### Title

Efficient Enzymatic Synthesis of Guanosine 5′-Diphosphate-Sugars and Derivatives




### Journal

Organic Letters, 15(21)

### ISSN

1523-7060



### Authors

Li, Lei
Liu, Yonghui
Wan, Yue
et al.



### Publication Date

2013-11-01

### DOI

10.1021/ol402585c

Peer reviewed

Published in final edited form as:
*Org Lett*. 2013 November 1; 15(21): 5528–5530. doi:10.1021/ol402585c.

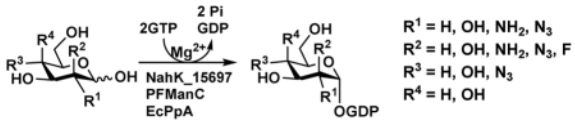
# Efficient Enzymatic Synthesis of Guanosine 5′-Diphosphate-Sugars and Derivatives

**Lei Li**[†,‡,¶], **Yonghui Liu**[†,¶], **Yue Wan**[†], **Yanhong Li**[§], **Xi Chen**[§], **Wei Zhao**[†], and **Peng George Wang**[†,‡]

State Key Laboratory of Medicinal Chemical Biology and College of Pharmacy, Nankai University, Tianjin 300071, Center for Diagnostics & Therapeutics and Department of Chemistry, Georgia State University, Atlanta, GA 30303, and Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, California 95616, USA

Wei Zhao: wzhao@nankai.edu.cn; Peng George Wang: pwang11@gsu.edu

## Abstract




An *N*-acetylhexosamine 1-kinase from *Bifidobacterium infantis* (NahK_15697), a guanosine 5′-diphosphate (GDP)-mannose pyrophosphorylase from *Pyrococcus furiosus* (PFManC), and an *Escherichia coli* inorganic pyrophosphatase (EcPpA) were used efficiently for a one-pot three-enzyme synthesis of GDP-mannose, GDPglucose, their derivatives, and GDP-talose. This study represents the first facile and efficient enzymatic synthesis of GDP-sugars and derivatives starting from monosaccharides and derivatives.

Glycosyltransferases are key enzymes responsible for the assembly of carbohydrates. Most of these enzymes require activated sugar-nucleotides as donor substrates. Thus, development of facile protocols for efficient synthesis of such molecules are of great significance and has been an active field of research.[1] Among guanosine 5′-diphosphate (GDP)-activated sugars, GDP-mannose (GDP-Man) is essential for the biosynthesis of mannosyl donor dolichol phosphate β-D-mannose (Dol-P-Man) involved in the synthesis of eukaryotic *N*-glycans, glycosylphospho-inositol (GPI) anchors, and O-mannosylated glycoproteins,[2] as well as bacterial cell-surface polysaccharides.[3] GDP-Man is also a fundamental metabolic intermediate for the biosynthesis of many other natural GDP-sugars, including GDP-mannuronic acid (GDP-ManA), GDP-L-fucose (GDP-Fuc), and GDP-6-deoxy-Talose (GDP-6deoxyTal) etc.[1] Other GDP-sugars, such as GDP-glucose (GDP-Glc) and GDP-glucosamine (GDP-Glc$NH_2$), are key intermediates in the biosynthesis of β1,4-glucans, glucosylglycerate, and legionaminic acid-containing glycoconjugates.[4]

Correspondence to: Wei Zhao, wzhao@nankai.edu.cn; Peng George Wang, pwang11@gsu.edu.
[†]Nankai University.
[‡]Georgia State University.
[§]University of California, Davis.
[¶]Contributed equally to this work.

