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TETRAHEDRON

Acyclic Analogues of Glucosamidine, 1-Deoxynojirimycin and N-(1,3-Dihydroxyprop-2-yl) Derivative of Valiolamine as Potential Glucosidase Inhibitors

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Abstract: The N-(β-hydroxyethyl)acetamidine (**4**) was prepared. Mesylation of **8** gave the 1,3-di-*O*-benzyl-2-*O*-methanesulfonyl-glycerol (**9**). Nucleophilic displacement of the mesyloxy group in **9** with ethanolamine and morpholine gave **11** and **12**, respectively. Their catalytic hydrogenation gave 2-deoxy-2-(1-hydroxyeth-2-yl)amino-glycerol (**5**) and 2-deoxy-2-(morpholin-4-yl)glycerol (**13**). The inhibition of the β-D-glucosidase enzyme from sweet almond by **4**, **5** and **13-16** has been studied.

Keywords: Acetamidine, Glycerol, Hydroxyethylamino, Morpholine, Glucosidase inhibitors.

INTRODUCTION

Important biological processes are carried out by carbohydrate processing enzymes and in particular by glycosidases.[1-3] Their roles in therapeutic and biotechnological applications as a consequence of modifying or blocking their action have attracted the attention of organic chemists and biochemists. These strateies have been applied to glycosidases involved in intestinal digestion, post-translational processing of glycoproteins, and lysosomal catabolism of glycoconjugates. Thus, a number of them show promise as antidiabetes,[4] antitumor metastasis,[5] and antiobesity drugs,[6] and as antifeedents[7-9] and antivirals.[10-13] The factors that govern the strength and the degree of selectivity of glycosidase inhibition are not clear and/or not well established.[14] Consequently there is a need for evaluating more examples with variant constitutions as glycosidase inhibitors. In this work, noncarbohydrate templates of bioactive carbohydrate molecules are designed for the investigation of their inhibition properties.

The amidine **1** is a competitive inhibitor[2,15-17] of sweet almond β–glucosidase, Jack bean α-mannosidase and bovine liver β-galactosidase. 1-Deoxynojirimycin,[2] also known as moranoline, has a pronounced potent competitive inhibition of endoplasmatic reticulum α-glucosidase II which is involved in glycoconjugate

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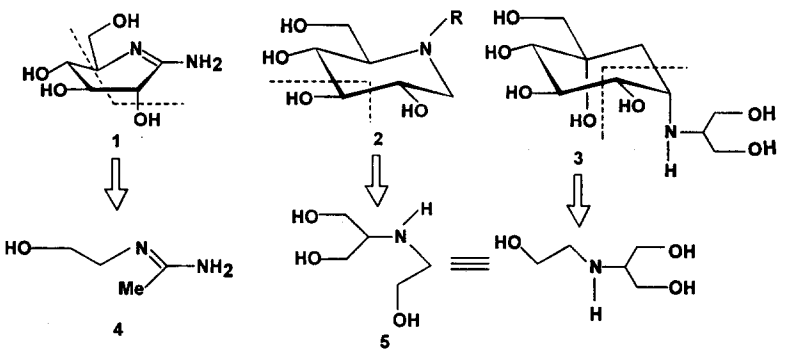
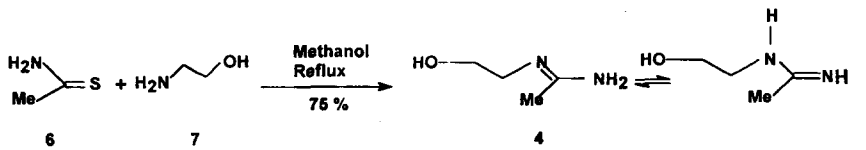
synthesis. It is useful against the HIV virus.[11] Its N-butyl derivative has a lipophilic nature and shows particularly potent antiviral activity.[18,19] A less toxic series of analogues has been developed by inserting oxygen atoms in the N-alkyl group.[20-23] Also the analogous 1-deoxy N-[(6-deoxy-1-*O*-methyl-α-D-glucopyranos-6-yl)]nojirimycin is a potent α-glucosidase inhibitor with important chemotherapeutic value.[24] 1-Deoxynojirimycin and its derivatives can be represented by formula **2**. Thus, the respective acyclic analogues of **1** and **2** which can be simply considered[25] as the triseco analogue of **1** and the diseco analogues of **2** by applying the disconnection between C-2-C-3, and C-4-C-5 in **1** and between C-2-C-3 and C-3-C-4 in **2** to offer **4** and **5**, respectively, are target compounds (Scheme 1). The design of **5** has been also encouraged by the fact that 2-amino-2-hydroxymethyl-1,3-propanediol and other amines showed a competitive inhibition of glycosidases.[26-29] Moreover, compound **5**, a mimic of a structural subunits of **2**, prepared as its hydrochloride by the alkylation of serinol, was found to be a good competitive inhibitor of yeast α-glucosidase.[30] Additionally, the N-(1,3-dihydroxyprop-2-yl) derivative **3** of valiolamine has been introduced as an effective therapeutic agent for diabetes.[31,32] Consequently, the hidden skeleton **5** in **3** is again an interesting target. The presence of basic groups in such designed targets which could enhance their binding to the enzyme as well as the simplicity and low cost of the synthesis of the target compounds render these targets attractive for synthesis. Thus, we have synthesized the amidine **4** and the N-(1,3-dihydroxyprop-2-yl) derivative **5** possessing a β-hydroxyethyl fragment as well as the analogue of **5** posessing a morpholine group. The glucosidase inhibition studies of these as well as the β-hydroxyethyl derivatives of some related saturated N-heterocyclic compounds have been investigated.



