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Synthesis of acyclic galactitol- and lyxitol-aminophosphonates as inhibitors of UDP-galactopyranose mutase

Weidong Pan, Christophe Ansiaux and Stéphane P. Vincent*

University of Namur (FUNDP), Département de Chimie, Laboratoire de Chimie Bio-Organique, rue de Bruxelles 51, B-5000 Namur, Belgium

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Abstract—UDP-galactopyranose mutase (UGM) catalyzes the isomerization of UDP-galacto*pyranose* (UDP-Gal*p*) into UDP-galacto*furanose* (UDP-Gal*f*), an essential step of the mycobacterial cell wall biosynthesis. Acyclic alditol-aminophosphonates in the D-galactose and D-lyxose series were designed as mimics of high energy intermediates of the UGM catalyzed isomerization. Interestingly, the D-lyxitol-aminophosphonate displayed better inhibition properties than the D-galactitol-aminophosphonate.
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Oligo-galacto*furanosides* (Gal*f*) are essential glycoconjugates of all mycobacteria including severe pathogens such as *Mycobacterium tuberculosis*.[1,2] The search for the biosynthetic origin of the Gal*f* residues has led to the discovery of an unusual enzymatic ring contraction: the interconversion of UDP-galactopyranose (UDP-Gal*p*) into UDP-galactofuranose (UDP-Gal*f*), the universal Gal*f* donor for all oligo-galactofuranosides. This ring contraction is catalyzed by a flavoenzyme, UDP-galactopyranose mutase (UGM) whose mechanism has recently attracted much attention.[3] Such an enzymatic isomerization is a key biocatalytic process not only because it is essential for the survival of mycobacteria,[4] but also because its mechanism has no precedents in enzymology.[5]

So far, several mechanisms have been proposed: a covalent catalysis[6] with the FAD cofactor playing the role of nucleophile[7,8] as well as single electron transfers from the FAD to generate an anomeric radical.[9] From the different mechanistic investigations published to date, several intermediates have been proposed. The inhibition of UGM by fluorinated substrate analogs, under non-reducing conditions, suggested that oxycarbenium **A** and **B** can reasonably be invoked as high-energy intermediates (Fig. 1).[10,11] Moreover, it was recently found that performing this enzymatic reaction in the presence of $NaBH_3CN$ led to the isolation of a covalent adduct between the FAD cofactor and the galactose residue: this experiment showed that an *acyclic* iminium such as **C** could also be implied as a high-energy intermediate.[8]

Therefore, we designed aminophosphonate **1** derived from D-galactose where the two key structural parameters of the high-energy intermediates were combined: the cationic character of intermediates **A** and **B** as well as the acyclic structure of the putative intermediate **C** (Fig. 1). Since in this design the amino functionality mimics a cation at the anomeric position (and not on the endocyclic oxygen), we anticipated that D-lyxose derivative **2** would be interesting to synthesize as well: this molecule displays the same stereochemical pattern than D-galactose but with an amino group in place of the anomeric carbon. The phosphonate functionality in molecules **1** and **2** was included in the design to provide strong coulombic interactions with the UGM amino acids, binding the pyrophosphate moiety of UDP-galactose.

The key step of our synthetic strategy was a reductive amination between dibenzyl aminophosphonate **4** (Scheme 1) and aldehydes **6**, **7** or **8** derived from D-galactose and D-lyxose (Scheme 2). Benzyl protective groups were chosen for the alditol and the phosphonate to insure a clean and quantitative final deprotection step. Aminophosphonate **4** was prepared in two steps.

Keywords: Enzymes; Tuberculosis; Aminophosphonates; Galactofuranose.