Supporting Information Available: Experimental details for cloning, over-expression, and purification of NahK_15697, PFManC, EcPpA, chemical synthesis of monosaccharide derivatives, and enzymatic synthesis of GDP-sugars and derivatives, as well as NMR and HRMS data and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

Chemical synthesis of sugar-nucleotides generally suffers from tedious protection/deprotection steps, low total yields, and long reaction times.[5] On the other hand, enzymatic approaches following *de novo* biosynthetic pathways requires multiple enzymes and laborious separation processes. Recently, salvage biosynthetic pathways of several sugar-nucleotides were discovered, which usually involve two enzyme-catalyzed steps: 1) a kinase-catalyzed formation of monosaccharide 1-phosphate from the corresponding monosaccharide and ATP; 2) a pyrophosphorylase-catalyzed formation of sugar-nucleotide and pyrophosphate by-product from nucleotide triphosphate and the monosaccharide 1-phosphate. Taking advantage of promiscuous enzymes involved in these pathways, efficient chemo-enzymatic approaches were developed for preparative-scale synthesis of sugar-nucleotides and their non-natural derivatives. For example, a bifunctional L-fucose 1-kinase/GDP-Fuc pyrophosphorylase (FKP) from *Bacteroides fragilis* was applied successfully for the synthesis of GDP-Fuc and derivatives.[6] In addition, monosaccharide 1-kinases and a promiscuous UDP-sugar pyrophosphorylase (BLUSP) were used efficiently for one-pot enzymatic synthesis of UDP-hexose and derivatives from simple hexose and derivatives.[7] Furthermore, a panel of UDP-HexNAc and derivatives were chemo-enzymatically prepared by combining an *N*-acetylhexosamine 1-kinase (NahK) and an UDP-N-acetylglucosomine pyrophosphorylase (GlmU or AGX1) in either a one-pot or a sequenctial manner.[8,9]

Nevertheless, such a simple synthetic route has not yet been developed for the synthesis of GDP-Man and other GDP-sugars, mainly due to the lack of suitable monosaccharide 1-kinases. As a result, chemically prepared or commercially available manose 1-phosphate and derivatives were generally used in the formation of GDP-sugars.[10,11,12] We recently found that a NahK from *Bifidobacterium infantis* ATCC15697 (NahK_15697) could phosphorylate a number of monosaccharides including mannose and derivatives.[13] Taking advantage of this and the promiscuity NahK_15697 and a GDP-Man pyrophosphorylase from *Pyrococcus furiosus* DSM3638 (PFManC),[12] we present here an efficient one-pot three-enzyme system for quick preparative-scale synthesis of GDP-sugars and their derivatives.

As shown in Scheme 1, three enzymes were used in one-pot to synthesize GDP-Man, GDP-Glc, their derivatives and GDP-Tal. The first enzyme was NahK_15697, which catalyzed the formation of monosaccharide 1-phosphates. The second enzyme was PFManC, which catalyzed the reversible formation of GDP-sugars and pyrophosphate from monosaccharide 1-phosphates and guanosine 5′-triphosphate (GTP). The last enzyme was an inorganic pyrophosphatase cloned from *Escherichia coli* (EcPpA).[14] It drove the reaction towards the formation of GDP-sugars by hydrolyzing the pyrophosphate by-product.

Genetic analysis showed that the DNA sequence of the archaeal enzyme PFManC contains numerous rare codons. To increase the heterologous protein expression level in *E. coli*, the DNA sequence of PFManC was codon optimized. The synthetic gene obtained by custom synthesis was cloned into pET22b(+) vector. The protein was overexpressed in *E. coli* BL21(DE3), yielding over 80 mg of PFManC per liter cell culture after purification.[15]

Besides GTP, it was reported that PFManC could also utilize ATP to form ADP-sugars.[12] In order to avoid unexpected by-product formation in the one-pot system, GTP, instead of ATP, was used as the phosphate donor for NahK_15697 (Scheme 1). To our delight, GTP was a suitable substrate for NahK_15697. As shown in Table S1 and Figure S2, except for Man4$N_3$ (**6**) which had a relatively low yield of 36%, NahK_15697 was able to use GTP as a phosphate donor for high-yield (>53%) phosphorylation of all other monosaccharides and derivatives tested including mannose (**1**) and its derivatives (**2**–**5**), talose (**7**), as well as glucose (**8**) and its C2-derivatives (**9**–**12**). The results confirmed previously reported broad substrate specificity of NahK toward both monosaccharides and phosphate donors.[8,13,16] We also tested a number of C6 modified substrates, including Rha (**25**), Rha4$N_3$ (**26**), PerNAc

(**27**), 6-deoxyTal (**28**), and ManA (**29**), but none was a suitable substrate (Table S1 and Figure S2) for NahK_15697 when either ATP or GTP was used as the phosphate donor. The results imply that the C6 hydroxyl group may play essential roles in substrate recognition by NahK_15697.

