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Synthesis and biological evaluation of galactofuranosyl alkyl thioglycosides as inhibitors of mycobacteria

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Abstract—As part of our research interest directed toward the development of antimycobacterial agents, we have investigated compounds based on galactofuranose (Gal*f*), an essential cell wall component of mycobacteria. The objective of this study was to explore structure activity relationships of Gal*f* thioglycosides with straight chain and branched aglycons. Acylated Gal*f* 9-heptadecyl thioglycoside was prepared by Lewis acid-catalyzed thioglycosidation of 1,2,3,5,6-penta-*O*-acyl-D-galactofuranose with 9-heptadecanethiol, and subsequently converted to the corresponding sulfone using *m*-CPBA. Both Gal*f* 9-heptadecyl thioglycoside and sulfone displayed in vitro inhibition (MIC) of the growth of *Mycobacterium smegmatis* below 5 μg/mL, while Gal*f* 1-octyl thioglycoside gave no inhibition at or below 32 μg/mL.

Keywords: Galactofuranose; Thioglycoside; Sulfone; Mycobacterium; *Mycobacterium smegmatis*; Inhibition

1. Introduction

As a result of growing resistance to antibiotics, many bacterial infections that were once controlled by antibacterial drug therapies are being revisited. This is apparent not only in third world societies, but also in the industrialized world due to the development of multi-drug resistant (MDR) strains of bacteria that spread diseases such as tuberculosis (TB). The proliferation of multi-drug resistant strains of *Mycobacterium tuberculosis* is fast becoming a global emergency as health practitioners are exhausting all current treatment options for MDR-TB.[1] The implementation of the Directly Observed Treatment, Short Course strategy (DOTS), where health workers ensure that the patient swallows every pill over the course of treatment, is considered the most comprehensive treatment for TB infection and a method to curtail further resistance.[2] But for MDR-TB infections, both the DOTS regime and conventional drugs are ineffective.[3] It is imperative, therefore, that the pursuit of more effective, less toxic, and preferably cheaper drugs to treat the illness be intensified.

As part of our medicinal chemistry program targeting the pathogen *M. tuberculosis*, we have investigated compounds based on galactofuranose (Gal*f*), an essential cell wall component of Mycobacteria.[4] We have recently reported the synthesis and anti-mycobacterial activity of a series of novel β-galactofuranosyl *N*,*N*-dialkyl sulfenamides **1** and sulfonamides **2**, in which the length of the alkyl chains was varied.[5] Alkyl chain length was found to influence the anti-mycobacterial activity, with inhibitory activity increasing with increasing chain length. In particular, the *N*,*N*-dioctyl derivatives **1** and **2** ($n = 7$) both showed in vitro inhibition (MIC) of mycobacterial growth below 5 μg/mL.[5] The objective of this study was to further explore structure–activity relationships of these Gal*f* derivatives by investigating

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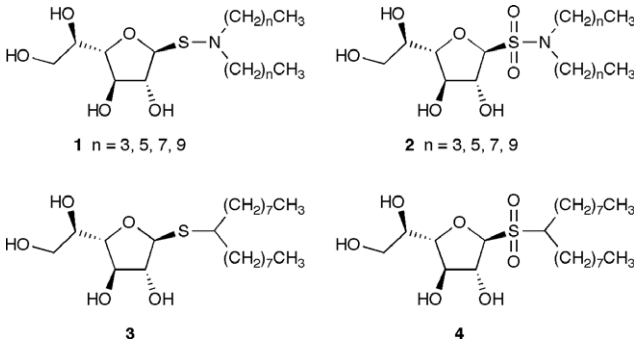
the influence of the type of aglycon linkage on antimycobacterial activity. Thus, we have prepared a branched-alkyl (9-heptadecyl) β-thioglycoside **3**, and the corresponding sulfone **4**, as direct S–C isosteres of the *N*,*N*-dioctyl sulfenamide and sulfonamide. In addition, to explore the necessity for a di-alkylated (branched) aglycon for activity, a straight chain thioglycoside of analogous (octyl) chain length was also prepared and evaluated. The thioglycoside linkage was chosen as S-glycosides have greater stability toward glycohydrolases than the analogous O-glycosides.[6–8]

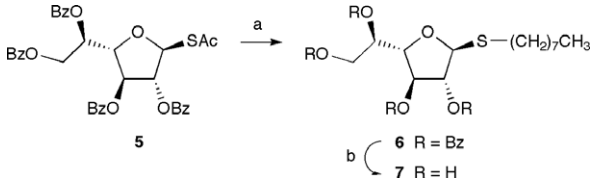


2. Results and discussion

2.1. Galactofuranosyl thioglycosides

A convenient method for the preparation of alkyl and sugar thioglycosides is the coupling of an anomeric thiolate ion with an alkyl halide or sulfonate ester. Previous work within our group,[9] as well as the work of Roy and co-workers,[10] on the synthesis of thioglycosides has shown that a reactive thiolate functionality can be readily generated by the selective de-S-acetylation of an anomeric thiolacetate under mild treatment with amine bases in DMF. This thiolate can couple efficiently with alkyl halides producing the desired thioglycosides.[9,10] It was therefore envisaged that the Gal*f* octyl and 9-heptadecyl β-thioglycosides would be readily accessible from the 1-*S*-acetyl-2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranose (**5**)[11] via coupling with the appropriate alkyl bromide. Thus, reaction of the Gal*f* β-thiolacetate derivative **5** with 1-bromooctane in DMF in the presence of diethylamine[9] gave the protected octyl β-thioglycoside **6** in good yield (Scheme 1). The anomeric purity (β) was maintained during the reaction, as anticipated.[9,10] Deprotection of the tetra-O-benzoylated derivative **6** using sodium methoxide in methanol gave the Gal*f* decyl β-thioglycoside **7** in excellent yield.

