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Synthesis of 2-Deoxy-2-C-Alkylglucosides of *myo*-Inositol as Possible Inhibitors of a *N*-Deacetylase Enzyme in the Biosynthesis of Mycothiol

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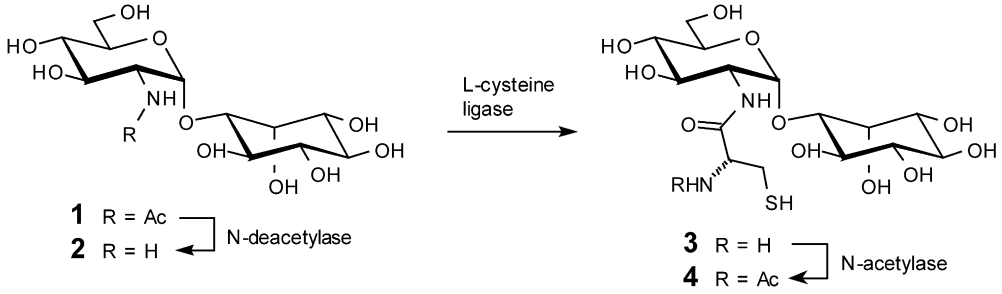
Abstract—Two new analogues of 1-D-1-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-*myo*-inositol, a biosynthetic intermediate in the production of mycothiol in the *Mycobacteria* have been synthesized. Both the 2-deoxy-2-C-(2′-hydroxypropyl)-D-glucoside **5**, and the 2-deoxy-2-C-(2′-oxopropyl)-D-glucoside **6**, are derived from fully benzylated 1-D-1-*O*-(2-C-allyl-2-deoxy)-D-glucopyranosyl)-*myo*-inositol **20**, readily assembled via a protected 2-C-allyl-2-deoxyglucosyl fluoride. Both **5** and **6** inhibit the incorporation of [^{3}H]inositol by whole cells of *Mycobacterium smegmatis* into a number of metabolites which contain inositol.

Mycothiol (1-D-1-*O*-[2-(*N*-acetamido-L-cysteinamido)-2-deoxy-α-D-glucopyranosyl]-*myo*-inositol) (**4**, Scheme 1) is a low-molecular weight thiol, produced only by actinomycetes,[1] and is of significance in that it appears to play an analogous role to glutathione in maintaining a reduced intracellular environment in these Gram-positive bacteria. Given the increasing need for new classes of antituberculars, mycothiol is of considerable contemporary interest, because of the pronounced structural differences between it and glutathione, and the role played by these thiol compounds in the detoxification of alkylating agents and other noxious chemicals.[2] Mycothiol was first isolated as the disulfide from *Streptomyces sp. AJ9463*[3] and as the bimane derivative and free thiol from *M. bovis,* which allowed the demonstration of a mycothioldisulfide reductase activity, by analogy to glutathione reductase.[4] We recently reported the chemical synthesis of **2** and its uptake by enzymes in a fraction from cell-free extracts of *Mycobacterium smegmatis* in the biosynthesis of mycothiol, or



Scheme 1. Biosynthesis of mycothiol.

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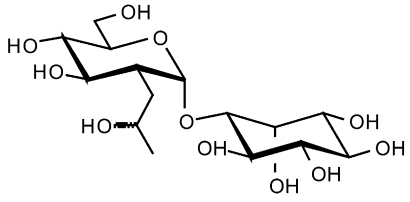
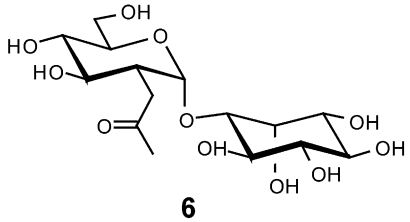
its precursor **3** in the absence of SCoA.[5] A total synthesis of mycothiol as its bimane derivative has also been published recently, providing further proof of structure.[6] The proposed biosynthetic pathway to mycothiol is summarized in Scheme 1.

The identification of key *pseudo*-disaccharide intermediates **1** and **2**[7] in the biosynthesis has led to our exploration of the possibility that analogues **5** and **6**, with modifications at C-2 of the glucosamine unit, may act as inhibitors of the *N*-deacetylase enzyme, thereby preventing the production of mycothiol and the survival of *M. tuberculosis*. We report here on efficient synthetic routes to these analogues, and results of preliminary evaluation of their biological activity.