The synthesis of GDP-sugars was carried out using the one-pot three-enzyme system shown in Scheme 1.[17] As listed in Table 1,[18] the system was quite efficient in synthesizing GDP-Man (**13**, 94%), GDP-Man$NH_2$ (**14**, 75%), GDP-Man$N_3$ (**15**, 81%), GDP-ManF (**17**, 84%), GDP-Glc (**20**, 72%), GDP-2-deoxyGlc (**21**, 76%), and GDP-Glc$NH_2$ (**22**, 80%) from corresponding mono-saccharides and derivatives (**1**–**3**, **5**, **8**–**10**). GDP-Man4$N_3$ (**18**), a potential non-radioactive probe for investigating the activity of mannosyltransferases,[11] was synthesized with a moderate yield of 33%, most likely due to the less optimal activity of NahK_15697 for Man4$N_3$ (**6**). The system also provided a moderate yield (47%) and a low yield (16%) for the formation of GDP-Tal (**19**) and GDP-Glc$N_3$ (**23**), respectively, which may be attributed by less optimal PFManC activity for Tal 1-phosphate and Glc$N_3$ 1-phosphate. On the other hand, the synthesis of GDP-ManNAc (**16**) and GDP-GlcNAc (**24**) using the one-pot three-enzyme system was not successful, suggested that substrates with bulkier groups at C2 position are not acceptable for PFManC.

Concerning the report that PFManC exhibited optimal activity at 80 °C, and was able to synthesize GDP-GlcNAc from GlcNAc 1-phosphate,[12] a one-pot two-step strategy was also tested for the preparation of GDP-ManNAc and GDP-GlcNAc. In general, reactions were firstly carried out in Tris-HCl buffer (100 mM, pH 8.0) containing ManNAc or GlcNAc (15 mM), GTP (35 mM), $MgCl_2$ (10 mM), and NahK_15697 (0.4 mg/mL). After incubation at 37 °C for 24 hr, PFManC (0.5 mg/mL) and excess of EcPpA were added and the reactions were allowed to proceed at 80 °C for up to 6 hr. Unfortunately, neither of the reactions resulted in detectable GDP-sugars. More experiments are required to further identify the substrate specificity of PFManC.

In conclusion, we have further investigated the substrate specificity of NahK_15697 and PFManC using chemically or enzymatically prepared compounds, and have developed an efficient one-pot three-enzyme system to quickly obtain GDP-Man, GDP-Glc, their non-natural derivatives, and GDP-Tal from simple monosaccharides and derivatives in preparative-scale. These structurally defined GDP-sugars and derivatives are excellent compounds for investigating the substrate specificity of glycosyltransferases (e. g. mannosyltransferases).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

