Synthesis of a novel pseudodisaccharide glycoside as a potential glycosidase inhibitor

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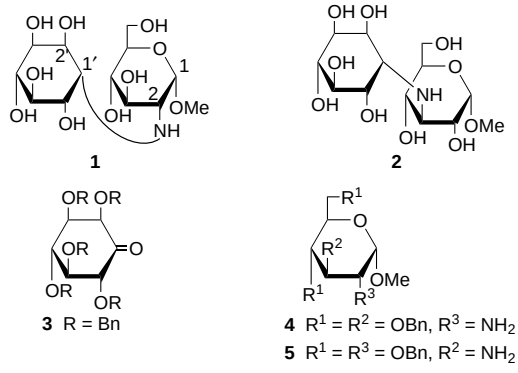
An *N*-linked pseudodisaccharide 2 containing an inositol moiety in place of a glycopyranosyl residue has been synthesised to mimic the disaccharide unit, 3-*O*-(α-D-glucopyranosyl)-D-glucopyranose, cleaved by Glucosidase II during glycoprotein processing; an attempt to prepare the analogous 2-*N*-linked compound 1 as an inhibitor of Glucosidase I led to the novel *N*-phenyl derivative 10 of 2-amino-2-deoxy-D-glucopyranose.

Glycoprotein processing, a key step in the formation of cell surface oligosaccharides, initially involves the action of Glucosidase I and II on the *N*-linked oligosaccharide $Glc_3Man_9GlcNAc_2$ for sequential removal of the α(1 → 2)- and α(1 → 3)-linked glucose residues.[1] Inhibition of either of these two key enzymes leads to incorrect modification of nascent oligosaccharides.[2] Malformation of gp 120 on the surface of the AIDS virus is the likely reason why glycosidase inhibitors such as 1-deoxynojirimycin (DNJ) or castanospermine exhibit anti-HIV activity.[3]

We wished to design potential glycosidase inhibitors which mimic the *disaccharide units* which form the substrates for Glucosidase I and II, 2-*O*-(α-D-glucopyranosyl)-D-glucopyranose and 3-*O*-(α-D-glucopyranosyl)-D-glucopyranose, respectively. There are clear advantages in introducing nitrogen into such structures at strategic points[4] but in view of the likely hydrolytic instability of simple *N*-linked mimics, which are glycosylamines, and the efficacy of *N*-linked monocarba-oligosaccharide inhibitors such as acarbose[5] and oligostatin,[6] we decided to prepare the corresponding *N*-linked pseudodisaccharides **1** and **2** in which the non-reducing glycosyl moiety is imitated by an inositol unit, which only fails in its ability to mimic the α-D-glucopyranose unit in having an OH in place of the usual 5-CH_2OH group and a –CH(OH)– group in place of the ring oxygen atom. We report an unexpected result in our attempt to synthesise **1**,§ the synthesis of **2**, and a



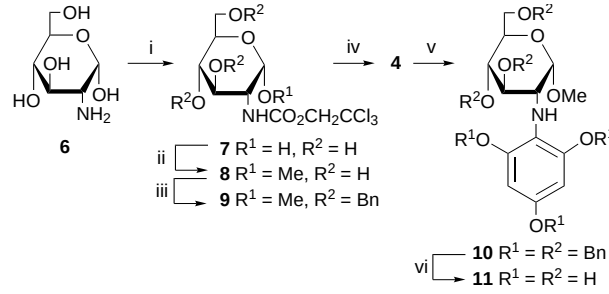
preliminary result on inhibition studies with **2** on yeast α-glucosidase.

Our proposed syntheses were based on the reaction between the chiral penta-*O*-benzylinosose **3**[7] and either methyl 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside **4** or methyl 3-amino-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranoside **5** to afford corresponding imines, which we aimed to reduce stereoselectively, creating a chiral centre which, in the final product **1** and **2**, mimics the α-anomeric link in the oligosaccharide.

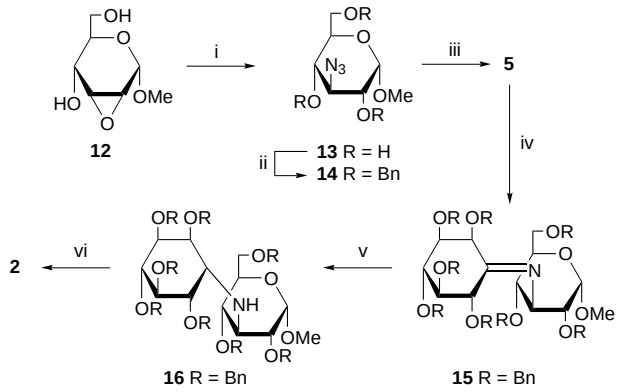
To prepare amine **4**, 2-amino-2-deoxy-D-glucopyranose **6** was *N*-protected by reaction with 2,2,2-trichloroethoxycarbonyl chloride, and the resultant amide **7** was converted by reflux in MeOH containing HCl into the corresponding methyl α-D-glycoside **8** (Scheme 1). Attempted *O*-benzylation of the latter compound using NaH–BnBr led to extensive decomposition, but treatment with benzyl trichloroacetimidate[8] under acidic catalysis gave the required protected benzyl ether **9** which was de-*N*-protected by treatment[9] with Zn/Cu couple in KH_2PO_4–THF–H_2O to give **4**.

