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Spirodiketopiperazines of mannofuranose: carbopeptoid α-amino acid esters at the anomeric position of mannofuranose

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Abstract

Epimeric mannofuranose anomeric aminoesters are prepared from readily available azidolactones and can act as building blocks for the incorporation of mannofuranose units into peptide chains [carbopeptoids]; alternative acylating conditions allow either rapid acylation of the more stable but kinetically hindered amine or reaction with the less hindered but less stable amine to allow control of the anomeric configuration of the products. This is exemplified by coupling of the aminoesters with glycine derivatives to give dipeptide equivalents, and subsequent cyclization to spiro diketopiperazines. Anomers with the nitrogen function *cis* to the 2,3-diol are more stable than those with nitrogen *trans*; mannofuranose derivatives are more stable than the mannopyranose isomers.

1. Introduction

It has been suggested that pyranose amino acid fragments exemplified by **1** can be used as peptide-bond linked carbohydrates (carbopeptoids) to provide extra diversity and carbohydrate structure in the search for ligand lead and drug discovery.[1] The same pyranose amino acid moiety **1** has been used as a flexible β-turn mimic and other aminopyranose carboxylates may be able to act as dipeptide isosteres, perhaps inducing β- and γ-turns.[2] Pyranose amino acid fragments such as **2** have also been used in the synthesis of peptide sugar hybrids.[3] Other pyranose derivatives have been studied as novel oligonucleotide backbone analogues,[4] building blocks for combinatorial synthesis,[5] and other peptide–carbohydrate hybrids.[6] Some

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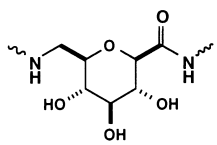
reports of solid-phase synthesis using such monomers have appeared.[7] Very few tetrahydrofuran related amino acids have been reported,[8] although the template properties of such materials would appear to constitute at least as interesting a set of materials as those derived from pyranoses, in terms of both structural diversity and conformational properties.



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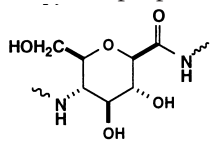

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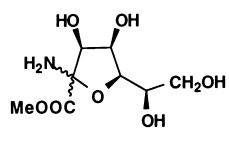

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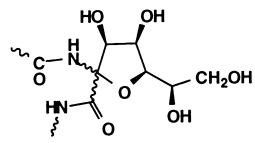

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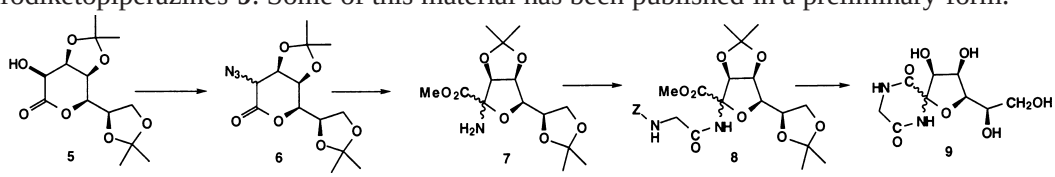
Amino acid moieties at the anomeric positions of sugars such as the mannofuranose aminoesters **3** are an example of a family of novel carbohydrate structures which could be incorporated into amide libraries **4**. Such structures are α,α-disubstituted amino acids and thus may induce secondary structures in relatively short sequences; they are also attractive components for peptide library synthesis in that they provide a novel set of amino acids with hydroxylic components. Additionally, such fragments allow the generation of families of materials with the specific structural information of an individual carbohydrate recognition site. We have reported in a number of preliminary and other communications the synthesis of glucose,[9] rhamnose,[10] galactose[11] and mannose[12] analogues with an α-amino acid moiety at the anomeric position of the sugar. The only other reports of such compounds are a different approach to the synthesis of mannose components such as **4** by Dondoni,[13] and in the synthesis of aminosialic acid analogues.[14] Such structures may be viewed as α-amino-*C*-glycosides of hexoses, so that a seven carbon sugar is required for their synthesis; the readily available lactone **5**[15] has been widely used for the synthesis of mimics of mannose[16] and for the synthesis of other highly functionalised compounds.[17] Many of these syntheses depend on the efficient conversion of **5** to the epimeric azides **6**;[18] this paper reports the conversion of the azides **6** to the epimeric amino esters **7**, coupling of the amines to a protected glycine to give the protected carbopeptoids **8**, and their subsequent elaboration to the spirodiketopiperazines **9**. Some of this material has been published in a preliminary form.[19]

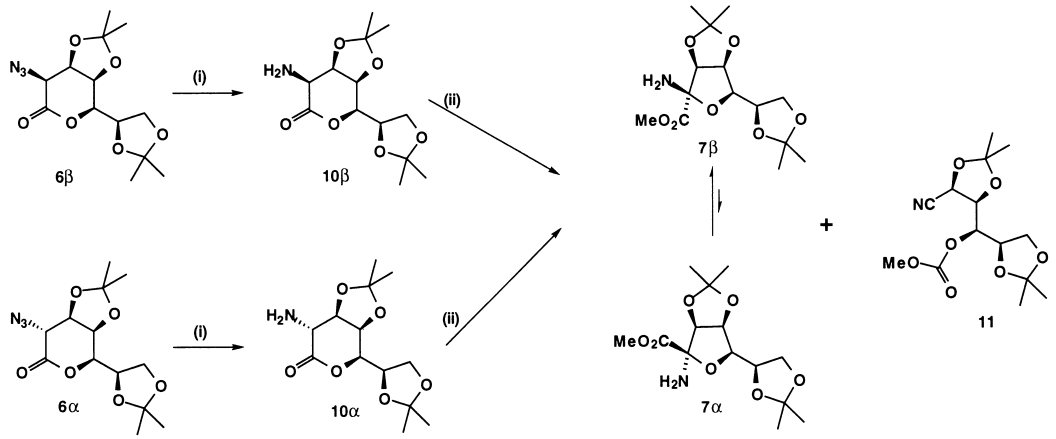


5 [Z = $PhCH_2O.CO$] 6 7 8 9

2. Results and discussion

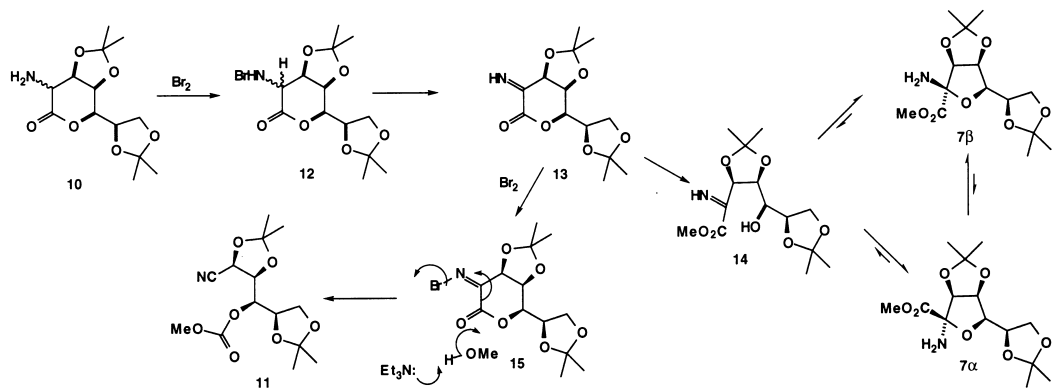
The synthesis of the protected amines **7** is shown in Scheme 1. Hydrogenation of the azides **6β** and **6α** in ethyl acetate in the presence of palladium black gave the amines **10β** and **10α** in 98% and 94% yields, respectively. Oxidation of **6β** by bromine in methanol, followed by addition of triethylamine, gave the epimeric amines **7α** and **7β** [60% and 12% yields, respectively] together with the nitrile **11** [17% yield], giving a combined yield of isolated oxidation products of 89%. Similar treatment of the epimeric amine **6α** gave the same three products in the same proportions.

A possible mechanism for this novel oxidative ring contraction is shown in Scheme 2 [there is no direct evidence for the existence of **13**, **14** and **15** as discrete intermediates]. Initial bromination of **10** might give **12** from which base catalysed elimination of HBr would form the imine **13**. It is possible that the initial bromination occurs at C-2 of the lactone, but overall substitution of H^+ by Br^+ followed by



Scheme 1. (i) H_2, Pd black, EtOAc; (ii) Br_2, Et_3N, AcONa, MeOH

HBr elimination would also form **13**. The formation of the same proportion of products from the epimeric amines **10** might then arise from the common intermediate **13**, capable of forming all three of the isolated products. Ring opening of iminolactone **13** by methanol would give an open chain hydroxyimine **14** which closes to form the epimeric amino esters **7α** and **7β**. Alternatively, further oxidation of the imine **13** by bromine would give the bromoimine **15**. Subsequent base-induced nucleophilic attack on **15** would then result in fragmentation to form the isolated nitrile **11**, rather than in ring opening.



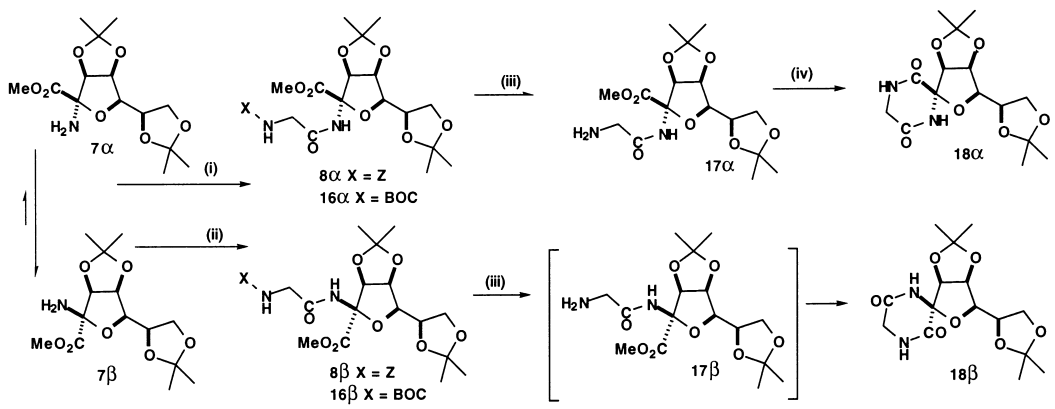
Scheme 2. Possible pathway for the formation of aminoesters **7** and nitrile **11**

Both epimeric amino esters **7** can be obtained in pure form; these provided the first examples of stable amino esters at the anomeric position of sugars. Their easy isolation probably owes much to the level of protection of the carbohydrate, and the use of non-nucleophilic solvents to study the materials. The **7β**:**7α** ratio of 4.8:1 isolated in the reaction appears to be close to the equilibrium position; in non-protic solvents, the equilibration is slow enough to allow the isolation of the individual anomers. The major epimer **7β** is less sterically hindered as the amino group is *cis* to the acetonide/diol group, whereas with the minor epimer **7α** the larger methyl ester group is more crowded. The assignment of the anomeric configuration of the structures is based on the NMR studies described later in the paper and is consistent with the evidence from the crystal structure and NMR data of related mannofuranose spirohydantoins.[20,21]

The epimeric amino esters **7** equilibrate even in non-polar solutions; all attempts to isolate deprotected equivalents of the aminoesters were unsuccessful. The open chain imine would be susceptible to nucleophilic attack to give a variety of products. However, acylation of the amines should give anomeric amides

which are likely to be much more stable; electron-withdrawing groups stabilise tetrahedral adducts, relative to trigonal C=X systems. Thus *N*-acylpyranosylamines are stable and isolable compounds which do not easily anomerise and are not subject to ready hydrolysis at the anomeric position. This is even more likely to be the case for *N*-acylated amino esters, such as **8** for example, in which both the *N*-acyl functionality and the ester are both electron withdrawing groups and will thermodynamically destabilise the open chain imine equivalent against the tetrahedral adducts. Such substitution will also kinetically inhibit any equilibration process.

