoxy*-2,6-*imino*-3-O-(4-*methoxybenzyl*)-D-*mannofuranoside* **10**α/**10**β.—Palladium black (380 mg) in a solution of the 1:1 triflate mixture **9**α/**9**β (4.8 g) in ethanol (50 cm^3) was shaken with hydrogen at room temperature and atmospheric pressure for 27 h. The mixture was filtered through Celite, and anhydrous sodium acetate (763 mg, 9.3 mmol) was added to the filtrate, which was then heated at 50 °C for 12 h. The orange solution was evaporated under reduced pressure to give an orange oil, which was dissolved in dichloromethane (100 cm^3) and the solution was washed with brine (100 cm^3), dried, and concentrated to give a mixture (~1:1) of the crude title compounds **10**α/**10**β (3.3 g) as an orange syrup. The syrup was used in the next stage without purification.

A small quantity of the 1:1 mixture **10**α/**10**β was separated by chromatography [MeOH–CH_2Cl_2 (1:99 → 1:24 gradient elution)] to yield the pure components.

α-*Anomer* **10**α; syrup which solidified on storage, m.p. 80–81 °C [R_f 0.42, MeOH–CH_2Cl_2 (1:19)] (Found: C, 68.6; H, 6.75; N, 3.8. $C_{22}H_{27}NO_5$ requires C, 68.55; H, 7.1; N, 3.6%); $[\alpha]_D$ +65.7 (*c* 0.3, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3340br (NH), 1610, 1510, 1455 and 1230br; δ(250 MHz) 2.83 (1 H, dd, J_{66} 13.4, J_{65} 9.5, C*H*HN), 3.16 (1 H, dd, J_{65} 6.7, CH*H*N), 3.22 (1 H, d, J_{23} 3.5, CHN), 3.40 (3 H, s, anomeric OMe), 3.71 (1 H, dd, 5-H), 3.81 (3 H, s, ArO*Me*), 4.21 (1 H, dd, J_{34} 6.1, 3-H), 4.39 (1 H, d, 4-H), 4.46 and 4.52 [2 × 1 H, 2 d, each *J* 12.3 (AB), ArCH_2], 4.39 and 4.53 [2 × 1 H, 2 d, each *J* 11.4 (AB), ArCH_2], 5.05 (1 H, s, C*H*OMe), 6.87 and 7.20 [2 × 2 H, 2 d, *J* 8.6 (A_2X_2), ArH] and 7.28–7.35 (5 H, m, ArH); *m/z* (CI) 386 (MH^+, 75%), 121 (C_8H_9O, 100) and 91 (C_7H_7, 72).

β-*Anomer* **10**β; oil [R_f 0.34, MeOH–CH_2Cl_2 (1:19)]; $[\alpha]_D$ −14.8 (*c* 0.31, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3350br (NH), 1610, 1510, 1455 and 1230br; δ(250 MHz) 2.76 (1 H, br s, D_2O-exch., NH), 3.13 (1 H, dd, J_{66} 12.3, J_{65} 6.8, C*H*HN), 3.26 (1 H, t, $J_{23} \sim J_{21} \sim$ 3.3, CHN), 3.28 (1 H, dd, J_{65} 9.6, CH*H*N), 3.55 (3 H, s, anomeric OMe), 3.73 (1 H, dd, 5-H), 3.81 (3 H, s, ArO*Me*), 3.95 (1 H, dd, J_{34} 5.9, 3-H), 4.34 (1 H, d, 4-H), 4.39 and 4.50 [2 × 1 H, 2 d, *J* 11.6 (AB), ArCH_2], 4.52 (2 H, s, ArCH_2), 5.04 (1 H, d, J_{12} 3.0, C*H*OMe), 6.86 and 7.18 [2 × 2 H, 2 d, *J* 8.6 (A_2X_2), ArH] and 7.28–7.35 (5 H, m, ArH); *m/z* (CI) 386 (MH^+, 75%), 121 (C_8H_9O, 100) and 91 (C_7H_7, 67).

Methyl 5-O-*Benzyl*-N-*benzyloxycarbonyl*-2,6-*dideoxy*-2,6-*imino*-3-O-(4-*methoxybenzyl*)-D-*mannofuranoside* **11**α/**11**β.—Saturated aq. sodium hydrogen carbonate (60 cm^3) was added to a stirred solution of the 1:1 mixture **10**α/**10**β (3.1 g) in 1,4-dioxane (100 cm^3). Benzyl chloroformate (1.79 cm^3, 12.6 mmol) was added to this mixture at room temperature and the whole was stirred for 3.5 h after which time the mixture was concentrated to remove the organic solvent. The remaining aqueous residue was extracted with dichloromethane (2 × 70 cm^3) and the combined extracts were dried, and evaporated at reduced pressure to give a brown oil. The oil was purified by chromatography [acetone–toluene; (1:99 → 1:17 gradient elution)] to afford a 1:1 mixture (^{1}H NMR) of the title compounds **11**α/**11**β (2.85 g, 59% from **7**α/**7**β) as a syrup. The 1:1 mixture was used in the next stage without purification.

A small quantity of the mixture **11**α/**11**β was separated by chromatography (eluent as above) to yield the pure components.

