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Sulfonium ions as inhibitors of the mycobacterial galactofuranosyltransferase GlfT2†

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The mycobacterial cell wall possesses a core galactan moiety composed of approximately 30 galactofuranosyl residues attached *via* alternating β-(1→5) and β-(1→6) linkages. A bifunctional galactofuranosyltransferase, GlfT2, is one of two essential enzymes for mycobacterial cell wall biosynthesis. The enzymatic reactions catalyzed by GlfT2 undoubtedly proceed by way of a transition state that has significant oxocarbenium-ion character. In this paper, a series of sulfonium ion compounds were designed and synthesized as analogues of the donor substrate, uridine diphosphate-galactofuranose, as potential inhibitors of GlfT2. The compounds contain moieties that mimic both galactofuranose and uridine diphosphate domains, and carry a permanent positive charge to mimic the oxocarbenium ion-like transition state. These compounds were evaluated against Glf2 using a coupled spectrophotometric assay, and some were shown to be weak inhibitors of the enzyme.

Introduction

Mycobacterium tuberculosis, the causative agent of tuberculosis, and other mycobacterial species possess a unique cell wall structure containing an array of carbohydrates and lipids.[1] The major constituent of this complex architecture is the mycolyl–arabinogalactan (mAG) complex, which provides the organism with significant protection from its environment.[2,3] A notable feature of the mAG complex is that all of the galactose and arabinose residues in the arabinogalactan (AG) domain are in the furanose ring form.[4] Glycoconjugates containing furanose residues are absent in humans and thus the glycosyltransferases involved in AG biosynthesis are of interest as targets for new antibacterial agents.[4–6] The pathway by which the mAG complex is assembled involves the sequential addition of sugar residues to a polyprenol bound intermediate by a number of glycosyltransferases.[3] Among the enzymes in this pathway that have been biochemically-characterized,[5,7,8] are two bifunctional galactofuranosyltransferases, GlfT1 and GlfT2. Both enzymes transfer galactofuranose (Gal*f*) from uridine diphosphate-galactofuranose (UDP-Gal*f*, **1**), to an acceptor oligosaccharide *via* an oxacarbenium ion transition state (**2**) to form the elongated oligosaccharide and in the process liberate UDP (Scheme 1).

Of these two galactofuranosyltransferases, GlfT2 has received the most investigation. The protein, an inverting glycosyltransferase, has been recombinantly expressed[7,9] and shown to possess a single active site that is capable of carrying out two distinct glycosyl transfer reactions leading to either β-Gal*f*-(1→5)-β-Gal*f* or β-Gal*f*-(1→6)-β-Gal*f* linkages.[10,11] The polymerase activity of GlfT2 proceeds *via* a processive mechanism[12] and a recent crystal structure has revealed a novel tetrameric structure that led to a postulated model for chain length control.[13] The design of effective inhibitors for GlfT2 has been limited to date, although this is an active area of investigation.[14–20]

A common strategy in developing inhibitors of enzymes that carry out glycosyl transfer reactions, in particular glycosidases, is to synthesize structures that mimic the positive character of the oxacarbenium ion transition state (**2**, Scheme 1).[21,22] Iminosugars, which carry a positive charge at physiological pH, have been widely studied and used as inhibitors of glycosyltransferases and glycosidases.[23] Another approach is to prepare molecules possessing a positively charged sulfur atom (sulfonium ions) to establish this electrostatic mimicry.[24–28]

Yuasa and coworkers developed the first carbohydrate-based sulfonium-ion derivative **3**[24] (Fig. 1), as a β-glucosidase inhibitor. Since then, other carbohydrate-based sulfonium-ion derivatives were synthesized as inhibitors of various glycosidases. For example, sulfonium ion derivatives of the iminosugar glycosidase inhibitors swainsonine and castanospermine, **4** and **5**, respectively, have been synthesized and evaluated for inhibitory activity against glycosidases.[25,26] These studies were validated when two naturally-occurring sulfonium ion glycosidase inhibitors, salacinol (**6**)[27] and kotalanol (**7**)[28] were isolated in 1997. Both salacinol and kotalanol have significant inhibitory activity toward α-glucosidases.[29,30] Due to their unique structural features and potential to become a lead drug candidate in the treatment of type II diabetes,[31] an increasing

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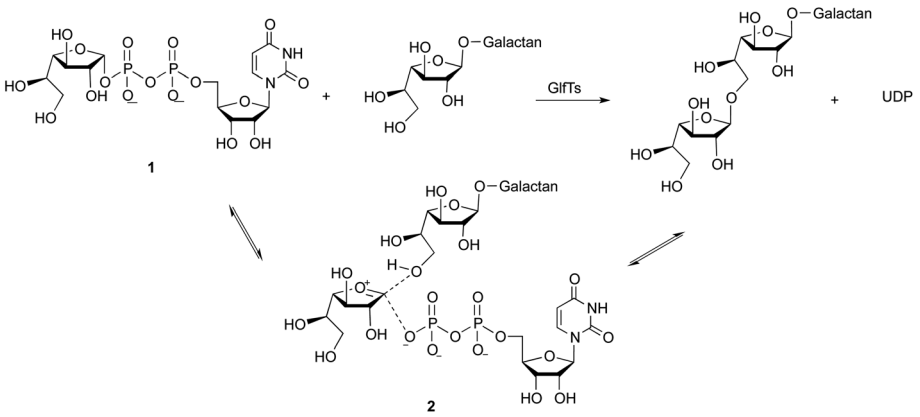


Scheme 1 Mycobacterial galactofuranosyltransferase-catalyzed galactan elongation.

