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Synthesis of methyl 5-*S*-alkyl-5-thio-D-arabinofuranosides and evaluation of their antimycobacterial activity

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Abstract—The emergence of drug resistant tuberculosis necessitates a search for new antimycobacterial compounds. The antigen 85 (ag85) complex is a family of mycolyl transferases involved in the synthesis of trehalose-6,6′-dimycolate and the mycolated hexasaccharide motif found at the terminus of the arabinogalactan in mycobacterium. Enzymes involved in the synthesis of cell wall structures like these are potential targets for the development of new antiinfectives. To potentially inhibit the ag85 complex, methyl 5-*S*-alkyl-5-thio-arabinofuranoside analogues were designed based on docking studies with ag85C derived from *Mycobacterium tuberculosis*. The target arabinofuranosides were then synthesized and the antibacterial activity evaluated against *Mycobacterium smegmatis* ATCC 14468. Two of the compounds, 5-*S*-octyl-5-thio-α-D-arabinofuranoside (**8**) and 5-*S*-octyl-5-thio-β-D-arabinofuranoside (**11**), showed MICs of 256 and 512 μg/mL, respectively. Attempts to directly evaluate acyltransferase inhibitory activity of the arabinofuranosides against ag85C are also described. In conclusion, a new class of antimycobacterial arabinofuranosides has been discovered.
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1. Introduction

Mycobacterium, including the human pathogen *Mycobacterium tuberculosis*, possesses a cell wall that differs significantly in structure from both Gram-negative and Gram-positive bacteria.[1] The unique hydrophobic properties of this envelope protect the bacterium from its environment and provides a barrier to the diffusion of commonly used hydrophilic antimicrobial agents.[2] The main structural element of the mycobacterial cell wall is the mycolyl-arabinogalactan-peptidoglycan (mAGP) complex.[1] The arabinan portion of this structure contains a 1,3-branched arabinofuranoside-based hexasaccharide motif that is approximately two-thirds mycolated via ester linkages at each of the four of the primary hydroxyls to form the mycolyl-arabinan domain (**1**, Fig. 1a).[3,4] The mycolyl moieties are high molecular weight α-alkyl, β-hydroxy fatty acids (classified as α-, methoxy-, and keto- mycolates, Fig. 1b) that form the inner leaflet of the outer lipid bilayer. Mycolyl esters are also found as glycoconjugates non-covalently associated with the outer leaflet. It is the mycolates that are credited for producing the hydrophobic character of the cell envelope.

Many antimycobacterial drugs work by interfering with the biosynthesis of key components of the cell wall.[5–7] This suggests that the other enzymes involved in the synthesis of cell wall structures may serve as targets for antimycobacterial drug development. Antigens 85A, B and C,[8] represent a family of homologous mycolyltransferases that are responsible for the synthesis of trehalose-6,6′-dimycolate (TDM) from trehalose-6-monomycolate (TMM)[9,10] and for transfer of mycolic acids from TMM to the arabinogalactan (AG).[2c,11] In light of the role ag85 plays in mycobacterial cell wall synthesis, we have initiated studies to identify inhibitors of the ag85 complex based on substrate analogues of the arabinan. The significance of these studies is heightened as a result of the resurgence in cases of TB, in particular, those of the multi-drug resistant variety.[1b,12,13]

An elaborated mechanism for mycolyl transfer to the arabinan by ag85 is shown in Figure 2 based on the work by Ronning et al.[14] The key element of the mechanism is the attack on the carbonyl carbon of TMM by Ser124. Trehalose leaves the binding site, but the

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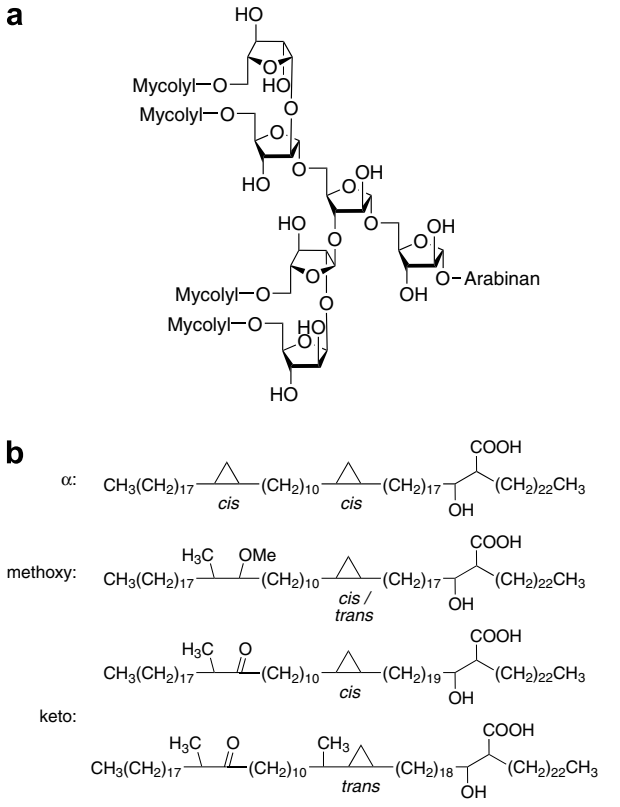


Figure 1. (a) The terminal arabinofuranosyl motif [β-D-Ara*f*-(1 → 2)-α-D-Ara*f*]2-3,5-α-D-Ara*f*-(1 → 5)-α-D-Ara*f* displayed at the non-reducing end of the arabinogalactan. Each terminal O-5 hydroxyl is a potential site for mycolyl ester formation. (b) The structures of the three classes of mycolic acids found in *Mycobacterium tuberculosis*.

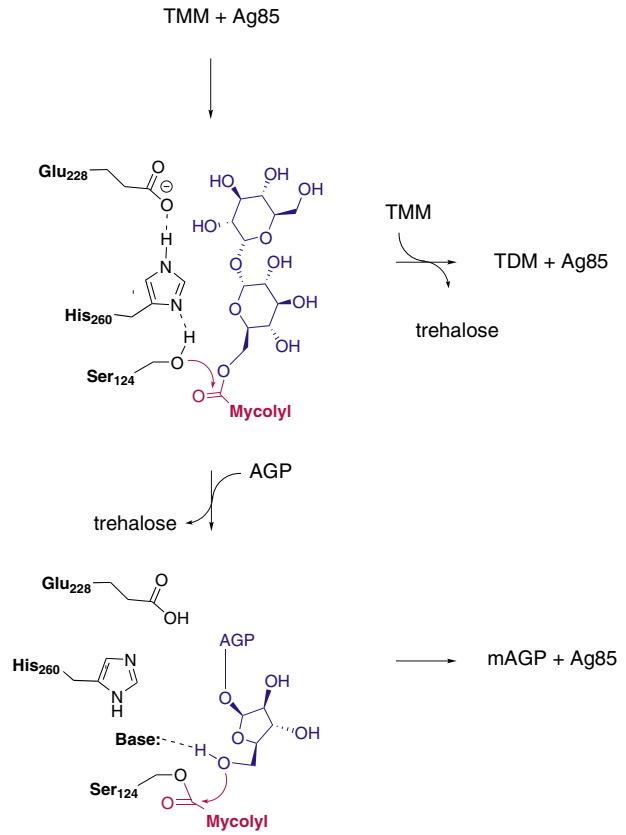


Figure 2. Proposed mechanism of mycolyl moiety (shown in red) transfer to TMM or AGP to form TDM and mAGP, respectively. Residues of the catalytic triad found in *M. tuberculosis* antigen 85C: Ser124, His260, and Glu228 are shown.

mycolyl moiety remains as part of an acyl enzyme intermediate. A second molecule of TMM, or presumably the terminus of the AGP, enters the substrate-binding pocket, which we refer to as carbohydrate-binding pocket I, and the mycolyl group is transferred producing TDM or mAGP, respectively. A hydrophobic tunnel adjacent to the catalytic site has also been proposed to accommodate the α-chain of the mycolate ester. A more extensive interfacial model for the mechanism has been developed by Anderson et al. using ag85B.[15] Aspects of this model highlight the possible role of a second carbohydrate-binding site (carbohydrate-binding pocket II, Fig. 3) and a hydrophobic channel which may assist the entry of the α-chain into the hydrophobic tunnel.[16] Using these studies as a guide we propose a model for the tetrahedral intermediate resulting from the attack of the terminal arabinofuranoside primary hydroxyl on the ag85 acyl enzyme intermediate (Fig. 4) analogous to the one proposed for the TMM-ag85 intermediate.[14] This model is consistent with X-ray studies that show a substrate-binding pocket large enough to accommodate two residues and an alkyl chain.

