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Castanospermine-trehazolin hybrids: a new family of glycomimetics with tuneable glycosidase inhibitory properties†

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Bicyclic azasugar glycomimetics related to castanospermine and trehazolin have been prepared from sugar carbodiimides via aminooxazoline intermediates; preliminary enzyme inhibition tests showed a marked dependence of the selectivity and potency towards α and β-glucosidases upon the nature of the exocyclic substituent.

Castanospermine (**1**) represents one of a number of plant derived alkaloidal sugar mimics (azasugars) exhibiting potent glycosidase inhibitory activity.[1] A diverse plethora of biological properties have been ascribed to **1** as a consequence of its specific inhibition of α- and β-glucosidases, including antiviral, anti-cancer and anti-diabetic properties.[2] Structure–activity studies have shown that the rigid bicyclic skeleton of **1**, that locks the homologous bond to C-5–C-6 in hexopyranoses, is responsible for the observed higher enzyme specificity as compared with the monocyclic analog 1-deoxynojirimycin (**2**).[3] Yet, the bridgehead location of the nitrogen atom in castanospermine prevents incorporation of *N*-substituents, a strategy that has led to potent and specific glycosidase inhibitors already approved for clinical trials in the piperidine series, such as *N*-butyl-1-deoxynojirimycin[4] **3** and the pseudodisaccharide derivative MDL 73945 **4**.[5] The four chiral centres in **1** have been systematically modified by inversion of the configuration, removal, replacement or esterification of the hydroxy groups. All of these modifications, however, resulted in a decreased glucosidase activity.[2]

Recently we found that a subtle change in the structure of azasugars, by replacing the imino sp^3 nitrogen atom by a pseudoamide-type nitrogen, with substantial sp^2 character, led to a new group of glycosidase inhibitors with high anomer specificity.[6] Interestingly, this electronic feature is also present in the natural trehalase inhibitor trehazolin (**5**), where the occurrence of a less basic isourea function‡ allows interaction with the key bilateral carboxylic groups in the active site of the enzyme as well as additional interactions with the aglyconic binding site, resulting in a remarkable enzyme specificity.[8] We report herewith the synthesis of glucose mimics endowed with the essential structural features of both castanospermine (*i.e.*, a bicyclic indolizidine framework with a hydroxylation profile analogous to that of D-glucose) and trehazolin (*i.e.*, a fused 2-aminooxazoline ring). We envisioned that incorporation of different substituents at the exocyclic nitrogen could be used to modulate the enzyme specificity of the new azasugars.

Our synthetic route relies on the ability of the masked aldehyde group of monosaccharides to act as the electrophile target for azole heterocycles and involves a D-glucofuranose aminooxazoline derivative as the precursor. A new methodology has been developed for the construction of the oxazoline ring that exploits the reactivity of sugar carbodiimides, avoiding the use of hazardous reagents (mercury salts, isocyanates) generally employed in the reported syntheses of trehazolin analogues *via* thiourea or urea intermediates.[8] Thus, Staudinger reaction of the 5-azido-5-deoxy-α-D-glucofuranose derivative **6**§ with triphenylphosphine followed by *in situ* aza-Wittig type coupling with butyl isothiocyanate afforded carbodiimide **7**.[10] Fluoride-catalysed hydrolysis of the silyl ether group proceeded with spontaneous intramolecular nucleophilic addition of the generated hydroxy group to the vicinal heterocumulene functionality, affording the required 2-aminooxazoline derivative **8** in 60% overall yield. Deacetylation followed by acid hydrolysis of the acetonide group led, first, to a mixture of the corresponding glucofuranose oxazolinium salt **9** as a mixture of the α and β anomers (NMR) which rearranged into the target bicyclic 2-oxacastanospermine derivative **11** through the open chain *aldehydo* form **10** upon neutralisation (Scheme 1).¶

Our next interest was the synthesis of the castanospermine–trehazolin hybrids **18** and **19**, having structures that are