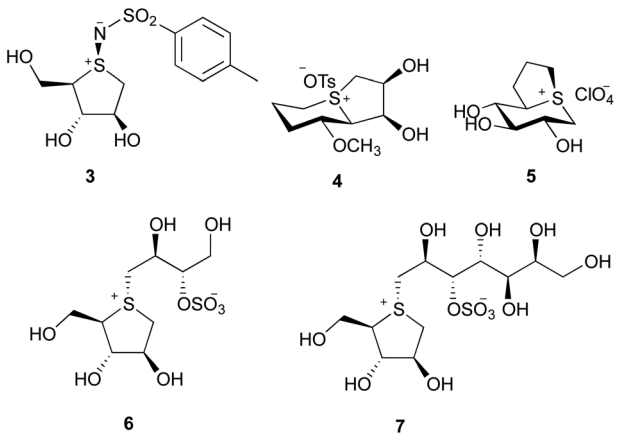
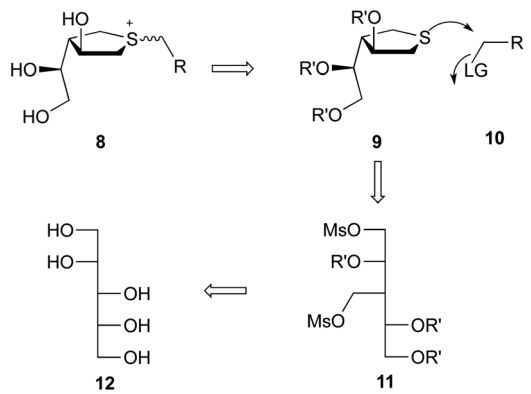


Fig. 1 Structures of some known sulfonium ion compounds.

amount of research on carbohydrate-based cyclic sulfonium compounds has since been carried out. Ponkoranol,[32] salaprinol[32] and de-*O*-sulfonated analogues[33–35] were also obtained from *Salacia* genus plants. Their diasteriomers, nitrogen derivatives, selenium derivatives[36–39] and maltose-extended analogues[40] have been chemically synthesized and their biological activities evaluated.



Scheme 2 Retrosynthetic analysis of sulfonium ions with general structure 8.

Although sulfonium ions have been demonstrated to inhibit glycosidases, reports of their ability to inhibit glycosyltransferases are less common. Intrigued by the possibility of developing inhibitors of GlfT2 based upon a sulfonium ion scaffold, we designed a route to a suitable mimic of the postulated transition state in this glycosylation reaction. We describe here the synthesis of a panel of compounds with the general structure **8** (Scheme 2) and an evaluation of their ability to inhibit GlfT2.

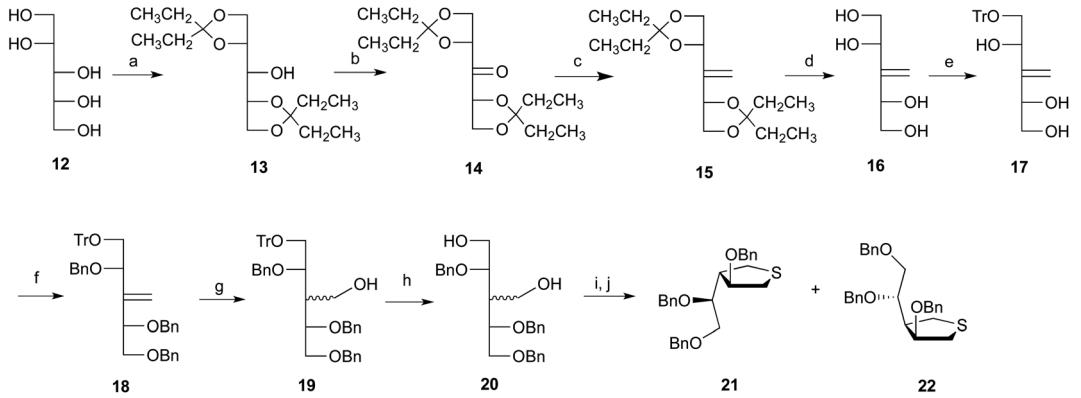
Results and discussion

Design considerations

Based on previous work on the synthesis of salacinol and derivatives[29–39] we considered that the most direct route to the target sulfonium ions was through an intermolecular S_N2 reaction between a cyclic sulfide (**9**, Scheme 2) and an appropriate electrophile (**10**). In selecting the cyclic sulfide scaffold to build the targets upon, it was necessary to choose a ring system in which both the ring oxygen and the hydroxyl group on C2 were removed to provide a stable species. Previous studies have demonstrated that GlfT2 will tolerate modification of the OH at C2 in UDP-Gal*f*.[14,40] The two substituents on the sulfide ring were designed to have the same stereochemistry as those of C3 and C4 on the galactofuranose ring. Therefore, we envisioned that **9** could be prepared by cyclization of the branched alditol derivative **11**, which in turn could come from commercially available D-arabinitol (**12**). With regard to the electrophile, we chose to explore a number of simple alkyl halides, as well as more complex structures that could better mimic the uridine diphosphate portion of the postulated transition state structure.

