Efficient and Concise Synthesis of βMan1–4GlcN Linkage of Pentasaccharide Core by Using 6-Nitro-2-benzothiazolyl α-Mannoside

Hiroki Mandai[†,††,†††] and Teruaki Mukaiyama*[†,††]

[†]*Center for Basic Research, The Kitasato Institute (TCI), 6-15-5 Toshima, Kita-ku, Tokyo 114-0003*

[††]*The Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641*

[†††]*Faculty of Phamaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510*

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Efficient and concise synthesis of βMan1–4GlcN of pentasaccharide core is established; direct β-mannosylations of 4-OH group of 2-deoxy-2-phthaloyl and 2-azide-2-deoxy glucose derivatives by using 6-nitro-2-benzothiazolyl α-mannoside proceeded smoothly to afford the desired β-mannosides in high yields.

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

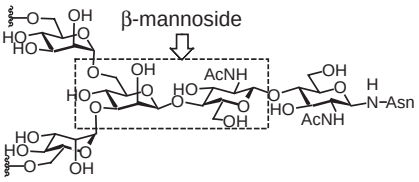
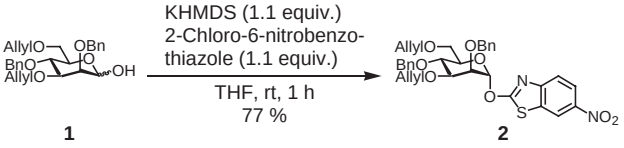


Figure 1. Common pentasaccharide core of N-linked glycans.

It was recently disclosed that 6-nitro-2-benzothiazolyl α-glucoside and α-mannoside, novel glycosyl donors, were reactive enough to construct β-saccharide linkages via S_N2-type process.[7] In this paper, we would like to report a general and effective method for β-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-α-D-mannopyranoside **2** from the precursor **1**[8] according to our previously reported procedure.[7] The condensation reaction proceeded smoothly at room temperature to afford 6-Nitro-2-benzothiazolyl α-mannoside **2** in 77% yield as a major product (Scheme 1). Although 6-nitro-2-benzothiazolyl β-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 α-mannoside by measurement of NMR spectrum that showed $^1J_{CH} = 177$ Hz between H-1 and C-1.[9]



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

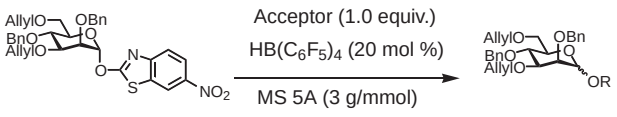
Mannosylation between mannosyl donor **2** (1.2 equiv.) and *p*-methoxyphenyl 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **3**[10] (1.0 equiv.) was carried out under previously reported conditions[7] and the desired β-mannoside **4β**[6] was obtained in 52% yield along with 25% yield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

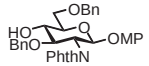
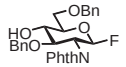
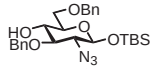
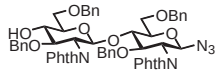
In order to improve the yield of the desired β-mannoside, optimization of several reaction conditions; that is, the molar ratios between mannosyl donor **2** and glycosyl accepter **3**, concentration of the reaction mixture, and an experimental procedure were further examined in detail. After the reaction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

All β-mannosylation reactions proceeded smoothly to afford the desired β-mannoside in higher yields compared with those shown in previously reported direct mannosylation method.[3,6] On the other hand, the mannosylation of glycosyl acceptor **5** having a fluorine atom on its reducing end gave disaccharide **8**[6] 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.

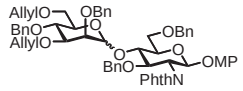
This problem was overcome by shortening of the reaction time to 0.2 h (Entry 4), however, β-stereoselevtivity was lower than the other glycosyl acceptors since the fluorine atom at anomeric position reduced the nucleophilicity of hydroxy group at C-4 position. The glycosyl acceptor **6** gave the best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

Table 1. β-Mannosylation with several glycosyl acceptors



Entry	Acceptor	Time/h	Mannoside	Yield/% (α/β)[a]
1[b]		0.5	**4**	77 (33/67)
2	**3**	1	**4**	99 (18/82)
3		1	**8**	71 (22/78)
4	**5**	0.2	**8**	91 (26/74)
5	 **6**	1	**9**	95 (13/87)
6	 **7**	1	**10**	95 (20/80)

[a]The α/β ratios were determined by isolations of both stereoisomers. [b]The reaction was carried out under previously reported conditions.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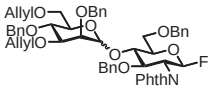
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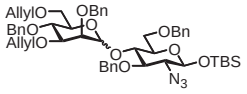
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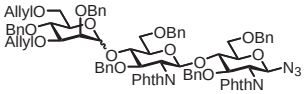
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the present method to afford the desired β-mannoside **9**$\boldsymbol{\beta}$ in 83% yield along with 12% of the α-one (Entry 5). Further, it was interesting to note that the chitobiose acceptor **7** gave the desired β-trisaccharide **10**$\boldsymbol{\beta}$[6] in 76% yield along with 19% of the α-one. The anomeric configurations of all mannosides were confirmed by $^1J_{CH}$ coupling constant measurement. Ogawa et al. utilized trisaccharide **10**$\boldsymbol{\beta}$ prepared from **4**$\boldsymbol{\beta}$ or **8**$\boldsymbol{\beta}$ in the total synthesis of pentasaccharide core of N-linked glycans after removal of protecting group of allyl ether.[6]

It is noted that an efficient and concise method for synthesis of βMan1–4GlcN, a part of the pentasaccharide core, was established by using 6-nitro-2-benzothiazolyl α-mannosyl donor **2**. This method was quite useful in direct mannosylation and provided several β-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

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13 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 CH_2Cl_2 (0.5 mL) was successively added $HB(C_6F_5)_4$ (0.05 M in toluene–Et_2O (1:1), 0.20 mL, 0.01 mmol)[14] at $-78\,^\circ$C and, 5 min later, a solution of mannosyl donor **1** (55.9 mg, 0.09 mmol) in CH_2Cl_2 (1.25 mL) added over 30 min. After the mixture was stirred for 1 h at $-78\,^\circ$C, the reaction was quenched by adding sat. aq. $NaHCO_3$. Then, the mixture was filtered through Celite and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine and dried over Na_2SO_4. After filtration and evapolation, the resulting residue was purified by preparative TLC (silica gel, toluene: EtOAc = 7:1) to afford β-mannoside **10**β (53.6 mg, 76%) and **10**α (13.6 mg, 19%).
14 $HB(C_6F_5)_4$ was generated accoding to literal procedure: H. Jona, H. Mandai, W. Chavasiri, K. Takeuchi, and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, **75**, 291 (2002).

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