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Synthesis of *Arabino* glycosyl triazoles as potential inhibitors of mycobacterial cell wall biosynthesis

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ABSTRACT

A series of *arabino* glycosyl triazoles with varying hydrophobic groups were synthesised as putative mimics of decaprenolphosphoarabinose (DPA) as potential inhibitors of mycobacterial cell wall biosynthesis. Biological testing against *Mycobacterium bovis BCG* revealed low to moderate anti-mycobacterial activity, with strong dependence on the identity of the hydrophobic side chain.

Inhibition of mycobacterial cell wall biosynthesis represents a significant therapeutic opportunity for the development of new anti-tubercular agents.[1] The cell walls of mycobacteria are complex, and there are therefore many biosynthetic steps that could in principle be targeted in order to compromise mycobacterial viability. Two cell wall polysaccharides that are unique to mycobacteria and which are crucial to mycobacterial survival and growth are lipoarabinomannan (LAM) and arabinogalactan (AG). Inhibition of the biosynthesis of either LAM or AG represents a potentially highly selective opportunity for therapeutic intervention, and the design and synthesis of such potential inhibitors has been a field of intense interest over recent years. Approaches have included[2] attempted inhibition of various glycosyl transferases[3] used for polysaccharide assembly, and also of other vital biosynthetic enzymes such as the Gal*p*/Gal*f* mutase which catalyses a crucial pyranose/furanose isomerisation during assembly of the galactan cell wall component.[4]

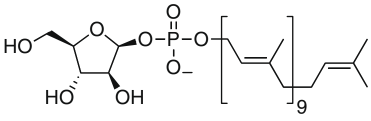

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Figure 1. Decaprenolphosphoarabinose **1** (DPA).

Particular interest has focused on the attempted discovery[5] of potent inhibitors of arabinosyl transferases, which are responsible for the stepwise assembly of the arabinan portions of both LAM and AG, and use decaprenol phosphoarabinose **1** (DPA, Fig. 1) as the glycosyl donor. Metabolically stable analogues of DPA may inhibit these arabinosyl transferases, and so in turn represent lead compounds for the discovery of new anti-tubercular agents. In order to design mimics of DPA that may be expected to display useful in vivo activity, two immediate considerations present themselves. Firstly replacement of the labile glycosyl phosphate with a stable isostere would be highly desirable. Secondly it may be expected that an alkyl chain of a certain minimum length would be required to mimic the large hydrophobic decaprenyl moiety of DPA. Various isosteric replacements for the glycosyl phosphate have been investigated[6] including β-*C*-glycosyl sulfones and β-*C*-phosphonates. Amongst several approaches[7] to inhibition of mycobacterial arabinan biosynthesis under investigation in this laboratory the potential use of a β-glycosyl triazole as the replacement for the glycosyl phosphate moiety of DPA appeared to be an attractive line of investigation.[8] The discovery that Cu(I) salts can efficiently catalyse the Huisgen 1,3-dipolar cycloaddition of alkynes and azides, reported simultaneously[9] by Meldal and Sharpless, has led to a resurgence in interest in the triazole functional group, and has popularised its use as an isosteric replacement in lead generation programs. Indeed this particular application of 'Click chemistry'[10] has become one of the mainstays of currently favoured approaches for the

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introduction of structural diversity in medicinal chemistry programs. The synthesis of glycosyl triazoles derived from anomeric azides has been extensively investigated, and glycosyl triazoles[11] have been demonstrated to posses a wide range of interesting biological activity. Pertinently to the anti-mycobacterial field of investigation other carbohydrate-derived triazoles in which the triazole moiety is not linked to the anomeric position have also generated interest, and a series of *arabino* and *ribo*-configured pentose triazoles in which azide was introduced at the 5-position before cycloaddition with a series of alkynes have very recently been synthesised as potential anti-tubercular agents.[12]

The synthesis of a series β-arabinofuranose glycosyl triazoles was therefore undertaken as putative mimics of DPA; herein a variety of hydrophobic groups of varying lengths were installed as mimics of the extended polyprenyl side chain in order to delineate structure activity relationships. Anti-bacterial activity of the ensuing compounds was tested in a variety of cell growth assays against both *Escherichia coli* and *Mycobacterium bovis BCG*.