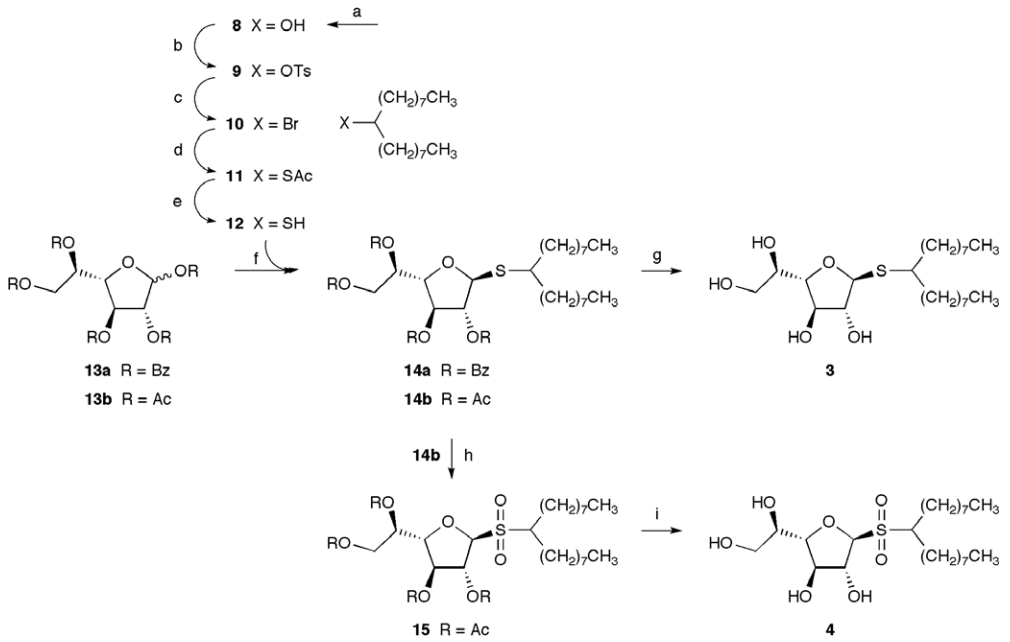
For the synthesis of the branched Gal*f* 9-heptadecyl thioglycoside **3**, the required secondary alkyl bromide, 9-bromoheptadecane (**10**), was prepared as shown in Scheme 2 (steps a–c). The starting 9-heptadecanol (**8**) was prepared by a Grignard reaction of 1-bromooctane with nonyl aldehyde to furnish the branched alcohol in



Scheme 1. Reagents and conditions: (a) $CH_3(CH_2)_7Br$, DMF, $HNEt_2$, 25 °C, 4 h, N_2 (75%); (b) (i) NaOMe, MeOH, 25 °C, 1 h, N_2; (ii) acidic ion exchange resin (91%).

67% yield. Conversion of alcohol **8** to the *p*-toluenesulfonate ester **9**, and subsequent reaction with lithium bromide in refluxing acetone, gave 9-bromoheptadecane (**10**). Reaction of Gal*f* β-thiolacetate derivative **5** with 9-bromoheptadecane (**10**) under reaction conditions identical to those described above for thioglycoside formation with 1-bromooctane (Scheme 1, step a), however, was not successful. Variation to the reaction solvent, reaction time (7 h to 11 days), and temperature (25–67 °C) was made but resulted only in the recovery of de-S-acetylated material. Failure to successfully prepare the desired 9-heptadecyl thioglycoside from Gal*f* β-thiolacetate **5** led us to pursue an alternate method of aglycon attachment.

Previously, de Lederkremer and co-workers[8] have shown that $SnCl_4$ catalyzed reaction of per-O-benzoylated galactofuranose **13a** with thiols could be employed to produce, stereoselectively, galactofuranosyl β-thioglycosides. To pursue this approach, 9-heptadecanethiol (**12**) was prepared by reaction of bromide **10** with potassium thiolacetate, followed by de-S-acetylation under Zemplén conditions of the resultant thiolacetate derivative **11** (Scheme 2, steps d and e). $SnCl_4$ mediated glycosidation of 1,2,3,5,6-penta-*O*-benzoyl-α/β-galactofuranose[12] (**13a**) with 9-heptadecanethiol (**12**) produced the 9-heptadecyl thioglycoside **14a** in good yield (80%). The ^1H NMR spectrum of the product, however, indicated there to be an approximately 1:9 α/β anomeric mixture of the thioglycoside **14a**, with overlapping resonances for H-1 at δ 5.65 and 5.66 ppm for the major and minor products, respectively. This was somewhat surprising as the $SnCl_4$ mediated thioglycosidation reaction used to afford **14a** has been reported[8] to give selectively the β-anomer (due to anchimeric assistance of C-2 benzoate) in glycosidation of **13a** with alkyl (*n*-Pr, *i*-Pr), benzyl, and aryl thiols. The assignment of the major product as the Gal*f* β-thioglycoside was made on the basis of small ^1H $J_{1,2}$ and $J_{2,3}$ coupling constants,[8,13] which were consistent with those reported by Marino et al.[8] for the O-benzoylated Gal*f* *i*-propyl β-thioglycoside ($J_{1,2} < 1$, $J_{2,3}$ 1.4 Hz[8]). The ^3J coupling constant (4.9 Hz) observed for the anomeric proton of the minor product, which constituted approximately 10% of the product (by ^1H NMR analysis), was consistent with literature values for a protected galactofuranosyl α-ano-



Scheme 2. Reagents and conditions: (a) (i) Mg, I_2, bunsen heat, N_2; (ii) $CH_3(CH_2)_7Br$, THF, 45 °C, 3 h, N_2; (iii) $CH_3(CH_2)_7CHO$, 50 °C, 2 h, N_2 (67%); (b) TosCl, DMAP, pyr, 0–25 °C, 8 h, N_2; (c) LiBr, acetone, reflux, 1.5 h (80% over 2 steps); (d) KSAc, acetone, reflux, 24 h, N_2 (82%); (e) (i) NaOMe, MeOH, 25 °C, 1.5 h, N_2; (ii) acidic ion exchange resin (84%); (f) $SnCl_4$, CH_2Cl_2, 0 °C, 2 h, N_2 (**14a** 80%, ~1:9 α/β); or $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0–25 °C, 3 h, N_2 (**14b** 84%, ~1:9 α/β); (g) (i) NaOMe, MeOH, 25 °C, 3 h, N_2; (ii) acidic ion exchange resin (82%); (h) *m*-CPBA, CH_2Cl_2, reflux, 2 h (80%); (i) aq K_2CO_3, MeOH, 25 °C, 15 min (96%).

mer.[12,13] Similar reaction yield and anomeric ratio of the product were obtained when carrying out the thioglycosidation with 1,2,3,5,6-penta-*O*-acetyl-α/β- galactofuranose[14] (**13b**) in place of the benzoylated derivative **13a**. De-O-benzoylation of 9-heptadecyl thioglycoside **14a** under Zemplén conditions gave the deprotected thioglycoside target compound **3**. The changes in ^3J coupling constants upon deprotection observed for the β-anomer, in particular the distinctive increases from ~1.3 to ~4.5 Hz in the $J_{1,2}$ and $J_{2,3}$ values, were consistent with those reported by Marino et al.[8] for deprotected Gal*f* β-thioglycosides.

