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Preliminary Communication

Synthesis and glycosidase inhibitory activity of 1-amino-3,6-anhydro-1-deoxy-D-sorbitol derivatives

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ABSTRACT

3,6-Anhydro-1-(aryl or alkylamino)-1-deoxy-D-sorbitol derivatives have been prepared in four steps from isosorbide, a by-product from the starch industry. The inhibitory activities of these new compounds have been evaluated towards 13 glycosidases. A first lead-compound was identified, which inhibited β-*N*-acetylglucosaminidase from bovine kidney (82% inhibition at 1 mM).

1. Introduction

Glycosidase enzymes represent an important class of biocatalysts involved in a wide range of anabolic and catabolic reactions [1]. In turn, specific inhibitors of glycosidases have shown high promises as probes for function studies of enzymatic events and as drugs for the treatment of viral or fungal infections [2], cancer and metabolic disorders such as diabetes [3]. The most potent glycosidase inhibitors known so far are amino-analogues of the natural carbohydrate substrates, which feature either an *endo* (in the ring) or an *exo*-nitrogen (out of the ring) functionality [4]. Iminosugars, the *endo*-nitrogen analogues, have been the most widely studied glycosidase inhibitors and a library of hundreds of compounds has been synthetized over the years, which has enabled the identification of very potent and specific inhibitors through SAR studies [5]. Deoxynojirimycin (DNJ **1**, Fig. 1), the imino-glucose derivative, is a very potent α-glucosidase inhibitor, some optimized analogues of which have found therapeutic applications to treat type II diabete or Gaucher's disease [6]. Much less is known about the biological potencies of carbohydrate analogues featuring an *exo* amino functionality, though some promising results have been obtained with preliminary models. Thus, amino-substituted α- and β-benzyl-*C*-glucosides **2** (K_i = 11 μM) and **3** (K_i = 70 μM) display potent inhibition against α- and β-glucosidase respectively, the former being as potent as the standard DNJ **1** (K_i = 9 μM) [7]. In like manner, valiolamine **4** is a potent "Nature-made" (*Streptomyces hygroscopicus* var. *limoneus*) inhibitor of sucrase (IC_{50} = 49 nM) [8] and oseltamivir **5** is active against neuraminidase (IC_{50} in the nanomolar range) and was approved for the treatment of influenza [9]. The inhibitory action of carbohydrate analogues, which are similar to either intermediates (iminosugars) or substrates (*exo*-amino compounds), is attributed particularly to the potent binding interactions of the nitrogen atom with the carboxylate residues present in the active site that are involved in the catalytic hydrolysis (Fig. 1).

In our search for new series of potent glycosidase inhibitors we focused on amino-substituted *C*-furanosides **6**, which belong to the *exo*-amino sugar analogues family (Fig. 2). Compounds **6** might also be regarded as oxa-analogues of iminosugars **7a–d** which are potent inhibitors of β-glucosidase (K_i = 13–40 μM) [10] and of iminosugar **7e**, a weak inhibitor of α-mannosidase from jack-bean (IC_{50} = 3–25 μg/mL) [10c]. The supplementary hydroxyl group in the structure of **6** could virtually induce binding interactions in the active site, which could counterbalance the loss of the intracyclic amino functionnality from **7**. Up to now, the potential of such compounds as glycosidase inhibitors has not been assessed. We describe herein the synthesis of *C*-furanosides **6a–e** and their biological evaluation towards 13 different glycosidases.

2. Results and discussion

2.1. Synthesis

Isosorbide is a low-cost biomass derived material that is industrially produced from sorbitol. New interest in the use of isosorbide

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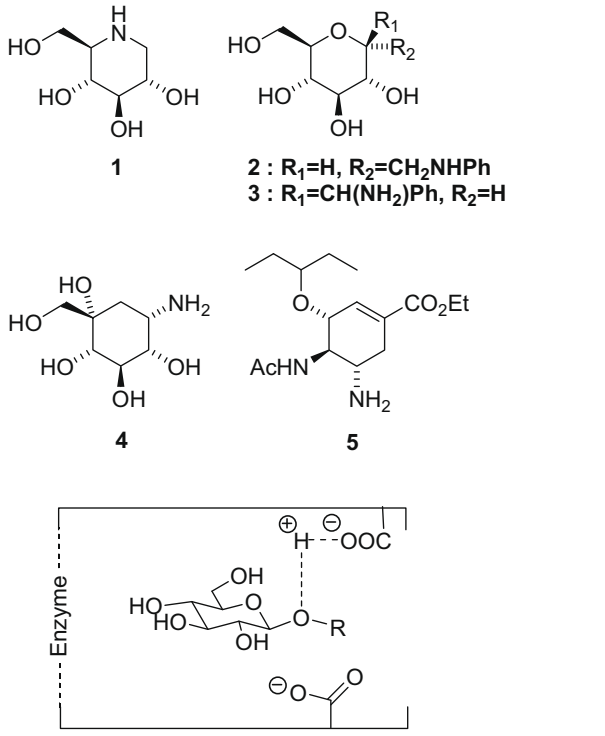


