Organic LETTERS

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Synthesis of a Novel UDP-carbasugar as UDP-galactopyranose Mutase Inhibitor

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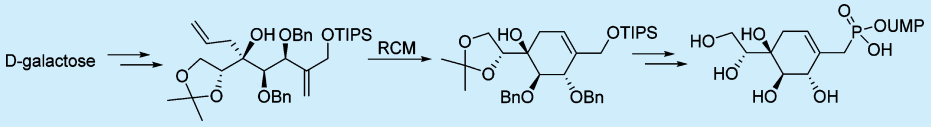
Supporting Information



ABSTRACT: The multistep synthesis of a novel UDP-*C*-cyclohexene, designed as a high energy intermediate analogue of the UDP-galactopyranose mutase (UGM) catalyzed isomerization reaction, is reported. The synthesis of the central carbasugar involved the preparation of a galactitol derivative bearing two olefins necessary for the construction of the cyclohexene ring by a ring-closing metathesis as a key step. Further successive phosphonylation, deprotection, and UMP coupling provided the target molecule. The final molecule was assayed against UGM and compared with UDP-*C*-Gal*f*, the C-glycosidic UGM substrate analogue.

Because of the emergence of extremely resistant strains, tuberculosis still threatens the world population.[1] Thus, novel strategies to fight *Mycobacterium tuberculosis* have recently been developed.[2] Among them, UDP-galactopyranose mutase (UGM)[3] has been validated as a new target because of its involvement in the biosynthesis of the mycobacterial cell wall.[4] Indeed, some UGM inhibitors have been shown to kill the bacterium,[5] a result that confirmed that the enzyme is essential for the survival of the pathogen.[4] UGM catalyzes the interconversion of UDP-galactopyranose (UDP-Gal*p*) **1** into UDP-galactofuranose (UDP-Gal*f*) **2** (Figure 1), the biosynthetic precursor of all galactofuranose-containing eukaryotic and prokaryotic glycoconjugates.[6,7] UGMs from major pathogens have been identified,[6a,8] but interestingly, this enzyme is absent in mammals, which may favor the discovery of selective therapeutic agents.

Moreover, UGM is a unique flavoenzyme whose structure(s) and mechanism have been extensively studied.[6b,9] Surprisingly, it was discovered that the FAD cofactor plays the role of catalytic nucleophile yielding covalent intermediates **5** and **6** (Figure 1) after the release of the UDP moiety.[6b,9i,10] Several crystal structures of UGM have been obtained, some of them in the presence of UDP or UDP-Gal*p*.[9b,11] However, many questions remain unsolved regarding both the mechanism and the binding modes of UGM with its substrate UDP-Gal*f* and the key high-energy intermediates. For instance, the S_N1/S_N2 nature of the substitutions occurring during this isomerization is still under debate,[6b,9h,i,10,12] with some experiments suggesting a cationic intermediate such as **4** and other data indicating a concerted process.[9e,13] Therefore, the design of new mechanistic probes that mimic UDP-Gal*f* or the transition states of this enzyme[9g,h,13,14] may help us to better understand its mechanism and the structural requirements for tight binding to this important therapeutic target.

Here we describe the synthesis and biochemical evaluation of a novel UDP-cyclitol **7** designed as a transition-state analogue of the UGM-catalyzed ring contraction. Our multistep synthesis involves the construction of a key cyclohexene scaffold through a ring-closing metathesis reaction.

As highlighted above, the UGM-catalyzed interconversion of UDP-Gal*p* **1** into UDP-Gal*f* **2** involves three important intermediates: the acyclic adduct **5** and the oxycarbeniums **3** and **4**. In order to obtain high-affinity mechanistic probes, several groups have designed UDP-galactose analogues mimicking either the charged intermediate **4**[14a,b,15] or UDP coupled to acyclic galactitols to mimic intermediate **5**.[9h,14g,h] From these studies it can be concluded that UGM has a much greater affinity for the furanose Gal*f* compared to the pyranose. In addition, the cationic character of the intermediates is probably not a critical parameter to mimic given the fact that cationic UDP-Gal*f* analogues never displayed strong inhibition profiles.[14a,15] On the other hand, the UDP-galactitol (acyclic form) derivatives such as molecules **8**[9h] and **9**[14h] showed strong inhibition profiles against UGM.