α-*Anomer* **11**α; syrup [R_f 0.59, acetone–toluene (1:4)] (Found: C, 69.5; H, 6.4; N, 2.9. $C_{30}H_{33}NO_7$ requires C, 69.35; H, 6.4; N, 2.7%); $[\alpha]_D$ +21.7 (*c* 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 1690 (C=O), 1230br, 1100 and 1040; δ(250 MHz) 2.96 and 3.02 (2 × 0.5 H, 2 dd, each pair J_{66} 13.1, J_{65} 9.3, C*H*HN), 3.38 and 3.39 (2 × 1.5 H, 2 s, anomeric OMe), 3.80 (3 H, s, ArO*Me*), 3.84–3.90 (1 H, m, 5-H), 4.16–4.54 (7 H, m), 4.58 and 4.75 (2 × 0.5 H, 2 d, each J_{23} 3.6, CHN), 4.99 (1 H,

s, C*H*OMe), 5.03–5.14 (2 H, m, ArCH_2), 6.83, 7.05 and 7.17 [4 H, total, 3 d, each *J* 8.5 (A_2X_2), ArH] and 7.25–7.34 (10 H, m, ArH); *m/z* (CI) 537 (MNH_4^+, 6%), 520 (MH^+, 32) and 121 (C_8H_9O, 100).

β-*Anomer* **11**β; syrup [R_f 0.42, acetone–toluene (1:4)] (Found: C, 69.3; H, 6.4; N, 2.8%); $[\alpha]_D$ −53.7 (*c* 1.2, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 1690 (C=O), 1230br, 1105br and 1040br; δ(250 MHz) 3.38 and 3.40 (2 × 0.5 H, 2 dd, each pair J_{66} 12.3, J_{65} 8.3, C*H*HN), 3.49 (3 H, s, anomeric OMe), 3.80–3.86 (4 H, m, ArO*Me* and 5-H), 3.91 and 4.01 (2 × 0.5 H, 2 dd, each pair J_{34} 5.9, J_{32} 3.9, 3-H), 4.19 and 4.39 (2 × 0.5 H, 2 dd. J_{65} 7.2, CH*H*N), 4.34 and 4.36 (2 × 0.5 H, 2 d, 4-H), 4.21–4.59 (4 H, m, ArCH_2), 4.64 and 4.84 (2 × 0.5 H, 2 t, each $J_{21} \sim J_{23} \sim 3.4$, CHN), 5.03 and 5.08 (2 × 0.5 H, 2 d, each J_{12} 3.1, C*H*OMe), 5.02–5.20 (2 H, m, ArCH_2), 6.82, 6.84, 7.05 and 7.16 [4 × 1 H, 4 d, each *J* 8.6 (A_2X_2), ArH] and 7.30–7.35 (10 H, m, Ph). ^1H–^1H correlations: δ 3.38 and 3.40 (6-H)–4.19 and 4.39 (6-H), 3.80–3.86 (5-H); 3.80–3.86 (5-H)–3.38, 3.40, 4.19 and 4.39 (6-H), 4.34 and 4.36 (4-H); 3.91 and 4.01 (3-H)–4.34 and 4.36 (4-H), 4.64 and 4.84 (2-H); 4.19 and 4.39 (6-H)–3.80 to 3.86 (5-H), 3.38 and 3.40 (6-H); 4.23 (ArCH_2)–4.41 (ArCH_2); 4.34 and 4.36 (4-H)–3.80 to 3.86 (5-H), 3.91 and 4.01 (3-H); 4.31 (ArCH_2)–4.55 (ArCH_2); 4.49 (ArCH_2)–4.59 (ArCH_2); 4.64 and 4.84 (2-H)–3.91 and 4.01 (3-H); 5.03 and 5.08 (1-H): 5.03 and 5.08 (1-H)–4.64 and 4.84 (2-H); 5.09 (ArCH_2)–5.17 (ArCH_2); 6.82 and 6.84 (PMB ArH)–7.05 and 7.16 (PMB ArH); 7.05 and 7.16 (PMB ArH)–6.82 and 6.84 (PMB ArH); *m/z* FAB (3-NOBA) 542 (MNa^+), 520 (MH^+) and 121 (C_8H_9O).

2-O-*Benzyl*-N-*benzyloxycarbonyl*-1,5-*dideoxy*-1,5-*imino*-4-O-(4-*methoxybenzyl*)-D-*mannitol* **12**.—A solution of the anomeric mixture **11**α/**11**β (5.81 g, 11.2 mmol) in TFA–water–THF (3:3:1, 126 cm^3) was stirred at room temperature for 1 h. The solution was poured slowly into a mixture of dichloromethane (500 cm^3), water (900 cm^3), and sodium hydrogen carbonate (170 g) and was stirred vigorously for 15 min. The layers were separated and the aqueous fraction was extracted with dichloromethane (200 cm^3). The combined organic extracts 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 (107 cm^3) and to the stirred solution, cooled to 0 °C, was slowly added aq. sodium borohydride (840 mg, 22.2 mmol in 17 cm^3). The temperature of the solution was maintained at 0 °C for 0.5 h and then allowed to reach ambient temperature during another 0.5 h. 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 (200 cm^3) and the solution was washed with water (200 cm^3). The aqueous fraction was re-extracted with dichloromethane (100 cm^3) and the combined organic extracts were dried, and evaporated under reduced pressure to afford an oil. Purification of the oil by chromatography [acetone–toluene (1:9 → 3:7 gradient elution)] afforded the *title compound* **12** (4.7 g, 83%) as a syrup [R_f 0.25, acetone–toluene (1:4)] (Found: C, 68.3; H, 6.3; H, 2.8. $C_{29}H_{33}NO_7$ requires C, 68.62; H, 6.55; N, 2.76%); $[\alpha]_D$ −18.0 (*c* 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3580br (OH), 3420br (OH), 1690 (CO) and 1230br; δ(250 MHz; CD_3COCD_3) 3.09–3.29 (2 H, becoming 1 H after D_2O-exch., m, C*H*HN and OH), 3.79 (3 H, s, OMe), 4.28 (1 H, br s, D_2O-exch., OH), 3.66–4.62 (11 H excluding OMe and OH, m), 5.11 (2 H, br s, PhCH_2), 6.87 [2 H, *J* 8.6 (A_2X_2), ArH] and 7.18–7.37 (12 H, m, ArH); *m/z* (EI) 507 (M^+, 2%), 476 ($M^+ - OCH_3$, 16), 121 (C_8H_9O, 100) and 91 (C_7H_7, 100).