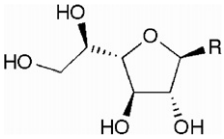
2.2. Galactofuranosyl sulfone

The metabolic fate for many sulfur containing drugs can often include oxidation at sulfur to form the corresponding sulfoxide and/or sulfone.[15] By contrast, antibacterial sulfone drugs per se require this level of oxidation at sulfur for activity.[16] Our previous investigation[5] of the effect of oxidation at sulfur in the sulfenamide series **1** on in vitro activity showed that the conversion of the *N*,*N*-dioctyl sulfenamide **1** ($n = 7$) to the analogous sulfonamide **2** did not diminish anti-mycobacterial potency. It was therefore of interest to synthesize the Gal*f* 9-heptadecyl sulfone for comparison with thioglycoside **3**. Preparation of sulfone **15** from per-O-acetylated 9-heptadecyl thioglycoside **14b** was readily achieved using *meta*-chloroperbenzoic acid (*m*-CPBA) as oxidant in dichloromethane under reflux (Scheme 2, step h). The desired protected 9-heptadecyl sulfone **15**, obtained in 80% yield after chromatography, was subsequently de-O-acetylated using potassium carbonate solution in methanol to give Gal*f* 9-heptadecyl sulfone **4** in high yield.

2.3. In vitro inhibition of mycobacterial growth

Thioglycosides **7** and **3**, and sulfone **4** were evaluated as in vitro inhibitors of *Mycobacterium smegmatis* (ATCC 14468). *M. smegmatis* is a rapidly growing mycobacterium that has been used as a surrogate microorganism for detecting the antimycobacterial activity of novel agents.[17] The compounds were screened for growth inhibitory activity, over the range 64–0.06 μg/mL, using a standard[18,19] broth microdilution assay for susceptibility testing against *M. smegmatis.* Inhibition data (MIC) for **7**, **3**, and **4**, as well as data previously reported[5] for the Gal*f* *N*,*N*-dioctyl sulfenamide **1** and sulfonamide **2** ($n = 7$), are given in Table 1. Both the 9-heptadecyl thioglycoside **3** and sulfone **4** displayed significant inhibitory potency (<5 μg/mL) against *M. smegmatis*, comparable to that of the sulfenamide and sulfonamide derivatives **1** and **2** ($n = 7$).[5] Why the activity of sulfone **4** is less than that of the parent thioglycoside **3** is unclear at this stage. The 9-heptadecyl thioglycoside **3** was the most active compound of the series, with an MIC of 1 μg/mL. Interestingly, the 'mono-alkylated' octyl thioglycoside **7** did

Table 1. Activity (MIC) of Gal*f* alkyl thioglycosides **7** and **3** and sulfone **4** against *M. smegmatis* (ATCC 14468)[a]



	R	MIC (μg/mL)[b]
7	$S(CH_2)_7CH_3$	>32
3[c]	$SCH[(CH_2)_7CH_3]_2$	1
4[c]	$S(O)_2CH[(CH_2)_7CH_3]_2$	2
1	$SN[(CH_2)_7CH_3]_2$	4[5]
2	$S(O)_2N[(CH_2)_7CH_3]_2$	2[5]

[a] Broth microdilution assay in MHBII broth.[19] 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.

[c] Ratio of anomers approximately 1:9 α/β.

not show any activity at the maximum concentration tested. As a comparison to these activities, the current anti-TB drug ethambutol is reported to have an MIC of 0.5 μg/mL against a representative strain of *M. smegmatis*.[20] The results presented here, in conjunction with the previously reported *N*,*N*-dialkyl sulfenamides and sulfonamides of varying alkyl chain length, suggest that effective in vitro inhibition of mycobacterial growth is achieved with a Gal*f* derivative that incorporates two octyl chains in the aglycon unit. While the precise mode of action of these compounds is under further investigation, other preliminary and on-going studies (unpublished data) to investigate membrane selectivity and consequently possible host cell toxicity suggest that these compounds can differentiate between bacterial and mammalian cell membranes.

In summary, the branched 9-heptadecyl thioglycoside, and the corresponding sulfone, of galactofuranose have been prepared to further explore structure–activity relationships related to the anti-mycobacterial activity observed previously[5] for β-galactofuranosyl *N*,*N*-dioctyl sulfenamide **1** and sulfonamide **2** ($n = 7$). In addition, a straight chain octyl β-thioglycoside was prepared to test the necessity for an aglycon unit with two alkyl chains for in vitro potency. The protected octyl β-thioglycoside **6** was readily synthesized from Gal*f* β-thiolacetate derivative **5**, however this synthetic approach was not successful for the branched 9-heptadecyl thioglycoside **14** which required Lewis acid-catalyzed synthesis from the 1-acyl derivative **13** and 9-heptadecanethiol. While the straight chain octyl β-thioglycoside **7** was not inhibitory in vitro against *M. smegmatis* at the concentrations tested, both the branched chain galactofuranosyl 9-heptadecyl thioglycoside **3** and sulfone **4** were active at below 5 μg/mL consistent with the activity shown by the *N*,*N*-dioctyl sulfenamide and sulfonamide. The broad-spectrum antibacterial activity of these compounds is also being explored and will be reported in due course.