We thus reasoned that UDP-galactose mimicking both the acyclic intermediate **5** and the galactofuranose substructure of **6** may display potent inhibition profiles and would be interesting to cocrystallize with UGMs. We thus designed UDP-carbasugar **7** as an inhibitor of UGM (Figure 1). Compared to UDP-galactitols **8** and **9**, the cyclohexene ring has a similarity to a galactofuranose, thanks in part to the geometry of the exocyclic C5−C6 diol, while maintaining some similarity with the acyclic intermediate **5**. Moreover, the cyclohexene, with two sp^2 hybridized carbons, may partially mimic the conformation of the oxycarbenium **4**. Globally, cyclohexene **7** gathers some important structural features of the transformation of the galactitol **5** to the furanose **6**.

Received: March 21, 2014
Published: April 18, 2014

   dx.doi.org/10.1021/ol500848q | *Org. Lett.* 2014, 16, 2462−2465

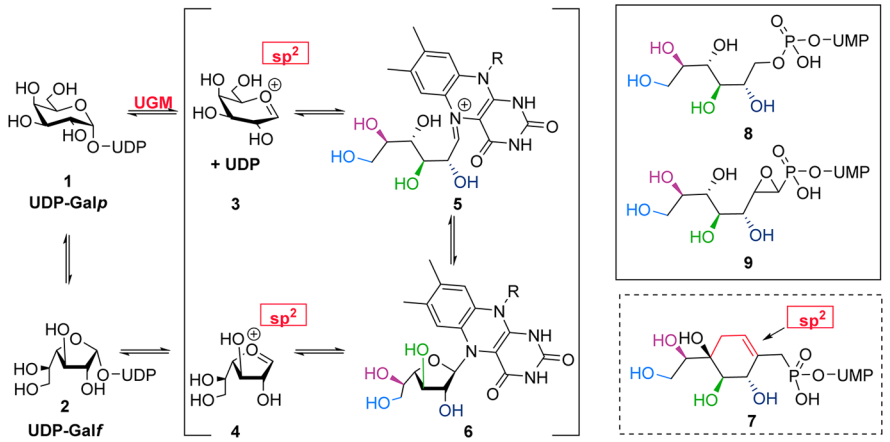


Figure 1. Mechanism of UGM and design of the new high energy intermediate analogue **7**.

Our retrosynthetic analysis of **7** is outlined in Figure 2. The key cyclohexene **10** would be obtained by ring-closing metathesis of

