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Synthesis and biological evaluation of new inhibitors of UDP-Gal*f* transferase—a key enzyme in *M. tuberculosis* cell wall biosynthesis†

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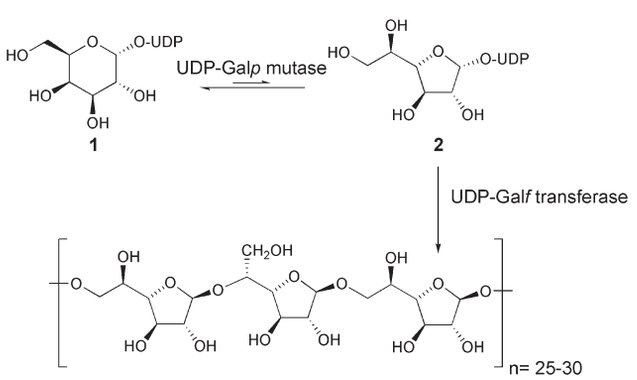
Two iminosugars have been designed and synthesized as potential inhibitors of UDP-Gal*f* transferase, an enzyme involved in *Mycobacterium tuberculosis* cell wall biosynthesis. The design is based on a proposed model of the transition state for the transferase reaction. One of the two racemic compounds is the first reported inhibitor of the target enzyme from *M. smegmatis*.

Today, one person dies every 10 seconds from tuberculosis (TB). The World Health Organization (WHO) has estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*, the causative agent of TB, and it has predicted that by 2020 one billion people will be newly infected if new anti-TB treatments are not developed.[1] The cell wall of mycobacteria is essential for cell growth and survival in the host,[2] and the importance of the cell wall integrity is confirmed by the effectiveness of treatments that disrupt cell wall biosynthesis (for example, isoniazid and ethambutol). D-Galactans are key components of the mycobacterial cell wall,[3] and since their main constituents (D-galactofuranose residues) are not found in mammalian metabolism, their biosynthesis constitutes a very attractive and accessible target for new anti-TB drugs without any deleterious side effects. The biosynthesis of these alternating β-1,5 and β-1,6 galactofuranosyl polymers involves two specific enzymes (Scheme 1): UDP-galactopyranose mutase (UDP-Gal*p* mutase EC 5.4.99.9)[4] catalyzing the interconversion of UDP-α-D-galactopyranose **1** (UDP-Gal*p*) into UDP-α-D-galactofuranose **2** (UDP-Gal*f*), and UDP-galactofuranosyl transferase (UDP-Gal*f* transferase) catalyzing the transfer of UDP-Gal*f* (donor) onto a growing oligosaccharide chain (acceptor).



Scheme 1 Mycobacterial galactan biosynthesis.

Unlike the mutase, which has been extensively studied,[5] little information is available on the transferase. To date, no inhibitor of this enzyme has been identified, and little mechanistic and structural information is available. It has recently been shown that UDP-Gal*f* transferase is a bifunctional enzyme capable of catalyzing both β-1,5 and β-1,6 linkages in *M. tuberculosis* (gene product of *Rv3808c*).[6]

Iminosugars—analogues of monosaccharides where the ring oxygen or the anomeric carbon has been replaced by a nitrogen—constitute a class of promising inhibitors of carbohydrate enzymes. In particular, they have been demonstrated to be potent inhibitors of glycosidases and glycosyltransferases.[7] We were interested in using iminosugars as potential inhibitors of UDP-Gal*f* transferase. Fleet and coworkers studied the inhibitory properties of compounds **4**, **5** and **6** (Fig. 1) against mycobacterial galactan biosynthesis, and concluded that **4** and **5** inhibited the biosynthesis, probably by their effect on UDP-Gal*p* mutase.[8]

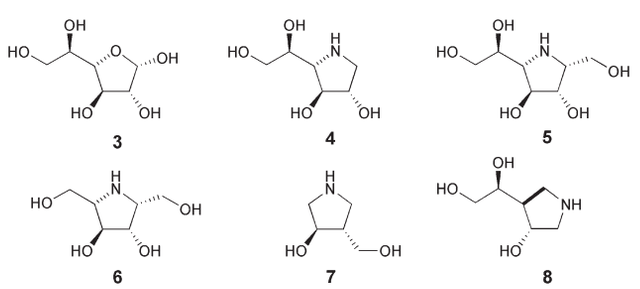


Fig. 1

Other examples of applications of iminosugars include inhibition of purine nucleoside phosphorylase by compound **7**,[9] and use of iminosugar **8** as part of a DNA-intercalating agent.[10]

