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Bioorganic & Medicinal Chemistry Letters 16 (2006) 928–933
Bioorganic & Medicinal Chemistry Letters

Synthesis of complex-type glycans derived from parasitic helminths

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Received 22 September 2005; revised 20 October 2005; accepted 28 October 2005
Available online 15 November 2005

Abstract—Chemical synthesis of complex-type glycans **1** and **2** derived from eggs of parasitic helminths, *Schistosoma mansoni* and *Schistosoma japonicum*, is described. These branched sugar chains were synthesized regio- and stereoselectively by using β-mannosylation, desilylation under high pressure, and glycosylation in frozen solvent as key transformations.

Schistosomes are parasitic helminths that chronically infect more than 200 million in developing countries.[1] Recent investigation revealed that they express asparagine-linked (N-linked) glycoprotein glycans having structures distinct from those of mammals (Fig. 1). Intriguingly, they have features in common with those of plant glycoproteins,[2] carrying D-xylose (Xyl) and/or L-fucose (Fuc) residues with unique linkage modes. These sugar residues are linked to β-linked mannose (Man) (Xylβ1 → 2Man) and innermost *N*-acetylglucosamine (GlcNAc) (Fucα1 → 3GlcNAc) residues, respectively. It has been shown that these structures are antigenic to human[3] and contribute in IgE binding to plant allergens.[4]

Infection with *Schistosoma mansoni* induces a T_H2-type immune response,[5] which was ascribed to carbohydrate functioning as adjuvants.[6] Curiously, individuals infected with the parasite acquire resistance to allergy, however.[7,8] So-called 'IgE blocking hypothesis' implies that the polyclonal IgE antibody that was produced after parasite infection saturates the IgE receptors on mast cells and blocks the binding of specific IgE antibody. On the other hand, rapid increase of allergic diseases in an urban area is explained by the 'hygiene hypothesis.' It advocates that a highly hygienic environment causes a drastically reduced infection, which promotes the outbreak of allergy. However, this theory obviously fails to explain the aforesaid relationship between parasite infection and allergy. More recently, a new theory has emerged, which advocates the role of IL-10 in an anti-inflammatory network for inhibiting the allergy.[8] Long-term parasite infection upregulates the production of this cytokine in the presence of regulatory T cells. However, precise understanding of the roles of glycoprotein glycans in these phenomena has been difficult to identify, because of the limited access to these molecules.

Our attention was focused on the synthesis of complex-type *N*-glycans **1** and **2**, which were found in eggs of parasites, *Schistosoma mansoni* and *Schistosoma japonicum* (Fig. 1).[9] In addition to their biomedical significance, these glycans are of synthetic interest, because of their complex pattern of branching. The construction of a triply branched structure on GlcNAc[a] and Man[c] seemed to be challenging. We describe herein the successful opening of the avenue toward mono- (**2**) and di-fucosylated (**1**) xylosyl glycans. Rate acceleration effect of frozen system solved the difficulty encountered in the introduction of Fuc to the 3-position of GlcNAc.

It was planned to prepare octasaccharide **3** from hexasaccharide donor **5** and Fucα1 → 6GlcNAc component **6**. Incorporation of an additional fucose residue onto **3** was to be conducted with thioglycoside **4**[10] to complete the nonasaccharide skeleton (Fig. 2). Imaginary discon-

Keywords: Glycoprotein; Complex-type glycans; Parasitic helminths; Glycosylation; Frozen solvent.
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doi:10.1016/j.bmcl.2005.10.100