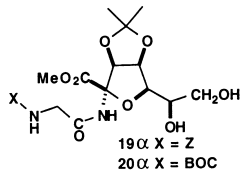
The less stable aminoester **7α** has the less hindered amine, and it was found that alternative acylating conditions could control the anomeric configuration of the product amide [Scheme 3]. Thus acylation of the thermodynamically more stable anomer **7β** with Z-glycine [Z=benzyloxycarbonyl], dicyclohexylcarbodiimide [DCC] and 1-hydroxybenzotriazole [HBT] in DMF gave **8α** as the major product [72% yield] with a small amount of **8β** [8% yield]. A similar product ratio was obtained from the coupling of the less stable aminoester **7α** under the same conditions giving **8α** [71% yield] and **8β** [6% yield]. It thus appears that, under these conditions, the amino esters equilibrate more rapidly than they are acylated, and that the less hindered — but less stable — amine **7α** is acylated faster. Similar results were obtained when BOC-glycine [BOC=*tert*-butyloxycarbonyl] was coupled with **7β** under the same conditions giving **16α** in 75% yield but **16β** in only 8% yield, whereas with **7α**, **16α** was produced in 72% yield and **16β** in 7% yield.



Scheme 3. (i) Z-Glycine or BOC-glycine, DCC, 1-hydroxybenzotriazole, DMF; (ii) Z-glycine, $ClCO_2Et$, Et_3N, CH_2Cl_2; (iii) H_2, Pd black, MeOH; (iv) ^tBuOK, THF

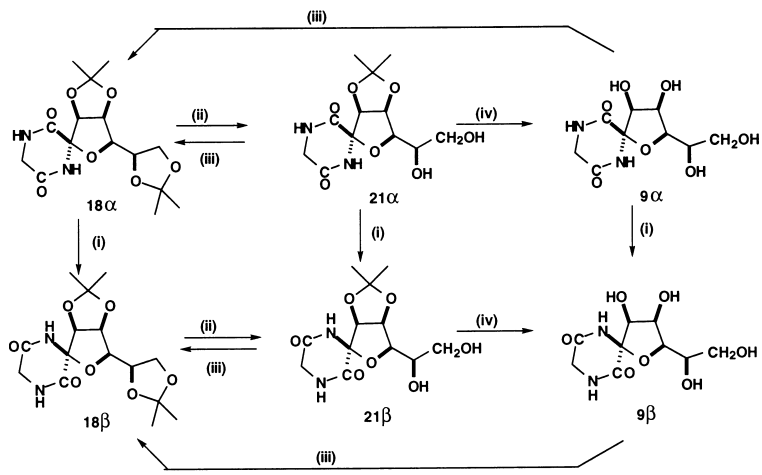
In marked contrast, equilibration of the anomeric amines **7** does not compete with the peptide coupling when Z-glycine is activated as the mixed anhydride with ethyl chloroformate. Under these conditions, where the more stable **7β** is coupled, the major product is **8β** [56% yield] with only traces of **8α** being formed. Thus the coupling reaction now occurs faster than equilibration to the more reactive anomeric amine **7α**. In no case was there any evidence to indicate that any of the coupled products underwent any equilibration under the reaction conditions.

The epimeric Z-protected dipeptides **8** were converted to the corresponding diketopiperazines **18** without any equilibration of the anomers taking place. Hydrogenation of the Z-protecting group in **8β** gave the diketopiperazine **18β** in 95% yield, indicating that the initially formed amine **17β** spontaneously cyclises. In contrast, hydrogenation of the anomeric Z-derivative **8α** afforded the amine **17α** isolated in 91% yield. The cyclisation of **17α** requires attack by the amine on a very hindered ester carbonyl group; treatment of **17α** with potassium *tert*-butoxide in tetrahydrofuran induces clean cyclisation to **18α** [89% yield] with no contamination by anomeric equilibration.



All these acylated derivatives of **7** are stable to any epimerisation at the anomeric position under a wide variety of conditions; thus the side chain acetonide can be removed from **8α** and **16α** by acetic acid in methanol to form **19α** and **20α** in yields of 80% and 82% respectively. The diols **19α** and **20α** were reprotected by reaction with 2,2-dimethoxypropane and acetone in the presence of *p*-toluenesulfonic acid to give **8α** and **16α** in quantitative yields.

No anomeric equilibration of the diketopiperazine moiety occurred under a wide range of treatments with acid. The diketopiperazines **18α** and **18β** can be partially deprotected by reaction with acetic acid in methanol to afford **21α** and **21β** in 88% and 87% yields, respectively [Scheme 4]. Treatment of **21α** and **21β** with aqueous trifluoroacetic acid resulted in complete deprotection to **9α** and **9β** in 92% and 80% yields. Furthermore the fully **9α** — and partially **21α** — deprotected furanoses can be converted to **18α** in excellent yields; **9β** and **21β** can be similarly deprotected to **18β**. No anomeric equilibration — nor any isomerisation to mannopyranose isomers — took place in any of these reactions, implying that there is no ring opening of the furanose ring under these conditions.



Scheme 4. (i) ^{t}BuOK, DMF; (ii) AcOH:MeOH (1:1); (iii) $Me_2C(OMe)_2$, Me_2CO, *p*-toluenesulfonic acid; (iv) $CF_3COOH:H_2O$ (1:1)

The relative stability of epimeric diketopiperazines was indicated by a remarkable series of equilibrations of the α-anomers to the β-anomers by treatment with potassium *tert*-butoxide in dimethylformamide at 100°C. Thus equilibration under these conditions caused epimerisation of the fully protected diacetonide **18α** to **18β** in 88% yield, of the monoacetonide **21α** to **21β** in 85% yield, and of the fully protected diacetonide **9α** to **9β** in 90% yield. These experiments clearly demonstrate that the β-anomers are thermodynamically more stable than the α-isomers. Moreover, although in **21** and **9** ring opening and closing takes place there is no capture of the intermediate imines by the side-chain secondary hydroxyl group to give mannopyranose isomers, and this strongly suggests that mannofuranose isomers are thermodynamically more stable than mannopyranose isomers under these conditions.

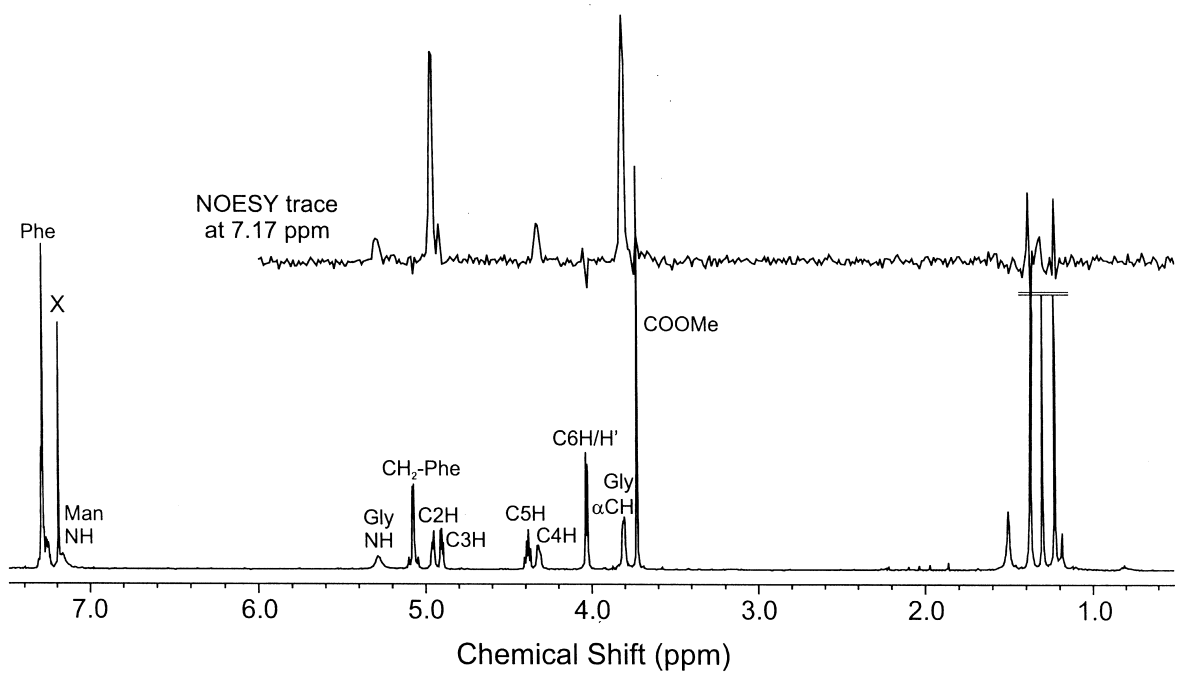
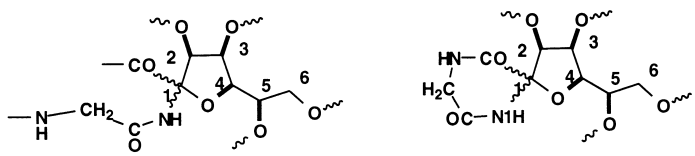


Fig. 1. *Lower trace*: the 1D ^{1}H NMR spectrum of **8α**, showing the resonance assignments. *Upper trace*: a trace through the phase-sensitive 2D NOESY spectrum, 400 ms mixing time, at 7.17 ppm (corresponding to the Man NH resonance)



Numbering of mannofuranose structures in NMR figures 1 - 5

The anomeric configurations of **8**, **9**, **18** and **21** were established by NMR studies. The 1D NMR spectra of **8α** and **8β** are shown in Figs 1 and 2, respectively.

