ELSEVIER

Carbohydrate Research 306 (1998) 539–544

CARBOHYDRATE RESEARCH

Synthetic study on a novel Asn-linked core structure: synthesis of a pentasaccharide α-D-Man-(1→3)-[α-D-Man-(1→6)]-β-D-Man-(1→4)-[β-D-GlcNAc-(1→6)]-β-D-GlcNAc→OMp[1]

Zhong-Wu Guo [a], Yukishige Ito [a], Yoshiaki Nakahara [a,*], Tomoya Ogawa [a,b,*]

[a] *The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-shi, Saitama, 351-01 Japan*

[b] *Graduate School of Agriculture and Life Sciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113 Japan*

Received 27 October 1997; accepted 16 January 1997

Abstract

Synthesis of a pentasaccharide α-D-Man-(1→3)-[α-D-Man-(1→6)]-β-D-Man-(1→4)-[β-D-GlcNAc(1→6)]-β-D-GlcNAc→OMp (**2**) is described. A comparison between the ^{1}H NMR data of **2** and those of a novel Asn-linked core structure **1** containing a new GlcNAc residue suggests an α-D-configuration for the new linkage. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: α-D-Man-(1→3)-[α-D-Man-(1→6)]-β-D-Man-(1→4)-[β-D-GlcNAc(1→6)]-β-D-GlcNAc→OMp; N-Linked core structure

1. Introduction

Most cell-surface proteins and many secretory proteins are N-glycoproteins [1], and their N-linked oligosaccharides play important biological roles [2]. Despite the fact that a huge number of different N-linked oligosaccharide structures have been disclosed, only four types of N-linked core structures have been described in mammalian cells before, namely: α-D-Man-(1→3)-[α-D-Man-(1→6)]-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-β-D-GlcNAc→Asn, α-D-Man-(1→3)-[α-D-Man-(1→6)]-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-[α-L-Fuc (1→6)]-β-D-GlcNAc→Asn, α-D-Man-(1→3)-[β-D-GlcNAc-(1→4)]-[α-D-Man-(1→6)]-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-β-D-GlcNAc→Asn and α-D-Man-(1→3)-[β-D-GlcNAc-(1→4)]-[α-D-Man-(1→6)]-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-[α-L-Fuc (1→6)]-β-D-GlcNAc→Asn. However, Stanley et al. [3] have recently isolated a novel N-linked core structure **1** with an additional GlcNAc attached to the core GlcNAc from Chinese hamster ovary (CHO) cell–LEC18. It was suggested that the new GlcNAc residue markedly alters the conformation of related oligosaccharides and thus causes the high

* Corresponding authors.

[1] Mp = p-meth

PII S0008-6215(98)00031-7

resistance of LEC18 cell to both pea lectin (PSA) and Lens culinaris agglutinin (LCA). Nevertheless, because in the ^{1}H NMR spectrum of **1**, the anomeric proton of the new GlcNAc residue is at rather low field (δ:5.21) and has a coupling constant of 5.5 Hz that is an intermediate value between the typical ones of α- and β-linked residues (3–4 Hz and 6.5–8.0 Hz, respectively), the configuration of the new linkage was therefore not determined. Our present work is to synthesize a part-structure **2** of the β-isomer of the novel core structure and to compare the spectroscopic properties of **2** to those of the natural structure **1**.