3. Experimental

3.1. General

TLC was carried out on aluminum plates coated with Silica Gel 60 F_{254} (E. Merck) with detection by ultraviolet (UV) light and/or either 5% H_2SO_4 in ethanol, ninhydrin, phosphomolybdic acid, or vanillin developing agents with heating. Compounds were purified by flash chromatography on Silica Gel 60 (0.040–0.063 mm), and in some cases further purified by HPLC using an Agilent HP1100. IR spectra were recorded using a Bruker Vector 22 spectrophotometer between NaCl windows. ^1H and ^{13}C NMR spectra were recorded in the deuterated solvent specified using a Bruker spectrometer at 300 and 75.5 MHz, respectively. Chemical shifts are given in parts per million (ppm) relative to the solvent used [$CDCl_3$: 7.26 (s) for ^1H, 77.0 (t) for ^{13}C; CD_3OD: 4.78 (s) for ^1H, 49.0 (sept) for ^{13}C; D_2O: 4.67 (s) for ^1H]. 2D ^1H–^1H COSY and ^1H–^{13}C HMQC experiments were run to confirm ^1H and ^{13}C assignments. Abbreviations of multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublets), q (quartet), quint (quintet), and (br) indicates a broad signal. LRMS (ESI) were obtained using a Bruker Esquire 3000. HRMS were obtained using either an electrospray method in positive mode or electron impact.

MIC determinations against *M. smegmatis* (ATTC 14468) were carried out by Micromyx LLC, Kalamazoo, MI, USA, in standard Mueller-Hinton II Broth using the standard broth microdilution method.[19]

3.2. Octyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (6)

To a solution of 1-*S*-acetyl-2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranose[11] (**5**) (1.56 g, 2.38 mmol), and 1-bromooctane (824 μL, 4.77 mmol, 2 M equiv), in dry DMF (50 mL) under N_2 was added diethylamine (740 μL, 7.15 mmol, 3 M equiv). The reaction mixture was stirred at rt for 4 h under N_2. After this time the volatile compounds were removed under reduced pressure. The residue was diluted in EtOAc (100 mL), washed once with 0.5 M HCl (100 mL), twice with water (100 mL), and dried (Na_2SO_4). The solution was filtered and the solvent removed under reduced pressure. The residue was purified by chromatography (hexane–EtOAc, 6:1) to furnish **6** as a pale yellow syrup (1.30 g, 75%). $R_f = 0.40$ (hexane–EtOAc, 6:1); IR (neat): ν 3036, 2958, 2857, 1726, 1452, 1279, 1250, 1110 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.26–8.12 (m, 20H, 4 × OCOPh),

6.09 (m, 1H, H-5), 5.67 (dd, 1H, $J_{3,2} = 0.9$, $J_{3,4} = 4.1$ Hz, H-3), 5.63 (br s, 1H, H-1), 5.55 (app t, 1H, $J_{2,1\approx2,3} = 1.4$ Hz, H-2), 4.83 (dd, 1H, $J_{4,3} = 4.6$, $J_{4,5} = 3.9$ Hz H-4), 4.75 (m, 2H, H-6 and H-6′), 2.69 (m, 2H, SCH_2), 1.65 (app quint, 2H, $J = 7.5$ Hz, S–CH_2–CH_2), 1.15–1.45 (m, 10H, $5 \times CH_2$), 0.89 (app t, 3H, $J = 6.5$, $J = 6.9$ Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.5, 166.1, 166.0, 165.8 ($4 \times$ O*C*OPh), 133.9, 133.8, 133.7, 133.5, 130.4, 130.4, 130.3, 130.1, 130.0, 129.9, 129.4, 129.3, 128.9, 128.8, 128.8, 128.7 ($4 \times$ OCO*Ph*), 88.9 (C1), 83.3 (C2), 81.5 (C4), 78.3 (C3), 70.7 (C5), 63.9 (C6), 32.2 (S–*C*H_2), 31.6 (S–CH_2–*C*H_2), 30.1, 29.6, 29.5, 29.3, 23.0 ($5 \times CH_2$), 14.5 (CH_3); LRMS (ESI) *m*/*z* 747 [(M+Na)$^+$ 100%]; HRMS calcd for $C_{42}H_{44}O_9S{\cdot}NH_4^+$: 742.3044; found, 742.3049.

3.3. Octyl 1-thio-β-D-galactofuranoside (7)

To a solution of octyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (**6**) (413 mg, 0.57 mmol) in dry MeOH (20 mL), under N_2 was added NaOMe solution (570 μL, 1 M solution in dry MeOH). The reaction mixture was stirred for 1 h, at rt, under N_2. After this time the solution was neutralized with Amberlite IR 120 (H^+) resin, filtered, and the solvent removed under reduced pressure. The residue was purified by chromatography (EtOAc–MeOH, 10:1) to yield **7** as a white amorphous solid (160 mg, 91%). $R_f = 0.43$ (EtOAc); ^{1}H NMR (CD_3OD): δ 5.02 (d, 1H, $J_{1,2} = 4.6$ Hz, H-1), 4.03 (dd, 1H, $J_{3,4} = 7.5$, $J_{3,2} = 4.9$ Hz, H-3), 3.91 (dd, 1H, $J_{4,5} = 3.0$, $J_{4,3} = 7.5$ Hz, H-4), 3.87 (app t, 1H, $J_{2,3\approx2,1} = 4.7$ Hz, H-2), 3.72 (m, 1H, H-5), 3.60 (m, 2H, H-6 and H-6′), 2.63 (m, 2H, SCH_2), 1.56–1.70 (m, 2H, CH_2 octyl chain), 1.23–1.47 (m, 10H, $5 \times CH_2$, octyl chain), 0.89 (app t, 3H, $J = 7.0$, $J = 6.5$ Hz, CH_3); ^{13}C NMR (CD_3OD): δ 90.8 (C-1), 83.9 (C-2), 82.9 (C-4), 78.4 (C-3), 72.2 (C-5), 64.7 (C-6), 33.0 (SCH_2), 32.0, 31.1, 30.4, 30.3, 30.0, 23.7 ($6 \times CH_2$ octyl chain), 14.4 (CH_3); LRMS (ESI) *m*/*z* 331 [(M+Na)$^+$ 100%]. Anal. Calcd for $C_{14}H_{28}O_5S$: C, 54.52; H, 9.15. Found: C, 54.34; H, 9.32.

