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•Research article•

Total synthesis of D-*glycero*-D-*mannno*-heptose 1*β*, 7-bisphosphate with 3-*O*-amyl amine linker and its monophosphate derivative

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[ABSTRACT] D-*Glycero*-D-*mannno*-heptose 1*β*, 7-bisphosphate (HBP*β*) is an important intermediate for constructing the core structure of Gram-negative bacterial lipopolysaccharides and was reported as a pathogen-associated molecular pattern (PAMP) that regulates immune responses. HBP*β* with 3-*O*-amyl amine linker and its monophosphate derivative D-*glycero*-D-*mannno*-heptose 7-phosphate (HP) with 1*α*-amyl amine linker have been synthesized as candidates for immunity study of HBP*β*. The *O*3-amyl amine linker of heptose was installed by dibutyltin oxide-mediated regioselective alkylation under fine-tuned protecting condition. The stereoselective installation of 1*β*-phosphate ester was achieved by NIS-mediated phosphorylation at low temperature. The strategy for installation of 3-*O*-amyl amine linker onto HBP derivative can be expanded to the syntheses of other conjugation-ready carbohydrates bearing anomeric phosphoester.

[KEY WORDS] HBP*β*-3-*O*-amyl amine; HP-1*α*-*O*-amyl amine; 3-*O*-Alkylation; NIS-mediated phosphorylation
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Introduction

Carbohydrate are essential components of biological process such as cell recognition, pathogen infection and the immune response [1]. In order to identify molecules that bind to carbohydrates [2] or to raise antibodies against specific glycans, carbohydrates with orthogonal linkers enable the conjugation or immobilization of carbohydrates. D-*Glycero*-D-*mannno*-heptose 1*β*, 7-bisphosphate (HBP*β*) (Fig. 1) is the precursor for heptose residues that make up the core structure of lipopolysaccharides (LPS) of Gram-negative bacteria [3]. HBP*β* can induce an immune response in mice as a cytosolic pathogen-associated molecular pattern (PAMP) [4]. The structure-activity relationship of HBP*β* in the immune response remains unknown. HBP derivative **1** equipped with an 3-*O*-amyl amine linker was designed to serve as carbohydrate antigen to induce HBP*β*-specific antibodies and identify immunologically important domains of HBP*β*. The amyl amine linker is placed at the C3 position instead of the anomeric position to retain the anomeric phosphate. D-*Glycero*-D-*mannno*-heptose 7-phosphate derivative **2** with a linker at the anomeric position will be prepared to determine whether 1*β*-phosphorylation is important for the immune response that recognizes HBP*β*.

Although HBP*β* had been synthesized enzymatically and chemically [5-6], the chemical synthesis of HBP*β* derivative **1** that includes a 3-*O*-amyl amine linker is still a challenging task. Firstly, D-*glycero*-D-*mannno*-heptose, the core monosaccharide scaffold, is a higher carbon chain than common pentose or hexoses. Secondly, phosphate groups placed at the C1 and C7 positions are less stable than other carbohydrate modifications such as acetamido and acetyl groups [7]. Anomeric phosphates are good leaving groups for glycosylation

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These authors have no conflict of interest to declare.

