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Synthesis and evaluation of sulfamide-type indolizidines as glycosidase inhibitors

Mahmoud Benltifa,[a] M. Isabel García Moreno,[b] Carmen Ortiz Mellet,[b,*] José M. García Fernández[c] and Anne Wadouachi[a,*]

[a]*Laboratoire des Glucides UMR CNRS 6219, Université de Picardie Jules Verne, 33 Rue Saint-Leu, F-80039 Amiens, France*
[b]*Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, E-41071 Sevilla, Spain*
[c]*Instituto de Investigaciones Químicas, CSIC, Americo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain*

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Abstract—A practical synthesis of reducing sulfamide-derived iminosugar glycomimetics related to the indolizidine glycosidase inhibitor family is reported. The polyhydroxylated bicyclic system was built from readily accessible hexofuranose derivatives through a synthetic scheme that involves 5,6-cyclic sulfamides. Further intramolecular nucleophilic addition of the sulfamide nitrogen atom to the masked aldehyde group of the monosaccharide in the open chain form afforded the target sugar mimics. By starting from D-glucose and D-mannose precursors, 2-aza-3,3-dioxo-3-thiaindolizidine derivatives with hydroxylation profiles that matched those of (+)-castanospermine and 6-*epi*-(+)-castanospermine were obtained. In vitro screening against a panel of glycosidases evidenced a high selectivity towards α-mannosidase.
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Glycosidases play a very important role in many biological processes, such as intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates. Consequently, compounds that can modify or inhibit these processes bear strong potential in therapies targeted at, for instance, cancer, viral infections, diabetes and glycosphingolipid storage disorders, being also of interest for mechanistic studies and purification of enzymes.[1]

Nitrogen-in-the-ring carbohydrate mimics (iminosugars and azasugars) have attracted increasing interest for such purposes. Their glycosidase inhibitory activity has been related to their capability to become protonated at physiological pH, thus mimicking the putative glycosyloxacarbenium cation postulated as an intermediate of glycosidase-catalyzed hydrolysis.[2]

However, while glycosidases exhibit an exquisite selectivity towards their respective substrates, iminosugars frequently behave as broad-range glycosidase inhibitors. Bicyclic derivatives generally show a higher selectivity towards isoenzymes as compared with monocyclic analogues. For example (+)-castanospermine (**1**), an indolizidine alkaloid with a hydroxylation profile of structural complementarity with D-glucose, is a potent and specific inhibitor of α- and β-glucosidases from different organisms and sub-cellular locations, for example, lysosomal or digestive.[3] Nonetheless, the lack of anomeric selectivity, probably related to the absence of a defined configuration at the pseudoanomeric carbon (C-5), remains particularly problematic in view of clinical applications.

We have previously reported the synthesis of a new family of glycomimetics in which the sp^3 amine-type nitrogen characteristic of azasugars has been replaced by a neutral or very weakly basic pseudoamide-type nitrogen, with substantial sp^2 character (sp^2-azasugars).[4] This structural change dramatically increases the anomeric effect at aminoacetal centres, thereby stabilizing axially oriented oxygen substituents. Interestingly, reducing sp^2-azasugar-type castanospermine analogues displayed a total anomeric selectivity towards α-glucosidase, the inhibition potency being strongly dependent on the nature of the cyclic pseudoamide group. Thus, the reducing

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* Corresponding authors. Tel.: +33 0322827527; fax: +33 0322827560; e-mail addresses: mellet@us.es; anne.wadouachi@u-picardie.fr

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urea and thiourea derivatives **2** and **3** behaved as very weak inhibitors of yeast α-glucosidase (K_i = 5.7 and 4.2 mM), while the corresponding carbamate and thiocarbamates **4** and **5** were the potent inhibitors of this enzyme (K_i = 40 and 2.2 μM, respectively).[5] Moreover, modifications at the exocyclic heteroatom in the five-membered ring (e.g., **6**), led to a sharp shift in enzyme specificity (K_i = 2.2 μM for β-glucosidase from bovine liver), pointing to the existence of critical interactions in the enzyme–inhibitor complex involving this region of the molecule[6] (Fig. 1). The biological activity was also very sensitive to the relative orientation of the hydroxyl groups, as already observed in the natural polyhydroxyindolizidine alkaloid series.[7]

In order to further explore the structure–activity relationships (SAR) in this family of compounds, the replacement of the (thio)urea segment in **2** and **3** into a sulfamide functional group seemed particularly intriguing. Sulfamides have been used for the design of pharmacological agents with many applications.[8] The sp^2 character of the nitrogen atoms in sulfamides must be significantly reduced as compared with (thio)ureas as a consequence of the higher difference in energy between the implied N and S orbitals. As a consequence, the geometry of the N atom in sulfamides can vary from trigonal planar to pyramidal.[9] On the other hand, the two oxygen atoms at the tetrahedral S atom are well suited to establish dipole–dipole or hydrogen bond interactions with protein residues located nearby. Actually, a comparative structural analysis of the interaction between cyclic urea and cyclic sulfamides with HIV-1 protease revealed notable structural differences.[10] Here we report the synthesis of the (+)-castanospermine analogue **7** and its 6-epimer **8**, the first examples of cyclic sulfamide-type glycomimetics (Fig. 2). Their structure and biological activity against a panel of glycosidases are discussed.

