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Derivatives of (2*R*,3*R*,4*S*)-2-Aminomethylpyrrolidine-3,4-diol are Selective α-Mannosidase Inhibitors

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Abstract—A collection of (2*R*,3*R*,4*S*)-3,4-dihydroxypyrrolidin-2-yl derivatives have been tested for their inhibitory activities toward 25 glycosidases. Competitive ($K_i = 7.4$ μM) and selective inhibition of α-mannosidase from jack bean has been found for (2*R*,3*R*,4*S*)-2-[(benzylamino)methyl]pyrrolidine-3,4-diol and other derivatives.

The specific inhibition of *N*-linked glycoprotein-processing α-mannosidases may provide a useful anti-cancer strategy.[1] Clinical trials have shown that swainsonine, a natural α-mannosidase inhibitor that contains a 4-amino-4-deoxy-mannofuranoside moiety[2,3] reduces solid tumors and hematological malignancies.[4] Simpler synthetic analogues are also potent α-mannosidase inhibitors.[3,5] Mannostatin A and B isolated from the soil microorganism *Streptoverticillum verticillus*[6] and a synthetic analogue[7] are probably the most potent inhibitors of α-mannosidases reported thus far.[5,8] Often, α-mannosidase inhibitors that are monosaccharide mimics[9,10] also inhibit other glycosidase types,[11] in particular α-L-fucosidases.[9,12] It is believed that enzyme selectivity could be improved if the iminosugar, which mimics the pyranosyl cation intermediate liberated during the hydrolytical process, would include some information of the glycosidic bond that is cleaved (α vs β, site of glycosidation of the aglycon) and of the aglycon itself (shape, electrostatic and/or lyophilic interaction with the enzyme). Such inhibitors could be disaccharide mimics such as dideoxy-imino-alditols linked to monosaccharides through non-hydrolyzable linkages.[13] For the moment, the syntheses of these disaccharide mimics are lengthy and do not offer the necessary flexibility for drug development. Furthermore, to become a drug, a good inhibitor must satisfy a number of conditions[14] such as membrane permeability, which often requires the presence of lyophilic groups. We show in this note that readily prepared (2*R*,3*R*,4*S*)-2-(benzylamino)-methyl-3,4-dihydroxypyrrolidine and analogues that can be viewed to mimic a transition or intermediate structure of the hydrolytic process in which the enzyme intervenes with two carboxylic groups (Fig. 1)[15] can be good inhibitors of α-mannosidases.[16]

Following Fleet's method,[17] we converted D-gulonolactone into **1** that was then transformed into aldehyde **2** (33% overall yield based on D-gulonolactone, seven steps).[18] Aldehyde **2** reacted with one equivalent of primary amine RNH_2 (b–k) in anhydrous 1,2-dichloroethane at 20 °C giving the corresponding imines that were reduced in situ with $NaBH(OAc)_3$ into the corresponding amines **3b**–**3k** (65–90% yield after aqueous work up with $NaHCO_3$ and purification by flash chromatography on silica gel).

Deprotection [CF_3COOH/H_2O (4:1) or HCl 4 M in H_2O, 20 °C, 1–2 h] provided the corresponding diamines **4b**–**4k** nearly quantitatively. Hydrogenolysis [H_2/$Pd(OH)_2$/charcoal, MeOH] of the benzylamino derivative **3i** furnished **3a** that was deprotected (HCl 4 M) to give diamine **4a**. Acetylation of **3i** (Ac_2O/pyridine/4-dimethylaminopyridine, 20 °C) gave **5i** (95% yield) that was hydrolyzed with CF_3COOH/H_2O 4:1 (20 °C) providing acetamide **6i** in good yield (Scheme 1). The

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known *cis*-pyrrolidine-3,4-diol (**7**)[19] was also included in our enzymatic assays (see below) together with triol **8**[5,20] (derived by $NaBH_4$ reduction of **2** and acidic hydrolysis) and tetrol **9** (obtained by acidic hydrolysis of **1**).[17]

We have tested compounds **4a**–**4k**, **6i**, **7**, **8** and **9** for their inhibitory activities toward 25 commercially available glycosidases. The data are summarized in the Table 1 for five β-galactosidases, five α-glucosidases, two β-glucosidases, and two α-mannosidases. Unless indicated otherwise, these compounds did not show any inhibitory activity at 1 mM concentration toward the following enzymes: α-galactosidases from *Aspergillus niger*, from *Escherichia coli* and from coffee bean, α-L-fucosidase from bovine epididymis, β-mannosidase from *Helix pomatia*, β-xylosidase from *A. niger*, β-*N*-acetylglucosaminidase from jack bean, from bovine epididymis A and B and α-*N*-acetylgalactosaminidase from chicken liver.