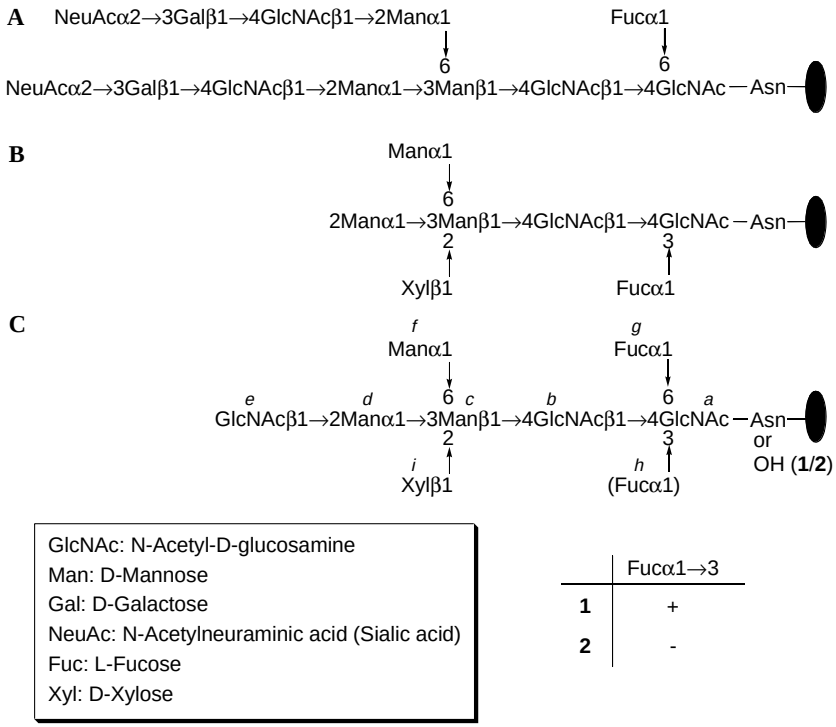


Figure 1. Typical structures of animal (**A**), plant (**B**), and helminth (**C**) derived complex-type glycans.

nection of hexasaccharide **5** led to smaller fragments **7**,[11] **8**, and **9**.

Synthesis of the fragment **9** was conducted as shown in Scheme 1. *p*-Methoxybenzyl (PMB)-assisted intramolecular aglycon delivery (IAD)[12] was used for the construction of β-manno glycoside. It was conducted between glucosamine derivative **10**[13] and thioglycoside **11**[12c] to afford disaccharide **13**, via mixed acetal **12**, in 68% yield as a single stereoisomer. Since the product had a C-2 hydroxy group liberated, it was directly used for the subsequent xylosylation with trichloroacetimidate **14**[14] to give trisaccharide in 67% yield. Subsequent desilylation under standard conditions (Bu_4NF, AcOH/THF) resulted in a substantial degree of Ac migration. This problem was circumvented by using HF–pyridine high-pressure conditions (1 GPa),[15] which cleanly gave **9** in 88% yield.

The latter compound was then used as a glycosyl acceptor for subsequent glycosylation with disaccharide donor **8**, which was synthesized by the glycosylation of **15**[16] with **16**[17] (Scheme 2). This glycosylation, promoted by MeOTf[18] in toluene, stereoselectively provided **17**, which was isolated in 94% yield. After acidic cleavage of the cyclohexylidene acetal, resultant **18** was coupled with mannosyl donor **7** to give hexasaccharide **19** in 77% yield.

Our next task was to exchange all benzyl ethers to acetyl groups. This seemingly straightforward transformation turned out to be problematic. Namely, catalytic hydrogenolysis under typical conditions [H_2, $Pd(OH)_2$, EtOH] was accompanied by the reduction of Phth groups; after acetylation, a mixture of desired product **20a** and over-reduction product(s) **20b** was obtained, from which the isolation of **20a** was devastatingly difficult. To our delight, $Pd(OH)_2$ catalyzed hydrogen transfer[19] in refluxing cyclohexene/EtOH/AcOH (2:1:1) proceeded cleanly to give **20a** with excellent purity after complete acetylation. Sequential treatments with hydrazine acetate and trichloroacetonitrile–DBU afforded initially designed donor **5**.[20]