The general synthetic strategy implemented to build the 2-aza-3,3-dioxo-3-thiaindolizidine skeleton relies on the ability of the carbonyl group of a monosaccharide in its open-chain aldehydo form (Fig. 3B) to act as an electrophile against a pseudoamide nitrogen atom located at 1,5-relative position. The hydroxylation profile in the final compound matches that of the monosaccharide template. Consequently, the preparation of the target derivatives **7** and **8** required D-gluco and D-manno precursors, respectively, selectively functionalized at C-5 (Fig. 3A).

Compound **7** was prepared in five steps from 5-azido-5-deoxy-1,2-di-*O*-isopropylidene-6-*O*-tetrahydropyranyl-

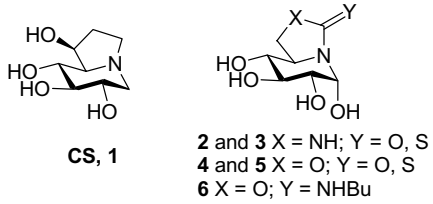


Figure 1. Structure of (+)-castanospermine and some pseudoamide-type analogues.

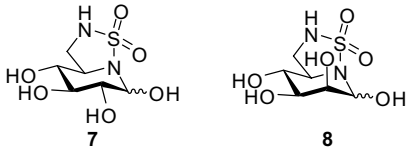


Figure 2. Structure of the sulfamide-type compounds prepared in this work.

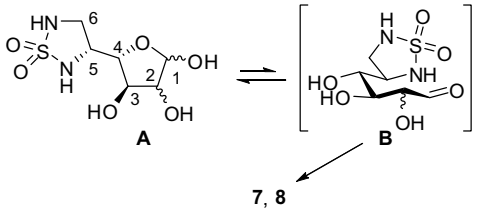
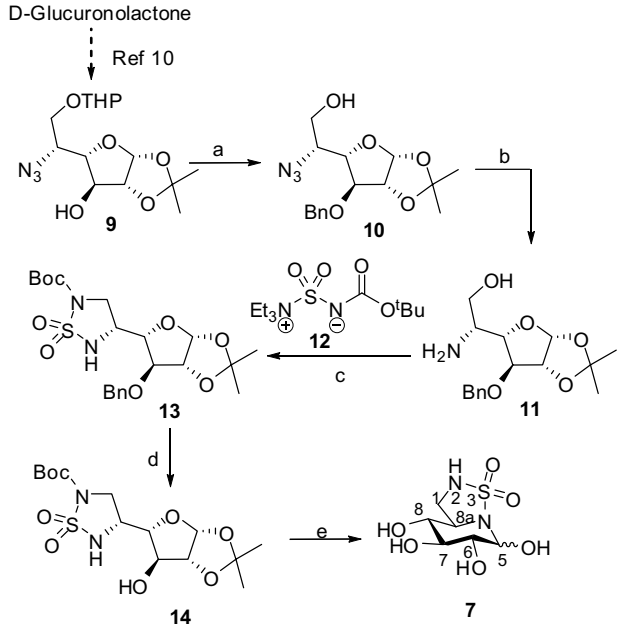


Figure 3. General retrosynthetic scheme for the preparation of sulfamide-type castanospermine analogues.

α-D-glucofuranose **9**, readily accessible from commercial D-glucuronolactone.[11] The reaction sequence involved benzylation of the secondary hydroxyl group followed by removal of the tetrahydropyranyl protecting group by acid hydrolysis to give the azido-alcohol **10** (Scheme 1). Reduction of the azido group under Staudinger conditions afforded the corresponding 5-amino-5-deoxy derivative **11**, which was transformed into the Boc-protected cyclic sulfamide **13** by treatment with the Burgess-type reagent *tert*-butyl *N*-(triethylammonium sulfonyl)carbamate **12**.[12] Debenzylation of **13** by catalytic hydrogenation (→**14**) followed by simultaneous hydrolysis of the Boc and isopropylidene groups with aqueous



Scheme 1. Reagents and conditions: (a) 1—BnBr, 60% NaH, DMF, 1 h, rt; 2—TsOH, 1:1 CH_2Cl_2–MeOH, 2 h, rt, 88% overall yield from **9**; (b) 1—PPh_3, 5:1 dioxane–MeOH; 2—30% NH_4OH aq 96%; (c) THF, 74 °C, 2 h, 30%; (d) H_2, Pd/C, 1:4 EtOAc–EtOH, 1 atm, 18 h; (e) 90% TFA–H_2O, rt, 30 min, 60% overall yield from **13**.

