Pergamon

Tetrahedron Letters 40 (1999) 8689–8692

TETRAHEDRON LETTERS

An Approach to Combinatorial Library Generation of Galactofuranose Mimics as Potential Inhibitors of Mycobacterial Cell Wall Biosynthesis: Synthesis of a Peptidomimetic of Uridine 5'-Diphosphogalactofuranose (UDP-Gal*f*)

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Received 9 July 1999; accepted 28 September 1999

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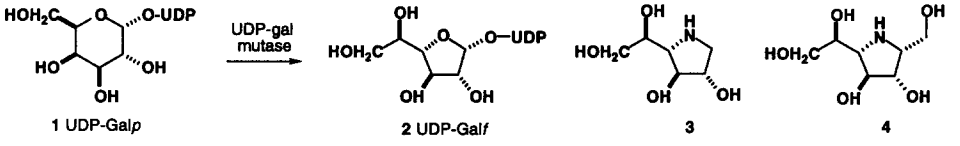
Abstract

An approach to the synthesis of amide libraries based upon an α-iminogalactofuranose template as potential inhibitors of mycobacterial cell wall biosynthesis is described. The synthesis of peptide analogues of uridine 5'-phosphogalactofuranose (UDP-Gal*f*) is also described.

Keywords: Amide, combinatorial chemistry, tuberculosis, galactofuranose, transferase, inhibition.

It is estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis*,[1] the causative agent of tuberculosis (TB) and the biggest killer by a single infectious agent in the world today.[2] The emergence of multiple-drug resistant strains has stimulated a search for novel chemotherapeutic strategies.[3]

The longevity of the TB bacilli can be ascribed to its unique cell wall structure[4] which incorporates a number of sugars - such as L-rhamnose, D-arabinofuranose and D-galactofuranose - which have no role in mammalian metabolism. Galactans, which are alternating D-galactofuranosyl β(1-5) β(1-6) linked chains, are essential cell-wall components.[5,6] *In vitro* studies have shown uridine 5'-diphosphogalactofuranose **2** (UDP-D-Gal*f*), formed by a contraction of UDP-D-Gal*p* **1** in a reaction catalyzed by UDP-Gal mutase, to be the donor for the galactosyl transferases involved in cell wall biosynthesis (Figure 1).[7,8] D-Galactofuranose has no role in human metabolism, so that inhibition by galactofuranose mimics of either (i) UDP-Gal mutase or (ii) any UDP-Gal*f* transferases responsible for processing of UDP-Gal*f* may provide new treatments for TB without any deleterious side effects.[9]



1 UDP-Gal*p* 2 UDP-Gal*f* 3 4

Figure 1

The α-imino-D-galactofuranose derivatives **3** and **4** were the first identified inhibitors of UDP-Gal mutase, but were inactive against the mycobacterial UDP-Gal*f* transferases.[10] Imino-sugar mimics of L-rhamnose[11] and D-arabinose[12] have been synthesized, and their biological activities documented. Studies on mimetics of L-rhamnose as potential inhibitors of TDP-rhamnose biosynthesis have been reported.[13] The key step in the synthesis of the α-imino-D-galactofuranose derivatives **3** and **4** was the basic methanolysis of the amino-triflate **7** (derived from the protected heptonolactone **5**) to afford an open chain amino-triflate **8** which undergoes closure from the C-2 amine onto the leaving group at C-5 with inversion of configuration to afford **9**, Figure 2.