Additional factors that favor ag85 as a target for the development of antimycobacterials are dealt with by Belisle et al. who show that the enzyme complex is required for the viability of mycobacterium.[8] Further, the conserved nature of the catalytic site of all three homologues of ag85 suggests that a single inhibitor will likely inhibit all members of the ag85 family.[14,15,17] Finally, known inhibitors of ag85 have been shown to compromise the cell wall, which can allow chemotherapy to be more effective.[8,18] Attempts to produce inhibitors of ag85 are limited, but some compounds show promise as leads. These include 6-azido-6-deoxytrehalose which also inhibits the growth of *Mycobacterium aurum*.[8] Alkyl phosphonates[19] designed to mimic the tetrahedral intermediate of ag85C also show activity against *Mycobacterium avium* and inhibit mycolyltransferase activity[20] with IC_{50}s in the low micromolar range. TDM mimics that contain long hydrocarbon chains have displayed strong activity against *Mycobacterium smegmatis* when combined with INH and are presumed to inhibit ag85,[21] while related compounds consisting of 6,6′-bis(sulfonamido) and *N*,*N*′-dialkylamino derivatives of trehalose were active against *M. tuberculosis* and *M. avium*.[22] Since ag85 enzymes are believed to recognize the terminal arabinofuranoside motif of the AGP we envisioned that compounds containing an arabinofuranoside and an alkyl component could act as substrate analogues. The rationale is illustrated in Figure 4, which shows that compounds linked through the primary carbon of an arabinofuranoside potentially overlap a portion of tetrahedral intermediate formed during mycolic acid transfer. A thioether was

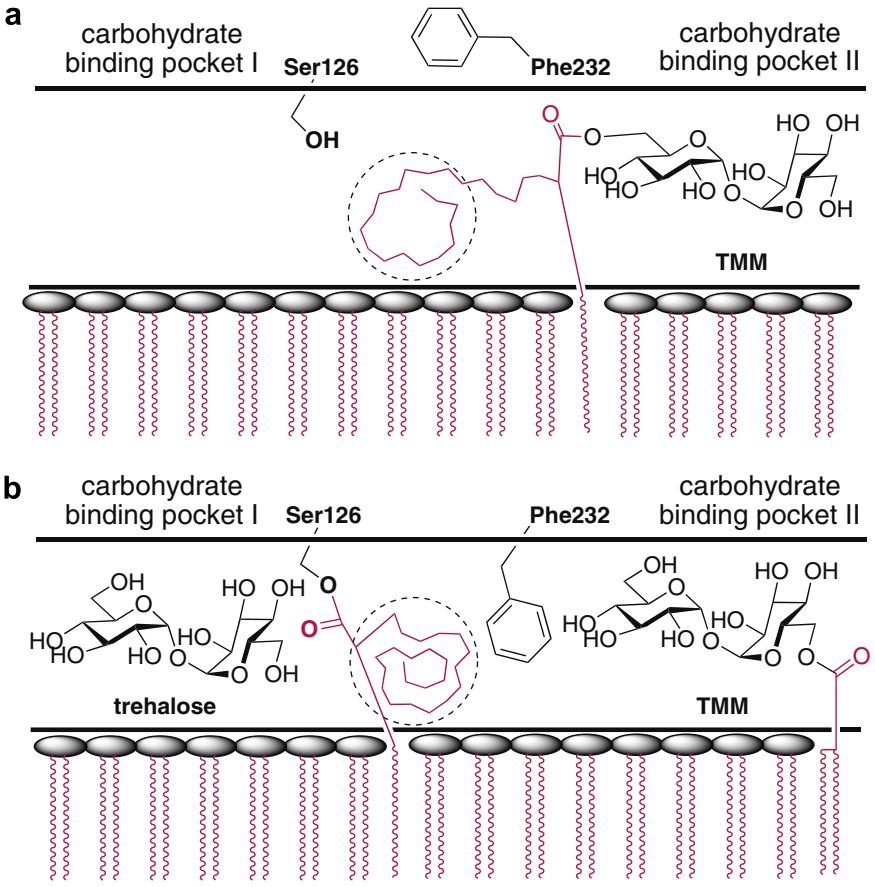


Figure 3. Selected steps of the interfacial model (Ref. 15) proposed for ag85. (a) TMM bound to the second carbohydrate-binding site with the α-chain located in the hydrophobic channel (parallel lines) of ag85, the α-chain is shown entering the hydrophobic tunnel (dashed circle). (b) The ag85 acyl-enzyme intermediate, with Phe232 covering the hydrophobic channel. The meromycolate branch of the mycolate (red) remains buried in the hydrophobic cell wall. Note: residue numbering is taken from the *M. tuberculosis* ag85B.

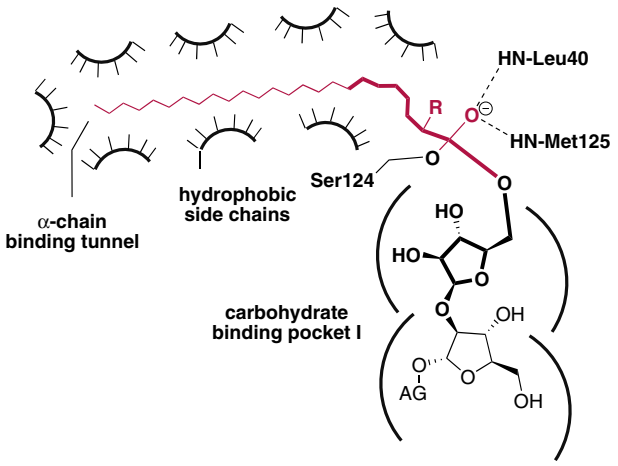


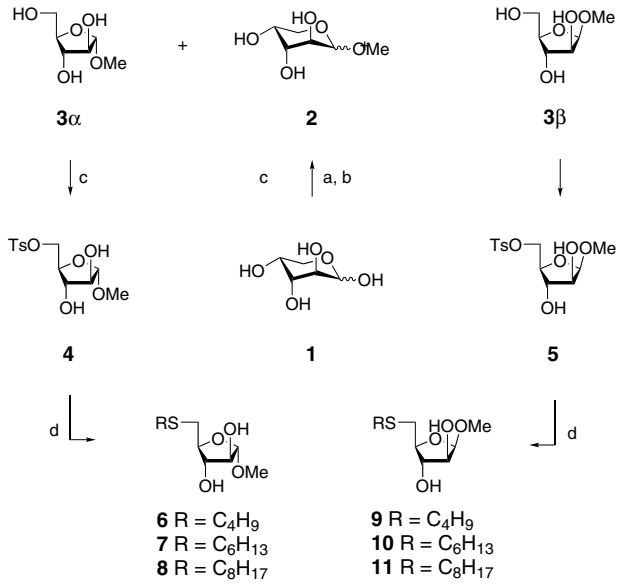
Figure 4. A model of the tetrahedral intermediate resulting from the attack of the terminal arabinofuranoside primary hydroxyl on the acyl enzyme intermediate of ag85. AG = arabinogalactan. R = meromycolate branch of a mycolic acid. The 5-*S*-alkyl-5-thio-D-arabinofuranosides inhibitors were designed based on a minimal structural domain defined by the atoms and bonds in bold.