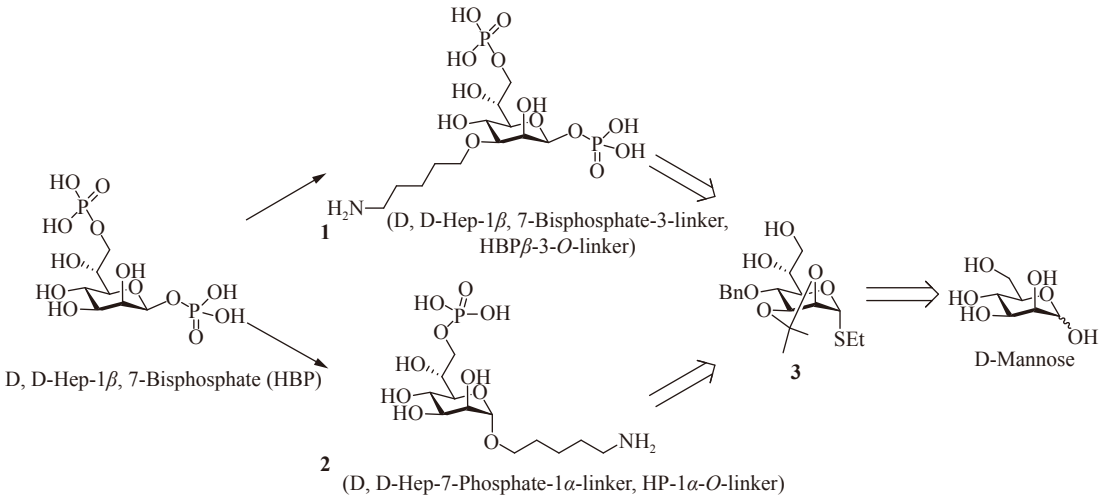


Fig. 1 Retrosynthetic analysis of HBP*β*-3-*O*-linker 1 and HP-1*α*-*O*-linker 2

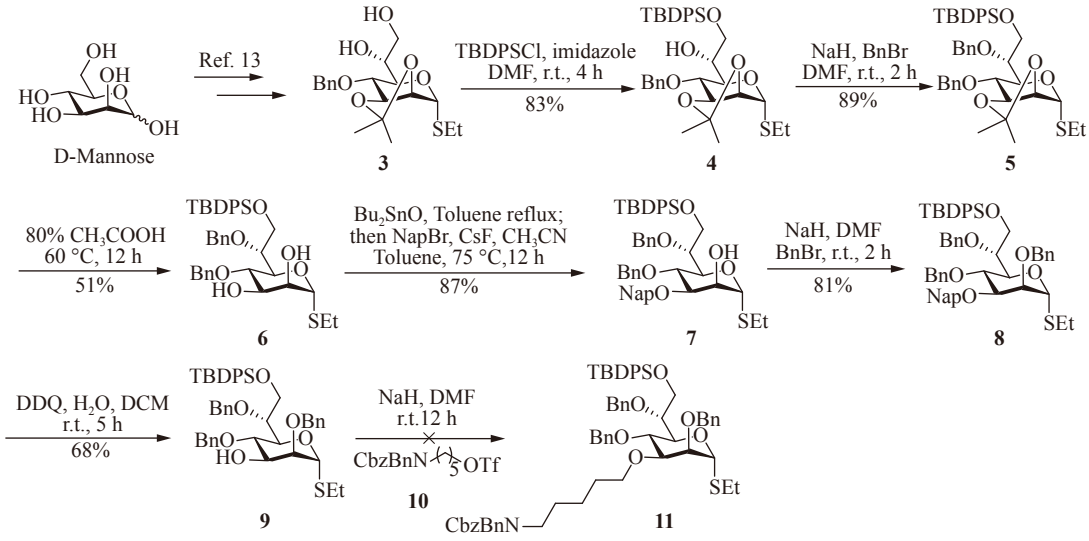
reactions [8]. Thirdly, the 1*β* phosphate ester linkage is difficult to construct on mannose-type sugars as a consequence of the anomeric and the neighboring-group participation effects [9]. While chemical syntheses of mannose 1*β*-phosphates and heptose 1*β*-phosphates have been studied for decades [10-12], still no general approach for the formation of mannose-type *β*-phosphate ester linkages exists. Finally, the alkylation of the 3-hydroxyl group is a challenging task due to possible steric effect and protecting group influence.

Result and Discussion

Synthesis of HBPβ-3-O-amyl amine 1

The retrosynthetic analysis reveals that both targets, HBP*β*-3-*O*-linker **1** and HP-1*α*-*O*-linker **2**, can obtained from common intermediate **3** (Fig. 1). In turn, D, D-Hep **3** can be synthesized from D-mannose using methods we established previously [13].