Synthesis the cyclic sulfide

The synthesis of the cyclic sulfide was achieved in a nine-step sequence, starting from **12** (Scheme 3). First, reaction of **12** with 3,3-dimethoxypentane in the presence of camphorsulfonic acid afforded a diacetal intermediate, **13**, which has the hydroxyl on C3 unprotected, in 72% yield. Oxidation of this hydroxyl group with SO_3·pyridine complex in DMSO proceeded in 84% yield to give the C_2-symmetric ketone **14**, which was subsequently



Scheme 3 *Reagents and conditions*: (a) 3,3-dimethoxypentane, CSA, THF, 72%; (b) $SO_3 \cdot Py$, DMSO, 84%; (c) Ph_3PCH_3I, NaHMDS, 93%; (d) CSA, CH_3OH 97%; (e) TrCl, DMAP, Et_3N, DMF, 63%; (f) BnBr, NaH, DMF, 87%; (g) $BH_3 \cdot (CH_3)_2S$, THF, H_2O_2, NaOH, 80%; (h) *p*-TSA, CH_3OH, CH_2Cl_2, 96%; (i) MsCl, Et_3N, CH_2Cl_2; (j) $Na_2S \cdot 9H_2O$, DMF, 2 steps 84%.

reacted with methyl triphenylphosphonium iodide and NaHMDS affording a 93% yield of olefin **15**.[41] Cleavage of the acetal protecting groups from **15** was achieved upon reaction with camphorsulfonic acid, providing compound **16** in 97% yield. This tetraol was then reacted with one equivalent of trityl chloride to produce the monoprotected compound **17** in 63% yield. The remaining hydroxyl groups were then protected as benzyl ethers by reaction of **17** with benzyl bromide and NaH to generate product **18** in 87% yield.

A hydroboration–oxidation sequence was explored to convert the alkene into a hydroxymethyl group. We initially used borane–dimethylsulfide ($BH_3 \cdot S(CH_3)_2$) complex, which gave an organoborane that was subsequently oxidized to the stereoisomers **19** by treatment with H_2O_2 under basic conditions. The isomers, which could not be separated, were obtained in total yield of 80% in a ratio of 1 : 1.4. To improve the stereoselectivity, other borane reagents ($BH_3 \cdot THF$, $BH_3 \cdot$pyridine, or $BH_3 \cdot NEt_3$) were examined, but none gave better results. More sterically-demanding hydroboration reagents, 9-BBN, disiamylborane and thexylborane, were also investigated. Unfortunately, the conversion of **18** into the corresponding organoborane was not successful when using these reagents.

The stereoisomeric mixture of **19** was treated with *p*-toluenesulfonic acid, giving **20**, also as an inseparable mixture of stereoisomers, in a combined yield of 96%. Treatment of the mixture of diols with methanesulfonyl chloride and triethylamine in CH_2Cl_2 produced the expected mesylated product, which was directly treated with sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$) in DMF at 100 °C to form, in 84% yield, a separable mixture of the cyclic sulfides **21** and **22**, in a ratio of 1.4 : 1. In the ring forming reaction, it was necessary to heat the solution slowly to 100 °C, to allow the $Na_2S \cdot 9H_2O$ to dissolve completely before the initiation of displacement. If heated quickly, dehydration of $Na_2S \cdot 9H_2O$ occurs before Na_2S dissolves in the solvent. It was found that substituted tetrahydrofuran side products **23** and **24** (Fig. 2) were generated from the reaction of **20** and when the reaction mixture was heated too rapidly.

It was not possible to determine the relative stereochemistry of two substituents on the cyclic thioethers **21** and **22** using NMR spectroscopy. In an effort to prove unequivocally the structures, we investigated the conversion of **21** and **22** into compounds that could be crystallized. Thus, **21** and **22** were oxidized by *m*-CPBA to give **25** and **26**, which were subsequently hydrogenolyzed over Pd–C to form **27** and **28** in 92% yield over two steps (Scheme 4). Neither **27** nor **28** were crystalline. However, oxidation of **28** with $NaIO_4$ produced an aldehyde that was condensed with 2,4-dinitrophenylhydrazine to give a yellow crystalline solid, **29**. From the crystal structure of **29** (Fig. S1,† CCDC 986599), the two substituents on the ring were

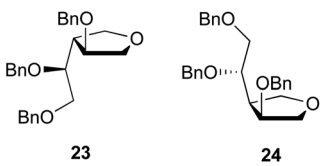
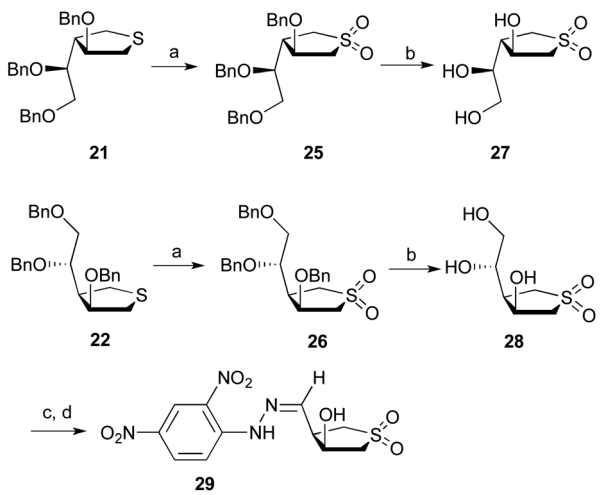


Fig. 2 Tetrahydrofuran byproducts **23** and **24**.



Scheme 4 *Reagents and conditions*: (a) *m*-CPBA, CH_2Cl_2, 94%; (b) H_2, Pd–C, HOAc, CH_3OH, 98%; (c) $NaIO_4$, $NaHCO_3$, THF, H_2O; (d) 2,4-dibitrophenylhydrazine, CH_3OH, 2 steps 30%.