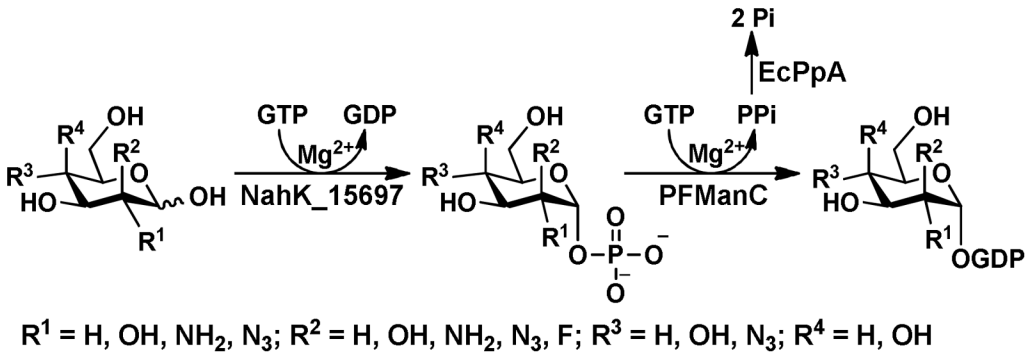
## Acknowledgments

This work was supported by National Natural Science Foundation of China (Grant No. 31100587 to L. Li, 21332006 to W. Zhao), NIH grants R01HD065122 (to X. Chen) and R01HD061935 (to P. G. Wang), NSF grant CHE-1012511 (to X. Chen), and Natural Science Foundation of Tianjin (Grant No. 12JCYBJC18600 to W. Zhao).

## References

1. (a) Thibodeaux CJ, Melancon CE 3rd, Liu HW. Angew Chem Int Ed. 2008; 47:9814–59.(b) Wagner GK, Pesnot T, Field A. Nat Prod Rep. 2009; 26:1172–94. [PubMed: 19693414]
2. (a) Essentials of Glycobiology. 2. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2008. (b) Loibl M, Strahl S. Biochim Biophys Acta. 2013; 1833:2438–46. [PubMed: 23434682]

3. (a) Goto M. Biosci Biotech Bioch. 2007; 71:1415–27.(b) Samuel G, Reeves P. Carbohydr Res. 2003; 338:2503–19. [PubMed: 14670712]
4. (a) Carpita NC. Plant Physiol. 2011; 155:171–84. [PubMed: 21051553] (b) Costa J, Empadinhas N, Da Costa MS. J Bacteriol. 2007; 189:1648–54. [PubMed: 17189358] (c) Schoenhofen IC, Vinogradov E, Whitfield DM, Brisson JR, Logan SM. Glycobiology. 2009; 19:715–25. [PubMed: 19282391]
5. (a) Coyne MJ, Reinap B, Lee MM, Comstock LE. Science. 2005; 307:1778–81. [PubMed: 15774760] (b) Kleczkowski L, Decker D, Milczynska M. Plant Physiol. 2011; 156:3–10. [PubMed: 21444645]
6. (a) Yi W, Liu X, Li Y, Li J, Xia C, Zhou G, Zhang W, Zhao W, Chen X, Wang PG. Proc Natl Acad Sci U S A. 2009; 106:4207–12. [PubMed: 19251666] (b) Wang W, Hu T, Frantom PA, Zheng T, Gerwe B, Del Amo DS, Garret S, Deidel RD 3rd, Wu P. Proc Natl Acad Sci U S A. 2009; 106:16096–101. [PubMed: 19805264]
7. Muthana MM, Qu J, Li Y, Zhang L, Yu H, Ding L, Halekan H, Chen X. Chem Commun. 2012; 48:2728–30.
8. (a) Cai L, Guan W, Kitaoka M, Shen J, Xia C, Chen W, Wang PG. Chem Commun. 2009; 45:2944–6.(b) Cai L, Guan W, Wang W, Zhao W, Kitaoka M, Shen J, O'Neil C, Wang PG. Bioorg Med Chem Lett. 2009; 19:5433–5. [PubMed: 19683921]
9. (a) Guan W, Cai L, Fang J, Wu B, Wang PG. Chem Commun. 2009; 45:6976–8.(b) Chen Y, Thon V, Li Y, Yu H, Ding L, Lau K, Qu J, Hie L, Chen X. Chem Commun. 2011; 47:10815–7.
10. (a) Watt GM, Flitsch SL, Fey S, Elling L, Kragl U. Tetrahedron: Asymmetry. 2000; 11:621–8.(b) Zou L, Zheng RB, Lowary TL. Beilstein J Org Chem. 2012; 8:1219–26. [PubMed: 23019451]
11. Marchesan S, Macmillan D. Chem Commun. 2008; 44:4321–4323.
12. Mizanur RM, Pohl NL. Org Biomol Chem. 2009; 7:2135–9. [PubMed: 19421452]
13. Li Y, Yu H, Chen Y, Lau K, Cai L, Cao H, Tiwari VK, Qu J, Thon V, Wang PG, Chen X. Molecules. 2011; 16:6396–407. [PubMed: 21799473]
14. Lahti R, Pitkaeranta T, Valve E, Ilta I, Kukko-kalske E, Heinonen J. J Bacteriol. 1988; 170:5901–7. [PubMed: 2848015] (b) See Supporting Information for details about cloning, over-expression and purification.
15. See Supporting Information for details about cloning, over-expression and purification of PFManC.
16. Nishimoto M, Kitaoka M. Appl Environ Microb. 2007; 73:6444–9.
17. See Supporting Information for reaction details.
18. All NMR and MS data are available in Supporting Information.