3.4. 9-Heptadecanethiol (12)

3.4.1. 9-Heptadecanol (8). Magnesium turnings (169 mg, 6.95 mmol) and iodine (10 mg, catalytic) were combined and heated over a Bunsen flame under N_2 until I_2 gas evolved. The flask was allowed to cool, and then dry THF (20 mL) was added followed by 1-bromooctane (1.0 mL, 5.79 mmol) and the mixture was stirred for 3 h at 45 °C under N_2. After this time, nonyl aldehyde (1.01 mL, 5.90 mmol) was added and the reaction mixture was stirred for a further 2 h, at 50 °C, under N_2. The reaction was then quenched with satd aq NH_4Cl, and the solvent was evaporated under reduced pressure. The residue was diluted with CH_2Cl_2 (300 mL), and washed with satd aq NaCl (200 mL) followed by water (200 mL). The organic layer was dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure. The residue was bonded to silica (dissolved in EtOAc, evaporated in the presence of silica) and purified by chromatography (hexane–EtOAc, 6:1) to furnish **8** as a white powder (995 mg, 67%). $R_f = 0.52$ (hexane–EtOAc, 6:1); ^{1}H NMR ($CDCl_3$): δ 3.58 (m, 1H, $OCHR_2$), 1.20–1.48 (m, 28H, $14 \times CH_2$), 0.87 (app t, 6H, $J = 6.4$, $J = 7.0$ Hz, $2 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ 72.0 (CH), 37.5, 31.9, 29.7, 29.6, 29.3, 25.7, 22.7 ($14 \times CH_2$), 14.1 ($2 \times CH_3$); LRMS (ESI) *m*/*z* 279 [(M+Na)$^+$ 12%], 449 (100); HRMS (−ve ion mode) calcd for $C_{17}H_{35}O$: 255.2688; found, 255.2687.

3.4.2. 9-Heptadecyl *p*-toluenesulfonate (9). 9-Heptadecanol (**8**) (580 mg, 2.26 mmol) and tosyl chloride (1.30 g, 6.82 mmol, 3 equiv) were dissolved in dry pyridine (10 mL) at 0 °C under N_2. 4-Dimethylaminopyridine (10 mg, catalytic) was added and the mixture was stirred for 10 min at 0 °C under N_2. After this time the ice bath was removed and the mixture was stirred for a further 8 h at rt under N_2. The solvent was then removed under reduced pressure, and the residue dissolved in CH_2Cl_2. The solution was washed with 1 M HCl (100 mL) and then with satd aq $NaHCO_3$ (100 mL) to neutrality. The organic layer was dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure. The residue contained crude 9-heptadecyl *p*-toluenesulfonate (**9**) which was used without further purification. $R_f = 0.60$ (hexane–EtOAc, 12:1); ^{1}H NMR ($CDCl_3$): δ 7.80 (d, 2H, $J = 8.3$ Hz, Ph), 7.33 (d, 2H, $J = 8.2$ Hz, Ph), 4.55 (quint, 1H, $J = 6.0$ Hz, CH), 2.46 (s, 3H, SPhC*H*$_3$), 1.50–1.70 (m, 4H, $2 \times CH_2$), 1.11–1.37 (m, 24H, $12 \times CH_2$), 0.89 (app t, 6H, $J = 6.6$, $J = 7.1$ Hz, $2 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ 129.6, 127.7 (Ph), 84.7 (CH), 34.1, 31.8, 29.4, 29.3, 29.2, 24.7, 22.7 ($14 \times CH_2$), 21.8 (SPhC*H*$_3$), 14.1 ($2 \times CH_3$); LRMS (ESI) *m*/*z* 433 [(M+Na)$^+$ 100%].

3.4.3. 9-Bromoheptadecane (10). Lithium bromide (1.96 g, 22.6 mmol) and 9-heptadecyl *p*-toluenesulfonate (**9**) (crude, from 2.26 mmol **8**) were taken up in dry acetone (30 mL) and stirred at reflux for 90 min under N_2. After this time the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (100 mL). The solution was washed once with satd aq NaCl (100 mL) and once with water (100 mL), dried (Na_2SO_4), filtered, and the solvent removed under reduced pressure. The residue was purified by chromatography (hexane) to furnish **10** as a clear oil (577 mg, 80% from **8**). $R_f = 0.97$ (hexane–EtOAc, 12:1); ^{1}H NMR ($CDCl_3$): δ 4.05 (m, 1H, $J = 5.7$ Hz, $J = 7.3$ Hz, CH), 1.21–1.88 (m, 28H, $14 \times CH_2$), 0.90 (app t, 6H, $J = 6.4$, $J = 7.0$ Hz, $2 \times CH_3$); ^{13}C NMR ($CDCl_3$): δ

59.1 (CH), 39.2, 31.8, 29.5, 29.2, 29.1, 27.6, 22.7 ($14\times CH_2$), 14.1 ($2\times CH_3$); LRMS (ESI): compound failed to ionize.

3.4.4. 9-*S*-Acetyl-9-heptadecanethiol (11). 9-Bromoheptadecane (**10**) (376 mg, 1.18 mmol) and potassium thiolacetate (270 mg, 2.36 mmol) were taken up in dry acetone (20 mL) and refluxed for 24 h under N_2. After this time the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (100 mL). The solution was washed once with aq NaCl (100 mL) and once with water (100 mL), dried (Na_2SO_4), and the solvent removed under reduced pressure. The residue was purified by chromatography (hexane) to furnish **11** as a peach colored oil (304 mg, 82%). $R_f = 0.26$ (hexane); ^{1}H NMR ($CDCl_3$): δ 3.52 (m, 1H, J 5.7 Hz, J 7.6 Hz, CH), 2.32 (s, 3H, $SCOCH_3$), 1.20–1.70 (m, 28H, $14\times CH_2$), 0.90 (app t, 6H, J 6.5, J 7.0 Hz, $2\times CH_3$); ^{13}C NMR ($CDCl_3$): δ 196.2 ($COCH_3$), 44.7 (CH), 34.8, 31.8 ($2\times CH_2$), 30.8 ($CO\mathit{C}H_3$), 29.5, 29.3, 26.8, 22.7 ($12\times CH_2$), 14.1 ($2\times CH_3$); LRMS (ESI) m/z 337 [$(M+Na)^+$ 100%]; HRMS calcd for $C_{19}H_{38}OS$: 314.2643; found, 314.2633.