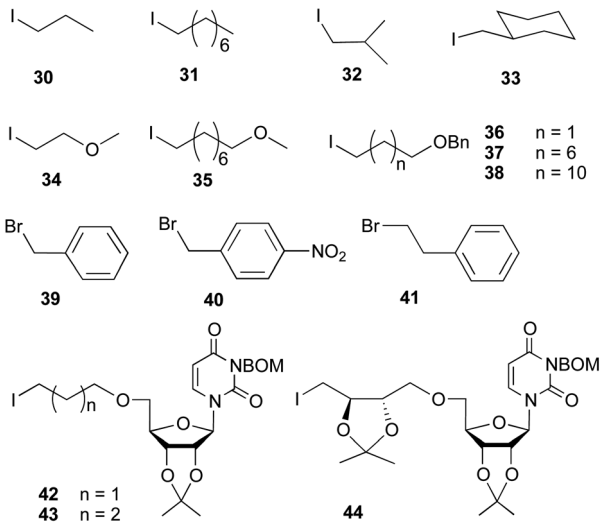


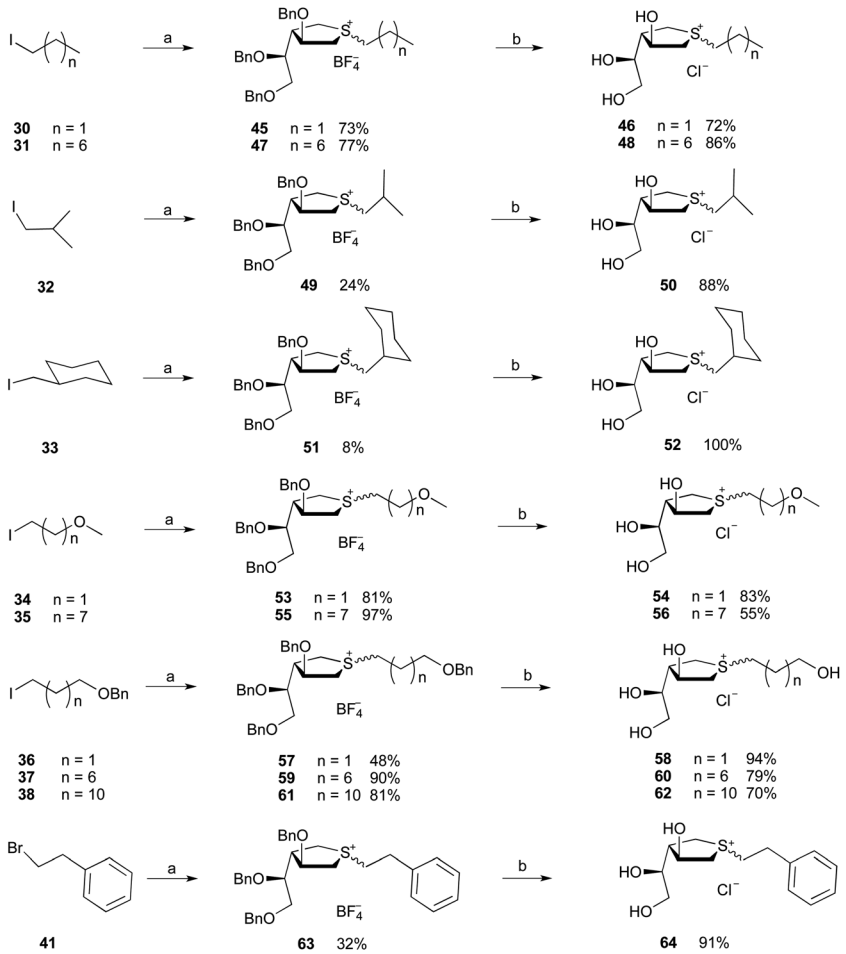
Fig. 3 Structures of halides **30–44**.

determined to be *syn* to each other. It can therefore be inferred that two substituents in **21** have an *anti* relationship. Thus, compound **21** has the "galactofuranose" configuration, and was used in the coupling reactions with alkyl iodides to prepare the sulfonium ion targets.

Preparation of alkyl iodides

It has been reported previously that alkyl iodides[37,42] and triflates[43] are good electrophiles in the synthesis of sulfonium ions because the leaving groups are weakly nucleophilic anions, which reduce the possibility of decomposition of the sulfonium ion *via* nucleophilic substitution reactions.[44] When these reactions are carried out with alkyl iodides in the presence of a silver salt (*e.g.*, $AgBF_4$), the iodine ion liberated in the reaction can be precipitated as AgI and thus is unable to act as a nucleophile. Based upon these reports, we chose alkyl iodides and bromides as the alkylating agents for the synthesis of the target molecules.

A range of alkyl iodides and bromides with diverse branching patterns were selected to prepare sulfonium ion analogues (Fig. 3). Compounds **30–33** contain linear or branched alkyl groups. **34** and **35** have an oxygen atom in the chain, which may form hydrogen-bonding interaction with the enzyme. Iodides **36–38** will provide the sulfonium ion analogs with a hydroxyl group after hydrogenolysis, which could also hydrogen bond with the enzyme. We also chose to synthesize sulfonium ion analogues containing benzylic groups from compounds **39–41**.

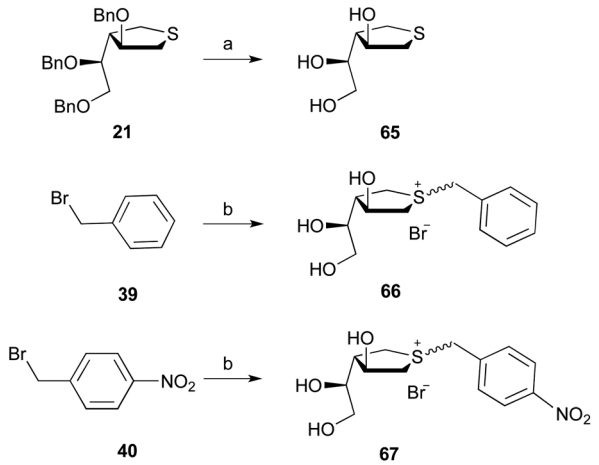


Scheme 5 *Reagents and conditions*: (a) **21**, $AgBF_4$, CH_3CN, 65 °C; (b) BCl_3, 1 M in CH_2Cl_2, then Amberlyst resin (Cl^-).