In both compounds, an NOE is observed from the Man NH resonance to the Man C2H resonance. This NOE is much larger in **8α** than **8β**. The average Man NH to Man C2H distances were calculated from the NOE build-up curves by using the C2H to C4H NOE as an internal calibration (Table 1). This gives a value of 2.4 Å for **8α** and 3.8 Å for **8β**. Figure 3 shows the variation in NH–C2H distance with rotation around the C1–N bond for the two anomeric configurations of **8** (either *cis* or *trans* H–C2–C1–N). A distance of 2.4 Å can only be obtained for the *cis* form, whilst 3.8 Å can only be obtained for the *trans* form. Thus, in **8α** the C2H proton is on the same side of the ring as the NH group (*cis*) whilst in **8β** they are on different sides (*trans*). This is confirmed by the observation of strong NOEs from the Man NH resonance to two of the protecting group methyl resonances for **8β** (Fig. 2).

The 1D NMR spectra of **9α** and **9β** are shown in Figs 4 and 5. In both spectra, four hydroxyl proton resonances can be identified. For **9α**, the hydroxyl resonances can be assigned to C2, C3, C5 and C6 confirming the presence of a furanose ring. A strong NOE is observed between N1H and C2H for **9α** (Fig. 4, giving an inter-proton distance of 2.7 Å), but not for **9β** (Fig. 5). The N1H to C2H distances determined by molecular modelling for the two epimers of **9** are 2.5 Å for *cis* and 3.9 Å for *trans*

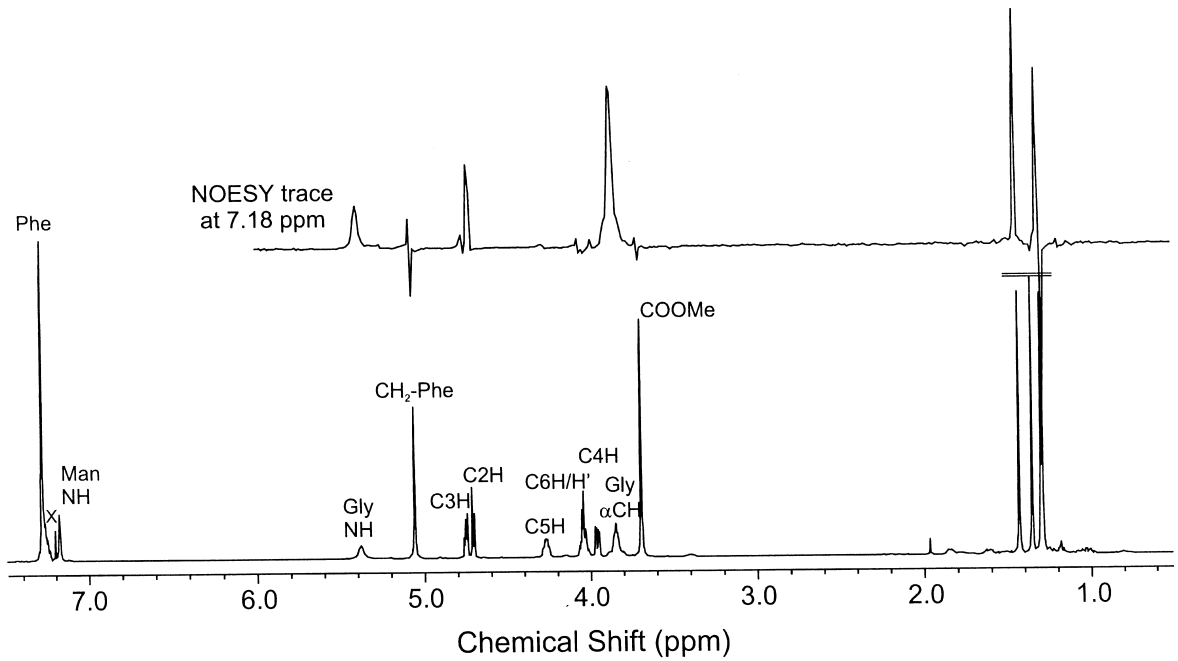


Fig. 2. *Lower trace*: the 1D ^{1}H NMR spectrum of **8β**, showing the resonance assignments. *Upper trace*: a trace through the phase-sensitive 2D NOESY spectrum, 400 ms mixing time, at 7.18 ppm (corresponding to the Man NH resonance)

Table 1
Calculated inter-proton distances for **8α** and **8β** based on NOE cross-peak intensity. The Man C2H–C4H distances, used as the internal calibration, were determined by molecular modelling to be 3.25 Å for *cis* H–C2–C1–N and 3.44 Å for *trans* H–C2–C1–N. The choice of which value to use has little effect on the calculated distances for NH to C2H. The C2H–NH distances determined by molecular modelling are shown in figure c as a function of the C2–C1–N–H torsion angle

Compound	Proton pair	NOE Intensity	Calibration distance (Å)	Calculated distance (Å)
8α	C2H -- C4H	0.021	3.25 (cis)	--
	C2H -- NH	0.130	--	2.40
8β	C2H -- C4H	0.045	3.44 (trans)	--
	C2H -- NH	0.024	--	3.83

H–C2–C1–N1. This confirms the *cis* H–C2–C1–N1 configuration for **9α** and the *trans* configuration for **9β**.

The anomeric configurations of **18α**, **18β**, **21α**, and **21β**, (data not shown) were determined in an identical fashion to **9α** and **9β**, giving *cis* H–C2–C1–N1 configurations for **18α** and **21α** and *trans* H–C2–C1–N1 configurations for **18β** and **21β**.

It is thus clear that *N*-acylated derivatives of mannofuranose structures containing an α-amino acid moiety at the anomeric position are stable configurationally other than under strongly basic or acidic conditions; additionally, the difference in reactivity between the anomeric amino esters **7** allows control of the configuration of the resulting acylated materials. Such compounds are suitable candidates for the generation of amide libraries at the anomeric positions of carbohydrates.

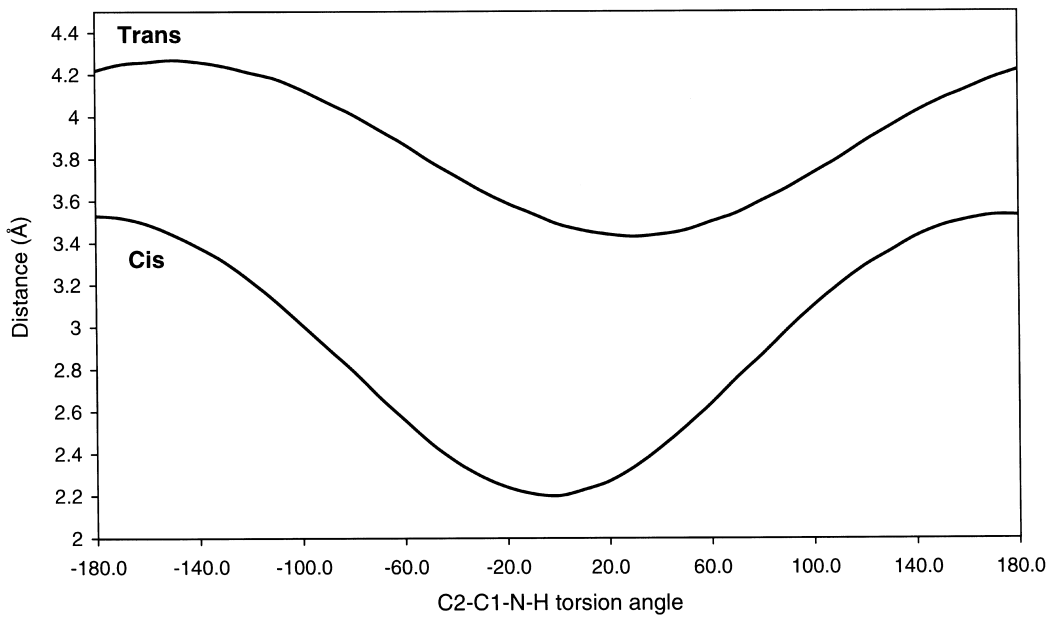


Fig. 3. Plots of NH to C2H distance (Å) versus torsion angle (rotation around the C1–N bond) for the two configurations of **8**. *Lower line* is for *cis* H–C2–C1–N (**8α**), *upper line* is for *trans* H–C2–C1–N (**8β**)

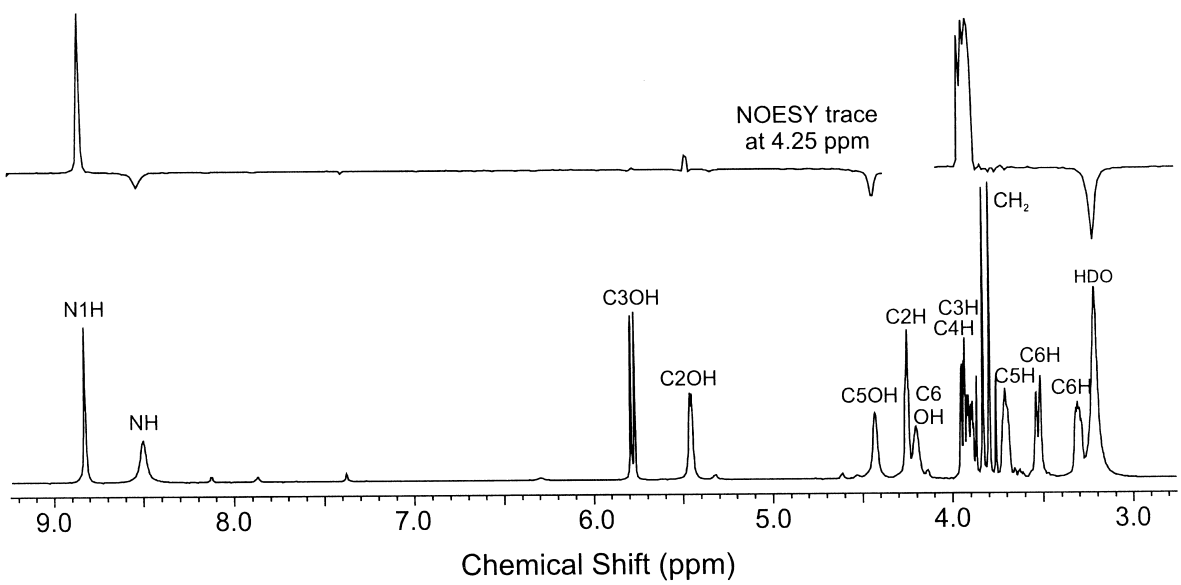


Fig. 4. *Lower trace*: the 1D ^{1}H NMR spectrum of **9α**, showing the resonance assignments. *Upper trace*: a trace through the phase-sensitive 2D NOESY spectrum, 400 ms mixing time, at 4.25 ppm (corresponding to the C2H resonance). The negative peaks are chemical exchange peaks involving the C7OH resonance

