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Synthesis of a Carbasugar Analogue of a Putative Intermediate in the UDP-Gal*p*-Mutase Catalyzed Isomerization

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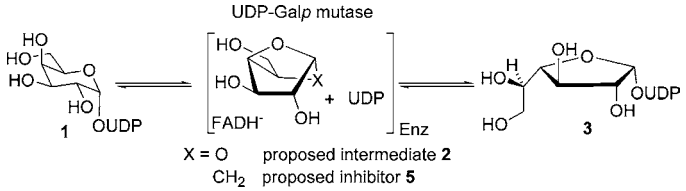
ABSTRACT



The synthesis of the carbasugar analogue of 1,4-*anhydro*-β-D-galactopyranose, a proposed intermediate in the reaction catalyzed by uridine diphosphate-α-D-Gal*p* mutase, in racemic form via Diels−Alder and Barton decarboxylation chemistry is reported. This compound was found not to inhibit the mutase from *Mycobacterium tuberculosis*, indicating that the enzyme does not possess a 1,4-*anhydro*-β-D-galactopyranose binding pocket.

Mycobacteria are pathogens responsible for several human diseases, including tuberculosis (TB) and leprosy.[1] Annually, TB is currently responsible for ~3 million deaths worldwide, and there is therefore an urgent need for effective new anti-TB drugs.[2] Uridine diphosphate-α-D-galactopyranose (UDP-Gal*p*) mutase (E.C. 5.4.99.9) is a flavoenzyme that catalyzes the interconversion of UDP-α-D-galactopyranose (UDP-Gal*p*) **1** and UDP-α-D-galactofuranose (UDP-Gal*f*) **3**. The latter is an essential biosynthetic precursor of the cell wall arabinogalactan component of *Mycobacterium tuberculosis*. It has been demonstrated that the action of this enzyme is essential for the organism's viability.[3] Galactofuranose is also found in nemotodes, algae, and fungi, including *Aspergillus*.[4] Its absence in mammals makes UDP-Gal*p* mutase an attractive target for anti-tuberculosis agents. The catalytic mechanism for this intriguing enzyme has not been fully elucidated despite a number of studies having been conducted.[5] On the basis of results obtained from positional isotope exchange studies, Barlow et al.[5a] proposed a mechanism that involved 1,4-*anhydro*-β-D-galactopyranose **2** as a putative intermediate in the reaction (Scheme 1).

This led Kovensky and Sinaÿ[6] to demonstrate that 1,4-*anhydro*-2,3,6-*tri*-*O*-benzyl-β-D-galactopyranose could be regioselectively ring-opened under acidic conditions using a variety of alcohols. This nonenzymatic study generated only furanoside product with the formation of predominately the

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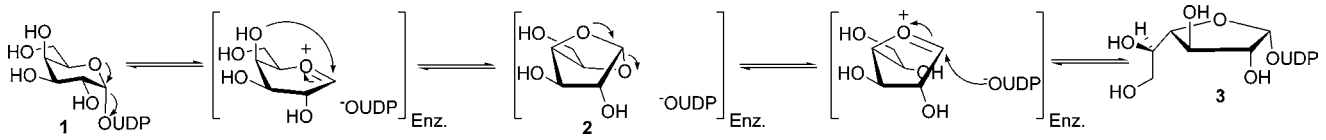
Scheme 1. Proposed Mechanism of Action for UDP-Gal*p* Mutase[5a]



β-anomer. More recently, Sinaÿ et al. reported that a UDP-*C*-β-D-1,4-*anhydro*galactopyranose **4** with the galactose moiety locked in a bicyclic $^{1,4}B$ boat conformation was a weak competitive inhibitor (91% inhibition at 1 mM) of the mutase from *E. coli* and suggested that this was good evidence for the validity of Scheme 1.[7] We have identified **5**, the carbasugar analogue of 1,4-*anhydro*-β-D-galactopyranose **2**, as a simple, stable compound that could also test the hypothesis of **2** being a low-energy enzyme-bound intermediate in the mutase-catalyzed reaction. Compound **5** could be used to dissect the contributions to binding made by a bicyclic $^{1,4}B$ boat conformation of the galactopyranose portion of **4** in the absence of UDP. Evidence for **2** being a valid intermediate in the enzyme-catalyzed reaction, as suggested by Scheme 1, will be provided by determining if **5** behaves as a competitive inhibitor of UDP-Gal*p* mutase. Here, we report the synthesis and biological testing of this compound in racemic form.