In addition, to mimic UDP-Gal*f*, iodides containing a uridine moiety were prepared. In previous molecular modeling studies by van Boom and coworkers, a five-atom linker between the uridine and the sugar moiety was proposed to provide the required distance to span a pyrophosphate moiety.[45] Thus, compounds **42–44**, which contain a uridine moiety and five or six atoms separated from the iodide were selected. The synthesis of these alkyl halides can be found in the ESI (Scheme S1†).

Coupling and deprotection reactions

Reactions with simple alkyl halides. With the cyclic sulfide and the various halides in hand, we explored their coupling to produce the targets (Schemes 5–7). These reactions were carried out as reported by Mohan *et al.*,[37] by reacting the two substrates in CH_3CN in the presence of $AgBF_4$ at 65 °C to give the sulfonium ion tetrafluoroborate salt. It was necessary to carry out the coupling reaction under an argon atmosphere to minimize the formation of the sulfoxide byproduct (Fig. S2†), presumably resulting from air oxidation of the sulfide.

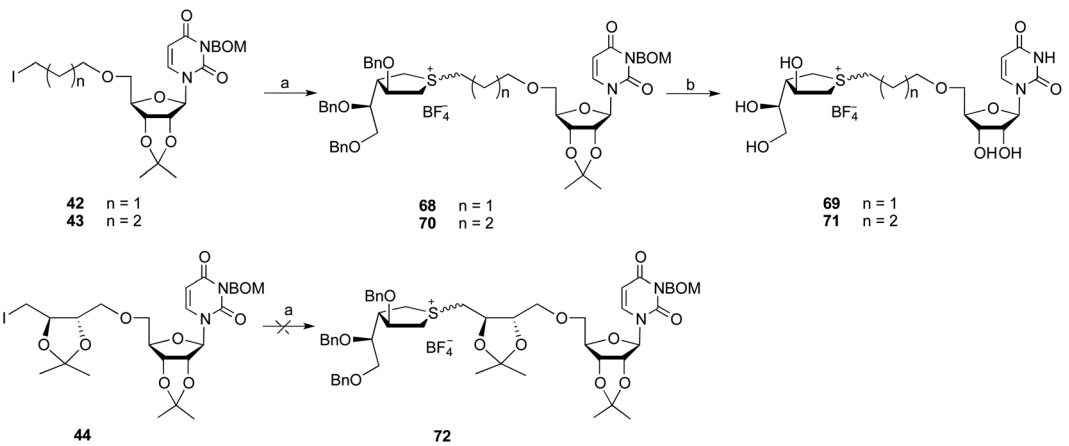


Scheme 6 *Reagents and conditions*: (a) Na, NH_3, 90%; (b) **65**, $CF_3CHOHCF_3$, **66** : 57%; **67** : 58%.

The coupling reactions of **21** with the unhindered primary alkyl iodides (**30**, **31**, **34**, **35** and **36–38**) gave the expected products in yields ranging from 48% to 97%. The more hindered alkyl iodides, **32** and **33**, gave much poorer yield of 24% and 8%, respectively. Similarly, 1-bromo-2-phenylethane (**41**) was converted into compound **63** in modest (32%) yield. As shown, the coupling reactions gave mixtures of *R*/*S* stereoisomers, which was expected[46–48] given the lack of substituents at the carbons adjacent to the sulfur. Such groups would be anticipated to influence the stereochemistry of the reaction. The ratios of stereoisomers ranged from 60 : 40 to 95 : 5, which were inseparable. Despite extensive efforts, determining the structure of the major isomer by NMR spectroscopy proved impossible, and none of the compounds were crystalline solids. Therefore, the products were characterized as mixtures. Subsequent, removal of the benzyl groups from the protected sulfonium ions was carried out upon treatment with boron trichloride (1 M in CH_2Cl_2) at −78 °C. The products were then treated with Amberlyst resin (Cl^- form) to convert the tetrafluoroborate salt into the corresponding chloride salt. The final chloride salts **46**, **48**, **50**, **52**, **54**, **56**, **58**, **60**, **62** and **64** were obtained in 55–100% yield over this two-step transformation.

Reactions with benzylic halides. An alternate approach was developed for the preparation of the sulfonium ion compounds from benzylic bromides **39** and **40**, as the products would not be expected to be stable to BCl_3-promoted debenzylation. Thus, as illustrated in Scheme 6, compound **21** was converted by Birch reduction into **65** in 90% yield. Subsequent reaction of **65** with benzyl bromide **39** in 1,1,1,3,3,3-hexafluoro-isopropanol[37] at 50 °C afforded **66** in 57% yield. Using the same conditions, reaction of **65** with *p*-nitrobenzyl bromide **40** produced **67** in 58% yield.

Reactions with uridine-derived iodides. The preparation of analogues containing the uridine moiety is shown in Scheme 7. Reaction of **21** with iodides **42** and **43** under the same conditions as described above afforded **68** and **70**, in 30% and 39% yield, respectively. Although the iodine was attached to linear



Scheme 7 *Reagents and conditions*: (a) **21**, $AgBF_4$, CH_3CN, 65 °C, **68** : 30%; **70** : 39%; (b) BCl_3, 1 M in CH_2Cl_2, then Amberlyst resin (Cl^-), **69** : 55%; **71** : 54%.