chosen to conveniently link the arabinose and alkyl components to form 5-*S*-alkyl-5-thio-D-arabinofuranosides. The proposed ag85 inhibitors also take advantage of the xenobiotic nature of the arabinofuranoside moiety which would minimize any potential non-specific activity in a mammalian system.[23]

2. Results

2.1. Synthesis of 5-*S*-alkyl-5-thio-D-arabinofuranosides

A synthetic strategy was developed to rapidly access a library of 5-*S*-alkyl-modified α/β-D-arabinofuranosides **6**–**11** (Scheme 1). The synthesis was initiated by subjecting commercially available D-arabinose with in situ generated hydrochloric acid by means of addition of acetyl chloride (0.22 N HCl) to anhydrous methanol, followed by heating at 90 °C (Scheme 1, Method A, Section 5) in an attempt to generate the methyl furanosides, **3α** and **3β**.[24] Under these glycosylation conditions, the reaction was complete within 0.5 h. However, along with methyl arabinofuranoside **3α** (20% yield), an undesired mixture of methyl arabinopyranosides **2** (64% yield), β/α = 2.3:1, ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O), δ_α 3.97 ($^3J_{H-1,H-2}$ = 6.6 Hz, H-1), δ_β 4.52 ($^3J_{H-1,H-2}$ = 3.0 Hz, H-1), was also formed. The lack of the β-methyl arabinofuranoside was a disappointment since we had speculated the β-anomer would be a more potent inhibitor of ag85, being structurally similar to the β-linked



Scheme 1. Reagents and conditions: (a) MeOH, 0.22 N CH_3COCl, 90 °C, 0.5 h; (b) i—MeOH, HCl, 0–4 °C, 2.5 days; ii—MeOH, CH_3COCl (0.8 equiv), 0–4 °C, 18 h; iii—purified by gravity column chromatography on silica gel using acetone/MeOH/$CHCl_3$ (1:1:8); (c) TsCl, Py, rt, 1.5 days; (d) appropriate alkane thiol, NaOMe, DMF, 1–3.5 h, rt.

arabinofuranoside found at the terminus of the arabinogalactan. Thus, in order to access the desired quantities of diastereomerically pure α/β methyl arabinofuranosides, we resorted to the conditions reported by Lowary and others,[25] who also accessed the furanosides, **3α/3β**, in a ratio of 1:1 using hydrochloric acid in anhydrous methanol at 0 °C. Using those conditions, the desired kinetically favored furanosides **3α** and **3β** were obtained in 85% yield in a 1.4:1 ratio. Although the conditions were effective at delivering the desired products, the reaction required 2.5 days to reach 85% conversion. The reaction time was shortened to 18 h by using acetyl chloride (0.8 equiv) in anhydrous methanol, instead of concentrated hydrochloric acid. The latter conditions afforded **3α** and **3β** in a combined yield of 89% on a 10.5 g scale, again with the α-anomer formed in excess of the β (Method B, Section 5). Formation of the furanosides was confirmed by ^{1}H NMR, where characteristic anomeric protons of **3α** and **3β** appeared at δ 4.58 with coupling constants of 2.0 and 4.4 Hz in DMSO-d_6 solvent, respectively. These coupling constants are consistent with related α- and β-arabinofuranosides where the $^3J_{H-1,H-2}$ of α-arabinofuranosides ranged from 0–3.0 Hz and δ(C-1) = 104–110 ppm and the $^3J_{H-1,H-2}$ for the β-isomers ranged from 3.0–5.0 Hz and δ(C-1) = 97–104 ppm.[26] Further evidence for the formation of the furanosides was provided by the triplet centered at δ 4.71 in **3α** and δ 4.61 in **3β** arising from the primary hydroxyl at C-5 (RCH_2–O*H*), which makes the furanosides straightforward to distinguish from the pyranosides, which show doublets for all of the hydroxyls (R_2CH–O*H*) in DMSO-d_6. The primary hydroxyl of **3α** was selectively tosylated using tosyl chloride in anhydrous pyridine to yield known methyl 5-*O*-tosyl-D-arabinofuranoside **4** in 60% yield.[27] Similarly, known tosylate **5** was obtained in 50% yield.[27] In both cases the triplets assigned to primary hydroxyl disappeared after tosylation. 5-*O*-Tosylate **4** was subjected to nucleophilic displacement with several *n*-alkanethiols (C_4, C_6, and C_8) in the presence of sodium methoxide in anhydrous DMF to afford the desired 5-*S*-alkyl derivatives **6–8** in 92%, 50%, and 98% yields, respectively. Under similar thiolation condition, 5-*O*-tosylate **5** generated thioethers **9–11** in 73%, 60%, and 73% yields, respectively. All compounds were characterized by ^{1}H and ^{13}C NMR, ^{1}H–^{1}H gCOSY, and HRMS. $^3J_{H-1,H-2}$ values of the desired α-series **6–8** (0 Hz) and β-series **9–11** (4.8 Hz) are in line with those reported in the literature as previously mentioned.[26] Similarly, δ(C-1) values for compounds **6–8** (109.0–109.1 ppm) and **9–11** (102.12–102.16 ppm) lie in the range reported for α- and β-arabinofuranosides, respectively.[26]

2.2. Screening of the compounds for antimycobacterial activity

Arabinofuranosides **6–11** were screened for their ability to inhibit the growth of *M. smegmatis* ATCC 14468 using a Kirby–Bauer disk diffusion assay.[28] INH was used as the positive control while DMSO, also the diluent, was used as a negative control. *M. smegmatis* was used as a fast growing, non-pathogenic surrogate for *M. tuberculosis*.[29] The strain has been previously used to screen TDM mimics and oxazolidinone congeners, *in lieu* of *M. tuberculosis*.[21,30] The diameters of the zones of inhibition (DZIs) are summarized in Table 1. Similar DZIs were obtained when the strains were grown using both Lennox and Middlebrook 7H10-OADC + 0.5% glycerol agars.

In order to evaluate the selectivity of the compounds for killing mycobacterium and obtain quantitative values for the growth inhibition, we determined the minimum inhibitory concentrations (MICs) of glycosides **6–11**[31] against *Bacillus subtilis* ATCC 6633, *M. smegmatis* ATCC 14468, and *Escherichia coli* ATCC 23722. Serial dilutions ranging from 0.25 μg/mL to 1.024 mg/mL were used to determine MICs in comparison to INH. The MICs are summarized in Table 2.

Table 1. Kirby–Bauer assay of glycosides **6–11** against *Mycobacterium smegmatis* ATCC 14468[a]

Compound	DZI (mm) (20 mg/mL)	DZI (mm) (10 mg/mL)
6	None	None
7	None	None
8	17.5	14.5
9	None	None
10	None	None
11	13.5	7.5
DMSO	None	
INH	23.0 (500 μg/mL) (regrowth noted)	

[a] Solutions of 20 mg/mL and 10 mg/mL for compounds **6–11** and 500 μg/mL for INH were prepared in DMSO, 10 μL of each solution was then applied to a susceptibility disk. DZI = diameter (in mm) of the zone of inhibition.