* Corresponding author. Tel.: +32 0 81 72 45 21; fax: +32 0 81 72 45 17; e-mail: stephane.vincent@fundp.ac.be

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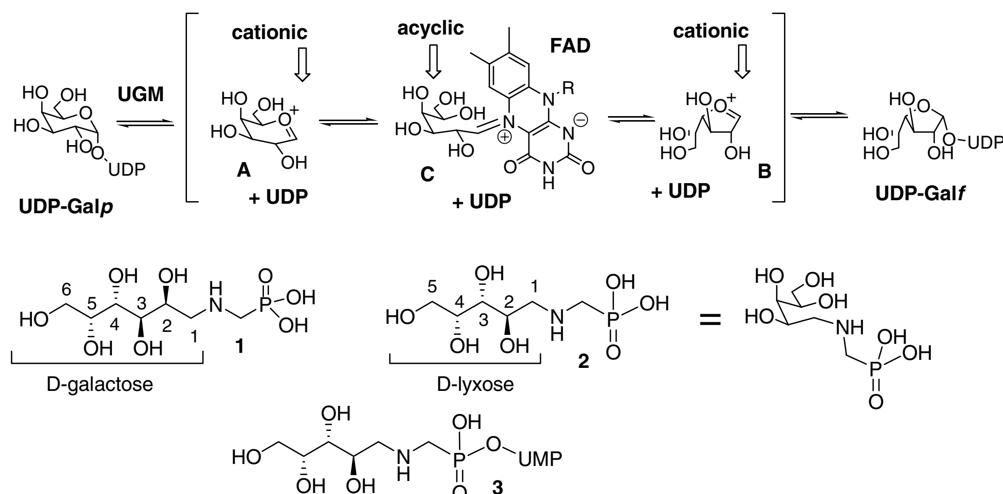
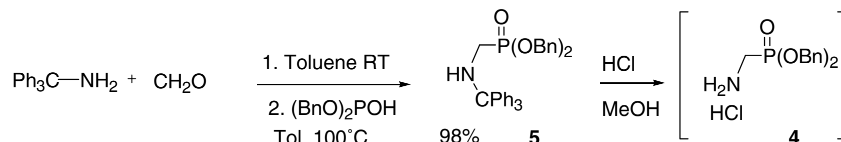
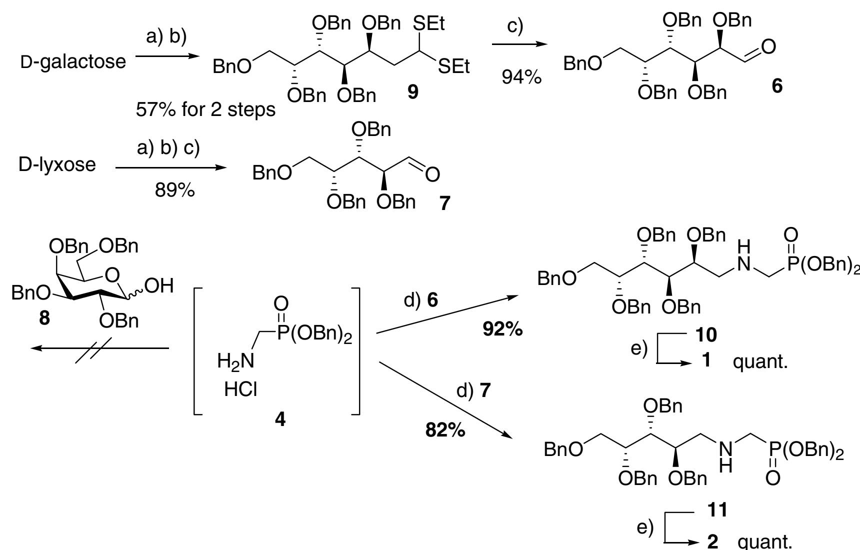


Figure 1. Design of acyclic aminophosphonates as a mimics of putative high-energy intermediates of the UGM catalyzed pyranose/furanose interconversion.



Scheme 1.



Scheme 2. Reagents and conditions: (a) C_2H_5SH, HCl, (b) BnBr, NaH, DMF, (c) $HgCl_2/H_2O$ acetone/H_2O, (d) $NABH(OAc)_3$ THF, rt, and (e) Pd/C, H_2 THF/H_2O.