Fig. 1. Structures of potent glycosidase inhibitors and catalytic hydrolysis of glycosides.

as a chemical building block has been driven by recent discoveries of the properties of some derivatives as polymer additives, surfactants or pharmaceuticals [11]. Isosorbide dinitrate (Isordil®) is used pharmacologically as a vasodilator in angina pectoris or in the treatment of congestive heart failure [12]. In this study, compounds **6a–e** could be readily prepared from isosorbide (Scheme 1) [13].

In a first step, isosorbide was reacted with *in situ* generated iodotrimethylsilane (NaI/TMSCl) to afford the pure iodohydrin **8** [14]. The treatment of **8** with sodium hydride gave epoxide **9** in 80% yield over two steps. In the next step, epoxide **9** was treated with a series of aliphatic or aromatic amines [15]. When the reaction was conducted in methanol at 40 °C with a twofold excess of amine, complete disappearance of the starting epoxide occurred. Whatever the amine used, the ring-opening was highly regioselective and only one product could be isolated from the reaction mixture deriving from the attack on the less substituted carbon of the epoxide. The structures of the so-formed β-aminoalcohols **10a–e** could be firmly deduced from their analytical data. For each compound, a signal close to 48–50 ppm was observed in ^{13}C NMR, which was characteristic of a primary CH_2–N carbon.

The *C*-furanosides **10a–e** were purified by column chromatography over silica gel and isolated in 66–98% yield (Scheme 1). Deprotection of the isopropylidene group was achieved by treatment of **10a–e** with a 1 m solution of HCl. The so-obtained hydrochlorides were purified by ion exchange chromatography to yield fully deprotected aminoalcohols **6a–e**.

2.2. Glycosidase inhibition assay

The inhibitory potencies of compounds **6a–e** were assayed towards a series of 13 commercial glycosidases (α-L-fucosidase from bovine kidney, α-galactosidase from coffee beans, β-galactosidase from *Escherichia coli* and from *Aspergillus orizae*, α-glucosidase from yeast and from rice, β-glucosidase from almonds, amyloglucosidase from *Aspergillus niger*, α-mannosidase from Jack beans, β-mannosidase from snail, β-xylosidase from *Aspergillus niger*, β-*N*-acetylglucosaminidase from Jack beans and from bovine kidney). The experiments were performed essentially as previously described [16]. Briefly, 0.01–0.5 unit/mL of enzyme (1 unit = 1 μmol of glycoside hydrolyzed/min), preincubated for 5 min at 20 °C with an aqueous solution of the inhibitor (final concentration 1 mM), and increasing concentrations of aqueous solution of the appropriate *p*-nitrophenyl glycoside substrate buffered to the optimum pH of the enzyme were incubated for 20 min at 37 °C (45 °C for the amyloglucosidases). The reaction was stopped by the addition of a 2.5 volume of 0.3 M sodium borate buffer pH 9.8. In a control experiment, the enzyme activity was assayed in the absence of inhibitor. The *p*-nitrophenolate formed was quantified at 405 nm, and the percentage inhibition in enzyme activity was calculated as follows:

$$\%\,\text{Inhibition} = \frac{\Delta\text{Absorbance (control)} - \Delta\text{Absorbance (test)}}{\Delta\text{Absorbance (control)}} \times 100$$

The results are reported in Table 1. Unlike polyhydroxypyrrolidines, the interaction of *C*-furanoside-like carbohydrate mimics with glycosidases has scarcely been studied. To our knowledge, no inhibition data are available in the literature and the results in Table 1 give a first insight into their potential. Firstly, we found that the inhibition profile of aminoalcohols **6a–e** was significantly different from that of the corresponding pyrrolidines **7**.The *N*-isopropyl derivative **6c** was completely inactive against all the tested enzymes. However, the presence of an aromatic substituent induced some effect in term of strength or selectivity. Thus, compounds **6a,b,d** were active against β-galactosidase from Escherichia coli (42–64% inhibition). The morpholino derivative **6e** was active (39%) against α-L-fucosidase in a selective manner.

