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Inhibition of UDP-Gal Mutase and Mycobacterial Galactan Biosynthesis by Pyrrolidine Analogues of Galactofuranose

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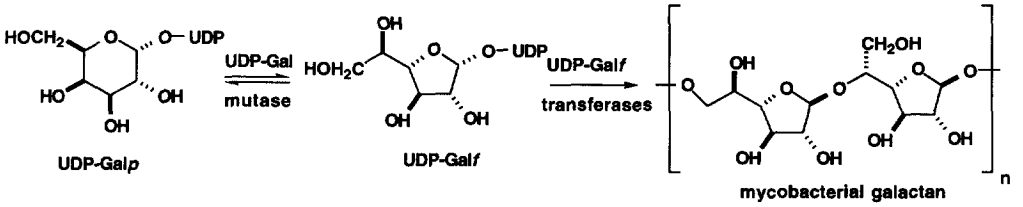
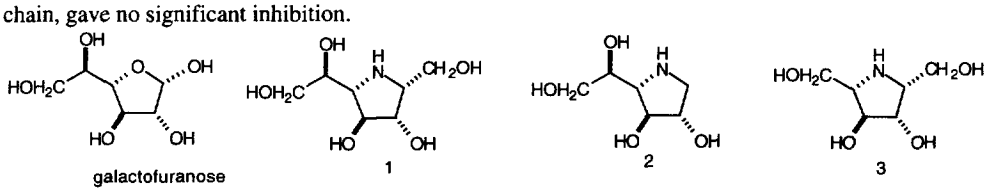
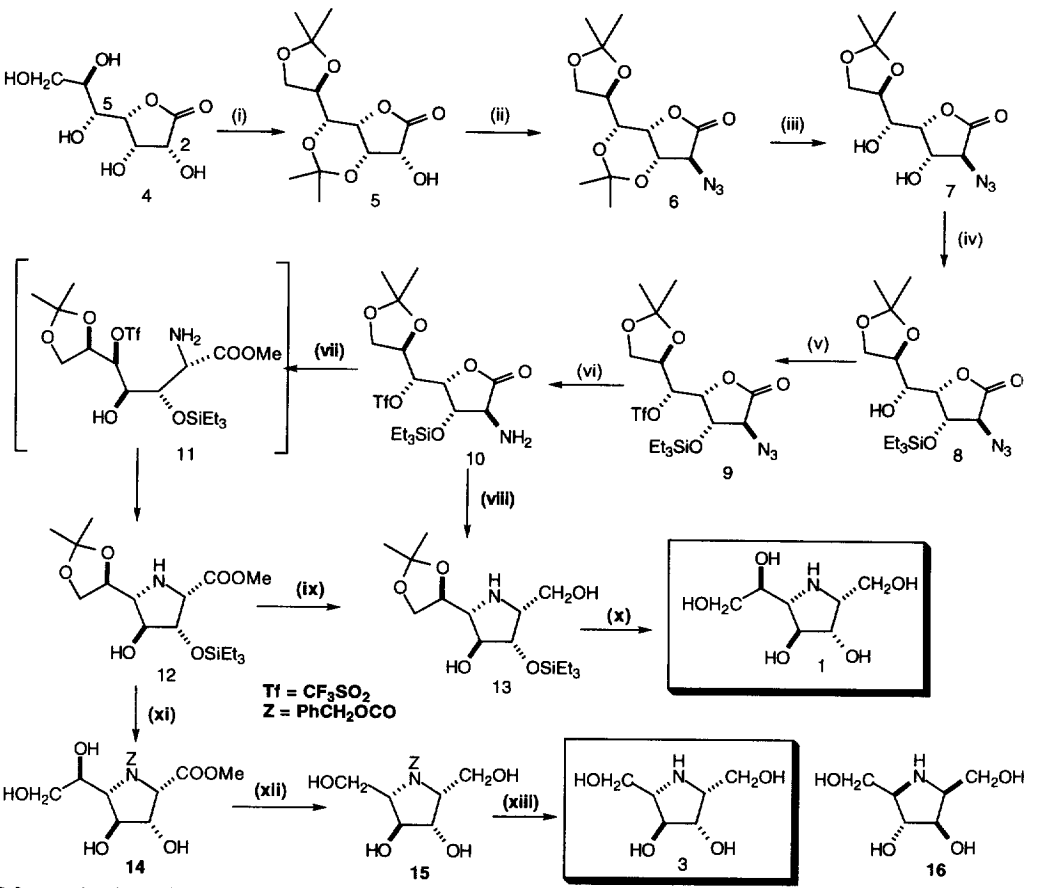
Abstract: Some pyrrolidine analogues of galactofuranose - synthesised from carbohydrate lactones - are the first known inhibitors of *E. coli K12* UDP-Gal mutase and mycobacterial galactan biosynthesis. This inhibition may form a new chemotherapeutic strategy for the treatment of human pathogens which contain integral galactofuranosyl structures such as tuberculosis and leprosy.