The simple *cis*-pyrrolidine-3,4-diol (**7**) is a weak inhibitor of several glycosidases including α-mannosidases. Potency and enzyme selectivity does not change very much on introducing a (2*R*)-hydroxymethyl substituent as in **8** (Table 1). Surprisingly, the introduction of a (2*R*,1′*R*)-1,2-dihydroxyethyl substituent as in **9** suppresses most inhibitory activity. The latter compound is a weak but selective inhibitor of β-glucosidase ($IC_{50} = 320\ \mu M$) from almond. In agreement with the hypothesis of Figure 1, diamine **4a** is a better inhibitor of α-mannosidases than **7**–**9**. With α-mannosidase from jack bean, $K_i = 53\mu M$ was measured [competitive inhibition with *p*-nitrophenyl α-D-mannopyranoside (Lineweaver–Burk plots)].[21] With the hope that alkylation of the primary amines moiety of **4a** might improve inhibitory activity and selectivity toward α-mannosidases, we tested **4b**–**4k**. Except for the *N*-α-thiophenylmethyl **4g** and *N*-benzyl

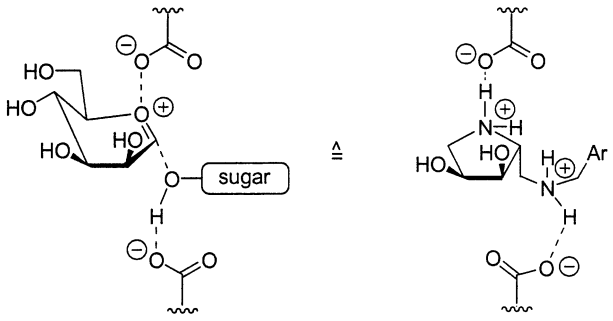
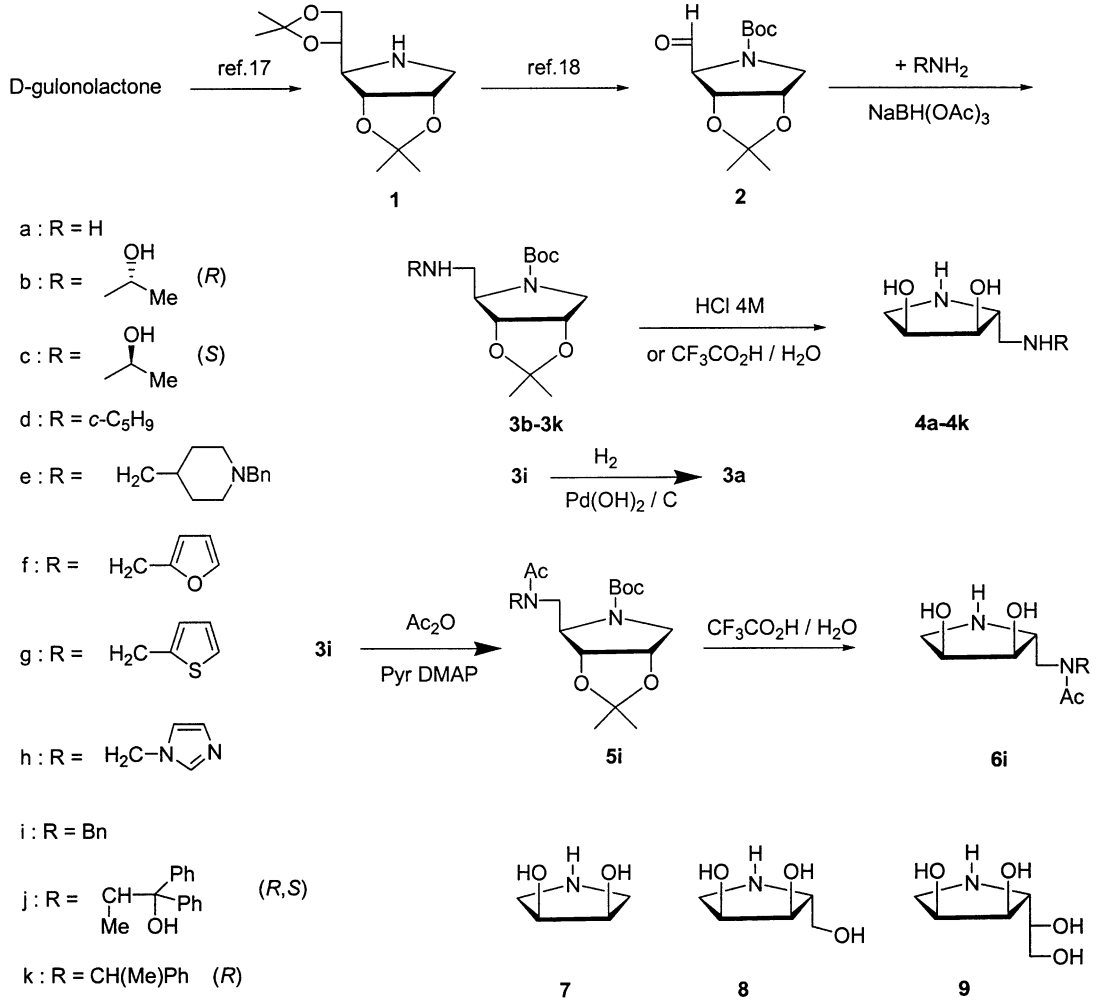