Storage of **4** with an equimolar quantity of inosose **3** in benzene over molecular sieves at 20 °C for several days gave no reaction, but repeated evaporation of benzene from the mixture at 60 °C under reduced pressure afforded a new product with a characteristic single at δ 6.25 in its ^{1}H NMR spectrum. 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 **10** 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.¶ Subjecting **10** to catalytic transfer hydrogenation conditions without careful exclusion of air led to the initial formation of a purple solution followed by extensive decomposition as evidence by TLC, presumably because of oxidation of the initially formed aminophloroglucinol derivative. Hydrogenation with subsequent manipulations under an atmosphere of argon gave material with the expected ^{1}H and ^{13}C NMR spectra of methyl 2-deoxy-2-(2,4,6-trihydroxyphenylamino)-α-D-glucopyranoside **11**.

In contrast, our related strategy for the preparation of **2** was successful. Amine **5** was prepared in three steps from methyl 2,3-anhydro-α-D-allopyranoside **12** by reaction with azide ion, followed by conventional benzylation (NaH–BnBr in DME) of the 3-azido-*gluco* product **13**, chromatographically isolated from the simultaneously formed 2-azido-*altro* isomer, and then



Scheme 1 *Reagents and conditions*: 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, 96%; iii, $CCl_3C(NH)OBn$, CH_2Cl_2–cyclohexane (1 : 2), TFA, 20 °C, 2.5 h, 78%; iv, Zn/Cu, THF–1 M aq. KH_2PO_4 (5 : 1), 20 °C, 8.5 h, 51%; v, **3**, 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)



Scheme 2 *Reagents and conditions*: i, NaN_3, $(NH_4)_2SO_4$, DMF, 110 °C, 3 h, 47%; ii, NaH, BnBr, DMF, 20 °C, 1 h, 91%; iii, Ph_3P, THF–H_2O (10 : 1), 80 °C, 7 h, 70%; iv, **3**, C_6H_6, 4 Å molecular sieves under N_2, 20 °C, 70 h; v, $NaBH_3CN$, DMF, 20 °C, 70 h and 100 °C, 1 h, 61%; vi, EtOH–cyclohexene (2 : 1), Pd black, reflux 24 h, 67%

reduction (Ph_3P–THF–H_2O) of the so-produced azido benzyl ether **14** (Scheme 2). Condensation of **5** with the protected chiral inosose **3** to give imine **15** occurred on storage of a mixture of the two reactants in a 1.2 : 1 molar ratio in benzene solution over molecular sieves at 20 °C for 70 h. Surprisingly, attempted formation of the imine **15** by an aza-Wittig reaction of **3** with the iminophosphorane obtained by treatment of azide **13** with PPh_3 under anhydrous conditions proved unsuccessful.

Reduction of imine **15** with sodium cyanoborohydride in DMF occurred with the required stereoselectivity, the major product being shown by ^{1}H NMR spectroscopy at 600 MHz to be the amine **16**. Thus, on the reasonable assumption that the inositol ring in **16** adopts a chair conformation with four equatorial substituents, an assumption supported by the vicinal coupling constants, the signal for the proton (1′-H) on the inositol carbon carrying the nitrogen substituent would be expected to be an overlapping doublet of doublets, each with $J \sim 3$ Hz, resulting in an apparent triplet with ~ 3 Hz splitting. The alternative stereoisomer would be expected to show for the same proton a true doublet of doublets with component splittings of ~ 9 and 3 Hz. The observed apparent triplet with a J value of 4.4 Hz confirmed structure **16**. Signal allocation for 1′-H on the inositol ring was confirmed by measurement of the ^{1}H NMR spectrum of the analogous deuterated compound obtained by reduction of imine **15** with sodium borodeuteride.

De-*O*-benzylation was achieved by catalytic transfer hydrogenation (cyclohexene–Pd black–EtOH) at reflux temperature for 24 h. Purification of the product by chromatography on silica gel with 1 : 1 EtOAc–MeOH gave the required pseudodisaccharide **2** as a foam. Inspection of the ^{1}H NMR spectrum of the crude hydrogenolysis product indicated a 'satellite' doublet at δ 4.64 to that of the anomeric proton at δ 4.67, with an integrated intensity $\leqslant$5% of the major signal, but no other difference from the spectrum of **2** could be observed. That the benzyl ether **16** appeared 'anomerically pure' results from the very small amount of the diastereoisomer present and extensive overlap of the absorption region for anomeric protons with that of the benzylic CH_2 resonances.

Preliminary inhibition studies on yeast α-glucosidase indicate that **2** is a much poorer inhibitor of the enzyme than DNJ. Thus, under identical conditions (30 °C, pH 6.5, PIPES buffer), an approximately 100-fold greater molar concentration of **2** was required compared to that for DNJ to bring about a 20% reduction in the rate of enzyme-catalysed release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside. This result reflects, most likely, a different inhibitory mechanism for **2** compared to that of DNJ;[5] the latter carries an endocyclic N-atom in contrast to **2**, in which the N-atom replaces the usual glycosidic O-atom.

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Notes and References

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§ All new compounds, except for the unstable imine **15**, gave satisfactory elemental analyses or high resolution mass spectral data and gave NMR spectra in accord with expected structures.

¶ Although the isomeric 3,4,5-tribenzyloxy isomer cannot be ruled out, the structural allocation is based on the combined considerations of: (i) a comparison of the observed ^{13}C NMR chemical shifts for Ar-C in the tetrasubstituted aromatic ring of **10** with those estimated for the two isomers; (ii) a comparison of the observed ^{1}H NMR chemical shift for Ar-H at δ 6.25 with those for 3,4,5-trimethoxyaniline (δ 5.91) and 2,4,6-trimethoxyaniline (δ 6.05), all measured in $CDCl_3$; (iii) the likely mechanism of elimination from the supposed intermediate imine.

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