2-O-*Benzyl*-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-1,5-*dideoxy*-1,5-*imino*-4-O-(4-*methoxybenzyl*)-D-*mannitol* **13**.—A solution of TBDMSCl (2.79 g, 18.5 mmol) in DMF (30 cm^3) was added during 5 min to a stirred, ice-cooled solution of the diol **12** (4.7 g, 9.3 mmol), imidazole (2.53 g, 37.2 mmol) and 4-(dimethylamino)pyridine (80 mg) in DMF (100 cm^3) under argon. The reaction mixture was warmed to room temperature and stirred for 18 h. The solution was evaporated at room temperature under reduced pressure to give an oil, which was dissolved in dichloromethane (200 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 (1:99 → 1:24 gradient elution)] to yield the *title compound* **13** (5.6 g, 97%) as an oil [R_f 0.21, acetone–toluene (1:50)] (Found: C, 67.5; H, 7.5; N, 2.5. $C_{35}H_{47}NO_7Si$ requires C, 67.6; H, 7.6; N, 2.25%); $[\alpha]_D$ −16.1 (*c* 1.0, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3580br (OH), 1690 (CO) and 1230br; δ(250 MHz) 0.01 and 0.03 (~6 H, masked by TMS, 2 s, $SiMe_2$), 0.86 (9 H, br s, SiBu^t), 2.50 and 2.84 (2 × 0.5 H, br s, D_2O-exch., OH), 3.00 and 3.05 (2 × 0.5 H, 2 t, J_{66} 11.8, C*H*HN), 3.80 (3 H, s, OMe), 3.73–4.12 (5 H excluding OMe, m), 4.29–4.68 (6 H, m), 5.14 and 5.18 [2 × 1 H, 2 d, *J* 12.5 (AB), CO_2CH_2] and 6.79–7.34 (14 H, m, ArH); *m/z* FAB (3 NOBA) 622 (MH^+) and 121 (C_8H_9O).

2-O-*Benzyl*-N-*benzyloxycarbonyl*-3-O-(*benzyloxycarbonylmethyl*)-6-O-(tert-*butyldimethylsilyl*)-1,5-*dideoxy*-1,5-*imino*-4-O-(4-*methoxybenzyl*)-D-*mannitol* **14**.—A 0.95 mol dm^{-3} solution of butyllithium in hexane (10.4 cm^3, 9.9 mmol) was added during 10 min to a stirred solution of compound **13** (5.6 g, 9.0 mmol) in dry THF (100 cm^3) at −55 °C under argon. After 15 min at this temperature the mixture was treated with a solution of benzyl *O*-(trifluoromethylsulfonyl)glycolate[13] (2.95 g, 9.9 mmol) in dry THF (35 cm^3) was added during 10 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 3 h. Sodium hydrogen carbonate (4 g) was then added to the reaction mixture followed, after 5 min, by water (30 cm^3). The mixture was concentrated, diluted with water, and extracted with dichloromethane (2 × 150 cm^3). The combined extracts were dried, and evaporated under reduced pressure to afford an oil, which was purified by chromatography [acetone–toluene; (1:99 → 1:24 gradient elution)]. Early fractions from the column yielded the title compound **14** (3.87 g, 56%) as a syrup [R_f 0.44, acetone toluene (3:97)]; $[\alpha]_D$ −20.5 (*c* 1.0 in $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 1750 (ester C=O), 1690 (amide C=O) and 1230br; δ(250 MHz) 0.0 (~6 H masked by TMS, br s, $SiMe_2$), 0.84 (9 H, br s, SiBu^t), 3.13 and 3.17 (2 × 0.5 H, 2 t, $J_{66} \sim J_{65} \sim 11.5$, C*H*HN), 3.79 (3 H, s, OMe), 3.76–4.67 (13 H excluding OMe, m), 5.06–5.20 (4 H, m, 2 × CO_2CH_2) and 6.78–7.36 (19 H, m, ArH); *m/z* FAB (3-NOBA) 792 (MNa^+) and 121 (C_8H_9O).