Figure 1. Design of dicationic mimics of a transition or intermediate structure of an α-mannosidase-catalyzed hydrolysis of an α-D-mannopyranoside.



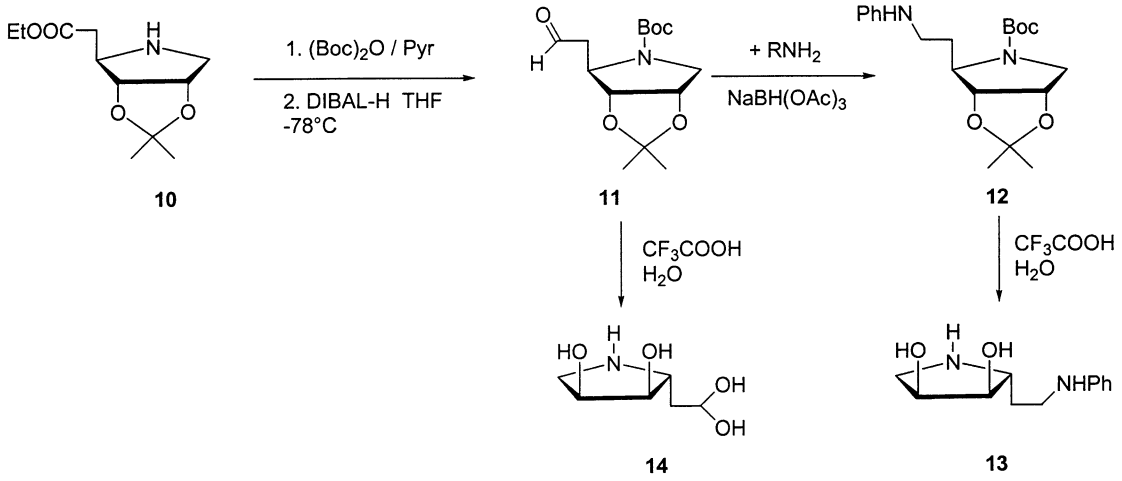
Scheme 1.

Table 1. Inhibitory activities of (2*R*,3*R*,4*S*)-3,4-dihydroxypyrrolidin-2-yl derivatives. Percentage of inhibitions at 1 mM concentration, IC_{50} and K_i in μM, when measured. Optimal pH, 35 °C[a,b]

