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Inhibition of Naringinase (L-Rhamnosidase) by Piperidine Analogues of L-Rhamnose: Scaffolds for Libraries Incorporating Trihydroxypipecolic Acids

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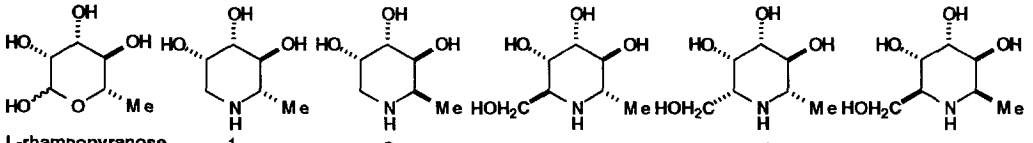
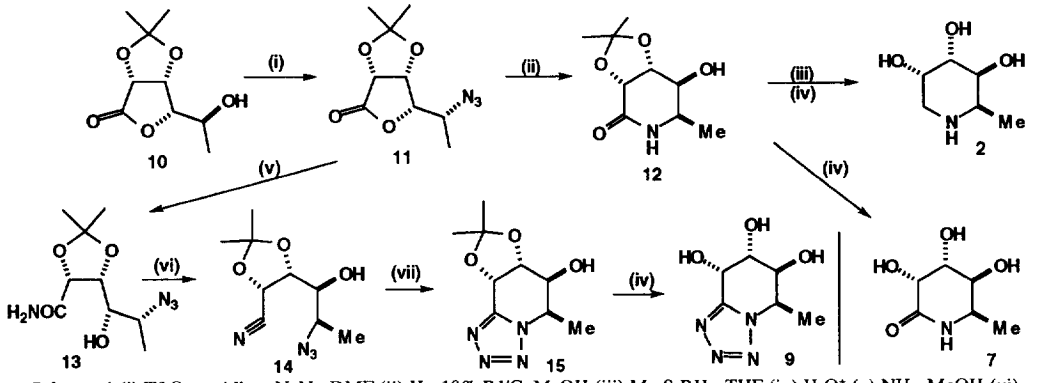
Abstract: L-Deoxyrhamnojirimycin **1** does not inhibit naringinase significantly but 5-*epi*-L-deoxyrhamnojirimycin **2** is a potent inhibitor. Conversely, α-C-glycosides of **1** are good inhibitors of L-rhamnosidase whereas those of **2** are not. Intermediate azabicyclic lactones are likely to be of use for the incorporation of a number of trihydroxypipecolic acids into peptide libraries.

The preceding paper[1] reports the synthesis and evaluation of a number of potent inhibitors of naringinase which are azafuranose analogues of L-rhamnose. Although aza-D-mannofuranose analogues give rise to many potent inhibitors of D-mannosidases, mannopyranose analogues such as deoxymannojirimycin and 6-*epi*castanospermine are usually only weak mannosidase inhibitors.[2] L-Deoxyrhamnojirimycin (LRJ) **1** was first synthesised from D-gulonolactone and reported to have no significant inhibition of naringinase.[3] Later, **1** was prepared by a sequence involving an aldolase reaction and stated to be a good inhibitor of naringinase.[4] Wong[5] repeated both the chemical and biochemical syntheses of **1** and found the materials to be identical by NMR and other spectroscopic data, but confirmed the earlier results that the sample from the chemical synthesis was a very poor inhibitor but that from the enzymic route was quite a good inhibitor of L-rhamnosidase; Wong suggested that the activity might be due to traces of an impurity in **1** which, on the basis of the synthesis, he proposed could be 5-*epi*-LRJ **2**.