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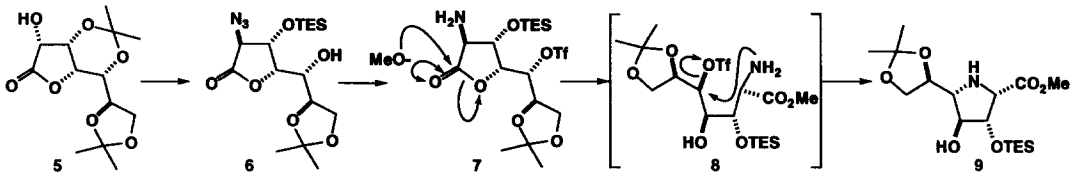
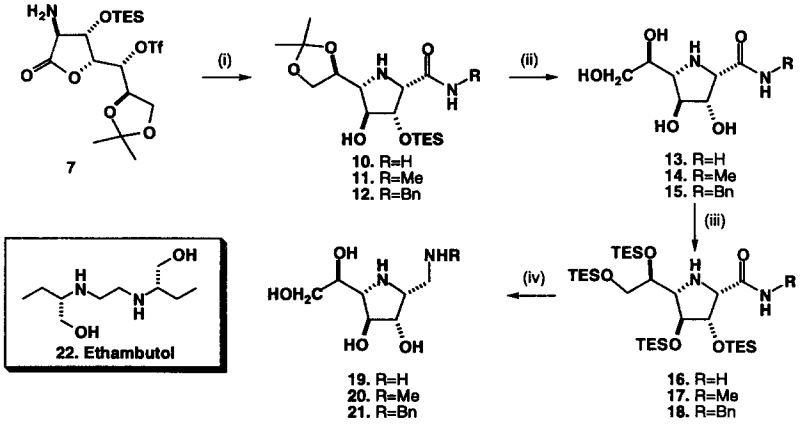


Figure 2

In this paper, an extension of this methodology is described, whereby the amino-lactone **7** is opened with nitrogen rather than oxygen nucleophiles leading to a novel series of iminosugar amides possessing D-galactofuranosyl stereochemistry. Thus, treatment of the amino-triflate **7** with ammonia, methylamine and benzylamine in THF at room temperature afforded the respective pyrollidine amides **10** (m.p. 214°C, $[\alpha]_D^{22}$ -37.0 (*c*, 1.0 in MeOH) [95% yield]), **11** ($[\alpha]_D^{22}$ -52.0 (*c*, 1.0 in MeOH) [98% yield]) and **12** ($[\alpha]_D^{22}$ -34.2 (*c*, 1.0 in MeOH) [91% yield]), scheme 1.



Scheme 1: (i) RNH_2, THF (ii) TFA, H_2O (iii) Et_3SiCl, Imidazole, DMF, 80°C (iv) $BH_3.SMe_2$, THF, reflux; then TFA, H_2O

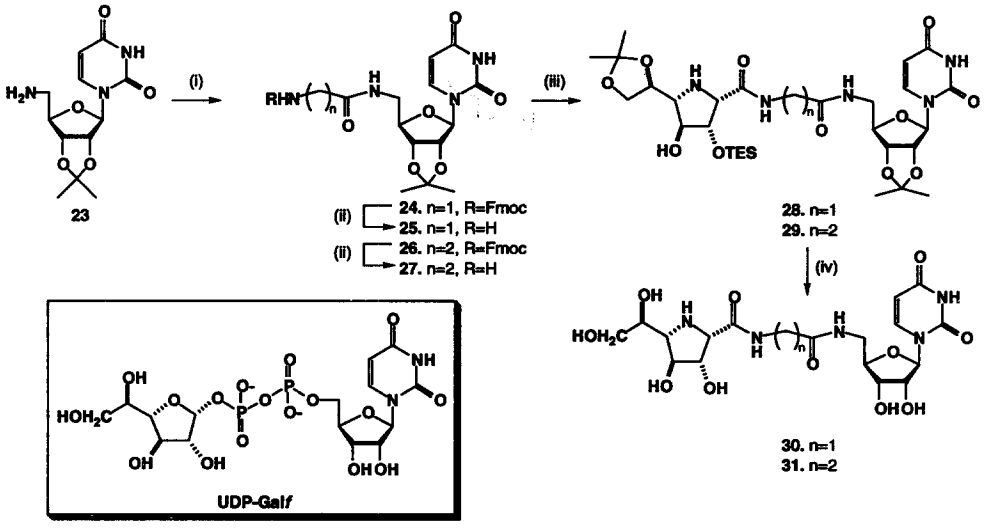
Treatment of the amides **10-12** with aqueous trifluoroacetic acid effected clean removal of the isopropylidene and triethylsilyl protecting groups, and subsequent purification by ion exchange chromatography yielded the deprotected amide **13** ($[\alpha]_D^{23}$ -60.3 (*c*, 1.0 in MeOH) [89% yield]), methylamide **14** ($[\alpha]_D^{23}$ -72.3 (*c*, 1.0 in MeOH) [95% yield]) and benzylamide **15** ($[\alpha]_D^{23}$ -43.2 (*c*, 1.0 in MeOH) [60% yield]). Initial attempts to effect reduction of the carbonyl functionality of the amides **10-12** with borane were troublesome, resulting in significant cleavage of the isopropylidene protecting group to a primary ether, although the silyl group was stable under these conditions. It was reasoned that exchange of the isopropylidene protection for silyl groups would facilitate isolation of the reduction product. Thus, exhaustive triethylsilylation of the deprotected amides **13-15** with triethylsilylchloride in the presence of imidazole in DMF afforded the tetrasilyl amide **16** ($[\alpha]_D^{23}$ -31.0 (*c*, 1.0 in $CHCl_3$) [44% yield]), tetrasilyl methylamide **17** ($[\alpha]_D^{23}$ -27.6 (*c*, 1.0 in $CHCl_3$) [70% yield]) and tetrasilyl benzylamide **18** ($[\alpha]_D^{23}$ -28.8 (*c*, 1.0 in $CHCl_3$) [74% yield]).[14] Reduction of the persilyl derivatives **16-18** with borane-dimethyl sulfide complex in refluxing THF occurred smoothly, and the protecting groups were removed without purification of the intermediate diamine (by treatment with aqueous trifluoroacetic acid). Purification by acidic ion exchange chromatography (Amberlite IR-120, H^+) gave the free diamines which were converted to their hydrochloride salts to afford diamine **19** ($[\alpha]_D^{23}$ -26.0 (*c*, 1.0 in H_2O) [93% yield]);[15] *N*-methyldiamine **20**, ($[\alpha]_D^{23}$ -18.0 (*c*, 1.0 in H_2O) [80% yield])[16] and *N*-benzyldiamine **21**, ($[\alpha]_D^{23}$ -12.8 (*c*, 1.0 in H_2O) [90% yield]).[17]

