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Synthesis of 2-carboxymethyl polyhydroxyazepanes and their evaluation as glycosidase inhibitors

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ABSTRACT

A series of diastereomeric tetrahydroxylated azepanes featuring a carboxymethyl group at the pseudo-anomeric position have been synthesized from a common unsaturated intermediate. *Syn*- and *anti*-dihydroxylation reactions were achieved to yield the target compounds after efficient one-step deprotection of carbamate, ester and acetonide groups simultaneously. Screening of these polyhydroxylated azepanes toward a range of commercially available glycosidases was performed and one of the stereoisomers showed potent and selective inhibition toward β-galactosidase ($IC_{50} = 21\ \mu M$).

1. Introduction

Glycosidases are fundamental carbohydrate-processing enzymes, which play a key role in a number of biological events such as digestion, biosynthesis of glycoproteins or the catabolism of glycoconjugates. Accordingly, the discovery of potent and selective glycosidase inhibitors has been the subject of intensive research during the last decades, either as tools to study the functional implication of this class of enzymes or, more recently, as potential therapeutic agents [1,2].

Five-membered, six-membered and bicyclic iminosugars represent valuable classes of glycosidase inhibitors, which were isolated from various natural sources [3]. The capability of these alkaloids to strongly bind to the catalytic site of glycosidases in a reversible manner was attributed to their ability to mimic the oxocarbenium-like intermediate occurring at the transition state of the reaction catalyzed by glycosidases [4]. Extensive synthetic effort has been devoted to the preparation of unnatural congeners of these various types of alkaloids by varying stereochemistry, substitution pattern or ring size in order to improve their potency as well as their selectivity [5–8]. As a result, some iminosugars have now entered clinical trials as potential drugs to treat type 2 diabetes, Gaucher's disease, viral infections or cancer [9–12]. Among the synthetic iminosugars, azepanes which feature a flexible 7-membered ring are promising lead structures for the development of new potent glycosidase inhibitors [13]. Polyhydroxylated azepanes have been shown to display strong affinities toward glycosidases with K_i's in the micro- or even nano-molar range (Fig. 1) [14–21].

The conformational flexibility of the seven-membered ring is assumed to assist the binding interactions to take place into the catalytic site, affording lower K_i's [22]. In addition, azepanes allow additional functionalization opportunities on the extra in-the-ring carbon when compared to pyrrolidines or piperidines, permitting supplementary binding interactions which could improve the inhibition activity. In light of this, we wished to prepare new series of polyhydroxylated azepanes of general structure **1** (Scheme 1), featuring a carboxymethyl substituent at the pseudo-anomeric position, *ie* at the carbon directly linked to nitrogen, in the aim of generating strong interactions with the active site residues, which are responsible for the acid/base catalysis (a pair of Glu or Asp carboxylic acids). Such compounds could be easily obtained from the known unsaturated azepane esters **2** or **3** [23], after stereocontrolled *syn*- or *anti*-dihydroxylation and deprotection, giving rise to a small library of diastereoisomeric azepanes allowing some SAR studies.

2. Results and discussion

The starting compounds **2** and **3** differ in structure only by the configuration at C-2. This variation of the position of the carboxymethyl substituent would allow the exploration of possible interactions with acid/base residues positioned on the α or on the β

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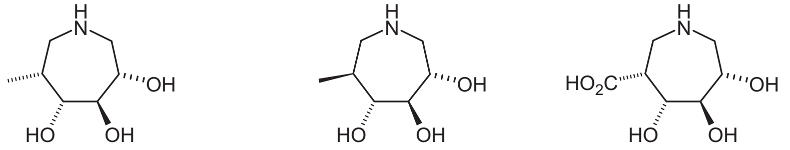
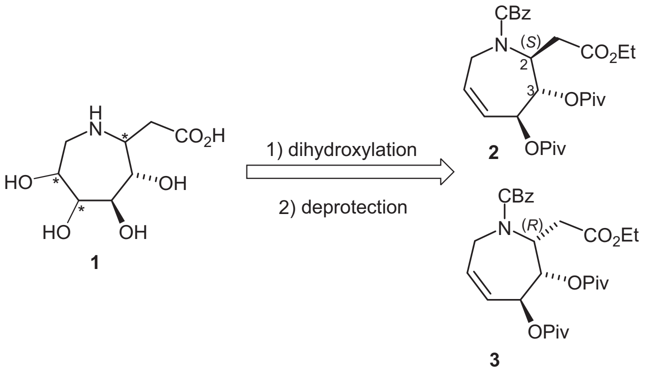


K_i = 40 μM (α-glucosidase) K_i = 35 μM (α-mannosidase)

K_i = 104 μM (β-galactosidase) K_i = 2.5 μM (β-glucosidase)

K_i = 0.041 μM (α-fucosidase)

Fig. 1. Some representative structures of previously reported polyhydroxylated azepanes.



