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Synthesis and evaluation of galactofuranosyl *N*,*N*-dialkyl sulfenamides and sulfonamides as antimycobacterial agents

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Abstract—The recent emergence of clinically oppressive superbugs, some with resistance to nearly all frontline drug therapies, has challenged our ability to combat such infectious organisms as *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB). Our medicinal chemistry program targeting this pathogen has identified several potent galactofuranose-based in vitro inhibitors of mycobacterial growth. The most potent compound, the Gal*f* *N*,*N*-didecyl sulfenamide **8d**, displayed anti-mycobacterial activity (MIC) of 1 μg/mL in a cell based assay against a representative strain of *Mycobacterium smegmatis*.

Of the seventy or so mycobacterial species,[1] the high morbidity and mortality associated with *Mycobacterium tuberculosis* infection makes this bacterium by far the most concerning of the mycobacterium genus. At present an estimated one-third of the world's population is infected with the TB bacillus.[2] With approximately nine million new cases each year, and an annual death toll of two million, *M. tuberculosis* is now recognised as the single most infectious pathogen worldwide.[2,3] Compounding this problem is the emergence of strains of the TB bacillus that are resistant to all major anti-TB drugs.[2] This grim outlook has intensified the quest to discover more effective, less toxic and preferably cheaper drugs to treat TB infection. Our medicinal chemistry program targeting this pathogen has focused on the development of compounds based on the carbohydrate D-galactofuranose (Gal*f*), an essential cell wall component of mycobacteria.[4]

The mycobacterial cell wall contains a mycolyl-arabinogalactan peptidoglycan (mAGP) complex; a highly cross-linked peptidoglycan layer supporting an arabinogalactan (AG) polymer which is capped with a tetramycolated hexa-arabinofuranoside cluster.[5] The arabinogalactan contains a galactan polymer made up of alternating β-(1,5) and β-(1,6) linked D-Gal*f* residues, with branching points to link the arabinofuranoside polymer. The thick glycolipid structure of the cell wall is known to be essential for the viability of mycobacteria and is also responsible for impermeability of the mycobacterial cell wall to many antibacterial drugs.[5] Although a number of groups have made concerted efforts to develop inhibitors targeting the arabinogalactan biosynthetic pathway,[6] to date there have been no reports of Gal*f*-based compounds that show significant anti-mycobacterial activity. Here we report the synthesis of a series of galactofuranosyl *N*,*N*-dialkyl sulfenamides and sulfonamides which show in vitro inhibition of mycobacterial growth.

Galactofuranosyl sulfenamides. We have previously described the preparation of the tetra-*O*-benzoylated galactofuranosyl *N*,*N*-diethyl sulfenamide **1**.[7] Initial biological evaluation of the corresponding deprotected *N*,*N*-diethyl sulfenamide **2** against *Mycobacterium smegmatis* in a disk susceptibility test showed that this compound indeed had some, albeit limited, antimycobacterial activity.[8] Given the highly waxy nature of the outer mycolate component of mycobacteria, it was possible that a polar compound, such as **2**, may have had difficulty in penetrating this layer. Based on this, we considered it worthwhile to construct a series of more hydrophobic derivatives of the Gal*f*-based sulfenamides

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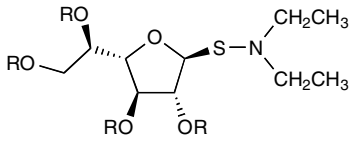
to potentially facilitate transport across the mycolate layer which may result in more potent compounds.