Enzyme/inhibitor	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**
β-Galactosidase									
E. coli	92%	36%	50%	ni	39%	ni	ni	49%	24%
Bovine liver	ni	ni	ni	ni	53% IC_{50} = 770	ni	ni	ni	26%
A. niger	24%	47%	61% IC_{50} = 480	26%	ni	39%	ni	41%	ni
A. orizae	60%	ni	32%	ni	ni	ni	ni	24%	ni
Jack bean	76%	39%	46%	21%	26%	34%	ni	36%	ni
α-Glucosidase									
Yeast (maltase)	84%	46%	59% IC_{50} = 410	30%	55% IC_{50} = 610	66% IC_{50} = 280	ni	55% IC_{50} = 610	ni
Rice (maltase)	53%	ni	ni	ni	ni	ni	ni	ni	ni
Baker yeast (isomaltase)	98%	84% IC_{50} = 250 K_i = 83 (C)	94% IC_{50} = 70 K_i = 77 (M)	92% IC_{50} = 170 K_i = 71 (M)	90% IC_{50} = 120 K_i = 40 (C)	94% IC_{50} = 65 K_i = 45 (M)	23%	90% IC_{50} = 120 K_i = 40 (C)	ni
A. niger (amyloglucosidase)	ni	ni	ni	ni	ni	ni	ni	ni	ni
Rhizopus mold (amyloglucosidase)	ni	ni	ni	ni	ni	ni	ni	ni	ni
β-Glucosidase									
Almond	97%	67% IC_{50} = 410	77% IC_{50} = 290 K_i = 340 (M)	62% IC_{50} = 500	70% IC_{50} = 430	82% IC_{50} = 140 K_i = 12 (C)	43%	70% IC_{50} = 420	68% IC_{50} = 370
Caldocellum saccharol.	93%	72% IC_{50} = 340	82% IC_{50} = 180 K_i = 170 (M)	29%	76% IC_{50} = 290 K_i = 120 (C)	75% IC_{50} = 250 K_i = 60 (M)	38%	76% IC_{50} = 290 Ki = 120 (C)	ni
α-Mannosidase									
Jack bean	81% IC_{50} = 170 K_i = 53 (C)	75% IC_{50} = 300 K_i = 59 (C)	66% IC_{50} = 490 K_i = 120 (C)	60% IC_{50} = 560	55% IC_{50} = 700	49%	89% IC_{50} = 85 K_i = 26 (C)	55% IC_{50} = 700	92% IC_{50} = 60 K_i = 7.4 (C)
Almond	51% IC_{50} = 1mM	39%	45%	53%	59% IC_{50} = 570	29%	68% IC_{50} = 350 K_i = 98 (C)	59% IC_{50} = 570	69% IC_{50} = 230 K_i = 71 (C)

Enzyme/inhibitor	**4j**	**4k**	**6i**	**7**	**8**	**9**[c]	**13**	**14**
β-Galactosidase								
E. coli	25%	34%	ni	41%	ni	ni	ni	48%
Bovine liver	84% IC_{50} = 140 K_i = 21 (M)	ni	ni	25%	51%	32%	ni	32%
A. niger	29%	ni	ni	39%	ni	ni	ni	91% IC_{50} = 140 K_i = 200 (M)
A. orizae	ni	ni	ni	53% IC_{50} = 790	ni	ni	ni	80% IC_{50} = 75 K_i = 75 (M)
Jack bean	22%	ni	ni	55% IC_{50} = 720	21%	ni	ni	84% IC_{50} = 110 K_i = 25 (M)
α-Glucosidase								
Yeast (maltase)	28%	ni	ni	ni	50%	ni	ni	ni
Rice (maltase)	ni	ni	ni	ni	30%	ni	ni	ni
Baker yeast (isomaltase)	46%	34%	33%	32%	73% IC_{50} = 330	ni	ni	28%
A. niger (amyloglucosidase)	ni	ni	ni	24%	96% IC_{50} = 31 K_i = 64 (M)	ni	ni	21%
Rhizopus mold (amyloglucosidase)	ni	ni	ni	47%	92% IC_{50} = 20 K_i = 44 (N)	ni	ni	40%
β-Glucosidase								
Almond	61% IC_{50} = 550	35%	69% IC_{50} = 430 K_i = 220 (M)	60% IC_{50} = 600	88% IC_{50} = 120 K_i = 53 (M)	73% IC_{50} = 320	ni	91% IC_{50} = 70 K_i = 27 (C)
Caldocellum saccharol.	72% IC_{50} = 320	49%	43%	ni	90% IC_{50} = 85 Ki = 19 (C)	40%	ni	90% IC_{50} = 80 K_i = 27 (C)
α-Mannosidase								
Jack bean	ni	66% IC_{50} = 440 K_i = 170 (C)	ni	70% IC_{50} = 400	54% IC_{50} = 800	ni	33%	81% IC_{50} = 180 K_i = 80 (M)
Almond	ni	48%	ni	40%	37%	ni	ni	56%

[a]For the conditions of measurements, see ref 21.
[b](C), competitive; (M), mixed type; (N), non-competitive inhibition; ni, no inhibition at 1 mM concentration.
[c]Inhibits also α-galactosidase from coffee bean (42% at 1 mM) and β-xylosidase from *A. niger* (42% at 1 mM).



