N-Linked carba-disaccharides as potential inhibitors of glucosidases I and II

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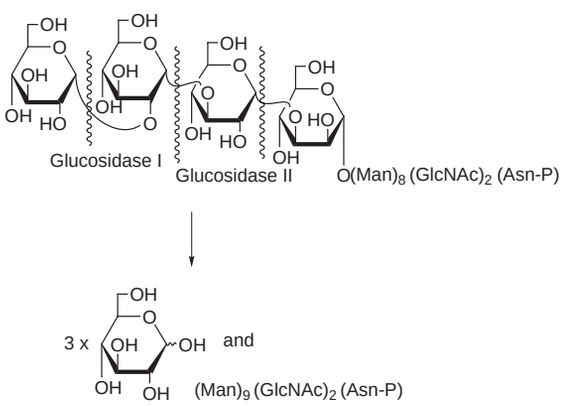
An N-linked pseudodisaccharide **2** containing an inositol moiety in place of a glycopyranosyl residue has been synthesised to mimic the disaccharide unit, 3-*O*-(α-D-glucopyranosyl)-D-glucopyranose, cleaved by glucosidase II during glycoprotein processing. Its inhibitory action towards yeast α-glucosidase was inferior to that of 1-deoxynojirmycin, a known inhibitor of glucosidase II. An attempt to prepare the analogous 2-N-linked compound **1** as an inhibitor of glucosidase I led to the novel N-phenyl derivative **11** of 2-amino-2-deoxy-D-glucopyranose.

Introduction

The enzymes glucosidase I and glucosidase II are involved in key steps in the processing of N-linked oligosaccharides (Scheme 1) by cleaving three terminal glucose residues from the



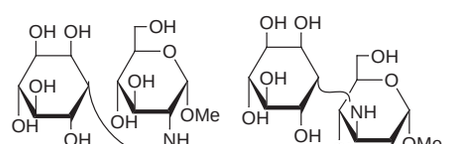
Scheme 1

tetradeca-oligosaccharide moiety $Glc_3Man_9GlcNAc_2$ of an important intermediate N-linked oligosaccharide.[1] Inhibitors of these enzymes such as 1-deoxynojirimycin (DNJ) (for glucosidase I and glucosidase II) and castanospermine (for glucosidase I) cause malformation of these oligosaccharides[2] and have shown interesting anti-HIV activity, which is thought to arise from prevention of successful completion of gp120 in the viral coat, and hence viral reproduction.[3] We considered that specificity and inhibitory activity towards these two enzymes might well be increased substantially by designing inhibitors which mimic the *two* carbohydrate residues immediately involved in the chain cleavage and further, that the exploration of inhibitors containing *exocyclic* nitrogen at C-1, rather than *endocyclic* nitrogen as found in 1-deoxynojirimycin and castanospermine, would be fruitful. However, since simple linkage of the two carbohydrate residues *via* nitrogen would afford a glycosylamine derivative which is likely to be hydrolytically unstable under physiological conditions, we sought to replace the glycosyl component in such a glycosylamine by a carbocyclic ring which might mimic to a great extent the topography of D-glucose and thus we arrived at the target molecules **1** and **2**. These mimic, respectively, the terminal pair and penultimate pair of carbohydrate residues in $Glc_3Man_9GlcNAc_2$ and may be designated N-linked carba-disaccharides. Consideration of the mechanism of glycosidase action[4] suggests that such compounds might preferentially occupy the catalytic site of the enzyme with the nitrogen atom at the site of initial protonation of a normal substrate. Importantly, such compounds resemble the terminal carba-disaccharide unit in the effective α-amylase inhibitor acarbose **3** in having a nitrogen bridge between the two rings, one of which is carbocyclic.[5,6] We now report full details of our work which was the subject of a preliminary communication.[7]

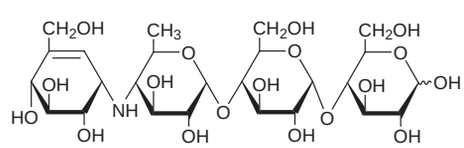
Results and discussion

The synthesis of **1** and **2** hinges on imine formation between the key chiral penta-*O*-benzylinosose **4**, accessible from the corresponding chiral protected *myo*-inositol[8] and either protected methyl 2-amino-2-deoxy- or methyl 3-amino-3-deoxy-α-D-glucopyranoside, **5** or **6**, respectively, followed by stereoselective reduction ($NaBH_3CN$) of the imine and then deprotection of the reduction product by catalytic hydrogenolysis of the *O*-benzyl groups.

Preparation of the chiral inosose **4** involves a 9-step synthesis from *myo*-inositol, in which the racemic 1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol is resolved[9] through ester formation with (*1S*,*4R*)-camphanic chloride and chromatographic separation



1 **2**



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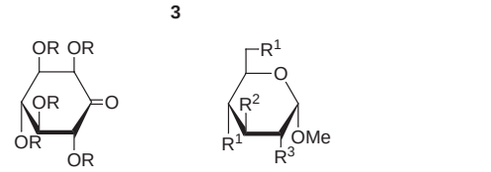

4 R = CH_2Ph **5** $R^1 = R^2 = OCH_2Ph$; $R^3 = NH_2$
6 $R^1 = R^3 = OCH_2Ph$; $R^2 = NH_2$

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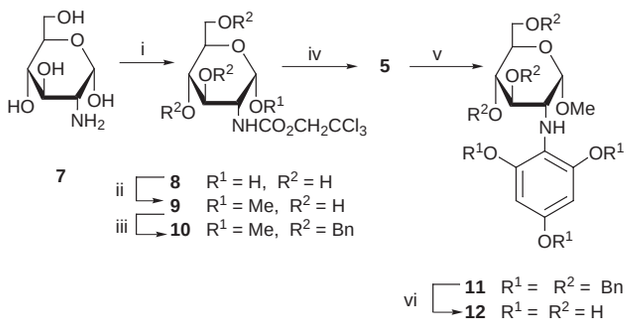
of the diastereoisomeric esters. Swern oxidation of the (−)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol gave (+)-2,3,5/4,6-pentabenzyloxycyclohexanone **4** as an analytically pure solid in 83% yield whose spectroscopic properties agree well with those reported[10] for the racemic compound. Characteristically, it showed carbonyl absorption at 1730 cm^{-1} and an absorption at δ_C 205.78 in its ^{13}C NMR spectrum.