3.4.5. 9-Heptadecanethiol (12). To a solution of 9-*S*-acetyl-9-heptadecanethiol (**11**) (219 mg, 0.66 mmol) in dry MeOH (10 mL) was added NaOMe solution (660 μL, 1 M solution in MeOH). The reaction mixture was stirred at rt for 1.5 h under N_2. After this time the solution was neutralized with Amberlite IR 120 (H^+) resin, filtered, and solvent removed under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed once with aq NaCl (100 mL), dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure. The residue was purified by chromatography (hexane) to furnish **12** as a pale yellow oil (160 mg, 84%). $R_f = 0.73$ (hexane); ^{1}H NMR ($CDCl_3$): δ 2.71 (m, 1H, CH), 1.12–1.63 (m, 28H, $14\times CH_2$), 1.29 (d, 1H, $J = 6.4$ Hz, CHS*H*), 0.82 (app t, 6H, $J = 6.6$, $J = 6.9$ Hz, $2\times CH_3$); ^{13}C NMR ($CDCl_3$): δ 41.2 (CH), 39.0, 31.9, 29.5, 29.4, 29.3, 27.1, 22.7 ($14\times CH_2$), 14.1 ($2\times CH_3$); LRMS (ESI): compound failed to ionize. Anal. Calcd for $C_{17}H_{36}S$: C, 74.92; H, 13.31. Found: C, 74.85; H, 13.56.

3.5. 9-Heptadecyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (14a)

To a solution of 1,2,3,5,6-penta-*O*-benzoyl-α/β-D-galactofuranose[12] (**13a**) (438 mg, 0.63 mmol) in dry CH_2Cl_2 (7 mL) was added tin(IV) chloride (80 μL, 0.68 mmol, 1.09 M equiv) and the solution was stirred for 10 min at 0 °C under N_2. 9-Heptadecanethiol (**12**) (150 mg, 0.55 mmol, 0.87 M equiv) was then added and the reaction mixture stirred for 2 h at 0 °C under N_2. After this time the solvent was removed under reduced pressure. The residue was diluted in CH_2Cl_2 (50 mL) and extracted with satd aq $NaHCO_3$ (100 mL), dried ($MgSO_4$), filtered, and the solvent removed under reduced pressure. The residue was bonded to silica (dissolved in EtOAc and evaporated in the presence of silica) and purified by chromatography (hexane–EtOAc, 6:1) to furnish **14a** (α/β ~ 1:9 by ^{1}H NMR analysis) as a clear syrup (373 mg, 80%, based on **12**). $R_f = 0.52$ (toluene–EtOAc, 20:1); IR (neat): ν 2929, 2856, 1726, 1456, 1284, 1249, 1178, 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$) β-anomer: δ 7.26–8.12 (m, 20H, $4\times CO_2Ph$), 6.10 (m, 1H, H-5), 5.66 (d, 1H, $J_{1,2} = 1.3$ Hz, H-1), 5.65 (br d, 1H, H-3 overlapped by H-1), 5.52 (app t, 1H, $J_{2,3\approx 2,1} = 1.3$ Hz, H-2), 4.82 (app t, 1H, $J = 4.3$, $J = 4.2$ Hz, H-4), 4.73 (m, 2H, H-6 and H-6′), 2.88 (m, 1H, $SCHR_2$), 1.12–1.71 (m, 28H, $14\times CH_2$, heptadecyl chain), 0.85 (m, 6H, $2\times CH_3$); α-anomer (partial spectrum): 5.96 (m, 1H, H-5), 5.87 (dd, 1H, $J_{3,2} = 3.5$, $J_{3,4} = 5.2$ Hz, H-3), 5.79 (dd, 1H, $J_{1,2} = 4.9$, $J_{2,3} = 3.6$ Hz, H-2), 5.66 (H-1 α, overlapped by H-1 and H-3 β); ^{13}C NMR ($CDCl_3$) β-anomer: δ 166.0, 165.7, 165.6, 165.4 ($4\times CO_2Ph$), 133.5, 133.4, 133.2, 133.1, 133.0, 130.0, 130.0, 129.9, 129.6, 129.5, 129.0, 129.0, 128.5, 128.4, 128.3, 128.3 (CO_2Ph), 87.8 (C-1), 86.1 (C-2), 83.1 (C-4), 81.1 (C-3), 70.4 (C-5), 63.5 (C-6), 46.7 ($SCHR_2$), 35.4, 34.8, 31.9, 29.6, 29.5, 29.3, 26.9, 26.7, 22.7 ($14\times CH_2$, heptadecyl chain), 14.1 ($2\times CH_3$); α-anomer: δ 86.2; LRMS (ESI) m/z 873 [$(M+Na)^+$ 100%]; HRMS calcd for $C_{51}H_{62}NaO_9S$: 873.4012; found, 873.3999.

3.6. 9-Heptadecyl 1-thio-β-D-galactofuranoside (3)

To a solution of 9-heptadecyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (**14a**) (α/β ~ 1:9) (310 mg, 0.36 mmol) in dry MeOH (15 mL) was addded NaOMe solution (360 μL, 1 M solution in MeOH). The reaction mixture was stirred for 3 h at rt under N_2. After this time the solution was neutralized with Amberlite IR 120 (H^+) resin, filtered, and solvent removed under reduced pressure. The residue was bonded to silica (dissolved in MeOH and evaporated in the presence of silica) and purified by chromatography (EtOAc) to furnish **3** as a white amorphous solid (131 mg, 82%). $R_f = 0.57$ (EtOAc); $[\alpha]_D^{25}$ −105.3 (c 1, MeOH); ^{1}H NMR (CD_3OD) β-anomer: δ 5.02 (d, 1H, $J_{1,2} = 4.4$ Hz, H-1), 3.98 (dd, 1H, $J_{3,2} = 4.8$, $J_{3,4} = 7.6$ Hz, H-3), 3.84 (dd, 1H, $J_{4,3} = 7.5$, $J_{4,5} = 2.7$ Hz, H-4), 3.82 (app t, 1H, $J = 4.5$, $J = 4.7$ Hz, H-2), 3.67 (dt, 1H, $J_{5,4} = 2.7$, $J_{5,6} = 6.2$ Hz, H-5), 3.53 (m, 2H, H-6 and H-6′), 2.72 (m, 1H, $SCHR_2$), 1.53 [m, 4H, $SCH(CH_2)_2$], 1.15–1.45 (m, 24H, $12\times CH_2$, heptadecyl chain), 0.84 (app t, 6H, $J = 6.4$, $J = 7.0$ Hz, $2\times CH_3$); α-anomer: 5.17 (d, 1H, $J_{1,2} = 3.9$ Hz, H-1); ^{13}C NMR (CD_3OD) β-anomer: δ 90.3 (C-1), 84.4 (C-2), 82.8 (C-4), 78.3 (C-3), 72.1 (C-5), 65.1 (C-6), 47.4 ($SCHR_2$), 36.6, 36.2, 33.1, 30.7, 30.5, 27.8, 23.8 ($14\times CH_2$, hepta-