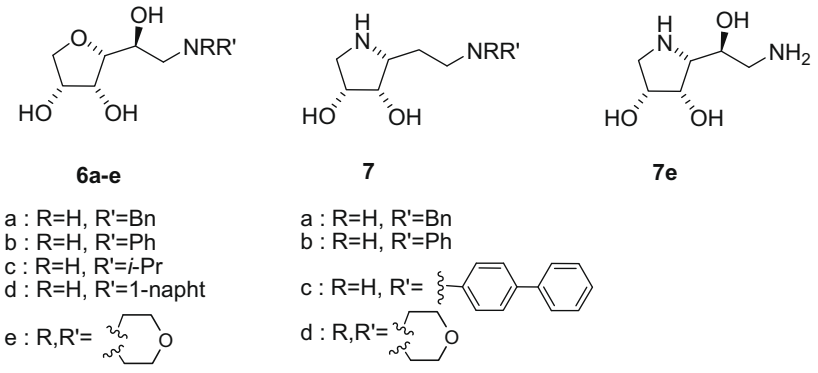
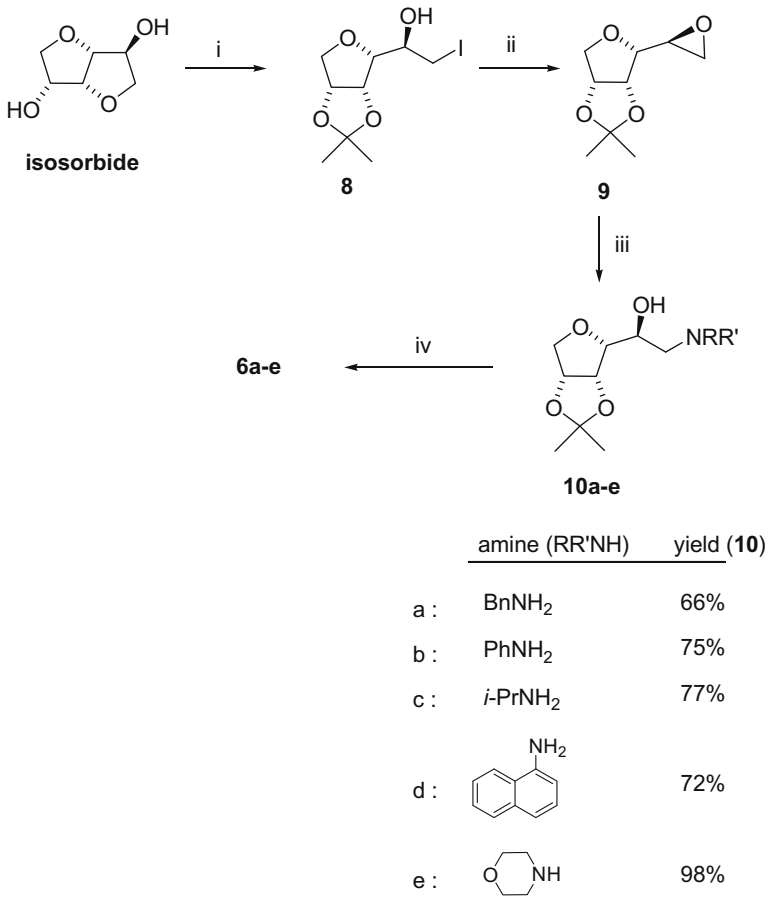


Fig. 2. Structures of compounds **6** and **7**.



Scheme 1. Synthesis of compounds **6a–e**. *Reagents and conditions*: (i) TMSCl, NaI, acetone, CH_3CN; (ii) NaH, THF (80%, 2 steps); (iii) RR'NH, MeOH, 40 °C (yield given on Scheme); and (d) HCl 1 M, rt (43–92%).

Surprisingly, in contrast to their analogues **7**, none of the tested compounds had any effect towards the glucose-processing enzymes (amyloglucosidase, α- or β-glucosidase). It is interesting to note that the "naked" diamine **11** was also completely inactive against β-glucosidase, whereas phenyl substituted pyrrolidine **7b** induced 94% inhibition at 1 mM (Fig. 3) [10]. Thus, both the aglycon part (the aromatic substituent) and the pyrrolidine framework are required to induce potent inhibition. Release of one of these two elements led to a dramatical loss in affinity. Finally the naphthyl derivative **6d** showed promising results towards *N*-acetylglucosaminidase from bovine kidney (82%). Compound **6a** (R = Bn) displayed also some activity (59%) against the same enzyme. Interestingly, the imino analogues **7a–d** had no effect on *N*-acetylglucosaminidase from bovine epididymis A and bovine epididymis B [10].