There are certain structural similarities in these diamines **19-21** to the anti-tuberculosis drug Ethambutol **22**, which has been shown to be an inhibitor of mycobacterial arabinogalactan biosynthesis.[18] However, the

diamines **19-21** were found to be inactive in the UDP-Gal mutase assay. Comparison between the galactitol **4**, a known UDP-Gal mutase inhibitor, and its amino congener **19** show that the introduction of a C-1 amino group in place of the hydroxyl functionality leads to a complete loss of inhibitory activity.

An alternative approach to the inhibition of mycobacterial galactose processing enzymes is mimicry of the activated glycosyl donor itself, UDP-Gal*f*. [19] This has been a successful strategy in the design of an α-1,3-galactosyltransferase inhibitor,[20] and has been extended to the design of inhibitors of other glycosylation enzymes.[21] It was proposed that the high-yielding nucleophilic ring opening of the amino triflate **7** by primary amines could be extended to facilitate the synthesis of peptidic analogues of UDP-Gal*f*. The use of an amino-acid linker between the nucleoside and iminogalactofuranose components should permit 'tailoring' of this bridging unit for the best 'fit', and replacement of the labile phosphodiester bonds with amides should increase stability and cell permeability of these compounds.

Thus, the amino-uridine derivative **23**[22] was coupled to Fmoc-Gly-Cl and Fmoc-β-Ala-Cl under biphasic conditions in the presence of sodium carbonate[23] to afford Fmoc-Gly amide **24** (m.p. 124°C, $[\alpha]_D^{22}$ -7.7 (*c*, 1.0 in MeOH) [87% yield]) and Fmoc-β–Ala amide **26** (m.p. 115°C, $[\alpha]_D^{22}$ -10.4 (*c*, 1.0 in MeOH) [93% yield]), Scheme 2. Removal of the *N*-terminal Fmoc groups with diethylamine in acetonitrile gave the free amines **25** and **27** respectively which were reacted directly with amino-triflate **7** at 60°C in THF in the presence of triethylamine. This gave the protected glycyl sugar-nucleoside **28** (m.p. 114°C, $[\alpha]_D^{22}$ -23.7 (*c* 1.0 in $CHCl_3$) [58% yield]) and the β-alanyl sugar-nucleoside **29** (m.p. 102°C, $[\alpha]_D^{22}$ -65.0 (*c*, 1.0 in MeOH) [67% yield]). Removal of the triethylsilyl and isopropylidene protecting groups with aqueous trifluoroacetic acid afforded the free iminosugar-peptidonucleosides **30** ($[\alpha]_D^{22}$ -6.9 (*c*, 1.0 in H_2O) [83% yield])[24] and **31** ($[\alpha]_D^{22}$ -6.5 (*c*, 1.0 in MeOH) [75% yield]).[25]