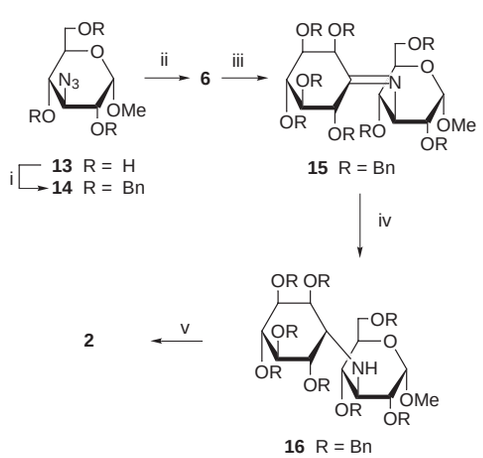
Synthesis of the protected 2-amino *gluco*-compound **5** required temporary N-protection with the 2,2,2-trichloroethoxycarbonyl (troc) group, introduced (Scheme 2) by reaction



Scheme 2 *Reagents and conditions* (*and yields*): i, CCl_3CH_2OCOCl, aq. $NaHCO_3$, 10 °C for 2 h then 20 °C for 19 h (72%); ii, 2.5% w/v HCl in MeOH, reflux 8.5 h, (65%); iii, $CCl_3C(NH)OCH_2Ph$, CH_2Cl_2–cyclohexane (1:2), CF_3SO_3H, 20 °C, 2.5 h (78%); iv, Zn/Cu, THF–1 M aq. KH_2PO_4 (5:1), 20 °C, 8.5 h (62%); v, **4**, C_6H_6 (addition and evaporation, × 5), 60 °C (79%); vi, EtOH–cyclohexene (2:1), Pd-black, reflux under Ar, 38 h, yield not determined (air-unstable product).

of D-glucosamine **7** with 2,2,2-trichloroethoxycarbonyl chloride in an aqueous solution of sodium hydrogen carbonate.[11] Fischer-type glycosidation of the so-formed carbamate **8** gave initially a mixture of α- and β-glycosides with the α-isomer **9** predominating as indicated by the integrated signals in the ^1H NMR spectrum for OMe, with the α-isomer resonating at higher field (δ_H 3.39) than the β-isomer (δ_H 3.50). As reaction time increased, the proportion of α-isomer increased until it was the major component of the crude product from which it could then be isolated in 65% yield by crystallisation. The glycoside **9** could not be benzylated under the usual conditions (NaH, $PhCH_2Br$) because of instability of the N-protecting group under basic conditions. However, *O*-benzylation was successfully achieved in an acid-catalysed process using benzyl trichloroacetimidate,[12] and removal of the troc group of the 3,4,6-tri-*O*-benzyl ether **10** with zinc powder in THF–potassium dihydrogen phosphate buffer[13] gave the free amine **5**.

For the synthesis of **6** (Scheme 3), methyl 3-azido-3-deoxy-α-



Scheme 3 *Reagents and conditions* (*and yields*): i, NaH, BnBr, DMF, 20 °C, 1 h (91%); ii, Ph_3P, THF–H_2O (10:1), 80 °C, 7 h (70%) or $LiAlH_4$, Et_2O, 35 °C, 1 h (75%); iii, **4**, C_6H_6, 4 Å molecular sieves under N_2, 20 °C, 70 h; iv, $NaBH_3CN$, DMF, 20 °C, 70 h and 100 °C, 1 h (61%); v, EtOH–cyclohexene (2:1), Pd-black, reflux 24 h (67%).

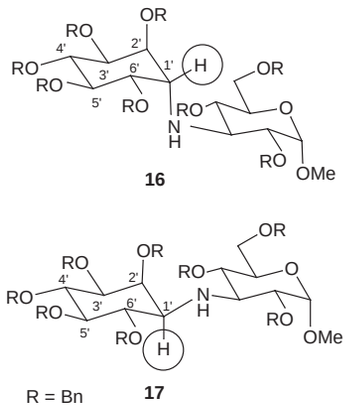


Fig. 1 Configurational isomers which can arise form reduction of the imine **15** showing stereo-dependence of $J_{1',2'}$ and $J_{1',6'}$.

D-glucopyranoside **13**, together with the methyl 2-azido-2-deoxy-α-D-altropyranoside were first prepared by a reported procedure[14] involving ring opening of methyl 2,3-anhydro-α-D-allopyranoside with azide ion and chromatographic separation of the isomeric azides. The ratio of the *gluco*- (2,3-diequatorial) to *altro*- (2,3-diaxial) isomers was 2.7:1, a much more favourable ratio with respect to the required *gluco*-isomer **13** than the 1:15 obtained[15] by a similar reaction on the corresponding 4,6-*O*-benzylidene derivative, which is conformationally constrained and therefore forced to follow a diaxial ring opening of the epoxide. Conventional benzylation of azide **13** gave the tri-*O*-benzyl azide **14** which, on reduction of the azido group by treatment with triphenylphosphine–THF–H_2O or with $LiAlH_4$ in diethyl ether gave the required protected 3-amino compound **6** in 70 and 75% yield, respectively.

Although a direct reductive alkylation of the amines **5** and **6** with the chiral inosose **4** would seem to offer the most direct route to compounds of the type **1** and **2**, problems were encountered on attempting to obtain reaction of amine **5** in this manner (see later) and yields were disappointing when the reaction was attempted with **6**. We investigated, therefore, an indirect two-step approach involving imine formation followed by cyanoborohydride reduction. With amine **6**, the first step was best conducted using the extremely mild conditions first reported by Taguchi and Westheimer[16] in which a solution of the carbonyl component and the amine in benzene is stored over molecular sieves at ambient temperature for 70 h; in this manner the imine **15** was obtained from **4** and **6** in ~60% yield and was then immediately reduced in DMF solution with sodium cyanoborohydride which could afford, in theory, one or both of the expected products **16** and **17**, which are depicted in their expected chair conformations in Fig. 1.