The disaccharide fragment **6** was prepared as depicted in Scheme 3. Thus, compound **21** was converted to 3-*O*-PMB derivative using trichloroacetimidate **22** and $La(OTf)_3$.[21] Subsequent cleavage of benzylidene acetal afforded diol **23**, which in turn was reacted with fucosyl donor **4** to give disaccharide **6** in 66% yield.[20] We also prepared difucosylated trisaccharide **26**, in order to explore the convergent route to **1**. To that end, **21** was glycosylated with tri-*O*-PMB-protected donor **24**[22] using $CuBr_2$–Bu_4NBr,[23] and the resultant disaccharide was converted to diol **25**. Subsequent fucosylation led to **26**. However, the reaction with hexasaccharide donor (e.g., **5**) did not provide any coupled product. Therefore, condensation with **5** was attempted using **6** as an acceptor, hoping that the steric hindrance was alleviated compared to **26** (Scheme 4). In fact, coupling under standard trichloroacetimidate activation conditions[24] proceeded in reasonable efficiency to afford octasaccharide **27**. Subsequently, it was completely deprotected to give monofucosylated octasaccharide **2** in 73% yield.[20]

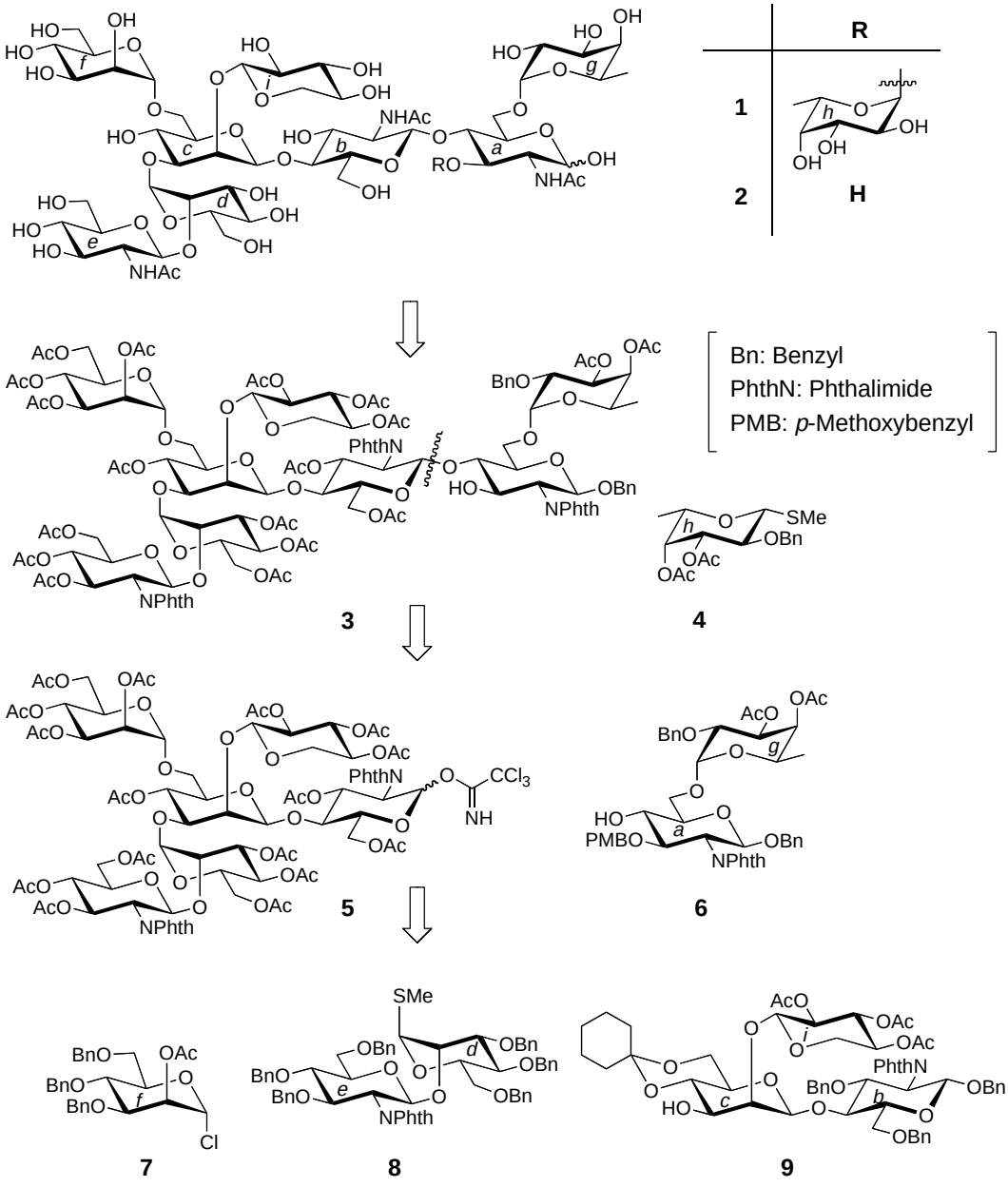
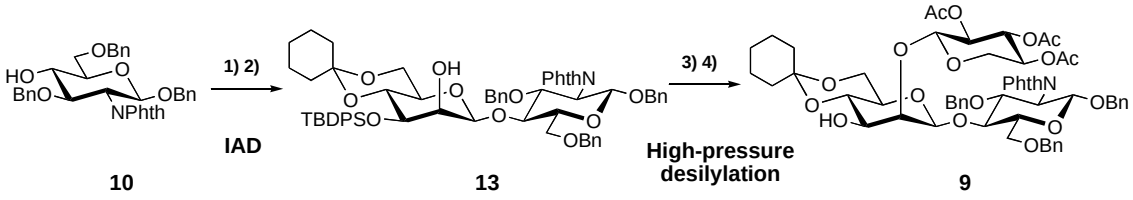
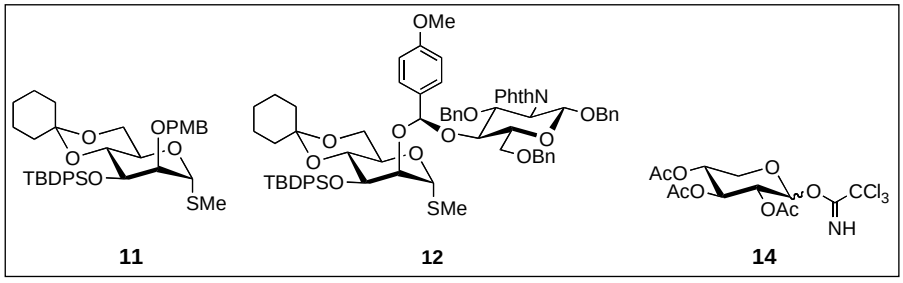