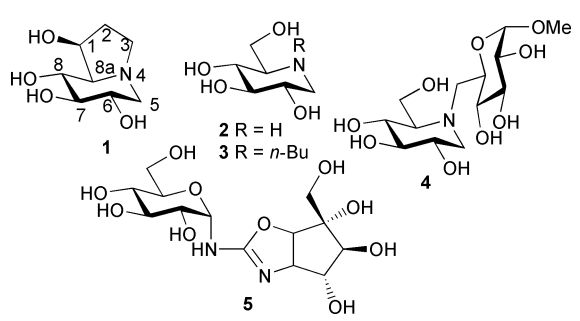
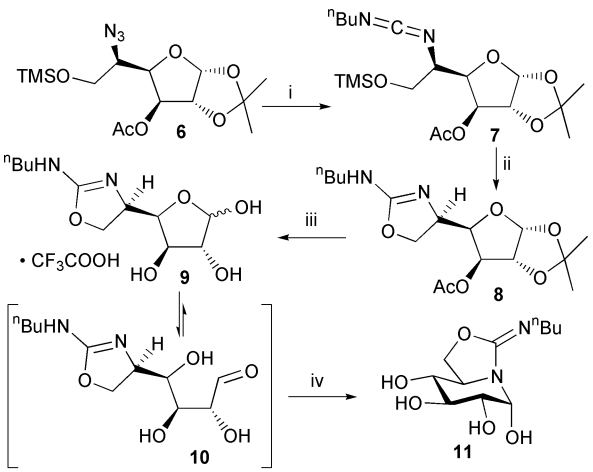


Fig. 1 Structures of castanospermine (**1**), 1-deoxynojirimycin (**2**), *N*-substituted 1-deoxynojirimycin derivatives (**3**, **4**) and trehazolin (**5**).



Scheme 1 *Reagents and conditions*: i, PPh_3, ^{n}BuNCS, toluene, 80 °C; ii, TBAF, THF, 0 °C, 25 min (60% overall); iii, NaOMe, MeOH, then TFA–water 9:1, co-evaporation with water; iv, IR 45 (OH^-) ion exchange resin (90 % overall).

† Electronic supplementary data (ESI) available: full characterization data for the new compounds **7–9**, **11**, **14–19**. See htpp://www.rsc.org/suppdata/cc/b2/b200162d/

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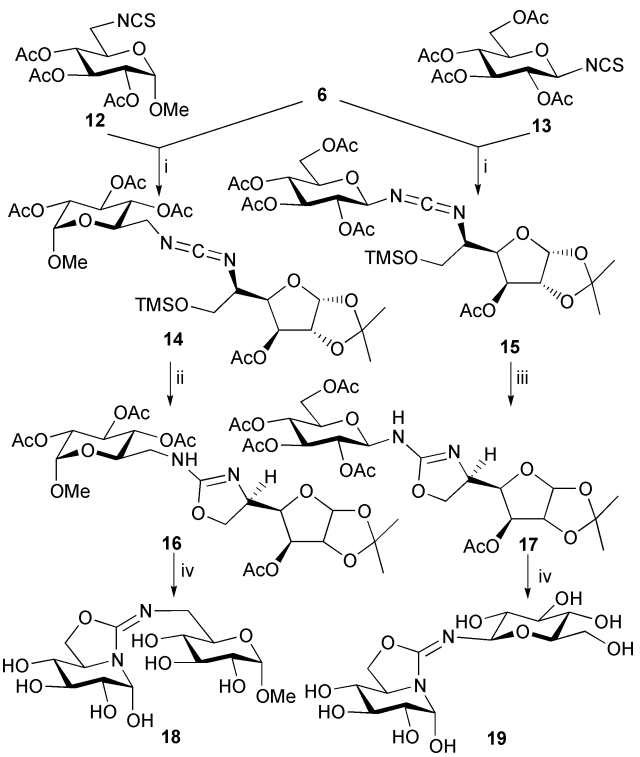
reminiscent of those of **4** and **5**. The tandem Staudinger–aza-Wittig reaction of **6** with the peracetylated methyl 6-deoxy-6-isothiocyanato-α-D-glucopyranoside[11] **12** afforded the (6→5)-carbodiimide linked pseudodisaccharide **14** which, after treatment with TBAF, gave the corresponding aminooxazoline **16** (68% overall). A much faster and cleaner reaction was observed when isothiocyanate **13**,[11] bearing an electron withdrawing glucopyranosyl substituent, was used. Generation of the cyclic isourea segment from the carbodiimide adduct **15** using TBAF was now accompanied by partial anomerisation of the resulting glucopyranosylamine derivative **17**, which was avoided by performing this step in the presence of acetic acid. Deacetylation of **16** and **17** and hydrolysis of the isopropylidene group under standard conditions followed by neutralisation of the reaction mixtures gave the corresponding 2-oxaindolizidines **18** and **19**, respectively, in high yield (Scheme 2).¶