2-O-*Benzyl*-N-*benzyloxycarbonyl*)-3-O-(*benzyloxycarbonyl methyl*)-6-O-(tert-*butyldimethylsilyl*)-1,5-*dideoxy*-1,5-*imino*-D-*mannitol* **15**.—DDQ (1.6 g, 7.03 mmol) was added in portions to a rapidly stirred mixture of compound **14** (3.76 g, 4.8 mmol) in a mixture of dichloromethane (48 cm^3) and water (2.8 cm^3) at room temperature. After 1.5 h, the mixture was filtered through Celite with a little more solvent. The filtrate was washed with saturated aq. sodium hydrogen carbonate (2 × 50 cm^3), dried, and evaporated under reduced pressure to give an oil, which was purified by chromatography [acetone–toluene (1:99 → 1:9 gradient elution)] to give the *title compound* **15** (2.72 g, 86%) as a syrup [R_f 0.13, acetone–toluene (1:19)] (Found: C, 66.4; H, 7.5; N, 2.1. $C_{36}H_{47}NO_8Si$ requires C, 66.5; H, 7.3; N, 2.2%); $[\alpha]_D$ −15.2 (*c* 0.25, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3600br (OH), 1750 (ester C=O), 1690 (amide C=O) and 1230br; δ(250 MHz) 0.0 (~6 H masked by TMS, br s, $SiMe_2$), 0.85 and 0.86 (9 H, 2 s, SiBu^t), 3.19 (1 H, br t, $J_{66} \sim J_{65} \sim 12.3$, C*H*HN), 3.80–3.86 (4 H, m), 4.25–4.30 (3 H, m), 4.34 (2 H, s), 4.53–4.70 (~2 H, m), 4.83 (1 H, br s, D_2O-exch., OH), 5.12 and 5.14 (2 × 2 H, 2 s,

2 × CO_2CH_2) and 7.30–7.34 (15 H, m, ArH); *m/z* FAB (3-NOBA) 672 (MNa^+).

2-O-*Benzyl*-N-*benzyloxycarbonyl*-3-O-(*benzyloxycarbonylmethyl*)-6-*O*-(*tert-butyldimethylsilyl*)-1,5-*dideoxy*-1,5-*imino*-4-O-(3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-2′-*phthalimido*-β-D-*glucopyranosyl*)-D-*mannitol* **17**.—Boron trifluoride–diethyl ether (120 mm^3, 0.96 mmol) was added to a stirred solution of the alcohol **15** (2.48 g, 3.82 mmol) and the imidate **16**[14] (3.3 g, 5.72 mmol) in dry dichloromethane (12 cm^3) at −20 °C under argon. After 1.5 h at −20 °C the mixture was treated with sodium hydrogen carbonate (300 mg) followed, after 5 min, by water (12 cm^3) and then the mixture was warmed to room temperature. The layers were separated and the aqueous fraction was extracted with dichloromethane (12 cm^3). The combined organic extracts were dried, and evaporated under reduced pressure to afford an oil, which was purified by chromatography [EtOAc–hexane (1:4 → 2:3 gradient elution)]. Evaporation of the early fractions from the column yielded an oily solid, which was stirred with diethyl ether–hexane (1:1; 8 cm^3) to give suspended needles of trichloroacetamide (500 mg). These were filtered off and the filtrate was evaporated under reduced pressure to produce recovered alcohol **15** (1.07 g, 42% recovery) as an oil [R_f 0.59, EtOAc–hexane (2:3)].

Evaporation of later fractions from the column yielded the *title compound* **17** (2.04 g, 50%) as a syrup [R_f 0.49, EtOAc–hexane (2:3)] (Found: C, 63.0; H, 6.2; N, 2.7. $C_{56}H_{66}N_2O_{17}Si$ requires C, 63.0; H, 6.2; N, 2.6%); $[\alpha]_D$ −24.4 (*c* 0.20, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 1780sh (Phth C=O, symm.), 1750 (ester C=O), 1720 (Phth C=O, asymm.), 1690sh (amide C=O) and 1230br; δ(250 MHz; resolution enhanced) −0.13, −0.10 and −0.03 (~6 H partly obscured by TMS, 3 s, $SiMe_2$), 0.80 and 0.87 (2 × 4.5 H, 2 s, Bu^t), 1.85, 2.01, 2.04 and 2.05 (9 H, 4 s, 3 × Ac), 2.98 and 3.04 (1 H, 2 dd, each pair J_{11} 11.3, J_{12} 7.2, C*H*HN), 3.48–4.57 (~17 H, m), 5.04–5.19 (3.5, H, 4′-H, part 2 × CO_2CH_2Ph), 5.45 and 5.48 (1 H, 2 d, each $J_{1',2'}$ 8.4, 1′-H), 5.76 and 5.85 (1 H, 2 dd, each pair *J* 10.6, 9.0, 3′-H), 7.08–7.35 (15 H, m, 3 × Ph) and 7.59–7.89 (4 H, m, Phth); *m/z* (3-NOBA) 1089 (MNa^+).