The known benzoylated *arabino* α-glycosyl bromide **2**[13] was reacted with sodium azide under phase transfer conditions to give the corresponding glycosyl azide (64% overall yield) as an anomeric mixture in which the desired β-anomer **3**[14] predominated (α:β ratio ~1:2), and from which pure β-anomer was easily obtained after purification by flash column chromatography (isolated yield of **3**, 43%). Modified Cu (I) catalysed Huigsen cycloaddition[15] of azide **3** with alkyne **4a** in which Cu(I) was generated in situ by the addition of sodium ascorbate to a solution of $CuSO_4$[16] proved sluggish, and therefore an alternative procedure was investigated. Reaction of azide **3** with alkyne **4a** and copper (I) iodide in toluene, in the presence of diisopropylethylamine (DIPEA) smoothly gave the desired triazole **5a**. Similar reaction of the benzoate protected azide **3** with the remainder of the alkynes **4b–f**[17] gave the corresponding β-glycosyl triazoles **5a–f**[18] in excellent yield. Finally de-protection with sodium methoxide in methanol yielded the triol triazoles **6a–f** for biological screening (Scheme 1).

Anti-mycobacterial testing of compounds **6a–f** was initially performed by a spot-test method using *M. bovis BCG* as a model for *M. tuberculosis*. *M. bovis BCG* cultures were spotted onto 6 well plates containing solid media and the test compounds at various concentrations. The cultures were then grown in an incubator at 37 °C for 7–14 days. The effect of test compounds on cell growth was then assessed by measuring the area of the circular mycobacterial spot cultures after photography using a Bio-Rad Gel-Doc 2000 system, a reduction in this value indicating anti-mycobacterial effects.[19] Whilst compound **6f** did not display any anti-mycobacterial effects at the highest concentration investigated (1 mg/mL) all of the other triazoles showed effects on mycobacterial cell growth. A second series of spot tests was performed on *E. coli* (strain JM109) in which none of the compounds **6a–f** displayed any anti-bacterial effects at the highest concentrations (1 mg/mL), indicating a selective anti-mycobacterial mode of action. The minimum inhibitory concentrations (MIC) of the anti-mycobacterial activity of compounds **6a–e** were then measured in a second series of tests on *M. bovis BCG* using the Alamar Blue microplate assay, in which the Alamar Blue dye changes from the oxidised indigo blue (and non-fluorescent) form to the reduced pink (and fluorescent) form in the presence of growing bacteria (Fig. 2).[20] The results are shown in Table 1 and Figure 2.

Several points are worthy of note, though general conclusions are somewhat harder to arrive at. First of all the complete lack of activity of the phenyl triazole **6f**, and the low activity of the hexyl triazole **6a** indicate that hydrophobicity of the side chain is impor-

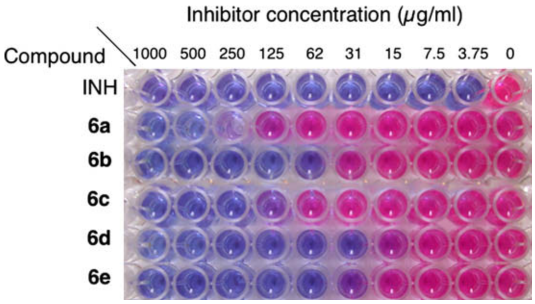
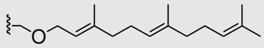
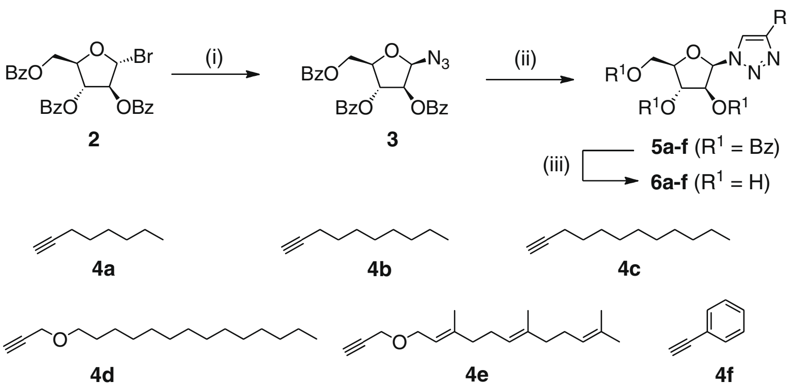


Figure 2. Alamar Blue assay of deprotected glycosyl triazoles **6a**–**e** and control with isoniazid (INH).