Scheme 2: (i) Fmoc Gly-Cl or Fmoc β–Ala-Cl, $CHCl_3$, 5% Na_2CO_3 (ii) Et_2NH, CH_3CN (iii) cmpd 7, Et_3N, THF, 60°C (iv) TFA, H_2O

In conclusion, it has been illustrated that the amino-triflate **7** can be considered as a masked polyhydroxylated proline synthon bearing D-galactofuranose stereochemistry *via* opening of the lactone functionality. This has been confirmed in the synthesis of the 1,2-diamines **19-21** and the novel iminosugar-peptidonucleoside analogues **30** and **31**. Exploitation of this chemistry should further allow the generation of compound libraries containing both uridinyl and galactofuranosyl iminosugar motifs.[26]

References and Notes

1 Centre for Disease Control, *Morbid. Mortal. Wkly. Rep.*, **1993**, *42*, 961.

2 Sudre, P.; Tendam, G.; Kochi A. *Bull. W. H. O.*, **1992**, *70*, 149.

3 Rouhi, A. M. *Chem. Eng. News*, **1999**, *77 (20)*, 52.

4 Chatterjee, D. *Curr. Opin. Chem. Biol.*, **1997**, *1*, 579.

5 Brennan, P. J.; Nikaido, H. *Annu. Rev. Biochem.*, **1995**, *64*, 29.

6 Lee, R. E.; Brennan, P. J.; Besra, G. S. *Curr. Top. Microbiol. Immunol.*, **1996**, *215*, 1.

7 Mikusova, K.; Mikus, M.; Besra, G. S.; Hancock, I.; Brennan, P. J. *J. Biol. Chem.*, **1996**, *271*, 7820.

8 Lee, R.; Monsey, D.; Weston, A.; Duncan, J. K.; Rithner, C.; McNeil, M.; *Anal. Biochem.*, **1996**, *242*, 1.

9 Maddry, J. A.; Suling, W. J.; Reynolds, R. C., *Res. Microbiol.*, **1996**, *147*, 106.

10 Lee, R. E.; Smith, M. D.; Nash, R. J.; Griffiths, R. C.; McNeil, M.; Grewal, R. K.; Yan, W.; Besra, G. S.; Brennan, P. J.; Fleet, G. W. J. *Tetrahedron Lett.*, **1997**, *38*, 6733.

11 Davis, B. G.; Hull, A.; Smith, C.; Nash, R.J.; Watson, A. A.; Winkler, D. A.; Griffiths, R. C.; Fleet, G. W. J., *Tetrahedron: Asymmetry*, **1998**, *9*, 2947.

12 Maddry, J. A.; Bansal, N.; Bermudez, L. E.; Comber, R. N.; Orme, I. M.; Suling, W. J.; Wilson, L. N.; Reynolds, R. C., *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 237.

13 Shilvock, J. P.; Wheatley, J. R.; Nash, R. J.; Watson, A. A.; Griffiths, R. C.; Butters, T. D.; Müller, M.; Watkin, D. J.; Winkler, D. A.; Fleet, G. W. J. *J. Chem. Soc., Perkin Trans. 1*, **1999**, in press.