D-Galactans are essential components of the *Mycobacterium tuberculosis* cell wall, the causative agent of tuberculosis.[1,2] The galactan is an alternating β(1-5) β(1-6) galactofuranosyl linked chain and it is believed that the flexibility of the β(1-6) galactofuranose is integral in maintaining cell wall structure and impermeability.[3] *In vitro* studies have shown UDP-galactofuranose (UDP-Gal*f*) to be the donor for the galactosyl transferases involved in cell wall biosynthesis (Figure 1).[4] UDP-Gal*f* is formed by the contraction of UDP-galactopyranose (UDP-Gal*p*) in a reaction catalysed by UDP-galactosyl mutase; the action of this enzyme has been recently described in *E. coli K12* and *Klebsiella pnuemoniae*.[5,6] There appears to be high homology of UDP-Gal mutases between species and the mycobacterial mutase is believed to be very similar. Galactofuranose has no role in mammalian metabolism, so that inhibition by galactofuranose mimics of either (i) UDP-Gal mutase or (ii) any UDP-Gal*f* transferases responsible for incorporation of UDP-Gal*f* into the cell wall may well be achieved without any harm to the mammalian host, providing a new approach to the treatment of tuberculosis and many other pathogens which contain galactofuranose.[6]



Piperidine and pyrrolidine analogues provide a powerful set of inhibitors of glycosidases.[7] However, there are an increasing number of examples of inhibition of other enzymes which are involved in carbohydrate metabolism. N-Butyldeoxynojirimycin has been identified as an inhibitor of a glucosyl transferases involved in glycosphingolipid biosynthesis,[8] a number of pyrrolidines and piperidines are effective as inhibitors of fucosyl transferases,[9] and DGDP **16**, the enantiomer of **3**, inhibits xylose isomerase.[10] This paper describes the synthesis of the galactofuranose pyrrolidine analogues **1**, [which has an α-hydroxymethyl group analogous to the UDP-donor], **2** and **3**. Both the galactofuranose mimics **1** and **2** caused inhibition of the biosynthesis of mycobacterial cells walls, probably by their effect on UDP-Gal mutase; **3**, with a shorter side chain, gave no significant inhibition.