Evidence that the reduction had proceeded with good stereoselectivity to give required product **16** was provided by the ^1H and ^{13}C NMR spectra but the complexity of the ^1H NMR spectrum precluded a definitive decision regarding the absence of a small amount of a second isomer, which later measurements on the deprotected material suggested must have been present in a minor amount (< 10%). Extensive investigation of the ^1H NMR spectrum at 600 MHz and a consideration of the conformations of **16** and **17** allowed allocation of structure **16** to the predominant reduction product on the basis of the coupling pattern observed for H-1 of the cyclitol. The key observation is the coupling pattern observed for H-1 in the cyclitol ring, which for **16** would be expected to be an apparent triplet ($J_{apparent}$ ~3 Hz) resulting from two similar coupling constants ($J_{1',2'}$ $J_{1',6'}$ ~3 Hz), and for **17** would be expected to be a double doublet ($J_{1',2'}$ ~3 Hz, $J_{1',6'}$ ~9 Hz). The observed signal, assignment confirmed by deuterium labelling, was an apparent triplet at δ_H 3.82 with $J_{apparent}$ ~4.4 Hz.

Deprotection of **16** using catalytic transfer hydrogenation[17] (Pd–cyclohexene–EtOH) gave the required pseudodisaccharide

2. The ^{1}H NMR spectrum was consistent with the expected structure, but alongside the doublet signal at δ 4.66 for the anomeric proton was a neighbouring doublet resonance at δ 4.46 of very low intensity with the same spacing, which we attribute to a small amount (~5%) of the stereoisomeric reduction product. The proportion of the latter could be reduced significantly, but with loss in overall yield, by careful re-chromatography on silica gel.

An attempt to repeat the reaction sequence for the preparation of **1** by reaction of **4** and **5** gave unexpected results. No reaction occurred on storage of a mixture of **4** and **5** in benzene over molecular sieves for several days but repeated evaporation of benzene from the mixture at 60 °C gave a product whose ^{1}H NMR spectrum contained a sharp two-proton singlet at δ 6.25 and a total of only *six* benzyl groups, pointing to aromatisation of the former cyclitol ring. NMR spectral data and elemental analysis indicated the compound to be methyl 3,4,6-tri-*O*-benzyl-2-(2,4,6-tribenzyloxyphenylamino)-2-deoxy-α-D-glucopyranoside **11** which can arise from an initially formed imine by elimination of two molecules of benzyl alcohol followed by aromatisation of the imino quinone so formed. The single aromatic resonance in the ^{1}H NMR spectrum requires that the isomeric 3,4,5-tribenzyloxy structure also be considered for the product but the assignment of the 2,4,6-tribenzyloxy structure is based on combined consideration of (i) comparison of the estimated ^{13}C chemical shifts for the two isomers with those measured (especially for Ar-*C*NHR: calculated for 2,4,6-isomer δ_C 120.2, calculated for 3,4,5-isomer δ_C 140.0, observed δ_C 121.38) (ii) the ^{1}H NMR chemical shifts of ArH in 2,4,6-trimethoxyaniline (δ_H 6.05) and 3,4,5-trimethoxyaniline (δ_H 5.91) compared with that observed (δ_H 6.25) (iii) the likely mechanism of formation from the supposed imine intermediate.

Careful catalytic transfer hydrogenolysis of **11** afforded a readily oxidised solid product, whose spectral properties supported the structure **12**.

The inhibitory properties of compound **2** against yeast α-glucosidase were compared with that of DNJ by measuring the rate of enzymic release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside in the absence and in the presence of inhibitor followed by standard Lineweaver–Burk analysis.[18] Compound **2** was a poor competitive inhibtor of the enzyme (K_i ~1.5 mM) in contrast to DNJ for which we obtained K_i 30 μM. Under identical conditions, to bring about a 20% reduction in the rate of enzymic hydrolysis of the aryl glycoside, an approximately 100-fold greater molar concentration of **2** was required compared with that for DNJ. The considerable difference in inhibitory properties of the two compounds towards the enzyme is perhaps not surprising in view of their likely different mechanisms of action as a result of the different positions of the nitrogen atoms in the two molecules. Protonated DNJ is likely to mimic the cyclic oxocarbenium intermediate of glycoside hydrolysis whereas protonated **2** mimics an earlier intermediate in the hydrolytic pathway, the O-1-protonated glycoside. Interestingly, recent reports suggest that nitrogen-containing inhibitors in which the N atom occupies the site of the carbon atom at the glycosidic centre (*e.g.*, C-1 in aldopyranoses) often show high inhibitory activity.[19] This carbon atom carries a formal positive charge in one of the resonance contributors of the intermediate oxocarbenium ion obtained. Clearly, comparative experiments with suitable inhibitors of a glycosidase, in which nitrogen replaces O-1, C-1 or ring-O of a glycoside, could provide useful information on the detailed mechanism of action of this class of enzymes.

In independent experiments performed by Dr W. McDowell, compound **2** was assayed for inhibitory properties against glucosidases I and II using rat liver microsomes possessing activity in these enzymes and a labelled substrate. Unfortunately, no inhibition of the activity of either of these enzymes was observed for **2** up to a concentration of 100 μg ml^{-1}, a result which is in accord with the poor inhibitory properties of **2** compared with DNJ against yeast α-glucosidase.