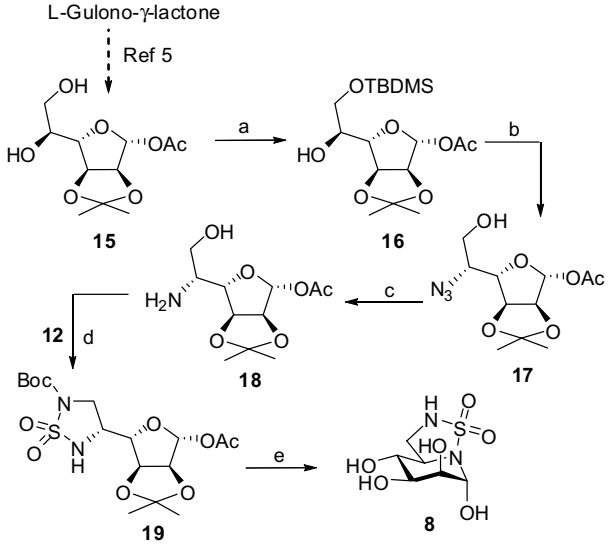
trifluoroacetic acid (TFA) proceeded with concomitant furanose → piperidine rearrangement[13] to afford the target bicyclic glucomimetic **7**[14] (Scheme 1).

To synthesize the 6-*epi*-(+)-castanospermine analogue **8**, commercial L-gulono-γ-lactone was first converted into the selectively protected furanose derivative **15** through a four-step reaction sequence as reported[6] (Scheme 2). The primary hydroxyl group of **15** was subsequently protected by formation of the corresponding *tert*-butyldimethylsilyl ether **16**. Sequential trifluoromethanesulfonylation of the remaining alcohol group, reaction with sodium azide and desilylation with tetra-*n*-butylammonium fluoride afforded the 5-azido-5-deoxy-D-mannofuranose derivative **17**, which was reduced to the key aminoalcohol precursor **18**. Sulfamidation by reaction with the Burgess-type reagent **12** provided the cyclic sulfamide **19**, which after removal of the acetyl and isopropylidene-protecting groups afforded the indolizidine mannomimetic **8**.[15]

The structure of compounds **7** and **8** was confirmed by their MS and their ^{1}H and ^{13}C NMR spectra in D_2O.[14,15] Although the α-anomer predominated, both the α- and β-anomers were now present in the solutions. This is radically different from that observed for the previously reported structures **2**–**6**, which existed exclusively in the α-configuration, being in agreement with the lower sp^2-character of the sulfamide nitrogen. The resonances of the pseudoanomeric carbons C5α and C5β (δ 83 to 74 ppm) were significantly upfield shifted from the expected values for reducing sugars, confirming the aminoacetal bicyclic structure.

The new sulfamide-type indolizidine glycomimetics were assayed against a panel of glycosidases including α-glucosidase (baker yeast), β-glucosidase (almonds), α-galactosidase (green coffee beans), β-galactosidase (bovine liver), α-mannosidase (Jack beans), β-mannosidase (snail), isomaltase (yeast), amyloglucosidase (*Aspergillus niger*), trehalase (pig kidney) and naringinase (*Penicillium decumbes*). Deceivingly, compound **7** did not bind to any of the glucoside-processing enzymes tested. It behaved instead as a modest but totally selective competitive inhibitor of α-mannosidase (K_i = 320 μM). This result is surprising considering that none of the pseudoamide-type (+)-castanospermine analogues **2**–**6** showed activity towards mannosyl hydrolases. The five-membered cyclic sulfamide group seems to be particularly well adapted for interacting with amino acids at the active site of this particular enzyme. The corresponding 6-*epi* diastereomer **8**, with a hydroxylation profile of structural complementarity with that of D-mannose, was twice as potent as an inhibitor of α-mannosidase (K_i = 150 μM) while keeping the total selectivity. This further stresses the important role of the sulfamide functionality in the biological activity, since neither the natural compound 6-*epi*-(+)-castanospermine nor the epimers at C-6 of compounds **2**–**6** are inhibitors of this enzyme.[5a,7]

In summary, we have developed an efficient synthetic route for the preparation of sulfamide-type indolizidine glycomimetics from commercial carbohydrate precursors. While the sulfamide group is able to provide stability to aminoacetal centres, the reduced sp^2 character of the bridgehead nitrogen in the bicyclic structure allows the presence of both the α and β-anomers in aqueous solution. Interestingly, the biological results indicate that the cyclic sulfamide moiety is well suited to interact with α-mannosidase, providing total selectivity towards this enzyme. Further research aiming at revealing the structural requirements governing such interactions are currently sought in our laboratories.



