Bioorganic Chemistry 58 (2015) 11–17

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

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

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ARTICLE INFO

Article history:
Received 15 July 2014
Available online 13 November 2014

Keywords:
Iminosugar
Azepane
Glycosidase
Inhibition
Dihydroxylation

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 μM).

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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http://dx.doi.org/10.1016/j.bioorg.2014.11.003
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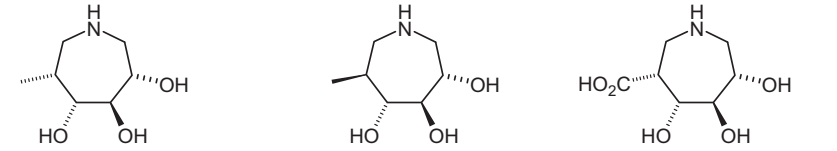
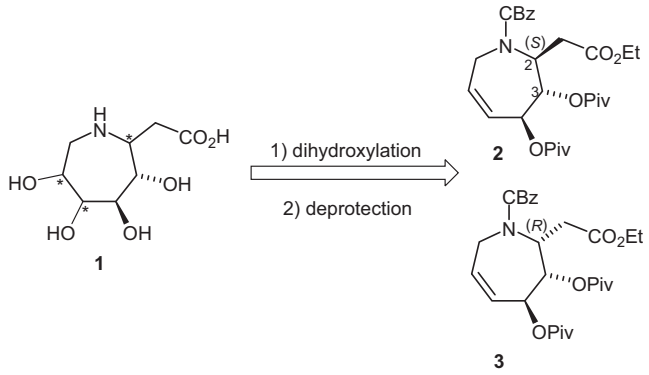


Fig. 1. Some representative structures of previously reported polyhydroxylated azepanes.

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 ^{1}H 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



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

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

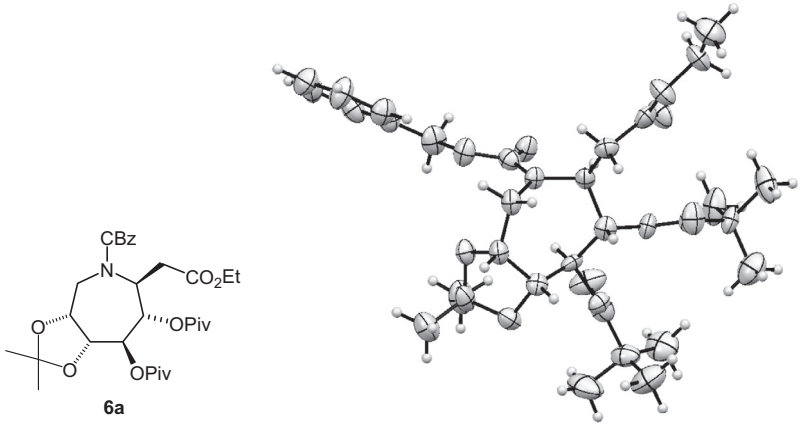
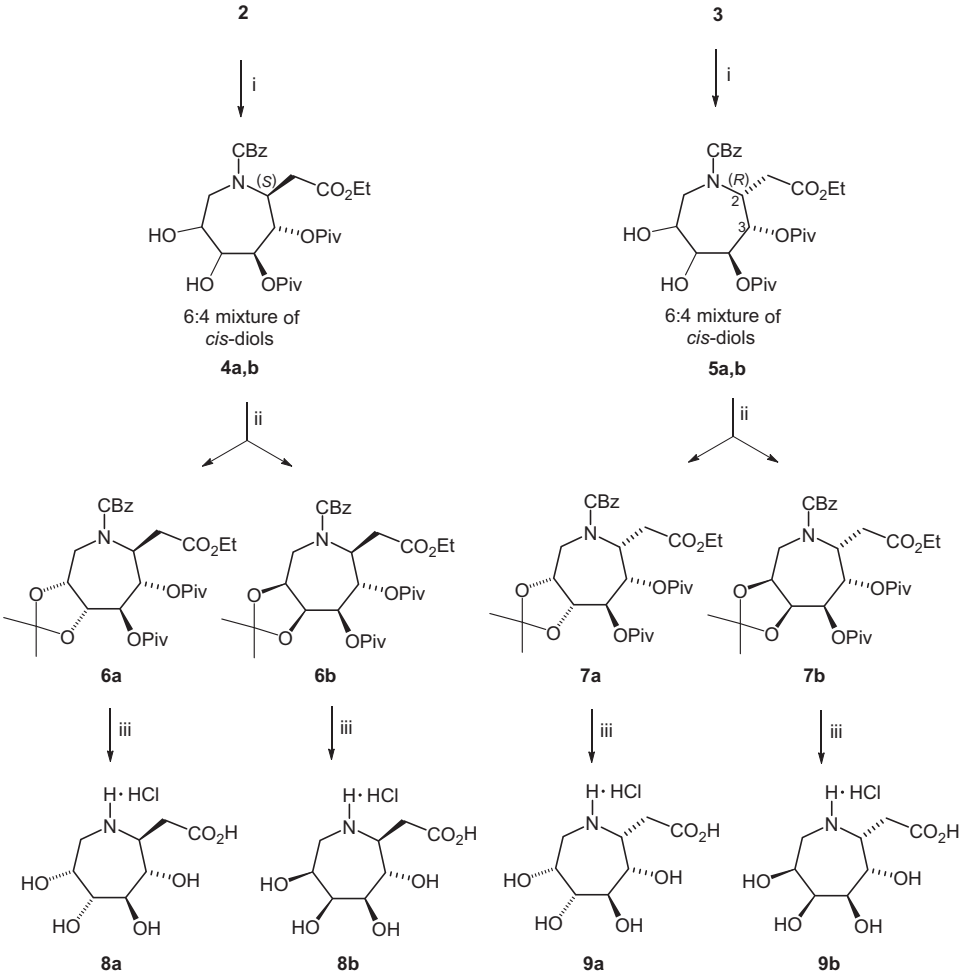