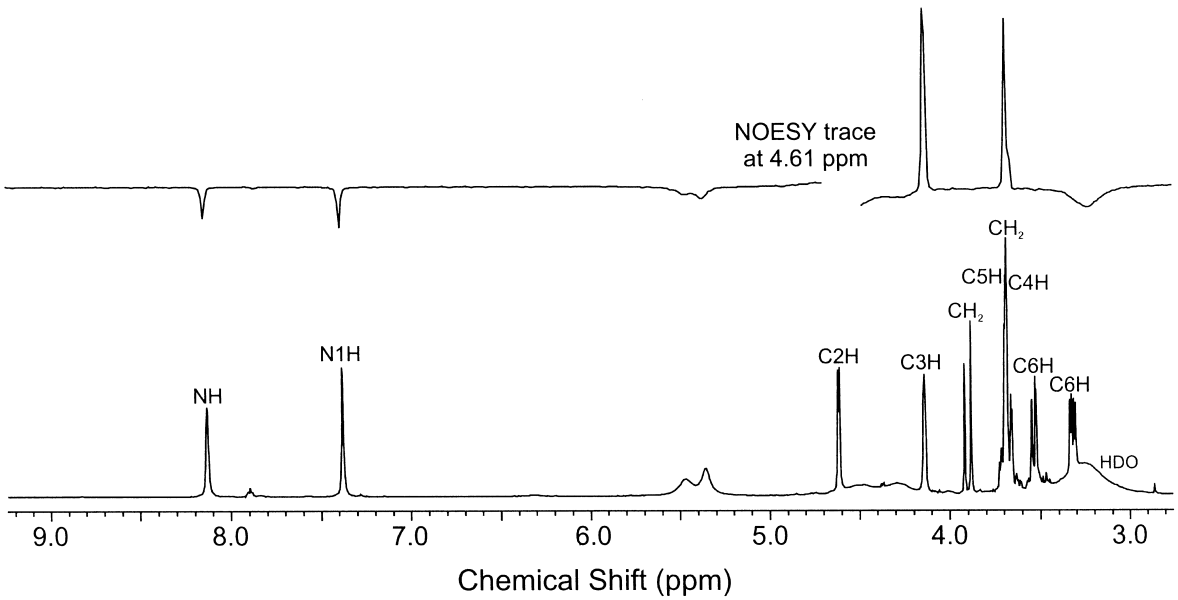


Fig. 5. *Lower trace*: the 1D ^{1}H NMR spectrum of **9β**, showing the resonance assignments. *Upper trace*: a trace through the phase-sensitive 2D NOESY spectrum, 400 ms mixing time, at 4.61 ppm (corresponding to the C2H resonance). The negative peaks are chemical exchange peaks involving the broad OH resonance at 4.5 ppm

3. Experimental

Melting points were recorded on a Kofler block and are corrected. Proton nuclear magnetic resonance (δ_H) spectra were recorded on a Bruker AM 500 (500 MHz) spectrometer, or where stated, on a Varian Gemini 200 (at 200 MHz). Carbon nuclear magnetic resonance (δ_C) spectra were recorded, on a Varian Gemini 200 (at 50.3 MHz) or, where stated, on a Bruker AM 500 (125 MHz) spectrometer; multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ-scale using residual solvent as an internal standard. For the NOE studies, ^{1}H NMR spectra on compounds **8α** and **8β** in $CDCl_3$, **9α**, **9β**, **21α** and **21β** in DMSO and **18α** and **18β** in CD_3CN were recorded on a Varian Unity 500, with a probe temperature of 30°C. Resonance assignments were obtained from the 1D and phase-sensitive 2D COSY spectra, referenced to the residual solvent signal (7.20 ppm for $CDCl_3$, 2.49 ppm for DMSO or 1.93 ppm for CD_3CN). In all cases except **9α**, the carbon backbone could be followed unambiguously. Unambiguous assignments for **9α** were obtained using ^{1}H–^{13}C correlations from HMQC and HMBC spectra. The only remaining ambiguities were in the stereo-specific assignments of the protecting group methyl resonances, which could be determined by the pattern of NOEs between these resonances and the mannose ring proton resonances. Phase-sensitive 2D NOESY spectra were recorded with mixing times of 100 to 800 ms without any random variation. Inter-proton distances were determined from the NOE cross-peak volumes using the two-spin approximation as previously described.[22] Molecular modelling was performed on a Silicon Graphics Indigo 2 workstation using Insight II and Discover software (MSI). The variation in internuclear distance with torsion angle was determined as previously described.[23] Infrared spectra were recorded on a Perkin–Elmer 1750 FT-IR spectrophotometer. Mass spectra were recorded on VG Micromass 20-250, ZAB 1F or Trio-1 GC–MS (DB-5 column) spectrometers using desorption chemical ionisation (DCI, NH_3), chemical ionisation (CI, NH_3), fast atom bombardment (FAB) or electrospray techniques, as stated. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Hydrogenations were

executed at atmospheric pressure under an atmosphere of hydrogen gas maintained by an inflated balloon. Microanalyses were performed by the microanalysis service of the Dyson Perrins Laboratory. Thin layer chromatography (t.l.c.) was carried out on aluminium sheets coated with $60F_{254}$ silica or glass plates coated with silica Blend 41. Plates were developed using a spray of 0.2% w/v cerium(IV) sulphate and 5% ammonium molybdate in 2 M sulphuric acid or 0.5% ninhydrin in methanol (for amines). Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; in particular, dichloromethane was refluxed over, and distilled from, calcium hydride, methanol was purchased dry in Aldrich Sure-Seal™ bottles, pyridine was distilled from, and stored over, potassium hydroxide, tetrahydrofuran was distilled, under nitrogen, from a solution dried with sodium in the presence of benzophenone. Hexane and ethyl acetate were distilled before use to remove involatile fractions. All solvents were removed *in vacuo*. The azidolactones **6** were prepared as previously described.[18]

3.1. 2-Amino-2-deoxy-3,4:6,7-di-O-*isopropylidene*-D-glycero-D-galacto-*heptono-1,5-lactone* ***10α***

A solution of azidolactone **6α** (1.01 g, 3.2 mmol) in ethyl acetate (10 ml) was stirred under an atmosphere of hydrogen in the presence of a catalytic amount of palladium black (*ca*. 8 mg). After 24 h the mixture was filtered through Celite. Crystallisation of the crude product by (ethyl acetate/hexane) yielded aminolactone **10α** as a white crystalline solid (900 mg, 98%), m.p. 124–127°C; $[\alpha]_D^{25}$ +87.6 (c 0.8, $CHCl_3$); ν_{max} (KBr): 3363, 3304 (NH), 1756 cm^{-1} (C=O); δ_H ($CDCl_3$): 1.38, 1.40, 1.43, 1.46 (12H, 4×s, 2×C(CH_3)$_2$), 3.96 (1H, br d, H-2), 4.12 (1H, dd, $J_{6,7}$ 4.2 Hz, $J_{7,7'}$ 9.1 Hz, H-7), 4.16 (1H, dd, $J_{6,7'}$ 6.0 Hz, $J_{7',7}$ 9.1 Hz, H-7′), 4.41 (2H, m, $J_{5,6}$ 8.4 Hz, $J_{6,7}$ 4.2 Hz, $J_{6,7'}$ 6.0 Hz, H-6, H-5), 4.64 (1H, dd, $J_{3,4}$ 1.9 Hz, $J_{4,5}$ 7.5 Hz, H-4), 4.93 (1H, dd, $J_{2,3}$ 8.6 Hz, $J_{3,4}$ 1.9 Hz, H-3); δ_C ($CDCl_3$): 24.0, 25.0, 25.9, 26.9 (4×q, C(CH_3)$_2$), 54.8 (d, C-2), 66.7 (t, C-7), 70.9, 73.0, 76.1, 76.4 (4×d, C-3, C-4, C-5, C-6), 109.9, 110.0 (2×s, $C(CH_3)_2$), 170.1 (s, C-1); m/z (CI, NH_3): 288 ($M+H^+$, 100) 305, ($M+NH_4^+$, 30%). Found: C, 54.67; H, 7.12; N, 4.62. $C_{13}H_{21}O_6N$ requires: C, 54.35; H, 7.37; N, 4.88%.

3.2. 2-Amino-2-deoxy-3,4:6,7-di-O-*isopropylidene*-D-glycero-D-talo-*heptono-1,5-lactone* ***10β***

A solution of azidolactone **6β** (1.04 g, 3.3 mmol) in ethyl acetate (20 ml) was stirred under an atmosphere of hydrogen in the presence of a catalytic amount of palladium black (*ca*. 8 mg). After 24 h the reaction mixture was filtered through Celite to give after crystallisation (ethyl acetate/hexane) aminolactone **10β** as a white crystalline solid (890 mg, 94%), m.p. 145–147°C (ethyl acetate/hexane); $[\alpha]_D^{25}$ +56.2 (c 1.1, $CHCl_3$); ν_{max} (KBr): 3374, 3319 (NH), 1752 cm^{-1} (C=O); δ_H ($CDCl_3$): 1.38, 1.39, 1.43, 1.46 (12H, 4×s, 2×C(CH_3)$_2$), 3.60 (1H, br d, sharp in D_2O, H-2), 4.03 (1H, dd, $J_{4,5}$ 1.7 Hz, $J_{5,6}$ 8.3 Hz, H-5), 4.08 (1H, dd, $J_{6,7}$ 3.9 Hz, $J_{7,7'}$ 9.2 Hz, H-7), 4.15 (1H, dd, $J_{6,7'}$ 6.1 Hz, $J_{7',7}$ 9.3 Hz, H-7′), 4.41 (1H, ddd, $J_{5,6}$ 8.2 Hz, $J_{6,7}$ 3.9 Hz, $J_{6,7'}$ 6.0 Hz, H-6), 4.69 (1H, dd, $J_{3,4}$ 7.8 Hz, $J_{4,5}$ 1.6 Hz, H-4), 4.77 (1H, dd, $J_{2,3}$ 2.9 Hz, $J_{3,4}$ 7.9 Hz, H-3); δ_C ($CDCl_3$): 24.1, 24.8, 25.7, 26.8 (4×q, C(CH_3)$_2$), 53.3 (d, C-2), 66.5 (t, C-7), 72.4, 72.6, 75.7, 76.4 (4×d, C-3, C-4, C-5, C-6), 109.9, 110.4 (2×s, $C(CH_3)_2$), 172.9 (s, C-1); m/z (CI, NH_3): 288 ($M+H^+$, 100%). Found: C, 54.31; H, 7.45; N, 4.53. $C_{13}H_{21}O_6N$ requires: C, 54.35; H, 7.37; N, 4.88%.