Regioselective *O*7-silylation of diol **3** in the presence of *tert*-butyldiphenylsiyl chloride (TBDPSCl) gave the silyl ether **4** in 83% yield (Scheme 1). The C6 hydroxyl group was benzylated to afford **5** in 89% yield. Subsequently, the 2- and 3-hydroxyl groups were deprotected by the treatment of 80% aqueous acetic acid at 60 ºC to give diol **6** in moderate yield. Regioselective installation of a 2-naphtylmethyl (Nap) group at the *O*3 position by treatment with dibutyltin oxide and 2-naphtylmethyl bromide gave compound **7** in 87% yield. The 2-*O*-benzylation was achieved in 81% yield with benzyl bromide and sodium hydride. *O*3-Nap was subsequently removed by 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) to give **9** in 68% yield. With alcohol **9** and triflate linker **10** in hand, *O*3-alkylation commenced. Coupling of **9** with linker **10** using sodium hydride as a promoter in anhydrous DMF failed to furnish target product **11** as only starting materials were recovered. Since the steric hindrance of the C3-hydroxyl group in **9** may be an obstacle for *O*3-alkylation, reaction conditions for heptose needed to be adjusted to de-

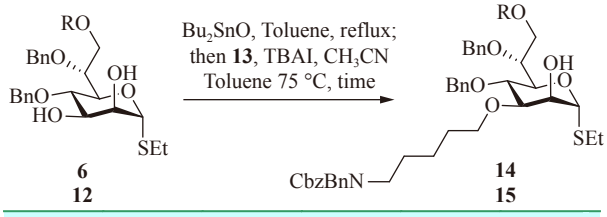


Scheme 1 Attempted synthesis of thioglycoside intermediate 11

crease steric crowding at the *O*3 position.

Inspired by the regioselective *O*3-benzylation and *O*3-naphtylmethylation of 2, 3-dihydroxyl-mannose mediated by dibutyltin oxide [14], the regioselective installation of 1-bromoamyl amine linker **13** [15] on 2, 3-dihydroxyl-heptose was studied (Table 1). Diol **6** bearing TBDPS at the *O*7 position was treated with dibutyltin oxide and amine linker **13** to afford compound **14** in 41% yield. It should be noticed that TBDPS is a bulky protecting group that may affect the reaction efficiency by steric hindrance. Diol **12** [13] bearing the smaller Nap group at the *O*7 position was synthesized to optimize this

Table 1 Regioselective installation of amyl amine linker at the C3 position



Entry	Hep	R	*t*/h	Product	Yield/%[a]
1	**6**	TBDPS	24	**14**	41
2	**12**	Nap	12	**15**	95

[a] all yield correspond to chromatographicallypurified product; **13**: CbzBnN$(CH_2)_5$Br

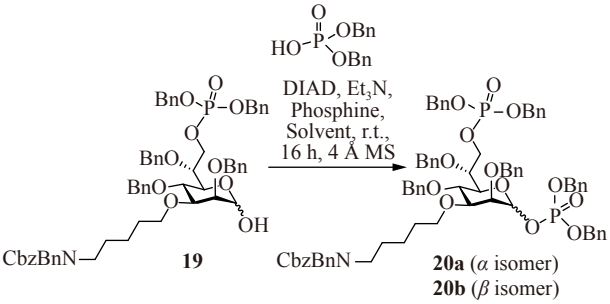
regioselective alkylation. The desired compound **15** containing 3-*O*-amyl amine linker was successfully obtained in 95% yield, demonstrating that the low reactivity of the C3 hydroxyl group can be mainly attributed to a steric effect.

Benzylation of **15** with benzyl bromide in the presence of sodium hydride was followed by removal of *O*7-Nap with DDQ (Scheme 2). Phosphorylation of alcohol **17** using 1*H*-tetrazole as activator and subsequent oxidation with *t*-BuOOH [16] afforded desired phosphate-diester-containing **18** in 61% yield. Subsequently, the anomeric thioethyl group in **18** was removed using *N*-bromosuccinimide to give hemiacetal **19** in 87% yield.

With hemiacetal **19** in hand, introduction of the phosphate group at the anomeric position under Mitsunobu reaction conditions [17] was studied (Table 2). Different phosphines and solvents were tested based on Fujumoto's previous work [5]. Unfortunately, biphosphate product **20** was produced in low yield and poor β-selectivity under these reaction conditions. The configuration of newly formed glycosidic bonds in **20a** and **20b** was clearly confirmed by anomeric proton $J_{C,H}$ coupling of 176 Hz (for α-isomer) and 160 Hz (for β-isomer). A comparison between compound **19** and reported substrates [5] of Mitsunobu reaction showed that the problems are likely caused by the presence of the 3-*O*-linker in **19**.