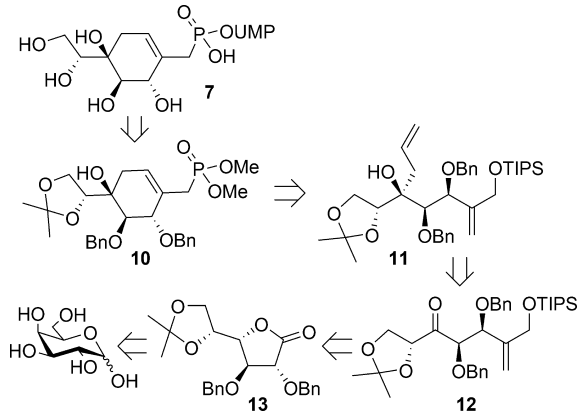


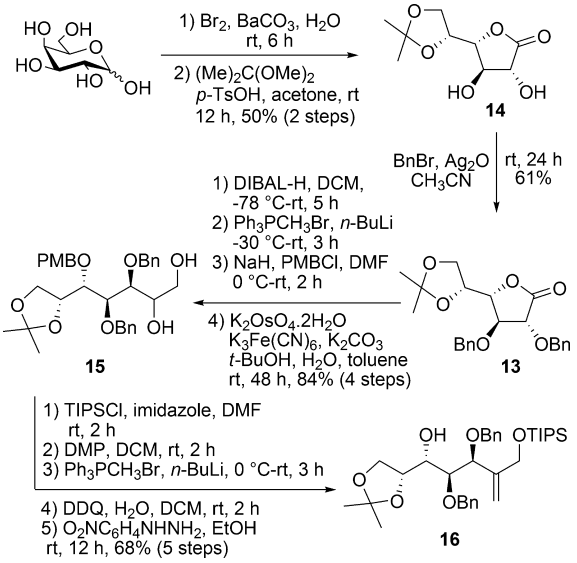
Figure 2. Retrosynthetic analysis.

the diene **11** which could possibly be prepared from the protected D-galactonolactone **13** via successive Wittig and Grignard reactions. We anticipated that lactone **13** would be easily accessible in a few steps from D-galactose.

Our first objective was the generation of diene **11**, precursor of the cyclohexene **10**. The synthesis began with oxidation of D-galactose in the presence of bromine followed by protection of the resulting D-galactono-1,4-lactone as an acetonide to afford the known lactone **14**[16] which, after benzylation, provided intermediate **13** in 61% yield (Scheme 1). The lactone **13** was then transformed into diol **15** in four steps. The reduction of the lactone by DIBAL-H was directly followed by a Wittig olefination without purification. A *p*-methoxybenzyl group was then installed prior to standard dihydroxylation resulting in the formation of epimeric diols **15** (in a ratio 87:13) in 84% yield over four steps after a single purification by silica gel chromatography.

In order to synthesize compound **16**, the diol **15** was regioselectively homologated: the silylation of the primary alcohol followed by Dess−Martin oxidation afforded the corresponding ketone. Olefination of the resulting carbonyl group under classical Wittig conditions, and subsequent deprotection of the *p*-methoxybenzyl ether with DDQ in DCM/H_2O gave **16** in 68% yield over four steps, with a single purification (Scheme 1). Noteworthy, after the cleavage of the

Scheme 1. Synthesis of Intermediate 16



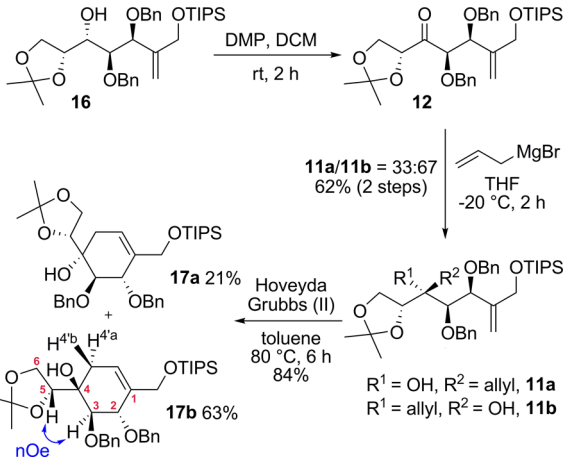
PMB group, the addition of 4-nitrophenylhydrazine was required to remove the byproduct 4-methoxybenzaldehyde which was found to be inseparable from alcohol **16**.

The intermediate **16** was oxidized by Dess−Martin periodinane (DMP) to give the ketone **12**, which was then treated by allylmagnesium bromide to provide a mixture of two diastereoisomers **11a** and **11b** in a ratio 33:67 with 62% yield over two steps (Scheme 2). This stereoselectivity was in accordance with Mulzer's study in which the same ratio was observed for the addition of allylmagnesium bromide (reagent) on related acyclic ketones bearing two different stereogenic centers on each side.[17] In this study, Mulzer had shown that the choice of protecting groups around the ketone strongly influenced the stereoselectivity of the Grignard addition. The absolute configurations in molecules **11a** and **11b** were at first assigned based on Mulzer's model[17] and were confirmed by NOE experiments performed on the cyclized derivatives **17a** and **17b**.