Table 2. MICs (μg/mL) of glycosides **6**–**11** against a panel of bacteria

Compound	*M. smegmatis* 14468	*B. subtilis* 6633	*E. coli* 23722
6	>1024	>1024	>1024
7	1024	>1024	>1024
8	256	128	>1024
9	>1024	>1024	>1024
10	1024	>1024	>1024
11	512	512	>1024
INH	2	Not determined	Not determined

2.3. Screening of the compounds for antigen 85C inhibitory activity

Attempts to evaluate the inhibitory activity of the glycosides against ag85C proved to be troublesome. Compounds **7**, **8** and **10**, **11** which showed antimycobacterial activity in the disk and broth dilution assays were dissolved in DMSO and added to a newly developed high-throughput enzymatic assay. The assay evaluates the mycolyltransferase activity of ag85C, from *M. tuberculosis*, using UV–vis absorption spectroscopy. A glucose-based substrate analogue was designed and synthesized with a chromophoric *p*-nitrophenol moiety as a signaling molecule. The assay couples the catalytic activities of ag85C and β-glucosidase to release the chromophore, *p*-nitrophenolate. The rate of *p*-nitrophenolate release is observed by direct measurement of the absorbance at 405 nm over time. By comparing the slope of the absorbance versus time curves, it is possible to evaluate the inhibitory activity of these compounds on ag85C. Unfortunately, under a variety of conditions evaluated the lipophilic compounds immediately precipitated upon addition to the aqueous assay system. Furthermore, increasing DMSO to a concentration required to keep the compounds in solution was found to inhibit ag85C activity. Compound **9**, which showed no activity in the antimicrobial assay, stayed in solution and could be evaluated using the mycolyltransferase assay. As expected, no inhibitory activity against ag85C was noted over a concentration range of 1–20 mM (Fig. 5).

3. Discussion

The ag85 complex is currently considered a potential target for the treatment of mycobacterial infection.[8,19–22] In light of that, we have initiated efforts to develop carbohydrate-based inhibitors of this enzyme. To date, the design of these inhibitors has focused on the synthesis of compounds related to trehalose, TMM, TDM, and small molecules designed to mimic the aliphatic, disaccharide, and tetrahedral intermediate domains present during TDM synthesis. Based on the observed reduction of cell wall bound mycolates after inhibition of ag85C and complementation studies in *Corynebacterium glutamicum*, it has been inferred that the terminus of the AG is a substrate for ag85 complex. Therefore, we hypothesized that arabinofuranosides which possess a C-5 aliphatic moiety could be used as a template for the design of substrate-based inhibitors of the ag85 complex. Our initial targets were simple methyl glycosides, which were accessed by a non-stereoselective kinetic glycosylation to afford a separable mixture of the α/β-methyl arabinofuranosides on a preparative scale. During the course of the synthesis of the arabinofuranosides we found acquiring the ^{1}H NMR spectra in dry DMSO-d_6 and examining the C-5 hydroxyl splitting to be an unambiguous aid in distinguishing furanoside from pyranoside isomers.

In our evaluation of the antimycobacterial activity of 5-*S*-alkyl-5-thio-D-arabinofuranosides, we made the assumption that *M. smegmatis* contained ag85 homologues sufficiently similar to ag85 from *M. tuberculosis* that it could serve as a surrogate in a growth inhibition assay. The presence of ag85A and ag85C in the closely related *M. smegmatis* (mc^{2}155) was identified using the microbial genome database from the National Center for Biotechnology Information. A multiple-sequence

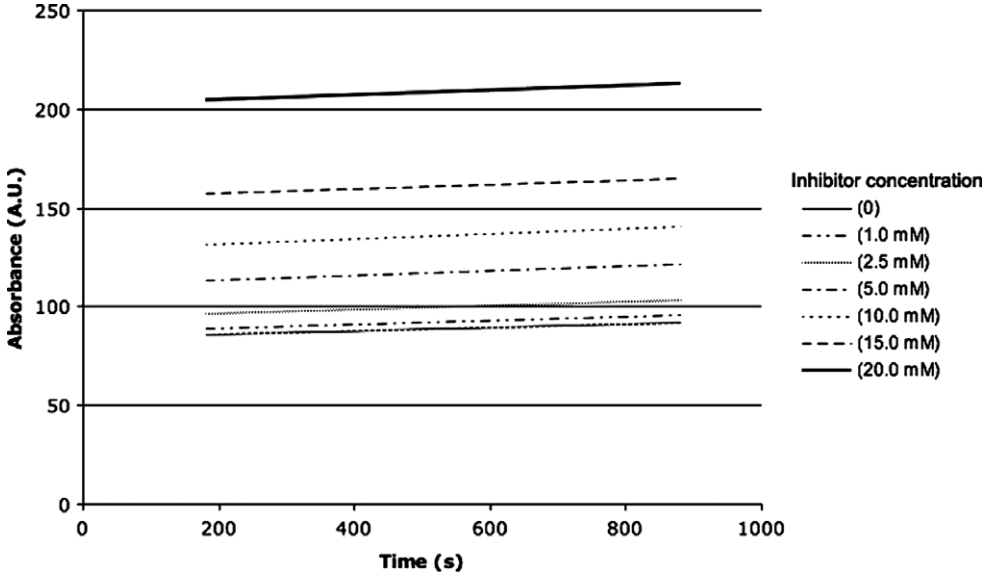


Figure 5. Absorbance versus time curve for compound **9** at different concentrations (1–20 mM).

alignment was performed between full-length ag85C *M. tuberculosis* (H37Rv) and ag85C and ag85A from *M. smegmatis* (mc^{2}155).[32] Ag85C from *M. tuberculosis* (H37Rv) was found to be 75.7% identical and 83.5% similar to ag85C from *M. smegmatis* (mc^{2}155). When ag85C from *M. tuberculosis* (H37Rv) was compared to ag85A from *M. smegmatis* (mc^{2}155), the sequences were 62.1% identical and 75.8% similar (Fig. 6). The residues representing the catalytic triad (red) are conserved for the three proteins compared. The carbohydrate-binding pocket I of *M. smegmatis* ag85A exhibits changes at two residues (blue) when compared to the *M. tuberculosis* ag85C proteins. However, these changes are conservative and should have little effect on binding site structure or electrostatics. Having established that *M. smegmatis* possesses ag85 sufficiently similar to *M. tuberculosis* to draw a potential correlation between killing and ag85 inhibition, we chose *M. smegmatis* 14468 as a non-pathogenic surrogate for *M. tuberculosis* to evaluate the structure–activity relationships of our antibacterial agents. This strain has been used previously for the evaluation of trehalose-based antimycobacterial agents making comparison of the results straightforward.[21]

A structure–activity relationship emerged on screening the 5-*S*-alkyl-5-thio-D-arabinofuranosides against *M. smegmatis*. Both the α and β glycosides with the C_8 alkyl chains displayed the lowest MICs, 256 and 512 μg/mL, respectively. The C_6 alkyl homologue also displayed activity at the highest concentration tested. The slightly higher activity for the α-anomer was surprising since the natural linkage of the terminal arabinofuranoside is a β-linkage. The results demonstrate a clear relationship between chain length and antimycobacterial activity. Longer alkyl chains were not employed since solubility was anticipated to be an issue. A hypothetical model for the interaction of arabinofuranoside **11** with ag85C is shown in Figure 7. The model was generated by manually overlaying the octyl chain of **11** onto the octyl chain of the octylthioglucoside present in the crystal structure of 1DQY.[14] It can be seen that the octyl chain and carbohydrate moiety are easily accommodated by the substrate-binding site of the

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M. tuberculosis Ag85C (H37Rv)      MTFFEQVRRLRSAATTLPRRLAIAAMGAVLVYGLVGTFGGPATAGAFSRP
M. smegmatis Ag85C (mc(2)155)      --------MRGIAAWKALRRFVIGALAALMLPGLIGFAGGSASAGAFSRP
M. smegmatis Ag85A (mc(2)155)      ---MKFVGRMRGAAAGLSRRLTVAVAAAAVLPGLVGVVGGSATAGAWSRP
                                              **    **:.:.. .* :: **:*  **.*:***:***

M. tuberculosis Ag85C (H37Rv)      GLPVEYLQVPSASMGRDIKVQFQGGGP--HAVYLLDGLRAQDDYNGWDIN
M. smegmatis Ag85C (mc(2)155)      GLPVEYLDVFSPSMNRDIRVQFQGGGP--HAVYLLDGLRAQDDYNGWDIN
M. smegmatis Ag85A (mc(2)155)      GLPVEYLEVPSAAMGRDIRVEFQSGGPGAPALYLLDGMRAREDQNGWDIE
                                   *******:* *.:*.***:*:**.***   *:*****:**::* *****:

