Tetrahedron 71 (2015) 5085–5090

Contents lists available at ScienceDirect

Tetrahedron

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

Synthesis of polyhydroxylated azetidine iminosugars and 3-hydroxy-*N*-methylazetidine-2-carboxylic acid from D-glucose

Pravin P. Lawande [a], Vyankat A. Sontakke [a], Roopa J. Nair [b], Ayesha Khan [c], Sushma G. Sabharwal [b], Vaishali S. Shinde [a,*]

[a] Garware Research Centre, Department of Chemistry, Savitribai Phule Pune University (formerly, University of Pune), Pune 411007, India
[b] Division of Biochemistry, Department of Chemistry, Savitribai Phule Pune University (formerly, University of Pune), Pune 411007, India
[c] Department of Chemistry, Savitribai Phule Pune University (formerly, University of Pune), Pune 411007, India

ARTICLE INFO

Article history:
Received 4 December 2014
Received in revised form 16 May 2015
Accepted 18 May 2015
Available online 22 May 2015

Keywords:
Azetidine
Iminosugars
Glycosidase inhibitory activity
Mitsunobu reaction
D-Glucose

ABSTRACT

The azetidine iminosugars, (2*S*,3*R*)-2-((*R*)-1,2-dihydroxyethyl)azetidin-3-ol **3**, (2*R*,3*R*)-2-(hydroxymethyl) azetidin-3-ol **4**, and (2*S*,3*R*)-3-hydroxy-*N*-methylazetidine-2-carboxylic acid **5** were synthesized from D-glucose derived 3-*N*-benzyloxycarbonyl-3-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose **8** using intramolecular Mitsunobu reaction as a key step. The glycosidase inhibitory activity of compounds **3**–**5** was screened against various enzymes. Amongst all synthesized compounds, the *N*-methylated compound **5** exhibited significant inhibitory activity against amyloglucosidase from *Aspergillus niger* in micro molar range.

1. Introduction

The polyhydroxylated nitrogen containing cyclic compounds commonly known as iminosugars, are found to be selective and promising glycosidase inhibitors.[1,2] Mechanistically, these small molecules act as mimic for the carbohydrates, thereby exhibiting remarkable biological activities. In an attempt to know the effect of ring size on biological activity, several iminosugars with varying ring size (5–8 membered) were either isolated or synthesized[3] and evaluated for various biological activities.[4]

Azetidines are four-membered aza-heterocycles[5] containing endocyclic nitrogen atom and known to show wide range of biological activity.[6] For example, L-azetidine-2-carboxylic acid (L-Aze) **1a** is naturally occurring non-proteinogenic amino acid found in plants[7] and is known to be ring contracted derivative of L-proline **2** (Fig. 1). Ozaki and co-workers[8] reported the enzymatic synthesis of *cis*-3-hydroxyazetidine-2-carboxylic acid **1b** from **1a**. Synthesis of polyhydroxylated azetidines is less accounted by the researchers due to the difficulties faced in the formation of enantiopure, highly strained four-membered ring. A number of synthetic methods have been developed for the formation of hydroxylated azetidine compounds starting from non-carbohydrate precursors,[9] however, there are few reports for synthesis using sugars as starting materials.[10] For example, Jager and co-workers synthesized azetidine iminosugars from 2-*O*-benzylglyceraldehyde[11] using [2+2] cycloaddition of acid chlorides with 2-*O*-benzylglyceraldehyde imines. Recently, Fleet and co-workers described the synthesis of azetidine iminosugars via nucleophilic double-displacement of *O*-triflate

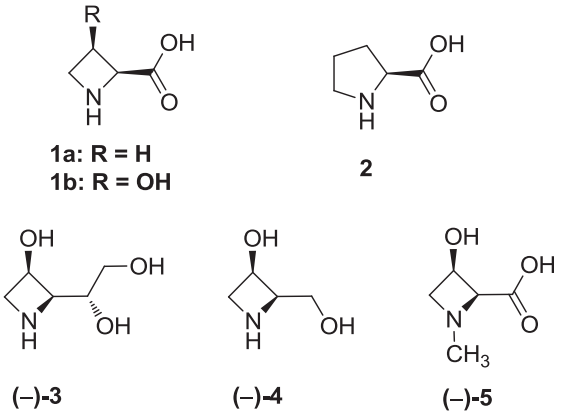


Fig. 1. Azetidine analogues and other related compounds.