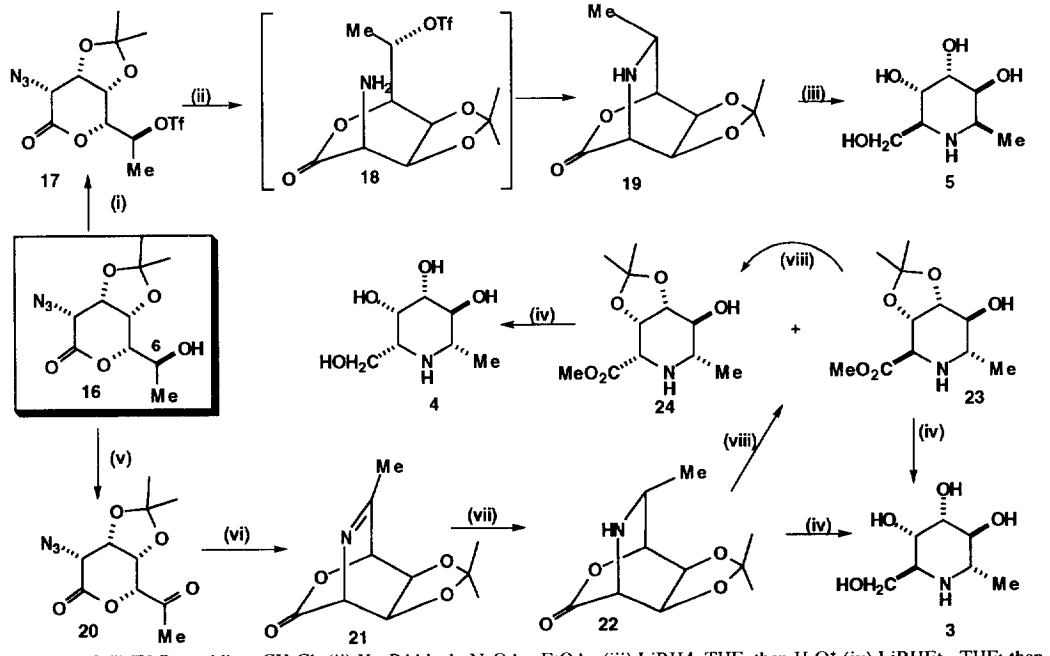


This paper reports the unambiguous synthesis of 5-*epi*-LRJ **2**, a related lactam **7** and tetrazole **9** from L-rhamnose and compares them as naringinase inhibitors with LRJ **1** and the corresponding lactam **6** and tetrazole **8**, prepared from D-gulonolactone by an identical route to that used for the enantiomer of **8** derived from L-gulonolactone.[6] Wong's suggestion that 5-*epi*-LRJ **2** might be a good inhibitor of naringinase is shown to be correct. Homologues of azasugars, such as the natural product homonojirimycin,[7] are also inhibitors of glycosidases.[8] This paper reports the synthesis, and effects on naringinase, of α- **3** and β- **4** homo-L-rhamnojirimycin, and of homo-5-*epi*-L-rhamnojirimycin **5**. Remarkably **3** is a powerful inhibitor of L-rhamnosidase whereas **5** has only a marginal inhibitory effect. Easily available bicyclic lactone intermediates in these syntheses provide intermediates that are convenient for the incorporation of the pipecolic acids into combinatorial libraries.



Scheme 1 (i) Tf_2O, pyridine; NaN_3, DMF (ii) H_2, 10% Pd/C, MeOH (iii) $Me_2S{:}BH_3$, THF (iv) H_3O^+ (v) NH_3, MeOH (vi) $(CF_3CO)_2O$, pyridine (vii) heat

For the synthesis of 5-*epi*-LRJ **2** [Scheme 1], the lactone **10**[9] was esterified with triflic anhydride in pyridine and the triflate then treated with sodium azide in DMF to afford the azide, **11**, m.p. 78-81 °C, $[\alpha]_D^{21}$ -118.8 (*c*, 1.33)[10] in 67% yield. Hydrogenation of the azidolactone **11** in methanol with 10% palladium on carbon caused reduction to the azide and spontaneous isomerisation to the lactam **12**, m.p. 151-152°C, $[\alpha]_D^{21}$ +39.0 (*c*, 0.52), 74% yield. Reduction of the lactam **12** with borane:dimethylsulphide in THF followed by acid hydrolysis with hydrochloric acid gave **2**[11] in 71% yield. Acid hydrolysis of **12** gave the lactam **7**, m.p. 192 - 193°C, $[\alpha]_D^{25}$ +66.9 (*c*, 0.86 in H_2O) in 89% yield. Reaction of the azidolactone **11** with ammonia in methanol gave the open chain amide **13**, m.p. 78-81°C, $[\alpha]_D^{23}$ +13.1 (*c*, 1.13 in acetone), 99% yield. Dehydration of **13** with trifluoroacetic anhydride in pyridine gave the nitrile **14** which on heating in toluene formed the protected tetrazole **15**, foam, $[\alpha]_D^{24}$ -23.4 (*c*, 0.84), 71% yield. The ketal was removed from **15** by acid hydrolysis to afford the unprotected tetrazole **9**, oil, $[\alpha]_D^{23}$ +24.3 (*c*, 0.6 in acetone) in 79% yield.