M. tuberculosis Ag85C (H37Rv)      TPAFEEYYQSGLSVIMPVGGQSSFYTDWYQPSQSN--GQNYTYKWETFLT
M. smegmatis Ag85C (mc(2)155)      TPAFEWFYQSGLSTIMPVGGQSSFYTDWYQPSKGN--GQDYTYKWETFLT
M. smegmatis Ag85A (mc(2)155)      LPTFEWFLNSGISVVMPVGGQSSFYSDWYKPACGSKDGGCKTYKWETFLT
                                    *:** : :**:*.:**********:***:*: ..  *   *********

M. tuberculosis Ag85C (H37Rv)      REMPAWLQANKGVSPTGNAAVGLSMSGGSALILAAYYPQQFPYAASLSGF
M. smegmatis Ag85C (mc(2)155)      QELPAWLEANRGVSRTGNAVVGLSMAGSAALTYAIYHPEQFIYASTLSGF
M. smegmatis Ag85A (mc(2)155)      QELPAWLAANRDVKPTGSAVVGLSMAGSSALMLAARHPQQFIYAASLSGT
                                   :*:**** **:.*. **.*.*****:*.:**  *  :*:** **::***

M. tuberculosis Ag85C (H37Rv)      LNPSEGWWPTLIGLAMNDSGGYNANSMWGPSSDP--AWKRNDPMVQIPRL
M. smegmatis Ag85C (mc(2)155)      LNPSEGWWPMLIGLAMNDAGGYNAESMWGPSTDP--AWKRNDPMVNINQL
M. smegmatis Ag85A (mc(2)155)      LNPSEGWWPMLIGISMGDAGGYKADDMWGSTNDPNNAWKANDPTENVATI
                                   ********* ***::*.*:***:*:.***.:.**  *** ***  ::  :

M. tuberculosis Ag85C (H37Rv)      VANNTRIWVYCGNGTPSDLGG-----DNIPAKFLEGLTLRTNQTFRDTYA
M. smegmatis Ag85C (mc(2)155)      VANNTRLWVYCGTGTPSELDAGVSGGNLLAAQFLEGLTLRTNLTFRDQYI
M. smegmatis Ag85A (mc(2)155)      ANNGTRIWVYCGNGKPGELGG-----TDLPAKFLEGFVCRTNSTFQEKYI
                                   . *.**:*****.*.*.:*..        :.*:****:. *** **:: *

M. tuberculosis Ag85C (H37Rv)      ADGGRNGVFNFPPNGTHSWPYWNEQLVAMKADIQHVLNGATPPAAPAAPA
M. smegmatis Ag85C (mc(2)155)      AAGGTNAVFNFPPNGTHTWNYWGQQLLEMKPDIQRVLGAQSAT-------
M. smegmatis Ag85A (mc(2)155)      EAGGKNGVFNFPQSGTHNWAYWGQQLQAMKPDLQRVLGATPTA-------
                                     ** *.***** .***.* **.:**  **.*:*:**.. ...

