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## Glycomimetic Inhibitors of Mycobacterial Glycosyltransferases: Targeting the TB Cell Wall

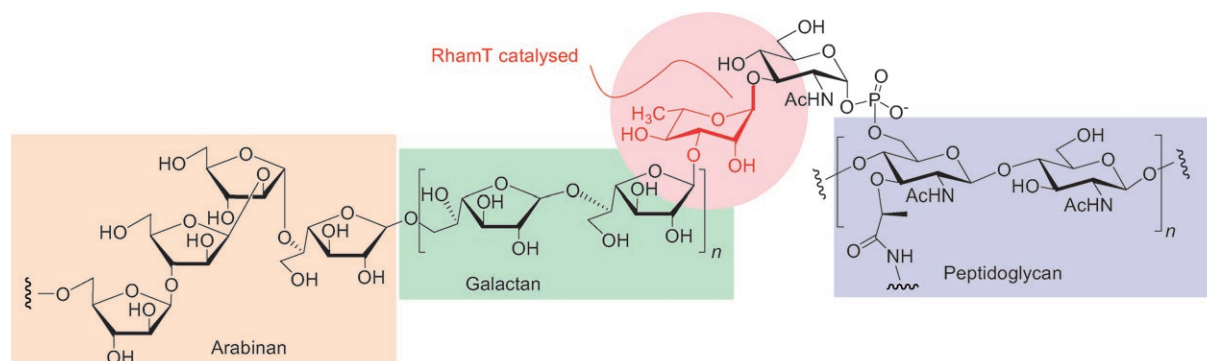
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Tuberculosis (TB) is the cause of more than a million deaths a year<sup>[1]</sup> and is believed to infect a third of the world's population.<sup>[2]</sup> Emergent multidrug-resistant strains<sup>[3]</sup> of *Mycobacterium tuberculosis*, the organism that causes the disease, and difficulties in treating immunocompromised individuals have further increased the urgency of the threat.<sup>[4]</sup> The cell wall of mycobacteria is formed by polysaccharides and lipids<sup>[5,6]</sup> essential for cell growth and survival in the host,<sup>[7]</sup> and the importance of its integrity is confirmed by the effectiveness of methods that disrupt cell wall biosynthesis.<sup>[8]</sup> The major polysaccharide region is joined to peptidoglycan by the so-called bridging region, which contains a critical and unique disaccharide phosphodiester linker (Scheme 1). The presence of an L-rhamnosyl residue in this linker region is a striking drug target opportunity,<sup>[9]</sup> as it is a sugar that is not found in mammalian cells. A key step in the proposed biosynthetic pathway of the bacterial cell wall<sup>[9,10]</sup> is the rhamnosyltransferase-mediated (RhamT) glycosylation of GlcNAc-diphosphoprenyl acceptor by the dTDP-Rha donor. Since this class of enzyme is not found in man, RhamT inhibition is an avenue for a potentially nontoxic treatment of TB.<sup>[8]</sup>

Iminosugars have been widely studied as inhibitors of carbohydrate-processing enzymes such as glycosidases<sup>[11,12]</sup> and glycosyltransferases (GTs).<sup>[13–15]</sup> However, inhibition of L-rhamnosyl-processing enzymes has not been widely explored<sup>[16,17]</sup> and there are no known Rham-T inhibitors. We report here a novel, ready and modular methodology to synthesise iminosugar L-rhamnomimetics that are effective inhibitors of L-rhamnose processing enzymes including importantly the *Mycobacterium* biosynthesis of the bridging disaccharide region.

Two parallel synthetic strategies (Scheme 2) allowed ready access to libraries of both  $\alpha$ - and  $\beta$ -pseudoanomers based on the L-rhamno-aza-C-glycoside<sup>[18]</sup> scaffold **1**. From the key divergent intermediate **3**,<sup>[16]</sup> through diastereoselective reduction or nucleophilic addition coupled with variation of the timing of substituent (R) introduction we could control both the pseudoanomeric configuration and identity of R in a wide-ranging manner. This has allowed us to map binding interactions and the effect of configuration in Rha-processing enzymes.

Imine intermediates to rhamnomimetics **1**  $\alpha$ -**h** and **1**  $\beta$ -**p** were readily accessed from azidocarbonyl precursors (**5** and **6**, respectively) through either intramolecular Staudinger aza-



Scheme 1. *Mycobacterium tuberculosis* cell-wall glycan and "linker" region.