Experimental

^{1}H NMR spectra were recorded at 270 MHz on a JEOL EX270 FT spectrometer, or at 600 MHz on a Varian Unity Inova spectrometer for samples in $CDCl_3$ unless stated otherwise, with Me_4Si as internal standard. ^{13}C NMR spectra were similarly recorded at 67.9 MHz on a JEOL EX270 FT spectrometer. Coupling constants (*J*-values) are given in Hz. Where appropriate, signal assignments were deduced by DEPT, COSY and HETCOR NMR experiments. Optical rotations were measured at ambient temperature with a Perkin-Elmer model 141 polarimeter for solutions in $CHCl_3$ unless stated otherwise and $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin-Elmer model 298 spectrophotometer. High-resolution mass spectra were recorded by the EPSRC Mass Spectrometry Service Centre at the University College of Swansea. Low-resolution mass spectra and elemental analyses were performed by Mr A. W. R. Saunders at the University of East Anglia. TLC was performed on silica gel (Machery-Nagel) SIL G-25UV$_{254}$ and compounds on developed plates were detected either by viewing with a UV lamp (254 nm), or by spraying with a 10% sulfuric acid, 1.5% molybdic acid, 1% ceric (Ce^{4+}) sulfate spray followed by heating to 150 °C. Column chromatography was performed on Matrex Silica 60 (70–200 mm mesh, Fisons) or Kieselgel 60 (70–230 mm mesh, Merck). Light petroleum refers to the fraction with boiling range 40–60 °C. Where mixed solvents were used, the ratios given are v/v. Tetrahydrofuran and diethyl ether were pre-dried by storage over sodium wire and obtained anhydrous by distillation from sodium metal and benzophenone once the blue colouration due to the ketyl radical had been achieved; dichloromethane was obtained anhydrous by distilling from calcium hydride; methanol was dried by distilling from the alkoxide (formed by reaction with activated magnesium) and storage over powdered 3 Å molecular sieves; toluene and benzene were dried by distillation from calcium hydride and stored over 4 Å molecular sieves; triethylamine was dried by distilling from calcium hydride immediately prior to use; pyridine was obtained anhydrous by distillation from calcium hydride and storage over potassium hydroxide pellets. Organic solutions were dried over anhydrous $MgSO_4$. Reactions were maintained at −78 °C by means of a solid CO_2–acetone bath, and at 0 °C by means of an ice-bath.

(−)-1,2,4,5,6-Penta-*O*-benzyl-*myo*-inositol[9] was prepared from *myo*-inositol, 2-(2′,2′,2′-trichloroethoxycarbonylamino)-2-deoxy-D-glucose[11] **8** from D-glucosamine **7**, and methyl 3-azido-3-deoxy-α-D-glucopyranoside[14] **13** from methyl 2,3-anhydro-α-D-glucopyranoside by the literature procedures. Yeast α-glucosidase (type IV from brewer's yeast) was obtained from Sigma Chemical Co.

(+)-2,3,5/4,6-Pentabenzyloxycyclohexanone 4

A solution of oxalyl dichloride (0.17 ml, 1.95 mmol) in dichloromethane (4.3 ml) was cooled to −78 °C and dimethyl sulfoxide (0.29 ml, 4.12 mmol) in dichloromethane (0.9 ml) was added dropwise from a syringe over *ca.* 5 min. The mixture was then stirred for 10 min at −78 °C and then (−)-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol (900 mg, 1.43 mmol) dissolved in dichloromethane (1.72 ml) was added dropwise during *ca.* 5 min. Stirring was continued at −78 °C for 30 min after which time triethylamine (1.19 ml, 8.58 mmol) was added. The cooling bath was removed and H_2O (5 ml) was added at room temperature. Stirring was continued for 10 min and the organic layer was then separated. The aqueous phase was extracted with dichloromethane (3 × 5 ml) and the combined organic layers were washed successively with brine (5 ml), 1% aq. HCl (5 ml), H_2O (4 ml), 5% aq. Na_2CO_3 (5 ml) and water (4 ml). The solu-

tion was dried, concentrated, and the residue was subjected to silica gel column chromatography [eluent: toluene–ethyl acetate (15:1)] to give *title chiral inosose* **4** (740 mg, 83%), mp 90–92 °C (Found: C, 78.2; H, 6.25. $C_{41}H_{40}O_6$ requires C, 78.3; H, 6.4%); $[\alpha]_D$ +9.2 (*c* 1); ν_{max}(Nujol)/cm^{-1} 1730 (CO), 1090 (COC), 745, 695 (Ar); δ_H (270 MHz) 3.44 (1H, dd, $J_{2,3}$ 2.7, $J_{3,4}$ 9.3, 3-H), 3.51 (1H, t, $J_{4,5} = J_{5,6} = 9.3$, 5-H), 3.97 (1H, d, 2-H), 4.28 (1H, t, 4-H), 4.41 (2H, s, OCH_2Ph), 4.44 and 4.60 (each 1H, d, J_{AB} 12.2, OCH_2Ph), 4.54 and 4.56 (each 1H, d, J_{AB} 11.9, OCH_2Ph), 4.69 (1H, d, 6-H), 4.77 and 4.83 (each 1H, d, J_{AB} 10.5, OCH_2Ph), 4.88 and 4.91 (each 1H, d, J_{AB} 10.5, OCH_2Ph), 7.26–7.32 (25H, m, 5 × Ph); δ_C (67.9 MHz) 72.61 (O*C*H$_2$Ph), 72.81 (O*C*H$_2$Ph), 73.36 (O*C*H$_2$Ph), 75.86 (O*C*H$_2$Ph), 75.99 (O*C*H$_2$Ph), 79.19 (3-C), 80.84 (4-C), 81.16 (2-C), 81.93 (5-C), 83.10 (6-C), 127.58–138.43 (30 C, Ar-C), 205.78 (CO).