Fig. 2. X-ray structure of compound **6a**.



Scheme 2. Synthesis of target polyhydroxylated azepanes **8–9**. (i) OsO_4 2.5% in *tert*-BuOH, NMO, EtOAc/H_2O 4:1 (78% for **4a,b**, 84% for **5a,b**); (ii) acetone/dimethoxypropane 5:1, *p*TSA cat. (96% for **6a,b**, 93% for **7a,b**); (iii) HCl 6 M/THF 4:1, 80 °C, 12 h (75% for **8a**, 65% for **8b**, 90% for **9a**, 83% for **9b**).

successful. Treatment of the unsaturated azepane **2** with *m*-CPBA in dichloromethane, as previously described for similar compounds [26], afforded a mixture of epoxides **12a,b** in low yield (<30%), whatever the conditions tested (equivalents of *m*-CPBA from 1.5 to 5.6, reaction time from 4 to 48 h, temperature from 0 °C to 40 °C). Finally, preparation of epoxides from unsaturated azepanes **2** and **3** was best carried out using dimethyldioxirane by reaction with Oxone® in acetone in the presence of an excess of sodium hydrogenocarbonate as described in the glycal series [27]. A large excess and a portionwise addition of Oxone® was required for a complete conversion of the starting material. In these conditions (10.5 equiv. Oxone® , 2 equiv per hour), epoxide **12a,b** was obtained in 42% yield. Results could be improved by addition of a phase transfer catalyst (Bu_4NHSO_4 0.5 equiv.) [28], which afforded epoxides **12a,b** and **13a,b** in 78% and 84% yield respectively from **2** and **3**.

