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Chemical Synthesis of D-*glycero*-D-*manno*-Heptose 1,7-Bisphosphate and Evaluation of Its Ability to Modulate NF-κB Activation

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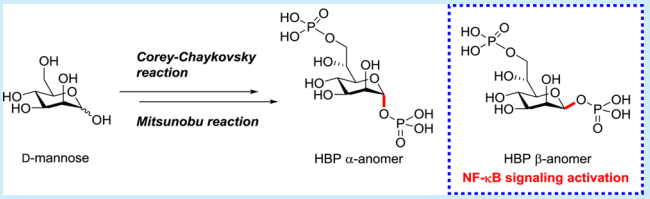
Supporting Information

ABSTRACT: D-*glycero*-D-*manno*-Heptose 1,7-bisphosphate (HBP) is the precursor for heptose residues found in Gram-negative bacterial membrane surface glycoproteins and glycolipids. HBP β-anomer was recently reported to be a pathogen-associated molecular pattern (PAMP) that regulates TIFA-dependent immunity. Herein, we report the chemical synthesis of HBP α- and β-anomers, which highlights a C-7 carbon homologation via the Corey−Chaykovsky reaction, and the introduction of a phosphate group at the anomeric position using the Mitsunobu reaction. Furthermore, NF-κB reporter assaying revealed that HBP β-anomer activates the NF-κB signaling pathway.



The D-*glycero*-D-*manno*-heptose 1,7-bisphosphate (HBP) is an important bacterial biosynthetic intermediate[1] that contains two phosphate groups at the C-1 and C-7 positions (Figure 1). HBP can exist as α- and β-anomers at the C-1

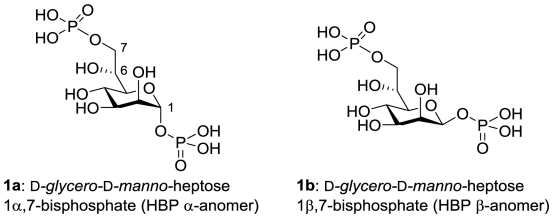


Figure 1. Structure of D-*glycero*-D-*manno*-heptose 1α,7-bisphosphate (**1a**) and 1β,7-bisphosphate (**1b**).

positions, both of which are derived from a common precursor, D-*glycero*-D-*manno*-heptose 7-phosphate. The α-anomer of HBP, D-*glycero*-D-*manno*-heptose 1α,7-bisphosphate (**1a**), is involved in the D-*glycero*-D-*manno*-heptose 1α-GDP pathway, which produces the precursor for incorporation into bacterial membrane surface glycoproteins[2] and capsular polysaccharides.[3] On the other hand, the β-anomer of HBP, D-*glycero*-D-*manno*-heptose 1β,7-bisphosphate (**1b**), is the intermediate in the L-*glycero*-D-*manno*-heptose 1β-ADP pathway,[4] which generates the precursor for heptose residues that make up the core of lipopolysaccharides (LPS) found in Gram-negative bacteria.

Recently, Gray-Owen et al. reported that D-*glycero*-D-*manno*-heptose 1β,7-bisphosphate (**1b**) from pathogenic *Neisseria* is a cytosolic pathogen-associated molecular pattern (PAMP)[5] that induces innate and adaptive immune responses.[6] In their study, HBP obtained through an enzymatic process activated the NF-κB signaling via regulation of TRAF-interacting protein with a forkhead-associated domain (TIFA)-dependent pathway. TIFA has previously been shown to be an activator of IKK/NF-κB signaling pathway by promoting the oligomerization and ubiquitin ligase activity of TRAF6.[7] Owing to the interesting structure of HBPs, as well as their immune stimulatory activities, we planned to develop a synthetic route to the chemically pure HBPs and to use them to elucidate their immune activities.