3.3. 2,3:5,6-Di-O-isopropylidene-4-methoxycarbonyl-mannononitrile **11**, methyl 2-amino-2,5-anhydro-3,4:6,7-di-O-isopropylidene-D-glycero-D-talo-heptonate **7β** and methyl 2-amino-2,5-anhydro-3,4:6,7-di-O-isopropylidene-D-glycero-D-galacto-heptonate **7α**

Bromine (0.42 ml, 8.2 mmol) was added to a stirred solution of aminolactone **10β** (2.36 g, 8.2 mmol) and sodium acetate (2.02 g, 24.6 mmol) in anhydrous methanol (40 ml). After 5 min t.l.c. (ethyl acetate:hexane, 1:1) indicated the presence of no starting material and triethylamine (2.29 ml, 16.4 mmol) was added. After 24 h the reaction mixture was preabsorbed onto silica; purification by flash chromatography (ethyl acetate:hexane, 1:1) yielded mannononitrile **11** as a white crystalline solid (440 mg, 17%), m.p. 109–110°C (hexane/ether); $[\alpha]_D^{20}$ +53.5 (c 0.8 $CHCl_3$); ν_{max} (KBr): 1761 cm^{-1} (C=O); δ_H (500 MHz and COSY, $CDCl_3$): 1.36, 1.39, 1.48, 1.60 (12H, 4×s, 2×C(CH_3)$_2$), 3.85 (3H, s, OCO_2CH_3), 3.96 (1H, dd, $J_{5,6}$ 6.1 Hz, $J_{6,6'}$ 8.3 Hz, H-6), 4.12 (1H, m, $J_{4,5}$ 8.0 Hz, $J_{5,6}$ 6.1 Hz, $J_{5,6'}$ 6.2 Hz, H-5), 4.16 (1H, dd, $J_{5,6'}$ 6.2 Hz, $J_{6,6'}$ 8.3 Hz, H-6′), 4.31 (1H, dd, $J_{2,3}$ 5.1 Hz, $J_{3,4}$ 7,8 Hz, H-3), 4.89 (1H, d, $J_{2,3}$ 5.1 Hz, H-2), 5.12 (1H, t, *J* 8.0 Hz, H-4); δ_C ($CDCl_3$): 25.2, 25.7, 25.9, 26.7, (4×q, 2×C(CH_3)$_2$), 55.5 (q, OCO_2CH_3), 66.1 (d, C-2), 67.4 (t, C-6), 74.0, 76.0, 77.5 (3×d, C-3, C-4, C-5), 111.0, 112.2 (2×s, 2×*C*(CH_3)$_2$), 116.8 (s, C-1), 155.0 (s, C=O); m/z (CI, NH_3): 316 ($M+H^+$, 60), 333 ($M+NH_4^+$, 100%). Found: C, 53.29; H, 6.81; N, 4.71. $C_{14}H_{21}O_7N$ requires: C, 53.33; H, 6.71; N, 4.44%.

Further elution of the column gave aminoester **7β** as a colourless oil (1.57 g, 60%); $[\alpha]_D^{20}$ −4.0 (c 0.5 $CHCl_3$); ν_{max} (KBr): 3430, 3347 (NH), 1738 cm^{-1} (C=O, ester); δ_H ($CDCl_3$): 1.37, 1.39, 1.44, 1.54 (12H, 4×s, 2×C(CH_3)$_2$), 3.71 (1H, dd, $J_{4,5}$ 3.0 Hz, $J_{5,6}$ 7.9 Hz, H-5), 3.80 (3H, s, CO_2CH_3), 3.99 (1H, dd, $J_{6,7}$ 4.4 Hz, $J_{7,7'}$ 8.7 Hz, H-7), 4.08 (1H, dd, $J_{6.7'}$ 6.3 Hz, $J_{7',7}$ 8.7 Hz, H-7′), 4.35 (1H, ddd, $J_{5,6}$ 7.8 Hz, $J_{6,7}$ 4.4 Hz, $J_{6,7'}$ 6.3 Hz, H-6), 4.86 (2H, m, H-3, H-4); δ_C ($CDCl_3$): 24.2, 25.6, 25.7, 26.8 (4×q, 2×C(CH_3)$_2$), 52.5 (q, CO_2CH_3), 66.9 (t, C-7), 73.2, 78.7, 80.5, 81.2 (4×d, C-3, C-4, C-5, C-6), 93.0 (s, C-2), 109.4, 112.8 (2×s, 2×*C*(CH_3)$_2$), 169.2 (s, C-1); m/z (CI, NH_3): 318 ($M+H^+$, 100%). Found: C, 52.86; H, 7.60; N, 4.21. $C_{14}H_{23}O_7N$ requires: C, 52.99; H, 7.31; N, 4.41%.

Further elution of the column gave aminoester **7α** as a white crystalline solid (313 mg, 12%), m.p. 102–104°C (hexane/ether); $[\alpha]_D^{20}$ +70.1 (c 0.5, acetonitrile); ν_{max} (KBr): 3903, 3854 (NH), 1737 cm^{-1} (C=O, ester); δ_H ($CDCl_3$): 1.32, 1.39, 1.44, 1.47 (12H, 4×s, 2×C(CH_3)$_2$), 3.80 (3H, s, CO_2CH_3), 4.10 (1H, dd, $J_{6,7}$ 4.3 Hz, $J_{7,7'}$ 8.8 Hz, H-7), 4.15 (1H, dd, $J_{6,7'}$ 6.1 Hz, $J_{7,7'}$ 8.8 Hz, H-7′), 4.20 (1H, dd, $J_{4,5}$ 3.5 Hz, $J_{5,6}$ 8.2 Hz, H-5), 4.51 (1H, ddd, $J_{5,6}$ 8.2 Hz, $J_{6,7}$ 4.3 Hz, $J_{6,7'}$ 6.1 Hz, H-6), 4.52 (1H, d, $J_{3,4}$ 5.8 Hz, H-3), 4.87 (1H, dd, $J_{3,4}$ 5.8 Hz, $J_{4,5}$ 3.5 Hz, H-4); δ_C (d_3 acetonitrile): 23.9, 24.4, 25.0, 26.0, (4×q, 2×C(CH_3)$_2$), 51.6 (q, CO_2CH_3), 66.5 (t, C-7); 73.0, 79.9, 80.3, 87.5 (4×d, C-3, C-4, C-5, C-6), 94.9 (s, C-2), 108.7, 112.7 (2×s, 2×*C*(CH_3)$_2$), 170.6 (s, C-1); m/z (CI, NH_3): 318 ($M+H^+$, 100%). Found: C, 53.30; H, 7.60; N, 4.40. $C_{14}H_{23}O_7N$ requires: C, 52.99; H, 7.31; N, 4.41%.

Oxidation of the epimeric amine **10α** (138 mg, 0.48 mmol) using a solution of bromine (0.48 mmol) in dichloromethane under the same conditions as above gave the same three products in the same proportions.

3.4. Methyl N-2-(benzyloxycarbonylglycylamino)-2,5-anhydro-3,4:6,7-di-O-isopropylidene-D-glycero-D-talo-heptonate **8α** and methyl N-2-(benzyloxycarbonylglycylamino)-2,5-anhydro-3,4:6,7-di-O-isopropylidene-D-glycero-D-galacto-heptonate **8β**

(a) From methyl β-aminoester **7β**: A solution of DCC (1.46 g, 7.11 mmol), HBT (0.97 g, 7.11 mmol) and Z-glycine (1.46 g, 7.11 mmol) in dry DMF (30 ml) was stirred at 0°C under nitrogen for 15 min. The reaction was then allowed to warm to room temperature and stirred for a further 45 min. Over this period a white precipitate of dicyclohexylurea gradually came out of the reaction mixture. The aminoester

7β (1.66 g, 5.14 mmol) in dry DMF (18 ml) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was filtered through Celite and the filtrate concentrated *in vacuo* to give a residue that was dissolved in dichloromethane (50 ml) and extracted with water (2×30 ml). The organic layers were combined and the solvents removed *in vacuo* to give a residue that was purified by flash chromatography (ethyl acetate:hexane, 2:1) to give peptide **8α** (1.92 g, 72% yield) as a white solid that was crystallised from acetonitrile but small quantities of dicyclohexylurea remained. ν_{max} (KBr): 3424, 3329 (N–H), 2986, 2953 (–CH), 1752, 1726, 1699 (C=O), 1543, 1215, 1068 cm^{-1}; δ_H (500 MHz, $CDCl_3$): 1.23, 1.31, 1.38 (3×s, 12H, 4×CH_3), 3.73 (s, 3H, –$COOCH_3$), 3.81 (b, 2H, Gly CH_2), 4.03 (m, 2H, Man C6H/H′), 4.32 (dd, $J_{3,4}$ 3.9 Hz, $J_{4,5}$ 7.8 Hz, 1H, Man C4H), 4.39 (m, 1H, Man C5H), 4.91 (dd, $J_{2,3}$ 5.5 Hz, 1H, Man C3H), 4.96 (d, 1H, Man C2H), 5.06, 5.09 (2×d, $J_{H,H'}$ 12.7 Hz, 2H, CH_2–Ph), 5.28 (b, 1H, Gly NH), 7.17 (s, 1H, Man NH), 7.3 (b, 5H, C_6H_5).