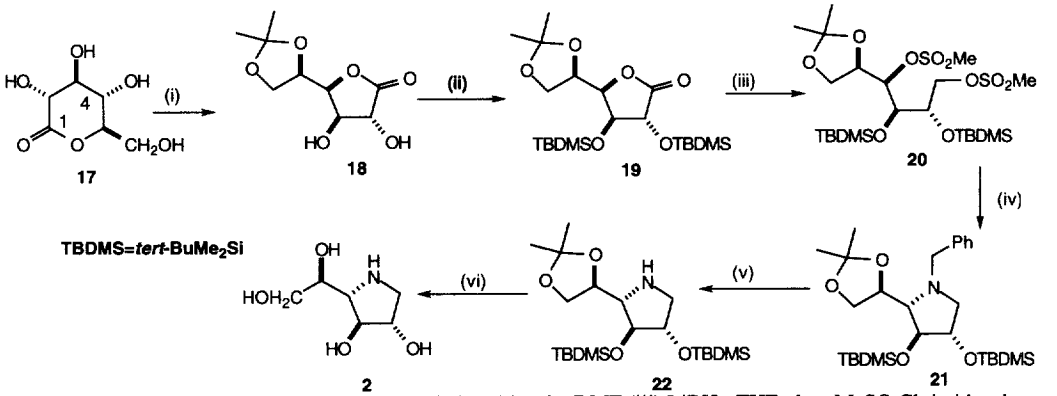


Scheme 1: (i) Ref. 11 (ii) Tf_2O, CH_2Cl_2, pyridine, -20°C; then NaN_3, DMF (iii) $CF_3COOH:H_2O$, 1:1; then $Me_2C(OMe)_2$, Me_2CO, CSA (iv) Et_3SiCl, imidazole, DMF (v) Tf_2O, CH_2Cl_2, pyridine (vi) H_2, Pd black, EtOAc (vii) NaOAc, MeOH (viii) $NaBH_4$, EtOH (ix) $LiEt_3BH$, THF (x) HCl, MeOH (xi) $PhCH_2OCOCl$, $NaHCO_3$, Et_2O; then $CF_3COOH:H_2O$, 1:1 (xii) HIO_4, THF; then $NaBH_4$, EtOH (xiii) H_2, Pd black, EtOH.

The synthesis of 2,5-imino-2,5-dideoxy-α-homogalactitol **1** from the readily available seven carbon lactone **4** requires introduction of nitrogen between C-2 and C-5 with inversion of configuration at both centres. The acetonide **5**[11] was esterified with triflic anhydride and then treated with sodium azide to afford the azide **6** m.p. 102°C, $[\alpha]_D^{22}$ -224 (*c*, 1.0 in $CHCl_3$) [66% yield]. Removal of both ketal protecting groups in **6** with aqueous trifluoroacetic acid followed by kinetic acetonation with dimethoxypropane in acetone in the presence of camphor sulfonic acid gave the diol **7** m.p. 136°C, $[\alpha]_D^{22}$ -164 (*c*, 1.0 in MeOH) [89% yield]. The most nucleophilic site in **7** is the C-3 hydroxyl group, so that reaction of **7** with triethylsilyl chloride in DMF in the presence of imidazole gave **8**, oil, $[\alpha]_D^{22}$ -120.6 (*c*, 1.0 in $CHCl_3$) [91% yield]; subsequent reaction of the remaining free alcohol in **8** with triflic anhydride in dichloromethane in the presence of pyridine afforded the relatively stable triflate **9**, oil, $[\alpha]_D^{22}$ -79.4 (*c*, 1.0 in $CHCl_3$) [89% yield]. Hydrogenation of the azide **9** in ethyl acetate in the presence of palladium black gave the aminotriflate **10**. Treatment of **10** with sodium acetate in methanol caused initial ring opening to an aminoester **11** which cyclised to give the methyl ester **12**, oil, $[\alpha]_D^{22}$ -16.7 (*c*, 1.0 in MeOH), in 94% yield. Reduction of **12** with superhydride in THF afford the diol **13**, $[\alpha]_D^{22}$ -16.7 (*c*, 1.0 in MeOH) in 87% yield; reduction of **10** with sodium borohydride in ethanol

gave **13** directly in 58% yield. The protecting groups in **13** were removed by HCl in methanol to give, after purification by ion exchange chromatography, α-homoiminogalactitol **1** [93% yield].[12]

The structural proof for **1** was provided by degradation of the side chain of **1** to form **3**. The amine **12** was first Z (benzyloxycarbonyl) protected using benzyl chloroformate in diethyl ether and aqueous sodium bicarbonate base. The acetonide and triethylsilyl protecting groups were removed by aqueous acetic acid to afford tetraol **14**, $[\alpha]_D^{22}$ -14.0 (*c*, 0.9 in MeOH) in 71% yield. Selective periodic acid cleavage of the side chain in **14** by 1.05 equivalents of periodic acid, followed by reduction of the resulting aldehyde and methyl ester by sodium borohydride in ethanol afforded **15**, $[\alpha]_D^{22}$ -3.3 (*c*, 1.0 in MeOH) in 56% yield. Hydrogenolysis of the Z-protecting group from the benzyl carbamate **15** by hydrogenation in ethanol in the presence of palladium black gave L-DGDP **3**[13] in 91% yield; this material had identical properties to an authentic sample[10] of the mirror image DGDP **16**, other than its rotation.[14]



