N-Thiocarbonyl azasugars: a new family of carbohydrate mimics with controlled anomeric configuration

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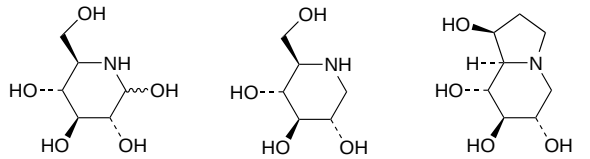
Bicyclic azasugar glycomimetics structurally related to the polyhydroxy-indolizine and -piperidine series incorporating a stereoelectronically controlled pseudoanomeric axial hydroxy group have been prepared by tautomeric rearrangement of cyclic thiocarbamate precursors; preliminary enzyme inhibition tests show an increased selectivity towards yeast α-glucosidase for the α-D-glucopyranose analogue as compared with castanospermine or nojirimicin.

Nitrogen-in-the-ring carbohydrate mimics (azasugars) represent an important group of tight-binding glycosyl hydrolase inhibitors useful for studies on glycoconjugate function, targeting and turnover.[1] An additional stimulus of great impact on the synthesis and use of these compounds comes from their application as chemotherapeutic agents for the treatment of several diseases such as diabetes, cancer, inflammation or viral replication, including the human immunodeficiency virus (HIV).[2] Although many potent azasugar glycosidase inhibitors are now available, they generally suffer from lack of specificity. Thus, nojirimycin **1**, a polyhydroxypiperidine derivative having an hydroxylation pattern of spatial similarity to that of D-glucose, and its 1-deoxy analogue **2** are good inhibitors of various α- as well as β-glycosidases. A higher enzyme specificity is observed for the configurationally related indolizidine alkaloid castanospermine **3**, which has been ascribed to the rigidity of the bicyclic structure that locks the homologous bond to C-5–C-6 in hexopyranoses.

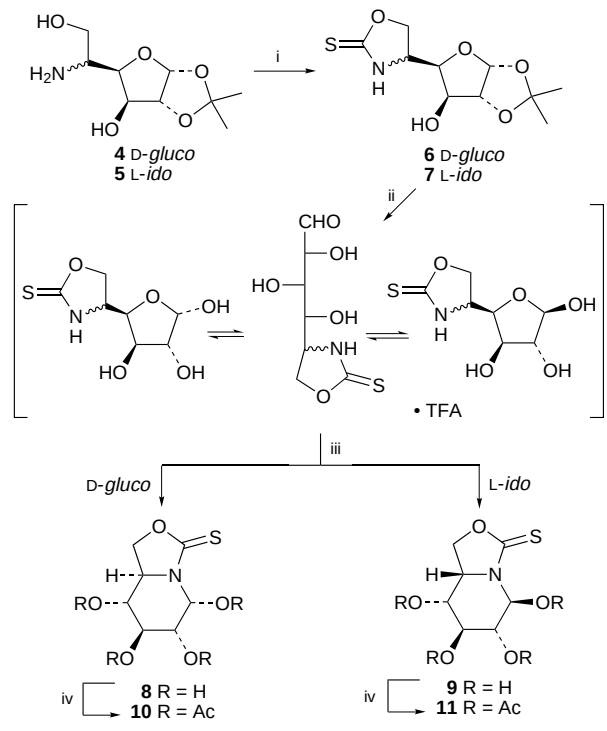


nojirimycin **1** 1-deoxynojirimycin **2** castanospermine **3**

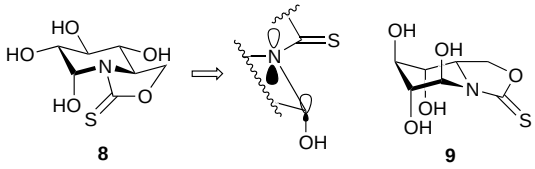
Neither of the above mentioned naturally occurring azasugars, nor virtually all the reported synthetic analogues, possess a defined configuration at the pseudoanomeric centre in aqueous solution. An ideal glycosidase ligand should, however, account for the stereocomplementarity of the leaving aglyconic oxygen atom with the key bilateral carboxylic acid and carboxylate groups in the active site of the enzyme, which is probably responsible for the α- or β-linkage specificity shown by many glycosidases.[3] Although the hydroxy group would be a universal surrogate for the natural glycosidic bond of the putative substrates, its hemiacetalic character results in rapid epimerization and low stability (*e.g.* **1**). In fact, indolizidine azasugars bearing a pseudoanomeric hydroxy substituent are unknown.[4]

We have previously shown that thioamide moieties in six-membered ring systems exert a definitive influence in the orientational properties of vicinal hydroxy groups.[5] Exclusively axial OH dispositions are allowed, probably due to a very efficient delocalization interaction between the π-type lone-pair orbital of the sp^2-hybridized N-atom in the ground state of *N*-thiocarbonyl functionalities and the σ* antibonding orbital of the contiguous C–O bond. We have now extended this concept to the preparation of carbohydrate mimics endowed with the favourable structural features of polyhydroxyindolizidines and incorporating a configurationally anchored pseudoanomeric hydroxy group.[6]

The synthetic strategy relies on the ability of the ambident *N*-thiocarbonyl group to act as a nitrogen nucleophile in coupling reactions with carbonyl compounds.[7] Thiocarbonylation of 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose **4**, available in eight steps from commercial D-glucofuranurono-3,6-lactone,[8] with carbon disulfide–DCC afforded the five-membered cyclic thiocarbamate **6** in excellent yield.§ Hydrolysis of the acetal protecting group under acidic conditions led to a mixture of the protonated α- and β-furanoses, as seen from a ^{13}C NMR spectrum of the crude reaction mixture, which after neutralisation rearranged to the fused bicyclic



Scheme 1 *Reagents and conditions*: i, CS_2, DCC, −10 °C, 6 h, 80–85%; ii, 9 : 1 TFA–H_2O, room temp., 2 h; iii, coevaporation with water, then IR 45 (OH^-) ion exchange resin, GPC (Sephadex G-10, 1 : 1 MeOH–H_2O), 75–90%; iv, 1 : 1 Ac_2O–pyridine, room temp., 16 h, quant.