M. tuberculosis Ag85C (H37Rv)      A
M. smegmatis Ag85C (mc(2)155)      -
M. smegmatis Ag85A (mc(2)155)      -
```

Figure 6. Sequence alignment of *M. tuberculosis* Ag85C (H37Rv) with *M. smegmatis* Ag85C (mc^{2}155) and *M. smegmatis* Ag85A (mc^{2}155). Multiple-sequence alignments were obtained with the ClustalW program (version 1.82).[32] Catalytic triad residues Ser, Glu, and His are indicated in red and selected carbohydrate-binding pocket I residues in blue. (*) indicates identical residues; (:) indicates residues that are conserved; (.) indicates residues that are semi-conserved; and (-) represents a gaps introduced to maintain the alignment. Residues in gray represent the portion of the sequence that is removed upon protein secretion.

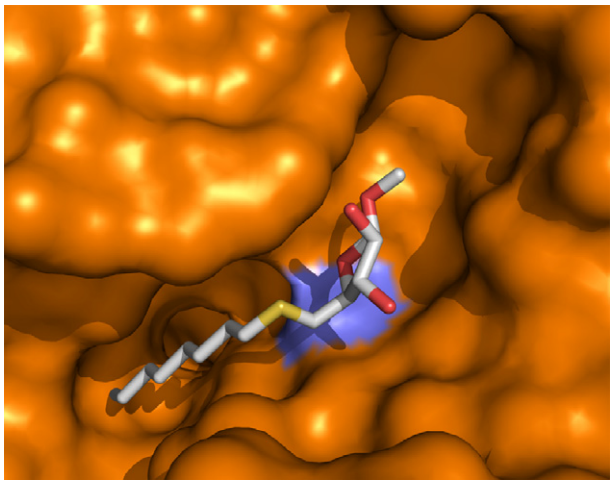

Figure 7. Methyl 5-*S*-octyl-5-thio-β-D-arabinofuranoside (**11**) docked within the active site of ag85C. The octyl chain was superimposed on the octyl chain of an octyl thioglucoside–ag85C complex (1DQY, Ref. 14). Additionally, hydrogen bonding seen in 1DQY between the thioglucoside and ag85C was used as a basis for positioning the arabinose moiety in the model. The catalytic nucleophile, Ser124, is highlighted in blue.

enzyme. Similar to the work of Wang et al., we also noted a lack of bacterial regrowth in the disk assay for compounds **8** (Fig. 8) and **11** (not shown) in comparison to INH.[21] This potentially indicates that the compounds were superior to INH in their ability to completely sterilize the zone of inhibition. We then evaluated the selectivity of the antimicrobial activity by including Gram-positive (*B. subtilis* 6633) and Gram-negative (*E. coli* 23722) strains as controls. Neither of these strains processes mycolic acids or have proteins similar to ag85.

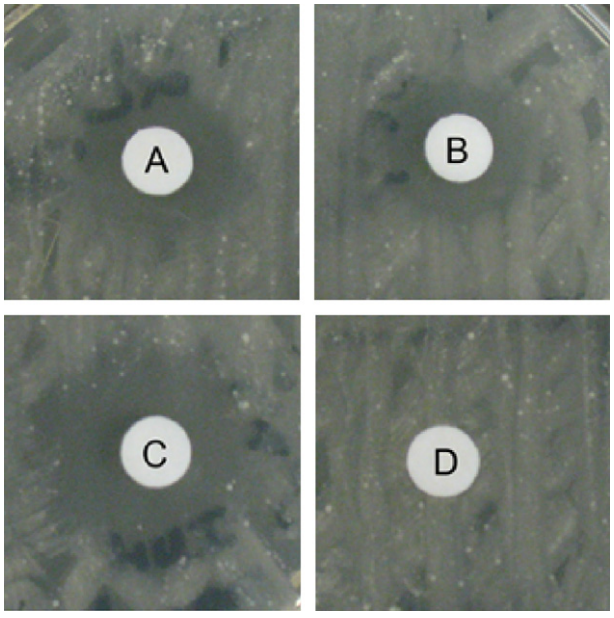

Figure 8. Kirby–Bauer diffusion assay after three days of *M. smegmatis* incubation for compound **8** and INH against dissolved in DMSO. (A) **8** 20 (20 mg/mL); (B) **8** (10 mg/mL); (C) INH (500 μg/mL); and (D) DMSO.

We were surprised that both compounds **8** and **11** showed MICs against *B. subtilis* (128 and 512 μg/mL, respectively) while the compounds showed no activity against *E. coli*. This non-specific activity raises the possibility that 5-*S*-alkyl-5-thio-D-arabinofuranosides, which have an amphipathic character, kill bacteria via a detergent effect independent of ag85 inhibition. Further, both mycobacterium[33–35] and *B. subtilis*[36] are reported to be sensitive to non-ionic surfactants. While detergency is one possible mode of action for the growth inhibition of *M. smegmatis*, it cannot rule out the possibility that the compounds act as inhibitors of ag85.

In an attempt to further address the mode of interaction of the compounds, an attempt was made to screen the arabinofuranoside library for the ability to inhibit ag85C using our acyltransferase assay. Existing assays to evaluate ag85 activity are cumbersome and require the isolation of TDM from bacteria and ^{14}C-labeled trehalose as an acceptor.[8,20] However, the most potent of our inhibitors (**8** and **11**) precipitated in the aqueous assay system; therefore, future efforts will focus on the generation of compounds with higher water solubility and have fewer structural features associated with amphipathic character.

4. Conclusion

We have synthesized a small library of arabinose-based compounds using a Fisher glycosylation protocol to obtain the kinetically formed methylfuranosides. Aliphatic chains were incorporated at C-5 of the methyl glycosides and their antimicrobial activity was evaluated against a panel of bacteria. Compounds with a C_8-chain were the most effective at inhibiting the growth of mycobacterium and activity was noted against a second Gram-positive strain. We attempted to evaluate the inhibitory activity of the compounds against ag85C and encountered solubility issues for compounds containing C_6 and C_8 side chains. Compound **9**, a C_4 congener, was examined over a range of concentrations in an acyltransferase assay and did not show inhibitory activity. Despite not having established a potent lead against the mycolyltransferase enzyme, the antibacterial nature of these compounds and their ability to prevent bacterial regrowth may prove useful for the development of a new class of carbohydrate-based anti-tubercular drugs. Work is underway to synthesize arabinose derived compounds that mitigate the solubility problem and to enhance the inhibitory activity against the ag85 complex.

5. Experimental

5.1. Materials

All fine chemicals such as D-arabinose, tosyl chloride, 1-butanethiol, 1-hexanethiol, and 1-octanethiol, INH, DMSO and anhydrous solvents such as anhydrous methanol, and DMF were purchased from Acros and used without further purification. Thiazolyl blue tetrazolium bromide (MTT) was purchased from Sigma–Al-

drich. All the other solvents were obtained from Fisher and used as received except pyridine, which was dried and distilled following the standard procedures.[37] Silica (230–400 mesh) for column chromatography was obtained from Sorbent Technologies; thin-layer chromatography (TLC) precoated plates were from EMD. TLCs (silica gel 60, f_{254}) were visualized under UV light or by charring (5% H_2SO_4–MeOH). Flash column chromatography was performed on silica gel (230–400 mesh) using solvents as received. ^{1}H NMR were recorded either on a Varian VXRS 400 MHz or an INOVA 600 MHz spectrometer in $CDCl_3$ or DMSO-d_6 using residual $CHCl_3$ and DMSO as internal references, respectively. ^{13}C NMR were recorded on the Varian INOVA 150 MHz spectrometer in $CDCl_3$ using the triplet centered at δ 77.273 as internal reference. High resolution mass spectrometry (HRMS) was performed on a Micromass Q-TOF2 instrument. Petri dishes (100 × 15 mm) were obtained from Fisher Scientific. LB broth, Middlebrook 7H9 broth, Lennox and Middlebrook 7H10 agar, glycerol, ADC and OADC enrichment, and blank susceptibility disks were obtained from Becton, Dickinson, and Company (Franklin Lakes, NJ). *B. subtilis* ATCC 6633, *E. coli* ATCC 23722, and *M. smegmatis* ATCC 14468 were obtained from the American Type Culture Collection (Manassas, VA).

5.2. Synthesis

5.2.1. Method A: methyl α-D-arabinofuranoside (3α) and methyl β-D-arabinofuranoside (3β). To a suspension of D-arabinose (5 g, 33.3 mmol) in anhydrous MeOH (60 mL), acetyl chloride (0.9 mL) was added dropwise at ambient temperature under N_2 atmosphere and the resulting solution was stirred at 90 °C. The reaction was monitored by TLC and appeared to stop after 0.5 h. The solution was allowed to attain room temperature and excess acid was neutralized by Na_2CO_3 (requisite amount, checked by pH-paper) and the resulting suspension was filtered through Celite using MeOH as solvent. Celite bed was washed successively with MeOH (3× 60 mL). The combined filtrate was concentrated to a small volume and the precipitated Na_2CO_3 was removed by filtration through analytical filter paper. The thick crude residue thus obtained was dissolved in MeOH, absorbed on silica gel and finally loaded over silica gel flash column (10 × 6.5 cm). Elution with 1:1:8 acetone/MeOH/$CHCl_3$ generated the methyl glycosides **3α** (1.1 g, 20%) as a colorless thick gum and a mixture of undesired pyranosides **2** (3.51 g, 64%) as colorless amorphous solid; silica gel TLC R_f = 0.3 (3:3:14 acetone/MeOH/$CHCl_3$).