5



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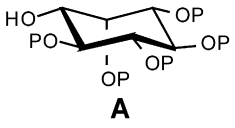
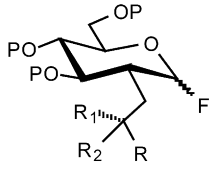
The synthetic approach was based on selection of key building blocks **A** through **D** as our initial targets. It was envisaged that suitably protected *myo*-inositol derivative **A** could be coupled with activated glycosyl donors **B** or **C**, which in turn were derivable from 2-deoxy-2-C-alkenylglucoside **D**.

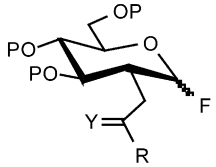


A



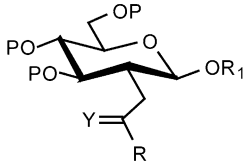
B

R = H, CH_3
R_1, R_2 = H, OR



C

R = H, CH_3
Y = CH_2, O



D

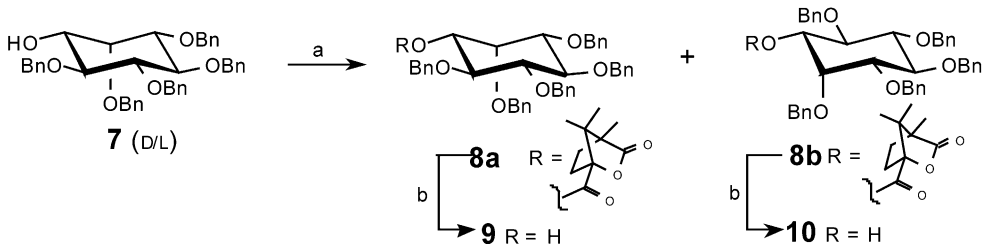
R = H, CH_3
Y = CH_2, O

From the range of reported methods for preparation of the enantiomerically pure derivative of *myo*-inositol[8–10] we opted for resolution of D,L-1,2,4,5,6-penta-*O*-benzyl-*myo*-inositol[10] **7** as its (*S*)-(−)-camphanate ester (Scheme 2). Esterification[11] of alcohol **7** with camphanic acid chloride gave in high yield a separable (SiO_2, CH_2Cl_2/Et_2O, 99:1) mixture of two diastereomers **8a** (mp: 140–144 °C) and **8b** (mp: 162–164 °C). Basic hydrolysis afforded the desired alcohols **9** ($[\alpha]_D$ +7.1 (*c* 1.0, $CHCl_3$) and **10** ($[\alpha]_D$ −8 (*c* 1.0, $CHCl_3$)[12] in excellent yield.

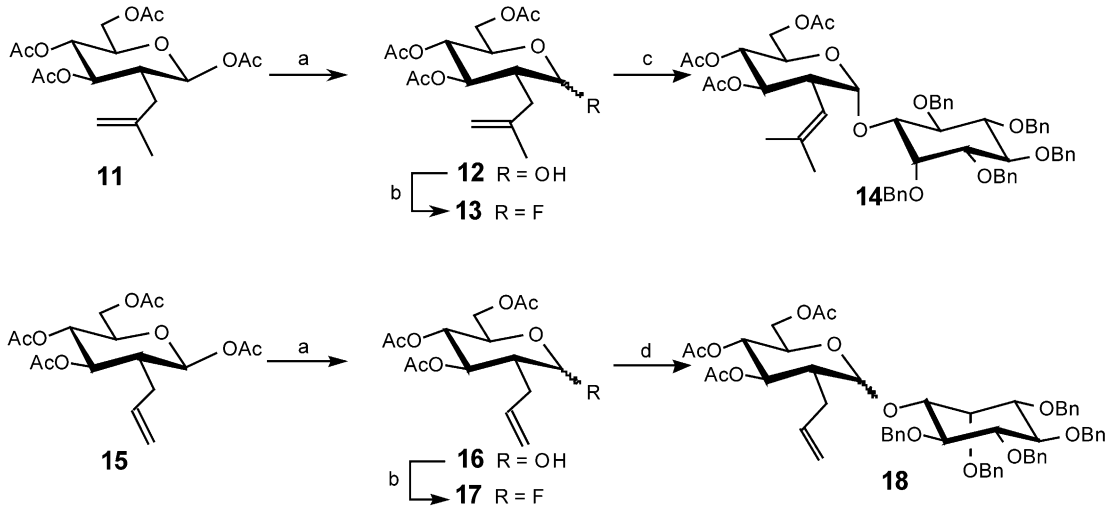
Attention then turned to preparation of suitable glycosyl donors. We reasoned that donors of the type **C** (Y = O) shown above, with a carbonyl group at position 2′ of the side chain would not be suitable because of the potential for participation in the glycosylation reaction leading to β-selectivity. Consequently, 2-C-alkenyl-glycosyl fluorides (**C**, Y = CH_2), obtainable from glycosides **D**, were targeted starting with preparation of 1,3,4,6-tetra-*O*-acetyl-2-C-methallyl-2-deoxy-β-D-glucopyranose **11**[13] (Scheme 3). Selective deacetylation[14] afforded alcohol **12** and subsequent fluorination with DAST[15] gave desired glycosyl donor **13** as a mixture of anomers (α:β = 2:1, 98%). Coupling of donor **13** with L-inositol acceptor **10** in the presence of BF_3·etherate[16] gave *pseudo*-disaccharide **14** in 73% yield with desired α-selectivity but undesired isomerisation of the side chain double bond.