Based on other glycosyltransferases studies,[11] we proposed a possible transition state structure for the transferase reaction (Fig. 2). According to this model, the anomeric carbon and the ring oxygen present a partial positive charge. Thus, there are two different approaches to mimic the transition state with iminosugars. The ring oxygen of Gal*f* (galactofuranose **3**) can be replaced by a nitrogen atom, which by protonation could mimic the oxocarbenium intermediate. Iminosugar **4** has been synthesised and tested by Fleet *et al.*; they concluded that it was inhibiting the mutase, and not the transferase.[8] Alternatively, the anomeric carbon could be replaced

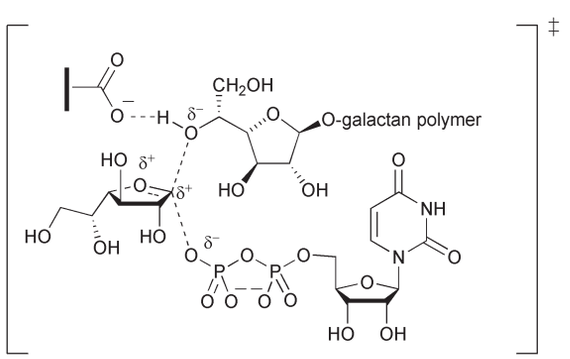


Fig. 2 Proposed transition state for UDP-Gal*f* transferase reaction.

† Electronic supplementary information (ESI) available: Experimental details. See http://www.rsc.org/suppdata/ob/b4/b411554f/

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by a nitrogen and the ring oxygen by a methylene group to give a 1-*N*-iminosugar. We believe that this type of iminosugar could be a more effective inhibitor of the transferase. For stability reasons, our target molecule is lacking the hydroxyl at C-2 as we anticipated that the cyclic hemiaminal **9** would be unstable. Therefore, we proposed (3*S*,4*R*)-4-[(1*S*)-1,2-dihydroxyethyl]pyrrolidin-3-ol **10** as a stable inhibitor of UDP-Gal*f* transferase (Fig. 3).

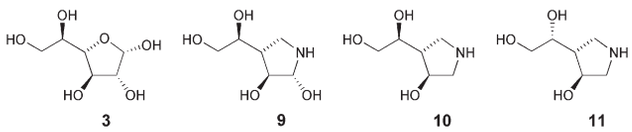
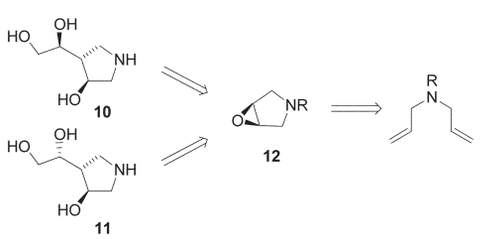


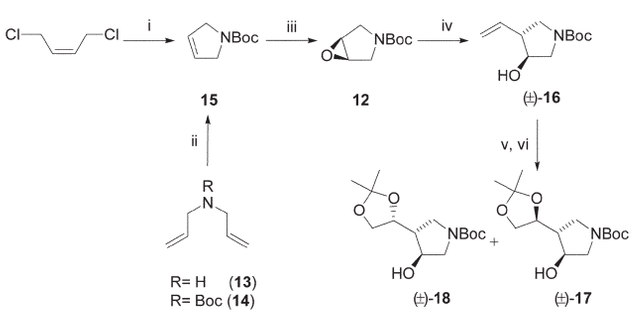
Fig. 3

In this paper, we report a rapid and efficient synthesis of racemic compound **10** and its diastereoisomer **11**. Our retrosynthetic strategy is highlighted in Scheme 2. During the course of our research, Pedersen and coworkers reported the synthesis of (3*R*,4*S*)-4-[(1*S*)-1,2-dihydroxyethyl]pyrrolidin-3-ol **8** and investigated its use as an intercalator pseudonucleotide.[10] Their synthesis was based on the reductive amination of a derivative of L-xylose with iminosugar **8** being obtained after 9 steps in 16% overall yield.



Scheme 2 Retrosynthesis of potential inhibitors **10** and **11**.

Our retrosynthetic strategy differs from the classical approach of using sugar derivatives as starting materials and instead uses 3,4-epoxypyrrolidine. Hansen and Bols reported the use of 3,4-epoxypyrrolidine **12** (with R = Boc) as the precursor for racemic 4-alkylpyrrolidin-3-ols.[9] Since we could have easy access to this type of epoxide, we decided to take advantage of this method. Thus the key *meso* epoxide **12** was prepared from *N*-Boc-diallylamine **14** over 2 steps involving ring closing metathesis (RCM)[12] and epoxidation (Scheme 3). Epoxide opening using vinylmagnesium chloride and CuBr·Me_2S in THF gave almost exclusively the desired *trans* product **16** compared to a 9 : 1 mixture of *trans* : *cis* products reported by Hansen and Bols when using Me_2S as the solvent.