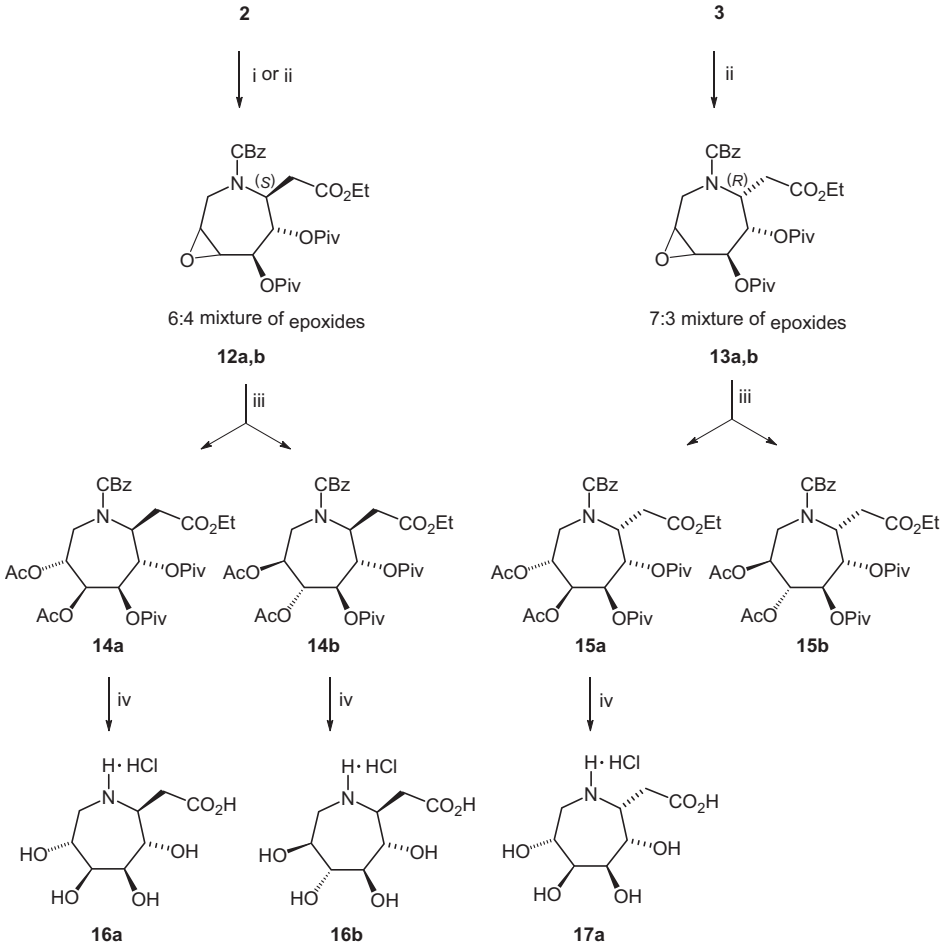
Surprisingly, the direct ring opening of oxiranes **12** or **13** under acid or base catalysis proved quite unsuccessful [26], thus a recent procedure using acetic anhydride as solvent in the presence of a catalytic amount of erbium triflate was used [29]. When applied to epoxide **12**, the reaction proceeded smoothly to give *trans*-diacetates **14a,14b** in good yield (87%) as a mixture of separable diastereomers (70:30). Starting from oxirane **13**, the reaction was much more selective and only one stereoisomer **15a** was isolated in 77% yield. Final deprotection was achieved as previously described by treatment with a mixture 6 M HCl/THF 4:1 during 12 h to afford target polyhydroxylated azepanes **16a,b-17a**.

Following the general rules established for the *cis*-diols **8**,**9**, the absolute configurations of the new generated stereocenters C-5 and C-6 in structures **16a,b** and **17a** were deduced from the relevant $J_{4,5}$ and $J_{5,6}$ listed in Table 2. Thus, the relative orientation of H-4/H-5 was *cis* in **16a** ($J_{4,5}$ = 0 Hz) and **17a** ($J_{4,5}$ = 3.5 Hz) and *trans* in **16b** ($J_{4,5}$ = 6.2 Hz). In a same manner, the *trans* orientation of H-5/H-6 deriving from the stereochemical outcome of the ring-opening reaction was confirmed by a $J_{5,6}$ of about 6 Hz.