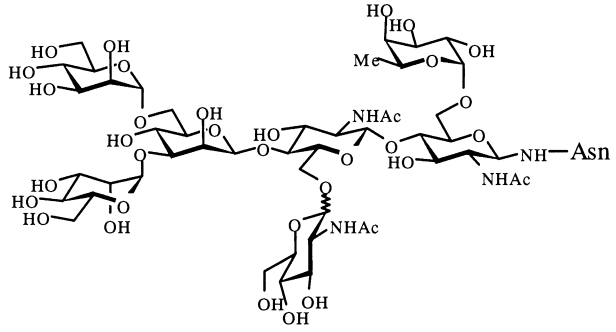
2. Results and discussion

First of all, four properly protected and activated monosaccharides **3**, **4**, **6**, and **9** were prepared according to the reported methods [4]. Then, as shown in Scheme 1, reaction of **3** and **4** at room temperature, promoted by AgOTf, gave **5** (71%) as the only product. In order to prepare compound **7** containing a β-mannosyl linkage, we employed a reaction using monosaccharide **6** as the mannosyl donor, disaccharide **5** as glycosyl acceptor, and silver alumina–silicate as promoter [5]. To the best of our knowledge, this is the first example trying to introduce a β-mannosyl residue to the C-4 hydroxyl group of GlcNAc carrying a glycosyl residue at C-6 by means of silver silicate promotion. Herein, compound **7** and its α-isomer were obtained in 1.0:1.4 ratio and 53% yield. It is noteworthy that the stereoselectivity for β-isomer was lower in this reaction than those in reactions between **6** and simple GlcNAc acceptors (β:α = 1.2–1.4:1 [4]), and, in addition, the reaction was slower and less efficient. Therefore, even when a large excess of glycosyl donor **6** (3.5 equiv) was employed, a substantial amount of glycosyl acceptor **5** (35%) was still recovered after overnight reaction. Compound **7** was deallylated using an iridium complex as catalyst [6]. Reaction of the resulting product **8** with **9** was stereoselectively promoted by silver trifluoromethanesulfonate to afford pentasaccharide **10** (84%). Dephthaloylation of **10** with ethylenediamine in *n*-butanol [7], followed by acetylation of the resulting product in acetic anhydride and methanol, was subsequently carried out in one pot to produce **11** in 74% yield. Reductive debenzylation of **11** in the presence of $Pd(OH)_2$ under a hydrogen atmosphere finally gave the desired product **2**, which has shown satisfactory MS and NMR results.

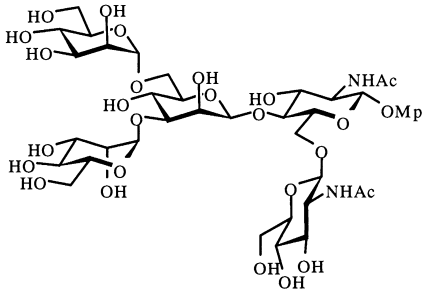
As it is clearly shown in the ^{1}H NMR spectrum of **2**, the anomeric proton signal of GlcNAc-β-(1→6) is at δ 4.55 with a coupling constant of 8.3 Hz. These data are quite similar to those of a typical β-linked GlcNAc (δ: 4.60, J 8.0 Hz) observed in all N-glycoproteins or N-glycopeptides. Our observation of the different coupling constants as well as the 0.7 ppm chemical shift difference between the anomeric protons for (1→6)-linked GlcNAc in the natural product **1** (δ: 5.21) and in synthetic part-structure **2**, together with the reported observation that the new (1→6)-linked GlcNAc was not accessible to β-hexosaminidase [3], suggested an α-D-configuration for the novel GlcNAc residue.