Further elution gave peptide **8β** (0.2 g, 7.5% yield) as a gum, ν_{max} (film): 3396 (–NH), 2987, 2938 (–CH), 1760, 1730, 1702 (C=O), 1499, 1261, 1214, 1069 cm^{-1}; δ_H (500 MHz, $CDCl_3$): 1.28, 1.30, 1.36, 1.43 (4×s, 12H, 4×CH_3), 3.70 (s, 3H, –$COOCH_3$), 3.85 (b, 2H, Gly CH_2), 3.96 (dd, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 9.2 Hz, 1H, Man C4H), 4.04 (m, 2H, Man C6H/H′), 4.27 (m, 1H, Man C5H), 4.71 (d, 1H, $J_{2,3}$ 6.1 Hz, Man C2H), 4.75 (dd, 1H, Man C3H), 5.05 (s, 2H, CH_2–Ph), 5.40 (b, 1H, Gly NH), 7.18 (s, 1H, Man NH), 7.3 (b, 5H, C_6H_5); δ_C (50.3 MHz, $CDCl_3$): 24.77, 25.10, 25.67, 26.88 (q, –CH_3), 44.38, 67.11, 67.29 (t, –CH_2–), 53.08 (q, –$COOCH_3$), 72.73, 79.85, 80.03, 83.26 (d, –CH–), 88.56, 109.43, 114.12, 136.05 (s, –C–), 128.05, 128.20, 128.50 (d, Ar–H), 156.57, 168.59, 169.10 (s, C=O); m/z (NH_3, CI, %): 509 [$(M+1)^+$, 10], 401 (30), 343 (72), 285 (20), 167 (21), 108 (85), 91 (100), 58 (38). Found: C, 56.85; H, 6.38; N, 5.63. $C_{24}H_{32}N_2O_{10}$ requires: C, 56.69; H, 6.34; N, 5.51%.

(b) From methyl α-aminoester **7α**: A solution of DCC (185 mg, 0.9 mmol), HBT (123 mg, 0.9 mmol) and BOC-Gly (185 mg, 0.9 mmol) in dry DMF (4 ml) was stirred at 0°C under nitrogen for 15 min. The reaction was then allowed to warm up to room temperature and stirred for a further 45 min. Over this period a white precipitate dicyclohexylurea gradually come out of solution. Aminoester **7α** (210 mg, 0.66 mmol) dissolved in dry DMF (2 ml) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was worked up as above and the crude products purified by flash chromatography (ethyl acetate:hexane, 2:1) to give peptide **8α** (239 mg, 71% yield) as a white solid that was crystallised from acetonitrile but was contaminated with small quantities of dicyclohexylurea. Further elution gave peptide **8β** (20 mg, 6% yield).

3.5. Methyl N-*2-(*tert-*butoxycarbonylglycylamino)-2,5-anhydro-3,4:6,7-di*-O-*isopropylidene*-D-glycero-D-talo-*heptonate* ***16α*** *and methyl* N-*2-(*tert-*butoxycarbonylglycylamino)-2,5-anhydro-3,4:6,7-di*-O-*isopropylidene*-D-glycero-D-galacto-*heptonate* ***16β***

(a) From methyl β-aminoester **7β**: A solution of DCC (0.61 g, 2.97 mmol), HBT (0.40 g, 2.97 mmol) and BOC-Gly (0.52 g, 2.97 mmol) in dry DMF (16 ml) was stirred at 0°C under nitrogen for 15 min. The reaction was then allowed to warm up to room temperature and stirred for a further 45 min during which time dicyclohexylurea gradually precipitated out of solution. Then aminoester **7β** (0.71 g, 2.26 mmol) in dry DMF (6 ml) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture was filtered through Celite and the filtrate evaporated *in vacuo* to give a residue that was dissolved in dichloromethane (50 ml) and extracted with water (2×30 ml). The organic layers were evaporated *in vacuo* to give a residue that was preabsorbed onto silica and purified by flash chromatography (ethyl acetate:hexane, 2:1) to give peptide **16α** (0.80 g, 75% yield) as a white solid that was crystallised from acetonitrile but always contained small quantities of dicyclohexylurea. ν_{max} (KBr): 3414, 3337 (N–H), 2987, 2937 (–CH), 1760, 1730, 1698 (C=O), 1550, 1369, 1213, 1164, 1087 cm^{-1}; δ_H (500 MHz,

$CDCl_3$): 1.31, 1.37, 1.45, 1.46 (4×s, 21H, 7×–CH_3), 3.76–3.84 (m, 5H), 4.08–4.14 (m, 2H), 4.31 (m, 1H), 4.45–4.49 (m, 1H), 4.97–4.99 (m, 1H), 5.02–5.04 (m, 1H), 5.25 (bs, 1H, –NH), 7.56 (bs, 1H, –NH); δ_C (50.3 MHz, $CDCl_3$): 24.54, 25.12, 25.32, 26.86, 28.24 (q, –CH_3), 44.48, 66.79 (t, –CH_2–), 52.99 (q, –$COOCH_3$), 73.23, 80.82, 82.86, 87.16 (d, –CH–), 80.33, 93.48, 109.08, 113.99 (s, –C–), 156.57, 167.66, 169.86 (s, C=O); m/z (NH_3, CI, %): 493 [$(M+19)^+$, 2], 492 [$(M+18)^+$, 8], 475 [$(M+1)^+$, 3], 459 (8), 436 (16), 419 (21), 375 (24), 225 (100).

Peptide **16β** (0.09 g, 8.6% yield) as a gum. ν_{max} (film): 3397 (–NH), 2984, 2935 (–CH), 1758, 1699 (C=O), 1496, 1371, 1165, 1070 cm^{-1}; δ_H (500 MHz, $CDCl_3$): 1.37, 1.40, 1.44, 1.46, 1.57 (5×s, 21H, 7×–CH_3), 3.78 (s, 3H, –$COOCH_3$), 3.78–3.86 (m, 2H, –CH_2–), 4.04 (dd, $J_{3,4}$ 4.4 Hz, $J_{4,5}$ 8.7 Hz, 1H, H_4), 4.10–4.16 (m, 2H, H_6 and $H_{6'}$), 4.35 (ddd, $J_{5,6}$ 6.0 Hz, $J_{5,6'}$ 4.6 Hz, 1H, H_5), 4.78 (d, $J_{2,3}$ 5.8 Hz, 1H, H_2), 4.83 (dd, 1H, H_3), 5.09 (bs, 1H, –NH), 7.30 (bs, 1H, –NH); δ_C (50.3 MHz, $CDCl_3$): 24.77, 25.10, 25.78, 26.84, 28.18 (q, –CH_3), 44.17, 67.30 (t, –CH_2–), 53.02 (q, –COO*CH*$_3$), 72.71, 79.81, 80.00, 83.33 (d, –CH–), 80.05, 88.46, 109.41, 114.06 (s, –C–), 155.72, 168.97, 169.06 (s, C=O); m/z (NH_3, CI, %): 475 [$(M+1)^+$, 4], 376 (12), 375 (75), 343 (18), 225 (100).

(b) From methyl α-aminoester **7α**: A solution of DCC (86 mg, 0.42 mmol), HBT (57 mg, 0.42 mmol) and *N-tert*-butoxycarbonylglycine (73 mg, 0.42 mmol) in dry DMF (2 ml) was stirred at 0°C under nitrogen for 15 min. The reaction was then allowed to warm up to room temperature and stirred for a further 45 min. Aminoester **7α** (100 mg, 0.32 mmol) dissolved in dry DMF (1 ml) was added and the mixture was stirred for 16 h at room temperature. The reaction mixture worked up as above and the residue purified by flash chromatography (ethyl acetate:hexane, 2:1) to give peptide **16α** (108 mg, 72% yield) as a white solid that was crystallised from acetonitrile contaminated with small quantities of dicyclohexylurea, together with peptide **16β** (10.5 mg, 7% yield).

3.6. Methyl N-*2-(benzyloxycarbonylglycylamino)-2,5-anhydro-3,4:6,7-di*-O-*isopropylidene*-D-glycero-D-galacto-*heptonate* ***8β*** *and methyl* N-*2-(benzyloxycarbonylglycylamino)-2,5-anhydro-3,4:6,7-di*-O-*isopropylidene*-D-glycero-D-talo-*heptonate* ***8α***

A stirred solution of Z-glycine (185 mg, 0.945 mmol), and triethylamine (132 μl) in THF (6 ml) and CH_3CN (6 ml), at −15°C was treated with ethyl chloroformate (90 μl) and the reaction mixture left until a precipitate formed. Then a solution of the aminoester **7β** (300 mg, 0.945 mmol) and triethylamine (132 μl) in DMF (6 ml) was slowly added. The resulting mixture stirred first at −10°C for 1 hour and then at room temperature for 4 hours, concentrated *in vacuo* and the solid residue was dissolved in dichloromethane. The organic solution was washed with 0.5 M solution of HCl, then 0.5 M solution of sodium bicarbonate, dried (Na_2SO_4) and the solvent removed *in vacuo* to give a residue that was preabsorbed onto silica and purified by flash chromatography (ethyl acetate:hexane, 1:1) to give peptide **8β** (268.65 mg, 56% yield) and traces peptide **8α**, both identical to previously prepared samples.

3.7. Methyl N-*2-(*tert-*butoxycarbonylglycylamino)-2,5-anhydro-3,4*-O-*isopropylidene*-D-glycero-D-talo-*heptonate* ***20α***

Acetic acid (10 ml) was added to a solution of **16α** (0.5 g, 1.05 mmol) in methanol (10 ml), and the solution was stirred at 50°C for 18 h. The reaction mixture was evaporated *in vacuo* and co-evaporated with toluene (2×15 ml) to give a residue that was purified by flash chromatography (ethyl acetate:methanol, 95:5) to afford the partially hydrolysed BOC-peptide **20α** (0.38 g, 82% yield) as a white solid, m.p. 224–225°C (acetonitrile); $[\alpha]_D^{20}$: +98.5 (c, 1 in methanol); ν_{max} (KBr): 3453, 3308 (–NH), 2990, 2931 (–CH), 1749, 1721, 1694 (C=O), 1543, 1276, 1164, 1080, 1062 cm^{-1}; δ_H (500

MHz, CD_3OD): 1.30, 1.40, 1.44 (3×s, 15H, 5×–CH_3), 3.61 (dd, 1H), 3.69–3.71 (m, 4H), 3.76 (dd, 1H), 3.87 (dd, 1H), 3.98–4.01 (m, 1H), 4.78–4.86 (m, 2H), 4.90–4.92 (dd, 1H); δ_C (50.3 MHz, CD_3OD): 25.11, 25.95, 28.68 (q, –CH_3), 44.24, 65.05 (t, –CH_2–), 52.98 (q, –COO*C*H_3), 70.35, 81.08, 81.64, 87.21 (d, –CH–), 80.72, 93.31, 114.73 (s, –C–), 158.34, 168.71, 172.31 (s, C=O); m/z (NH_3, CI, %): 452 [$(M+18)^+$, 2], 396 (3), 378 (5), 335 (6), 75 (100). Found: C, 49.92; H, 7.02; N, 6.46. $C_{18}H_{30}N_2O_{10}$ requires: C, 49.77; H, 6.91; N, 6.45%.

*3.8. Reprotection of partially deprotected BOC-peptide **20α** to fully protected **16α***

2,2-Dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (27 mg) were added to a solution of **20α** (50 mg, 0.12 mmol) in analytical grade acetone (2 ml). The reaction mixture was stirred at room temperature for 12 h and then sodium hydrogen carbonate (34 mg) was added and the reaction stirred for a further 5 min. The mixture was filtered through Celite and the filtrate evaporated *in vacuo* to give peptide **16α** (54 mg, quantitative yield), identical to the material described above.

