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Synthesis of D-*glycero*-D-*manno*-heptose 1,7-bisphosphate (HBP) featuring a β-stereoselective bis-phosphorylation

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ABSTRACT

D-*glycero*-D-*manno*-Heptose 1,7-bisphosphate (HBP) plays a unique role in bacteriology. We describe in this study a very efficient synthesis of HBP, featuring a highly 6-D-selective construction of the heptose scaffold as well as a double phosphorylation step installing, in a single operation and in a β-stereoselective manner, the 1- and the 7-phosphates.

The preparation of "higher-carbon sugars" such as heptoses has been investigated for more than a century and has witnessed all the major (r)evolutions of organic chemistry that occurred during the 20th century. The discovery of natural heptoses stimulated the synthetic chemists to develop robust methodologies to synthesize them and demonstrate their structures, including in natural products such as the spicamycin,[1] miharamycins,[2,3] and desferrisalmycin.[4] Furthermore, the discovery that the *glycero*-D-*manno*-heptoses are common subunits of lipopolysaccharides (LPS) and capsular saccharides of many pathogenic bacteria, intensified the research by offering new applications: synthetic heptosides could be designed, for instance, as antimicrobial agents or synthetic vaccines.[5–7] The main synthetic methods to construct heptosides have been reviewed by Oscarson,[8] Kosma,[9] and more recently by our group.[10]

The biosynthesis of L,D-heptosides has attracted a strong attention because of their occurrence in the LPS of most gram-negative bacteria, including in major human pathogens.[11] Sedoheptulose-7-phosphate **1**, a product of the central metabolism, is the biosynthetic precursor of the bacterial heptoses (Fig. 1).[12,13] The first enzyme GmhA isomerizes **1** into D-*glycero*-D-*manno*heptose 7-phosphate **2** which is then phosphorylated by the kinase HldE yielding heptose bisphosphate **3** (HBP), the object of the present study. After hydrolysis of the terminal phosphate of **3**, intermediate **4** is transformed into nucleotide-sugar **5**. A regioselective epimerization is then promoted by HldD, yielding ADP-L-heptose **6**, the donor substrate of heptosyltransferases (WaaC, WaaF and WaaQ) that will further construct the LPS molecule.[14,15]

A similar pathway has been evidenced for the D,D-heptosylated oligosaccharides and the 6-deoxy-heptosides found in some bacterial O-antigens as well as in Gram-positive bacteria. The kinase HddA phosphorylates the same D,D-heptose **2** to give the α-anomer **7** with opposite stereoselectivity than HldE. This intermediate 7-phosphate is then dephosphorylated by the phosphatase GmhB and transformed into GDP-heptose **9** that can be further processed.

Our group has explored the synthesis of natural and non-natural heptosides as inhibitors of three enzymes of the LPS D,D-heptose pathway: GmhA, HldE and WaaC.[16–19]

Very recently, the group of Gray-Owen discovered that the intermediate D-*glycero*-D-*manno*-heptose 1,7-bisphosphate **3** (HBP) plays a very unique role in bacteriology, distinct from the LPS biosynthesis. Indeed, it was shown that HBP, once released from pathogenic bacteria such as *Nesseiria menengitidis*, can trigger an innate and adaptive immune response in mammals.[20] These remarkable and novel biological properties along with our continuous efforts in generating glycomimetics of heptose-phosphates pushed us to envisage the synthesis of HBP. This molecule can be used as substrate of GmhB to develop enzymatic assays to discover novel inhibitors of the D,D-heptose biosynthetic pathway, but it can also be exploited to study the bacterial immunology.

Interestingly, when we started this study, there was only an enzymatic synthesis of HBP but no chemical synthesis.[21] Very recently, we developed a methodology for the β-stereoselective phosphorylation of D-mannosides, including D-heptosides.[22,23]