Methyl 3-azido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranoside 14

Methyl 3-azido-3-deoxy-α-D-glucopyranoside **13** (100 mg, 0.46 mmol) was dissolved in DMF (2 ml), under nitrogen atmosphere, and sodium hydride (110 mg of a 60% dispersion in mineral oil, previously washed with hexane, 2.73 mmol) was added cautiously, portionwise. After being stirred for 40 min, the mixture was cooled (0 °C, ice-bath) and benzyl bromide (0.48 ml, 4.1 mmol) was added dropwise. After 10 min, the ice-bath was removed and the stirring was continued for 10 min at room temperature. Methanol (0.6 ml) was then added and the mixture was evaporated to dryness *in vacuo*. The residue was purified by silica gel column chromatography [eluent: first light petroleum and then hexane–ethyl acetate (7:3)] to give, as a viscous oil, *title compound* **14** (203 mg, 91%) (Found: C, 68.6; H, 6.3; N, 8.5. $C_{28}H_{31}N_3O_5$ requires C, 68.7; H, 6.4; N 8.6%); $[\alpha]_D$ +58.2 (*c* 1); ν_{max}(film)/cm^{-1} 2100 (N_3), 1100 and 1040 (COC), 730 and 700 (Ar): δ_H (270 MHz) 3.33 (3H, s, OCH_3), 3.37 (1H, dd, $J_{1,2}$ 3.3, $J_{2,3}$ 9.9, 2-H), 3.43 (1H, t, $J_{3,4} = J_{4,5} = 9.9$, 4-H), 3.58 (1H, dd, $J_{5,6a}$ 3.6, $J_{6a,6b}$ 11.7, 6a-H), 3.66–3.74 (2H, m, 5-H and 6b-H), 3.89 (1H, t, 3-H), 4.42 and 4.78 (each 1H, d, J_{AB} 10.7, OCH_2Ph), 4.45 and 4.60 (each 1H, d, $J_{A,B}$ 12.0, OCH_2Ph), 4.63 and 4.76 (each 1H, d, J_{AB} 12.2, OCH_2Ph), 4.58 (1H, d, 1-H), 7.18–7.39 (15H, m, 3 × Ph); δ_C (67.9 MHz) 55.20 (OCH_3), 65.46 (3-C), 68.04 (6-C), 69.64 (5-C), 73.17 (O*C*H$_2$Ph), 73.58 (O*C*H$_2$Ph), 74.80 (O*C*H$_2$Ph), 76.13 (4-C), 77.82 (2-C), 97.32 (1-C), 127.85–138.12 (18 C, Ar-C).

Methyl 3-amino-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranoside 6

Method 1. Triphenylphosphine (352 mg, 1.34 mmol) and water (0.4 ml) were added to a solution of azide **14** (224 mg, 0.46 mmol) in THF (4 ml) and the mixture was heated at 80 °C for 7 h and then concentrated. The residue was purified by silica gel column chromatography [eluent: diethyl ether–triethylamine (10:1)] to give *amine* **6** (148 mg, 70%) as a colourless oil.

Method 2. A solution of the azide **14** (160 mg, 0.33 mmol) in dry diethyl ether (0.7 ml) was added dropwise to a suspension of $LiAlH_4$ (30 mg, 0.79 mmol) in dry diethyl ether (0.7 ml). The reaction mixture was heated under reflux for 1 h and then cooled (0 °C) and the remaining $LiAlH_4$ was destroyed and inorganic salts were precipitated by the successive addition of H_2O (0.03 ml), 15% aq. NaOH (0.03 ml) and then H_2O (0.1 ml). The mixture was stirred for 30 min, filtered and the solid washed with diethyl ether. The combined organic filtrate was dried, concentrated and the residue was purified as described in method 1 to give oily amine **6** (113 mg, 75%) (Found: C, 72.4; H, 7.05; N, 3.1. $C_{28}H_{33}NO_5$ requires C, 72.55; H, 7.2; N, 3.0%); $[\alpha]_D$ +51.5 (*c* 0.5); ν_{max}(film)/cm^{-1} 3380 (NH_2), 1100 and 1030 (COC), 740 and 700 (Ar); δ_H (270 MHz) 1.68 (2H, br s, NH_2), 3.34 (3H, s, OCH_3), 3.33–3.38 (2H, m, 2- and 3-H), 3.42–3.47 (1H, m, 4-H), 3.64–3.77 (3H, m, 5-H, 6a-H, 6b-H), 4.50 and 4.64 (each 1H, d, J_{AB} 11.9, OCH_2Ph), 4.52 and 4.62 (each 1H, d, J_{AB} 10.9, OCH_2Ph), 4.64 (2H, s, OCH_2Ph), 4.63 (1H, 1-H), 7.18–7.35 (15H, m, 3 × Ph); δ_C (67.9 MHz) 53.76 (3-C), 55.14 (OCH_3), 68.60 (6-C), 69.86 (5-C), 72.81 (O*C*H$_2$Ph), 73.53 (O*C*H$_2$Ph), 74.53 (O*C*H$_2$Ph), 78.95 (4-C), 79.87 (2-C), 97.03 (1-C), 127.65–138.12 (18 C, Ar-C).

Methyl 2,4,6-tri-*O*-benzyl-3-[(1*S*,2*S*,3*S*,4*R*,5*R*,6*S*)-2,3,4,5,6-pentabenzyloxycyclohexylamino]-3-deoxy-α-D-glucopyranoside 16