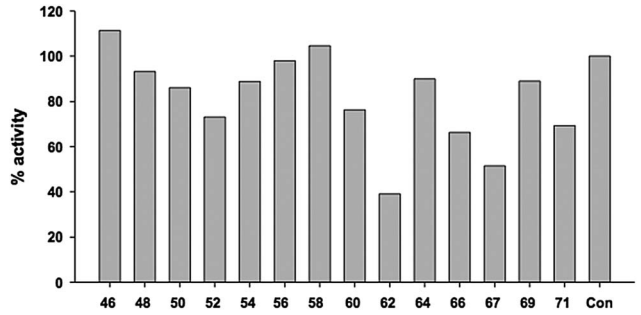


Fig. 4 Inhibition activity of the sulfonium ion analogues against GlfT2 at concentration of 4 mM.

alkyl group, the uridine-derived iodides showed substantially lower reactivity that the simple alkyl iodides **35** and **36**. Treatment of **68** and **70** with BCl_3 led to cleavage of all of the benzyl and BOM groups, as well as the isopropylidene acetal, to afford the expected products **69** and **71** in moderate yields of 55% and 54%, respectively. Iodide **44**, which possess an isopropylidene acetal in the linker, proved extremely unreactive. Even after extended reactions times none of the desired substation product **72** was observed.

Evaluation of sulfonium ions targets as inhibitors of GlfT2

The sulfonium ion analogues of UDP-Gal*f* were investigated as potential inhibitors of GlfT2, using a reported coupled spectrophotometric assay.[49,50] In these assays, an acceptor trisaccharide β-D-Gal*f*-(1→5)-β-D-Gal*f*-(1→6)-β-D-Gal*f*-octyl and the donor substrate UDP-Gal*f* (**1**) are incubated with a potential inhibitor. To obtain an indication of the potency of the various compounds, the analogues were initially screened at a concentration of 4 mM against the enzyme. The percentage activities compared to the no-inhibitor control are shown in Fig. 4. Under these conditions, most of the compounds show weak levels of inhibition against the enzyme, with the most potent compound, **62**, leading to about a 60% inhibition of activity. Neither of the compounds possessing the uridine moiety, **69** or **71**, demonstrated strong inhibition. Given the low levels of inhibition activity, additional studies to determine exact K_i values for these compounds were not carried out.

Conclusions

In summary, we have synthesized a panel of sulfonium ions that were designed as potential inhibitors of the mycobacterial galactofuranosyltransferase GlfT2. The synthesis of the targets involved the preparation of a sulfide, **21**, from D-arabinintol and its subsequent coupling with a range of alkyl halides followed by cleavage of the benzyl protecting groups under Lewis acidic conditions. The ability of these compounds to prevent GlfT2-mediated transfer of a galactofuranose residue to an acceptor reveled low levels of activity suggesting the limited potential of this class of compounds as inhibitors of mycobacterial AG biosynthesis.

Experimental section

Synthetic chemistry

General procedure for the preparation of sulfonium ions. To a solution of the cyclic sulfide **21** (1.0 equiv.) in dry CH_3CN (3 mL) was added the alkyl halide (1.0 equiv.) and $AgBF_4$ (1.0 equiv.). The reaction mixture was covered with aluminum foil and stirred at 65 °C for 24 h. The mixture was then cooled to rt, concentrated and the resulting residue was purified by chromatography (toluene–CH_3OH, 30 : 1) to give the sulfonium ion as mixture of isomers, which were inseparable.

General procedure for the deprotection of benzyl groups. To a solution of benzylated sulfonium ion (1.0 equiv.) in CH_2Cl_2 (2 mL) was added BCl_3 (1 M solution in CH_2Cl_2, 4 mL) at −78 °C under an Ar atmosphere. The mixture was stirred at −78 °C for 2 h and then warmed slowly to 0 °C and stirred for another 30 min. The solution was bubbled with air to remove the excess BCl_3 and then was added CH_3OH (3 mL). The solution was concentrated and the residue was coevaporated with CH_3OH (2 × 5 mL). The deprotected product was then dissolved in CH_3OH (5 mL) and stirred with ion exchange resin Amberlyst (Cl^- form) for 2 h and filtered. The filtrate was concentrated and the resulting oil was passed through a short Iatrobead column (CH_2Cl_2–CH_3OH 5 : 1 → 1 : 1) to give the target products.

(2*S*,4*S*)-3-Methylenepentane-1,2,4,5-tetraol (16). A solution of **15** (1.88 g, 6.62 mmol) and CSA (0.14 g, 0.6 mmol) in CH_3OH (12 mL) and CH_2Cl_2 (6 mL) was heated at reflux for 16 h, cooled to rt, neutralized with Et_3N and then concentrated. The resulting residue was purified by chromatography (CH_2Cl_2–CH_3OH 19 : 1 → 7 : 1) to give **16** as a colorless oil (0.95 g, 97%): $R_f = 0.33$ (CH_2Cl_2–CH_3OH, 5 : 1); $[\alpha]_D^{20}$ +26.7 (*c* 1.0, CH_3OH); ^1H NMR (400 MHz, CD_3OD): δ 5.28 (s, 2H), 4.14 (dd, $J = 4.1$, 7.3 Hz, 2H), 3.60 (dd, $J = 4.1$, 11.4 Hz, 2H), 3.48 (dd, $J = 7.3$, 11.4 Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD): δ 151.1, 113.0, 74.4, 67.1; HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_6H_{12}O_4Na$: 171.0628, found: 171.0628.

