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α-Rhamnosidase inhibitory activities of polyhydroxylated pyrrolidine

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Abstract—We designed and synthesized polyhydroxylated pyrrolidines **1**–**12** from L-tyrosine, L-phenylalanine, and D-tyrosine through iodine-mediated intramolecular cyclization followed by Woodward–Prevost reaction. The synthetic polyhydroxylated pyrrolidines were identified with structure-based inhibitory activity and selective inhibitory activity against α-rhamnosidase. (2*S*,3*S*,4*R*)-deacetyl anisomycin **7** was the best inhibitor among the 12 polyhydroxylated pyrrolidines because it possesses the same stereoconfiguration at C1, C2, C3 as α-L-rhamnopyranoside. An investigation into the nature of the inhibition showed that the synthetic pyrrolidines are competitive inhibitors. They also did not have remarkable inhibitory activity against seven glycosidases (α-glucosidase, α-mannosidase, α-amylase, β-glucosidase, β-galactosidase, β-amylase, and invertase).

1. Introduction

α-Rhamnosidase (EC. 3.2.1.40) hydrolyzes both natural and synthetic rhamnosides liberating L-rhamnose. To date, it has been found in *Rhamus dahurica* seed,[1] *Aspergillus niger*,[2] animal tissue,[3] and several bacteria.[4] Furthermore, α-rhamnosidase is involved in both the cleavage *O*-antigen tetrasaccharides,[5] the first phase of the bacterial invasion of a host cell, and also hydrolysis of rhamnogalacturonans which have a key role as immunomodulators, enhancing the cytotoxicity of human NK cells.[6] Hence, inhibitors of this enzyme are considered to be potential therapeutic agents for bacillary dysentery[5a] and cancer.[6b] Though the physiological functions of α -rhamnosidase are not completely understood, potent rhamnosidase inhibitors may be used as probes both to investigate the function of rhamnosidase and also for the development of potential therapeutic agents. Nitrogen-in-the-ring carbohydrate mimics (azasugars) have proved to be highly potent glycosidase inhibitors useful for studies into glycoconjugate function, targeting, and turnover.[7] The majority of azasugars used as inhibitors have been based on logical designs aimed specifically at a limited range of enzymes: glucosidase, mannosidase, and galactosidase. On the contrary, there are a few reports establishing the synthesis and evaluation of inhibitors of α-rhamnosidase.

Although inhibitors based on piperidine analogues of L-rhamnose[8] and tetrazole analogues[9] have been reported, pyrrolidine inhibitors are rarely screened for α-rhamnosidase inhibition.[10] In spite of this, pyrrolidine-based inhibitors are typically much more effective than piperidine analogues of L-rhamnose.[11] Recently, pyrrolidine analogues showed a potent inhibitory activity against α-rhamnosidase, which could be explained on the basis of structural similarities and differences between the analogues and L-rhamnose. Chapman et al[10b] suggested that a stereochemical rational for the inhibition shown by the pyrrolidines could be given in terms of the configurational similarities between OH-3,4 and C5 of L-rhamnose and OH-3,4 and C2 of polyhydroxy pyrrolidines (Fig. 1). These suggestions have some associated doubts because the study was based on only a few stereoisomers. In a preliminary

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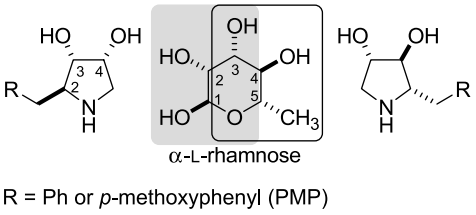
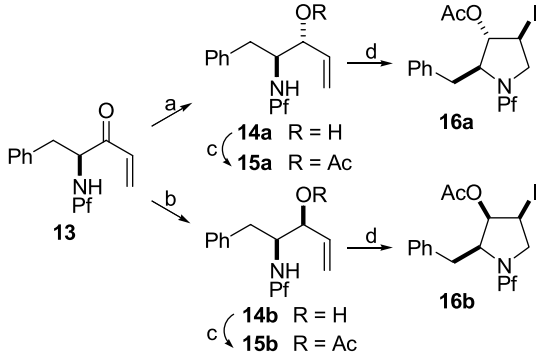


Figure 1. Design of stereoisomer of polyhydroxylated pyrrolidine as a potent α-rhamnosidase inhibitor.