The second key step of this synthesis was the generation of the cyclohexene ring by ring-closing metathesis. While this reaction has been already used for a general access to various carbasugars,[18] including cyclohexenes,[19] the synthesis of hindered trisubstituted cyclohexenes remains complex and poorly described in the literature. Recently, Kiessling et al.

dx.doi.org/10.1021/ol500848q | *Org. Lett.* 2014, 16, 2462−2465

Scheme 2. Synthesis of the Cyclohexene Ring

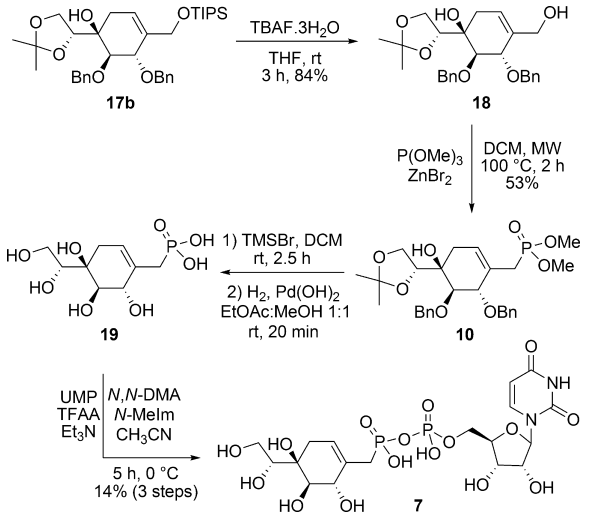


have reported the synthesis of epimers of shikimic acid using this powerful procedure.[19a] We thus investigated this approach starting from the mixture of epimeric dienes **11(a,b)**, and it was found that 0.12 equiv of Hoveyda−Grubbs (II) catalyst and a portionwise addition were necessary for the reaction to go to completion. After 6 h, cyclohexenes **17a** and **17b** were isolated in 21% and 63% yields, respectively (Scheme 2).

The structures of the diastereoisomers **17a** and **17b** were confirmed by NOE experiments performed on product **17b** in $CDCl_3$ which revealed different correlations between H5 and H3, H4′b and H2, and finally the two correlations H4′a−H3 and H4′a−H5. These results confirmed the absolute configuration of compound **17b** at C-4.

With the cyclohexene scaffold in hand, we focused on the synthesis of the target nucleotide-sugar analogue **7**. Cleavage of the silyl ether in **17b** with TBAF in THF gave the alcohol **18** in 84% yield (Scheme 3). Initial investigations on Michaelis−

Scheme 3. Synthesis of Final UDP-carbasugar 7

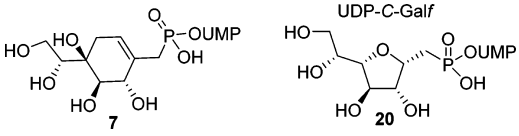


Becker reaction between a sulfonate or a bromide, generated from **18**, and diethyl phosphite failed under various experimental conditions.[20] Fortunately, the Michaelis−Arbusov reaction was successful, but only when the specific conditions recently reported in the literature were applied:[21] the treatment of **18** with trimethyl phosphite and zinc bromide as Lewis acid under microwave irradiation led to a complete conversion after 2 h and the formation of **10** in 53% isolated yield, along with the expected byproduct $(Me)P(O)(OMe)_2$. Compound **10** was then deprotected efficiently with an excess of TMSBr in CH_2Cl_2 followed by hydrogenolysis catalyzed by $Pd(OH)_2$. The desired phosphonate **19** was quantitatively obtained after maximum 20 min of reaction time to avoid the double bond saturation.