(2*S*,4*S*)-3-Methylene-5-(trityloxy)pentane-1,2,4-triol (17). Compound **16** (0.96 g, 6.49 mmol) and DMAP (0.08 g, 0.65 mmol) were dissolved in DMF (30 mL) and Et_3N (1 mL). To this mixture was added a solution of trityl chloride (1.80 g, 6.49 mmol) in DMF (20 mL) dropwise. The reaction mixture was stirred overnight to give a cloudy solution, to which CH_3OH (5 mL) was added. The solution was concentrated and the resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2–CH_3OH 30 : 1 → 5 : 1) to give **17** as an oil (1.60 g, 63%): $R_f = 0.63$ (CH_2Cl_2–CH_3OH, 9 : 1); $[\alpha]_D^{20}$ +9.4 (*c* 1.9, CH_2Cl_2); ^1H NMR (400 MHz, CD_3OD): δ 7.47–7.44 (m, 6H), 7.31–7.26 (m, 6H), 7.23–7.19 (m, 3H), 5.24 (s, 1H), 5.20 (s, 1H), 4.24 (dd, $J = 5.4$, 5.7 Hz, 1H), 4.03 (dd, $J = 3.5$, 7.2 Hz, 1H), 3.52 (dd, $J = 3.5$, 11.4 Hz, 1H), 3.35 (dd, $J = 7.2$, 11.4 Hz, 1H), 3.21–3.14 (m, 2H), 2.95 (s, 1H), 2.84 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 151.1, 145.5, 130.0, 128.8, 128.1, 112.7, 88.2, 74.3, 72.8, 69.2, 67.2; HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{26}O_4Na$: 413.1723, found: 413.1724.

(2*S*,4*S*)-3-Methylene-5-(trityloxy)-1,2,4-tribenzyloxypentane (18). Compound **17** (94 mg, 0.24 mmol) and benzyl bromide (0.15 g, 0.88 mmol) were dissolved in DMF (3 mL) and cooled to 0 °C. To this mixture was added NaH (36 mg, 0.88 mmol, 60% in

mineral oil) slowly and the solution was stirred for 1 h at 0 °C. The excess reagents were then quenched by the addition of H_2O and the solution was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4) and concentrated, and the resulting residue was purified by chromatography (EtOAc–hexane 1 : 15) to give **18** as an oil (0.14 g, 87%): $R_f = 0.56$ (EtOAc–hexane 1 : 4); $[\alpha]_D^{20}$ +27.5 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47–7.21 (m, 30H), 5.44 (s, 1H), 5.42 (s, 1H), 4.66 (d, J = 12.1 Hz, 1H), 4.51–4.35 (m, 4H), 4.21 (d, J = 12.1 Hz, 1H), 3.92 (dd, J = 3.1, 7.3 Hz, 1H), 3.84 (dd, J = 3.5, 7.3 Hz, 1H), 3.45 (dd, J = 7.3, 10.6 Hz, 1H), 3.38 (dd, J = 7.2, 10.4 Hz, 1H), 3.34 (dd, J = 3.5, 10.6 Hz, 1H), 3.10 (dd, J = 3.1, 10.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.2, 144.1, 138.7, 138.4, 138.3, 128.8, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.4, 127.3, 126.9, 115.3, 86.8, 79.1, 78.6, 73.3, 73.1, 70.9, 70.6, 66.9; HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{46}H_{44}O_4Na$: 683.3131, found: 683.3131.

(2*S*,3*S*)-3,4-Bis(benzyloxy)-2-((*S*)-1-(benzyloxy)-2-(trityloxy)-ethyl)butan-1-ol and (2*R*,3*S*)-3,4-bis(benzyloxy)-2-((*S*)-1-(benzyloxy)-2-(trityloxy)ethyl)butan-1-ol (19). To a solution of **18** (4.33 g, 6.56 mmol) in THF (30 mL) at 0 °C was added $BH_3 \cdot S(CH_3)_2$ (10.0 mL, 19.8 mmol, 2 M in THF), and then the solution was warmed to rt and stirred for 16 h under argon. The solution was then added dropwise to a mixture of H_2O–THF–NaOH (2M)–H_2O_2 (30%) (1 : 1 : 3 : 1.5, 20 mL) at 0 °C and stirred for 1 h. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (EtOAc–hexane 1 : 6) to give a inseparable mixture of stereoisomers **19** with ratio 60 : 40, as as a colorless oil (3.60 g, 80%): $R_f = 0.34$ (EtOAc–hexane 1 : 4); HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{46}H_{46}O_5Na$: 701.3238, found: 701.3240. The compound was used in the next reaction without further characterization.

(2*S*,3*R*)-2-(Benzyloxy)-3-((*S*)-1,2-bis(benzyloxy)ethyl)butane-1,4-diol and (2*S*,3*S*)-2-(benzyloxy)-3-((*S*)-1,2-bis(benzyloxy)ethyl)-butane-1,4-diol (20). To a solution of **19** (3.51 g, 5.18 mmol) in CH_2Cl_2 (10 mL) and CH_3OH (10 mL) was added *p*-TsOH (0.35 g, 1.84 mmol). The mixture was stirred for 3 h, neutralized with Et_3N and concentrated. The resulting residue was purified by chromatography (EtOAc–hexane 1 : 2) to give inseparable stereoisomers **20** as a colorless oil (2.16 g, 96%): R_f = 0.29 (EtOAc–hexane 1 : 1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.22 (m, 15H), 4.71 (d, J = 11.5 Hz, 0.6H), 4.68 (d, J = 11.5 Hz, 0.4H), 4.63–4.52 (m, 3H), 4.42 (d, J = 11.5 Hz, 0.8H), 4.37 (d, J = 11.6 Hz, 0.6H), 4.34 (d, J = 11.6 Hz, 0.6H), 4.12 (ddd, J = 2.8, 5.3, 5.3 Hz, 0.6H), 3.98 (dd, J = 4.8, 8.6 Hz, 0.4H), 3.95–3.64 (m, 7H), 2.62 (br, 2H), 2.12 (dddd, J = 3.6, 3.6, 5.2, 7.2 Hz, 0.4H), 2.04 (dddd, J = 2.9, 2.9, 4.2, 5.9 Hz, 0.6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.3, 138.1, 138.0, 137.9, 128.6, 128.5, 128.4, 128.0, 127.9, 127.7, 127.8, 127.6, 79.2, 78.0, 77.8, 76.9, 73.6, 73.4, 73.0, 72.9, 72.0, 71.7, 71.6, 69.7, 63.4, 60.8, 60.5, 60.1, 44.5, 43.8; HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{32}O_5Na$: 459.2142, found: 459.2140.