4-O-(2′-*Acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-3-O-*carboxymethyl*-D-*mannitol** **18**.—A solution of compound **19** (see below) (1.24 g, 1.46 mmol) and hydrazine monohydrate (0.7 cm^3) in ethanol (50 cm^3) was heated under reflux for 21 h. Upon cooling, a solid precipitated out. The mixture was evaporated under reduced pressure to give an oily solid and the residue was reconcentrated with toluene (2 × 30 cm^3). The residue was kept at room temperature for 2 h *in vacuo* and then stirred at room temperature for 18 h with acetic anhydride (24 cm^3) and pyridine (34 cm^3). The solution was evaporated under reduced pressure to afford an oil and the residue was re-evaporated from toluene (2 × 30 cm^3). The residue was dissolved in dichloromethane (30 cm^3) and the solution was stirred for 5 min with ion-exchange resin [CG 120 (H-form), 1.8 g]. The resin was removed by filtration and the filtrate was washed with water (20 cm^3), dried, and evaporated under reduced pressure to give an oil, which was purified by chromatography [graded elution: acetone–toluene; (1:9 → 1:1 gradient; EtOAc–EtOH (9:1); EtOAc–EtOH–water (7:2:1)] to yield the title compound **18** (704 mg, 54%) as a foam [R_f 0.39, EtOAc–EtOH (9:1)]; $[\alpha]_D$ −7.9 (*c* 0.14, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3440br (NH), 1750 (ester C=O), 1690br (carboxy, amide C=O) and 1220br; δ(250 MHz) 0.04 and 0.06 (6 H, 2 s, $SiMe_2$), 0.89 (9 H, s, Bu^t), 1.83, 2.03, 2.04 and 2.10 (total 12 H, 4 s, 4 × Ac), 3.00 (1 H, br dd, J_{66} ~13.3, J_{65} ~11.2, C*H*HN), 3.61–4.74 (~15 H, m), 4.96–5.17 (5 H, m), 5.50 (1 H, br d, *J* 9.1, D_2O-exch., NH) and 7.26–7.39 (10 H, m, ArH); *m/z* (CI) 889 (MH^+, 13%), 831 (M − C_4H_9, 3), 134 (90) and 117 (100).

2-O-*Benzyl*-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-4-O-[2-(2-*carboxybenzamido*)-2-*deoxy*-β-D-*glucopyranosyl*]-3-O-*carboxymethyl*-D-*mannitol* **19**.—Aq. potassium hydroxide (1 mol dm^{-3}; 15 cm^3, 15 mmol) was added dropwise to a stirred solution of compound **17** (2.44 g, 2.29 mmol) in a mixture of 1,4-dioxane (13 cm^3) and methanol (3.3 cm^3) at room temperature. After 1 h the solution was acidified to pH 2.5 (pH meter) with dil. hydrochloric acid and the cloudy mixture was extracted successively with dichloromethane (2 × 50 cm^3) and then with ethyl acetate (50 cm^3). The combined organic extracts were dried, and evaporated under reduced pressure to give a foam, which was purified by chromatography [graded elution with EtOAc–EtOH (4:1); EtOAc–EtOH–water (7:2:1)] to afford the title compound **19** (1.92 g, 97%) as a foam [R_f 0.24, EtOAc–EtOH–water (7:2:1)]; $[\alpha]_D$ −12.6 (*c* 0.1, Me_2SO); ν_{max}(KBr)/cm^{-1} 3399br (OH), 1700 (amide C=O), 1670sh (carboxy C=O), 1251, 1120 and 1080; δ[250 MHz; $(CD_3)_2SO$–D_2O] 0.0 (~6 H, masked by TMS, br s, $SiMe_2$), 0.71 and 0.77 (total 9 H, 2 s, Bu^t), 2.92 (1 H, br t, J_{66} ~ J_{65} ~13.0, C*H*HN), 3.15–3.21 (2 H, m), 3.49–4.09 (~9 H partly obscured by HOD, m), 4.26 (1 H, br t, *J* 7.5), 4.40–4.70 (4 H, m), 4.85–5.05 (2 H, m, CO_2CH_2Ph) and 7.24–7.62 (14 H, m, ArH); *m/z* FAB (THIOG; negative ion mode) 867 (M − H^-, 100%) and 849 (M − H_3O^-, 50).

N-{[4-O-(2′-*Acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-N-*benzyloxycarbonyl*-6-O-(tert-*butyldimethylsilyl*)-1,3,5-*trideoxy*-1,5-*imino*-D-*mannitol*-3-*yl oxy*]*acetyl*}-L-*alanyl*-D-*glutamic Acid Dibenzyl Ester* **20**.—HOBt hydrate (60 mg, 0.44 mmol) was added to a stirred solution of L-alanyl-D-glutamic acid dibenzyl ester hydrochloride† (207 mg, 0.48 mmol) and compound **18** (326 mg, 0.37 mmol) in DMF (3 cm^3) at room temperature. This was followed by the successive additions of *N*-methylmorpholine (45 mm^3, 0.41 mmol) and a solution of DCC (92 mg, 0.44 mmol) in THF (3 cm^3). The solution was stirred for 18 h and was then evaporated under reduced pressure to give an oil, which was triturated with tetrachloromethane (5 cm^3). The suspended *N*,*N*′-dicyclohexylurea was filtered off and the filtrate was diluted with dichloromethane (10 cm^3) and washed with water (10 cm^3). After being dried, the organic extract was evaporated under reduced pressure to afford an oil and this purified by chromatography [acetone–toluene (1:19 → 3:7 gradient elution)] to give the title compound **20** (300 mg, 64%) as a foam [R_f 0.30, acetone–toluene (3:7)]; $[\alpha]_D$ −14.7 (*c* 0.6, $CHCl_3$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420br (NH), 3320br (NH), 1740 (ester C=O), 1680, 1660sh and 1230br; δ(250 MHz) 0.03 and 0.05 (6 H, 2 s, $SiMe_2$), 0.87 (9 H, s, Bu^t), 1.07 and 1.13 (total 3 H, 2 d, each *J* 7.2, ala Me), 1.80, 2.02, 2.03 and 2.10 (total ~12 H overlapping, 4 s, 4 × Ac), 2.0–2.46 (4 H overlapping, m, glu CH_2CH_2), 3.02 (1 H, br t, J_{66} ~ J_{65} ~ 13.0, C*H*HN), 3.61–4.69 (~18 H, m), 4.93–5.19 (8 H, m, 3′- and 4′-H and 3 × CO_2CH_2Ph), 5.56 (1 H, d, *J* 9.1, D_2O-exch., N*H*Ac), 6.89 (1 H, d, *J* 7.8, D_2O-exch., glu NH), 7.26–7.39 (20 H, m, 4 × Ph) and 7.71 and 7.83 (total 1 H, 2 d, each *J* 7.5, D_2O-exch., ala NH); *m/z* FAB (3-NOBA) 1291 (MNa^+), 1269 (MH^+) and 330 ($C_{14}H_{20}NO_8$).