A solution of ketone **4** (270 mg, 0.43 mmol) and amine **6** (250 mg, 0.54 mmol, 20% excess) in dry benzene (1.0 ml), maintained under nitrogen atmosphere, was stirred in the presence of 4 Å molecular sieves (300 mg, powdered) for 3 days. The mixture was filtered and the solid washed with dry benzene. The filtrate was concentrated *in vacuo* and the resulting crude imine **15** was diluted in DMF (5.0 ml) and $Na(CN)BH_3$ (185 mg) was introduced in one portion. The reaction mixture was stirred for 3 days at room temperature and 1 h at 100 °C. DMF was removed under reduced pressure and the residue was partitioned (water–dichloromethane). The organic phase was separated and the aqueous phase extracted with dichloromethane. The combined organic phase was dried, and concentrated *in vacuo*. Purification of the residue by column chromatography [eluent: hexane–ethyl acetate (3:1)] gave *title compound* **16** (282 mg, 61%) as a viscous, colourless oil (Found: C, 76.65; H, 6.8; N, 1.35. $C_{69}H_{73}NO_{10}$ requires C, 77.0; H, 6.8; N, 1.3%); $[\alpha]_D$ +34 (*c* 0.5); ν_{max}(film)/cm^{-1} 3310 (NH), 1065 (COC), 740 and 695 (Ar); δ_H (600 MHz) 3.20–3.35 (2H, m, 2- and 3-H), 3.33 (3H, s, OCH_3), 3.43–3.47 (1H, m, 4-H), 3.62 (1H, dd, $J_{5,6a}$ 1.9, $J_{6a,6b}$ 10.8, 6a-H), 3.68 (1H, dd, $J_{5,6b}$ 3.5, 6b-H), 3.77 (1H, br dt, $J_{4,5}$ 10.0, 5-H), 3.81 (1H, t, $J_{3',4'} = J_{4',5'} = 9.8$, 4′-H*), 3.82 (1H, t, $J_{1',2'} = J_{1',6'} = 4.4$, 1′-H), 3.91 (1H, t, $J_{5',6'}$ 9.8, 5′-H*), 3.94 (1H, dd, 6′-H), 4.03 (1H, br t, $J_{2',3'}$ 3.0, 2′-H), 4.20 (1H, dd, 3′-H), 4.29 and 4.57 (each 1H, d, J_{AB} 12.0, OCH_2Ph), 4.39 and 4.65 (each 1H, d, J_{AB} 11.2, OCH_2Ph), 4.46 and 4.61 (each 1H, d, J_{AB} 12.0, OCH_2Ph), 4.54 and 4.59 (each 2H, d, J_{AB} 11.5, OCH_2Ph), 4.54 and 4.64 (each 1H, d, J_{AB} 12.0, OCH_2Ph), 4.67 (1H, d, $J_{1,2}$ 3.6, 1-H), 4.76 and 4.79 (each 1H, d, J_{AB} 10.8, OCH_2Ph), 4.80 and 4.89 (each 1H, d, J_{AB} 10.8, OCH_2Ph), 7.03–7.38 (40H, m, 8 × Ph); δ_C (67.9 MHz) 55.02 (OCH_3), 56.72 (1′-C), 58.29 (3-C), 68.64 (6-C), 69.81 (5-C), 72.82 (O*C*H$_2$Ph), 72.93 (O*C*H$_2$Ph), 72.99 (2 × O*C*H$_2$Ph), 73.20 (O*C*H$_2$Ph), 73.56 (O*C*H$_2$Ph), 75.59 (O*C*H$_2$Ph), 75.65 (O*C*H$_2$Ph), 76.94 (2′-C), 79.35 (6′-C), 79.89 (4-C), 80.43 (2- and 3′-C), 82.31 (4′- or 5′-C), 82.53 (5′- or 4′-C), 97.41 (1-C), 127.00–139.40 (48 C, Ar-C).

* These assignments may be reversed, with corresponding re-allocation of *J*-values.

Methyl 3-deoxy-3-[(1*S*,2*S*,3*S*,4*R*,5*R*,6*S*)-2,3,4,5,6-pentahydroxycyclohexylamino]-α-D-glucopyranoside 2

Pd-black catalyst (35 mg) was added to a solution of **16** (250 mg, 0.23 mmol) in ethanol–cyclohexene [6.9 ml (2:1)]. The mixture was heated under reflux for 24 h, cooled, filtered, and the filtrate was concentrated *in vacuo*. The residue was purified first by silica gel column chromatography [eluent: MeOH–ethyl acetate (1:1)] to give compound **2** in crude form. A second purification by ion exchange resin IRA 400 (eluent: MeOH) afforded **2** (55 mg, 67%) as a foam; $[\alpha]_D$ +92.4 (*c* 0.4, MeOH); ν_{max}(Nujol)/cm^{-1} 3387 (OH, NH), 1040 (CO); δ_H (600 MHz; CD_3OD) 2.78 (1H, t, $J_{2,3} = J_{3,4} = 9.8$, 3-H), 3.25 (1H, t, $J_{4,5}$ 9.8, 4-H), 3.26 (1H, br t, $J_{1',2'} = J_{1',6'} = 4.5$, 1′-H), 3.32 (1H, dd, $J_{1,2}$ 3.7, 2-H), 3.41 (3H, s, OCH_3), 3.47 (1H, t, $J_{3',4'} = J_{4',5'} = 9.1$, 4′-H*), 3.51 (1H, m, 5-H), 3.53 (1H, t, $J_{5',6'}$ 9.1, 5′-H*), 3.63 (1H, dd, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 11.8, 6a-H), 3.67 (1H, dd, $J_{2',3'}$ 3.2, 3′-H), 3.77 (2H, 2 × dd, $J_{5,6b}$ 2.3, 6b-H and 6′-H), 4.04 (1H, t, 2′-H), 4.66 (1H, d, 1-H), 4.84 (s br, OH); δ_C (67.9 MHz; CD_3OD) 55.45 (OCH_3), 62.44 (1′-C), 62.82 (6-C), 64.27 (3-C), 72.20 (4-C), 72.55 (2- and 6′-C), 72.72 (3′-C), 73.24

(2′-C), 74.32 (5-C), 74.57 (4′- or 5′-C), 75.27 (5′- or 4′-C), 101.05 (1-C); *m/z* (FAB) 356 $[M + H]^+$ (Found: $[M + H]^+$ 356.1560. $C_{13}H_{26}NO_{10}$ requires *m/z*, 356.1557.

* These assignments may be reversed, with corresponding re-allocation of *J*-values.