3. Experimental

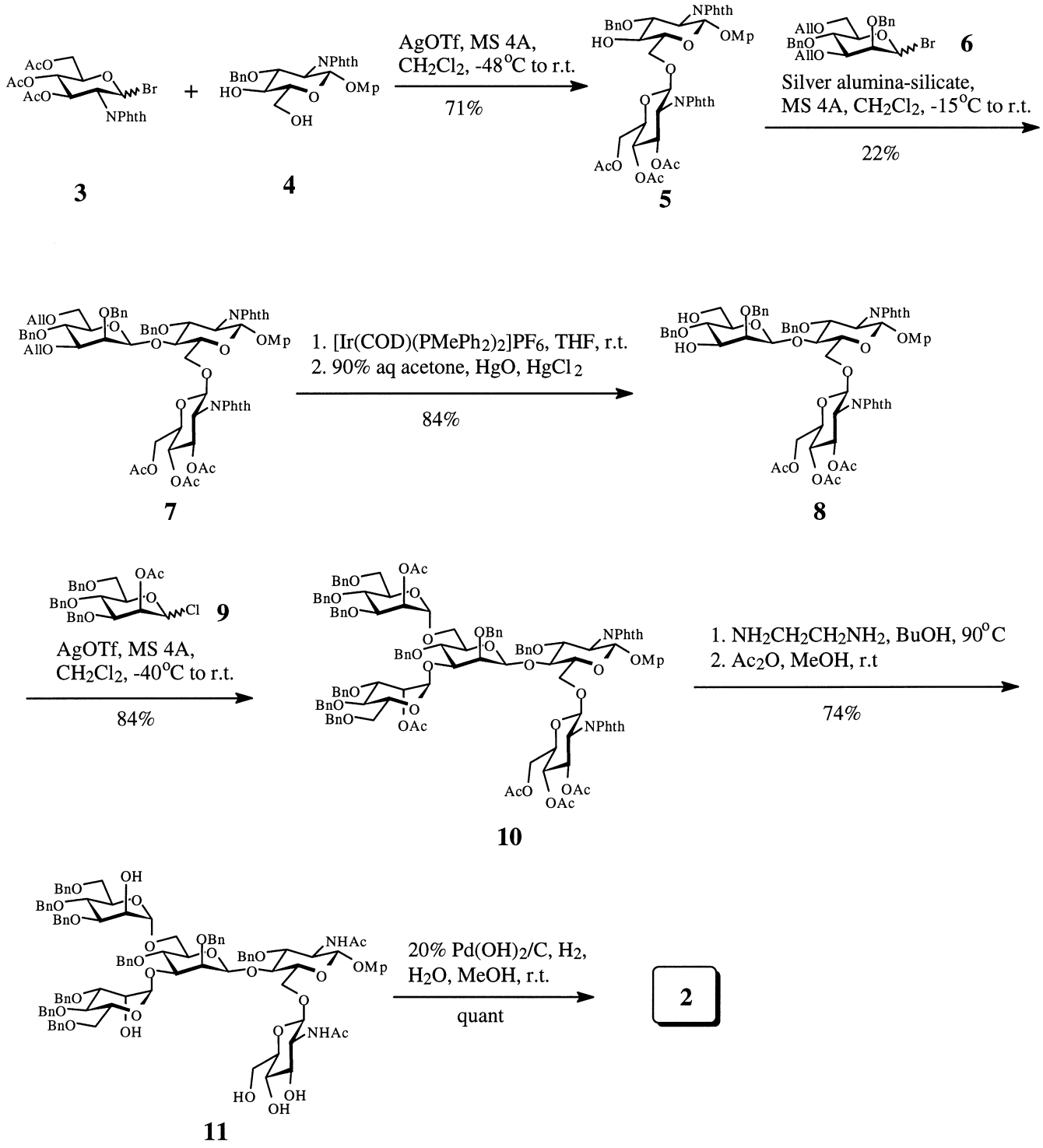
General methods.—Optical rotations were measured at 23 ± 2 °C with a Jasco DIP 370 polarimeter. ^{1}H NMR spectra were recorded with a Jeol EX 270 or a Jeol α600 spectrometer for solutions in $CDCl_3$ with TMS as the internal standard



1



2



Scheme 1.

unless otherwise indicated. ^{1}H and ^{13}C signals were assigned according to their 1D spectra and 2D ^{1}H–^{1}H or ^{1}H–^{13}C COSY spectra. Analytical TLCs and preparative TLCs were performed on precoated Silica Gel 60 $F_{25}4$ glass plates (E. Merck). Silica gel column chromatography (CC) was performed with Silica Gel 60 (E. Merck). Molecular sieves (MS) were purchased from Nakarai Chemical Co. and were activated at 180 °C under vacuum immediately prior to use. All reactions except hydrogenations were performed in anhydrous solvent under a dry N_2 atmosphere.