Although a number of synthetic studies of naturally occurring heptose derivatives have been reported thus far,[8] the synthesis of D-*glycero*-D-*manno*-heptose 1,7-bisphosphate has yet to be reported, except for the enzymatic synthesis reported by Dunaway-Mariano et al.[3] The pivotal steps toward chemical synthesis are homologation of the C7 carbon, construction of the C6 chiral center, and introduction of the C1 phosphate group. In particular, the construction of the β-mannosidic *O*-phosphate ester linkage in **1b** is a major challenge. A few published reports have focused on the synthesis of these phosphate moieties, including synthetic studies of mannose 1β-phosphates[9] and *glycero*-*manno*-heptose 1β-phosphates.[9c,10] Sabesan reported that a condensation between chlorophosphates and 2,3,4,6-*O*-acyl-protected sugar derivatives in the presence of DMAP gave the mannose 1β-phosphate.[9a] Crich

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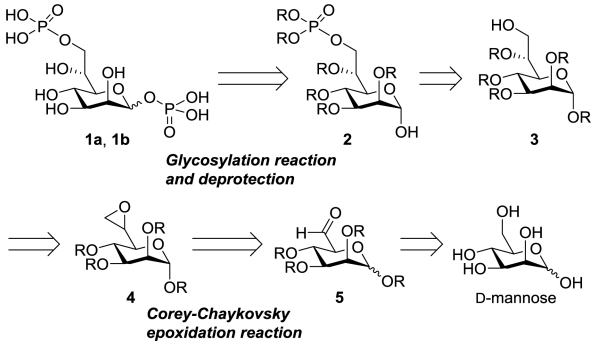
developed a β-selective phosphorylation of mannosyl sulfoxide containing 4,6-*O*-benzylidene protecting group.[9b] In general, glycosyl phosphates are known to be unstable and are thus sometimes used as glycosyl donors.[11] In addition, it was found that the anomeric phosphate linkage was prone to anomerization in the presence of Lewis acids, which are generally used in glycosylation reactions.[12] Therefore, there was a need to develop a suitable reaction for generating *glycero-manno*-heptose 1β-phosphates.

Our retrosynthetic analysis of HBPs is shown in Scheme 1. We planned to introduce the unstable C-1 phosphate group

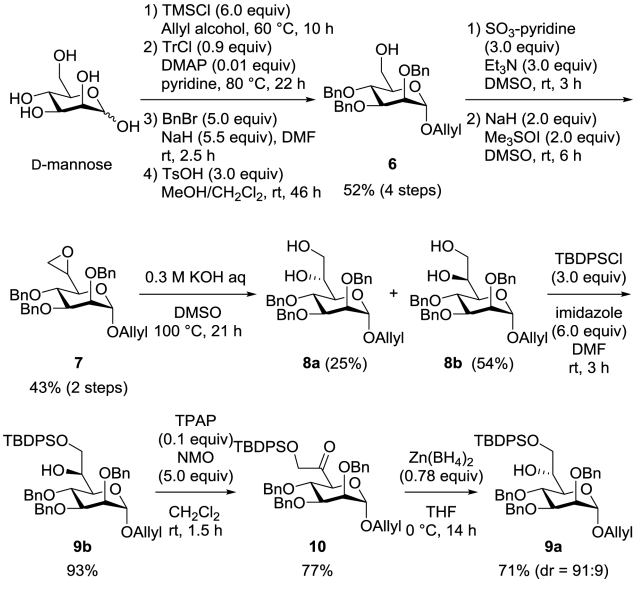
Scheme 1. Retrosynthetic Analysis of HBPs (1a and 1b)



during the late stages of our synthesis and to investigate a mild phosphorylation reaction. The hemiacetal **2** could be obtained through ring-opening of epoxide **4**, followed by phosphorylation of the hydroxyl group of **3**. The epoxide **4** could be accessed by a Corey−Chaykovsky epoxidation reaction on the aldehyde **5**, which could be prepared from D-mannose.