Scheme 1

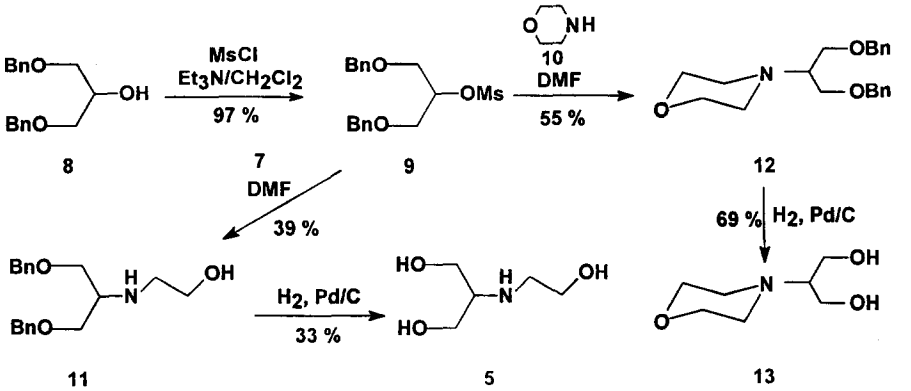
RESULTS AND DISCUSSION

The synthesis of the target compound N-(β-hydroxyethyl)acetamidine (**4**) was achieved by the reaction of ethanolamine (**7**) with thioacetamide (**6**) in refluxing methanol (Scheme 2). Its analytical data were in accordance with the structure (See experimental section).



Scheme 2

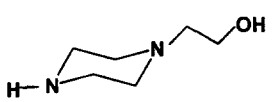
1,3-Di-*O*-benzylglycerol (**8**) was selected for the synthesis of the two target compounds **5** and **13**. Thus, mesylation of **8** with mesyl chloride in the presence of triethylamine gave 1,3-di-*O*-benzyl-2-*O*-mesylglycerol (**9**)[33] (Scheme 3). Nucleophilic displacement of the mesyloxy group was carried out by ethanolamine in N,N-dimethylformamide (DMF) to give the β-hydroxy ethylamino derivative **11**, hydrogenolytic debenzylation of which with Pd/C as catalyst gave target compound **5**. The structure of **11** was confirmed by the presence of a multiplet in its ^{1}H NMR spectrum at δ 3.52 corresponding to the two CH_2 groups of the ethanolamine residue. The disappearance of the aromatic protons in the ^{1}H NMR spectrum of **5** indicated the successful debenzylation. Similarly, the corresponding 2-morpholine derivative **12** was prepared by using morpholine for the displacement of the mesyloxy group in **9** to give **12**, catalytic hydrogenation of which gave **13**. The presence of the morpholine ring in **12** was confirmed by the presence of a triplet at δ 3.61 corresponding to the two NCH_2 and a triplet at δ 3.68 corresponding to the two OCH_2 groups. The structure of **13** was confirmed by the disappearance of the aromatic protons signals in its ^{1}H NMR spectrum.