decyl chain), 14.5 ($2\times CH_3$); LRMS (ESI) *m/z* 457 [$(M+Na)^+$ 100%]; HRMS calcd for $C_{23}H_{46}NaO_5S$: 457.2964; found, 457.2959. Anal. Calcd for $C_{23}H_{46}O_5S$·MeOH: C, 61.76; H, 10.80. Found: C, 61.69; H, 10.89.

3.7. 9-Heptadecyl *S*,*S*-dioxo-(2,3,5,6-tetra-*O*-acetyl)-1-thio-β-D-galactofuranoside (15)

3.7.1. 9-Heptadecyl 2,3,5,6-tetra-*O*-acetyl-1-thio-β-D-galactofuranoside (14b). To a solution of 1,2,3,5,6-penta-*O*-acetyl-D-galactofuranose[14] (**13b**) (1.49 g, 3.82 mmol) in dry CH_2Cl_2 (40 mL) was added $BF_3{\cdot}OEt_2$ (580 μL, 4.58 mmol, 1.2 M equiv) and the solution was stirred for 10 min at 0 °C under N_2. 9-Heptadecanethiol (**12**) (1.04 g, 3.82 mmol) was then added and the reaction mixture stirred for 3 h at rt under N_2. After this time the solvent was removed under reduced pressure. The residue was then diluted in CH_2Cl_2 (50 mL) and extracted with satd aq $NaHCO_3$ (100 mL), dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The residue was purified by chromatography (hexane–EtOAc, 4:1) to furnish **14b** (α/β ~ 1:9 by ^1H NMR analysis) as a clear syrup (1.93 g, 84%). $R_f = 0.59$ (hexane–EtOAc, 4:1); ^1H NMR ($CDCl_3$) β-anomer: δ 5.41 (m, 1H, H-5), 5.32 (m, 1H, H-1), 5.08 (app t, 1H, $J_{2,3\approx2,1} = 2.4$ Hz, H-2), 5.01 (dd, 1H, $J_{3,2} = 2.1$, $J_{3,4} = 6.0$ Hz, H-3), 4.39 (dd, 1H, $J_{4,5} = 3.3$, $J_{4,3} = 6.0$ Hz, H-4), 4.31 (dd, 1H, $J_{6,5} = 4.5$, $J_{6,6'} = 12.0$ Hz, H-6), 4.18 (dd, 1H, $J_{6',5} = 7.2$, $J_{6',6} = 11.7$ Hz, H-6′), 2.78 (m, 1H, $SCHR_2$), 2.14, 2.11, 2.10, 2.05 ($4\times$ s, 4×3H, $4\times OCOCH_3$), 1.21–1.65 (m, 28H, $14\times CH_2$, heptadecyl chain), 0.89 (m, 6H, $2\times CH_3$); α-anomer: 5.33 (H-1 α, overlapped by H-1 β); ^{13}C NMR ($CDCl_3$) β-anomer: δ 170.5, 170.0, 169.7 ($4\times OCOCH_3$), 87.4 (C-1), 82.2 (C-2), 79.1 (C-4), 76.6 (C-3), 69.1 (C-5), 62.7 (C-6), 46.7 ($SCHR_2$), 35.3, 34.7, 31.9, 29.6, 29.5, 29.3, 26.8, 26.7, 22.7 ($14\times CH_2$, heptadecyl chain), 20.8, 20.7 ($4\times OCOCH_3$), 14.1 ($2\times CH_3$); LRMS (ESI) *m/z* 625 [$(M+Na)^+$ 100%].

3.7.2. 9-Heptadecyl *S*,*S*-dioxo-(2,3,5,6-tetra-*O*-acetyl)-1-thio-β-D-galactofuranoside (15). To a solution of 9-heptadecyl 2,3,5,6-tetra-*O*-acetyl-1-thio-β-D-galactofuranoside (**14b**) (401 mg, 0.67 mmol) in dry CH_2Cl_2 (20 mL) was added *m*-CPBA (3 M equiv, 345 mg, 2.00 mmol) and the solution was refluxed for 2 h. After this time the mixture was diluted to 80 mL with CH_2Cl_2 and quenched with satd aq $NaHCO_3$ (80 mL). The organic layer was separated and dried (Na_2SO_4), filtered, and the solvent evaporated under reduced pressure. The residue was purified by chromatography (hexane–EtOAc, 3:1) to furnish **15** as a clear syrup (336 mg, 80%). $R_f = 0.3$ (hexane–EtOAc, 3:1); IR (neat): *ν* 2930, 2857, 1754, 1373, 1310, 1228, 1051 cm^{-1}; ^1H NMR ($CDCl_3$) β-anomer: δ 5.96 (app t, 1H, $J_{1,2\approx2,3} = 3.9$ Hz, H-2), 5.27 (m, 1H, H-5), 5.22 (dd, 1H, $J_{3,4} = 7.4$, $J_{3,2} = 4.1$ Hz, H-3), 5.01 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.62 (dd, 1H, $J_{4,5} = 3.8$, $J_{4,3} = 7.4$ Hz, H-4), 4.32 (dd, 1H, $J_{6,6'} = 11.7$, $J_{6,5} = 5.2$ Hz, H-6), 4.14 (dd, 1H, $J_{6',6} = 11.7$, $J_{6',5} = 6.9$ Hz, H-6′), 3.10 [m, 1H, $S(O)_2CH$], 2.13, 2.10, 2.09, 2.05 ($4\times$ s, 4×3H, $4\times OCOCH_3$), 1.60–2.05 (m, 4H, $2\times CH_2$, heptadecyl chain), 1.19–1.55 (m, 24H, $12\times CH_2$, heptadecyl chain), 0.87 (m, 6H, $2\times CH_3$); ^{13}C NMR ($CDCl_3$) β-anomer: δ 170.3, 170.1, 169.8, 169.0 ($4\times OCOCH_3$), 91.5 (C-1), 81.8 (C-4), 76.1 (C-3), 75.6 (C-2), 68.4 (C-5), 61.9 (C-6), 60.1 ($S(O)_2CH$), 31.8, 29.6, 29.5, 29.3, 29.2, 28.1, 26.7, 26.6, 26.3, 22.6 ($14\times CH_2$, heptadecyl chain), 20.7, 20.7, 20.6 ($4\times OCOCH_3$), 14.1 ($2\times CH_3$); LRMS (ESI) *m/z* 657 [$(M+Na)^+$ 100%]. Anal. Calcd for $C_{31}H_{54}O_{11}S$: C, 58.65; H, 8.57. Found: C, 58.85; H, 8.75.