Our preparation of the required key intermediate **9a** via the epoxide **7** derived by the Corey−Chaykovsky reaction is outlined in Scheme 2. The alcohol **6** was easily prepared from D-mannose in 52% yield over four steps. The oxidation of **6** gave the corresponding aldehyde, which was subsequently allowed to react with NaH and Me_3SOI to provide the epoxide **7** in 43% yield as a 61:39 diastereomixture. With the requisite

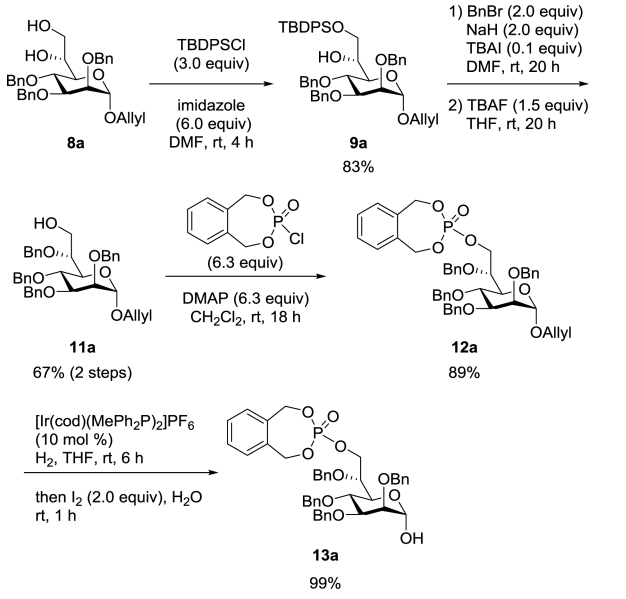
Scheme 2. Synthesis of Key Intermediate 9a



carbon framework, **7**, in hand, we next performed the epoxide ring-opening reaction with KOH in DMSO to give the diols **8a** and **8b**,[13] which were easily separated by SiO_2 column chromatography. The absolute configuration of the C6 chiral center was confirmed by conversion to MTPA esters[14] (see the Supporting Information), which indicated that the diol **8a** contained the desired C6 chiral center. Thus, we investigated the stereoconversion of the C6 chiral center. We were also able to convert undesired **8b** to the key intermediate **9a**, possessing the desired C6 chiral center, in the following manner. Treatment of the diol **8b** with TBDPSCl gave the alcohol **9b**, which was oxidized with TPAP to generate the ketone **10**. Subsequent diastereoselective reduction with $Zn(BH_4)_2$[8e] gave the desired isomer **9a** (71%, dr = 91:9).

Next, we examined the conversion of **8a** to the hemiacetal **13a** (Scheme 3). The introduction of a silyl group to **8a**

Scheme 3. Synthesis of Hemiacetal 13a



provided **9a**. The benzyl protection of **9a** and subsequent removal of the TBDPS group gave the alcohol **11a**. The formation of a phosphoester at the C7 position produced **12a**, and subsequent removal of the allyl group with an Ir catalyst generated the hemiacetal **13a**.

With the phosphorylation precursor in hand, we performed model studies to introduce the phosphate group at the anomeric position[11] using mannose derivatives. Initially, we investigated the phosphorylation of 2,3,4,6-*O*-benzyl-protected model substrates with chlorophosphates under the Sabesan conditions,[9a] but the desired products were not obtained. Next, we performed the phosphorylation reaction using gold catalysts,[15] which have unique reactivities, different from Lewis acids such as BF_3OEt_2 or TMSOTf (eq 1). The treatment of model substrate **14** with either $Ph_3PAuOTf$ or $Ph_3PAuBAr_4$[F16] gave only the α-anomer, **15a**, in yields of 94% and 35%, respectively. We attempted the phosphorylation reaction under basic conditions, using a glycosyl iodide,[17] which was easily prepared from **16**. The reaction of glycosyl iodide **17** with dibenzyl phosphate and Et_3N in CH_2Cl_2 favored production of the β-anomer **15b** (eq 2, α/β = 30:70). Furthermore, we investigated the Mitsunobu reaction[18] of model substrate **18**, shown in Table 1. Reaction of **18** with