Alternatively, NIS-mediated glycosylation of thioglycoside **18** with dibenzyl phosphate [18] was chosen to form the

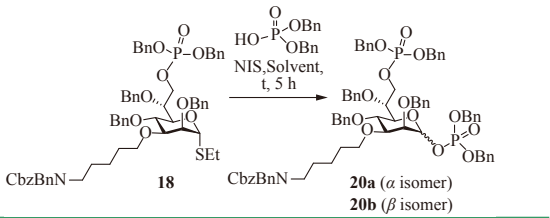
Table 2 Efforts toward 1β-phosphate group *via* a Mitsunobu reaction



Entry	Phosphine	Solvent	Yield/%[a]	**20a**/**20b** (α/β[b])
1	*n*-Bu_3P	DCM	15	13 : 1
2	*n*-Bu_3P	CH_3CN	trace[c]	/
3	*n*-Bu_3P	Toluene	7	12 : 1
4	Ph_3P	DCM	11	12 : 1

[a] All yield correspond to chromatographically purified product; [b] The ratio is determined after isolation; [c] Trace amount of **20a** and **20b** was detected by LC-MS

Table 3 Synthesis of compound 20a and 20b



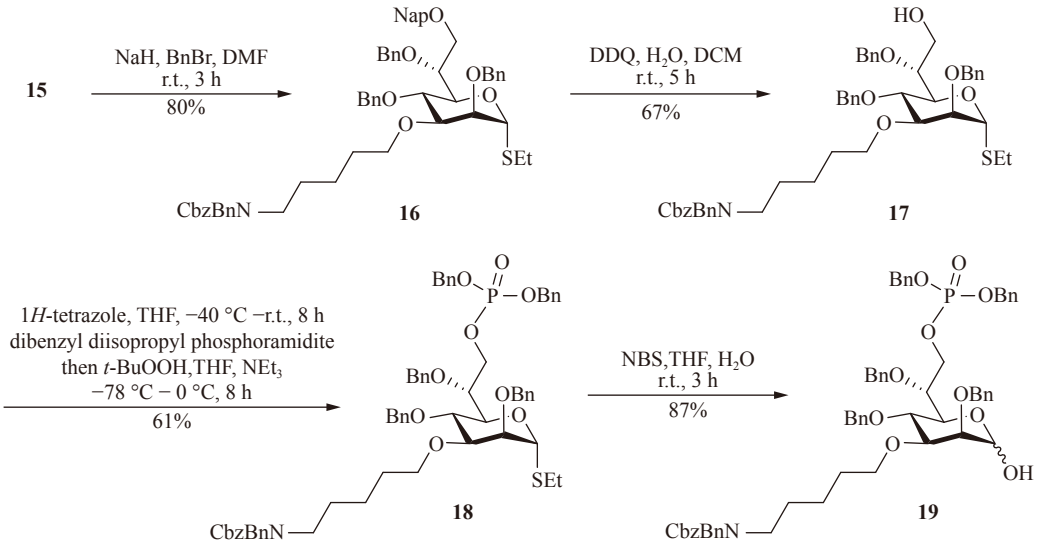
Entry	T[a]/°C	Solvent	Yield/%[b]	**20a**/**20b** (α/β[c])
1	0	DCM	64	6 : 1
2	0	CH_3CN	21	7 : 1
3	−20	DCM	91	2.5 : 1

[a] T, reaction temperature; [b] All yield correspond to chromatographically purified product; [c] The ratio is determined after isolation