14 All new compounds have satisfactory microanalysis and/or high resolution mass spectrometry data.

15 Selected data for diamine **19**: δ_H(500MHz, D_2O) 4.25 (1H, app d, J 3.5, H-3), 4.12 (1H, m, H-4), 3.96 (1H, ddd, $J_{2,1}$ 6.6, $J_{2,1'}$ 6.2, $J_{2,3}$ 3.8, H-2), 3.91 (1H, ddd, $J_{6,5}$ 8.4, $J_{6,7'}$ 4.8, $J_{6,7}$ 3.4, H-6), 3.69 (1H, dd, $J_{7,7'}$ 12.3, $J_{7,6}$ 3.4, H-7), 3.58 (1H, dd, $J_{7',7}$ 12.3, $J_{7',6}$ 4.8, H-7'), 3.51 (1H, dd, $J_{1,1'}$ 14.0, $J_{1,2}$ 6.6, H-1), 3.52 (1H, dd, $J_{5,6}$ 8.5, $J_{5,4}$ 3.7, H-5), 3.40 (1H, dd, $J_{1',1}$ 13.9, $J_{1',2}$ 6.2, H-1'); δ_C(125MHz, $CDCl_3$) 77.1 (d), 75.6 (d), 70.1 (d), 69.0 (d), 63.6 (t), 59.2 (d), 36.3 (t).

16 Selected data for methyl diamine **20**: δ_H(500MHz, D_2O) 4.26 (1H, dd, $J_{3,2}$ 3.6, $J_{3,4}$ 1.4, H-3), 4.13 (1H, dd, $J_{4,5}$ 3.3, $J_{4,3}$ 1.5, H-4), 4.01 (1H, ddd, $J_{2,1}$ 6.7, $J_{2,1'}$ 6.2, $J_{2,3}$ 3.7, H-2), 3.91 (1H, m, H-6), 3.69 (1H, dd, $J_{7,7'}$ 12.3, $J_{7,6}$ 3.4, H-7), 3.59 (1H, dd, $J_{7',7}$ 12.4, $J_{7',6}$ 4.2, H-7'), 3.57 (1H, dd, $J_{1,1'}$ 13.8, $J_{1,2}$ 6.7, H-1), 3.52 (1H, dd, $J_{5,6}$ 8.4, $J_{5,4}$ 3.4, H-5), 3.45 (1H, dd, $J_{1',1}$ 13.7, $J_{1',2}$ 6.2, H-1'), 3.72 (3H, s, NMe); δ_C(125MHz, $CDCl_3$) 77.1 (d), 75.7 (d), 70.1 (d), 69.0 (d), 63.6 (t), 58.4 (d), 45.4 (t), 34.7 (q).

17 Selected data for benzyl diamine **21**: δ_H(500MHz, D_2O) 7.49 (5H, m, Ph), 4.35 (2H, s, CH_2Ph), 4.34 (1H, dd, $J_{3,2}$ 3.6, $J_{3,4}$ 1.6, H-3), 4.21 (1H, dd, $J_{4,5}$ 3.4, $J_{4,3}$ 1.6, H-4), 4.01 (1H, ddd, $J_{2,1}$ 6.2, $J_{2,1'}$ 6.2 $J_{2,3}$ 3.7, H-2), 3.91 (1H, ddd, $J_{6,7}$ 3.5, $J_{6,7'}$ 4.8 $J_{6,5}$ 8.3, H-6), 3.77 (1H, dd, $J_{7,7'}$ 12.3, $J_{7,6}$ 3.5, H-7), 3.71 (1H, dd, $J_{1,1'}$ 13.8, $J_{1,2}$ 6.2, H-1), 3.67 (1H, dd, $J_{7',7}$ 12.3, $J_{7',6}$ 4.8, H-7'), 3.60 (2H, m, H-5, H-1'); δ_C(125MHz, $CDCl_3$) 130.8 (d), 130.1 (d), 77.0 (d), 75.7 (d), 70.1 (d), 69.0 (d), 63.5 (d), 58.9 (t), 43.5 (t).

18 Lee, R. E.; Mikusova, K.; Brennan, P. J.; Besra, G. S. *J. Am. Chem. Soc.*, **1995**, *117*, 11829.

19.Schroder, P. N.; Giannis, A. *Angew. Chem., Int. Ed.*, **1999**, *38*, 1379.

20 Kim, Y. J.; Ichikawa, M.; Ichikawa, Y. *J. Am. Chem. Soc.*, **1999**, *121*, 5829.

21 Müller, B.; Schaub, C.; Schmidt, R. R. *Angew. Chem., Int. Ed.*, **1998**, *37*, 2893.

22 Wang, R.; Steensma, D. H.; Takaoka, Y.; Yun, J. W.; Kajimoto, T.; Wong, C. –H. *Bioorg. Med. Chem.*, **1997**, *5*, 661.