Figure 2. Design of synthetic blocks.

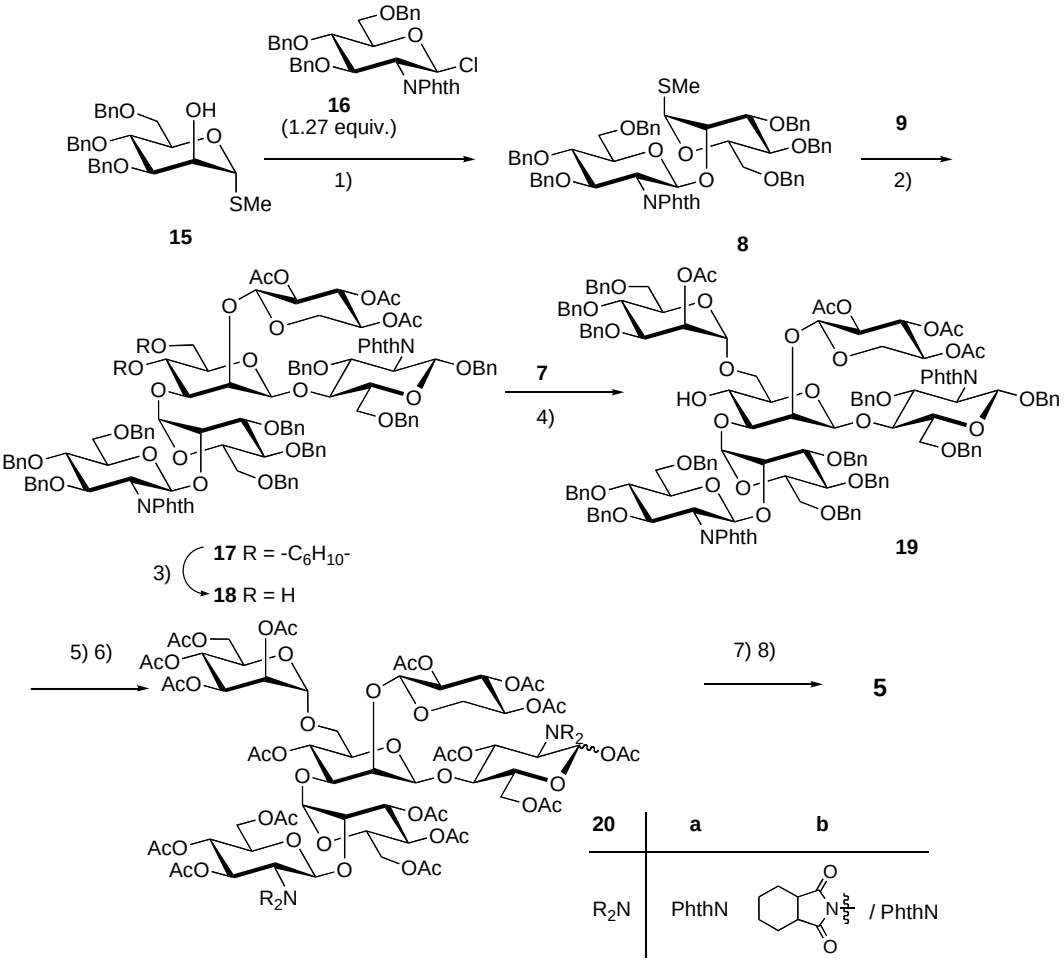
In order to synthesize nonasaccharide **1**, the PMB group of **27** was removed by using DDQ in the presence of $Mn(OAc)_3$[25] to give **3**. Further introduction of a fucose residue to **3** was proven to be challenging. Reaction with **4** in the presence of MeOTf and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) resulted in <50% conversion, even under forcing conditions. In this particular case, incomplete glycosylation was quite annoying, because all attempts to separate fucosylated product **28** from unreacted **3** failed. Recently, we reported the dramatic acceleration of glycosylation reactions in frozen solvent,[26] when thioglycoside was used as the donor and activated with MeOTf. Gratifyingly, our attempt to apply this protocol (MeOTf, DTBMP, *p*-xylene, 4 °C) to promote the coupling between **3** and **4** was extremely successful, giving the coupled product **28** in 83% yield. Although formation of a small amount of β-isomer could not be ruled out, thus obtained **28** was stereochemically homogeneous within the detection limit of 400 MHz ^{1}H NMR. Complete deprotection was conducted in four steps to give nonasaccharide **1**[20] in 87% yield.

In conclusion, first synthesis of complex-type *N*-glycans **1** and **2** found in egg of parasites, *S. mansoni* and *S. japonicum*, has been accomplished. Future studies are in progress to synthesize other oligosaccharides, which lack xylose and/or fucose from **1** to reveal the structure activity relationship of plant- and helminth-derived oligosaccharides.