Scheme 3

Inhibition studies: The assay method[34] was based on the treatment of o-nitrophenyl β-D-glucopyranoside (ONPG) with β-D-glucosidase from sweet almond and measuring the continuous release of o-nitrophenol. The Michaelis-Menten constant (K_M) at pH 6.8 was determined to be 0.07 M. The kinetic parameters were determined from the Lineweaver-Burk plots.[35] In Table 1 the inhibition constants (K_i) of **4** and **5** are compared

with those of the related compounds **13-16** and the important known[17,36] inhibitors **1** and **2**. The values of K_i (~10^{-4} M) indicate that **4, 5** and **13-16** are less effective inhibitors of the β-glucosidase enzyme than compounds **1** and **2**, but they are of the same magnitude as that of volioline[1] (K_i 8.1 x 10^{-4} M). Compounds **4, 5** and **13-16** exhibit uncompetitive inhibition whereas the lead compounds **1** and **2** are competitive. Moreover, although volioline has a low potent inhibition towards β-glucosidase from sweet almond it has a strong inhibition of intestinal sucrase-isomaltase. Only few examples of uncompetitive inhibition of β-glucosidases or glucanases have been repoerted in literature.[37-40] Therefore, totally different inhibition mechanisms seem to be operative. The inhibition properties towards α-glucosidase enzymes have not yet been measured; **5** showed good competitive inhibition of yeast α-glucosidase.[30]



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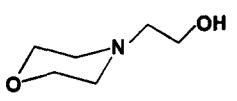

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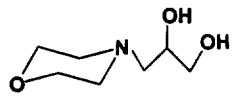

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Table 1. Inhibition constants and type of inhibition of the target compounds by β-glucosidase from sweet almond

Inhibitor No.	K_i [M]	Type of inhibition	Inhibitor No.	K_i [M]	Type of inhibition
1[17]	8.4 x 10^{-6}	Competitive	**13**	7.3 x 10^{-4}	Uncompetitive
2 (R = H)[36]	1.8 x 10^{-5}	Competitive	**14**	7.1 x 10^{-4}	Uncompetitive
4	3.8 x 10^{-4}	Uncompetitive	**15**	6.2 x 10^{-4}	Uncompetitive
5	9.1 x 10^{-4}	Uncompetitive	**16**	6.4 x 10^{-4}	Uncompetitive

EXPERIMENTAL PART

General Procedure. All reactions were monitored by TLC on Silica gel 60 F_{254} (Merck) with detection by charring with sulfuric acid. Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography. ^{1}H NMR spectra (250 MHz) were recorded with a Bruker spectrometer using Me_4Si as the internal standard. ^{13}C NMR spectra were recorded with a Bruker 250 MHz instrument at 62.9 MHz. Mass spectra were recorded using electron ionization (EI) on a Varian MA 311A spectrometer and fast atom bombardement (FAB) on a Kratos MS 50 spectrometer. Microanalysis were performed at the Fakultät für Chemie, Universität Konstanz, Germany.

N-(β-hydroxyethyl)acetamidine (4). A solution of thioacetamide (**6**) (5 g, 66.6 mmol) and ethanolamine (**7**) (8.12 g, 133 mmol) in methanol (50 mL) was heated under reflux with stirring at 80 °C for 5 h. The solvent was

evaporated under reduced pressure and the residue was purified by FC using EtOAc-MeOH (4:1) to give **4** as an oil (5.1 g, 75%); ^{1}H NMR (250 MHz, $CDCl_3$), δ: 2.58 (s, 3H, CH_3), 2.95 (s, 1H, OH), 3.82-3.88 (m, 4H, 2 x CH_2), 8.14 (br s, 2H, NH_2); ^{13}C NMR (62.9 MHz, $CDCl_3$), δ: 33.98 (CH_3), 48.25 (CH_2), 60.16 (CH_2), 201.72 (C=N); MS (EI): m/z (%): 102 (M^+, 11), 76 (41), 59 (100). Anal. Calcd. for $C_4H_{10}N_2O$: C, 47.03; H, 9.86; N, 27.42. Found: C, 46.90; H, 9.88; N, 27.13.