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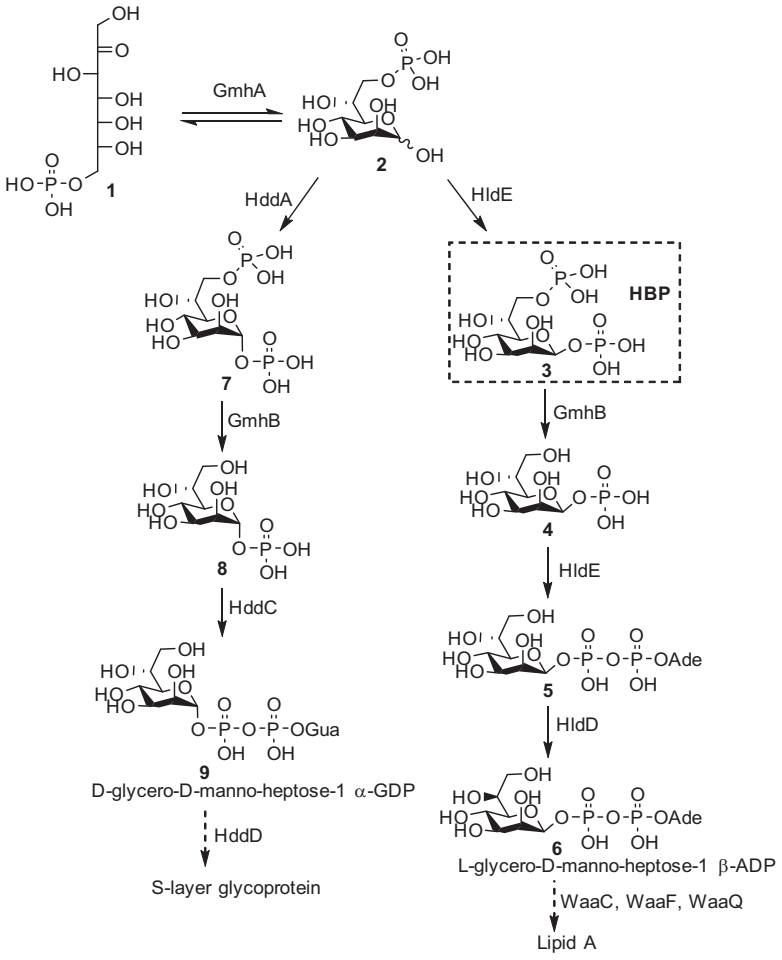


Fig. 1. D-*glycero*-D-*manno*-Heptose 1α-GDP biosynthetic pathway and L-*glycero*-D-*manno*-heptose 1β-ADP pathway. Ade = adenosine, Gua = guanosine.

Our next challenge was thus the synthesis of the biologically relevant HBP molecule.

We have to mention that, while we were writing this manuscript, a chemical synthesis of HBP was disclosed by the group of Fujimoto.[24] Here, we describe our own approach and emphasize the differences in the synthetic strategies.

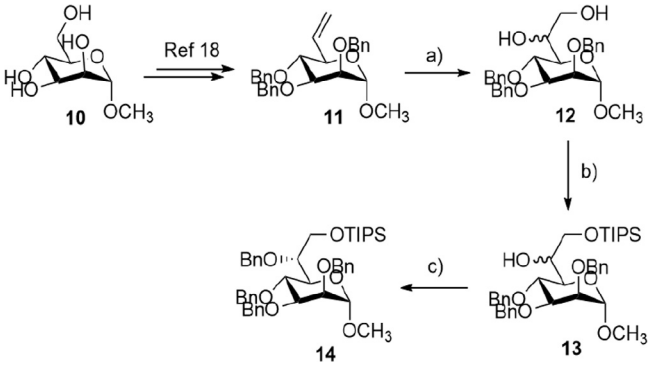
Results

As mentioned above, many strategies have been exploited to generate heptoses from hexoses. We selected the pathway depicted in Scheme 1 because of its high stereoselectivity in favor of the desired 6-D epimer **12**.[16,18,25]

In our synthesis of fluorinated analogues of heptosides,[16] we had also explored the opening of an epoxide derived from an alkene similar to **11**, but we had found that this strategy is slightly L-selective, as recently exploited by Fujimoto et al.[24] Therefore, we privileged a dihydroxylation of **11** that eventually lead to a high 9/1 stereoselectivity in favor of the D isomer.[16,18]

The two epimers **12D** and **12L** could not be separated after the dihydroxylation step. This epimeric mixture was thus engaged in the two subsequent steps yielding pure **14** as a single D stereoisomer. It is important to note that the two epimers could only be separated at the stage of molecule **14**, but not **12** and **13**. The intermediate **14** features now a differentiated 7-position opening the way for phosphorylation studies.