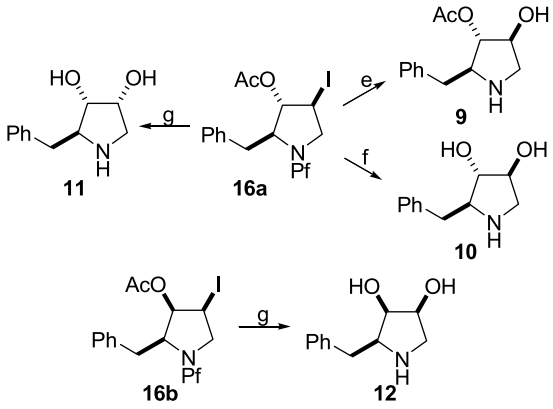
study, we have established a new stereodivergent synthetic approach to dihydroxylated pyrrolidines based on the Woodward–Prevost reaction[12] leading to the synthesis of four polyhydroxylated pyrrolidines **1**–**4** from D-tyrosine.[13] Such a stereoselective process leading to stereoselective synthesis of a range of stereoisomers of a pyrrolidine is of utmost utility and importance for the development of structure-based glycosidase inhibitors. To further facilitate the discovery of new specific α-rhamnosidase inhibitors, we tried to synthesize polyhydroxylated pyrrolidines through our newly developed stereodivergent synthetic protocols.[12] This approach led to the discovery of the most potent pyrrolidine inhibitors of α-rhamnosidase to date.

2. Synthesis

Recently, we reported a stereodivergent synthesis of anisomycin derivatives **1**–**4** from D-tyrosine[13] and these stereoiosmers **5**–**8** were synthesized from L-tyrosine as the same synthetic protocols using the Woodward–Prevost reaction as a key step.[12] Here we described the syntheses of pyrrolidine **9**–**12** as shown in Schemes 1 and 2, starting from L-phenylalanine. Compound **13** was obtained in five steps with the final step being a Swern oxidation.[12] The unsaturated ketone **13** was stereoselectively reduced to give either: **14a** using (*S*)-BINAL; or **14b** using BH_3-$(CH_3)_2S$. Each of the enantiomerically pure amino alcohols **14a** and **14b** was acetylated, and the ensuing stereoselective iodine-mediated intramolecular cyclization gave **16a** and **16b**, respectively. Finally, the *trans*-iodoacetated pyrrolidine was heated in the presence of



Scheme 1. Reagents and conditions: (a) (*S*)-BINAL, THF, −78 °C; (b) BH_3-$(CH_3)_2S$, toluene, −78 °C; (c) Ac_2O, Et_3N, CH_2Cl_2, rt; (d) I_2, sat. $NaHCO_3$/THF/Et_2O (2/1/1), rt.



Scheme 2. Reagents and conditions: (e) $AgOCOCF_3$, toluene, reflux and H_2, Pd/C, EtOAc, rt; (f) AgOAc, toluene, reflux, $LiAlH_4$, THF, 0 °C, and H_2, Pd/C, EtOAc, rt; (g) $AgBF_4$, wet toluene, rt, $LiAlH_4$, THF, 0 °C, and H_2, Pd/C, EtOAc, rt.

AgOAc under Prevost conditions to afford the corresponding diacetylated pyrrolidine. Subsequent deprotection of the acetyl group and Pf (9-phenyl fluoren-9-yl) group led to *trans*-dihydroxylated pyrrolidine **10**.

Acetylated pyrrolidine **9** was obtained by reaction under Prevost conditions with $AgOCOCF_3$ followed by hydrogenation with Pd/C. Moreover, **16a** was treated with $AgBF_4$ under Woodward conditions, followed by reductive cleavage of the acetate with $LiAlH_4$ and hydrogenation with Pd/C to generate compound **11**.

Pyrrolidine **12**, possessing an all-*cis*-configuration, was obtained from *cis*-iodoacetated pyrrolidine **16b** through the same set of reactions as compound **11**. Structures of the all final compounds **1**–**12** were confirmed by spectroscopic data. The spectroscopic data of compounds **1**–**4**, **9**, and **10** were consistent with previous data.[12–14]

(2*S*,3*S*,4*S*)-Anisomycin (5). $[\alpha]_D$ +19.8 (*c* 0.7, MeOH). ^1H NMR (300 MHz; $CDCl_3$) δ 1.95 (1H, s), 2.02 (3H, s), 3.03 (1H, dd, J = 14.2, 8.7 Hz), 3.24 (1H, dd, J = 14.2, 7.2 Hz), 3.32 (2H, m), 3.71 (1H, m), 3.79 (3H, s), 4.31 (1H, m), 4.93 (1H, m), 6.90 (2H, d, J = 8.5 Hz), 7.22 (2H, d, J = 8.5 Hz).

(2*S*,3*S*,4*S*)-Deacetyl anisomycin (6). $[\alpha]_D$ −20.3 (*c* 1.0, MeOH). ^1H NMR (300 MHz; $CDCl_3$) δ 2.76 (1H, dd, J = 13.8, 8.1 Hz), 2.96 (2H, m), 3.12 (2H, m), 3.77 (3H, s), 3.79 (1H, m), 4.06 (1H, m), 6.87 (2H, d, J = 8.6 Hz), 7.19 (2H, d, J = 8.6 Hz).