* *Systematic numbering*: 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5-*O*-benzyl-*N*-benzyloxycarbonyl-1-*O*-(*tert*-butyldimethylsilyl)-4-*O*-carboxymethyl-D-mannitol.

† See footnote * in Results and Discussion section.

N{[4-O-(2′-*Acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-1,3,5-*trideoxy*-1,5-*imino*-D-*mannitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-D-*glutamic Acid* **21**.—A solution of compound **20** (112 mg, 88 μmol) in glacial acetic acid (4 cm^3)–water (2 cm^3) with suspended palladium black (140 mg) was shaken at room temperature for 24 h with hydrogen at atmospheric pressure. The catalyst was removed by filtration and washed with the same solvent (3 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 reconcentrated from toluene (2 × 10 cm^3) to give the title compound **21** (76 mg) as a crude oil. This material was used in the next stage without purification.

A small quantity of crude material **21** was purified by chromatography [graded elution: ethyl EtOAc–EtOH (7:3); EtOAc–EtOH–water (7:2:1; 5:3:2)]. The isolated product was dissolved in water (2 cm^3) and freeze dried to yield compound **21** as an amorphous solid [R_f 0.23, EtOAc–EtOH–water (5:3:2)]; $[\alpha]_D$ −18.1 (*c* 0.1, water); ν_{max}(KBr)/cm^{-1} 3365br, 1741 (ester C=O), 1653, 1550, 1241br and 1044; δ(250 MHz; D_2O) 1.42 (3 H, d, *J* 7.1, ala Me), 1.96, 2.00, 2.02 and 2.05 (12 H, overlapping, 4 s, 4 × Ac), 1.85–2.16 (~2 H overlapping, m, CH_2CH_2CO), 2.26 (2 H, br t, *J* 7.1, CH_2CH_2CO), 3.19 (1 H, d, J_{11} 13.0, CHHN), 3.26–3.35 (1 H, m, piperidine CHN), 3.42 (1 H, dd, J_{12} 2.9, CHHN), 3.67–4.52 (13 H, m), 4.81 (~1 H partially obscured by HOD suppression), 4.99 (1 H, t, $J_{4'5'}$ = $J_{4'3'}$ = 10.0, 4′-H) and 5.26 (1 H, dd, *J* 9.5 and 10.3, 3′-H); *m*/*z* FAB (THIOG) 751 (MH^+) and 126 ($C_6H_8NO_2$).

N-{[4-O-(2′-*Acetamido*-2′-*deoxy*-β-D-*glucopyranosyl*)-1,3,5-*trideoxy*-1,5-*imino*-D-*mannitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-D-*glutamic Acid* **22**.—Conc. aq. ammonium hydroxide (specific gravity 0.88; 0.3 cm^3) was added to a solution of the crude glycopeptide **21** (76 mg) in methanol (3 cm^3). The flask was stoppered and the solution was stirred at room temperature for 24 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; 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* **22** (43.5 mg, 79% from compound **20**) as an amorphous solid [R_f 0.40, EtOAC–EtOH–water (1:1:1)] (Found: *m*/*z* 625.2628. $C_{24}H_{40}N_4O_{15}$ requires MH^+, 625.2568); $[\alpha]_D$ −13.2 (*c* 0.1, water); ν_{max}(KBr)/cm^{-1} 3411br, 1718w, 1647 and 1558; δ(250 MHz; D_2O) 1.42 (3 H, d, *J* 7.2, ala Me), 2.04 (3 H, s, Ac), 1.90–2.24 (2 H, m, glu CH_2CH_2CO), 2.42 (2 H, t, *J* 7.3, glu CH_2CH_2CO), 3.21 (1 H, d, J_{11} 13.2, CHHN), 3.28–3.55 (5 H, m, 3′-, 4′- and 5′-H, piperidine CHCH$_2$OH and CHHN), 3.66–3.82 (5 H, m, 2′-, 6′-H, piperidine CH_2OH and $CHOCH_2$), 3.93 (1 H, dd, $J_{6'6'}$ 12.5, $J_{6'5'}$ 3.6, 6′-H), 4.16 (1 H, t, *J* 9.0, piperidine CHOCH), 4.30 (2 H, s, OCH_2CO), 4.28–4.35 (1 H, m, glu $CHCO_2H$), 4.44 (1 H, q, *J* 7.2, ala CH), 4.45 (1 H, br s, piperidine CHOH) and 4.69 (1 H, d, $J_{1'2'}$ 7.9, 1′-H). ^1H–^1H Correlations: 9-H–8-H; 11-H–12-H, 10-H; 1-H–1-H (*gem*, δ 3.42, d), 2-H; 5-H (δ 3.35)–6-H, 4-H (very weak); 5′-H (δ 3.36)–4′-H (δ 3.40), 6′-H (δ 3.79), 6′-H (δ 3.93); 4′-H–5′-H, 3′-H (δ 3.50); 1-H (δ 3.42)–1-H, 2-H; 3′-H–4′-H, 2′-H (δ 3.69); 2′-H–3′-H, 1′-H; 6-H (δ 3.68, dd)–6-H (*gem*, δ 3.80, d), 5-H; 3-H (δ 3.77)–4-H, 2-H; 6′-H (δ 3.79)–5′-H, 6′-H (*gem*); 6-H (δ 3.80)–5-H, 6-H (*gem*); 6′-H–5′-H, 6′-H (*gem*); 4-H–5-H (very weak), 3-H; 10-H–11-H; 8-H–9-H; 2-H–1-H, 3-H; 1′-H–2′-H; *m*/*z* FAB (THIOG) 625 (MH^+) and 126 ($C_6H_8NO_2$).

