

# Efficient and Concise Synthesis of $\beta$ Man1–4GlcN Linkage of Pentasaccharide Core by Using 6-Nitro-2-benzothiazolyl $\alpha$ -Mannoside

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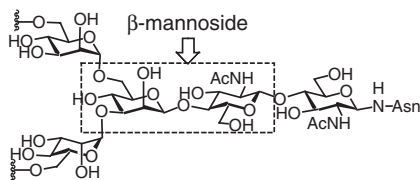
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Efficient and concise synthesis of  $\beta$ Man1–4GlcN of pentasaccharide core is established; direct  $\beta$ -mannosylations of 4-OH group of 2-deoxy-2-phthaloyl and 2-azide-2-deoxy glucose derivatives by using 6-nitro-2-benzothiazolyl  $\alpha$ -mannoside proceeded smoothly to afford the desired  $\beta$ -mannosides in high yields.

N-linked glycans are known to play numerous important biological roles in cellular interactions.<sup>1</sup> They are generally divided into three classes; namely, high-mannose, complex, and hybrid types depend on structures of oligosaccharide chains.<sup>1</sup> All types of N-linked glycans have the common pentasaccharide core including  $\beta$ Man(1→4)GlcNAc linkage (Figure 1). Although many methods to synthesize common pentasaccharide core have been reported,<sup>2</sup> there are only a few efficient and versatile methods for construction of  $\beta$ Man(1→4)GlcNAc linkage because of its synthetic difficulties. Crich's direct coupling method was thought to be the best in forming  $\beta$ Man(1→4)-GlcNAc linkage when 2-azide-2-deoxy glucose<sup>3</sup> or 2-deoxy-2-sulfonamide chitobiose<sup>4</sup> derivative having reactive 4-OH group was used. While,  $\beta$ -mannosylation of less reactive 4-OH of 2-deoxy-2-phthaloyl glucose derivatives afforded the desired products in moderate yields.<sup>5,6</sup> Thus formed disaccharide was used in further elongation of  $\beta$ -saccharide linkage because its reducing end was effectively activated by neighboring effect of 2-PhthN group.<sup>6</sup>

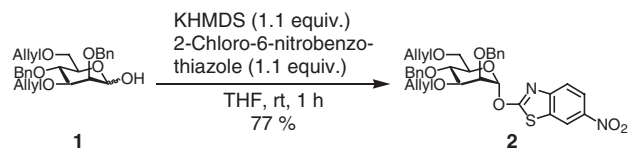


**Figure 1.** Common pentasaccharide core of N-linked glycans.

It was recently disclosed that 6-nitro-2-benzothiazolyl  $\alpha$ -glucoside and  $\alpha$ -mannoside, novel glycosyl donors, were reactive enough to construct  $\beta$ -saccharide linkages via  $S_N2$ -type process.<sup>7</sup> In this paper, we would like to report a general and effective method for  $\beta$ -mannosylation of 4-OH group of 2-deoxy-2-phthaloyl and 2-azide-2-deoxy glucose derivatives to prepare the part of pentasaccharide core.

The present study started from the preparation of 6-nitro-2-benzothiazolyl 3,6-di-*O*-allyl-2,4-di-*O*-benzyl- $\alpha$ -D-mannopyranoside **2** from the precursor **1**<sup>8</sup> according to our previously reported procedure.<sup>7</sup> The condensation reaction proceeded smoothly at room temperature to afford 6-Nitro-2-benzothiazol-

yl  $\alpha$ -mannoside **2** in 77% yield as a major product (Scheme 1). Although 6-nitro-2-benzothiazolyl  $\beta$ -mannoside was detected by thin layer chromatography, it was too labile to isolate in pure form. The anomeric configuration of **2** was determined to be  $\alpha$ -mannoside by measurement of NMR spectrum that showed  $^1J_{CH} = 177$  Hz between H-1 and C-1.<sup>9</sup>