Scheme 2. Reagents and conditions: (a) TBDMSCl, pyridine, 3 h, rt, 98%; (b) 1—Tf_2O, CH_2Cl_2, −10 °C, 30 min; 2—NaN_3, DMF, 75% overall yield from **16**; 3—TBAF (1M/THF), THF, 2 h 30 min, rt, 85%; (c) H_2, Pd/C, MeOH, 2 h; (d) THF, 74 °C, 2 h, 30%; (e) 1—NaOMe, 1:1 $CHCl_3$–MeOH, 3 h, 96%; 2—90% TFA–H_2O, 0 °C, 30 min, 62%.

Acknowledgments

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13. *General procedure for the furanose → indolizidine rearrangement of the cyclic sulfamide:* The cyclic sulfamide was treated with TFA–H_2O (9:1) at 0 °C for 30 min, concentrated under reduced pressure, coevaporated several times with water, neutralized with Amberlite IRA-68 (HO^-) ion-exchange resin and subjected to column chromatography using (10:1:1) → (5:1:1) CH_3CN–H_2O–NH_4OH as eluent to give the sulfamide-type indolizidine.
14. *Compound* **7**: α/β ratio 1:0.47; yield 40 mg (60%); R_f 0.43 (6:3:1 CH_3CN–H_2O–NH_4OH). ^1H NMR (500 MHz, D_2O) δ 5.20 (d, 1H, $J_{5,6}$ = 3.7 Hz, H-5α), 4.37 (d, 1H, $J_{5,6}$ = 8.0 Hz, H-5β), 3.66 (m, 1H, H-8aα), 3.61 (dd, 1H, $J_{1a,1b}$ = 11.1 Hz, $J_{8a,1a}$ = 6.4 Hz, H-1aα), 3.60 (m, 1H, H-1aβ), 3.56 (m, 1H, H-7β), 3.55 (t, 1H, $J_{6,7} = J_{7,8}$ = 9.1 Hz, H-7α), 3.48 (dd, 1H, H-6α), 3.34 (t, 1H, $J_{8,8a}$ = 9.1 Hz, H-8α), 3.33 (m, 2H, H-6β, H-8β), 3.26 (dd, 1H, $J_{8a,1b}$ = 7.8 Hz, H-1bα), 3.24 (m, 2H, H-8aβ, H-1bβ); ^{13}C NMR (125.7 MHz, D_2O) δ 82.3 (C-5β), 75.4 (C-6β), 75.0 (C-8β), 74.8 (C-5α), 73.2 (C-8α), 72.5 (C-7α), 72.0 (C-6α), 58.2 (C-8aβ), 55.3 (C-8aα), 43.8 (C-1α), 43.7 (C-1β); HRMS (FAB): Calcd for $C_6H_{12}N_2O_6NaS$: 263.031378, found *m/z* 263.029947.
15. *Compound* **8**: α/β ratio 1:0.15; yield 8.5 mg (62%); R_f 0.36 (7:3 CH_2Cl_2–MeOH). ^1H NMR (300 MHz, D_2O), α anomer: δ 5.22 (d, 1 H, $J_{5,6}$ = 2.9 Hz, H-5), 3.80 (dd, 1H, $J_{6,7}$ = 3.2 Hz, $J_{7,8}$ = 9.4 Hz, H-7), 3.74 (m, 1H, H-8[a]), 3.71 (m, 2H, H-8, H-1a), 3.66 (t, $J_{8,8a}$ = 9.4 Hz, H-8), 3.34 (m, 1H, H-1b); ^{13}C NMR (75 MHz, D_2O), α anomer: δ 76.7 (C-5), 71.1 (C-6), 70.7 (C-8), 70.2 (C-7), 55.9 (C-8a), 44.3 (C-1); β anomer: δ 80.78 (C-5), 73.5, 73.3, 70.2 (C-6, C-7, C-8), 57.2 (C-8a), 44.1 (C-1); HRMS: Calcd for $C_6H_{12}N_2O_6NaS$: 263.0314, found *m/z* 263.0323.