Scheme 2: (i) Me_2CO, *p*TSA, (ii) *tert*-$BuMe_2SiCl$, imidazole, DMF (iii) $LiBH_4$, THF; then $MeSO_2Cl$, imidazole, pyridine (iv) $PhCH_2NH_2$, 120°C, 4 days (v) H_2, Pd black, EtOH (vi) HCl, MeOH

The synthesis of the azafuranose analogue of galactofuranose, 1,4-imino-1,4-dideoxy-galactitol, **2** requires joining C-1 of gluconolactone **17** with C-4 accompanied by inversion of configuration at C-4 [Scheme 2]. Gluconolactone **17** was treated with acetone and *p*-toluenesulfonic acid (*p*TSA) to give the 5,6-acetonide[15] **18** in 39% yield. Reaction of **18** with *tert*-butyldimethylsilyl chloride in DMF in the presence of imidazole to give the fully protected lactone **19** $[\alpha]_D^{22}$ 41.1 (*c*, 1.1 in $CHCl_3$) [73% yield]. Reduction of **19** with lithium borohydride in THF gave the corresponding diol which on treatment with methanesulfonyl chloride in pyridine in the presence of DMAP afforded the dimesylate **20** $[\alpha]_D^{22}$ -5.8 (*c*, 1.0 in $CHCl_3$) in 79% yield. Reaction of **20** in benzylamine at 120°C gave initial nucleophilic displacement of the primary mesylate by the amine followed by intramolecular cyclisation with inversion of configuration of the secondary mesylate to give the fully protected pyrrolidine **21** $[\alpha]_D^{22}$ +42.0 (*c*, 1.0 in $CHCl_3$) in 91% yield. Hydrogenolysis of the benzyl group in **21** in ethanol gave **22** $[\alpha]_D^{22}$ +12.2 (*c*, 1.0 in $CHCl_3$) in 80% yield which on deprotection with HCl in methanol and purification by ion exchange chromatography (Amberlite IR120 H^+ form, eluted with 1M aqueous ammonia) gave the iminogalactictol **2** in 62% yield; the data for both the free base[16] **2** and the corresponding hydrochloride[17] were consistent with that previously reported.[18]

In vitro studies of mycobacterial galactan biosynthesis demonstrate that **1** and **2** inhibit incorporation of radioactive label from [^{14}C]-UDP-Gal*p* into mycobacterial galactan [Table].[19] In contrast, **3** was only weakly active suggesting that the two carbon side chain of galactofuranose is necessary for significant inhibition. Both **1** and **2** inhibit the interconversion of UDP-Gal*p* to UDP-Gal*f* by *E. coli K12* mutase with the reverse reaction, UDP-Gal*f* to UDP-Gal*p* being more sensitive to inhibition than the forward reaction. Although piperidine mimics bearing D-*galacto* stereochemistry at the secondary hydroxyl substituents are extremely powerful inhibitors of galactosidases, the pyrrolidine equivalents are not;[20] thus both **1** and **2** have

negligible inhibitory activity against galactosidases - for example, **1**, **2** and **3** all give less than 20% inhibition of the activity of green coffee bean α-galactosidase at 750uM.[21] It may be therefore that analogues such as **1** and **2** are fairly specific in their interactions with enzymes that handle galactofuranose.