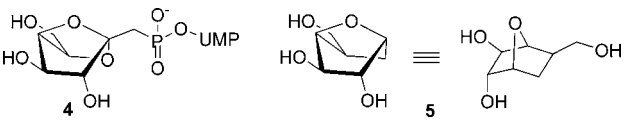
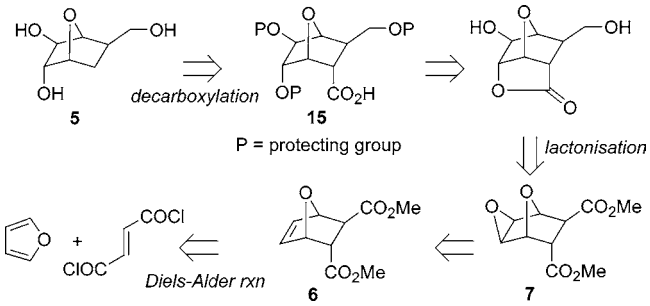


Figure 1.

The 7-oxabicyclo[2.2.1]heptane skeleton was accessible via a Diels−Alder reaction of excess furan and methyl acrylate in the presence of zinc iodide at 40 °C.[8a] After 3 days, this gave 15% of the desired *exo*-isomer as the predominate product (2:1 *exo*:*endo*). Separation of this diastereoisomer from the *endo*-form was achieved using SiO_2 flash chromatography, albeit with some decomposition on the column. A sufficient quantity of the *exo*-isomer was generated for epoxidation with *m*-CPBA to give methyl (±)-3,8-dioxatricyclo[3.2.1.02,4]octane-6-carboxylate.[8b] However, despite investigating both nucleophiles (i.e., KOH in DMSO/H_2O) and protic acids (i.e., $HClO_4$ in hexafluoro-2-propanol), it was not possible to selectively open the epoxide without decomposition of the oxanorbornane system. Therefore, this approach was abandoned.

Kunstmann et al. have previously shown that a hydroxymethyl or carboxylate group in the 3-*endo* position could perform an intramolecular 5-*exo*-tet reaction on the 5,6-*exo*-epoxide to produce an *exo*-hydroxylactone.[9] On the basis of this literature precedent, we designed the retrosynthesis shown in Scheme 2. This route would allow rapid access to

Scheme 2



the correct diastereoisomer of our target molecule with the opportunity to resolve the Diels−Alder adduct **6** using pig liver esterase[10] to produce the desired carbasugar as a single enantiomer.

The Diels−Alder reaction to prepare the desired *trans* diester was investigated with both dimethyl fumarate and fumaryl dichloride dienophiles, and with and without Lewis acid catalysts (zinc iodide; boron trifluoride). The best yields were obtained by mixing fumaryl dichloride and furan together stoichiometrically for 30 min at 0 °C to give a pale yellow solid that was then added portionwise to a solution of anhydrous methanol and triethylamine in diethyl ether at 10 °C. This gave the desired *trans*-dimethyl ester **6** as a very pale yellow crystalline solid in quantitative yield (Scheme 3). Compound **6** was converted into its *exo*-epoxide using a freshly distilled solution of dimethyl dioxirane (DMDO) in acetone.[11]

The DMDO solution was added at 0 °C, and the reaction was left for 16 h at room temperature before the solvent was removed and the residual solid purified by flash chromatography on neutral alumina to give the *exo*-epoxide **7** as a white solid in 80% yield. Treatment of this solid with aqueous sodium hydroxide solution (10% w/v) at 0 °C produced a yellow solution after stirring overnight at room temperature. This was then cooled to 0 °C and neutralized

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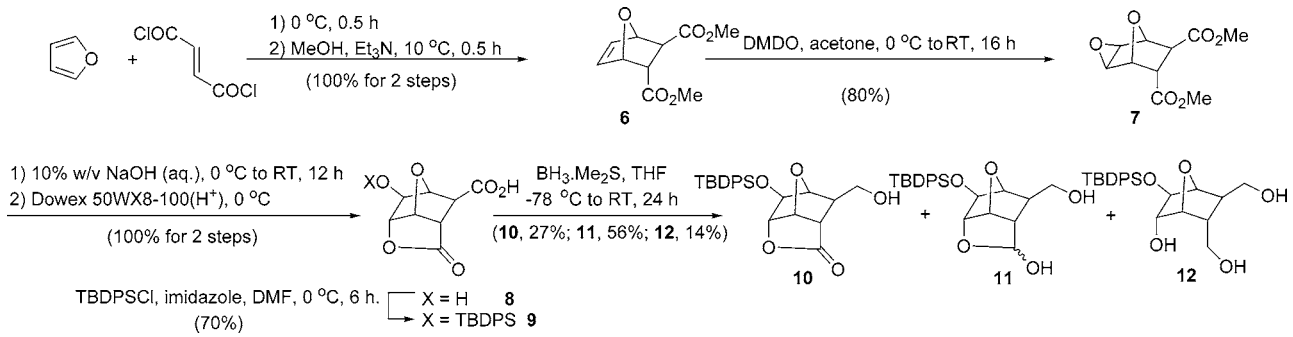
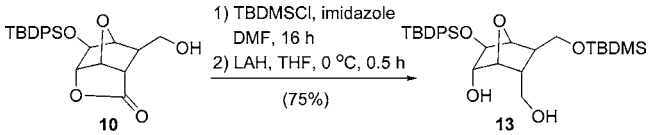
Scheme 3