3.9. Methyl N-*2-(benzyloxycarbonylglycylamino)-2,5-anhydro-3,4*-O-*isopropylidene*-D-glycero-D-talo-*heptonate **19α***

Acetic acid (4 ml) was added to a solution of the fully protected peptide **8α** (100 mg, 0.2 mmol) in methanol (4 ml), and the solution was stirred at 50°C for 18 h. The reaction mixture was evaporated *in vacuo* and co-evaporated with toluene (2×10 ml) to give a residue that was purified by flash chromatography (ethyl acetate:methanol, 95:5) to afford the partially hydrolysed peptide **19α** (74 mg, 80% yield) as a white solid, m.p. 228–229°C (acetonitrile); $[\alpha]_D^{20}$: +92.5 (c, 0.8 in methanol); ν_{max} (KBr): 3328 (–NH and –OH), 2948 (–CH), 1755, 1731, 1698 (C=O), 1551, 1264, 1233, 1078 cm^{-1}; δ_H (500 MHz, CD_3OD): 1.30, 1.40 (2×s, 6H, 2×–CH_3), 3.62 (dd, 1H), 3.70–3.78 (m, 6H), 3.88 (dd, 1H), 3.98–4.02 (m, 1H), 4.77–4.91 (m, 2H), 5.09 (s, 2H), 7.30–7.37 (m, 5H, 5×Ar–H); δ_C (50.3 MHz, CD_3OD): 23.57, 24.43 (q, –CH_3), 43.09, 63.57, 66.44 (t, –CH_2–), 51.57 (q, –COO*C*H_3), 68.88, 79.55, 80.23, 85.80 (d, –CH–), 91.92, 113.43, 136.89 (s, –C–), 127.71, 127.84, 128.27 (d, Ar–H), 157.89, 167.62, 171.04 (s, C=O); m/z (NH_3, CI, %): 469 [$(M+1)^+$, 6], 378 (27), 361 (45), 303 (100). Found: C, 54.13; H, 6.03; N, 5.86. $C_{21}H_{28}N_2O_{10}$ requires: C, 53.83; H, 6.03; N, 5.98%.

*3.10. Reprotection of **19α** to fully protected Z-peptide **8α***

2,2-Dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (27 mg) was added to a solution of mono-acetonide **19α** (50 mg, 0.11 mmol) in analytical grade acetone (2 ml). The reaction mixture was stirred at room temperature for 12 h; sodium hydrogen carbonate (34 mg) was then added and the reaction mixture stirred for a further 5 min. The mixture was filtered through Celite and the filtrate evaporated *in vacuo* to give peptide **8α** (54 mg, quantitative yield) as a white solid, identical to the material above.

*3.11. Methyl [*N-*(2-glycylamino)-2,5-anhydro-3,4:6,7-di*-O-*isopropylidene*-D-glycero-D-talo-*heptonoyl] glycinate **17α***

Palladium black (50 mg) was added to a solution of peptide **8α** (0.5 g, 0.5 mmol) in methanol (30 ml) and the reaction mixture stirred for 2 h at room temperature under an atmosphere of hydrogen. The reaction mixture was then filtered through Celite and the filtrate evaporated *in vacuo* to give a residue that was purified by flash chromatography (chloroform:methanol, 85:15) to give aminoester **17α** (336

mg, 91% yield) as a white solid, m.p. 155–157°C (chloroform); $[\alpha]_D^{20}$: +100.8 (c, 0.25 in MeOH); ν_{max} (KBr): 3191 (–NH), 2989 (C–H), 1756, 1705 (C=O), 1556, 1375, 1266, 1214, 1070 cm^{-1}; δ_H (500 MHz, $CDCl_3$): 1.32, 1.38, 1.46 (3×s, 12H, 4×–CH_3), 3.41 (s, 2H), 3.82 (s, 3H, –$COOCH_3$), 4.09–4.15 (m, 2H), 4.37 (dd, $J_{3,4}$ 3.8 Hz, $J_{4,5}$ 7.4 Hz, 1H, H_4), 4.46–4.50 (m, 1H), 5.02 (dd, $J_{2,3}$ 5.8 Hz, 1H, H_3), 5.15 (d, 1H, H_2), 8.52 (bs, 1H, –NH); δ_C (50.3 MHz, $CDCl_3$): 24.55, 24.75, 25.31, 26.71 (q, –CH_3), 42.63, 66.61 (t, –CH_2–), 52.89 (q, –COO*CH*$_3$), 73.09, 80.45, 81.46, 86.39 (d, –CH–), 92.78, 109.19, 113.94 (s, –C–), 167.92, 170.60 (s, C=O); m/z (NH_3, CI, %): 389 [$(M+19)^+$, 2], 376 [$(M+2)^+$, 14], 375 [$(M+1)^+$, 100], 343 (45), 317 (46), 285 (60), 259 (56), 183 (48), 101 (73).

3.12. 1-(R)-(3′,6′-Diketopiperazine)-1,4-anhydro-2,3:5,6-di-O-isopropylidene-D-mannose **18α**

(a) From methyl glycinate **17α**: Potassium *tert*-butoxide (195 mg, 1.74 mmol) was added to a solution of aminoester **17α** (0.5 g, 1.34 mmol) in dry THF (250 ml) and the reaction mixture was stirred for 24 h at room temperature under nitrogen. The solution was evaporated *in vacuo* to give a residue that was purified by flash chromatography (ethyl acetate:methanol, 95:5) to give the protected diketopiperazine **18α** (405 mg, 89% yield) as an amorphous solid, $[\alpha]_D^{20}$: +113.5 (c, 1 in MeOH); ν_{max} (KBr): 3259 (–NH), 2987, 2936 (–CH), 1693 (C=O), 1375, 1214, 1070 cm^{-1}; δ_H (500 MHz, CD_3CN): 1.28, 1.32, 1.40, 1.42 (4×s, 12H, 4×–CH_3), 3.70 (dd, $J_{5,6}$ 4.1 Hz, $J_{6,6'}$ 17.5 Hz, 1H, H_6), 3.95–3.99 (m, 1H), 4.05–4.10 (m, 2H), 4.23 (dd, $J_{4,5}$ 7.3 Hz, $J_{3,4}$ 3.3 Hz, 1H, H_4), 4.36–4.40 (m, 1H, H_5), 4.86 (d, $J_{2,3}$ 5.7 Hz, 1H, H_2), 4.90 (dd, 1H, H_3), 6.54 (bs, 1H, –NH), 7.15 (bs, 1H, –NH); δ_C (50.3 MHz, CD_3CN): 25.01, 25.26, 25.54, 26.93 (q, –CH_3), 45.78, 67.14 (t, –CH_2–), 73.73, 81.17, 82.05, 89.34 (d, –CH–), 92.52, 109.68, 114.83 (s, –C–), 164.77, 167.43 (s, C=O); m/z (NH_3, CI, %): 344 [$(M+2)^+$, 6], 343 [$(M+1)^+$, 30], 327 (12), 286 (14), 285 (100). Found: C, 52.78; H, 6.55; N, 7.94. $C_{15}H_{22}N_2O_7$ requires: C, 52.63; H, 6.48; N, 8.18%.

(b) From monoacetonide **21α**: To a solution of the monoacetonide **21α** (26 mg, 0.08 mmol) in analytical grade acetone (2 ml), 2,2-dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (26 mg) was added. The reaction mixture was stirred at room temperature for 9 h, then neutralised with sodium hydrogen carbonate (52 mg), stirred for a further 5 min, filtered through Celite and the filtrate evaporated *in vacuo* to give diketopiperazine **18α** (29 mg, quantitative yield) as a white solid, identical to the material above.

(c) From fully deprotected **9α**: The unprotected diketopiperazine **9α** (20 mg, 0.08 mmol) in analytical grade acetone (2 ml) and 2,2-dimethoxypropane (1 ml) was stirred with *p*-toluenesulfonic acid (27 mg) at room temperature for 5 h and then neutralised with sodium hydrogen carbonate (34 mg). The reaction mixture was worked up as above to give the diacetonide **18α** (24 mg, 90% yield) as a white solid.

3.13. 1-(S)-(3′,6′-Diketopiperazine)-1,4-anhydro-2,3:5,6-di-O-isopropylidene-D-mannose **18β**

(a) From the Z-protected peptide **8β**: Palladium black (5 mg) was added over a solution of peptide **8β** (50 mg, 0.05 mmol) in methanol (3 ml) and the reaction mixture stirred for 2 h at room temperature under an atmosphere of hydrogen. The reaction mixture was then filtered through Celite and the filtrate evaporated *in vacuo* to give a residue that was purified by flash chromatography (chloroform:methanol, 85:15) to give diketopiperazine **18β** (32 mg, 95% yield) as an amorphous white solid, $[\alpha]_D^{20}$: +96 (c, 0.5 in MeOH); ν_{max} (KBr): 3231 (–NH), 2988, 1695 (C=O), 1455, 1381, 1216, 1063, 1046 cm^{-1}; δ_H (500 MHz, CD_3CN): 1.30, 1.35, 1.36, 1.52 (4×s, 12H, 4×–CH_3), 3.80 (dd, $J_{5,6}$ 3.8 Hz, $J_{6,6'}$ 18.1 Hz, 1H, H-6), 3.93–4.08 (m, 4H), 4.27–4.31 (m, 1H), 4.88 (dd, $J_{2,3}$ 6.0 Hz, $J_{3,4}$ 3.8 Hz, 1H, H-3), 4.94 (d, 1H, H-2), 6.69 (bs, 1H, –NH), 6.82 (bs, 1H, –NH); δ_C (50.3 MHz, CD_3CN): 24.47, 25.35, 26.01, 26.94 (q, –CH_3), 45.17, 66.99 (t, –CH_2–), 73.98, 80.37, 81.29, 81.60 (d, –CH–), 88.29, 109.46, 118.27 (s, –C–),

165.27, 167.27 (s, C=O); m/z (NH_3, CI, %): 360 [$(M+18)^+$, 5], 343 [$(M+1)^+$, 76], 285 (100), 267 (48). Found: C, 52.61; H, 6.44; N, 8.15. $C_{15}H_{22}N_2O_7$ requires: C, 52.63; H, 6.48; N, 8.18%.