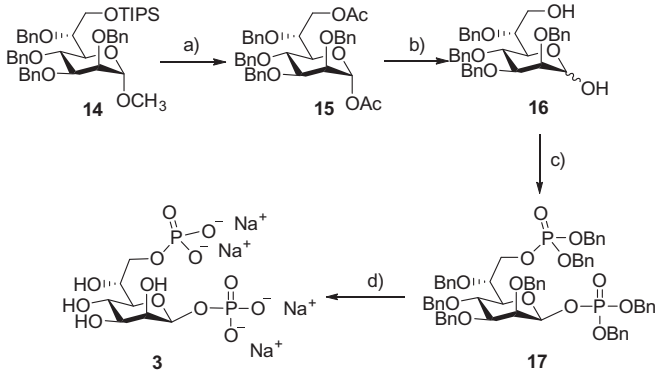
The key step of our synthetic strategy was to perform a double phosphorylation of the heptose scaffold at the 7- and 1-positions,



Scheme 1. Reagents and conditions: a) $K_2OsO_4(H_2O)_2$, K_2CO_3, $K_3Fe(CN)_6$, water/*t*-BuOH/toluene, 0 °C-R.T, 89%, D/L = 9:1; b) TIPSCl, imidazole, THF, R.T, 93%, D/L = 9:1; c) NaH, BnBr, DMF, R.T, 84%, the D/L = 9:1 epimers were separated at that stage.

simultaneously. To do so, we transformed intermediate **14** into **16** by an acetolysis yielding intermediate bis-acetate **15** that was de-acetylated to provide **16** in high yield (Scheme 2).

The β-stereoselective phosphorylation of *manno*-configured sugars is a real synthetic challenge. We have recently realized a methodological study to synthesize β-1-phosphates of a broad range of carbohydrates, including mannosides and heptosides.[22,23] Applied to intermediate **16**, our own methodology only provided bis-phosphate in low yield and poor stereoselectivity. This result



Scheme 2. Reagents and conditions: a) conc. H_2SO_4, $AcOH/Ac_2O$, 0 °C, 67%; b) MeONa, MeOH, R.T, 90%; c) dibenzyl phosphate, tris(4-chlorophenyl)phosphine, Et_3N, DEAD, THF, α:β = 1:2, 53% (β); d) H_2, $Pd(OH)_2/C$, 1,4-dioxane/H_2O, Dowex 50 (Na+).

corroborates the literature data on the challenging β-mannosylation reactions whose stereochemical outcome highly depends on the functional group at the 6-position, in a remote fashion. Moreover, this bisphosphorylation step was complicated by the fact that the resulting bis-phosphate showed some unstability, especially during the silica gel purification step.

In 2011, we had synthesized a series of D,D-heptose-7-phosphate analogues and we had found that a Mitsunobu phosphorylation at the position 7 was particularly efficient.[18] Mitsunobu type phosphorylations at the anomeric position of carbohydrates are not so usual, but we decided to explore this strategy. To our great pleasure, we could obtain bisphosphate **17** in good yield (60%) and satisfactory α/β selectivity (α/β = 1:3), using the standard PPh_3/DEAD Mitsunobu conditions. However, we must specify that the separation of the desired β-anomer by standard silica gel chromatography was extremely difficult. To optimize the yield, we eventually found that using tris(4-chlorophenyl)phosphine in place of PPh_3 facilitated the purification. Under these conditions, bisphosphate **17** was obtained in 53% yield with a α/β selectivity of 1/2, a yield and a stereoselectivity that have to be considered very satisfactory based on literature data.[8–10] The β configuration was ascertained by NMR techniques as in our previous studies.[23] In particular, we found that the ^1J(C1-H1) of the α anomer was 176.4 Hz and 163.9 Hz for the β anomer. Moreover, through-space NOE correlations between H1 and H5 of the β anomer confirmed this assignment.

We have to mention that Fujimoto et al. also developed a Mitsunobu phosphorylation at the anomeric position.[24] However their synthetic scheme involves two distinct and subsequent phosphorylation techniques and a protective group manipulation between the two phosphorylations. While this manuscript was in the review/revision process, Zamyatina et al. also disclosed an efficient synthesis of HBP **3**.[26] Here again, two subsequent phosphorylation steps were required. Interestingly, in their approach, acetates were selected as protective groups and the anomeric phosphorylation using a Mitsunobu reaction was not successful. This result is typical of the strong influence of protective groups on the carbohydrate reactivity.

The subsequent deprotection of **17** could be classically achieved by a global hydrogenolysis of all benzyl groups. The analytical data of the final molecule corresponded well with literature data.[21,24]

In conclusion, we disclosed in this study a very efficient synthesis of HBP, featuring a highly 6-D-selective construction of the heptose scaffold as well as a double phosphorylation step installing, in a single operation and in a β-stereoselective manner, the 1- and the 7-phosphates.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.08.005.

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