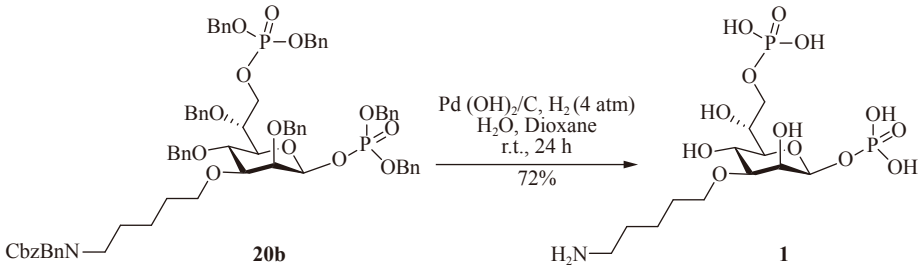
anomeric phosphate group (Table 3). The coupling of **18** and dibenzyl phosphate under 0 ºC using DCM as solvent gave **20** in 64% yield (α/β = 6 : 1). Surprisingly, the glycosylation in CH_3CN, a well-known β-selectivity-enhancing solvent, at the same temperature gave biphosphate **20** in lower yield and β-selectivity. Significantly, the yield and β-selectivity of anomeric phosphorylation in DCM was promoted by decreasing the reaction temperature to −20 ºC. The final global deprotection of desired **20b** was achieved under $Pd(OH)_2/C$ and H_2 condition to give **1** in 72% yield (Scheme 3).

Synthesis of HB-1α-amyl amine ***2***

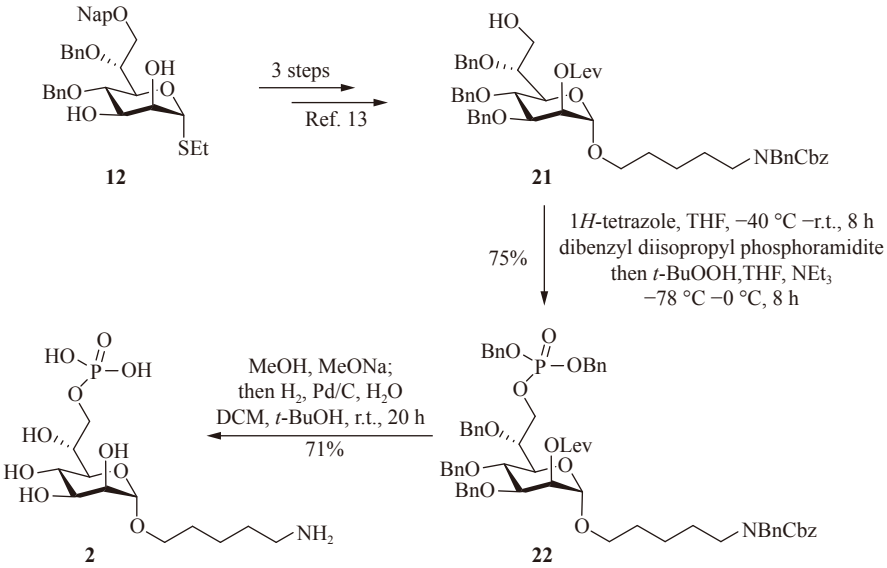
Compound **21** [13] was readily synthesized in three steps from **12** (Scheme 4). The phosphate ester at C7 was installed under the same phosphorylation condition as during the synthesis of **18** to give **22** in 75% yield. The final deprotection was subsequently performed in the presence of Pd/C and H_2



Scheme 2 Synthesis of hemiacetal 19



Scheme 3 Global deprotection of 20b



Scheme 4 Synthesis of monophosphate HBP derivative 2

to give **2** in 71% yield.

Conclusion

The carbohydrate antigens D-*glycero*-D-*mannno*-heptose 1*β*, 7-bisphosphate **1** containing a 3-*O*-amyl amine linker and D-*glycero*-D-*mannno*-heptose 7-phosphate **2** equipped with an *O*-amyl amine linker at the anomeric position were synthesized from D-mannose. Installation of the amyl amine linker at C3 position of heptose was achieved efficiently by dibutyltin oxide-mediated regioselective alkylation under fine-tuned protecting condition. The 1*β* anomeric phosphoester was obtained by optimized NIS-mediated phosphorylation

condition in good yield and stereoselectivity. The synthetic HBP derivatives **1** and **2** can be conjugated and immobilized *via* the amine linker to facilitate subsequent biological studies. The synthesis of HBP derivative **1** equipped with an 3-*O*-amyl amine linker will serve well for the syntheses of other conjugation-ready carbohydrates bearing anomeric phosphoester.