with Dowex 50WX8-100 (H^+-form) resin. The desired lactone **8** was isolated in quantitative yield by filtering off the ion-exchange resin and was then purified by recrystallizing from warm acetone. The 2-hydroxyl of **8** was then selectively protected in the presence of the carboxylate using *tert*-butyldiphenyl silyl chloride and imidazole in anhydrous DMF followed by treatment with a solution of potassium carbonate in MeOH/THF (1:5 v/v) to give **9** in 70% yield. The carboxylate of **9** was reduced in the presence of the lactone using borane-dimethyl sulfide in THF. Using 1.3 equiv of the borane, 80% of the lactone **10** was produced. The free hydroxyl group of **10** was protected as its *tert*-butyldimethylsilyl ether prior to reduction of the lactone functionality to give diol **13** using LAH in THF (Scheme 4).

Scheme 4



However, we were then unable to selectively protect the secondary alcohol in **13**, in the presence of the primary one using a variety of standard protecting groups. Therefore, an alternative route via lactol **11** was explored. This was prepared by treating **9** with excess borane−dimethyl sulfide complex in THF to give three products, lactone **10**, lactol **11**, and triol **12**. Lactol **11** was obtained as a white foam in 56% yield and was separated from the lactone and triol byproducts by SiO_2 flash chromatography. The lactol was then converted into its hydrazone using *N*,*N*-dimethylhydrazine and *p*-TsOH (Scheme 5). The hydrazone diol was reacted without purification with acetic anhydride, triethylamine, and DMAP to give the hydrazone diacetate **14**. This could be purified by SiO_2 flash chromatography to give the desired product as an oil in 90% yield over the two steps. Some decomposition of the hydrazone to the aldehyde and dimethylhydrazine was observed during chromatography.

With all of the required hydroxyl groups protected, the hydrazone was quantitatively converted back to the aldehyde by stirring with copper(II) chloride in sodium phosphate buffer (1.0 M, pH 7.0)/THF for 12 h at room temperature. The aldehyde **15** was oxidized to its carboxylic acid using pyridinium dichromate in anhydrous DMF to give **16** as a colorless oil in 95% yield.

Barton decarboxylation of **16** was achieved following the procedure of Eaton et al.[12] Oxalyl chloride was used to convert the free carboxylic acid into its acid chloride in DCM. Following removal of the solvent, the residual solid was taken up in anhydrous benzene and added dropwise to a refluxing suspension of 2-mercaptopyridine *N*-oxide, DMAP, and *tert*-butyl thiol in anhydrous benzene that was irradiated with a 300 W tungsten lamp (Osram Wotan vita-lux). The decarboxylation proceeded efficiently to give **17** as an oil in 84% yield after purification by column chromatography on silica. The target molecule **5** was obtained by treatment of **17** first with TBAF in THF to remove the silyl protecting group. This generated a mixture of two products **18** and **19** in 95% overall yield. These proved to be the desired diacetate **18** and its isomer **19** in which the acetyl group had migrated from the *endo*- to the *exo*-hydroxyl. This was proven by treating a portion of this mixture with acetic acid, DMAP, and triethylamine to give the peracetylated compound **20** as the only product. The remainder of the residue from the TBAF reaction was treated with a methanolic solution of potassium carbonate for 14 h at room temperature.

The solution was neutralized with Dowex 50WX8-100 resin (H^+-form) and was then lyophilized to give **5** as a white powder. The overall yield of this route was ~25% over 11 steps. The 7-oxanorbornane core of **5** has previously been shown to be stable under physiological conditions and is found in a number of natural products and their analogues, including protein phosphatase inhibitors based on norcantharidin,[13] as well as being exploited extensively in the "naked" sugar methodology developed by Vogel.[14] Oxa-ring opening of **5** to give new carbasugar derivatives[14] as glycosidase inhibitors is currently under investigation.