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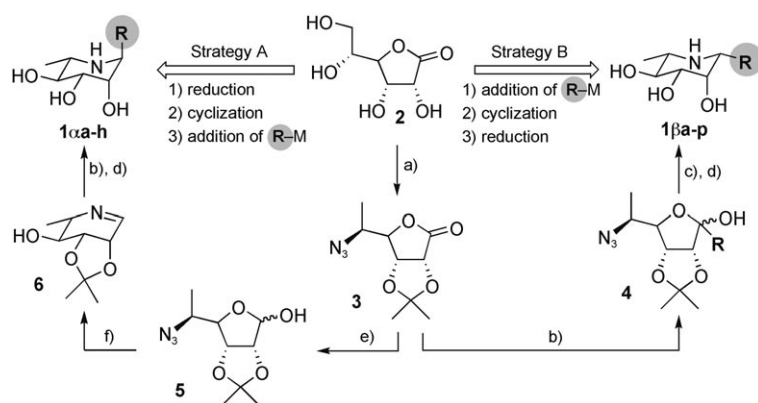
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Wittig ring-closing metathesis or hydrogenolytic reductive cyclisation, respectively. Substituents were introduced at an early (by nucleophilic addition to azidolactone **3**) or late stage (by nucleophilic addition to cyclic polyhydroxyimine **6**).

Azidolactone **3** was prepared from D-gulonolactone in six steps.<sup>[16]</sup> Representative substituents were introduced to the **1**  $\beta$  scaffold through nucleophilic addition including the use of lithiated alkoxymethyl nucleophiles.<sup>[19]</sup> Few examples of nucleophilic additions to 5-azido 1,4-lactones have been reported,<sup>[20]</sup> pleasingly all additions (Table 1) selectively yielded corresponding azidolactols (**4 b–p**, 68–100%) with no sign of overaddition.<sup>[21–28]</sup> Hydrogenation gave protected L-rhamnoperidines



**Scheme 2.** Synthesis of the  $\alpha,\beta$ -aza-C-rhamnomic. Reagents and conditions: a) 5 steps: i) 2,2-dimethoxypropane, PTSA, acetone. ii) AcOH-H<sub>2</sub>O. iii) CBr<sub>4</sub>, PPh<sub>3</sub>, THF. iv) H<sub>2</sub>, Pd-C, EtOH, NEt<sub>3</sub>. v) Tf<sub>2</sub>O, py, DCM, -40 °C then NaN<sub>3</sub>, DMF. b) RM, THF, -78 to 60 °C or -78 ° to RT; c) H<sub>2</sub>, Pd-C, EtOH; d) TFA/H<sub>2</sub>O; e) DIBAL-H, THF, -78 °C; f) Ph<sub>2</sub>P(CH<sub>2</sub>)<sub>2</sub>PPh<sub>2</sub>, THF, 50 °C. See Table 1 for range of R and yields. PTSA = *p*-toluenesulfonic acid; Tf = trifluoromethanesulfonyl; DCM = dichloromethane; DMF = dimethylformamide; DIBAL-H = diisobutylaluminium hydride.

**Table 1.** Organometallic additions: reaction conditions and yields.

RM	Side chain		Method <sup>[a]</sup>	Yield <sup>[b]</sup> [%]	
	1 $\alpha$	1 $\beta$		1 $\alpha$	1 $\beta$
1	<i>i</i> Bu <sub>2</sub> AlH	a	A	100	
2	MeMgCl	b	B	91	
3	EtMgBr	c	B	98	
4	EtMgBr	c	A	67	
5	VinylMgBr	d	B	93	
6	VinylMgBr	d	A	81	
7	AllylMgBr	e	B		72
8	AllylMgBr	e	A <sup>[d]</sup>	75	
9	EthynylMgBr	f	A	82	
10	PhMgCl	g	B	92	
11	PhMgBr	g	A <sup>[e]</sup>	31	
12	2-NaphthylMgBr	h	B	99	
13	2-NaphthylMgBr	h	A <sup>[e]</sup>	24	
14	<i>n</i> BuLi	i	B	96	
15	OctylMgBr	j	B	97	
16	MeOPhMgBr	k	B	99	
17	BnMgBr	l	B	82	
18	MeOPhCH <sub>2</sub> MgCl	m	B	69	
19	BiphenylMgBr	n	B	95	
20	PhenanthrylMgBr	o	B	70	
21	MeOPhCH <sub>2</sub> OCH <sub>2</sub> Li <sup>[c]</sup>	p	B	68	

[a] Method A: imine formation using Ph<sub>2</sub>P(CH<sub>2</sub>)<sub>2</sub>PPh<sub>2</sub> in THF, 50 °C, 15 min prior to addition to Grignard solution. Method B: organometallic (1.2–1.3 equiv) additions in THF -78 °C to -60 °C, 1–2 h then hydrogenation. [b] Isolated yields of **1** from **3** over 3–4 steps; configuration determined by NOESY spectroscopy. [c] RLi prepared in situ with MeOPhCH<sub>2</sub>OCH<sub>2</sub>SnBu<sub>3</sub> and *n*BuLi. [d] Imine formation also with PBU<sub>3</sub> (1.2 equiv), THF, 30 min, rt. [e] Imine formation was with PPh<sub>3</sub> (3.0 equiv), THF, 50 °C, 3 h. See the Supporting Information for full details.

as single diastereoisomers in high yields (82–92%) by reduction of the azido group and subsequent intramolecular reduc-

tive amination. Isopropylidene deprotection using TFA followed by ion-exchange gave **1a**<sup>[16]</sup> and novel aza-C- $\beta$ -rhamnomic **1 $\beta$ b–p** (70–98%; Scheme 2).