*Table: Inhibition of iminosugars **1-3** against mycobacterial galactan biosynthesis[4] and UDP-Gal mutase[5]*

Compound 200 μg/ml	% Inhibition of mycobacterial galactan biosynthesis	% Inhibition of UDP-Gal*p* to UDP-Gal*f*	% Inhibition of UDP-Gal*f* to UDP-Gal*p*
1	63	64	67
2	56	36	81
3	16	-	-

In summary, this paper reports the synthesis of some pyrrolidine analogues of galactofuranose which provide the first examples of specific inhibitors of mycobacterial galactan biosynthesis probably by inhibition of the mycobacterial UDP-Gal mutase. This may provide a novel strategy for the study of mycobacterial cell wall biosynthesis and initiate a new approach to the treatment of tuberculosis and other related diseases.[22]

REFERENCES

1. Hueber, R.E., Castro, K.G., *Annu. Rev. Med.*, 1995, **46**, 47.
2. Brennan, P.J., Nikaido, H., *Annu. Rev. Biochem.*, 1995, **64**, 29.
3. Lee, R.E., Brennan, P.J., Besra, G.S., *Curr. Top. in Microbiol. Immunol.*, 1996, **215**, 1.
4. Mikusova, K., Mikus, M., Besra, G.S., Hancock, I., Brennan, P.J., *J. Biol. Chem*, 1996, **271**, 7820.
5. Lee, R., Monsey, D., Weston, A., Duncan, J.K., Rithner, C., McNeil, M., *Anal. Biochem.* 1996, **242**, 1.
6. Koplin, R., Brisson, J-R., Whitfield, C., *J. Biol. Chem.*, 1997, **272**, 4121.
7. Winchester, B., Fleet, G.W.J., *Glycobiology*, 1992, **2**, 199.
8. Platt, F.M., Neises, G.R., Dwek, R.A., Butters, T.D., *J. Biol. Chem.*, 1994, **269**, 8362
9. Qiao, L., Murray, B.W., Shimazaki, M., Schultz, J., Wong, C-H., *J. Am. Chem. Soc.*, 1996, **118**, 7653.
10. A. Collyer, J. D. Goldberg, H. Viehmann, D. M. Blow, N. G. Ramsden, G. W. J. Fleet, F. J. Montgomery and P. Grice, *Biochemistry*, 1992, **31**, 12211.
11. Brimacombe, J. S., Tucker, L. C. N., *Carbohydr. Res.*, 1966, **2**, 341.
12. Selected data for homogalactitol **1**: oil $[\alpha]_D^{22}$ -18.2 (*c*, 1.0 in H_2O); δ_H (D_2O) 4.09 (1H, dd, $J_{3,4}$ 3.1, $J_{3,2}$ 5.3, H-3), 3.92 (1H, dd, $J_{4,5}$ 5.6,$J_{4,3}$ 3.1, H-4), 3.80 (1H, m, H-6), 3.76 (1H, dd, $J_{1,2}$ 5.8, $J_{1,1'}$ 11.5, H-1), 3.68 (1H, dd, $J_{1',2}$ 6.4, $J_{1,1'}$ 11.6, H-1'), 3.66 (1H, dd, $J_{6,7}$ 4.1, $J_{7,7'}$ 11.8, H-7), 3.55 (1H, dd, $J_{6,7'}$ 7.3, 11.8, H-7'), 3.31 (1H, dt, $J_{2,3}$ 5.8, $J_{1,2}$ 6.0, H-2), 2.95 (1H, dd, $J_{4,5}$ 5.5, $J_{5,6}$ 5.5, H-5); δ_C (D_2O) 81.0 (d), 78.9 (d), 72.7 (d), 66.9 (d), 65.5 (t), 62.7 (d), 61.9 (t).