Methyl 2-(2′,2′,2′-trichloroethoxycarbonylamino)-2-deoxy-α-D-glucopyranoside 9

Acetyl chloride (1 ml, 14.08 mmol) was added dropwise to a suspension of 2-(2′,2′,2′-trichloroethoxycarbonylamino)-2-deoxy-D-glucose **8** (1.0 g, 2.82 mmol) in dry methanol (20 ml). The mixture was heated under reflux for 8.5 h, after which time TLC [ethyl acetate–methanol (9:1)] showed that most of the starting material was consumed. The reaction mixture was cooled, and then neutralised by portionwise addition of lead carbonate (1.41 g). Stirring was continued for 1 h and then the mixture was filtered through a kieselguhr pad and the filtrate was concentrated to dryness. Recrystallisation of the crude product, containing mostly α-isomer, from toluene gave *title compound* **9** (678 mg, 65%) as a crystalline solid, mp 135–138 °C (Found: C, 31.3; H, 4.45; N, 3.5; Cl, 27.6. $C_{10}H_{16}NO_7Cl_3 \cdot H_2O$ requires C, 31.1; H, 4.7; N, 3.6; Cl, 27.5%); $[\alpha]_D$ +75.4 (*c* 0.3, MeOH); ν_{max}(Nujol)/cm^{-1} 3350 (OH, NH), 1730 (CO), 1055 (COC), 820 (CCl_3); δ_H (270 MHz; $CDCl_3$ + CD_3OD) 3.39 (3H, s, OCH_3), 3.46 (1H, t, $J_{3,4} = J_{4,5} = 9.2$, 4-H), 3.53–3.59 (1H, m, 5-H), 3.60–3.70 (2H, m, 2-, 3-H), 3.73 (1H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 11.9, 6a-H), 3.85 (1H, dd, $J_{5,6b}$ 2.7, 6b-H), 4.68, 4.73, 4.78, 4.83 (2H, AB q, J_{AB} 12.0, OCH_2CCl_3), 4.75 (1H, d, $J_{1,2}$ 2.7, 1-H); δ_C (67.9 MHz; $CDCl_3$ + CD_3OD) 55.39 (OCH_3), 56.45 (2-C), 62.02 (6-C), 71.35 (4-C), 72.39 (3-C), 72.61 (5-C), 75.12 (OCH_2CCl_3), 95.84 (OCH_2CCl_3), 99.18 (1-C), 155.61 (CO).

Methyl 3,4,6-tri-*O*-benzyl-2-(2′,2′,2′-trichloroethoxycarbonylamino)-2-deoxy-α-D-glucopyranoside 10

Triflic acid (0.01 ml) was added to a stirred suspension of compound **9** (100 mg, 0.27 mmol) and benzyl trichloroacetimidate (0.3 ml, 1.62 mmol) in cyclohexane–dichloromethane (2:1, 2.7 ml) and the mixture was stirred for 2.5 h. The reaction mixture was then filtered and the filtrate washed successively with saturated aq. $NaHCO_3$ and water. The dried solution was evaporated to dryness and the residue was purified by column chromatography [eluent: first hexane–ethyl acetate (9:1) and then (7:3)]. The product **10** (135 mg, 78%) was obtained as a viscous oil which crystallised spontaneously to give a solid, mp 59–62 °C; $[\alpha]_D$ +57.4 (*c* 0.8); ν_{max}(film)/cm^{-1} 3350 (NH), 1735 (CO), 1055 (COC), 825 (CCl_3), 740, 705 (Ar); δ_H (270 MHz) 3.34 (3H, s, OCH_3), 3.60–3.80 (5H, m, 3-, 4-, 5-H, 6-H_2), 3.86–4.10 (1H, m, 2-H), 4.51 and 4.62 (each 1H, d, J_{AB} 11.4, OCH_2Ph), 4.51 and 4.78 (each 1H, d, J_{AB} 10.5, OCH_2CCl_3), 4.63 and 4.77 (each 1H, d, J_{AB} 12.2, OCH_2Ph), 4.70 and 4.81 (each 1H, d, J_{AB} 11.2, OCH_2Ph), 4.73 (1H, d, $J_{1,2}$ 3.6, 1-H), 7.16–7.33 (15H, m, 3 × Ph); δ_C (67.9 MHz) 55.03 (OCH_3 and 2-C), 68.35 (6-C), 70.69 (5-C), 73.40 (OCH_2Ph), 74.59 (OCH_2Ph), 74.89 (OCH_2CCl_3), 75.12 (OCH_2Ph), 78.20 (3- or 4-C), 80.44 (4- or 3-C), 95.36 (OCH_2CCl_3), 98.61 (1-C), 127.67–138.00 (18 C, Ar-C), 154.17 (CO); *m/z* (EI) 546 $[M - C_7H_7]^+$ (Found: $[M - C_7H_7]^+$ 546.0846. $C_{24}H_{27}Cl_3NO_7$ requires *m/z*, 546.0853).

Methyl 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside 5

Activated zinc dust [20] (1.09 g) was added to a stirred solution of compound **10** (440 mg, 0.69 mmol) in THF (9.4 ml) and 1.0 M potassium dihydrogen phosphate solution (1.9 ml). After stirring for 9 h the reaction mixture was filtered through a kieselguhr pad, concentrated and the residue was re-diluted with dichloromethane and treated with pyridine (0.8 ml). The mixture was stirred for 2 h and then washed with water, dried and evaporated to dryness. The crude material was purified by chromatography [eluent: diethyl ether–triethylamine (5:1)] to give *title compound* **5** (198 mg, 62%) as a colourless oil; $[\alpha]_D$ +101.4 (*c* 0.3); ν_{max}(film)/cm^{-1} 3400 (NH), 1050 (COC), 740 and 700 (Ar); δ_H (270 MHz) 1.46 (2H, s br, NH_2), 2.81 (1 H, dd, $J_{1,2}$ 3.4, $J_{2,3}$ 9.2, 2-H), 3.35 (3H, s, OCH_3), 3.55 (1H, t, $J_{3,4} = J_{4,5} = 9.2$, 4-H), 3.63 (1H, t, 3-H), 3.65–4.00 (3H, m, 5-H, 6-H_2), 4.52 and 4.64 (each 1H, d, J_{AB} 12.3, OCH_2Ph), 4.52 and 4.78 (each 1H, d, J_{AB} 10.9, OCH_2Ph), 4.69 and 4.95 (each 1H, d, J_{AB} 11.5, OCH_2Ph), 4.74 (1H, d, 1-H), 7.15–7.36 (15H, m, 3 × Ph); δ_C (67.9 MHz) 55.05 (OCH_3), 55.90 (2-C), 68.64 (6-C), 70.83 (5-C), 73.51 (OCH_2Ph), 74.66 (OCH_2Ph), 75.54 (OCH_2Ph), 78.79 (3-C), 84.06 (4-C), 100.64 (1-C), 127.56–138.57 (18 C, Ar-C); *m/z* (EI) 463 $[M]^+$ (Found: $[M]^+$, 463.2359. $C_{28}H_{33}NO_5$ requires *M*, 463.2359).