(b) From equilibration of the α-anomer **18α**: Potassium *tert*-butoxide (30 mg, 0.27 mmol) was added to a solution of the epimeric diketopiperazine **18α** (500 mg, 1.46 mmol) dissolved in dry DMF. The reaction mixture was heated at 100°C under an atmosphere of nitrogen for 12 h. The solvent was then removed *in vacuo* to give a residue that was purified by flash chromatography (ethyl acetate:MeOH, 95:5) to afford diketopiperazine **18β** (440 mg, 88% yield) identical to the material described above.

(c) From the monoacetonide **21β**: *p*-Toluenesulfonic acid (27 mg) was added to a solution of diketopiperazine **21β** (20 mg, 0.07 mmol) in acetone (2 ml) and 2,2-dimethoxypropane (1 ml). The reaction mixture was stirred at room temperature for 5 h and then sodium hydrogen carbonate (34 mg) was added and the reaction stirred for a further 5 min. The mixture was filtered through Celite and the filtrate evaporated *in vacuo* to give diketopiperazine **18β** (23 mg, quantitative yield) as a white solid.

(d) From the deprotected spirodiketopiperazine **9β**: To a solution of diketopiperazine **9β** (20 mg, 0.08 mmol) in analytical grade acetone (2 ml), 2,2-dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (26 mg) was added. Work-up as described above afforded the protected diketopiperazine **18β** (21 mg, 80% yield).

*3.14. 1-(*R*)-(3′,6′-Diketopiperazine)-1,4-anhydro-2,3-*O*-isopropylidene-*D*-mannose **21α***

A solution of diketopiperazine diacetonide **18α** (250 mg, 0.7 mmol) in a mixture of acetic acid and water (1:1) (20 ml) was stirred at 50°C for 2 h. The reaction mixture was evaporated *in vacuo* and the residue co-evaporated twice with toluene (25 ml) and purified by flash chromatography ($CHCl_3$:MeOH, 4:1) to give diketopiperazine **21α** (195 mg, 88% yield) as a white solid, m.p. 125°C, decomposed (methanol); $[\alpha]_D^{20}$: +120.5 (c, 1 in MeOH); ν_{max} (KBr): 3344 (–NH and –OH), 2940, 1691 (C=O), 1420, 1377, 1216, 1090 cm^{-1}; δ_H (500 MHz, CD_3OD): 1.31, 1.45 (2×s, 6H, 2×–CH_3), 3.68 (dd, $J_{5,6}$ 4.8 Hz, $J_{6,6'}$ 11.7 Hz, 1H, H-6), 3.75 and 4.08 (ABq, J_{AB} 17.5 Hz, 2H, –CH_2–), 3.79 (dd, $J_{5,6'}$ 2.7 Hz, 1H, H-6′), 4.05–4.08 (m, 1H, H-5), 4.18 (dd, $J_{4,5}$ 8.9 Hz, $J_{3,4}$ 3.3 Hz, 1H, H-4), 4.90 (d, $J_{2,3}$ 5.6 Hz, 1H, H-2), 5.01 (dd, 1H, H-3); δ_C (50.3 MHz, CD_3OD): 23.57, 24.28 (q, –CH_3), 44.56, 63.53 (t, –CH_2–), 68.62, 80.42, 80.54, 88.14 (d, –CH–), 91.82, 114.19 (s, –C–), 166.46, 168.39 (s, C=O); m/z (NH_3, CI, %): 320 [$(M+18)^+$, 20], 303 [$(M+1)^+$, 96], 245 (100).

*3.15. 1-(*S*)-(3′,6′-Diketopiperazine)-1,4-anhydro-2,3-*O*-isopropylidene-*D*-mannose **21β***

(a) By epimerization of α-anomer **21α**: The epimeric diketopiperazine **21α** (200 mg, 0.7 mmol) was dissolved in dry DMF (10 ml) and treated with potassium *tert*-butoxide (46 mg, 0.4 mmol). The reaction mixture was heated at 100°C under an atmosphere of nitrogen for 12 h and then evaporated *in vacuo* to give a residue that was purified by flash chromatography (ethyl acetate:MeOH, 85:15) to give diketopiperazine **21β** (170 mg, 85% yield) as a white solid, m.p. 216–218°C (methanol); $[\alpha]_D^{20}$: +88.8 (c, 0.5 in MeOH); ν_{max} (KBr): 3350 (N–H and –OH), 2940, 1686 (C=O), 1418, 1373, 1215, 1090 cm^{-1}; δ_H (500 MHz, D_2O): 1.41, 1.56 (2×s, 6H, 2×–CH_3), 3.58 (dd, $J_{5,6}$ 5.5 Hz, $J_{6,6'}$ 12.2 Hz, 1H, H-6), 3.74 (dd, $J_{5,6'}$ 2.7 Hz, 1H, H-6′), 3.89–3.92 (m, 1H, H-5), 3.98 and 4.19 (ABq, J_{AB} 18.7 Hz, 2H, –CH_2–), 4.05 (dd, 1H, H-4), 5.06–5.09 (m, 2H, H-3 and H-2); δ_C (50.3 MHz, D_2O): 24.23, 25.56 (q, –CH_3), 44.73, 63.88 (t, –CH_2–), 69.85, 79.09, 80.92, 81.35 (d, –CH–), 88.37, 114.75 (s, –C–), 166.16, 169.92 (s, C=O); m/z (NH_3, CI, %): 320 [$(M+18)^+$, 71], 303 [$(M+1)^+$, 95], 245 (73), 183 (100).

(b) By hydrolysis of the diacetonide **18β**: A solution of the diacetonide **18β** (40 mg, 0.12 mmol) in a mixture of acetic acid and water (1:1) (4 ml) was stirred at 50°C for 2 h. The reaction mixture

was evaporated *in vacuo* and the residue coevaporated twice with toluene (3 ml) and purified by flash chromatography ($CHCl_3$:MeOH, 80:20) to give diketopiperazine **21β** (31 mg, 87% yield) identical to the material above.

*3.16. 1-(*R*)-(3′,6′-Diketopiperazine)-1,4-anhydro-*D*-mannose **9α***

The monoacetonide **21α** (150 mg, 0.48 mmol) was dissolved in a mixture of trifluoroacetic acid:water (1:1) (6 ml) and stirred at room temperature for 1 h. The mixture was then evaporated *in vacuo*, coevaporated twice with toluene (5 ml) and the residue crystallised from MeOH/ethyl acetate to give diketopiperazine **9α** (120 mg, 92% yield), m.p. 190°C, decomposed (methanol); $[\alpha]_D^{20}$: +2 (c, 0.1 in MeOH); ν_{max} (KBr): 3277 (–NH and –OH), 2917, 1689 and 1655 (C=O), 1457, 1338, 1092, 1081 cm^{-1}; δ_H (500 MHz, DMSO): 3.30 (bdd, $J_{5,6}$ 6.1 Hz, $J_{6,6'}$ 11.3 Hz, 1H, C6H), 3.53 (bd, 1H, C6H), 3.71 (b, 1H, C5H), 3.77–3.85 (m, 2H, CH_2), 3.90 (ddd, $J_{2,3}$ 4.5 Hz, $J_{3,4}$ 2.5 Hz, $J_{3,3OH}$ 11 Hz, 1H, C3H), 3.94 (dd, $J_{4,5}$ 8.8 Hz, 1H, C4H), 4.20 (b, 1H, C6OH), 4.25 (t, $J_{2,2OH}$ 4.5 Hz, 1H, C2H), 4.42 (b, 1H, C5OH), 5.46 (d, 1H, C2OH), 5.77 (d, 1H, C3OH), 8.50 (b, 1H, NH), 8.83 (s, 1H, N1H); δ_C (125 MHz, DMSO): 43.8 (t, CH_2), 63.1 (t, C6), 68.6 (d, C5), 71.6 (d, C3), 79.5 (d, C2), 81.8 (d, C4), 90.4 (s, C1), 165.2, 166.3 (2×s, 2×CO); m/z (NH_3, CI, %): 263 [$(M+1)^+$, 19], 143 (81), 127 (100).

*3.17. 1-(*S*)-(3′,6′-Diketopiperazine)-1,4-anhydro-*D*-mannose **9β***

(a) By epimerization of the unprotected α-anomer **9α** The epimeric diketopiperazine **9α** (100 mg, 0.4 mmol) was dissolved in dry DMF (5 ml) and potassium *tert*-butoxide (45 mg, 0.4 mmol) added. The reaction mixture was heated at 100°C under an atmosphere of nitrogen for 12 h and then evaporated *in vacuo* to give a residue that was evaporated *in vacuo* and then coevaporated twice with toluene (5 ml) and crystallised from MeOH/ethyl acetate/hexane to afford diketopiperazine **9β** (90 mg, 90% yield) as a white solid, m.p. 151–153°C (methanol); $[\alpha]_D^{20}$: +10.8 (c, 0.5 in MeOH); ν_{max} (KBr): 3389 and 3247 (–NH and –OH), 2937, 1693 (C=O), 1443, 1215, 1087, 1049 cm^{-1}; δ_H (500 MHz, DMSO): 3.32 (dd, $J_{5,6}$ 5.2 Hz, $J_{6,6'}$ 11.2 Hz, 1H, C6H), 3.54 (dd, $J_{5,6'}$ 1.9 Hz, 1H, C6H), 3.67 (m, 1H, C4H), 3.67, 3.90 (2×d, $J_{H,H'}$ 17.8 Hz, 2H, CH_2), 3.69 (m, 1H, C5H), 4.14 (m, 1H, C3H), 4.61 (d, $J_{2,3}$ 4.1 Hz, 1H, C2H), 4.29, 4.51, 5.36, 5.47 (4×b, 4H, 4×OH), 7.38 (s, 1H, N1H), 8.13 (b, 1H, NH); δ_C (125 MHz, DMSO): 44.0 (t, CH_2), 62.9 (t, C6), 69.4 (d, C5), 70.8 (d, C3), 72.5 (d, C2), 80.2 (d, C4), 87.4 (s, C1), 164.2, 166.3 (2×s, 2×CO); m/z (NH_3, CI, %): 280 [$(M+18)^+$, 12], 263 [$(M+1)^+$, 28], 245 (15), 143 (96), 138 (81), 127 (45), 120 (100). Found: C, 40.91; H, 5.52; N, 10.53. $C_9H_{14}N_2O_7$ requires: C, 41.22; H, 5.34: N, 10.69%.

(b) By hydrolysis of the monoacetonide **21β**: The monoacetonide **21β** (20 mg, 0.07 mmol) was dissolved in a mixture of trifluoroacetic acid:water (1:1) (1 ml) and stirred at room temperature for 1 h. The mixture was then evaporated *in vacuo*, coevaporated twice with toluene (2 ml) and the residue crystallised from MeOH/ethyl acetate to give diketopiperazine **9β** (14 mg, 80% yield) identical to the material above.

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