(3*R*,4*S*)-3-(Benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)tetrahydrothiophene (21) and (3*R*,4*R*)-3-(benzyloxy)-4-((*S*)-1,2-bis(benzyloxy)ethyl)tetrahydrothiophene (22). To a solution of isomers **20** (1.85 g, 4.24 mmol) and Et_3N (2 mL) in dry CH_2Cl_2 (40 mL) at −30 °C was added methanesulfonyl chloride (0.90 mL, 10.32 mmol). The reaction mixture was warmed to 0 °C steadily and stirred for 1 h and the excess reagent was quenched by the addition of ice. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution (3 × 10 mL), followed by brine, and then dried (Na_2SO_4) and concentrated. The residue was coevaporated twice with toluene and dissolved in dry DMF (30 mL). To this solution was added $Na_2S \cdot 9H_2O$ (1.25 g, 5.21 mmol) and the mixture was heated at 100 °C for 2 h. After being cooled to rt, the mixture was diluted with Et_2O and the organic layer was washed with H_2O (3 × 10 mL), followed by brine and then dried (Na_2SO_4) and concentrated. The resulting residue was purified by chromatography (EtOAc–hexane 1 : 10) to give **21** (0.90 g) and **22** (0.64 g) (1.4 : 1, 84% in total) both as yellowish oils: (**21**) R_f = *0.40* (EtOAc–hexane 1 : 6); $[\alpha]_D^{20}$ −95.2 (*c* 1.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37–7.26 (m, 15H), 4.74 (d, J = 11.7 Hz, 1H), 4.55–4.49 (m, 3H), 4.45 (d, J = 11.7 Hz, 1H), 4.38 (d, J = 11.7 Hz, 1H), 4.07 (ddd, J = 6.3, 7.9, 7.9 Hz, 1H), 3.86 (ddd, J = 5.0, 5.0, 5.0 Hz, 1H), 3.60 (dd, J = 5.0, 10.1 Hz, 1H), 3.56 (dd, J = 5.0, 10.1 Hz, 1H), 3.04–3.96 (m, 2H), 2.84–2.74 (m, 2H), 2.42 (dddd, J = 3.7, 7.9, 8.3, 9.2 Hz, 1H); ^{13} C NMR (125 MHz, $CDCl_3$): δ 26.9, 33.8, 50.2, 72.2, 72.3, 73.2, 73.7, 76.0, 82.0, 127.5, 127.6, 127.7, 127.8, 128.3, 128.4, 138.1, 138.7; HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{30}O_3SNa$: 457.1808, found: 457.1805. (**22**) R_f = 0.33 (EtOAc–hexane 1 : 6); $[\alpha]_D^{20}$ −76.2 (*c* 0.37, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.26 (m, 15H), 4.71 (d, J = 11.3 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.59 (d, J = 11.8 Hz, 1H), 4.55–4.53 (m, 2H), 4.45 (d, J = 11.3 Hz, 1H), 4.38 (d, J= 1.6 Hz, 1H), 3.92 (ddd, J = 2.9, 4.2, 10.0 Hz, 1H), 3.73 (dd, J = 2.9, 10.6 Hz, 1H), 3.48 (dd, J = 4.2, 10.6 Hz, 1H), 3.14–3.08 (m, 1H), 2.94–2.90 (m, 1H), 2.81–2.74 (m, 2H), 2.45 (dddd, J = 3.0, 7.3, 10.0, 12.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.7, 138.4, 138.1, 128.4, 128.3, 127.7, 127.6, 127.5, 81.1, 77.4, 73.5, 72.3, 70.9, 70.7, 51.9, 34.8, 30.4; HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{30}O_3SNa$: 457.1808, found: 457.1810.

Evaluation of inhibitory activity

Inhibition activity test. Solutions of KCl (2 M), $MgCl_2$ (1 M), and MOPS (1 M, pH 7.6) were prepared in de-ionized distilled (MilliQ, MQ) water, filtered and stored at 4 °C. Recombinant GlfT2, prepared and stored as previously reported were used in the assay.[7] On the day of experiment, donor analogues were reconstituted in filtered MQ water to give a 32 mM stock. Solutions of 15 mM NADH, 5 U mg^{-1} PK, 16.8 U mg^{-1} LDH, and 40 mM UDP-Gal*f* were prepared in 50 mM MOPS (pH 7.6); 100 mM PEP was prepared in 250 mM MOPS (pH 7.6); 40 mM trisaccharide was prepared in filtered MQ water. All solutions were stored on ice during operation. Reactions to screen the ability of analogues to inhibit GlfT2 were initiated with the addition of GlfT2 (0.5 μg) to assays to give a final volume of 40 μL containing 50 mM MOPS (pH 7.6), 50 mM KCl, 20 mM $MgCl_2$, 1.1 mM NADH, 3.5 mM PEP, 7.5 U PK, 16.8 U LDH, 2 mM trisaccharide acceptor, 4 mM analogues and 0.75 mM donor UDP-Gal*f*. Reactions were incubated at 37 °C and monitored at 340 nm at 10–15 s intervals for 20 min using a Spectra Max 340 PC microplate reader. The inhibition screening assays were repeated at two-times linking enzyme levels (15 U PK and 33.6 U LDH), to rule out inhibition of the linking enzymes by the analogues.

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