The cyclohexene phosphonate **19** was then coupled to an activated form of UMP to generate UDP-carbasugar **7**, which was obtained in 14% yield after size-exclusion chromatography and reversed-phase HPLC. As for the synthesis of UDP-galactofuranose **2**,[22] the activation of UMP as a *N*-methylimidazolium salt gave the best results. The low yield can be explained by the fact that once formed, the UDP-carbasugar can decompose into a cyclic phosphonate and UMP.[23] This intramolecular reaction occurs not only during the reaction but also during the purification steps. This side reaction was also observed for other phosphonate analogues of UDP-galactose **1** and **2**.[14e,24]

In order to study the inhibition profile of UDP-cyclohexene **7**, we used UDP-*C*-α-Gal*f* **20**[24] (the *C*-glycosidic analogue of UGM's substrate UDP-α-Gal*f*) and UDP **21** for comparison purposes (Table 1). Indeed, UDP is often used in the literature as

Table 1. Inhibition Percentages and K_d of 7, 20, and 21



inhibitor	% inhibition[a] (HPLC)	K_d[b] (μM, FP)
UDP (21)	39.1 ± 8.0	45 ± 1.1
20	10.3 ± 6.1	517 ± 1.4
7	29.4 ± 5.7	870 ± 1.4

[a]Inhibition assay conditions: [Inhibitor] = 1 mM, reduced enzyme [UGM_{Kp}] = 12 nM, [UDP-Gal*f*] = 105 μM, [dithionite] = 12.5 mM. [b]Fluorescence polarization assay conditions: [fluorescent probe] = 15 nM, [I] = 10 μM to 2 mM, nonreduced enzyme [UGM_{Kp}] = 500 nM.

a control inhibitor.[9h] The comparison with **20** was also useful because the replacement of the *exo*-anomeric oxygen atom by a methylene group may affect the mode of interactions with UGM.[11a]

Two complementary enzymatic assays were carried out on UGM of *Klebsiella pneumoniae* (UGM_{Kp}) which is the most studied UGM enzyme for inhibition studies.[9h,14b,15,25] UGM is a flavoenzyme whose cofactor must be reduced to be kinetically competent. Thus, we realized first a competition assay against the substrate UDP-Gal*f* **2** under reducing conditions.[9h] In this assay, the conversion of **2** into its isomer **3** is monitored by HPLC in presence and absence of inhibitors. The decreases in reaction rates are translated into inhibition percentages that have been gathered in Table 1.

From this first series of experiments, we noticed that the carbasugar **7** has an inhibition level intermediate between UDP **21** and UDP-*C*-Gal*f* **20**. This result shows that when UGM is catalytically active, the enzyme has a stronger affinity for the cyclohexene analogue **7** than for the galactofuranose subunit of **20**. Therefore, these results indicate that, to mimic high-energy intermediates of the UGM catalyzed reaction, it is appropriate, to design molecules in which the galactose unit is indeed between a furanose state as in **6** (Figure 1) and an acyclic state as in **5**.

However, to obtain a more complete picture of the binding properties of carbasugar **7**, we also measured the fluorescence

dx.doi.org/10.1021/ol500848q | *Org. Lett.* 2014, 16, 2462−2465

polarization (FP)[26] on UGM_{Kp} under nonreducing conditions. This assay is easier to perform, but it only gives an estimation of the affinities of the molecules because the enzyme is not in its catalytically active state. The inhibition profile has been found in the following order: UDP > UDP-*C*-Gal*f* > **7**, thus showing a reversal of affinities compared to the HPLC assay. The latter result was not surprising since major affinity changes of UDP-galactose analogues toward UGM as a function of the redox state of the enzyme have been observed several times in the literature.[9e,24,25,14i]

In summary, we have explored a new synthetic pathway for the generation of the UDP-carbasugar **7** starting from D-galactose and by using ring closing-metathesis as a key step for the transformation of acyclic galactitol into the cyclohexene ring. When assayed against the reduced enzyme, the final cyclohexene **7** displayed a better affinity than the galactofuranoside **20**, which constitutes important information for the future design of transition-state analogues.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by an ARC grant from the Académie Louvain (A.R.C. 08/13-012 for S.B. and I.G.) and by FNRS (Chargé de Recherche for A.T., PDR T.0170.13 for C.S.).

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