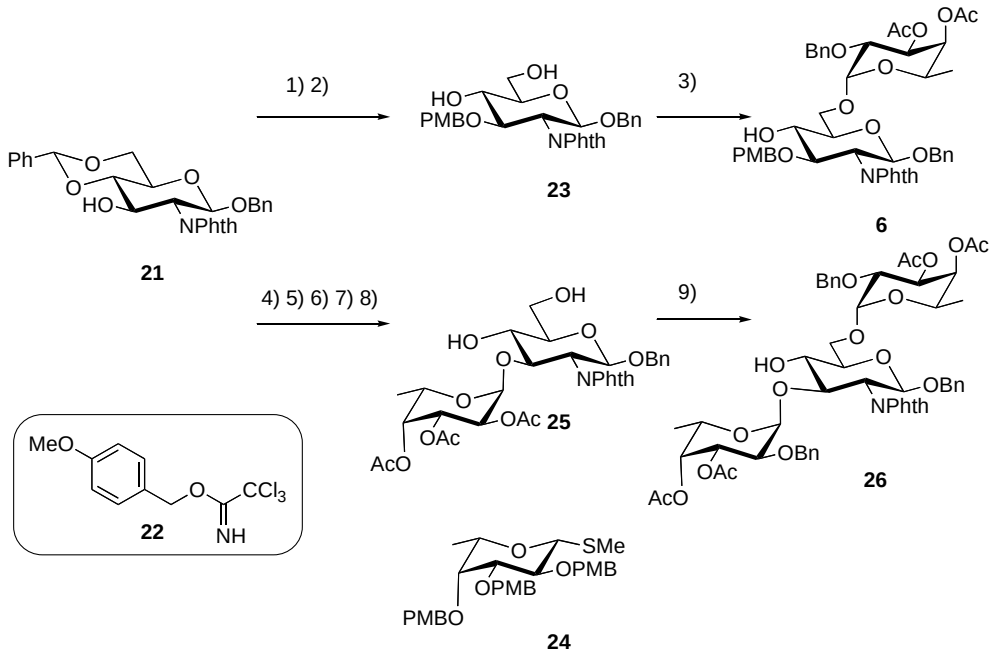




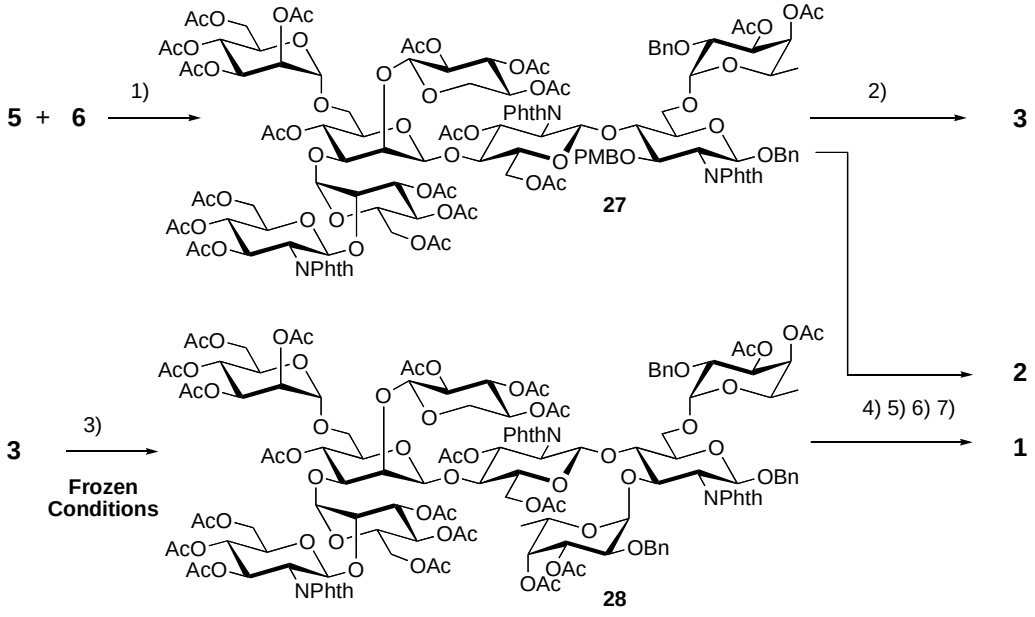
Scheme 1. Reagents and conditions: (1) **11** (1.2 equiv), DDQ (1.25 equiv), MS 4A, CH_2Cl_2, rt, 1.5 h; (2) MeOTf (3.5 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (4 equiv), MS 4A, $ClCH_2CH_2Cl$, 45 °C, 24 h, 68% (two steps); (3) **14** (3 equiv), TMSOTf (2 equiv), CH_2Cl_2, MS 4A, −40 °C, 2 h, 67%; (4) HF–Pyr, DMF, 1 GPa, 12 h, 88%.