Table 1
Inhibition of glycosidases by compounds **6** (% inhibition at 1 mM concentration).

	6a	6b	6c	6d	6e
α-L-fucosidase (bovine kidney)	–[a]	–	–	–	39
α-Galactosidase (coffe beans)	–	–	–	–	–
β-Galactosidase (escherichia coli)	42	56	–	64	–
β-Galactosidase (aspergillus oryzae)	–	–	–	–	–
α-Glucosidase, (yeast)	–	–	–	–	–
α-Glucosidase, (rice)	–	–	–	–	–
Amyloglucosidase (*Aspergillus niger*)	–	–	–	–	–
β-Glucosidase (almonds)	–	–	–	–	–
α-Mannosidase (Jack beans)	–	–	–	–	–
β-Mannosidase (snail)	19	–	–	–	–
β-Xylosidase (*Aspergillus niger*)	–	–	–	–	–
β-*N*-acetylglucosaminidase (Jack beans)	17	–	–	–	–
β-*N*-acetylglucosaminidase (bovine kidney)	59	–	–	82	–

[a] No inhibition at 1 mM.

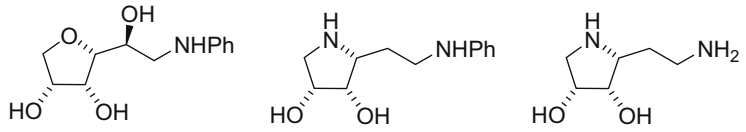
3. Conclusion

In conclusion, we have developed a short synthesis of a series of amino-*C*-furanosid derivatives as potential glycosidase inhibitors. The compounds **6a–e** have been prepared in four steps starting from isosorbide, a by-product from the starch industry. Generally, these aminosugar analogues presented low inhibitory properties towards glycosidases. However, the naphthyl derivative **6d** displayed a significant activity towards β-*N*-acetylglucosaminidase, a target enzyme for the development of antibacterial agents.

4. Experimental

4.1. General

Reactions were monitored by TLC (Macherey–Nagel Polygram®sil G/UV_{254}), detection being carried out by spraying with an alcoholic solution of $KMnO_4$ (2%)/Na_2CO_3 (4%) followed by heating. Column chromatography was performed on silica gel (Merck, 230–400 mesh). NMR spectra were recorded on a Bruker AC-250 spectrometer (250 MHz for ^1H and 62.5 MHz for ^{13}C) or Bruker Avance 400 spectrometer (400 MHz for ^1H). Chemical shifts are expressed as parts per million downfield from the internal standards tetramethylsilane for D_2O or CD_3OD solutions. Multiplicities are indicated by



6b (0%) **7b** (94%) **11** (0%)

Fig. 3. Inhibition potencies of **6b**, **7b** and **11** towards β-glucosidase.

s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), sept (septuplet) or br s (broadened singlet). Optical rotations were recorded on a Perkin–Elmer 343 polarimeter at 20 °C. High resolution mass spectra were performed on Q-TOF Micro micromass positive ESI (CV = 30 V).

4.2. Preparation of 3,6-anhydro-D-sorbitol derivatives

4.2.1. General procedure

A solution of epoxide **8** (1 equiv) and amine (2 equiv) was heated at 40 °C in methanol (c = 0.2 M) for 16 h. The solvent was then removed and the crude product was purified by column chromatography (eluent: CH_2Cl_2/MeOH (95/5)). An aqueous solution of hydrogen chloride (1 m concentration) was added to the residue and the solution was stirred overnight. Evaporation of the solvents gave a crude material which was subjected to ion exchange chromatography (Dowex 50WX-8 resin). Elution with 0.8 M NH_4OH gave pure **6** as a yellowish oil.

4.2.2. 3,6-Anhydro-1-(benzylamino)-1-deoxy-D-sorbitol 6a

Yield: 81%. $[\alpha]_D^{20} = -11.8$ (c 0.52, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.35–7.10 (m, 5H), 4.40 (dt, 1H, J = 6.7, 4.8), 4.19 (dd, 1H, J = 4.6, 4.5), 4.02 (ddd, 1H, J = 8.5, 6.6, 3.7), 3.94 (dd, 1H, J = 9.0, 6.7), 3.88–3.76 (m, 3H), 3.68 (dd, 1H, J = 9.0, 6.6), 2.80 (dd, 1H, J = 12.7, 3.7 Hz), 2.55 (dd, 1H, J = 12.7, 8.5); ^{13}C NMR (62.5 MHz, D_2O) δ 139.1, 129.1, 129.0, 127.9, 82.5, 71.7, 71.3, 70.5, 69.2, 52.6, 50.3; HRMS (ESI) Calcd for $C_{13}H_{20}NO_4$ ([M+H]$^+$), 254.1392. Found: 254.1392.