1,3-Di-*O*-benzyl-2-*O*-methanesulfonyl-glycerol (9). Methanesulfonyl chloride (2.57 mL, 33.2 mmol) was added dropwise to a stirred solution of 1,3-di-*O*-benzylglycerol (**8**) (4.54 g, 16.7 mmol) and triethylamine (3.5 mL) in dichloromethane (12.5 mL) at -30 °C. After 30 min the reaction mixture was poured onto a mixture of ice-cooled 1 M HCl (12.5 mL) and $CHCl_3$ (10 mL) and the organic layer was processed as reported[33] to give a syrup (5.7 g, 97%) which was used without any further purification; ^{1}H NMR (250 MHz, $CDCl_3$), δ: 2.90 (s, 3H, CH_3), 3.56-3.67 (m, 4H, 2 x CH_2), 4.44 (s, 4H, 2 x CH_2), 4.80-4.88 (m, 1H, CH), 7.17-7.30 (m, 10H, Ar-H).

Nucleophilic displacement studies on 1,3-di-*O*-benzyl-2-*O*-methanesulfonyl-glycerol. *General Procedure.* A solution of 1,3-di-*O*-benzyl-2-*O*-methanesulfonyl-glycerol (**9**) (2.8 g, 8 mmol) in DMF (3 mL) was treated with morpholine or ethanolamine (5 mL) and the mixture was heated under reflux for 25-30 h. The solvent was removed in vacuo to give a syrup which was dissolved in chloroform (100 mL) and washed with water. The chloroform layer was dried over Na_2SO_4 and evaporated to give a syrup which was purified by FC using light petroleum-EtOAc (5:1).

1,3-Di-*O*-benzyl-2-deoxy-2-(1-hydroxyeth-2-yl)amino-glycerol (11). Yield 0.98 g (39%) as an oil; R_f 0.56 (light petroleum-EtOAc = 6:4); ^{1}H NMR (250 MHz, $CDCl_3$), δ: 2.88 (brs, 2H, OH, NH), 2.80 (m, 2H, CH_2), 2.98 (m, 1H, CH), 3.52 (m, 4H, 2 x CH_2), 3.58 (m, 2 H, CH_2), 4.51 (s, 4H, 2 x CH_2), 7.18-7.31 (m, 10H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$), δ: 48.84 (CH_2), 56.92 (CH_2), 61.29 (CH), 70.47 (2 x CH_2), 73.32 (2 x CH_2), 127.67, 128.38, 138.17 (Ar-C). Anal. Calcd for $C_{19}H_{25}NO_3$: C, 72.35; H, 7.98; N, 4.44. Found: C, 72.15; H, 7.94; N, 4.45.

1,3-Di-*O*-benzyl-2-deoxy-2-(morpholin-4-yl)glycerol (12). Yield 1.5 g (55%) as an oil; R_f 0.82 (light petroleum-EtOAc = 6:4); ^{1}H NMR (250 MHz, $CDCl_3$), δ: 2.68 (t, 4H, J = 4.6 Hz, 2 CH_2), 2.84 (m, 1H, CH), 3.61 (t, 4H, J = 3.6 Hz, 2 x NCH_2), 3.68 (t, 4H, J = 3.7 Hz, 2 x OCH_2), 4.50 (s, 4H, 2 x CH_2), 7.21-7.33 (m, 10H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$), δ: 50.80 (2 x CH_2), 63.72 (CH), 67.51 (2 x CH_2), 68.28 (2 x CH_2), 73.24 (2 x CH_2), 73.24 (2 x CH_2), 127.56, 127.60, 128.34 (Ar-C): MS (EI): m/z (%): 341 (M^+, 16), 235 (13), 220 (70). Anal. Calcd for $C_{21}H_{27}NO_3$: C, 73.87; H, 7.96; N, 4.10. Found: C, 73.44; H, 7.78; N, 3.88.

Catalytic hydrogenation of 1,3-di-*O*-benzyl-2-deoxy-2-substituted amino-glycerol. *General Procedure.* To a solution of **11** or **12** (1 g) in ethyl acetate (35 mL) and methanol (35 mL) was added 10% Pd/C (0.3 g). The

reaction mixture was stirred under hydrogen overnight and then additional of Pd/C (0.1 g) was added, and hydrogenation was continued overnight. The mixture was filtered through Celite and the filtrate was concentrated and purified by FC using EtOAc.