Scheme 2. Reagents and conditions: (1) AgOTf (2.5 equiv), $ClCH_2CH_2Cl$/toluene (2:1), MS 4A, −40 °C, 1 h, 63%; (2) MeOTf (4.5 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (4.5 equiv), MS 4A, toluene, 50 °C, 12 h, 94%; (3) $TsOH{\cdot}H_2O$, MeCN, rt, 9 h, 91%; (4) **7** (1.2 equiv), AgOTf (2.4 equiv), MS 4A, $ClCH_2CH_2Cl$/toluene (2:1), −30 °C to rt, 2.5 h, 77%; (5) $Pd(OH)_2$, cyclohexene/EtOH/AcOH (2:1:1), reflux, 60 h; (6) Ac_2O, pyridine, rt, 6 h, 93%, two steps; (7) $N_2H_4{\cdot}AcOH$ (1.3 equiv), DMF, rt, 2 h; (8) Cl_3CCN, DBU (0.95 equiv), rt, 12 h, 75%, two steps.



Scheme 3. Reagents and conditions: (1) **22** (3 equiv), $La(OTf)_3$ (0.12 equiv), CH_2Cl_2, rt, 20 h, 79%; (2) $TsOH{\cdot}H_2O$ (3.5 equiv), MeCN/MeOH (1:1), rt, 11 h, 78%; (3) **4** (1.2 equiv), MeOTf (3.6 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (3.6 equiv), MS 4A, cyclopentyl methyl ether, rt, 22 h, 66%; (4) **24** (2 equiv), $CuBr_2$ (3 equiv), Bu_4NBr (3 equiv), MS 4A, $ClCH_2CH_2Cl$/DMF (5:1), rt, 30 h, 75%; (5) DDQ (6 equiv), CH_2Cl_2/H_2O (9:1), rt, 2 h; (6) NaOMe (1 equiv), MeOH/THF (5:1), 50 °C, 2 h; (7) Ac_2O, DMAP, Pyr, rt, 6 h; (8) TFA, CH_2Cl_2/H_2O (20:1), 71% (four steps); (9) **4** (1.4 equiv), MeOTf (4.2 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (4.2 equiv), MS 4A, cyclopentyl methyl ether, rt, 2 h, 92%, α:β = 11:1.



Scheme 4. Reagents and conditions: (1) TMSOTf (0.2 equiv), CH_2Cl_2, MS 4A, −78 to −40 °C, 4.5 h, 56%; (2) DDQ (1.5 equiv), $Mn(OAc)_3{\cdot}2H_2O$ (4.5 equiv), CH_2Cl_2, rt, 36 h, 65%; (3) **4** (3 equiv), MeOTf (7.5 equiv), DTBMP (4.5 equiv), MS 4A, *p*-xylene, 4 °C, 2 d, 83%; (4) $H_2NCH_2CH_2NH_2$, *n*-BuOH, 85 °C, 12 h; (5) Ac_2O, pyridine, rt, 6 h; (6) NaOMe, MeOH, rt, 12 h; (7) $Pd(OH)_2$, H_2, aq MeOH, rt, 12 h, 87% (**1**), 73% (**2**), four steps.