N-{[4-O-(2′-*Acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-2-O-*benzyl*-N-*benzyloxycarbonyl*-6-O-(*tert*-*butyldimethylsilyl*)-1,3,5-*trideoxy*-1,5-*imino*-D-*mannitol*-3-*yl oxy*]*acetyl*}-L-*alanyl*-γ-D-*glutamyl*-(N$^{\varepsilon}$-*benzyloxycarbonyl*)-L-*lysine Dibenzyl Ester* **23**.—In a similar manner to that described for the preparation of compound **20**, the acid **18** (231 mg) and L-alanyl-γ-D-glutamyl-ε-(*N*-benzyloxycarbonyl)-L-lysine dibenzyl ester hydrochloride* (236 mg) afforded, after chromatography [acetone–toluene (1:9 → 1:1 gradient elution)] the *title compound* **23** (263 mg, 66%) as a syrup [R_f 0.55, acetone–toluene (2:3)] (Found: C, 62.1; H, 6.7; N, 5.1. $C_{79}H_{102}N_6O_{23}Si$ requires C, 61.95; H, 6.7; N, 5.5%); $[\alpha]_D$ −7.7 (*c* 0.25, $CHCl_3$); ν_{max}($CHCl_3$)/cm^{-1} 3420br, 3320br, 1735 (ester C=O), 1675br and 1215br; δ(250 MHz) 0.01, 0.04, 0.05 and 0.10 (6 H, 4 s, $SiMe_2$), 0.88 and 0.92 (9 H, 2 s, Bu^t), 1.04 and 1.08 (3 H, 2 d, each *J* 6.96, ala Me), 1.80, 2.02, 2.05 and 2.07 (~12 H overlapping, 4 s, 4 × Ac), 1.25–1.84 (~6 H, overlapping, m, lys $CH_2CH_2CH_2CH_2N$), 2.00–2.25 (4 H, overlapping, m, glu CH_2CH_2), 2.97–3.16 (3 H, m, CHHN, lys CH_2N), 3.63–4.71 (~19 H, m), 4.93–5.21 (11 H reduces to 10 H after D_2O-exch., m, 3′- and 4′-H, NH, 4 × CO_2CH_2Ph), 5.63 (1 H, d, *J* 9.2, D_2O-exch., NHAc), 6.97 (1 H, d, *J* 7.0, D_2O-exch., NHCO), 7.13–7.43 (~25 H, m, 5 × Ph) and 7.83 (1 H, d, *J* 6.77, D_2O-exch., NHCO); *m*/*z* FAB (3-NOBA) 1553 (MNa^+) and 91 (C_7H_7).