* Corresponding author. Tel.: +91 20 25601395; fax: +91 20 25691728; e-mail address: vsshinde@chem.unipune.ac.in (V.S. Shinde).

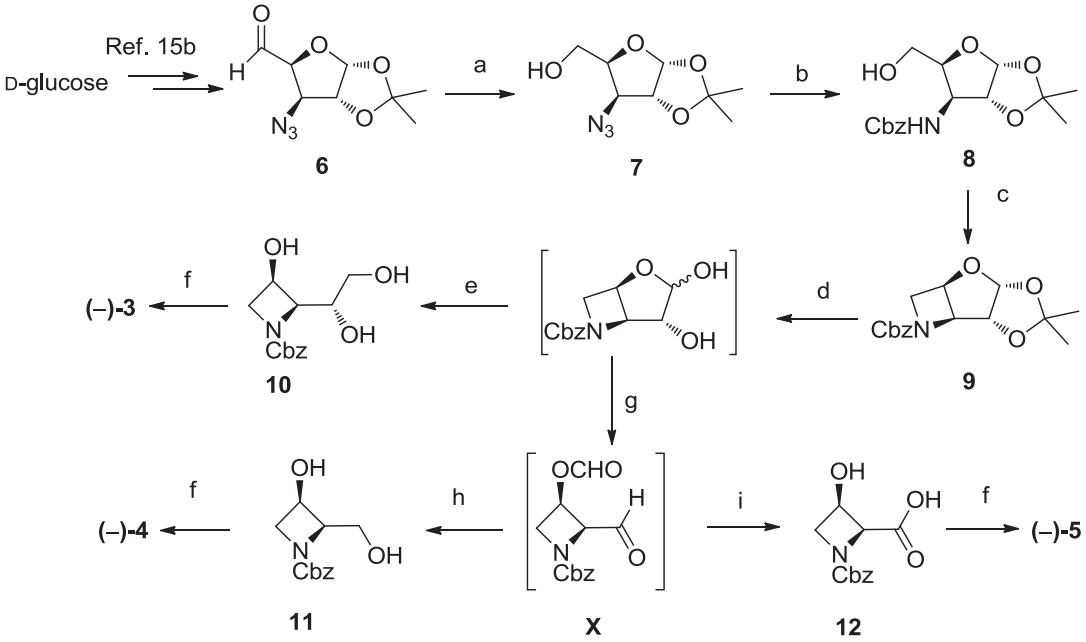
http://dx.doi.org/10.1016/j.tet.2015.05.057
0040-4020/

derivatives of pentoses[12] as well as hexoses[13] by amines. Georg and co-workers described synthesis of *N*-alkylated azetidine iminosugars from glyceraldehyde and tested for inhibitory activity against glucosyltransferase and other glycosidases.[14]

As a part of our continuing efforts in the synthesis of bioactive molecules from D-glucose,[15] we report herein, synthesis of azetidine iminosugars namely, (2*S*,3*R*)-2-((*R*)-1,2-dihydroxyethyl)azetidin-3-ol **3**, (2*R*,3*R*)-2-(hydroxymethyl)azetidin-3-ol **4**, and (2*S*,3*R*)-3-hydroxy-*N*-methylazetidine-2-carboxylic acid **5** and their glycosidase inhibitory activity. In our approach (as shown in Scheme 1), we constructed azetidine skeleton of target molecules by using intramolecular Mitsunobu reaction of the C5–OH group with C3-protected amino (from C3-azido) functionality of D-glucose. Our results in this direction are described herein.

Dowex (H^+) gave hemiacetal that was directly subjected to oxidative cleavage using sodium metaperiodate, followed by reduction using sodium borohydride in methanol to afford *N*-Cbz protected diol **11** as a thick liquid. Finally, hydrogenolysis using 10% Pd/C in methanol at 10 psi afforded **4** in 57% yield as a sticky solid.