**Scheme 1.** Synthesis of mannosyl donor **2**.

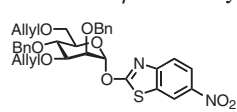
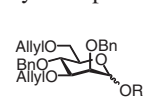
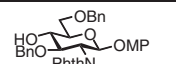
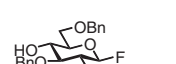
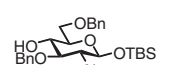
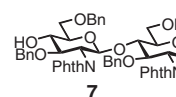
Mannosylation between mannosyl donor **2** (1.2 equiv.) and *p*-methoxyphenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- $\beta$ -D-glucopyranoside **3**<sup>10</sup> (1.0 equiv.) was carried out under previously reported conditions<sup>7</sup> and the desired  $\beta$ -mannoside **4**<sup>6</sup> was obtained in 52% yield along with 25% yield of the  $\alpha$ -one (Table 1, Entry 1). This low  $\beta$ -selectivity was thought that less reactive 4-OH group of glycosyl acceptor **3** generated the undesired oxonium ion intermediate which lead to formation of  $\alpha$ -mannoside.<sup>7</sup>

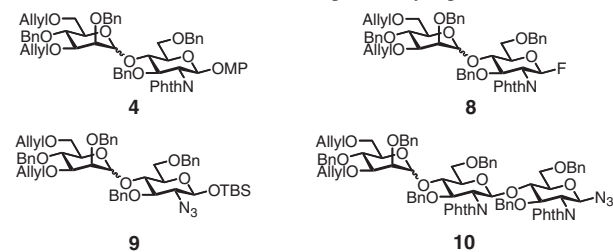
In order to improve the yield of the desired  $\beta$ -mannoside, optimization of several reaction conditions; that is, the molar ratios between mannosyl donor **2** and glycosyl acceptor **3**, concentration of the reaction mixture, and an experimental procedure were further examined in detail. After the reaction conditions were optimized, the scope of this mannosylation reaction was studied. Direct  $\beta$ -mannosylation of several glycosyl acceptors such as 4-OH of 2-deoxy-2-phthaloyl glucose derivatives **3**, **5**,<sup>6</sup> and **7**<sup>11</sup> or 4-OH of 2-azide-2-deoxy glucose derivative **6**<sup>12</sup> were carried out under the optimized reaction conditions (Table 1, Entries 2–6).<sup>13</sup>

All  $\beta$ -mannosylation reactions proceeded smoothly to afford the desired  $\beta$ -mannoside in higher yields compared with those shown in previously reported direct mannosylation method.<sup>3,6</sup> On the other hand, the mannosylation of glycosyl acceptor **5** having a fluorine atom on its reducing end gave disaccharide **8**<sup>6</sup> in 71% yield (Entry 3). The reason for this decrease in yield was explained by considering that the fluorine atom attached to disaccharide **8** was liberated by the interaction with  $HB(C_6F_5)_4$  catalyst during this mannosylation reaction.

This problem was overcome by shortening of the reaction time to 0.2 h (Entry 4), however,  $\beta$ -stereoselectivity was lower than the other glycosyl acceptors since the fluorine atom at anomeric position reduced the nucleophilicity of hydroxy group at C-4 position. The glycosyl acceptor **6** gave the best result in

**Table 1.**  $\beta$ -Mannosylation with several glycosyl acceptors

		Acceptor (1.0 equiv.) $\text{HB}(\text{C}_6\text{F}_5)_4$ (20 mol %) MS 5A (3 g/mmol) $\text{CH}_2\text{Cl}_2$ , $-78^\circ\text{C}$ , 1 h		
Donor <b>2</b> (1.8 equiv.)				Mannoside
Entry	Acceptor	Time/h	Mannoside	Yield/% ( $\alpha/\beta$ ) <sup>a</sup>
1 <sup>b</sup>		0.5	<b>4</b>	77 (33/67)
2	<b>3</b>	1	<b>4</b>	99 (18/82)
3		1	<b>8</b>	71 (22/78)
4	<b>5</b>	0.2	<b>8</b>	91 (26/74)
5		1	<b>9</b>	95 (13/87)
6	<b>6</b>	1	<b>10</b>	95 (20/80)
7				

<sup>a</sup>The  $\alpha/\beta$  ratios were determined by isolations of both stereoisomers.<sup>b</sup>The reaction was carried out under previously reported conditions.

the present method to afford the desired  $\beta$ -mannoside **9 $\beta$**  in 83% yield along with 12% of the  $\alpha$ -one (Entry 5). Further, it was interesting to note that the chitobiose acceptor **7** gave the desired  $\beta$ -trisaccharide **10 $\beta$**  in 76% yield along with 19% of the  $\alpha$ -one. The anomeric configurations of all mannositides were confirmed by  $^1J_{\text{CH}}$  coupling constant measurement. Ogawa et al. utilized trisaccharide **10 $\beta$**  prepared from **4 $\beta$**  or **8 $\beta$**  in the total synthesis of pentasaccharide core of N-linked glycans after removal of protecting group of allyl ether.<sup>6</sup>

It is noted that an efficient and concise method for synthesis of  $\beta\text{Man}1\text{--}4\text{GlcN}$ , a part of the pentasaccharide core, was established by using 6-nitro-2-benzothiazolyl  $\alpha$ -mannosyl donor **2**. This method was quite useful in direct mannosylation and provided several  $\beta$ -di- or trisaccharides in high yields.