Scheme 3 *Reagents and conditions*: i, $BocNH_2$, NaH, DMF, 57%; ii, $Cl_2Ru(PCy_3)_2$, CH_2Cl_2, 75%; iii, *m*-CPBA, CH_2Cl_2, 0 °C, 86%; iv, $CH_2CHMgCl$, CuBr·Me_2S, THF, 60%; v, AD-Mix β, ^{t}BuOH, H_2O, 95%; vi, 2,2-DMP, *p*-TsOH, CH_2Cl_2.

In order to introduce the stereogenic center at C-7, we used Sharpless dihydroxylation conditions (with commercially available AD-Mix). AD-Mix β gave an inseparable mixture of diastereomers in a ratio of 2 : 1. Conversion of the 1,2-diols into the corresponding 1,2-acetonides allowed separation of the mixture by column chromatography on silica. Determination of the relative stereochemistry of the more polar, crystalline product **17** proved possible by X-ray crystallographic analysis (Fig. 4).‡

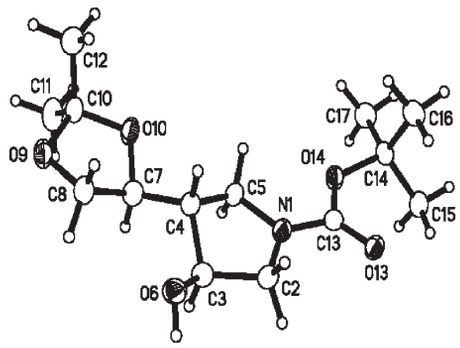
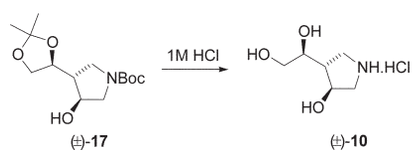


Fig. 4 ORTEP plot of **17**.

The final products were obtained by removal of the protecting groups in a single step using 1 M hydrochloric acid (Scheme 4). 1-*N*-Iminosugars **10** and **11** were purified by C18 silica-gel chromatography (20% MeOH in H_2O), and obtained in 24 and 13% overall yield respectively. They gave satisfactory HRMS and NMR data as reported in the electronic supplementary data.



Scheme 4

The racemic compounds were tested against mycobacterial galactan biosynthesis (*M. smegmatis*)[13] and compound **11** showed moderate activity (IC_{50} = 4.8 mM, Fig. 5),[14] similar to that reported for other iminosugars with glycosyltransferases.[15] Unexpectedly, the compound with the same relative stereochemistry as the natural substrate **10** was not as active (40% inhibition at 8 mM).

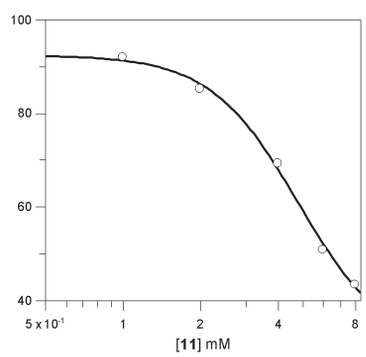


Fig. 5 Determination of the IC_{50} value for **11** (IC_{50} = 4.8 mM). The curve drawn is the best fit for the data to the 4-parameter equation.[17]

Given that UDP-Gal*f* transferase is able to catalyze the formation of both β-1,5 and β-1,6 glycoside linkages, it would suggest that there is some flexibility in this region of the active site that could account for this observation. We found that compounds **10** and **11** did not show significant inhibitory activity against UDP-Gal*p* mutase (*M. smegmatis*) using the formaldehyde release assay described by McNeil *et al.*[16] This might suggest that the development of a significant amount of positive charge at the anomeric carbon does not occur in the UDP-Gal*p* mutase catalyzed reaction, in accordance with the recently proposed mechanism involving a covalent flavin–galactose intermediate.[5d,f] An enantioselective version of our synthesis is currently being developed and will be reported in due course. Compounds **10** and **11** are also being elaborated to incorporate UDP and acceptor mimics. It is anticipated that these should result in more potent inhibitors of UDP-Gal*f* transferase.

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Notes and references

‡ Crystal data. $C_{14}H_{25}NO_5$, $M = 287.35$, monoclinic, $a = 14.668(2)$, $b = 5.7076(8)$, $c = 18.373(3)$ Å, $\beta = 95.266(3)°$, $U = 1531.7(7)$ Å^3, $T = 150$ K, space group $P2_1/n$ (alt. $P2_1/c$, no. 14), $Z = 4$, μ(Mo-Kα) = 0.094 mm^{-1}, 9434 reflections measured, 3464 unique ($R_{int} = 0.024$) which were used in all calculations. The final R_1 [2821 $F > 4\sigma(F)$] = 0.0396, $wR(F^2)$ [all data] was 0.113. CCDC reference numbers 241487. See http://www.rsc.org/suppdata/ob/b4/b411554f/ for crystallographic data in .cif or other electronic format.

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