3. Glycosidase inhibition

Azepanes **8–9** and **16–17** were assayed for their inhibitory activity toward a range of six commercially available glycosidases (Table 3). Introduction of a carboxylic acid group onto a polyhydroxylated azepane has been shown to induce a very potent inhibition of L-fucosidase from bovine kidney even if the role of the carboxylic acid remained unexplained [15]. The conformational flexibility of the seven-membered ring should be responsible for the favorable interactions with the enzyme.

Unfortunately, our compounds did not significantly inhibit the following glycosidases at 1 mM: rice α-glucosidase, almond β-glucosidase, α-mannosidase from jack bean, α-fucosidase from bovine kidney and α-rhamnosidase from *Aspergillus niger*. Inhibition was observed only toward β-galactosidase from *Aspergillus orizae*. Two prepared polyhydroxylated azepanes in the *cis*-diol series displayed a selective and moderate β-galactosidase inhibition: compounds **8b** (IC_{50} = 279 μM) and **9a** (IC_{50} = 215 μM). Finally,



Scheme 3. Synthesis of target polyhydroxylated azepanes **16–17**. (i) *m*-CPBA excess, dichloromethane, rt, 12 h (33% for **12a,b**); (ii) Oxone®excess, $NaHCO_3$ excess, Bu_4NHSO_4 cat., EtOAc/H_2O 4:1 (78% for **12a,b**, 84% for **13a,b**); (iii) Ac_2O excess, $Er(OTf)_3$ cat. (87% for **14a,b**, ratio **14a**/**14b** 70:30, 77% for **15a,b**, ratio**15a**/**15b** 95:5); (iv) HCl 6 M/THF 4:1, 80 °C, 12 h (50% for **16a**, 8% for **16b**, 83% for **17a**).

Table 1
Selected vicinal $^3J_{H,H}$ for azepanes **8–9**.

		R = OH[a] (**10a**)	R = CO_2H[b] (**8a**)	R = OH[a] (**10b**)	R = CO_2H[b] (**8b**)	R = OH[a] (**11a**)	R = CO_2H[b] (**9a**)	R = OH[a] (**11b**)	R = CO_2H[b] (**9b**)
$^3J_{H,H}$	$J_{3,4}$ (Hz)	8.4	9.3	8.8	9.1	2.7	nd	nd	7.8
	$J_{4,5}$ (Hz)	8.4	7.8	1.9	4.5	6.3	10.2	2.9	0
	$J_{5,6}$ (Hz)	2.4	0	1.4	0	0[c]	1.2	2.0	0

[a] As deduced from the $^3J_{H-H}$ of their respective enantiomers, given in Ref. [10].
[b] This work (see experimental part).
[c] Corrected (Pr. Y. Blériot, personal communication).

Table 2
Selected vicinal $^3J_{H,H}$ for azepanes **16–17**.

		16a	16b	17a
$^3J_{H,H}$	$J_{3,4}$ (Hz)	8.5	8.0	10.1
	$J_{4,5}$ (Hz)	0	6.2	3.5
	$J_{5,6}$ (Hz)	5.9	nd	6.6

best result was obtained with azepane **17a** in the *trans*-diol series with a quite good inhibition of the same β-galactosidase (IC_{50} = 21 μM).

4. Conclusions

In conclusion, we have synthesized a series of tetrahydroxylated azepanes displaying a methylene carboxylic group at the

Table 3
Inhibitory activity of azepanes **8–9** and **16–17** (percentage inhibition at 1 mM).

	8a	8b	9a	9b	16a	16b	17a
α-glucosidase Rice	NI	NI	40%	NI	34%	NI	NI
β-glucosidase Almonds	NI	NI	28%	NI	NI	NI	5%
α-mannosidase Jack beans	5%	NI	54%	5%	48%	15%	NI
α-fucosidase Bovine kidney	18%	4%	59%	6%	48%	15%	11%
β-galactosidase Aspergillus orizae	39%	80% (279 μM)	83% (215 μM)	20%	24%	72%	100% (21 μM)
α-rhamnosidase Aspergillus niger	NI	NI	29%	NI	NI	NI	10%

All the assays were done in duplicate with less than 10% variability.
NI = no inhibition at 1 mM concentration of the inhibitor.
IC_{50} in brackets.

pseudo anomeric position from previously reported unsaturated azepanes. A selective *syn*-dihydroxylation in standard conditions followed by an acetal formation allowed the separation of the *cis*-diastereomers. Formation of an epoxide followed by a ring-opening of the oxirane in the presence of acetic anhydride led to the preparation of the *trans*-distereomers.