(2*S*,3*S*,4*R*)-Deacetyl anisomycin (7). $[\alpha]_D$ −19.1 (*c* 1.0, MeOH). ^1H NMR (300 MHz; $CDCl_3$) δ 2.75 (1H, dd, J = 13.8, 8.1 Hz), 2.92 (2H, m), 3.11 (2H, m), 3.76 (4H, m), 4.05 (1H, m), 6.86 (2H, d, J = 8.3 Hz), 7.19 (2H, d, J = 8.6 Hz).

(2*S*,3*R*,4*S*)-Deacetyl anisomycin (8). $[\alpha]_D$ −1.0 (*c* 0.28, MeOH). ^1H NMR (300 MHz; $CDCl_3$) δ 3.14 (1H, dd, J = 14.1, 8.0 Hz), 3.33 (1H, m), 3.41 (1H, dd, J = 14.0,

6.7 Hz), 3.58 (1H, m), 3.88 (1H, m), 4.01 (3H, s), 4.23 (1H, m), 4.62 (1H, m), 7.13 (2H, d, $J = 8.5$ Hz), 7.49 (2H, d, $J = 8.5$ Hz).

(2*S*,3*S*,4*R*)-2-Benzyl-3,4-dihydroxy pyrrolidine (11). $[\alpha]_D$ −16.5 (*c* 1.0, MeOH). ^{1}H NMR (300 MHz; $CDCl_3$) δ 2.81 (1H, dd, $J = 13.5$, 8.1 Hz), 2.89 (1H, m), 3.08 (3H, m), 3.77 (1H, m), 4.05 (1H, m), 7.29 (5H, m).

(2*S*,3*R*,4*S*)-2-Benzyl-3,4-dihydroxy pyrrolidine (12). $[\alpha]_D$ −0.9 (*c* 0.4, MeOH). ^{1}H NMR (300 MHz; $CDCl_3$) δ 2.90 (1H, dd, $J = 13.6$, 7.6 Hz), 3.01 (1H, m), 3.17 (2H, m), 3.49 (1H, m), 3.95 (1H, m), 4.32 (1H, m), 7.27 (5H, m).

3. Biological activity

The polyhydroxylated pyrrolidines shown in Figure 2 were assessed as inhibitors of α-rhamnosidase, and the results are listed in Table 1. A comparison of the results for Gp II and Gp III shows that the inclusion of a *p*-methoxy group on the phenyl ring did slightly affect the activity. On the other hand, the potent inhibition of α-rhamnosidase shown by deacetylated pyrrolidine analogues **6** ($IC_{50} = 3.7$ μM) and **10** ($IC_{50} = 4.3$ μM) are in contrast to the weak activity shown by monoacetylated compounds **5** ($IC_{50} = 57.9$ μM) and **9** ($IC_{50} = 11.2$ μM) which possess the same stereochemistry as **6**.

Compounds **5**–**12** are stronger inhibitors than **1**–**4**. These results suggest that the 2*S*-configuration of pyrrolidines which were synthesized from L-amino acids, showed more inhibitory activity than the corresponding 2*R*-derived pyrrolidines from D-amino acids. However, all-*cis*-configured pyrrolidine **8** ($IC_{50} = 137.3$ μM) and **12** ($IC_{50} =$ 168.1 μM) both have relatively small inhibitory activity when compared with other 2*S*-configured pyrrolidines.

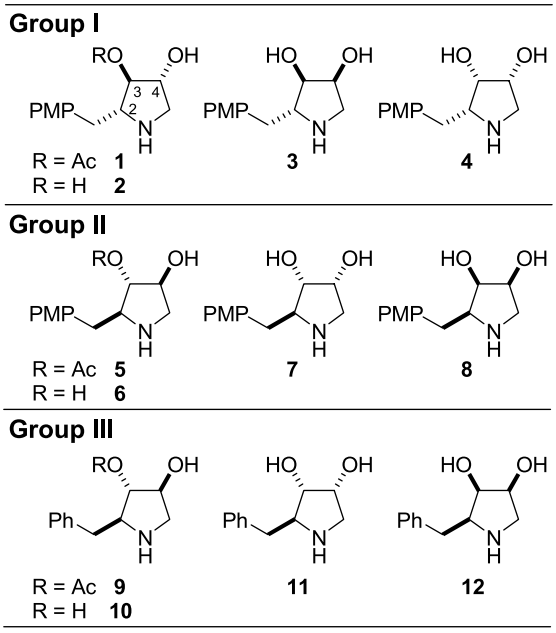


Figure 2. Target compounds for selective α-rhamnosidase inhibition.