2-Deoxy-2-(1-hydroxyeth-2-yl)amino-glycerol (5). Yield 0.14 g (33%) as an oil; R_f 0.26 (light petroleum-EtOAc = 1:1); ^{1}H NMR (250 MHz, DMSO-d_6), δ: 2.50 (m, 1 H, OH), 2.77 (m, 1 H, CH), 3.23-3.44 (m, 8 H, 4 x CH_2), 3.69 (m, 1H, OH), 5.40 (brs, 1H, NH); ^{13}C NMR (62.9 MHz, DMSO-d_6), δ: 56.43 (2 x CH_2), 57.01 (CH_2), 58.15 (CH), 66.94 (CH_2); MS (EI): m/z (%): 315 (M^+, 6), 284 (70), 220 (20), 206 (76), 194 (100). Anal. Calcd for $C_5H_{13}NO_3$: C, 44.43; H, 9.69; N, 10.36. Found: C, 44.13; H, 9.61; N, 10.17.

2-Deoxy-2-(morpholin-4-yl)glycerol (13). Yield 0.33 g (69%) as an oil; R_f 0.3 (light petroleum-EtOAc = 1:1); ^{1}H NMR (250 MHz, $CDCl_3$), δ: 2.63-2.74 (m, 5H, CH, 2 x CH_2), 3.65-3.73 (m, 10H, 4 x CH_2, 2 x OH); ^{13}C NMR (62.9 MHz, $CDCl_3$), δ: 49.49 (2 x CH_2), 59.05 (2 x CH_2), 66.04 (CH), 67.19 (2 x CH_2); MS (EI): m/z (%): 161 (M^+, 14), 130 (100). Anal. Calcd for $C_7H_{15}NO_3$: C, 52.15; H, 9.37; N, 8.68. Found: C, 52.06; H, 9.39; N, 8.58.

Inhibition studies. The inhibitory activity of compounds **4, 5,** and **13-16** on the hydrolysis of o-nitrophenyl β-D-glucopyranoside was determined.

(a) Materials. Buffer substances (potassium dihydrogenphosphate and disodium hydrogenphosphate) were purchased from Fluka and used as received. β-D-Glucosidase (Sweet almond) and o-nitrophenyl β-D-glucopyranoside were obtained from Boehringer Mannheim.

(b) Preparation of solutions. 1. *The buffer solution.* Potassium dihydrogenphosphate (9.07 g) was dissolved in distilled water and diluted to 1 L at 20 °C to give 0.07 M solution (A). Disodium hydrogenphosphate (11.8 g) was dissolved in distilled water and dilution was completed to 1 L at 20 °C to give 0.07 M solution (B). Then, 50 mL of A was mixed with 50 mL of B for use. 2. *Enzyme solution.* 1 mg of β-D-glucosidase was dissolved in the buffer solution (5 mL, pH 6.8) and used for assay without further dilution. 3. *The substrate solution.* ONPG (82.2 mg) was dissolved in the buffer solution (6.50 mL), (c = 42 mM), for enzyme assay. 4. *The inhibitor solution.* Compound **4**, **5**, or 13–16 (16 mg) was dissolved in the buffer solution (50 mL), (c = 1.80 mM). Inhibitor concentrations of 0.856, 0.571, 0.285 and 0.142 mM were used to determine the K_i value.[41] At each inhibitor concentration, six substrate concentrations 20, 10, 5, 3, 2.5 and 2 mM were used.

(c) Procedure for enzyme assays: To a 1.00 mL disposable cuvette was added buffer solution (500 μL) and ONPG-solution (500 μL). The solution was thermally equilibrated at 30 °C. The reaction was started by addition of 50.0 μL of β-D-glucosidase solution. Liberation of ONPG[36] was monitored using a PU 8740 UV/VIS-spectrophotometer, for 4 min (λ = 405 nm), and the initial hydrolysis rate was calculated according to the following equation: $1/V = 1/[S]\ (K_m/V_{max}) + 1/V_{max}$.

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