The failure to obtain the 2-C-methallylglucoside prompted a modified approach utilizing the known 2-deoxy-2-C-allylglucoside **15**[17b] which was converted as before via anomeric deacetylation and fluorination to the glycosyl fluoride **17** (Scheme 3). Treatment of acceptor **9** with donor **17** and BF_3·etherate as promotor gave *pseudo*-disaccharide **18** as an inseparable mixture of anomers (α:β = 8:1 from NMR), and in an approximate yield of 60% after removal of unreacted acceptor. Zemplén deacetylation of **18** allowed for separation and recovery of α-glycoside **19** by careful silica column chromatography in 36% overall yield from inositol acceptor **9** (Scheme 4). Benzylation of **19** gave fully protected pseudo-disaccharide **20** (94%, $[\alpha]_D$ +39.4 (*c* 2.7, $CHCl_3$), *m*/*z* (FABMS) 1171.3 ($C_{71}H_{74}O_{10}$.^{85}Rb requires *m*/*z* 1171.4). Evidence for the α-glycosidic bond included a signal at δ 5.09 (d, *J* = 3.3 Hz) for H-1 in the 300 MHz ^{1}H NMR spectrum, and a signal at δ 94.92 for C-1 in the ^{13}C NMR spectrum. Diagnostic signals for the allyl group were also clearly identified in both the ^{1}H and ^{13}C NMR spectra.

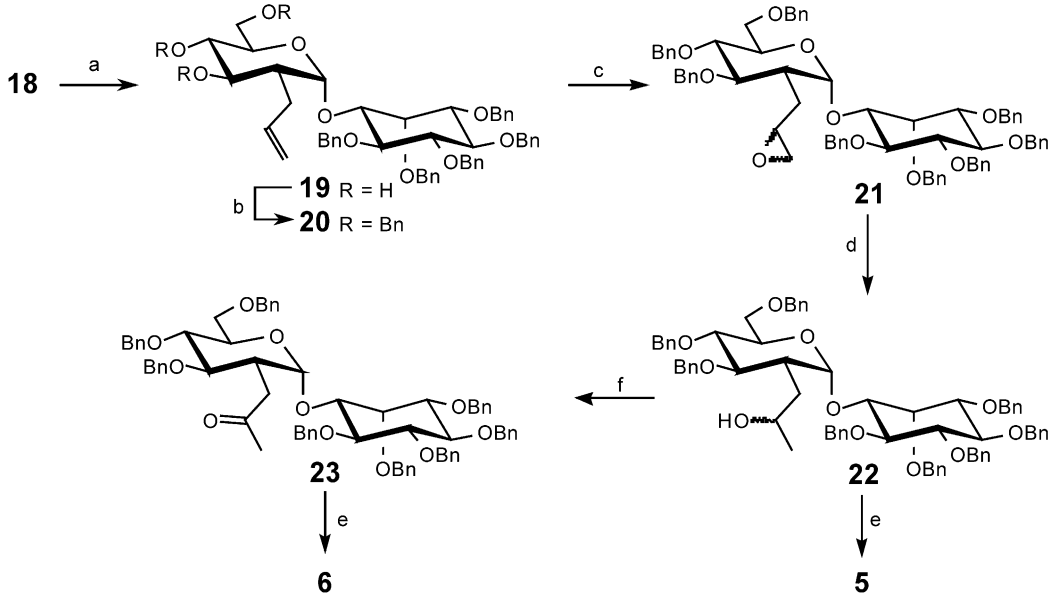
The most practical route to the oxygenated alkyl side-chains proved to be via epoxidation of the olefin followed by reduction to the secondary alcohol and oxidation as required (Scheme 4). Treatment of **20** with MCPBA[18] proceeded slowly and completion of reaction was ensured by periodic addition of further equivalents of MCPBA over 24 h to give a mixture (1:1) of inseparable diastereoisomers **21**. Regioselective reductive opening[19] of the epoxide **21** with $LiAlH_4$ in THF gave secondary alcohols **22**, again inseparable, in an acceptable yield. Global debenzylation afforded the target 2-deoxy-2-C-(2′-hydroxypropyl)-D-glucoside **5** as a solid; *m*/*z* (FABMS) 384.1 ($C_{15}H_{28}O_{11}$ requires *m*/*z* 384.4); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.11 (H-1), 4.24 (H-2′), 1.20 (CH_3); δ_C (400 MHz; $CDCl_3$; Me_4Si) 95.46 (C-1), 22.50 (C-CH_3).