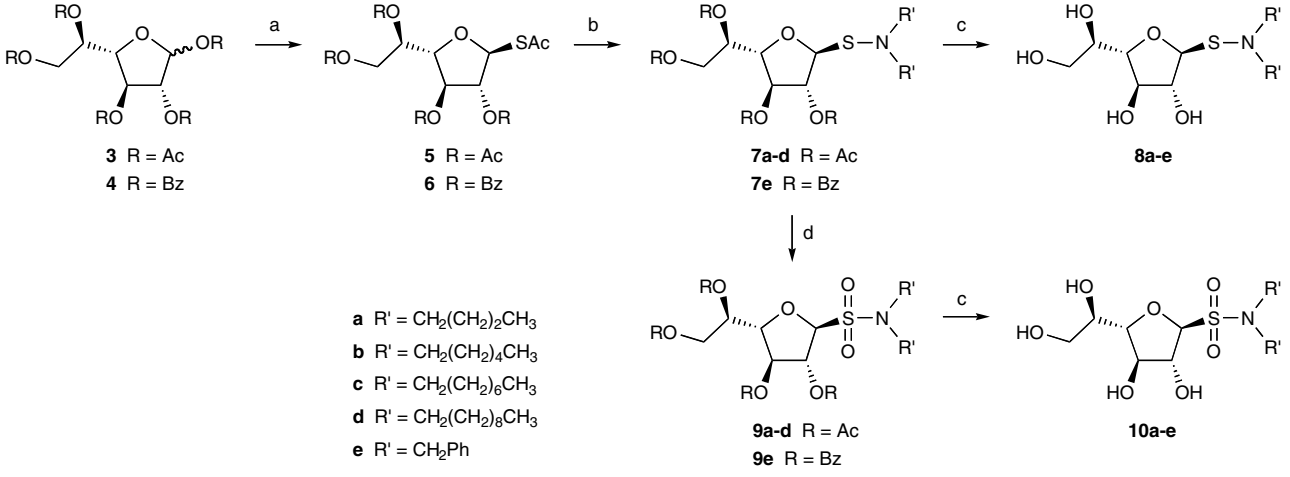
1 R = Bz
2 R = H

The synthetic approach taken to prepare the series of Gal*f* *N*,*N*-dialkyl sulfenamides,[9] based on the method used for the synthesis of **1**,[7] is shown in Scheme 1. As starting materials, tetra-*O*-acetylated[10] and benzoylated[11] Gal*f* **3** and **4** were prepared according to literature procedures, with the synthesis of the acetylated derivative **3**, via methyl α/β-D-galactofuranoside, being by far the higher yielding. Subsequent $SnCl_4$-catalysed reaction of **3** and **4** with thiolacetic acid provided the Gal*f* thiolacetate derivatives **5**[9] and **6**,[7] respectively, in high yield. The β-sulfenamides **7a**–**e** were synthesised from the galactofuranosyl thiolacetates, with apparent high anomeric stereoselectively, as previously described for the *N*,*N*-diethyl sulfenamide **1**, but with some variation to the reaction solvent. The reaction solvent (DMF) used in the synthesis of **1**[7] did not provide the desired target for reactions with the longer chain secondary amines, due to their lower solubility. Replacing DMF as reaction solvent with a 1:1 DMF/THF mixture resulted in moderate yields of the sulfenamides from the long chain aliphatic secondary amines (e.g., 35% yield of **7d** after reaction for 18 h). However reaction with dibenzylamine was not successful and returned only the de-*S*-acetylated starting material after 40 h. A report by Illyes et al. describing the formation of glucopyranosyl sulfenamides by reaction of a glucopyranosyl disulfide or methanethiolsulfonate with amines in methanol[12] led us to trial the use of methanol as solvent in our system. When applied to the synthesis of the tetra-*O*-benzoylated Gal*f* *N*,*N*-dibenzyl sulfenamide, the reaction was complete within 12 h, and the product **7e** was obtained in high yield (Table 1). Re-synthesis of the aliphatic derivatives **7a**–**d** using methanol as solvent resulted in good yields (53–75%) and significantly shortened reaction times of approximately 2 h.

Deprotection of the tetra-*O*-acylated derivatives **7a**–**e** to give the Gal*f* *N*,*N*-dibutyl **8a**, dihexyl **8b**, dioctyl **8c**, didecyl **8d**, and dibenzyl **8e** sulfenamides was achieved using one equivalent of sodium methoxide in methanol followed by careful neutralisation of the reaction mixture with Amberlite® IR-120 (H^+) resin to pH 7.5–8.0. Slight decomposition of the sulfenamides to lower R_f material was observed if the solution was acidified to pH 6–7. The decomposition products were characterized by ^1H NMR and mass spectrometric analysis to be, not surprisingly, the free amine, and what appeared to be a galactosyl thiol, both products resulting from cleavage of the sulfenamide bond. This is in line with literature reports[13] of the susceptibility of sulfenamides to hydrolysis under acidic conditions. Slight decomposition of the sulfenamides was also observed during flash chromatography on silica gel, possibly due to the acidic nature of silica gel. This decomposition could be minimised using 2% triethylamine in the eluent, to furnish the deprotected sulfenamides in 50–75% yield.

Galactofuranosyl sulfonamides. A potential pathway for in vivo metabolism of the glycosyl sulfenamides could be oxidation at sulfur[14] to form the corresponding sulfonamides. To investigate the effect of oxidation at sulfur on in vitro activity, the Gal*f* sulfenamides were oxidised to the corresponding sulfonamides **9a**–**e** (Scheme 1, Table 1).

The relatively straightforward conversion of the sulfenamides to the sulfonamide form was carried out by reaction of the acylated sulfenamide **7** with an excess of the oxidising agent, *meta*-chloro perbenzoic acid (*m*-CPBA),[13,15] in dichloromethane at reflux for 2 h (14 h for the dibenzyl sulfenamide) (Scheme 1). The sulfonamides **9a**–**e** were obtained in good yield. De-*O*-acylation using sodium methoxide in methanol gave the



Scheme 1. Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, HSAc, 0 °C to rt, 6 h, N_2 (**5** 96%)[9] or $SnCl_4$, CH_2Cl_2, HSAc, 0 °C to rt, 1 h, N_2 (**6** 90%)[7]; (b) HNR'_2, $BrCH(COOEt)_2$, MeOH, rt, **7a**–**d** 2 h, **7e** 12 h, Ar; (c) NaOMe, MeOH, rt, 0.5–2 h, N_2; (d) *m*CPBA, CH_2Cl_2, reflux, **9a**–**d** 2 h, **9e** 14 h.