Scheme 1. General strategy for the preparation of the target polyhydroxylated azepanes **1**.

face in the catalytic site of glycosidases. Unsaturated azepanes **2–3** were prepared from d-xylose in a 9-step sequence, following the method described by us in a previous paper [23]. Then, *syn*-dihydroxylation of unsaturated azepanes **2–3** was intended. Under the standard Upjohn conditions (OsO_4, NMO), compounds **2** and **3** reacted smoothly to yield in each case an unseparable 6:4 mixture of diastereomeric *cis*-diols **4a,b**, or **5a,b** in 78% and 84% yield respectively. Separation of both isomers could be achieved after protection of the *cis*-diols **4a,b** (respectively **5a,b**) as their acetonides **6** by reaction with acetone and dimethoxypropane in the presence of *p*-toluenesulfonic acid as the catalyst (93–96% yield). Silica gel chromatography of the crude material afforded the four fully protected azepanes **6a, 6b, 7a** and **7b** in a pure state. At this stage, determination of the relative configuration of the newly formed stereocenters by analysis of vicinal coupling constants proved difficult, due to the presence of the carbamate protecting-group leading to a 50:50 mixture of rotamers in the corresponding NMR spectra. Nevertheless, the determination of the stereochemistry of the major compound **6a** was unambiguously established by X-ray crystallography (Fig. 2). Moreover, the configurations of the stereocentres in the seven-membered ring were confirmed by NMR analysis of the corresponding deprotected congeners (*vide infra*). Final removal of the different protecting groups was achieved in a one-step procedure by treatment of azepanes **6** or **7** with a mixture of 6 M HCl/THF 4:1 at 80 °C during 12 h. Target polyhydroxylated azepanes **8–9** were generally obtained in good yields (65–90%) by this simple procedure (see Schemes 2 and 3).

Analysis of the $^3J_{H-H}$ coupling constants in compounds **8** and **9** allowed determination of the configuration at C-5 and C-6, the stereocenters generated during osmylation [24]. The conformational flexibility of azepanes has been studied in several reports using ^1H NMR spectroscopy assisted by molecular modeling analysis [25]. Actually, their average spatial structures result from the contribution of a set of two or three major conformers. For this reason, the correlation between *J* couplings and the relative position of the ring hydrogens is not as obvious as for 6-membered rings. Nevertheless, analysis of several examples from the literature showed that, in most cases, a *trans*-relationship between two vicinal protons led to a large coupling constant $^3J_{H,H}$ above 7 Hz whereas a *cis*-relationship led to a $^3J_{H,H}$ usually below 3 Hz. With this empirical rule in mind, a good correlation was observed between *J* couplings and configuration for the known compounds **10a,b** and **11a,b**, a series of analogues of azepanes **8** and **9** in which the carboxymethyl substituent was replaced by a hydroxymethyl moiety (Table 1) [24]. Such a correlation was also found in compound **8a**, the absolute configuration of which has been ascertained by X-ray crystallography of the precursor **6a** ($J_{4,5}$ = 8.4 Hz, *trans*; $J_{5,6}$ = 2.4 Hz, *cis*). Accordingly, the absolute configuration of the other isomers **8b, 9a, 9b** was deduced from the relevant $J_{4,5}$ and $J_{5,6}$ affording the given structures.

Preparation of the *trans*-diols was then undertaken by ring-opening of a cyclic sulfate or an epoxide. Formation of a cyclic sulfate from diol **4a**, either in a one-step reaction using SO_2Cl_2 or in a two-step procedure *via* the formation of a cyclic sulfite was not

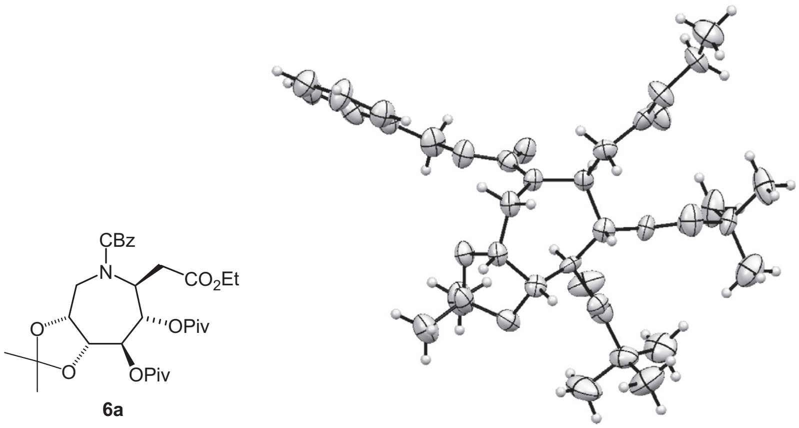


Fig. 2. X-ray structure of compound **6a**.