Scheme 2 (i) Tf_2O, pyridine, CH_2Cl_2 (ii) H_2, Pd black, NaOAc, EtOAc (iii) LiBH4, THF, then H_3O^+ (iv) $LiBHEt_3$, THF; then HCl, MeOH (v) PCC, CH_2Cl_2, molecular sieve (vi) $(EtO)_3P$, THF, reflux (vii) $NaCNBH_3$, MeCOOH (viii) NaOAc, MeOH

The readily available azidolactone **16**[12] is a common starting material for all of the C-azaglycosides **3**, **4** and **5** [Scheme 2]. The synthesis of **5** requires formation of a bond between nitrogen and C-6 of the lactone with inversion of configuration. Esterification of the free alcohol in **16** with triflic anhydride and pyridine in dichloromethane gave the triflate **17** which on hydrogenation in ethyl acetate in the presence of palladium black and sodium acetate gave the corresponding amine **18** which spontaneously cyclised to the lactone, **19**, m.p. 98-100°C, $[\alpha]_D^{21}$ +23.2 (*c* 1.0), in an overall yield of 61%.[13] Reduction of the aminolactone **19** with lithium borohydride in THF, followed by treatment with acidic ion exchange resin, gave the deprotected α-homo-*epi*-LRJ **5**[14] in 92% yield.

In order to retain the configuration at C-6 of the lactone **16** during the formation of the piperidine ring, the alcohol **16** was oxidised with pyridinium chlorochromate in dichloromethane in the presence of molecular sieve to afford the ketone **20**, m.p. 123-4°C, $[\alpha]_D^{21}$ -8.2 (*c* 0.92) in 82% yield. Treatment of the azidoketone **20** with triethyl phosphite at reflux in THF induced an intramolecular aza-Wittig reaction to form the bicyclic imine **21**, 183-4°C, $[\alpha]_D^{21}$ -175.8 (*c* 1.06), in 61% yield. Reduction of the imine with sodium cyanoborohydride in acetic acid resulted in hydride delivery from the least hindered side of the iminium ion to give the bicyclic aminolactone **22**, m.p. 118-9°C; $[\alpha]_D^{21}$ +32.8 (*c* 1.34), 83% yield; the structure of **22** was firmly established by X-ray crystallographic analysis, showing that the ring had been formed with overall retention of configuration.[15] Treatment of **22** with superhydride in THF followed by treatment with hydrogen chloride in methanol gave α-homoLRJ **3**[16] in 80% yield. Ring opening of the lactone **22** by sodium acetate in methanol gave a mixture of **23**, m.p. 128-9°C, $[\alpha]_D^{24}$ -23.7 (*c*, 0.60) together with **24** m.p. 175-6°C, $[\alpha]_D^{23}$ +44.9 (*c* 0.81); the proportion of products depends on the length of time of the reaction. It is clear that the initially formed **23** is less stable than **24** in which both the ester and methyl groups are equatorial; furthermore, **23** can be isomerised to **24** under the reaction conditions without appreciable elimination taking place. Superhydride reduction of **24**, followed by work up with hydrogen chloride in methanol, gave β-homoLRJ **4**[17] in 63% yield. Similar treatment of **23** afforded **3** in 71% yield. Both the bicyclic lactones **19** and **22** undergo rapid and efficient ring opening reactions with amines and should provide access to libraries containing trihydroxypipecolic acid.[18]

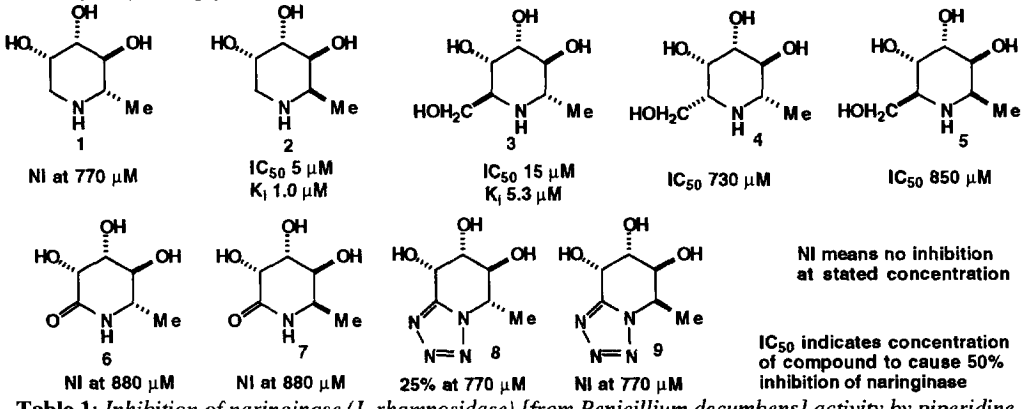


Table 1: *Inhibition of naringinase (L-rhamnosidase) [from Penicillium decumbens] activity by piperidine analogues of rhamnose in the hydrolysis of p-nitrophenyl-α-L-rhamnopyranoside*