The condensation of dibenzylphosphite with an intermediate imine generated from formaldehyde and tritylamine afforded intermediate **5** in 98% yield. Deprotection of the trityl group under acidic conditions produced **4** that was found difficult to purify by chromatography or recrystallization. Thus, we engaged it in reductive aminations without purification.

Disappointingly, reductive amination between **4** and the known lactol **8** (obtained in four steps from D-galactose) gave the expected aminophosphonate in a non-satisfactory 19% yield after optimization. We then reasoned that the same reaction involving acyclic aldehydes **6** and **7** in place of a lactol should proceed more efficiently. Thus, aldehyde **6** was generated from the corresponding diethyl dithioacetal **9** using standard conditions. The same three-step sequence was also applied to D-lyxose. Aldehydes **6** and **7** were obtained in 54% and 89% overall yields, respectively, from the corresponding unprotected carbohydrates. The reductive

amination between **4** and **6** or **7** required some optimization in order to obtain satisfactory yields. Several reaction parameters were screened: the solvent, the temperature, the use of primary amine **4** as a hydrochloride salt or a free amine, the reducing agent and the addition of weak acids such as acetic acid to promote the imine formation. We mainly found that $NaBH(OAc)_3$ gave significantly better yields than $NaBH_3CN$ under comparable and optimized conditions, and that the amine had to be used as its hydrochloride salt.[12] Despite the presence of a polar secondary amine functionality, benzylated phosphonates **10** and **11** could easily be purified by standard silica gel chromatography and were isolated in 92% and 82% yield, respectively. Hydrogenolysis of **10** and **11** finally afforded pure target molecules **1** and **2** in quantitative yield.[13]

The inhibition of the UGM catalyzed reaction by these two final molecules was then assessed by a competition assay: the kinetic of isomerization of UDP-Gal*f* into UDP-Gal*p* was followed by HPLC in the presence and absence of inhibitor. UGM from *Escherichia coli* was overexpressed and purified as previously described.[25] The inhibitions were measured under reducing conditions, using freshly prepared sodium dithionite (20 mM). The UDP-Gal*f* and enzyme concentrations were 150 μM and 15 nM, respectively.[14]

To control the assay, we also tested UDP as an inhibitor under the same conditions. Since the K_d of UDP with UGM has been measured, this inhibitor can now be considered as a standard.[11,15–17] At a concentration of 2 M of inhibitors we found that UDP, **1** and **2** inhibited the reaction at 36%, 0% and 11%, respectively. In the three cases the inhibition levels were weak, but to our surprise D-lyxose derivative **2** reproducibly displayed better inhibition properties than D-galactose aminophosphonate **1**.

To date, several groups have described cationic analogs of D-galacto *furanose* designed as inhibitors of either UGM or the galactan biosynthesis. The groups of Fleet,[18,19] Thomas,[20,21] and Martin[22,23] described the synthesis of iminosugars while Pinto and co-workers described the synthesis of sulfonium and selenonium derivatives.[17] The published inhibitory activities of these molecules are usually poor, as in the case of aminophosphonates **1** and **2**. The structural difference of the previously described molecules is that they are cationic analogs of galactofuranose whereas molecules **1** and **2** are acyclic molecules (1-amino-alditols) and possess a phosphonic acid functionality, thus mimicking a putative transient intermediate of the enzymatic reaction. As in the case of the conformational and mechanistic probes we published earlier,[24–26] the presence of a UDP moiety within the structure of the inhibitor seems to be a prerequisite to ensure a tight binding with UGM.

Very recently, Liu and co-workers described the synthesis of a non cationic UDP-galactitol in which the galactose mimic is also acyclic.[11] This substrate analog was designed as a mechanistic probe and displayed binding properties ($K_d = 46$ μM and 54% inhibition at 2.5 mM) in the range of that of UDP ($K_d = 14$ μM and 51% inhibition at 2.5 mM).

Therefore, we envision now to synthesize and test UDP-aminophosphonate **3** (Scheme 1) that should display strong inhibition properties due to the presence of a UDP moiety, a cationic amino group and an acyclic D-galactitol or D-lyxitol side-chain.