3.8. 9-Heptadecyl *S*,*S*-dioxo-1-thio-β-D-galactofuranoside (4)

To a solution of 9-heptadecyl *S*,*S*-dioxo-(2,3,5,6-tetra-*O*-acetyl)-1-thio-β-D-galactofuranoside (**15**) (230 mg, 0.36 mmol) in MeOH (20 mL) was added K_2CO_3 solution (5 equiv, 250 mg, 1.8 mmol, dissolved in 0.5 mL water). The reaction mixture was stirred for 15 min at rt. After this time the reaction mixture was filtered through Celite, and the filtrate evaporated under reduced pressure. The residue was purified by chromatography (EtOAc) to furnish **4** as a white amorphous solid (162 mg, 96%). $R_f = 0.59$ (EtOAc); ^1H NMR (CD_3OD) β-anomer: δ 4.82 (d, 1H, $J_{1,2} = 5.3$ Hz, H-1), 4.64 (dd, 1H, $J_{2,3} = 6.2$, $J_{2,1} = 5.3$ Hz, H-2), 4.18 (dd, 1H, $J_{3,4} = 8.8$, $J_{3,2} = 6.2$ Hz, H-3), 4.04 (dd, 1H, $J_{4,5} = 2.6$, $J_{4,3} = 8.8$ Hz, H-4), 3.69 (m, 1H, H-5), 3.59 (m, 2H, H-6 and H-6′), 3.19 (m, 1H, CH), 1.60–2.01 (m, 4H, $SCH(CH_2)_2$), 1.24–1.60 (m, 24H, $12\times CH_2$), 0.90 (app t, 6H, $J = 6.4$, $J = 7.0$ Hz, $2\times CH_3$); ^{13}C NMR (CD_3OD) β-anomer: δ 94.5 (C-1), 84.8 (C-4), 77.9 (C-3), 77.5 (C-2), 71.4 (C-5), 64.4 (C-6), 60.6 [$S(O)_2CHR_2$], 33.0, 30.7, 30.6, 30.4, 30.4, 28.8, 27.6, 27.5, 27.3, 23.8 ($14\times CH_2$), 14.5 ($2\times CH_3$); LRMS (ESI) *m/z* 489 [$(M+Na)^+$ 100%]; HRMS calcd for $C_{23}H_{46}NaO_7S$: 489.2862; found, 489.2855.

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References

1. World Health Organisation, *World Health Report*, 2004.
2. *Multidrug-resistant Tuberculosis*; Bastian, I., Portaels, F., Eds.; Kluwer Academic: Dordrecht, 2000.
3. Stokstad, E. *Science* **2000**, *287*, 2391.
4. Daffe, M.; Brennan, P. J.; McNeil, M. *J. Biol. Chem.* **1990**, *265*, 6734–6743.
5. Owen, D. J.; Davis, C. B.; Hartnell, R. D.; Madge, P. D.; Thomson, R. J.; Chong, A. K. J.; Coppel, R.; von Itzstein, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2274–2277.
6. Witczak, Z. *Curr. Med. Chem.* **1999**, *6*, 165–178.
7. Driguez, H. *ChemBioChem* **2001**, *2*, 311–318.
8. Marino, C.; Marino, K.; Miletti, L.; Alves, M. J. M.; Colli, W.; de Lederkremer, R. M. *Glycobiology* **1998**, *8*, 901–904.
9. Bennett, S.; von Itzstein, M.; Kiefel, M. J. *Carbohydr. Res.* **1994**, *259*, 293–299.
10. Park, W. K. C.; Meunier, S. J.; Zanini, D.; Roy, R. *Carbohydr. Lett.* **1995**, *1*, 179–184.
11. Owen, D. J.; von Itzstein, M. *Carbohydr. Res.* **2000**, *328*, 287–292.
12. D'Accorso, N. B.; Thiel, I. M. E.; Schüller, M. *Carbohydr. Res.* **1983**, *124*, 177–184.
13. Velty, R.; Benvegnu, T.; Gelin, M.; Privat, E.; Plusquellec, D. *Carbohydr. Res.* **1997**, *299*, 7–14.
14. Chittenden, G. J. F. *Carbohydr. Res.* **1972**, *25*, 35–41.
15. Smith, R. V.; Rosazza, J. P. *Biotechnol. Bioeng.* **1975**, *17*, 785–814.
16. Baca, A. M.; Sirawaraporn, R.; Turley, S.; Sirawaraporn, W.; Hol, W. G. J. *J. Mol. Biol.* **2000**, *302*, 1193–1212.
17. Mitscher, L. A.; Baker, W. R. *Pure Appl. Chem.* **1998**, *70*, 365–371.
18. National Committee for Clinical Laboratory Standards (NCCLS), *Susceptibility Testing of Mycobacteria, Nocardiae and Other Aerobic Actinomycetes; Approved Standard*, 2003, Document M24-A.
19. National Committee for Clinical Laboratory Standards (NCCLS), *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*, 2003, Document M7-A6.
20. Alcaide, F.; Pfyffer, G. E.; Telenti, A. *Antimicrob. Agents Chemother.* **1997**, *41*, 2270–2273.