The castanospermine analogues **11**, **18** and **19** existed in D_2O solution as single diastereomers of high stability, which is notably different from that known for sp^3 reducing azasugars. The high field shift of the C-1 resonance confirmed the aminoacetalic bicyclic structure, whereas the vicinal $^3J_{H,H}$ values around the piperidine ring unambiguously pointed to the *R* configuration for the new stereocentre, with the pseudoanomeric hydroxy group in axial position, fitting the anomeric effect. This result is in agreement with the existence of a very strong and stabilising interaction between the π-type lone-pair of the endocyclic nitrogen atom of the isothiourea grouping and the σ* antibonding orbital of the contiguous C–O bond. This orbitalic interaction is probably responsible for both stability and conformational integrity.[6] To the best of our knowledge, these are the first examples of ring-modified castanospermine analogues bearing exocyclic substituents.

The presence of a pseudoanomeric hydroxy group anchored in the axial position was expected to be translated into an increased selectivity towards α-glucosidases. Actually, the *N*-β-D-glucopyranosyl derivative **19** showed inhibition constants (K_i) against yeast α-glucosidase (15 μM) and almond β-glucosidase (387 μM) indicative of a reverse selectivity as compared to castanospermine (>1500 and 1.5 μM, respectively).[12] Yet, a dramatic influence of the nature of the exocyclic substituent was observed. Thus, the C-6 linked isomer **18** was a weak inhibitor for both enzymes (K_i 463 and 336 μM, respectively), whereas the *N*-butyl analogue **11** inhibited β-glucosidase (K_i 30 μM) three-fold more potently than α-glucosidase (K_i 86 μM). These results underline the importance of secondary interactions in glycosidase binding, even overpowering other effects related to the glyconic binding site. It is likely that the preparation and screening of libraries of castanospermine–trehazolin hybrids, by modifying both the configuration of the starting monosaccharide template and the nature of the substituent, will allow identification of still more selective glycosidase inhibitors. Work in this direction is currently under way in our laboratories.



Scheme 2 *Reagents and conditions*: i, PPh_3, toluene, 80 °C; ii, TBAF, THF, 0 °C, 25 min (68% overall); iii, TBAF, THF, AcOH 0 °C, 25 min (75% overall); iv, NaOMe, MeOH, then TFA–water 9:1; co-evaporation with water, then IR 45 (OH^-) ion exchange resin (86–95% overall).

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Notes and references

‡ A pK_a value in the range 9.74–7.59 has been reported (*c.f.*, ref. 7).

§ Obtained from the known 5-azido-5-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (*c.f.*, ref. 9) by selective silylation of the primary OH group with TMSCl–hexamethyldisilazane and subsequent acetylation.

¶ All new compounds gave satisfactory microanalytical, NMR (^1H and ^{13}C) and MS data in accord with the proposed structures. *Selected data* for **11**: $[\alpha]_D$ −2.0 (*c* 1, H_2O); δ_H (300 MHz, D_2O, *J*/Hz) 5.53 (1 H, d, $J_{1,2}$ 3.9, H-1); δ_C (75 MHz, D_2O) 158.7 (C=N), 74.9 (C-1). For **18**: $[\alpha]_D$ +99.0 (*c* 1, H_2O); δ_H (500 MHz, D_2O, *J*/Hz) 5.35 (1 H, d, $J_{1,2}$ 3.9, H-1), 4.78 (1 H, d, $J_{1,2}$ 3.8, H-1'); δ_C (125.7 MHz, D_2O) 161.4 (C=N), 100.5 (C-1'), 74.3 (C-1). For **19**: $[\alpha]_D$ +5.9 (*c* 1, H_2O); δ_H (500 MHz, D_2O, *J*/Hz) 5.34 (1 H, d, $J_{1,2}$ 4.0, H-1), 4.65 (1 H, d, $J_{1',2'}$ 8.7, H-1′); δ_C (125.7 MHz, D_2O) 156.9 (C=N), 86.6 (C-1′), 74.9 (C-1). The notation of atoms for NMR data has been kept consistent with that of D-glucose.

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