8 **9**

Fig. 1 Ring conformation of compounds **8** and **9** showing the OH orientational pattern. The π-electron stabilization interaction responsible of the strong anomeric effect is also represented for the pseudo $^4C_1$ chair conformation of the six-membered ring (*e.g.* **8**).

azasugar **8** by intramolecular nucleophilic addition of the thiocarbamate *N*-atom to the masked aldehydo group of the monosaccharide (Scheme 1).

The ^{1}H and ^{13}C NMR spectra of **8** in D_2O showed the presence of a single tautomeric form having the *R* configuration at the pseudoanomeric centre, *i.e.* a molecular framework analogous to that of α-D-glucopyranose (Fig. 1). No traces of the *S*-epimer or furanoid tautomers were detected even after conventional acetylation (→ **10**), corroborating the total stereoelectronic control induced by the vicinal thiocarbamate segment. The potential of this approach to design glycomimetics bearing a pseudoanomeric oxygen atom with defined configurational and conformational properties is further illustrated by the preparation of the L-*ido* derivative **9** from the corresponding C-5 epimeric amino sugar[8] **5** following a similar reaction sequence (Scheme 1). The all-OH-axial bicyclic tautomer with *S* configuration at the pseudoanomeric centre (Fig. 1) was exclusively detected in D_2O solution or after acetylation (→ **11**).¶ Both the vicinal $^3J_{H,H}$ coupling constants and computational calculations‖ support a rigid conformation for the bicyclic skeleton of **8** close to that of castanospermine **3**. Preliminary glycosyl hydrolase inhibition tests showed, however, a totally different scenario. Considering the most widely used glycosyl hydrolases, sweet almond β-glucosidase and yeast α-glucosidase, the inhibition constants for **8** (150 000 and 90 μmol dm^{-3}, respectively)** indicated a reverse linkage specificity as compared to **3** (1.5 and > 1500 μmol dm^{-3}) or **1** (0.89 and 6.3 μmol dm^{-3}).[1a] Although the latter is a one order of magnitude stronger inhibitor of yeast α-glucosidase than **8**, the selectivity ratio for **8** is above 10^4-fold higher.

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Footnotes and References

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§ The use of thiophosgene or *N*,*N*′-thiocarbonyldiimidazole as thiocarbonylation reagents resulted in lower yields of **6** due to formation of thiocarbamate and thiocarbonate derivatives involving the remaining unprotected OH-3. For previous reports on the reactivity of unprotected amino sugars towards thiocarbonylation reagents see ref. 9.

¶ All new compounds gave satisfactory microanalytical, NMR (^{1}H and ^{13}C) and mass spectral data in accord with the proposed structures. *Selected data* for **8**: $[\alpha]_D$ +0.8 (*c* 0.8, H_2O); δ_H(500 MHz, D_2O, *J*/Hz) 5.78 (1 H, d, $J_{1,2}$ 4.0, H-1), 4.77 (1 H, t, $J_{5,6b} = J_{6a,6b}$ = 9.2, H-6a), 4.56 (1 H, dd, $J_{2,3}$ 9.6, H-2), 4.48 (1 H, dd, $J_{5,6b}$ 7.3, H-6b), 4.12 (1 H, ddd, $J_{4,5}$ 9.6, H-5), 3.70 (1 H, t, $J_{3,4}$ 9.6, H-3), 3.50 (1 H, t, H-4). For **9**: $[\alpha]_D$ +64 (*c* 0.8, H_2O); δ_H(500 MHz, D_2O, *J*/Hz) 5.48 (1 H, d, $J_{1,2}$ 1.4, H-1), 4.62 (1 H, dd, $J_{5,6b}$ 9.4, $J_{6a,6b}$ 7.9, H-6a), 4.50 (1 H, td, $J_{4,5}$ 6.0, $J_{5,6b}$ 7.9, H-5), 4.44 (1 H, t, H-6b), 3.77 (1 H, t, $J_{2,3} = J_{3,4}$ = 3.4, H-3), 3.72 (1 H, dd, H-2), 3.61 (1 H, t, H-4). For clarity of presentation, the notation of atoms for NMR data has been kept consistent with the parent monosaccharides.

‖ Molecular mechanics calculations were performed using MM2* as integrated in MACROMODEL 5.5, using the GB/SA continuum solvent model for H_2O.

** β-Glucosidase from almonds and α-glucosidase from yeast were purchased from Aldrich. The inhibition constants were determined spectrophotometrically (410 nm) at 37 °C at pH 5 and 7, respectively. The Michaelis constants K_M obtained for the corresponding *p*-nitrophenyl β- and α-glucosides were 3.5 and 0.25 mmol dm^{-3}, respectively.

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