A one-step full deprotection was achieved in acid conditions to yield the tetrahydroxylated azepanes in both series. The seven polyhydroxylated azepanes were evaluated as inhibitors toward various glycosidases. Best results were obtained for the selective inhibition of *A. orizae* β-galactosidase, azepanes **8b** and **9a** being moderate inhibitors while azepane **17a** displayed a more potent inhibition (IC_{50} = 21 μM).

Introduction of other functional groups at the pseudo-anomeric position such as amino, amido, tetrazole or phosphonate groups to prepare compounds designed as potential inhibitors of glycosidases, glycosyltransferases or glycogen phosphorylase will be undertaken.

5. Experimental section

5.1. General methods

Reactants and reagents were purchased from Aldrich and Sigma and were used without further purification. Silica gel F254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic solution of phosphomolybdic acid, followed by heating. Flash column chromatography was performed over silica gel M 9385 (40–63 μm) Kieselgel 60. NMR spectra were recorded on Bruker AC 250 (250 MHz for ^{1}H, 62.5 MHz for ^{13}C) or 500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in parts per million (ppm) and were calibrated to the residual solvent peak. Coupling constants are in Hz and splitting pattern abbreviations are: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. Optical rotations were determined at 20 °C with a Perkin–Elmer Model 241 polarimeter in the specified solvents. High Resolution Mass Spectra (HRMS) were performed on Q-TOF Micro micromass positive ESI (CV = 30 V). FTIR spectra were recorded with an IR^{TM} plus MIDAC spectrophotometer and are expressed in cm^{-1}. Elemental analyses were performed with a Perkin Elmer CHN 2400 apparatus.

5.2. General procedure for syn-dihydroxylation reaction

Unsaturated azepanes **2** or **3** (425 mg, 1.19 mmol) were dissolved in a mixture EtOAc/H_2O 4:1 (2 mL) and *N*-methylmorpholine *N*-oxide (2.2 eq., 311 mg, 2.62 mmol) was slowly added under argon atmosphere at 0 °C. Osmium tetroxide (2.5% solution in *t*-BuOH, 2.2 eq., 0.8 mL, 2.92 mmol) was then slowly added and the reaction mixture was stirred at room temperature for 12 h. Excess osmium tetroxide was reduced by addition of a sodium sulfite solution. The crude mixture was extracted by EtOAc (5 mL) and washed by a sodium sulfite solution (3 × 5 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. Purification by silica gel chromatography (PE/diethyl ether 6:4) afforded compounds **4a,b** (from **2**) and **5a,b** (from **3**) as slight yellow oils (**4a,b** 394 mg, 78% 57/43; **5a,b** 424 mg, 84%, 61/39).

5.3. General procedure for acetal formation

The mixture of diastereomers **4a,b** or **5a,b** (124 mg, 0,225 mmol) was dissolved in 1,2 mL of acetone/2,2-dimethoxypropane (v/v, 5/1). Catalytic amount of TsOH (7 mg, 0,037 mmol) was added and the mixture was stirred for 3 h at rt. After neutralization with $NaHCO_3$ (10 mg, 0119 mmol), filtration and evaporation, compounds **6a,b** and **7a,b** were purified by silica gel chromatography (PE/diethyl ether: 60/40).