Table 1. Yields (unoptimised) for Gal*f* *N*,*N*-diakyl sulfenamides and sulfonamides

R′		Yield (%)		Yield (%)
$CH_2(CH_2)_2CH_3$	**7a**	53	**8a**	47[a]
$CH_2(CH_2)_4CH_3$	**7b**	65	**8b**	74
$CH_2(CH_2)_6CH_3$	**7c**	75	**8c**	68
$CH_2(CH_2)_8CH_3$	**7d**	69	**8d**	54
CH_2Ph	**7e**	89	**8e**	50[a]
$CH_2(CH_2)_2CH_3$	**9a**	62	**10a**	92
$CH_2(CH_2)_4CH_3$	**9b**	69	**10b**	78
$CH_2(CH_2)_6CH_3$	**9c**	63	**10c**	75[a]
$CH_2(CH_2)_8CH_3$	**9d**	87	**10d**	75
CH_2 Ph	**9e**	52	**10e**	76[a]

[a] Yield for de-O-acylation of benzoylated derivative.

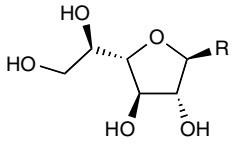
target compounds **10a**–**e** in good to excellent (75–90%) isolated yield. Purification by column chromatography on silica gel was successful, with the desired products eluted without decomposition.

Increased stability to acidic hydrolysis of the sulfonamides over the corresponding sulfenamides was indicated by their resistance to decomposition on silica gel support. In addition, where gradual decomposition of dioctyl sulfenamide **8c** occurred upon exposure to mild acid, as monitored by ^{1}H NMR spectroscopy, the corresponding sulfonamide **10c** was resistant to decomposition under the same conditions.

Biological evaluation. The series of galactofuranosyl *N*,*N*-dialkyl sulfenamides **8a**–**e** and sulfonamides **10a**–**e** were evaluated as inhibitors of *M. smegmatis* (ATCC 14468). *M. smegmatis* is a rapidly growing mycobacterium that has been used as a surrogate microorganism for detecting antimycobacterial activity of novel agents.[16] Initial screening against *M. smegmatis* carried out using a disk susceptibility test assay[17] (data not shown) indicated a lack of significant activity for the compounds with the shorter (*N*,*N*-dibutyl) alkyl chains. Subsequently, compounds **8b**–**e** and **10b**–**e** were screened for growth inhibitory activity, over the range 64–0.06 μg/mL, using a standard broth microdilution assay for susceptibility testing against *M. smegmatis*.[18] Inhibition data (MIC) are given in Table 2. As suggested by the initial disk diffusion assay, the shorter alkyl chain *N*,*N*-dihexyl derivatives had lower potency than the *N*,*N*-dioctyl, and in the sulfenamide series *N*,*N*-didecyl, derivatives. In the sulfenamide series both the *N*,*N*-dioctyl (**8c**, MIC 4 μg/mL) and *N*,*N*-didecyl (**8d**, MIC 1 μg/mL) derivatives showed potent growth inhibitory activity. As a comparison, the current anti-TB drug ethambutol is reported to have an MIC of 0.5 μg/mL against a representative strain of *M. smegmatis*.[19] In addition, the didecyl sulfenamide **8d** displayed antimycobacterial activity at levels of less than 5 μg/mL against various species including *M. tuberculosis*, *M. fortuitum*, and *M. abscessus*.[9] Interestingly, of the sulfonamide derivatives, only the *N*,*N*-dioctyl derivative (**10c**, MIC 2 μg/mL) showed significant activity, with the *N*,*N*-didecyl derivative **10d** inactive up to at least 32 μg/mL.

In summary, a series of galactofuranosyl *N*,*N*-dialkylated sulfenamides and sulfonamides have been synthesised and evaluated as in vitro inhibitors of mycobacterial growth. A number of these compounds displayed strong inhibition of mycobacterial growth with MIC values below 5 μg/mL. The resulting compounds provide interesting templates that may be promising leads for further development as antibacterial agents.

Table 2. Activity (MIC) of Gal*f* sulfenamides **8b**–**e** and sulfonamides **10b**–**e** against *M. smegmatis* (ATCC 14468)[a]



	R	MIC (μg/mL)[b]
Sulfenamides		
8b	$SN[(CH_2)_5CH_3]_2$	> 64
8c	$SN[(CH_2)_7CH_3]_2$	4
8d	$SN[(CH_2)_9CH_3]_2$	1
8e	$SN[CH_2Ph]_2$	16
Sulfonamides		
10b	$S(O)_2N[(CH_2)_5CH_3]_2$	64
10c	$S(O)_2N[(CH_2)_7CH_3]_2$	2
10d	$S(O)_2N[(CH_2)_9CH_3]_2$	> 32
10e	$S(O)_2N[CH_2Ph]_2$	> 64

[a] Broth microdilution assay in MHBII broth.[18] Incubation at 30 °C for 72 h.

[b] MIC values are given as the lowest concentration of compound that completely inhibited growth of the bacterium.

Acknowledgments

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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.2007.01.068.

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