N-{[4-O-(2′-*Acetamido*-3′,4′,6′-*tri*-O-*acetyl*-2′-*deoxy*-β-D-*glucopyranosyl*)-1,3,5-*trideoxy*-1,5-*imino*-D-*mannitol*-3-*yloxy*]-*acetyl*}-L-*alanyl*-γ-D-*glutamyl*-L-*lysine* **24**.—In a similar manner to that described for the preparation of compound **21**, the tripeptide **23** (100 mg) was hydrogenolysed over palladium black to give, after chromatography (gradient elution as before) the *title compound* **24** (40 mg, 70%) as a freeze-dried solid [R_f 0.45, EtOAc–EtOH–water (1:1:1)] (Found: C, 48.7; H, 6.9; N, 9.6. $C_{36}H_{58}N_6O_{19}$ requires C, 49.2; H, 6.65; N, 9.6%); $[\alpha]_D$ −32.7 (*c* 0.2, water); ν_{max}(KBr)/cm^{-1} 3378br, 1742 (ester C=O), 1654, 1593br, 1550sh and 1239br; δ(250 MHz; D_2O) 1.43 (3 H overlapping, d, *J* 7.2, ala Me), 1.32–1.48 (2 H overlapping, m, $CH_2CH_2[CH_2]_2N$), 1.59–1.83 (4 H, m, $CH_2CH_2CH_2CH_2N$), 1.99, 2.02, 2.03 and 2.07 (12 H, overlapping, 4 s, 4 × Ac), 1.92–2.19 (2 H, overlapping, m, glu CH_2CH_2CO), 2.28 (2 H, br t, *J* 8.0, glu CH_2CO), 2.63–2.72 (1 H, br, m, $CHCH_2OH$), 2.77 (1 H, d, *J* 14.1, piperidine CHHN), 2.97 (2 H, t, *J* 7.5, lys CH_2N), 3.07 (1 H, dd, *J* 2.5, piperidine CHHN), 3.59–3.65 (2 H, m, CHHOH, piperidine $CHOCH_2$), 3.73 (1 H, dd, *J* 2.6 and 11.4, CHHOH), 3.82–3.89 (1 H, m, 5′-H), 3.89 (1 H, br t, *J* 8.2, piperidine CHOCH), 3.93 (1 H, dd, $J_{2'1'}$ 8.4, $J_{2'3'}$ 10.4, 2′-H), 4.07–4.33 (7 H, m), 4.52 (1 H, q, *J* 7.2, ala CHMe), 4.91 (1 H, d, anomeric CH), 5.00 (1 H, dd, $J_{4'3'}$ 9.4, $J_{4'5'}$ 10.1, 4′-H) and 5.28 (1 H, dd, 3′-H). ^1H–^1H Correlations: 9-H–8-H, 15-H–16-H, 14-H, 16-H–15-H, 17-H, 14-H–15-H, 14-H (*gem*), 13-H (δ 4.11–4.17), 11-H–11-H (*gem*), 12-H, 10-H (δ 4.26–4.31); 12-H–11-H; 5-H–6-H, 4-H (very weak); 1-H–1-H (*gem*), 2-H (δ 4.25); 17-H–16-H; 3-H–4-H, 2-H; 6-H–6-H (*gem*), 5-H; 5′-H–6′-H (δ 4.10, 4.28, 2 d, each *J* ~12), 4′-H; 4-H–3-H, 5-H (very weak); 2′-H–1′-H, 3′-H; 6′-H–5′-H, 6′-H (*gem*); 13-H–14-H; 8-H (δ 4.2, d, *J* ~14)–8-H (*gem*, δ 4.3); 2-H–1-H, 3-H; 10-H–11-H; 8-H–9-H; 1′-H–2′-H; 4′-H–5′-H, 3′-H; 3′-H–2′-H, 4′-H; *m*/*z* FAB (THIOG) 879 (MH^+), 446 and 126 ($C_6H_8NO_2$).

N-{[4-O-(2′-*Acetamido*-2′-*deoxy*-β-D-*glucopyranosyl*)-1,3,5-*trideoxy*-1,5-*imino*-D-*mannitol*-3-*yloxy*]*acetyl*}-L-*alanyl*-γ-D-*glutamyl*-ε-L-*lysine* **25**.—In a similar manner to that described for the preparation of compound **22**, compound **24** (32 mg) was *O*-deacetylated to give, after chromatography (gradient elution as before), the *title compound* **25** (16.5 mg, 61%) as a freeze-dried, amorphous solid [R_f 0.14, EtOAc–EtOH–water (1:1:1)] (Found: *m*/*z* 753.3535. $C_{30}H_{52}N_6O_{16}$ requires MH^+, 753.3518); $[\alpha]_D$ −24.4 (*c* 0.14, water); δ(400 MHz; D_2O) 1.50 (3 H, overlapping, d, *J* 7.3, ala Me), 1.45–1.54 (2 H overlapping, m, $CH_2CH_2[CH_2]_2N$), 1.71–1.79 (3 H, m, CHHCH$_2$CH$_2$CH$_2$N),

* See footnote * in Results and Discussion section.

1.85–1.91 (1 H, m, CH*H*[CH_2]$_3$N), 2.12 (3 H, s, NH*Ac*), 2.00–2.22 (2 H, m, glu C$H_2$$CH_2$CO), 2.38 (2 H, t, *J* 7.8, glu CH_2CO), 2.71 (1 H, br s, C*H*CH_2O), 2.83 (1 H, d, J_{11} 14.0, piperidine C*H*HN), 3.06 (2 H, t, *J* 7.5, lys CH_2N), 3.13 (1 H, dd, J_{12} 3.3, piperidine CH*H*N), 3.38–3.42 (1 H, m, 5′-H), 3.47 (1 H, dd, $J_{4'3'}$ 9.2, $J_{4'5'}$ 9.4, 4′-H), 3.59 (1 H, dd, $J_{3'2'}$ 10.1, $J_{3'4'}$ 8.9, 3′-H), 3.69 (1 H, dd, J_{32} 2.7, J_{34} 8.7, piperidine C*H*OCH_2), 3.73–3.79 (3 H, m, 2′- and 6′-H, C*H*HOH), 3.83 (1 H, dd, J_{66} 11.5, J_{65} 3.2, CH*H*OH), 3.88 (1 H, d, $J_{6'6'}$ 11.5, 6′-H), 3.96 (1 H, t, $J_{45} \sim J_{43} \sim$ 9.1, piperidine C*H*OCH), 4.23 (1 H, dd, *J* 7.9 and 4.64, lys C*H*CO_2H), 4.26 (1 H, dd, *J* 8.0 and 4.7, glu C*H*CO_2H), 4.29 (1 H, br s, piperidine C*H*OH), 4.35 (2 H, br s, OCH_2CO), 4.55 (1 H, q, *J* 7.3, ala CH), and 4.77 (~1 H partly obscured by HOD, anomeric CH); *m/z* FAB (THIOG) 753 (MH^+).

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