5.2.2. Method B: methyl α-D-arabinofuranoside (3α) and methyl β-D-arabinofuranoside (3β). To a suspension of D-arabinose (6 g, 39.96 mmol) in anhydrous MeOH (100 mL), HCl (2 mL) was added dropwise at 0–4 °C under N_2 atmosphere and the resulting solution was stirred at 0–4 °C. The reaction was monitored by TLC and appeared to stop after 2.5 days. Following the similar work up and purification protocols as described in Method A, desired methyl glycosides **3α** and **3β** were obtained in 85% yield, of which **3α** is 3.25 g (49.6%, colorless gum), and **3β** is 2.27 g (34.6%, yellow gum); silica gel TLC $R_{f3\alpha}$ = 0.43, $R_{f3\beta}$ = 0.30 (3:3:14 acetone/MeOH/$CHCl_3$). When the reaction was carried out in the presence of acetyl chloride in anhydrous methanol at 0 °C on a 10.5 g scale, 89% desired products were isolated in the same way as described above in 18 h.

5.2.3. Methyl 5-*O*-*p*-toluenesulfonyl-α-D-arabinofuranoside (4) and methyl 5-*O*-*p*-toluenesulfonyl-β-D-arabinofuranoside (5). To a well-stirred solution of **3α** (0.79 mg, 4.82 mmol) in anhydrous pyridine (15 mL) was added a solution of (*p*)-toluenesulfonyl chloride (0.97 g, 5.1 mmol) in anhydrous pyridine (15 mL) under N_2 atmosphere. The resulting solution was stirred at ambient temperature and the reaction was monitored by TLC and appeared to stop after 24 h. Excess tosyl chloride was hydrolyzed by adding ice cubes (two pieces) and stirring was continued for 0.5 h. The reaction mixture was poured into water (80 mL) and extracted with ethyl acetate (4× 30 mL). The combined organic phases were washed with brine, and aqueous phase was back extracted. Resulting organic phases were dried (anhydrous Na_2SO_4), filtered and the filtrate was concentrated under reduced pressure to get the crude material. Purification of the crude material by silica gel flash chromatography (10 × 4.5 cm) with 3:3:14 acetone/$CHCl_3$/hexanes afforded tosylate **4** as a yellow gum; yield: 60% (0.92 g); silica gel TLC R_f = 0.6 (1:4 MeOH/$CHCl_3$). Similarly, **3β** (0.85 g, 5.18 mmol) was converted to corresponding tosylate **5** in 50% yield (0.83 g) as a yellow gum; silica gel TLC R_f = 0.56 (1:1:8 acetone/MeOH/$CHCl_3$).

5.2.4. General procedure for the synthesis of 6–11. To a suspension of NaOMe (2.5 equiv/mmol of substrate: obtained by the reaction of metallic sodium with little excess of anhydrous MeOH and removing excess solvent under reduced pressure and thereby drying it in high vacuum pump) in anhydrous DMF (2 mL/mmol) was added appropriate alkanethiol (5 equiv/mmol of substrate) under N_2 atmosphere. The resulting mixture was stirred at ambient temperature for 15 min to get a clear solution. A solution of the appropriate tosylate (starting material) in DMF (3 mL/mmol) was added dropwise. The resulting solution was allowed to stir at the same temperature under N_2 atmosphere and monitored by TLC and appeared to stop at 1–3.5 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured into water and extracted with ethyl acetate (4× 40 mL). The combined organic layers were washed with brine, aqueous phases were back extracted, and resulting organic phase was dried (anhydrous Na_2SO_4), filtered and the filtrate was concentrated in vacuo to get the crude material. Purification of the crude material by silica gel flash chromatography (10 × 3.5 cm) with acetone/$CHCl_3$/hexanes (3:3:14 for **7**, **8**, **10** and **11** or 2:2:3 for **6** and **9**) produced the title compounds.

5.2.5. Methyl 5-*S*-butyl-5-thio-α-D-arabinofuranoside (6). Compound **4** (0.25 g, 0.786 mmol) was reacted with butanethiol (0.337 mL, 3.14 mmol) to afford **6** in 1 h as a colorless gum following the general procedure described above. Purification was accomplished by silica

gel flash column (10 × 3.5 cm) with 2:2:3 acetone/$CHCl_3$/hexanes; yield: 92% (0.17 g); silica gel TLC $R_f = 0.2$ (1:1:3 acetone/$CHCl_3$/hexanes); ^1H NMR (600 MHz, $CDCl_3$): δ 0.92 (t, 3H, $J = 7.8$ Hz, CH_3), 1.40 (sextet, 2H, CH_2), 1.58 (quintet, 2H, CH_2), 2.59 (d, 1H, $J = 9.6$ Hz, OH-3), 2.65 (ddd, 2H, $J = 3.0$, 7.8, 10.2 Hz, SCH_2–), 2.85 (dd, 1H, $J = 4.2$, 14.4 Hz, H-5), 3.27 (d, 1H, $J = 9.0$ Hz, OH-2), 3.40 (s, 3H, OCH_3), 3.88 (dd, 1H, $J = 3.0$, 10.2 Hz, H-3), 4.05 (d, 1H, $J = 9.0$ Hz, H-2), 4.25 (m, 1H, H-4), 4.90 (s, 1H, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.8 (CH_3), 22.10 (CH_2), 31.9 (CH_2), 33.5 (CH_2), 34.87 (CH_2), 55.2 (OCH_3), 80.73 (CH), 80.8 (CH), 84.99 (CH), 109.1 (C-1); mass spectrum (HRMS), $m/z = 259.0980$ $(M+Na)^+$ ($C_{10}H_{20}O_4NaS$ requires 259.0980).

5.2.6. Methyl 5-*S*-hexyl-5-thio-α-D-arabinofuranoside (7). Compound **4** (0.25 g, 0.786 mmol) was reacted with hexanethiol (0.442 mL, 3.14 mmol) to afford **7** in 1 h as a colorless gum following the general procedure described above. Purification was accomplished by silica gel flash column (10 × 3.5 cm) with 3:3:14 acetone/$CHCl_3$/hexanes; yield: 50% (0.104 g); silica gel TLC $R_f = 0.21$ (1:1:3 acetone/$CHCl_3$/hexanes); ^1H NMR (600 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 7.2$ Hz, CH_3), 1.29 (m, 4H, 2× CH_2), 1.37 (quintet, 2H, CH_2), 1.59 (quintet, 2 H, CH_2), 2.63 (d, 1H, $J = 9.6$ Hz, OH-3), 2.64 (m, 2H, S–CH_2–), 2.84 (dd, 1H, $J = 3.6$, 14.4 Hz, H-5), 2.90 (m, 1H, H-5), 3.30 (d, 1H, $J = 9.0$ Hz, OH-2), 3.40 (s, 3H, OCH_3), 3.87 (dd, 1H, $J = 3.0$, 9.6 Hz, H-3), 4.04 (d, 1H, $J = 8.4$ Hz, H-2), 4.24 (m, 1H, H-4), 4.90 (s, 1H, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.20 (CH_3), 22.70 (CH_2), 28.65 (CH_2), 29.77 (CH_2), 31.56 (CH_2), 33.66 (CH_2), 34.84 (CH_2), 55.17 (OCH_3), 80.69 (CH), 80.98 (CH), 84.62 (CH), 109.03 (C-1); mass spectrum (HRMS), $m/z = 287.1299$ $(M+Na)^+$ ($C_{12}H_{24}O_4NaS$ requires 287.1293).

5.2.7. Methyl 5-*S*-octyl-5-thio-α-D-arabinofuranoside (8). Compound **4** (0.15 g, 0.471 mmol) was reacted with octanethiol (0.287 mL, 1.65 mmol) to afford **8** in 1 h as a colorless gum following the general procedure described above. Purification was accomplished by silica gel flash column (10 × 3.5 cm) with 3:3:14 acetone/$CHCl_3$/hexanes; yield: 98% (0.154 g); silica gel TLC $R_f = 0.20$ (1:1:3 acetone/$CHCl_3$/hexanes); ^1H NMR (600 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 7.2$ Hz, CH_3), 1.28 (m, 8H, 4× CH_2), 1.37 (quintet, 2H, CH_2), 1.59 (quintet, 2H, CH_2), 2.60 (d, 1H, $J = 10.2$ Hz, OH-3), 2.64 (ddd, 2H, $J = 1.8$, 7.2, 9.0 Hz, SCH_2–), 2.85 (dd, 1H, $J = 4.2$, 14.4 Hz, H-5), 2.90 (dd, 1H, $J = 5.4$, 14.4 Hz, H-5), 3.27 (d, 1H, $J = 9.0$ Hz, OH-2), 3.40 (s, 3H, OCH_3), 3.87 (dd, 1H, $J = 3.0$, 9.6 Hz, H-3), 4.04 (d, 1H, $J = 8.4$ Hz, H-2), 4.24 (m, 1H, H-4), 4.90 (s, 1H, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.27 (CH_3), 22.81 (CH_2), 29.0 (CH_2), 29.35 (2× CH_2), 29.82 (CH_2), 31.98 (CH_2), 33.67 (CH_2), 34.82 (CH_2), 55.18 (OCH_3), 80.68 (CH), 80.98 (CH), 84.53 (CH), 109.0 (C-1); mass spectrum (HRMS), $m/z = 315.1602$ $(M+Na)^+$($C_{14}H_{28}O_4NaS$ requires 315.1606).