5.4. General procedure for epoxidation of unsaturated azepanes *2–3*

Compound **2** or **3** (246 mg, 0.47 mmol) was dissolved in a mixture EtOAc/water 4:1 (15 mL). Tetrabutylammonium hydrogenosulfate (0.5 eq, 114 mg, 0.24 mmol) was added to the reaction mixture. Then, Oxone® (10.5 eq, 1.63 g, 4.94 mmol) and sodium hydrogenocarbonate (21 eq, 829 mg, 9.87 mmol) were successively added portionwise (0.5 eq Oxone® and 1 eq sodium hydrogenocarbonate every 30 min). The reaction mixture was stirred during 4 days. Ethyl acetate was added (20 mL) and the crude mixture was washed with a saturated sodium chloride solution (3 × 20 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. The residue was purified by silica gel chromatography (PE/diethyl ether: 40/60). Compounds **12a,b** (from **2**) and **13a,b** (from **3**) were obtained as slight yellow oils (**12a,b** 394 mg, 78% 60/40; **13a,b** 424 mg, 84%, 70/30).

5.5. General procedure for acetolysis of epoxides *12–13*

Mixture of diastereomers **12a,b** or **13a,b** (101 mg, 0.225 mmol) was dissolved in acetic anhydride (3.9 mL, 200 eq, 40 mmol). Erbium triflate (0.1 eq, 12 mg, 0.037 mmol) was added and the reaction mixture was stirred during 24 h. Ethyl acetate was added (10 mL) and the crude mixture was washed by a saturated sodium hydrogenocarbonate solution (3 × 10 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated. Diastereomers were purified by silica gel chromatography (PE/diethyl ether: 40/60). Compounds **14a** and **14b** and **15b** were obtained as slight yellow oils (**14a** major diastereomer, 73 mg, 87%, 70/30; **14b** minor diastereomer, 31 mg, 87%, 30/70; **15a** major diastereomer, 92 mg, 77%, 95/5). Minor compound **15b** was not isolated.

Synthetic intermediates **4–7** in the *cis*-diol series and **12–15** in the *trans*-diol series were obtained as 50/50 mixture of rotamers due to the presence of the CBz group which results in line-broadening and duplication of signals in the corresponding ^{1}H NMR

spectra. Characterization of these compounds is detailed in the Supporting Information.

5.6. General procedure for the total deprotection of the polyhydroxylated azepanes

Compound **6**, **7**, **16** or **17** (100 mg, 0.19 mmol) was dissolved in 1.2 mL of HCl 6 N/THF (v/v, 4/1). The mixture was stirred for 12 h at 80 °C. Evaporation of the volatiles gave an oily residue which was washed successively with diethyl ether (3 mL), chloroform (3 × 3 mL) and chloroform/isopropanol (v/v, 1/1) affording the target azepanes as colorless foams. (**8a** 36 mg, 75%; **8b** 31 mg, 65%; **9a** 43 mg, 90%; **9b** 39 mg, 83%; **16a** 24 mg, 50%; **16b** 4 mg, 8%; **17a** 39 mg, 83%).

5.6.1. (2S,3S,4R,5R,6R)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **8a**

R_f = 0.37 [$CHCl_3$/MeOH/H_2O : 6/4/1]; $[\alpha]_{20}^{D}$ = +10 (*c* = 0.28, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.59 (1H, dd, *J* 9.3, 10.2 Hz, H-3), 4.47 (1H, ddd, *J* 8.8, 10.2, 10.8 Hz, H-2), 4.30 (1H, dd, *J* 1.7, 7.8 Hz, H-6), 3.89 (1H, dd, *J* 7.8, 9.3 Hz, H-4), 3.69 (1H, d, *J* 7.8 Hz, H-5), 3.59 (1H, dd, *J* 8.4, 13.5 Hz, H-7), 3.34 (1H, dd, *J* 1.7, 13.5 Hz, H-7), 3.12 (1H, dd, *J* 8.8, 17.4 Hz, H-8), 3.02 (1H, dd, *J* 10.8, 17.4 Hz ; H-8); ^{13}C NMR (D_2O, 125 MHz) δ (ppm) 174.1 (C_q, C-9), 79.8 (CH, C-3), 75.1 (CH, C-5), 73.9 (CH, C-4), 67.0 (CH, C-6), 51.1 (CH, C-2), 44.4 (CH_2, C-7), 33.3 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M + H]^+$ = 222.0978, found : 222.0978.