Table 1
Inhibitory effects of deprotected glycosyl triazoles **6a**–**f** against *M. bovis BCG*

Compound	R′	MIC[a] (μg/mL)
INH	–	0.1
6a	$-(CH_2)_5CH_3$	500
6b	$-(CH_2)_7CH_3$	62
6c	$-(CH_2)_9CH_3$	250
6d	$-CH_2O(CH_2)_{13}CH_3$	31
6e		62
6f	–Ph	No activity

[a] MIC, minimum inhibitory concentration; the lowest concentration of the compound which inhibited the growth of *M. bovis BCG* >90% from the Alamar Blue assay. Isoniazid (INH) was used as a control (MIC 0.1 μg/mL).



Scheme 1. Reagents and conditions: (i) NaN_3 (5.0 equiv), Bu_4NHSO_4 (1.0 equiv), 1:1 DCM-$NaHCO_3$ (aq), 43%; (ii) **4a–f** (5–10 equiv), CuI (1.0 equiv), DIPEA (1.0 equiv), 110 °C, 72–92%; (iii) $NaOCH_3$ (0.4 equiv), CH_3OH-THF (2:1 v/v), 92–100%.

tant for biological activity.[21] These results agree with the lack of activity previously reported for *arabino-C*-glycosyl phenyl sulfones,[3a] but the fact that the octyl triazole **6b** is more active that the corresponding decyl triazole **6c** indicates that there is not a simple correlation between biological activity and the length of an alkyl side chain; an observation in agreement with a previous report by Lowary[6b] on a study of *arabino-C*-glycosyl sulfones. It is also notable in the current study that the most potent compound, ether **6d** which contains a tetradecyl side chain, is approximately twice as active as the corresponding farnesyl ether **6e**, though the latter more closely resembles the natural substrate DPA. The significance of these results remains unclear, particularly in view of the modest anti-mycobacterial activity observed herein; even the most potent compound **6d** is at least two orders of magnitude less active than isoniazid (INH). The synthesis of more derivatives will be necessary in order to access more potent compounds and to further delineate precise structure–activity relationships.

In conclusion the synthesis of a variety of β-*arabino* glycosyl triazoles possessing various hydrophobic chains was achieved using modified Click chemistry. These glycosyl triazoles displayed weak to moderate yet selective anti-mycobacterial activity in assays against *M. bovis BCG*; activity was strongly dependent on the nature of the hydrophobic group.

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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.2008.09.082.

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18. See Supporting Information for full experimental details and complete characterisation of all new compounds. Selected data: **6d**, a pale yellow solid; mp 67–68 °C; $[\alpha]^{25}_{D}$ −19.6 (*c* 0.65, CH_3OH); δ_H (400 MHz, $CDCl_3$), 0.9 (t, *J* 6.8 Hz, CH_3), 1.24–1.36 (m, 24H, 12× CH_2), 1.54–1.61 (2H, m, CH_2), 3.50 (t, *J* 6.5 Hz, OCH_2), 3.81 (1H, dd, $J_{4\text{-}5}$ 5.1 Hz, $J_{5\text{-}5'}$ 12.1 Hz, H-5′), 3.86 (1H, dd, $J_{4\text{-}5'}$ 3.4 Hz, H-5″), 3.94–3.98 (1H, m, H-4′), 4.28 (1H, at, *J* 5.5 Hz, H-2′), 4.34 (1H, at, *J* 5.5 Hz, H-3′), 4.58 (s, 2H, $C{=}CCH_2$), 6.32 (1H, d, $J_{1\text{-}2}$ 5.5 Hz, H-1′), 8.15 (1H, s, $C{=}CH$); δ_C (125 MHz, CD_3OD), 14.5 (q, CH_3), 23.8 (t, CH_2), 27.3 (t, CH_2), 30.5 (t, CH_2), 30.6 (t, CH_2), 30.7 (t, CH_2), 30.8 (2× t, 2× CH_2), 30.8 (2× t, 2× CH_2), 30.8 (2× t, 2× CH_2), 30.9 (t, CH_2), 33.1 (t, CH_2), 62.6 (t, C-5′), 64.7 (t, OCH_2), 71.7 ($C{=}CCH_2$), 75.9 (d, C-2′), 78.8 (d, C-3′), 85.9 (d, C-1′), 124.9 ($C{=}CH$), 145.6 ($C{=}CH$); HRMS (ES^+) Calcd. for $C_{22}H_{41}N_3O_5$ (MNa^+) 450.2938. Found 450.2938.
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