Methyl 3,4,6-tri-*O*-benzyl-2-(2′,4′,6′-tribenzyloxyphenylamino)-2-deoxy-α-D-glucopyranoside 11

Compound **5** (195 mg, 0.42 mmol) and ketone **4** (214 mg, 0.34 mmol) were dissolved in dry benzene (3 ml) and the solution was concentrated to dryness at 60 °C and maintained under reduced pressure at this temperature for 3 h. The residue was re-dissolved in benzene (3 ml) and the process repeated four times. The residue was purified by column chromatography [eluent: hexane–ethyl acetate (3:1)] to give *title compound* **11** (230 mg, 79%) as a viscous oil (Found: C, 76.6; H, 6.4; N, 1.6. $C_{55}H_{55}NO_8$ requires C, 77.0; H, 6.45; N, 1.6%); $[\alpha]_D$ +28.3 (*c* 0.7); ν_{max}(film)/cm^{-1} 1050 (COC), 740, 700 (Ar); δ_H (270 MHz) 3.29 (3H, s, OCH_3), 3.58 (1H, t, $J_{3,4} = J_{4,5} = 9.4$, 4-H), 3.66 (1H, dd, $J_{5,6a}$ 1.7, $J_{6a,6b}$ 10.3, 6a-H), 3.73 (1H, dd, $J_{5,6b}$ 3.7, 6b-H), 3.76–3.91 (2H, m, 3-, 5-H), 4.14 (1H, dd, $J_{1,2}$ 3.3, $J_{2,3}$ 9.9, 2-H), 4.46 and 4.74 (each 1H, d, J_{AB} 10.8, OCH_2Ph), 4.50 and 4.60 (each 1H, d, J_{AB} 12.2, OCH_2Ph), 4.69 (2H, s, OCH_2Ph), 4.85 (2H, s, OCH_2Ph), 4.97 and 5.00 (each 1H, d, J_{AB} 12.1, OCH_2Ph), 4.98 (2H, s, OCH_2Ph), 4.99 (1H, d, 1-H), 6.25 (2H, s, 3′- and 5′-H), 6.93–7.40 (30H, m, 6 × Ph); δ_C (67.9 MHz) 55.05 (OCH_3), 58.63 (2-C), 68.91 (6-C), 70.47 (5-C), 70.58 (OCH_2Ph), 70.65 (2 × OCH_2Ph), 73.35 (OCH_2Ph), 74.66 (OCH_2Ph), 74.75 (OCH_2Ph), 78.59 (3-C), 83.71 (4-C), 94.87 (3′- and 5′-C), 100.32 (1-C), 121.38 (1′-C, Ar-CNHR), 126.84–139.02, (36 C, Ar-C), 149.79 (2′- and 6′-C), 152.63 (4′-C).

Methyl 2-deoxy-2-(2′,4′,6′-trihydroxyphenylamino)-α-D-glucopyranoside 12

Compound **11** (134 mg, 0.16 mmol), under argon atmosphere, was dissolved in ethanol–cyclohexene (2:1 v/v; 4.3 ml), a catalytic amount of Pd-black was added, and the mixture heated under reflux for 38 h. The catalyst was separated by decantion and the solution was transferred to a flask while being maintained constantly under an argon atmosphere. The solvent was removed by distillation *in vacuo* with intermittent introduction of argon. The pale yellow solid **12** (identified solely by its NMR spectra) was obtained in quantitative yield and was used directly to prepare the NMR sample, also under inert atmosphere; δ_H (270 MHz; CD_3OD) 3.40 (3H, s, OCH_3), 3.42 (1H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 3.57–3.66 (1H, m, 5-H), 3.64 (1H, dd, $J_{1,2}$ 3.3, $J_{2,3}$ 10.8, 2-H), 3.71 (1H, dd, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 11.9, 6a-H), 3.82 (1H, dd, $J_{5,6b}$ 2.3, 6b-H), 4.04 (1H, dd, 3-H), 4.63 (1H, d, 1-H), 4.90 (br s, OH), 6.00 (2H, s, 3′- and 5′-H); δ_C (67.9 MHz; CD_3OD) 55.93 (OCH_3), 62.17 (6-C), 64.58 (2-C), 71.12 (3-C), 71.94 (4-C), 74.08 (5-C), 95.63 (3′- and 5′-C), 96.92 (1-C), 102.47 (1′-C, Ar-*C*NHR), 153.88 (2′- and 6′-C), 161.02 (4′-C).

Enzyme assays

Inhibition experiments on yeast α-glucosidase (type IV from brewer's yeast) were performed as described previously [21] at 30 °C and at pH 6.5 in PIPES–NaOAc buffer through measurement of the rate of liberation of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside by monitoring the absorp-

tion of phenoxide anion at 400 nm. Plotting a graph of the slopes of Lineweaver–Burk reciprocal plots of $1/v$ against 1/[S], obtained in the presence of increasing amounts of inhibitor, against corresponding inhibitor concentrations [I], to which they are linearly related, yields an inhibition constant (K_i) from the intercept of this graph on the [I]-axis.[18]

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