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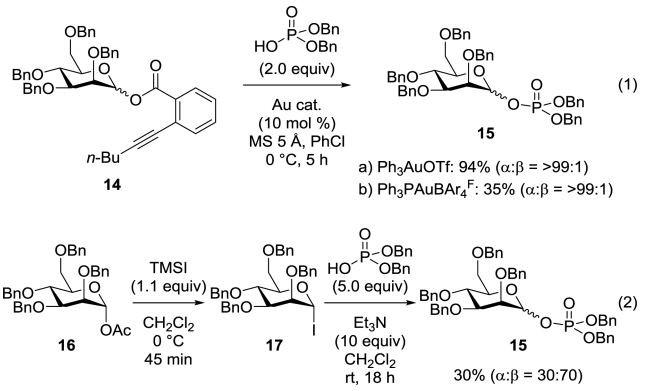
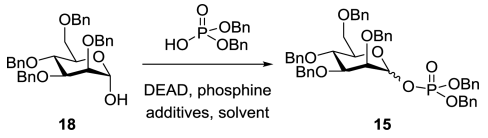


Table 1. Phosphorylation Reaction of the Model Substrate 18 under Mitsunobu Conditions



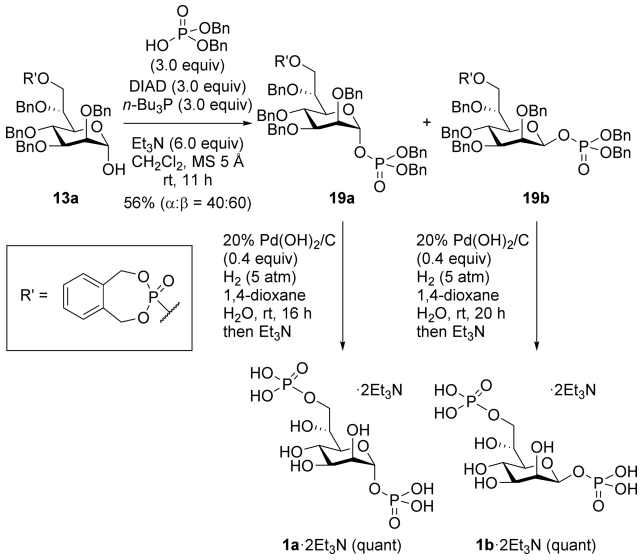
entry[a]	phosphine	additive	solvent	yield[b] (%)	α/β[c]
1	PPh_3		CH_2Cl_2	trace	
2	PPh_3	MS 5 Å	CH_2Cl_2	trace	
3	PPh_3	Et_3N, MS 5 Å	CH_2Cl_2	71	66:34
4[d]	PPh_3	Et_3N, MS 5 Å	CH_2Cl_2	61	65:35
5	PPh_3	Et_3N, MS 5 Å	dioxane	45	78:22
6	PPh_3	Et_3N, MS 5 Å	MeCN	ca. 61	71:29
7	PPh_3	Et_3N, MS 5 Å	toluene	62	61:39
8	PPh_3	DIEA, MS 5 Å	CH_2Cl_2	ca. 70	64:36
9	PPh_3	NMM, MS 5 Å	CH_2Cl_2	3	70:30
10	n-Bu_3P	Et_3N, MS 5 Å	CH_2Cl_2	74	59:41

[a]Reactions were carried out with substrate (0.055 mmol), dibenzyl phosphate (3.0 equiv), DEAD (3.0 equiv), and amine additive (6.0 equiv) at room temperature. [b]Yields of the isolated products. [c]Determined by ^1H NMR analysis. [d]The reaction was carried out at 0 °C. DEAD = diethyl azodicarboxylate; DIEA = diisopropylethylamine; NMM = *N*-methylmorpholine.

dibenzyl phosphate, DEAD, and PPh_3 in CH_2Cl_2 gave a trace amount of the desired product **15** (entry 1). The presence of MS 5 Å did not improve the yield of the desired product (entry 2). The addition of Et_3N had a significant effect on the reaction course, providing **15** at 71% yield as α/β = 66:34 (entry 3). The lower selectivity could result from formation of an oxocarbenium species in the transition state leading to an S_N1-type reaction. The reaction at 0 °C did not change the selectivity (entry 4). Changing the solvent and additive did not enhance the yield of the desired product (entries 5−8). The use of NMM as an additive was ineffective (entry 9), which indicated that the pK_a values of the amine additives could affect the reactions. The use of n-Bu_3P instead of PPh_3 did improve the yield and β-selectivity (entry 10, 74%, α/β = 59:41).