(±)-6-Hydroxymethyl-7-oxa-bicyclo[2.2.1]heptan-2-*exo*, 3-*endo*-diol **5** was tested against purified UDP-Gal*p* mutase

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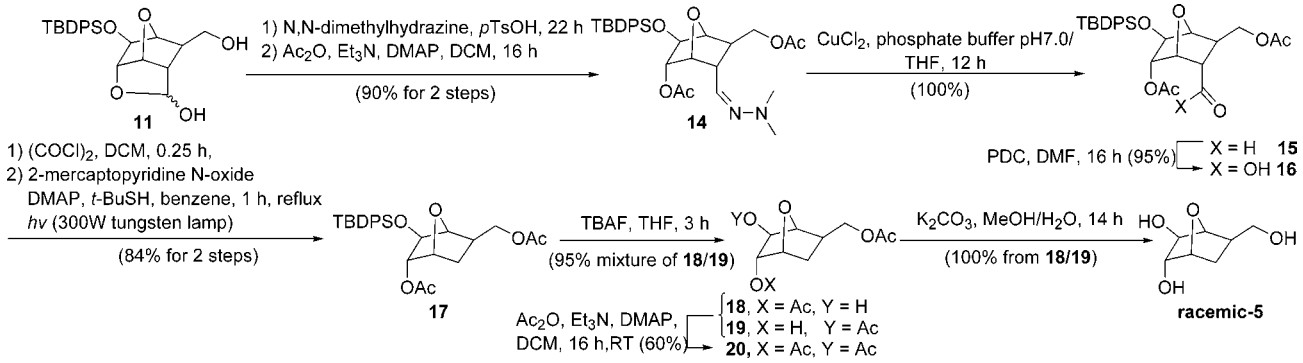
Scheme 5



from *Mycobacterium tuberculosis* in the reverse direction in which UDP-Gal*f* is converted to UDP-Gal*p* using an HPLC method developed by Lee and co-workers.[15] At a concentration of 100 μM, **5** was found to not inhibit the enzyme. The failure of **5** to competitively inhibit UDP-Gal*p* mutase provides evidence that the enzyme does not have a binding pocket with a high affinity for 1,4-*anhydro*-β-D-galactopyranose **2**. On the basis of Pauling's theory of enzymes operating by transition-state stabilization,[16] it would be expected that the mutase would have a higher affinity for molecules resembling the transition states involved in the formation of putative intermediate **2**, such as compound **5**. The lack of affinity of **5** for the mutase can therefore be interpreted as evidence that **2** is not an intermediate in the UDP-Gal*p* mutase catalyzed reaction, as proposed in Scheme 1.[5a]

A comparison of the binding affinities of UDP-Gal*f* (K_m = 22 μM),[7a] UDP-Gal*p* (K_m = 600 μM),[17] and UDP (K_i = 37 μM)[18] for the UDP-Gal*p* mutase from *Klebsiella pneumoniae* (in reduced form) indicates that most of the substrate binding affinity of the enzyme is directed toward the UDP portion of these molecules. This explains why compound **4** is a competitive inhibitor of UDP-Gal*p* mutase as it retains the UDP "handle" that dominates its binding. The contribution of the appended mimic of D-galactose locked in a bicyclic $^{1,4}B$ boat conformation is inconsequential, as reflected by the dissection of the interactions into those provided by compound **5** and UDP. While it may have been possible that the tether between the UDP and 1,4-*anhydro*-β-D-galactopyranose mimic in **4** was not optimal for it to occupy a putative 1,4-*anhydro*-β-D-galactopyranose active-site binding pocket resulting in this compound having a similar binding affinity to UDP/UDP-Gal*f*/UDP-*C*-Gal*f*,[7a] compound **5** is not restricted in this way and is free to scan the mutase surface. In conclusion, the lack of an 1,4-*anhydro*-β-D-galactopyranose binding pocket on the mutase strengthens the argument for the proposed alternative radical/nucleophilic flavin mechanisms.[5d-f]

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Supporting Information Available: Experimental procedures including those for the enzyme assay along with spectroscopic and other data for compounds **5**−**20**.[19] This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The crystallographic data (excluding structure factors) for compounds **6** (CCDC 279233), **8** (CCDC 273320), and **12** (CCDC 279269) have been deposited with the CCDC. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/data_request/cif or by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.; fax: +44 (0)1223 336033.