To achieve the synthesis of (2*S*,3*R*)-3-hydroxy-*N*-methylazetidine-2-carboxylic acid **5**, the hemiacetal obtained from **9** was treated with sodium metaperiodate, followed by the Pinnick oxidation[15b] using sodium chlorite and hydrogen peroxide to afford *N*-Cbz protected acid **12**. Final hydrogenolysis of compound **12** in the presence of 10% Pd/C in methanol failed to furnish expected free amine. However, we obtained *N*-methylated azetidine-2-carboxylic acid **5** in 50% yield as a sticky solid. The structure was confirmed on the basis of HRMS, NMR, and 2D-HSQC spectral data. The presence



Scheme 1. Reagent and conditions: (a) $NaBH_4$, $MeOH/H_2O$, 0 °C, 2 h, 97%; (b) (i) H_2, Pd/C, MeOH, rt, 3 h, 10 psi; (ii) $NaHCO_3$, CbzCl, $MeOH/H_2O$, 0 °C, 4 h, 93%; (c) PPh_3, DEAD, Dry DCM, rt, 3 h, 65%; (d) Dowex 50WX4-200 (H^+ form), 1,4-dioxane/H_2O, 60 °C, 24 h, 93%; (e) $NaBH_4$, THF/H_2O, −10 °C, 1 h, 88%; (f) H_2, Pd/C, MeOH, rt, 10 psi, 12 h, 81% for **3**, 57% for **4** and 50% for **5**; (g) $NaIO_4$, acetone/H_2O, 0 °C, 6 h; (h) $NaBH_4$, THF:H_2O, −10 °C, 1 h, 56% (from hemiacetal); (i) $NaClO_2$, NaH_2PO_4, 30% H_2O_2, aq MeCN, 0 °C to rt, 10 h, 66% (from hemiacetal).

2. Result and discussion

2.1. Synthesis

Required azido-aldehyde **6** was synthesized from commercially available D-glucose as reported earlier (Scheme 1).[15b] The aldehyde group in **6** was reduced by using sodium borohydride in aq methanol at 0 °C to give azido alcohol **7** in 97% yield. Reduction of C3-azido functionality in **7** using 10% Pd/C in methanol afforded corresponding amine, which was protected as carbamate derivative **8** by treatment with benzyl chloroformate (CbzCl) in the presence of sodium bicarbonate. Further, reaction of **8** with triphenylphosphine and diethyl azodicarboxylate (DEAD) under Mitsunobu condition gave key azetidine ring **9** in 65% yield as a white solid. Deprotection of 1,2-acetonide group of **9** with the Dowex (H^+) resin afforded anomeric mixture of hemiacetals (α/β=2:1; as evident from the ^1H NMR spectrum of the crude product), which was reduced by using sodium borohydride in THF/H_2O to give *N*-Cbz protected triol **10**. In the final step, hydrogenolysis of **10** using 10% Pd/C in methanol afforded (2*S*,3*R*)-2-((*R*)-1,2-dihydroxyethyl)azetidin-3-ol **3** in 81% yield.

Synthesis of (2*R*,3*R*)-2-(hydroxymethyl)azetidin-3-ol **4** was achieved from **9**. Thus, deprotection of 1,2-acetonide group using of sharp singlet at 2.92 ppm, corresponding to three protons integration in the ^1H NMR and a peak at 40.2 ppm in the ^{13}C NMR spectrum indicated the presence of $-NCH_3$ group.[16]

2.2. Glycosidase inhibitory activity

The glycosidase inhibitory activity of newly synthesized compounds **3**–**5** was studied against various glycosidases viz.: β-glucosidase, α-mannosidase, and α-galactosidase (isolated from almond seeds), α-mannosidase, β-mannosidase, and *N*-acetyl-β-D-glucosaminidase (isolated from Jack bean seeds), α-glucosidase (isolated from rice seeds and yeast, procured from Sigma Chemical Co), β-glucosidase and β-galactosidase (isolated from Bovine liver), α-galactosidase (isolated from coffee bean seeds), β-galactosidase (*Aspergillus oryzae* from Sigma Chemical Co), α-fucosidase and *N*-acetyl-β-D-glucosaminidase (isolated from Bovine kidney), α-amylase (isolated from *Geobacillus* JN704808) and amyloglucosidase (*Aspergillus niger* procured from Sigma Chemical Co). The 50% inhibitory concentration (IC_{50}) and inhibitory constant (K_i) values were determined for the test compounds and are summarized in Table 1. Amongst these compounds, **4** showed a weak competitive inhibition of β-galactosidase (bovine liver) with IC_{50} 7.5±0.05 mM and K_i 2.66 mM.