We went on to investigate phosphorylation reactions of the heptose derivative **13a**. The reaction using the corresponding glycosyl iodide was carried, but the desired bisphosphate was not obtained (data not shown). As shown in Scheme 4, β-Selective phosphorylation of **13a** was performed under the optimized conditions (Table 1, entry 10) to generate the desired bisphosphate **19** at 54% yield as α/β = 40:60 (which was determined by ^1H NMR analysis of crude product, and the diastereomeric ratio of isolated product was α/β = 53:47, due

Scheme 4. Total Synthesis of HBP (1a and 1b)



to slight amomerization during the preparation process). The anomeric configuration of **19a** and **19b** was confirmed by comparison of the $^1J_{CH}$ coupling at the anomeric carbon (175.7 and 162.5 Hz, respectively). The α and β anomers (**19a** and **19b**) were easily separated by SiO_2 column chromatography. Finally, we attempted to remove all of the protective groups from **19a** and **19b**. The reaction of **19b** with $Pd(OH)_2/C$ in MeOH under H_2 atmosphere provided the desired products. However, an undesired side product containing a methoxy group at the anomeric position (substitution of the phosphate to the methoxy group) was observed. After investigation of the solvents, we found that 1,4-dioxane−H_2O was the optimal solvent for producing the HBP α- and β-isomers (**1a** and **1b**), which were converted to the Et_3N salt forms, respectively. The structures of the Et_3N salt forms of **1a** and **1b** were determined by 1D and 2D NMR spectroscopy and mass spectrometry. There were no obvious contradictions between these data (Et_3N salt forms) and the previous reported spectra[3] (ammonium salt forms).

With HBP α- and β-anomers **1a** and **1b** in hand, we sought to determine their immune activities using NF-κB activation assays in digitonin-permeabilized HEK293T cells.[19] As shown in Figure 2, HBP β-anomer **1b** exhibited the NF-κB activation in a dose-dependent manner, which agrees with a previous study which found that cytosolic detection of HBP activates NF-κB, using HBP obtained through an enzymatic process. In contrast, HBP α-isomer **1a** displayed no activity in our assay, which indicates that the α-isomer is potentially useful as a negative control in the functional analysis of HBP.

In conclusion, we have successfully accomplished the first chemical synthesis of D-*glycero*-D-*manno*-heptose 1,7-bisphosphate, including both α- and β-anomers. The key features of our synthesis include the Corey−Chaykovsky epoxidation reaction for homologation of the C7 carbon and the phosphorylation under Mitsunobu condition for constructing a β-mannosyl phosphate. We have also demonstrated that a synthetic form of the HBP β-anomer activated the NF-κB signaling pathway. We expect to use synthetic HBP α- and β-anomers as chemical tools to elucidate immune signals such as TIFA-dependent immunity.

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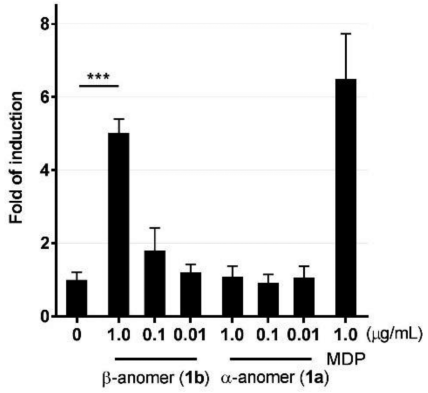

Figure 2. NF-κB activation assays for HBP α- and β-anomers, **1a** and **1b**, in digitonin-permeabilized HEK293T cells. HEK293T cells cotransfected with an NF-κB luciferase reporter plasmid and a control β-galactosidase plasmid were permeabilized with digitoin before being stimulated with HBP α-isomer (**1a**), β-anomer (**1b**), and MDP (Nod2 ligand) for 4 h. Luciferase activities were normalized against β-galactosidase activity. Fold induction was calculated on the basis of the normalized luciferase activity of unstimulated cells. ***$P < 0.001$ (two-tailed Student's *t*-test). Error bars: mean ±1 SD for triplicate experiments. A typical data set of two experiments is shown.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b01158.

Experimental procedures and characterization of the products (PDF)

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Notes

The authors declare no competing financial interest.

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