13. Selected data for L-DGDP **3** oil $[\alpha]_D^{22}$ -23.6 (*c*, 2.0 in H_2O); δ_H (D_2O) 4.07 (1H, dd, $J_{3,4}$ 2.9, $J_{2,3}$ 5.1, H-3), 3.83 (1H, dd, $J_{4,5}$, 5.2, $J_{3,4}$ 2.9, H-4), 3.75 (1H, dd, $J_{1,1'}$ 11.4, $J_{1',2}$ 6.0, H-1'), 3.70 (1H, dd, $J_{6,6'}$ 11.6, $J_{5,6'}$ 4.9, H-6'), 3.63 (1H, dd, $J_{1,1'}$ 11.4, $J_{1,2}$ 6.6, H-1), 3.62 (1H, dd, $J_{6,6'}$ 11.6, $J_{5,6}$ 6.3, H-6), 3.30 (1H, q, J 5.9, H-2), 2.99 (1H, q, J 5.5, H-5); δ_C (D_2O) 79.6 (d), 77.7 (d), 65.7 (d), 62.7 (t), 61.7 (d), 60.6 (t).
14. Lui, K.K.-C., Kajimoto, T., Chen, L., Zhong, Z., Ichikawa, Y and Wong, C.-H. *J. Org. Chem.*, 1991, **56**, 6280 gives rotation for DGDP **3** $[\alpha]_D^{23}$ +25.75 (*c*, 4.0 in H_2O)
15. Chittenden, G. J., *Recl. Trav. Chim. Pays-Bas*, 1988, **107**, 455.
16. Selected data for iminogalactitol **2** Free Base oil; $[\alpha]_D^{22}$ +3.0 (*c*, 1.0 in H_2O) Ref 17 gives +2.8 (*c*, 2.0 in H_2O), δ_H (D_2O) 4.02 (1H, ddd, $J_{2,3}$ 3.7, $J_{1',2}$ 3.8, $J_{1,2}$ 5.7, H-2), 3.82 (1H, dd, $J_{2,3}$ 3.7, $J_{3,4}$ 6.0, H-3), 3.73 (1H, ddd, $J_{5,6'}$ 7.4, $J_{4,5}$ 5.1, $J_{5,6}$ 4.0, H-5), 3.58 (1H, dd, $J_{6,6'}$ 11.8, $J_{5,6}$ 3.9 H-6), 3.47 (1H, dd, $J_{6,6'}$ 11.8, $J_{5,6'}$ 7.4, H-6'), 3.00 (1H, dd, $J_{1,1'}$ 12.2, $J_{1,2}$ 5.7, H-1), 2.81 (1H, dd, $J_{4,5}$ 5.6, $J_{3,4}$ 5.8, H-4), 2.78 (1H, dd, $J_{1,1'}$ 12.2, $J_{1',2}$ 3.9, H-1'); δ_C (D_2O) 79.7(d), 77.8(d), 71.8(d), 66.1(d), 64.3 (t), 51.3 (t).
17. Hydrochloride salt of **2** mp 102°C ($CHCl_3$:MeOH) $[\alpha]_D^{22}$ -25.3 (*c*, 1.0 in MeOH); δ_H (CD_3OD) 4.18 (1H, ddd, $J_{1',2}$ 4.5, $J_{1,2}$ 2.6, $J_{2,3}$ 2.6, H-2), 4.11 (1H, dd, $J_{3,4}$ 3.2, $J_{2,3}$ 2.9, H-3), 3.92 (1H, ddd, $J_{4,5}$ 7.1, $J_{5,6}$ 4.3, $J_{5,6'}$ 4.2, H-5), 3.72 (1H, dd, $J_{6,6'}$ 11.6, $J_{5,6'}$ 4.1, H-6'), 3.65 (1H, dd, $J_{6,6'}$ 11.6, $J_{5,6}$ 4.4, H-6), 3.47 (1H, dd, $J_{4,5}$ 7.0, J_{34} 3.6, H-4), 3.43 (1H, dd, $J_{1',2}$ 4.6, $J_{1,1'}$ 11.9, H-1'), 3.23 (1H, dd, $J_{1,2}$ 2.5, $J_{1,1'}$ 11.9, H-1); δ_C (CD_3OD) 78.1, 76.1, 70.3, 69.2, 65.0, 51.5.
18. Bernotas, R. C., *Tetrahedron Lett.*, 1990, **31**, 469.
19. The assays were conducted as in reference 4 with UDP-[14C]Gal replacing UDP-[14C]GlcNAc, and with subsequent isolation and counting of the polymer. The amount of inhibition is dependent on the protein concentration.
20. Lundt, I., Madsen, R., Daher, S. A., and Winchester, B., *Tetrahedron*, 1994, **50**, 7513.
21. For details of the assays, see Evans, S.V., Fellows, L.E., Bell, E.A., *Phytochemistry*, 1983, **22**, 768.
22. All isolable new compounds have satisfactory CHN microanalytical or high resolution mass spectral data.

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