Acknowledgments

We thank Ms. A. Takahashi for technical assistance and Dr. T. Chihara and his staff for elemental analysis and high-pressure reaction apparatus. Financial support from the Japan Society for the Promotion of Science [Grant-in-Aid for Creative Scientific Research No. 17GS0420] is acknowledged.

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20. ^{1}H NMR: Compound **6** (400 MHz, $CDCl_3$) δ 7.76–7.50 (br, 4H, Ar), 7.35–7.25 (m, 5H, Ar), 7.06–6.99 (m, 5H, Ar), 6.90 (d, 2H, J = 8.5 Hz, Ar), 6.40 (d, 2H, J = 8.5 Hz, Ar), 5.33 (dd, 1H, J = 3.4, 10.2 Hz, H-3^{Fuc}), 5.30–5.29 (m, 1H, H-4^{Fuc}), 5.10–5.08 (m, 1H, H-1^{GlcN}), 4.88 (d, 1H, J = 3.7 Hz, H-1^{Fuc}), 4.72 (d, 2H, J = 12.2 Hz, $ArCH_2$), 4.63–4.57 (m, 2H, $ArCH_2$), 4.41 (d, 1H, J = 12.2 Hz, $ArCH_2$), 4.38 (d, 1H, J = 12.2 Hz, $ArCH_2$), 4.26–4.21 (br, 1H, H-5^{Fuc}), 4.17–4.13 (m, 2H, H-2^{GlcN}, H-3^{GlcN}), 3.95 (dd, 1H, J = 4.2, 11.2 Hz, H-6a^{GlcN}), 3.88–3.83 (m, 3H, H-4^{GlcN}, H-6b^{GlcN}, H-2^{Fuc}), 3.60–3.54 (m, 4H, H-5^{GlcN}, ArOMe), 3.33 (d, 1H, J = 3.4 Hz, OH), 2.14 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.10 (d, 3H, J = 6.6 Hz, H-6^{Fuc}). Compound **5** (400 MHz, $CDCl_3$) δ 8.63 (s, 1H, NH), 6.57 (d, 1H, J = 8.8 Hz, H-1^{GlcN}), 5.90 (t, 3H, J = 9.8 Hz); Compound **2** (400 MHz, D_2O) δ 5.17 (d, 0.67H, J = 3 Hz, H-1$^{\alpha GlcN}$), 5.13 (s, 1H, H-1$^{\alpha Man}$), 4.91 (s, 1H, H-1$^{\alpha Man}$), 4.90–4.88 (m, 1H, H-1^{Fuc}), 4.87 (s, 1H, H-1$^{\beta Man}$), 4.67–4.64 (m, 2H), 4.51 (d, 1H, J = 8.5 Hz, H-1), 4.44 (d, 1H, J = 7.8 Hz, H-1), 4.25 (br, 1H, H-2$^{\beta Man}$), 1.22–1.20 (m, 3H, J = 6.6 Hz, H-6^{Fuc}). Compound **1** (400 MHz, D_2O) δ 5.14 (br, 1H, H-1$^{\alpha Man}$), 5.11 (d, 1H, J = 3.9 Hz, H-1^{Fuc}), 5.06 (d, 0.6H, J = 3.4 Hz, H-1$^{\alpha GlcNAc}$), 4.92 (d, 1H, J = 3.9 Hz, H-1^{Fuc}), 4.91 (br, 1H, H-1$^{\beta Man}$), 4.71–4.66 (m, 2H), 4.51 (d, 1H, J = 8.5 Hz, H-1), 4.45 (d, 1H, J = 7.6 Hz, H-1), 4.25 (br, 1H, H-2$^{\beta Man}$), 1.27 (d, 3H, J = 6.6 Hz, H-6$^{Fuc\text{-}h}$), 1.22 (d, 1.2H, J = 6.6 Hz, H-6$^{Fuc\text{-}g}$), 1.20 (d, 1.8H, J = 6.6 Hz, H-6$^{Fuc\text{-}g}$).
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