Table 1. Inhibitory activities of pyrrolidines against α-rhamnosidase[a]

Compound No.		α-Rhamnosidase	
		IC_{50} (K_i, μM)	Inhibition type
Group I	**1**	NI[b]	NT[c]
	2	209.4	NT
	3	183.8	NT
	4	NI	NT
Group II	**5**	57.9 (51.5)	Competitive
	6	3.7 (2.9)	Competitive
	7	3.2 (2.6)	Competitive
	8	137.3	NT
Group III	**9**	11.2 (10.0)	Competitive
	10	4.3 (3.4)	Competitive
	11	3.6 (2.9)	Competitive
	12	168.1	NT

[a] Inhibitory activity were tested with Ref. 15.
[b] NI: no inhibition, less than 30% inhibition at the concentration of 200 μM.
[c] NT: Not tested.

Compound **7**, (2*S*,3*S*,4*R*)-deacetylanisomycin, possessing the same configuration at C1, C2, and C3 as α-L-rhamnopyranoside (Fig. 3), has the best inhibitory activity ($IC_{50} = 3.2$ μM).

These results show that C2 hydrophobic substituents on the pyrrolidine ring have a role as aglycone. And the stereochemistry of C2 and C3 of pyrrolidine were strongly related with the binding affinity in the active site of α-rhamnosidase, while the one of C4 were not.

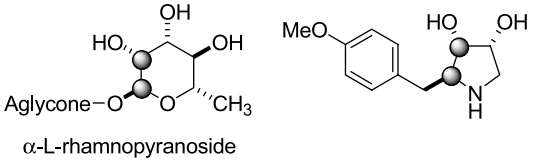


Figure 3. α-L-Rhamnoside and compound **7** as the best inhibitor.

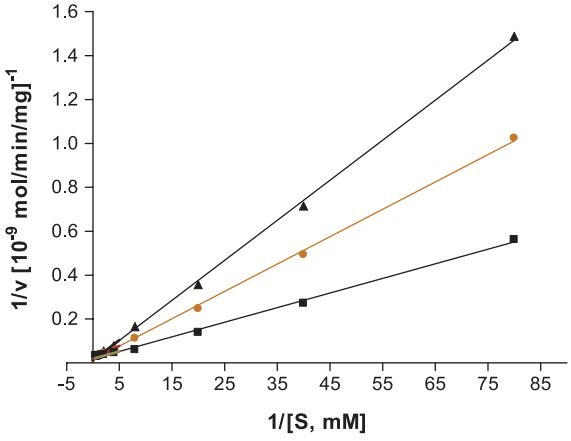


Figure 4. Lineweaver–Burk plot analysis of the inhibition kinetics of α-rhamnosidase inhibitory effects by compound **7** [■, control; ●, 5 μM; ▲, 10 μM inhibitor].

We examined the type of inhibition of this enzyme by **5**–**7** and **9**–**11** through a Lineweaver–Burk plot of α-rhamnosidase kinetics, which showed that all the tested pyrrolidines were competitive inhibitors of α-rhamnosidase (Fig. 4).

Except for the inhibition of β-amylase by compound **9** that showed 46.6% inhibition at 200 μM concentration, all synthetic pyrrolidines do not demonstrate inhibitory activity at 200 μM against seven glycosidases[16] (α-glucosidase from Bakers yeast, α-mannosidase from Jack Beans, α-amylase from *Bacillus licheniformis*, β-glucosidase from Almonds, β-galactosidase from *Escherichia coli*, β-amylase from Barley and invertase from Bakers yeast), using this data we could state that **5**–**7** and **9**–**11** are highly selective and potent inhibitors of α-rhamnosidase.

In conclusion, the synthesized 12 polyhydroxylated pyrrolidines were identified with structure-based inhibitory activity and specific inhibitory activity for α-rhamnosidase. The compound **7** (2*S*,3*S*,4*R*)-deacetyl anisomycin, had the best inhibitory activity because it possesses the same stereoconfiguration at C1 and C2 as α-L-rhamnopyranoside.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.06.051.

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15. Enzyme activity test: α-L-rhamnosidase activity was assayed using *p*-nitrophenyl α-L-rhamnopyranoside (PNLR) as a substrate and α-rhamnosidase from *P. decumbens* according to the method of Chapman et al.[10b] The reaction mixture, containing 50 μL of enzyme solution (33 μg/mL), 100 μL of 2.5 mM *p*-nitrophenyl α-L-rhamnopyranoside, 50 μL of sample solution and 50 μL of 50 mM phosphate buffer (pH 6.7) were incubated at 30 °C for 20 min. After the addition of 100 μL of 0.4 M NaOH to stop the reaction, absorbance of the mixture at 405 nm was determined.
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