10 11 12 14 13

Scheme 2.

derivatives **4i** all diamines **4b**–**4h** showed inhibitory activities similar to that of **4a**. The bulky *N*-(3-hydroxy-2-methyl-3,3-diphenylpropyl) derivative **4j**, apparently, cannot enter in the active site of the α-mannosidases. The best inhibitory activity ($K_i = 7.4$ μM, competitive) was found for **4i** toward α-mannosidase from jack bean.

This diamine also presents the best selectivity toward α-mannosidases. Substitution of the benzyl group of **4i** by a (1′*R*)-α-methylbenzyl group decreases the inhibitory activity by a factor of 25. Apparently, the diamino moiety of **4i** is necessary for this compound to bind to α-mannosidases as the acetamide **6i** did not inhibit these enzymes (bulk of the acetamido moiety?). In order to test the importance of the (2*R*)-aminomethyl group of **4** in a possible electrostatic interaction with a carboxylic group of the α-mannosidases, we prepared **13**, a structural isomer of **4i** in which the amine moiety of the side chain is further apart from the pyrrolidine base than in **4a**–**k**. In agreement with our working hypothesis (Fig. 1), **13** is a much weaker α-mannosidase inhibitor than **4i** (Table 1).

Interestingly, this compound ignores other glycosidases tested. Diamine **13** was prepared as shown in Scheme 2, starting from the known ester **10**.[22] Amine protection, followed by reduction with DIBAL-H (THF, −78 °C) gave an intermediate aldehyde **11** that was reacted with one equivalent of aniline and $NaBH(OAc)_3$ in 1,2-dichloroethane. This provided **12** that was deprotected on acidic treatment (CF_3COOH/H_2O, 20 °C) to give **13** (88% yield). Acidic treatment of **11** (CF_3COOH/H_2O) furnished **14** that was also submitted to the enzymatic assays (Table 1). Pyrrolidine **14** is a moderate inhibitor of α-mannosidase from jack bean with a mixed-type of inhibition. As for compounds **7** and **8**, **14** inhibits several other glycosidases.

A new lead for the selective inhibition of α-mannosidases has been found with (2*R*,3*R*,4*S*)-2-[(benzylamino)-methyl]pyrrolidine-3,4-diol.[23] Work is underway to apply parallel synthesis to generate further diamines **4** and to introduce (5*R*)-hydroxymethyl and other substitution on their pyrrolidine rings.

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23. Selected data for **4i**: ^{1}H NMR (400 MHz, MeOD): 7.54 (m, 5H); 4.37 (ddd, $^3J=1.6$, 4.2, 9.0 Hz, H-4); 4.29 (m, 2H, H-1″); 4.16 (dd, $^3J=4.2$, 9.0 Hz, H-3); 3.73 (ddd, $^3J=4.2$, 8.6, $^2J=13.4$ Hz, H-2); 3.52 (dd, $^3J=4.2$, $^2J=13.1$ Hz, H-5); 3.46 (dd, $^3J=8.6$, $^2J=13.4$ Hz, H-1′); 3.37 (dd, $^3J=1.6$, $^2J=13.1$ Hz, H-5). ^{13}C NMR (101 MHz, MeOD): 134.2, 132.1, 131.7, 76.6, 71.7, 59.6, n54.4, 52.8, 49.6. CI-MS (NH_3): 223 (100), 222 (43), 211 (4), 120 (22), 91 (25), 77 (29). Selected data for **13**: ^{1}H NMR (400 MHz, MeOD): 7.06–7.12 (m, 2H); 6.58–6.66 (m, 3H); 4.05 (m, H-4); 3.60 (dd, $^3J=5.3$, 7.8 Hz, H-3); 3.18–3.26 (m, 3H, H-2′a, H-2′b, H-5a); 3.01 (td, $^3J=7.9$ Hz); 2.80 (dd, $^3J=3.4$, $^2J=12.2$ Hz, H-5b) ; 1.96 (m, H-1′a); 1.69 (m, H-1′b).^{13}C NMR (101 MHz, MeOD): 150.1, 130.0, 118.2, 114.3, 78.3, 72.2, 61.3, 52.4, 42.7, 33.8. $[\alpha]_{589}^{25}=+37$ (*c* 1.2, CH_3OH).