5.2.8. Methyl 5-*S*-butyl-5-thio-β-D-arabinofuranoside (9). Compound **5** (0.389 g, 1.223 mmol) was reacted with butanethiol (0.656 mL, 6.12 mmol) to afford **9** in 3.5 h as a colorless gum following the general procedure described above. Purification was accomplished by silica gel flash column (10 × 3.5 cm) with 2:2:3 acetone/$CHCl_3$/hexanes; yield: 73% (0.212 g); silica gel TLC $R_f = 0.37$ (2:2:1 acetone/$CHCl_3$/hexanes); ^1H NMR (600 MHz, $CDCl_3$): δ 0.92 (t, 3H, $J = 7.2$ Hz, CH_3), 1.41 (sextet, 2H, CH_2), 1.59 (m, 2H, CH_2), 2.56 (d, 1H, $J = 9.6$ Hz, OH-2), 2.59 (t, 1H, $J = 7.2$ Hz, SCH_2–), 2.65 (dd, 1H, $J = 7.8$, 13.2 Hz, H-5), 2.69 (d, 1H, $J = 3.0$ Hz, OH-3), 2.84 (dd, 1H, $J = 6.0$, 13.8 Hz, H-5), 3.44 (s, 3H, OCH_3), 3.91 (m, 1H, H-4), 4.03 (ddd, 1H, $J = 3.0$, 6.6, 9.6 Hz, H-3), 4.09 (m, 1H, H-2), 4.80 (d, 1H, $J = 4.8$ Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.77 (CH_3), 22.04 (CH_2), 31.78 (CH_2), 32.20 (CH_2), 36.76 (CH_2), 55.38 (OCH_3), 78.06 (CH), 79.63 (CH), 81.17 (CH), 102.14 (C-1); mass spectrum (HRMS), $m/z = 259.0975$ $(M+Na)^+$($C_{10}H_{20}O_4NaS$ requires 259.0980).

5.2.9. Methyl 5-*S*-hexyl-5-thio-β-D-arabinofuranoside (10). Compound **5** (0.458 g, 1.44 mmol) was reacted with hexanethiol (1.01 mL, 7.20 mmol) to afford **10** in 3.5 h as a colorless gum following the general procedure described above. Purification was accomplished by silica gel flash column (10 × 3.5 cm) with 3:3:14 acetone/$CHCl_3$/hexanes; yield: 60% (0.227 g); silica gel TLC $R_f = 0.45$ (2:2:1 acetone/$CHCl_3$/hexanes); ^1H NMR (600 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 6.6$ Hz, CH_3), 1.29 (m, 4H, 2× CH_2), 1.38 (quintet, 2H, CH_2), 1.60 (m, 2H, CH_2), 2.47 (d,1H, $J = 9.6$ Hz, OH-2), 2.54 (d, 1H, $J = 3.0$ Hz, OH-3), 2.58 (t, 2H, $J = 7.2$ Hz, SCH_2–), 2.64 (dd, 1H, $J = 8.4$, 13.2 Hz, H-5), 2.85 (dd, 1H, $J = 6.0$, 13.8 Hz, H-5), 3.45 (s, 3H, OCH_3), 3.91 (m, 1H, H-4), 4.02 (ddd, 1H, $J = 3.0$, 6.6, 10.2 Hz, H-3), 4.09 (m, 1H, H-2), 4.81 (d, 1H, $J = 4.8$ Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.08 (CH_3), 22.59 (CH_2), 28.60 (CH_2), 29.66 (CH_2), 31.48 (CH_2), 32.51 (CH_2), 36.74 (CH_2), 55.32 (OCH_3), 78.01 (CH), 79.51 (CH), 81.18 (CH), 102.12 (C-1); mass spectrum (HRMS), $m/z = 287.1297$ $(M+Na)^+$($C_{12}H_{24}O_4NaS$ requires 287.1293).

5.2.10. Methyl 5-*S*-octyl-5-thio-β-D-arabinofuranoside (11). Compound **5** (0.325 g, 1.02 mmol) was reacted with octanethiol (0.889 mL, 5.11 mmol) to afford **11** in 2.5 h as a colorless gum following the general procedure described above. Purification was accomplished by silica gel flash column (10 × 3.5 cm) with 3:3:14 acetone/$CHCl_3$/hexanes; yield: 73% (0.219 g); silica gel TLC $R_f = 0.39$ (1:1:3 acetone/$CHCl_3$/hexanes); ^1H NMR (600 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 6.6$ Hz, CH_3), 1.28 (m, 8H, 4× CH_2), 1.37 (quintet, 2H, CH_2), 1.59 (m, 2H, CH_2), 2.49 (d, 1H, $J = 9.6$ Hz, OH-2), 2.57 (d, 1H, $J = 2.4$ Hz, OH-3), 2.58 (t, 2H, $J = 7.8$ Hz, SCH_2–), 2.64 (dd, 1H, $J = 8.4$, 13.2 Hz, H-5), 2.85 (dd, 1H, $J = 6.0$, 13.2 Hz, H-5), 3.45 (s, 3H, OCH_3), 3.91 (m, 1H, H-4), 4.03 (ddd, 1H, $J = 3.0$, 6.6, 9.6 Hz, H-3), 4.09 (m, 1H, H-2), 4.81 (d, 1H, $J = 4.8$ Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.18 (CH_3), 22.73 (CH_2), 29.00 (CH_2), 29.29 (CH_2), 29.31(CH_2), 29.75 (CH_2), 31.90 (CH_2), 32.55 (CH_2), 36.78 (CH_2), 55.35 (OCH_3), 78.04 (CH), 79.58 (CH), 81.21 (CH), 102.16 (C-1);

mass spectrum (HRMS), $m/z = 315.1607$ $(M+Na)^+$ ($C_{14}H_{28}O_0NaS$ requires 315.1606).

5.3. Kirby–Bauer disk diffusion assay

Disk diffusion assays were adapted from methods outlined by the NCCLS and Wang et al.[21,28] *M. smegmatis* ATCC 14468 was inoculated into Middlebrook 7H9 containing 0.2% glycerol and ADC enrichment, respectively. The inocula were incubated for approximately 24 h at 37.5 °C and 160 rpm to yield an $OD_{600} = 0.27$. The bacteria were plated on agar (Lennox or Middlebrook 7H10 containing 0.5% glycerol and OADC Enrichment) using sterile cotton-tipped applicators. Aliquots (10 μL) of arabinose derivatives dissolved in DMSO (20 mg/mL and 10 mg/mL) and INH (500 μg/mL) were applied to 6 mm diameter sterile paper disks. The plates were incubated at 37 °C for 24 h and then analyzed. The plates were returned to the incubator and then analyzed six days after the initial incubation to check for additional changes. The DZIs (mm) were recorded using a ruler.

5.4. Broth macrodilution assay

MIC values against three test organisms, *E. coli* ATCC 23722, *B. subtilis* ATCC 6633, and *M. smegmatis* ATCC 14468, were obtained using broth macrodilution assay using either Mueller-Hinton or Middlebrook 7H9 containing 0.2% glycerol and ADC enrichment (*M. smegmatis* ATCC 14468), adapted from NCCLS procedures.[31] Bacterial cultures equivalent to a 0.5 McFarland turbidity standard were prepared in the appropriate broth to give a final inoculum density of 5.0×10^5 cfu/mL and the solutions incubated at 37 °C for 36 h. To aid in the visualization of the bacteria, 20 μL of 10 mg/mL solution (MeOH solvent) of thiazolyl blue tetrazolium bromide (MTT) was added.[38] All assays were run in duplicate.

5.5. Antigen 85C acyltransferase assay

A master mix of sodium phosphate buffer C, β-glucosidase, and dextrose (0.1 M, 3 μL per reaction) was prepared. Master mix (85.84 μL) was added to each well of the assay, followed by 10 μL of a 10% v/v dimethyl sulfoxide stock solution of substrate (0.1–2.5 mM), and 1 μL of a dimethyl sulfoxide solution of arabinofuranoside **9**. The C_6 and C_8 homologues were not soluble in the aqueous well solution and therefore could not be assayed. To initiate catalysis 4.16 μL of a 45 μg/mL solution of purified antigen 85C in 10 mM Tris–HCl buffer, pH 7.5, 2 mM ethylenediaminetetraacetic acid and 1 mM dithiothreitol were added. The change of absorbance of the reaction solution at 405 nm was followed by absorbance measurement (absorbance plate reader Spectramax® 340PC by Molecular Devices) every 20 s for 1 h. All assays were prepared and performed at room temperature. The data were exported from the Molecular Devices SoftMax® Pro5 software and analyzed using Microsoft Excel® to obtain initial velocity values for each reaction.

5.6. Modeling studies

The putative inhibitor **11** was created and minimized using Chem3D Ultra (CambridgeSoft). This molecule was positioned in the active site of ag85C using the program Coot.[39] The manual positioning of the inhibitor within the active site was based on the crystal structure of *n*-octyl-β-D-thioglucoside bound to ag85C (accession code 1VA5).[14] Care was taken to conserve the hydrogen-bonding pattern between the carbohydrate moiety and residues L40, R41, and W262 forming the carbohydrate-binding site. The non-specific hydrophobic interactions were less of a concern, but attempts were made when positioning the carbons of the aliphatic chain to maintain interactions with residues F150, W158, I162, A165, and L227 of the acyl chain binding pocket.

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