The results of studies on the inhibition of naringinase (L-rhamnosidase) from *Penicillium decumbens* by the piperidine analogues are summarised in Table 1; all inhibition of naringinase by compounds **1** - **9** was competitive.[19] LRJ **1** showed no inhibition at 750 μM whereas 5-*epi*-LRJ **2** was a strong inhibitor of naringinase with K_i 1.0 μM. This would be entirely consistent with Wong's proposal that small amounts of **2** may also be formed in the enzymic route to **1** and this would account for the observed inhibition of naringinase by such samples. **2** was also a mild inhibitor of almond emulsin β-glucosidase [60% at 970 μM]. Some surprising results were obtained for the homologues. Thus, although **1** gave no inhibition of the L-rhamnosidase, α-homoLRJ **3** is a potent inhibitor [K_i 5.3 μM]. β-HomoLRJ **4** is a much weaker inhibitor but

is a powerful inhibitor of coffee bean α-galactosidase with IC50 4 μM; this will be discussed elsewhere. In contrast to the potent inhibition shown by **2**, homo-*epi*-LRJ **5** only has weak inhibitory effects on L-rhamnosidase. Both **3** and **5** at 800 μM showed weak inhibition of green coffee bean α-galactosidase, *E. coli* β-galactosidase and Jack Bean α-mannosidase. No inhibition of naringinase was found by either of the two lactams **6** and **7** or by the *epi*-tetrazole **9**. Only very weak inhibition of naringinase was observed for the pyranose tetrazole **8** [25% inhibition at 770 μM] in marked contrast to the furano-tetrazole analogue[3] which has K_i 56 μM; **8** is also a weak inhibitor of almond emulsin β-glucosidase [44% at 770 μM].

In summary, Wong's proposal that an impurity, probably **2**, in the enzymic synthesis of **1** could cause the difference in the properties of apparently the same material from two different sources looks correct. Some piperidine analogues of L-rhamnose are very good inhibitors of naringinase, so that both pyranose and furanose analogues of rhamnose are recognised by the enzyme. This is in contrast to most D-mannosidases where azafuranose mimics are good inhibitors, but azapyranose analogues usually are much weaker. Naringinase is a convenient enzyme to study in regard to epitopes of L-rhamnose, and this work may provide clues for finding inhibitors of enzymes which are involved in the incorporation of rhamnose into mycobacterial cell walls[20] in novel approaches to the treatment of tuberculosis.[21,22]