4.2.3. 3,6-Anhydro-1-(phenylamino)-1-deoxy-D-sorbitol 6b

Yield: 43%. $[\alpha]_D^{20} = -15.9$ (c 0.71, CH_3OH); ^{1}H NMR (400 MHz, D_2O) δ 7.30–7.24 (m, 2H), 6.88–6.81 (m, 3H), 4.37 (dt, 1H, J = 6.6, 4.8), 4.28 (dd, 1H, J = 4.6, 4.5), 4.05 (ddd, 1H, J = 8.6, 6.5, 3.6), 3.95 (dd, 1H, J = 9.0, 6.7), 3.87 (dd, 1H, J = 6.4, 4.3), 3.69 (dd, 1H, J = 9.0, 6.7), 3.40 (dd, 1H, J = 13.6, 3.6 Hz), 3.10 (dd, 1H, J = 13.6, 8.6); ^{13}C NMR (62.5 MHz, D_2O) δ 147.9, 130.0, 119.5, 115.1, 81.9, 71.7, 71.5, 70.5, 68.7, 50.1, 46.9; HRMS (ESI) Calcd for $C_{12}H_{18}NO_4$ ([M+H]$^+$), 240.1236. Found: 240.1240.

4.2.4. 3,6-Anhydro-1-(isopropylamino)-1-deoxy-D-sorbitol 6c

Yield: 92%. $[\alpha]_D^{20} = -9.7$ (c 0.7, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.39 (dt, 1H, J = 6.7, 4.8), 4.24 (dd, 1H, J = 4.5), 3.97–3.92 (m, 2H), 3.81 (dd, 1H, J = 6.4, 4.3), 3.68 (dd, 1H, J = 9.0, 6.6), 2.88 (sept, 1H, J = 6.3), 2.81 (dd, 1H, J = 12.7, 3.6 Hz), 2.63 (dd, 1H, J = 12.6, 8.7); ^{13}C NMR (75 MHz, D_2O) δ 82.6, 71.7, 71.4, 70.5, 69.3, 48.5, 48.4, 21.5, 21.3; HRMS (ESI) Calcd for $C_9H_{20}NO_4$ ([M+H]$^+$), 206.1392. Found: 206.1387.

4.2.5. 3,6-Anhydro-1-(naphthylamino)-1-deoxy-D-sorbitol 6d

Yield: 76%. $[\alpha]_D^{20} = -5.1$ (c 0.59, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.0–7.92 (m, 1H), 7.76–7.70 (m, 1H), 7.42–7.63 (m, 2H), 7.30–7.26 (m, 1H), 7.16 (d, 1H, J = 8.2), 6.69 (d, 1H, J = 7.6), 4.30–4.22 (m, 3H), 4.0–3.88 (m, 2H), 3.82–3.76 (m, 1H), 3.55 (dd, 1H, J = 12.7, 4.9), 3.33 (dd, 1H, J = 12.7, 7.1); ^{13}C NMR (75 MHz, D_2O) δ 145.9, 137.0, 131.4, 129.3, 128.6, 127.6, 126.5, 122.8, 120.7, 107,5, 82.9, 75.7, 75.2, 74.2, 70.3, 50.3; HRMS (ESI) Calcd for $C_{16}H_{20}NO_4$ ([M+H]$^+$), 290.1392. Found: 290.1386.

4.2.6. 3,6-Anhydro-1-(morpholino)-1-deoxy-D-sorbitol 6e

Yield: 88%. $[\alpha]_D$ = +1.4 (c 0.74, CH_3OH); ^{1}H NMR (250 MHz, D_2O) δ 4.39 (dt, 1H, J = 4.8, 6.7), 4.25 (t, 1H, J = 4.6), 4.08 (m, 1H), 3.95 (dd, 1H, J = 6.7, 9.0), 3.82–3.67 (m, 6H), 2.55–2.32 (m, 6H) ; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 81.6, 70.1, 69.9, 69.0, 65.8, 2 × 65.0, 59.6, 2 × 51.9; Calcd for $C_{10}H_{20}NO_5$ ([M+H]$^+$), 234.1341. Found: 234.1345.

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