Scheme 2. Reactions and conditions: (a) Camphanic acid chloride (1.2 equiv), Et_3N, DMAP, CH_2Cl_2, rt, 24 h, 99%; (b) KOH, EtOH, reflux, 2 h, 94%.



Scheme 3. Reactions and conditions: (a) Hydrazine acetate (2 equiv), DMF, 60 °C, 30 min, 98% (**12**), 92% (**16**); (b) DAST (3 equiv), THF, rt, 30 min, 90% (α:β = 2:1); (c) **10** (1 equiv), **13** (1.4 eq), $BF_3{\cdot}Et_2O$ (5 equiv), 4 Å ms, rt, 2 h, 73% (α:β > 99:1); (d) **9** (1 equiv), **17** (1.6 equiv), $BF_3{\cdot}Et_2O$ (5 equiv), 4Å ms, rt, 2 h, (α:β = 8:1).



Scheme 4. Reactions and conditions: (a) NaOMe, MeOH–CH_2Cl_2, rt, 1 h, 36% from **9**; (b) NaH, BnBr, THF, reflux, 20 h, 94%; (c) MCPBA (70%) (6 equiv), CH_2Cl_2, rt, 24 h, 83% (1:1);. (d) LAH (10 equiv), THF, 0 °C, 1 h, 82% (1:1); (e) H_2, 10% Pd/C, EtOAc–MeOH, rt, 3 days, >90%; (f) TPAP (cat), NMO (1.5 equiv), CH_2Cl_2, ms, rt, 25 min, 90%.