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10. Unless otherwise stated, all specific rotations were measured in chloroform.
11. Data for 5-epi-LRJ **2** $[\alpha]_D^{24}$ +6.3 (*c*, 0.96 in H_2O); δ_H (D_2O, 500MHz, pH 8) 0.94 (3H, d, H-6, *J* 6.9Hz), 2.59 (1H, dd, H-1, *J* 10.3Hz, *J* 12.8Hz), 2.71 (1H, dd, H-1', *J* 4.7Hz, *J* 12.9Hz), 2.91 (1H, m, H-5), 3.61 (1H, dd, H-4, *J* 2.0Hz, *J* 4.4Hz), 3.77 (1H, ddd, H-2, *J* 3.4Hz, *J* 4.4Hz, *J* 10.0Hz), 3.81 (1H, m, H-3); δ_C (D_2O, 50MHz) 15.7 (q, C-6), 44.8 (t, C-1), 49.3 (d, C-5), 66.6, 71.3, 72.9 (d x 3, C-2, C-3, C-4).
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13. The structure of lactone **19** was firmly established by X-ray crystallographic analysis of a cyclohexylidene derivative.
14. Data for homo-epi-LRJ **5** $[\alpha]_D^{25}$ -38.1 (*c*, 0.9 in H_2O, pH 8); ν_{max} (thin film)/cm^{-1}: 3401 (br OH, NH); δ_H (500 MHz; D_2O, pH 9): 1.11 (3H, d, $J_{6,7}$ 6.8, H-7), 2.93 (1H, dt, *J* 4.0, *J* 10.7, H-2), 3.20 (1H, q, *J* 6.8, H-6), 3.71-3.75 (3H, m), 3.77 (1H, dd, *J* 3.1, *J* 10.7), 3.98 (1H, t, *J* 3.5); δ_C (50 MHz; D_2O, pH 9): 15.9 (q, C-7), 61.0 (t, C-1), 49.3, 55.9 (2d, C-2, C-6), 65.4, 71.4, 72.5 (3d, C-3, C-4, C-5); *m/z* (DCI; NH_3): 178 (MH^+, 100%).
15. Details of the X-ray structure of **22** will be provided in the full paper.
16. Data for α-homoLRJ **3** oil$[\alpha]_D^{25}$ +15.0 (*c*, 0.9 in H_2O, pH 8), δ_H (500MHz; D_2O, pH 8) 1.05 (3H, d, $J_{6,7}$ 6.3, H-7), 2.59 (1H, dq, $J_{5,6}$ 9.4, $J_{6,7}$ 6.3, H-6), 2.96 (1H, ddd, $J_{1,2}$ 7.7, $J_{1',2}$ 7.1, $J_{2,3}$ 2.3, H-2), 3.26 (1H, dd, $J_{5,6}$ 9.4, $J_{4,5}$ 9.5, H-5), 3.51 (1H, dd, $J_{4,5}$ 9.5, $J_{3,4}$ 3.3, H-4), 3.56 (1H, dd, $J_{1,1'}$ 11.7, $J_{1',2}$ 7.1, H-1'), 3.62 (1H, dd, $J_{1,1'}$ 11.7, $J_{1,2}$ 7.7, H-1), 3.91 (1H, dd, $J_{2,3}$ 2.3, $J_{3,4}$ 3.3, H-3);); δ_C (50 MHz; D_2O, pH 10): 17.2 (q, C-7), 59.3 (t, C-1), 51.2, 59.7 (2d, C-2, C-6), 69.3, 71.8, 73.6 (3d, C-3, C-4, C-5); *m/z* (Electrospray) 178 (MH^+, 100%).
17. Data for β-homoLRJ **4** oil, $[\alpha]_D^{24}$ +12.1 (*c* 0.95 in H_2O, pH 8); δ_H (500MHz; D_2O, pH 8) 1.07 (3H, d, $J_{6,7}$ 6.4, H-7), 2.45 (1H, dq, $J_{5,6}$ 9.6, $J_{6,7}$ 6.4, H-6), 2.73 (1H, ddd, $J_{1,2}$ 6.9, $J_{1',2}$ 6.7, $J_{2,3}$ 1.3, H-2), 3.21 (1H, dd, $J_{5,6}$ 9.6, $J_{4,5}$ 9.7, H-5), 3.40 (1H, dd, $J_{4,5}$ 9.7, $J_{3,4}$ 3.2, H-4), 3.49 (1H, dd, $J_{1,1'}$ 11.2, $J_{1',2}$ 6.7, H-1'), 3.53 (1H, dd, $J_{1,1'}$ 11.2, $J_{1,2}$ 6.9, H-1), 3.90 (1H, dd, $J_{2,3}$ 1.3, $J_{3,4}$ 3.2, H-3); δ_C (50MHz; D_2O, pH 8) 17.7 (q, C-7), 55.6, 58.9 (2d, C-2, C-6), 61.9 (t, C-1), 69.8, 74.5, 75.4 (3d, C-3, C-4, C-5); *m/z* (APCI) 176 ($M-H^+$, 100%).
18. The details of ring opening of the lactones with amines will be presented in a full paper.
19. Naringinase (Sigma) (0.25 μg/ml) was assayed against 5mM *p*-nitrophenyl-α-L-rhamnopyranoside (Sigma) at pH 4.0 (K_m 1.1 mM). The compounds were also assayed for potential inhibition of α-glucosidase (Brewers yeast, rabbit gut), β-glucosidase (almond emulsin, rabbit gut, rabbit liver), α-galactosidase (green coffee bean,), β-galactosidase (*E.coli*, rabbit gut, rabbit liver), α-mannosidase (Jack Bean), β-N-acetylglucosaminidase (Jack Bean, bovine), xylanase (*Trichoderma viride*), pectinase (*Aspergillus niger*), and rabbit gut sucrase, maltase, trehalase and lactase; there was no significant inhibition of any of these enzymes other than where stated in the text. Details of the enzyme assays will be given in a full paper.
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