Experimental

All detailed experimental data were provided in supplementary material, and can be requested by sending E-mail to the corresponding author.

References

[1] Muthana S, Cao H, Chen X. Recent progress in chemical and chemoenzymatic synthesis of carbohydrates [J]. *Curr Opin Chem Bio*, 2009, **13**(5): 573-581.

[2] Vliegenthart JF. Carbohydrate based vaccines [J]. *FEBS Lett*, 2006, **580**(12): 2945-2950.

[3] Kneidinger B, Marolda C, Graninger M, *et al*. Biosynthesis pathway of ADP-L-glycero-beta-D-manno-heptose in *Escherichia coli* [J]. *J Bacteriol*, 2002, **184**(2): 363-369.

[4] Gaudet RG, Sintsova A, Buckwalter CM, *et al*. Cytosolic detection of the bacterial metabolite HBP activates TIFA-dependent innate immunity [J]. *Science*, 2015, **348**(6240): 1251-1255.

[5] Inuki S, Aiba T, Kawakami S, *et al*. Chemical synthesis of D-*glycero*-D-*manno*-heptose 1, 7-bisphosphate and evaluation of its ability to modulate NF-*κ*B activation [J]. *Org Lett*, 2017, **19**(12): 3079-3082.

[6] Wang L, Huang H, Nguyen HH, *et al*. Divergence of biochemical function in the HAD superfamily: D-glycero-D-manno-heptose-1, 7-bisphosphate phosphatase (GmhB) [J]. *Biochemistry*, 2010, **49**(6): 1072-1081.

[7] Liu Y, Chan YM, Wu J, *et al*. Chemical synthesis of a bisphosphorylated mannose-6-phosphate *N*-glycan and its facile mono-conjugation with human carbonic anhydrase II for *in vivo* fluorescence imaging [J]. *Chembiochem*, 2011, **12**(5): 685-690.

[8] Garcia AB, Gin DY. Synthesis of glycosyl-1-phosphates *via* dehydrative glycosylation [J]. *Org Lett*, 2000, **2**(14): 2013-2138.

[9] Li T, Tikad A, Pan W, *et al*. beta-Stereoselective phosphorylations applied to the synthesis of ADP- and polyprenyl-beta-mannopyranosides [J]. *Org Lett*, 2014, **16**(21): 5628-5631.

[10] Zamyatina A, Gronow S, Puchberger M, *et al*. Efficient chemical synthesis of both anomers of ADP L-glycero- and D-glycero-D-manno-heptopyranose [J]. *Carbohydr Res*, 2003, **338**(23): 2571-2589.

[11] Sabesan S, Neira S. Synthesis of glycosyl phosphates and azides [J]. *Carbohydr Res*, 1992, **223**(7): 169-185.

[12] Crich D, Dudkin V. Efficient, diastereoselective chemical synthesis of a *β*-mannopyranosyl phosphoisoprenoid [J]. *Org Lett*, 2000, **2**(24): 3941-3943.

[13] Zou X, Qin C, Pereira C, *et al*. Synergistic glycosylation as key to the chemical synthesis of an outer core octasaccharide of helicobacter pylori [J]. *Chem Eur J*, 2018, **24**(12): 2868-2872.

[14] Qin C, Schumann B, Zou X, *et al*. Total synthesis of a densely functionalized *Plesiomonas shigelloides* serotype 51 aminoglycoside trisaccharide antigen [J]. *J Am Chem Soc*, 2018, **140**(8): 3120-3127.

[15] Mandal SS, Duncombe L, Ganesh NV, *et al*. Novel solutions for vaccines and diagnostics to combat brucellosis [J]. *ACS Cent Sci*, 2017, **3**(3): 224-231.

[16] Liu L, Pohl NLB. A fluorous phosphate protecting proup with applications to carbohydrate synthesis [J]. *Org Lett*, 2011, **13**(7): 1824-1827.

[17] Mitsunobu O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products [J]. *Synthesis*, 1981, **1**: 1-28.

[18] Schumann B, Pragani R, Anish C, *et al*. Synthesis of conjugation-ready zwitterionic oligosaccharides by chemoselective thioglycoside activation [J]. *Chem Sci*, 2014, **5**(5): 1992-2002.

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