The synthesis of the corresponding aza-C- $\alpha$ -mimetics was achieved using organometallic additions<sup>[29–32]</sup> to partially protected cyclic imine **6**, accessed by the Staudinger aza-Wittig reaction.<sup>[33]</sup> Optimal yields were obtained from addition of imine to Grignards (inverse addition<sup>[31]</sup>), as representative, model nucleophiles, (see Table 1, Method B) which successfully yielded adducts despite the presence of an unprotected hydroxy group (4-OH) in **6**. Excellent diastereoselectivities were observed in all of the additions (*de* > 98%), consistent with a conformation enforced by 2,3-O-isopropylidene that favours nucleophile approach from the resulting convex  $\alpha$  face. Subsequent TFA deprotection yielded the aza-C- $\alpha$ -rhamnomic **1 $\alpha$ c–h** (85–95%).

Having readily generated a range of rhamnomic **1** as potential enzyme inhibitors, all the compounds were assayed against a variety of representative prokaryotic and eukaryotic glycosidases.<sup>[34]</sup> all showed some inhibition against  $\alpha$ -rhamnosyl processing enzyme naringinase at 1 mM. In particular, the naphthyl derivatives **1 $\alpha$ h** and **1 $\beta$ h** were found to be potent and selective competitive inhibitors with *K<sub>i</sub>* = 1.0 and 0.26  $\mu$ M, respectively. Importantly, this inhibition is highly selective: no inhibition was exhibited by other glycosidases capable of binding a range of sugar configurations found in mammalian glycobiology ( $\beta$ -glucosidase,  $\alpha$ - and  $\beta$ -mannosidase,  $\alpha$ - and  $\beta$ -galactosidase,  $\alpha$ -fucosidase). Excitingly, this suggested that the use of such compounds in mammalian systems may be possible without concomitant side-effect inhibition of host enzymes.

Following this exciting confirmation of the configurational mimicry of **1 $\alpha$ c–h** and **1 $\beta$ a–n** in Rha-processing systems, we moved to evaluate inhibition<sup>[35]</sup> of mycobacterial systems.<sup>[8,36,37]</sup> Interestingly, of all the compounds, unsubstituted **1a** was the most potent (38% at 100  $\mu$ M). Reasonable inhibition was also exhibited only by those bearing  $\beta$ -alkyl and  $\beta$ -aryl pseudoanomeric substituents (**1 $\beta$ b** 25%, **1 $\beta$ c** 32% **1 $\beta$ i** 19%, **1 $\beta$ j** 26%, **1 $\beta$ m** 23%, and **1 $\beta$ p** 30%), including the naphthyl analogue **1 $\beta$ h** (25%). To the best of our knowledge these are the first rhamnose-like inhibitors of mycobacterial RhamT to date and suggest some early potential for success in targeting this enzyme. This inhibition is configurationally sensitive and selective: no inhibition was displayed by any of the **1 $\alpha$**   $\alpha$ -series and none of the compounds inhibited (see the Supporting Information) the RmlC/D enzymes that are dTDP-6-deoxy-4-ketoglucose epimerase/dTDP-Rha synthase, respectively, responsible for the formation of the donor sugar dTDP- $\beta$ -L-Rha.

The striking lack of activity by any of the  $\alpha$ -configured mimetic series **1 $\alpha$**  and the inhibition by only members of the  $\beta$  series **1 $\beta$**  towards the mycobacterial system suggests mimicry of the donor substrate dTDP- $\beta$ -L-Rha, or of a transition state in which a group is found at the  $\beta$  face of a rhamnosyl unit. Indeed, addition of pure, synthetic (for synthesis see the Supporting Information) rhamnosyltransferase substrate dTDP- $\beta$ -

Rha to the mycobacterial assay system reduced inhibition in a manner consistent with competition with the inhibition of **1a**. This substrate protection/rescue suggested competitive inhibition by **1a** of the donor site of the L-rhamnosyltransferase.

Some of the most active compounds (for example, **1βj** (*n*-octyl), **1βm** (*p*-methoxybenzyl)) bear large substituents at their β face. In contrast, phenyl-(**1βg**)- and *p*-methoxy-phenyl-(**1βk**)-substituted compounds showed little or no inhibitory activity. Together these structure–activity data suggest a potentially deep but narrow-necked hydrophobic pocket at the β face within the inhibition (donor) site.

In conclusion, this paper describes the efficient synthesis of α- and β-homonojirimycin analogues of L-rhamnopyranose. These inhibitors are directly effective against the activity of isolated mycobacterial membrane, with promising but, as yet, unoptimised, inhibitory activity towards a mycobacterial pathway involving rhamnosyltransferase, a validated enzyme target in the fight against tuberculosis.

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 [34] These included β-glucosidase (sweet almonds), α-mannosidase (jack beans), β-mannosidase (snail acetone powder), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*), α-fucosidase (bovine kidney), and α-rhamnosidase (*Penicillium decumbens*, naringinase).  
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