5.6.2. (2S,3S,4R,5S,6S)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **8b**

R_f = 0.39 [$CHCl_3$/MeOH/H_2O : 6/4/1]; $[\alpha]_{20}^{D}$ = +27 (*c* = 0.26, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.89 (1H, dd, *J* 9.1, 9.7 Hz, H-3), 4.36 (1H, dd, *J* 2.7, 8.4 Hz, H-6), 4.21 (1H, d, *J* 4.5 Hz, H-5), 4.10 (1H, ddd, *J* 8.6, 9.7, 10.6 Hz, H-2), 4.10 (1H, dd, *J* 4.5, 9.1 Hz, H-4), 3.56 (1H, dd, *J* 8.4, 13.5 Hz, H-7), 3.35 (1H, dd, *J* 2.7, 13.5 Hz, H-7), 3.16 (1H, dd, *J* 8.6, 17.3 Hz, H-8), 3.05 (1H, dd, *J* 10.6, 17.3 Hz, H-8); ^{13}C NMR (D_2O, 125 MHz) 174.4 (C_q, C-9), 81.6 (CH, C-3), 72.9 (CH, C-5), 72.3 (CH, C-4), 68.3 (CH, C-6), 52.3 (CH, C-2), 46.3 (CH_2, C-7), 33.9 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M+H]^+$ = 222.0978, found : 222.0972.

5.6.3. (2R,3S,4R,5R,6R)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **9a**

R_f = 0.32 [$CHCl_3$/MeOH/H_2O : 6/4/1]; $[\alpha]_{20}^{D}$ = +16 (*c* = 0.72, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.84–4.81 (1H, m, H-3), 4.34 (1H, ddd, *J* 1.5, 6.7, 9.5 Hz, H-2), 4.27–4.25 (1H, m, H-4), 4.22–4.19 (1H, m, H-6), 3.79 (1H, dd, *J* 1.2, 10.2 Hz, H-5), 3.53 (1H, dd, *J* 3.8, 14.4 Hz, H-7), 3.44 (1H, dd, *J* 9.5, 19.2 Hz, H-8), 3.24 (1H, d, *J* 14.4 Hz, H-7), 2.88 (2H, dd, *J* 1.5, 19.2 Hz, H-8); ^{13}C NMR (D_2O, 125 MHz) 175.4 (C_q, C-9), 82.9 (CH, C-3), 69.8 (CH, C-5), 67.5 (CH, C-4), 66.3 (CH, C-6), 55.5 (CH, C-2), 50.7 (CH_2, C-7), 34.2 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M+H]^+$ = 222.0978, found : 222.0976.

5.6.4. (2R,3S,4R,5S,6S)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **9b**

R_f = 0.34 [$CHCl_3$/MeOH/H_2O : 6/4/1]; $[\alpha]_{20}^{D}$ = +21 (*c* = 0.69, MeOH); ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.97 (1H, dd, *J* 6.1, 7.8 Hz, H-3), 4.41 (1H, dd, *J* 6.1, 8.9 Hz, H-2), 4.21 (1H, sl, H-5), 4.14–4.12 (1H, m, H-6), 4.10 (1H, d, *J* 7.8 Hz, H-4), 3.47 (1H, dd, *J* 8.9, 19.2 Hz, H-8), 3.41 (1H, dd, *J* 4.8, 13.1 Hz, H-7), 3.20 (1H, dd, *J* 11.6, 13.1 Hz, H-7), 2.83 (1H, d, *J* 19.2 Hz, H-8); ^{13}C NMR (D_2O, 125 MHz) 175.5 (C_q, C-9), 83.0 (CH, C-3), 76.1 (CH, C-5), 70.8 (CH, C-4), 66.8 (CH, C-6), 56.2 (CH, C-2), 45.8 (CH_2, C-7), 34.1 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M+H]^+$ = 222.0978, found : 222.0986.