23 Beyermann, M.; Bienert, M.; Niedrich H. *J. Org. Chem.*, **1990**, *55*, 721.

24 Selected data for peptidonucleoside **30**. δ_H(500MHz, D_2O) (pH 4.1) 7.53(1H, d, $J_{2,1}$ 8.1, H-2), 5.78 (1H, d, $J_{1,2}$ 8.1, H-1), 5.68 (1H, d, $J_{3,4}$ 4.4, H-3), 4.62 (1H, d, $J_{9,10}$ 6.0, H-9), 4.44 (1H, dd, $J_{10,9}$ 6.1, $J_{10,11}$ 3.6, H-10), 4.24 (1H, app t, $J_{4,3}$ 4.4, $J_{4,5}$ 5.2, H-4), 4.11 (1H, dd, $J_{11,12}$ 4.7, $J_{11,10}$ 3.8, H-11), 3.98 (3H, m, H-5, H-6, H-13), 3.91 (2H, AB system, H-8,8'), 3.67 (1H, dd, $J_{14,14'}$ 12.2, $J_{14,13}$ 3.5, H-14), 3.56 (1H, dd, $J_{14',14}$ 12.2, $J_{14',13}$ 4.7, H-14'), 3.53 (1H, dd, $J_{12,13}$ 7.1, $J_{12,11}$ 4.9, H-12); 3.48 (1H, dd, $J_{7,7'}$ 14.6 , $J_{7,6}$ 4.3 , H-7), 3.44 (1H, dd, $J_{7',7}$ 14.5, $J_{7',6}$ 6.0, H-7'); δ_C(125MHz, D_2O) 171.6 (s), 166.8 (s), 166.6 (s), 151.9 (s), 142.7 (d), 102.7 (d), 90.9 (d), 82.1 (d), 76.3 (d), 75.9 (d), 73.4 (d), 70.8 (d), 68.4 (d), 65.9 (d), 63.3 (t), 62.2 (d), 43.1 (t), 41.0 (t).

25 Selected data for peptidonucleoside **31**: δ_H(500MHz, D_2O) 7.60 (1H, d, $J_{2,1}$ 8.1, H-2), 5.80 (1H, d, $J_{1,2}$ 8.1, H-1), 5.70 (1H, d, $J_{3,4}$ 4.4, H-3), 4.50 (1H, d, $J_{10,11}$ 5.8, H-10), 4.39 (1H, dd, $J_{11,10}$ 5.8, $J_{11,12}$ 3.3, H-1), 4.31 (1H, dd, $J_{4,3}$ 4.4, $J_{4,5}$ 5.2, H-4), 4.14 (1H, app t, J 4.0 H-12), 4.02 (3H, m, H-5, H-6, H-14), 3.72 (1H,dd, $J_{15,15'}$ 12.2, $J_{15,14}$ 3.6, H-15), 3.61 (1H, dd, $J_{15',15}$ 12.2, $J_{15',14}$ 4.8, H-14'), 3.55 (1H, dd, $J_{13,14}$ 7.1, $J_{13,12}$ 4.5, H-13); 3.49 (1H, dd, $J_{7,7'}$ 14.6 , $J_{7,6}$ 4.2 , H-7), 3.46 (2H, t, $J_{9,8}$ 6.6, H-9), 3.44 (1H, dd, $J_{7',7}$ 14.4, $J_{7',6}$ 6.5, H-7'), 2.52 (2H, t, $J_{8,9}$ 6.6, H-8); δ_C(125MHz, D_2O) 174.9 (s), 166.9 (s), 166.2 (s), 143.2 (d), 103.1 (d), 91.5 (d), 82.7 (d), 76.9 (d), 76.2 (d), 73.8 (d), 71.3 (d), 68.8 (d), 66.5(d), 63.8 (t), 62.8 (d), 41.5 (t), 37.1 (t), 35.8 (t).

26 This work has been supported by GlaxoWellcome, EPSRC and BBSRC