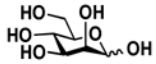
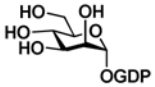
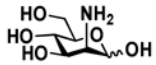
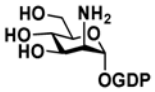
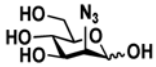
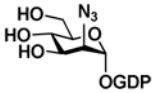
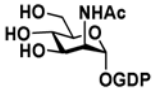
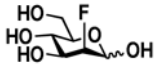
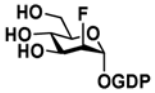
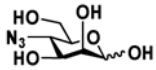
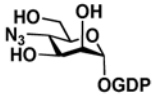
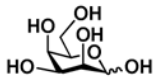
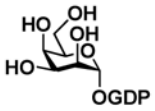
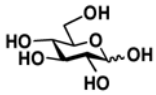
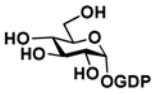
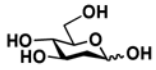
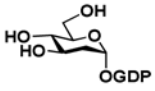


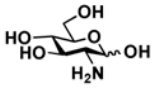
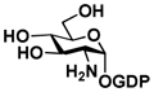
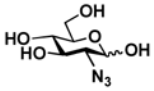
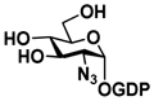
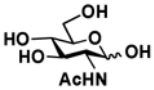
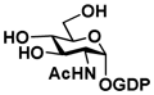

**Scheme 1. One-pot three-enzyme synthesis of GDP-sugars**

$R^1$ = H, OH, $NH_2$, $N_3$; $R^2$ = H, OH, $NH_2$, $N_3$, F; $R^3$ = H, OH, $N_3$; $R^4$ = H, OH

## Table 1

Synthesis of GDP-sugars using the one-pot three-enzyme system shown in Scheme 1

| substrate | product | yield [a] (%) | scale [b] (mg) |
|---|---|---|---|
|  **1** Man |  **13** GDP-Man | 94 | 102 |
|  **2** $ManNH_2$ |  **14** GDP-$ManNH_2$ | 75 | 84 |
|  **3** $ManN_3$ |  **15** GDP-$ManN_3$ | 81 | 92 |
|  **4** ManNAc |  **16** GDP-ManNAc | ND[c] | |
|  **5** ManF |  **17** GDP-ManF | 84 | 91 |
|  **6** $Man4N_3$ |  **18** GDP-$Man4N_3$ | 33 | 37 |
|  **7** Talose |  **19** GDP-Tal | 47 | 51 |
|  **8** Glc |  **20** GDP-Glc | 72 | 78 |
|  **9** 2-deoxyGlc (2-deoxyMan) |  **21** GDP-2-deoxyGlc | 76 | 80 |

| substrate | product | yield [a] (%) | scale [b] (mg) |
|---|---|---|---|
|  |  | 80 | 87 |
| **10** $GlcNH_2$ | **22** GDP-$GlcNH_2$ | | |
|  |  | 16 | 18 |
| **11** $GlcN_3$ | **23** GDP-$GlcN_3$ | | |
|  |  | ND | |
| **12** GlcNAc | **24** GDP-GlcNAc | | |

[a] Isolated yields from P-2 column.

[b] The mass of isolated product.

[c] ND, not detected.