5.6.5. (2S,3S,4R,5S,6R)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **16a**

R_f = 0.40 [$CHCl_3$/MeOH/H_2O : 6/4/1] ; ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.89 (1H, dd, *J* 6.6, 8.5 Hz, H-3), 4.35 (1H, d, *J* 8.5 Hz, H-4), 4.22 (1H, ddd, *J* 1.5, 6.6, 9.5 Hz, H-2), 4.12 (1H, d, *J* 5.9 Hz, H-5), 4.03 (1H, dt, *J* 2.2, 5.9 Hz, H-6), 3.39 (1H, dd, *J* 9.5, 19.4 Hz, H-8), 3.32 (2H, d, *J* 2.2 Hz, H-7), 2.75 (1H, dd, *J* 1.5, 19.4 Hz, H-8); ^{13}C NMR (D_2O, 125 MHz) 176.2 (C_q, C-9), 83.4 (CH, C-3), 74.6 (CH, C-5), 69.2 (CH, C-4), 66.6 (CH, C-6), 56.0 (CH, C-2), 49.5 (CH_2, C-7), 34.8 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M+H]^+$ = 222.0978, found : 222.0983.

5.6.6. (2S,3S,4R,5R,6S)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **16b**

R_f = 0.33 [$CHCl_3$/MeOH/H_2O : 6/4/1] ; ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.86 (1H, dd, *J* 8.0, 8.8 Hz, H-3), 4.26 (1H, dd, *J* 8.8 Hz, H-2), 4.13–4.11 (1H, m, H-5), 4.04 (1H, d, *J* 6.2, 8.0 Hz, H-4), 4.00–3.95 (1H, m, H-6), 3.58 (1H, dd, *J* 8.8, 19.4 Hz, H-8), 3.30 (1H, dd, *J* 5.0, 13.4 Hz, H-7), 3.12 (1H, dd, *J* 11.5, 13.4 Hz, H-7), 2.73 (1H, d, *J* 19.4 Hz, H-8); ^{13}C NMR (D_2O, 125 MHz) 174.4 (C_q, C-9), 78.7 (CH, C-3), 71.3 (CH, C-4), 69.7 (CH, C-5), 66.7 (CH, C-6), 51.3 (CH, C-2), 42.9 (CH_2; C-7), 33.6 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M+H]^+$ = 222.0978, found : 222.0974.

5.6.7. (2R,3S,4R,5S,6R)-2-Carboxymethyl-3,4,5,6-tetrahydroxyazepane hydrochloride **17a**

R_f = 0.26 [$CHCl_3$/MeOH/H_2O : 6/4/1] ; ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.70–4.67 (1H, m, H-3), 4.38 (1H, dt, *J* 9.3, 11.1 Hz, H-2), 4.28 (1H, ddd, *J* 1.5, 5.3, 6.6 Hz, H-6), 4.17 (1H, dd, *J* 3.5, 6.6 Hz, H-5), 4.04 (1H, dd, *J* 3.5, 10.1 Hz, H-4), 3.57 (1H, dd, *J* 1.5, 14.4 Hz, H-7), 3.36 (1H, dd, *J* 5.3, 14.4 Hz, H-7), 3.05 (1H, dd, *J* 8.8, 17.3 Hz, H-8), 2.97 (1H, dd, *J* 11.1, 17.3 Hz, H-8); ^{13}C NMR (D_2O, 125 MHz) 174.4 (C_q, C-9), 78.7 (CH, C-3), 71.3 (CH, C-4), 69.7 (CH, C-5), 66.7 (CH, C-6), 51.3 (CH, C-2), 42.9 (CH_2; C-7), 33.6 (CH_2, C-8); HRMS (*m*/*z*, ESI) calculated for $C_8H_{16}NO_6$: $[M+H]^+$ = 222.0978, found : 222.0982.

Acknowledgments

This work was supported by the "Contrat de Plan Etat-Région" (GLYCOVAL, PentoRaf and PlAneT programs). The authors are grateful to the "Ministère de l'Enseignement Supérieur et de la Recherche" and FEDER for financial support and a doctoral fellowship (H.T.) and to the "Fondation du Site Paris-Reims" for a post-doctoral fellowship (S.G.). The authors also warmly thank Ms A. Martinez for NMR spectra recording and for helpful discussions, F. Lachaud and the ICMMO (Orsay, Paris XI University) for X-ray data collection.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.11.003.

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