Further studies for synthesis of pentasaccharide core of N-linked glycans are now in progress.

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## References and Notes

- R. A. Dwek, *Chem. Rev.*, **96**, 683 (1996).
- G. Arsequell and G. Valencia, *Tetrahedron: Asymmetry*, **10**, 3045 (1999); J. J. Gridley and H. M. I. Osborn, *J. Chem. Soc., Perkin Trans. 1*, **2000**, 1471; B. Ernst, G. W. Hart, and P. Sinay, "Carbohydrate in Chemistry and Biology, Part 1," WILEY-VCH, Weinheim etc. (2000), p 320; A. A.-H. Abdel-Rahman, S. Jonke, E. S. H. El Ashry, and R. R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, **41**, 2972 (2002); C.-H. Wong, "Carbohydrate-Based Drug Discovery," WILEY-VCH, Weinheim etc. (2003), p 253.
- V. Dudkin and D. Crich, *Tetrahedron Lett.*, **44**, 1787 (2003).
- V. Dudkin, J. S. Miller, and S. J. Danishefsky, *Tetrahedron Lett.*, **44**, 1791 (2003).
- D. A. Crich and V. Dudkin, *J. Am. Chem. Soc.*, **123**, 6819 (2001).
- I. Matsuo, Y. Nakahara, Y. Ito, T. Nukada, Y. Nakahara, and T. Ogawa, *Bioorg. Med. Chem.*, **3**, 1455 (1995); Z.-W. Guo, Y. Nakahara, and T. Ogawa, *Tetrahedron Lett.*, **38**, 4799 (1997).
- T. Mukaiyama, T. Hashihayata, and H. Mandai, *Chem. Lett.*, **32**, 340 (2003); T. Hashihayata, H. Mandai, T. Mukaiyama, and H. Mandai, *Chem. Lett.*, **32**, 442 (2003); T. Hashihayata and T. Mukaiyama, *Heterocycles*, **61**, 51 (2003); T. Hashihayata, H. Mandai, and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, **77**, 169 (2004).
- T. Ogawa, T. Kitajima, and T. Nukada, *Carbohydr. Res.*, **123**, C5 (1983).
- K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, **1974**, 293.
- T. Nakano, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, **243**, 43 (1993).
- M. R. Pratt and C. R. Bartizan, *J. Am. Chem. Soc.*, **125**, 6149 (2003).
- C. Metadata and T. Ogawa, *Carbohydr. Res.*, **234**, 75 (1992).
- A typical experimental procedure was as follows: To a stirred suspension MS 5A (150 mg) and glucosyl acceptor **7** (49.4 mg, 0.05 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) was successively added  $\text{HB}(\text{C}_6\text{F}_5)_4$  (0.05 M in toluene-Et<sub>2</sub>O (1:1), 0.20 mL, 0.01 mmol)<sup>14</sup> at  $-78^\circ\text{C}$  and, 5 min later, a solution of mannosyl donor **1** (55.9 mg, 0.09 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.25 mL) added over 30 min. After the mixture was stirred for 1 h at  $-78^\circ\text{C}$ , the reaction was quenched by adding sat. aq.  $\text{NaHCO}_3$ . Then, the mixture was filtered through Celite and the aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$ . The combined organic layer was washed with brine and dried over  $\text{Na}_2\text{SO}_4$ . After filtration and evaporation, the resulting residue was purified by preparative TLC (silica gel, toluene: EtOAc = 7:1) to afford  $\beta$ -mannoside **10 $\beta$**  (53.6 mg, 76%) and **10 $\alpha$**  (13.6 mg, 19%).
- $\text{HB}(\text{C}_6\text{F}_5)_4$  was generated according to literal procedure: H. Jona, H. Mandai, W. Chavasiri, K. Takeuchi, and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, **75**, 291 (2002).