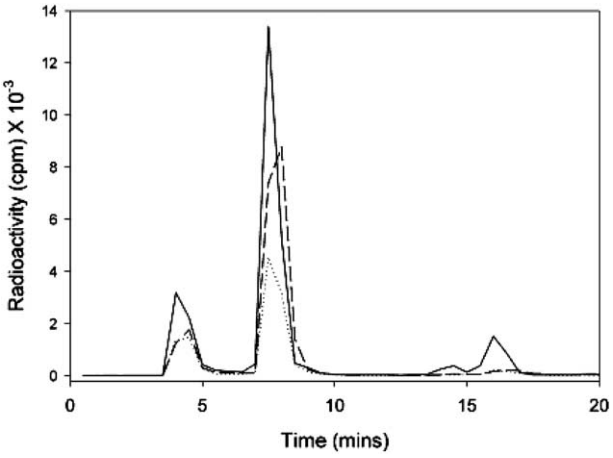


Figure 1. Effect of **5** on the incorporation of [^{3}H]inositol into acid soluble metabolites of *Mycobacterium smegmatis*: *M. smegmatis* was grown in Middle brook medium containing 5% glycerol. [^{3}H]Inositol was added when the culture reached an absorbance of 0.44 at 600 nm. Cells were incubated for 3.5 h in the presence of two different amounts of **5** and was then harvested and sonicated in 0.25M $HClO_4$ to obtain the acid soluble fraction, which was chromatographed on a Vydac 201HS54 octadecilesilane column. Following injection of the sample the column was eluted for 5 min at 100%A, then for 25 mins with a linear gradient to 70%B and for 5 min with a linear gradient to 100%B (A: 0.1% TFA, B: 0.1%TFA in a 6:4 (v/v) mixture of acetonitrile and water. The flow rate was 0.8 mL/min and 0.5 min fractions were collected and counted. The traces represent a radiolabel recovered in the eluate for a control culture lacking inhibitor (solid line), and for cultures labeled in the presence of 20 μg/mL (Broken line) or 200 μg/mL (dotted line) **5**.

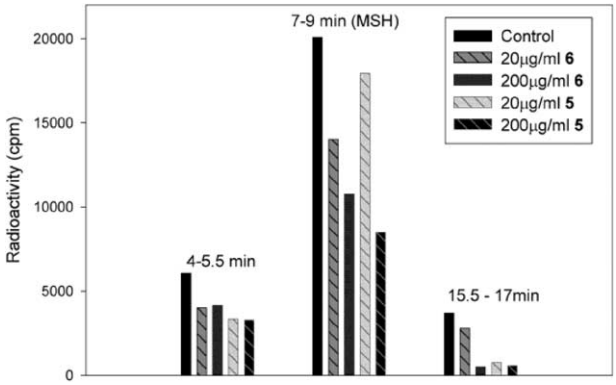


Figure 2. Comparison of the radiolabel recovered in the three major HPLC peaks after incubation of *M. smegmatis* in the presence of two different levels of **5** and **6**. (MSH; mycothiol.)

Oxidation of **22** with TPAP using NMO as co-oxidant[20] gave ketone **23** in excellent yield. ($[\alpha]_D$ +38.5 (*c* 2.2, $CHCl_3$); ν_{max}/cm^{-1} 1712 (C=O); δ_H (400 MHz; $CDCl_3$; Me_4Si) 5.28 (d, $J=3.6$, H-1), 4.17 (H-2′), 1.62 (CH_3), δ_C (400 MHz; $CDCl_3$; Me_4Si) 207.78 (CH_2COCH_3), 94.26 (C-1), 29.68 (CH_3)). Global deprotection gave the target 2-deoxy-2-C-(2′-oxopropyl)-D-glucoside **6** as a solid. (δ_H (300 MHz; D_2O; dioxane) 4.84 (d, $J=3.3$, H-1), 3.87 (t, $J=2.5$, H-2′), 2.02 (CH_3), δ_C 214.47 (C=O), 94.96 (C-1), 29.48 (CH_3).)

Only limited quantities of **5** and **6** were available for biological tests, but preliminary results suggest that these compounds are biologically active. While compounds **5** and **6** did not inhibit the growth of *Mycobacterium smegmatis* in vitro, a pronounced inhibition of incorporation of [^{3}H]inositol by whole cells into inositol containing metabolites was observed (Fig. 1). While each of the major radiolabeled peaks in Fig. 1 probably comprise a mixture of [^{3}H]inositol labeled compounds the peak eluted at 4–5.5 min was found to contain inositol monophosphate as determined by coelution with an authentic standard on a Partisil SAX10 anion exchange column, while the peak eluted at 7–9 min contain mycothiol as determined by reaction with sulfhydryl reagents and coelution with authentic mycothiol as the 7-diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin derivative. The [^{3}H]inositol labeled compound(s) eluted at 15.5–17 min have not as yet been characterised, but incorporation of radiolabel into this fraction was maximally inhibited at 20 μg/mL of **5** (Fig. 2). At this level of the inhibitor the transport of radiolabel into the organisms was unaffected as compared to a control which lacked inhibitors. It is evident, therefore, that **5** and **6** inhibit the incorporation of [^{3}H]inositol into a number of metabolites which contain inositol. The identification of these metabolites, more detailed enzymological studies and an evaluation of the effects of **5** and **6** on the susceptibility of mycobacteria to various stresses will be of much interest. In addition, continuing work will exploit the availability of crucial synthetic intermediates **20** and **21** which provide a platform for the synthesis of a variety of other carba-analogues of mycothiol and its biosynthetic precursors.

Acknowledgements

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