Work is in progress to complete the synthesis of UDP-aminophosphonate **3** and test it as an inhibitor of UGM.

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12. Aminophosphonate **5** (146 mg, 0.273 mmol) was suspended in a solution of HCl (3 mL, 1 M) in MeOH (3 mL). The mixture was refluxed at 50 °C for 10 min and cooled down to room temperature. The resulting solution was diluted with MeOH (8 mL), concentrated at 20 °C to remove the solvents and dried under vacuum for 2 h. To this residue was added a solution of aldehyde **6** (280 mg, 0.44 mmol) in anhydrous THF (16 mL) followed by $NaBH(OAc)_3$ (231 mg, 1.09 mmol) and the mixture was stirred vigorously overnight at room temperature. Then, the suspension was filtered through a short pad of silica gel and the solvents were evaporated under reduced pressure. The residue was purified by Flash Column Chromatography (cyclohexane/EtOAc, 3:1) to afford **10** as a colorless oil (229 mg, 92%, R_f 0.65 with cyclohexane/EtOAc 1:1).
13. Analytical data for **1**: $[\alpha]_D^{20}$ −11.3 (*c* 0.9 in H_2O); ^1H NMR (400 MHz, D_2O): $\delta = 4.12$ (ddd, $J_{2,3} = 0.9$ Hz, $J_{2,1a} = 4.3$ Hz, $J_{2,1b} = 9.1$ Hz, 1H; H-2), 3.79 (td, $J_{5,6} = 8.4$ Hz, $J_{5,4} = 0.9$ Hz, 1H; H-5), 3.49 (m, 4H; H-6, H-4 and H-3), 3.22 (ABX, $J_{1a,1b} = 13.3$ Hz, $J_{1a,2} = 4.3$ Hz,

$J_{1b,2} = 9.1$ Hz, 2H; H-1), 3.08 (d, $J_{1',P} = 12.8$ Hz, 2H; H-1′); ^{13}C NMR (101 MHz, D_2O): $\delta = 70.4$ (C-3 or C-4), 69.8 (C-5), 69.2 (C-4 or C-3), 65.4 (C-2), 63.1 (C-6), 52.1 (d, $J_{1,P} = 5.7$ Hz, C-1), 44.0 (d, $J_{1',P} = 138.7$ Hz, C-1′); ^{31}P NMR (161.8 MHz, D_2O): $\delta = 8.73$; MS (ESI): *m/z*: 298 $[M+Na]^+$; HRMS(ESI): *m/z*: calcd for $C_7H_{18}O_8NPNa$: 298.0668; found, 298.0664. Analytical data for **2**: $[\alpha]_D^{20}$ +7.1 ($c = 1.0$ in H_2O); ^{1}H NMR (400 MHz, D_2O): $\delta = 3.87$ (td, $J_{2,3} = J_{2,1b} = 8.0$ Hz, $J_{2,1a} = 3.0$ Hz, 1H; H-2), 3.73 (t, $J_{4,5} = 6.7$ Hz, 1H; H-4), 3.48 (d, $J_{4,5} = 6.7$ Hz, 2H; H-5), 3.40 (m, 2H; H-3 and H-1a), 3.08 (m, 1H; H-1b), 3.04 (d, $J_{1',P} = 12.4$ Hz, 2H; H-1′); ^{13}C NMR (101 MHz, D_2O): $\delta = 72.1$ (C-3), 69.8 (C-4), 66.2 (C-2), 62.8 (C-5), 51.8 (d, $J_{1,P} = 5.8$ Hz, C-1), 44.3 (d, $J_{1',P} = 137.0$ Hz, C-1′); ^{31}P NMR (161.8 MHz, D_2O): $\delta = 9.27$; MS (ESI): *m/z*: 268 $[M+Na]^+$; HRMS(ESI): *m/z*: calcd for $C_6H_{16}O_7NPNa$: 268.0562; found, 268.0564.

14. For inhibition assay and HPLC conditions see Refs. 24,25.

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