p-*Methoxyphenyl 3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl-(1→6)-3*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**5**).—To a stirred mixture of **4** (447 mg, 0.85 mmol), AgOTf (400 mg, 1.56 mmol) and 4A MS (1.5 g) in dry CH_2Cl_2 (8 mL) was added a solution of **3** (551 mg, 1.11 mmol) in CH_2Cl_2 (5 mL) at −48 °C. The mixture was gradually warmed up to room temperature and stirred overnight. It was filtered through a Celite pad, and the filtrate was concentrated in vacuo followed by purification by silica gel CC (2.5:1 toluene–EtOAc) to give **5** (560 mg, 71%) as a white solid and recovered **4** (124 mg). **5**: R_f 0.36 (1.6:1.0 toluene–EtOAc); ^{1}H NMR (270 MHz): δ 7.88–7.66 (m, 8 H, H-aromatic^{Phth}), 7.02–6.97 (m, 5 H, H-aromatic^{Bn}), 6.80–6.71 (m, 4 H, H-aromatic^{Mp}), 5.72 (dd, 1 H, $J_{3,4}$ 9.0 Hz, $J_{2,3}$ 10.4 Hz, H-3^2), 5.64 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^1), 5.54 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1^2), 5.15 (dd, 1 H, $J_{4,5}$ 10.1 Hz, H-4^2), 4.64, 4.48 (2 d, 1 H each, *J* 12.1 Hz, H-Bn), 4.35 (dd, 1 H, H-2^2), 4.30–4.23 (m, 3 H, H-3^1, H-2^1, H-6^2), 4.17 (dd, 1 H, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.2 Hz, H-6′^{2}), 4.08 (dd, 1 H, $J_{5,6}$ 2.2 Hz, $J_{6,6'}$ 11.4 Hz, H-6^1), 3.91 (dd, 1 H, $J_{5,6'}$ 2.0 Hz, H-6′^{1}), 3.73 (s, 3 H, OMe), 3.63–3.54 (m, 3 H, H-5^1, H-4^1, H-5^2), 2.56 (d, 1 H, *J* 3.6 Hz, OH), 2.13, 2.03, 1.87 (3 s, 3 H each, 3 Ac); ^{13}C NMR: δ 97.67 (C-1^2), 96.57 (C-1^1), 78.53 (C-3^1), 74.83 (C-5^1), 74.34 (Cα-Bn), 72.54 (C-4^1), 71.95 (C-5^2), 70.82 (C-3^2), 68.74 (C-4^2), 68.29 (C-6^1), 61.59 (C-6^2), 55.51 (C-O*Me*), 55.17 (C-2^1), 54.32 (C-2^2), 20.78, 20.52, 20.43 (3 *Me*C=O); FABMS: Calcd for $C_{48}H_{46}N_2O_{17}$: *m*/*z* 922.3. Found: *m*/*z* 945.3 $(M + Na)^+$.

p-*Methoxyphenyl 3,6-di*-O-*allyl-2,4-di*-O-*benzyl*-β-D-*mannopyranosyl-(1→4)-[3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl-(1→6)]-3*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**7**).—To a stirred mixture of compound **5** (550 mg, 0.6 mmol), silver alumina–silicate (1.4 g, 4.2 mmol) and 4A MS (1.4 g) in CH_2Cl_2 (10 mL) was added a solution of compound **6** (1.05 g, 2.1 mmol) in CH_2Cl_2 (5 mL) at −15 °C. The mixture was allowed to warm up to 0 °C in 1 h and then to room temperature overnight. Solids were filtered off, and the solution was concentrated to dryness in vacuo. The resulting residue was separated by silica gel CC (5:1 toluene–EtOAc) to afford compound **7** (177 mg, 22%), its α-isomer **7α** (252 mg, 31%) and recovered **5** (190 mg), all as white solids. **7**: $[\alpha]_D$ +45.1° (*c* 0.2, $CHCl_3$); R_f 0.40 (3:1 toluene–EtOAc); ^{1}H NMR (600 MHz): δ 7.81–6.73 (m, 27 H, H-aromatic), 5.95, 5.77 (2 m, 1 H each, 2 CH_2=C*H*−), 5.69 (dd, 1 H, $J_{3,4}$ 8.8 Hz, $J_{2,3}$ 10.2 Hz, H-3$^{2'}$), 5.64 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^1), 5.49 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1$^{2'}$), 5.38 (m, 1 H, J_t 17.1 Hz, CH_2=), 5.23–5.15 (m, 2 H, CH_2=), 5.15 (dd, 1 H, $J_{4,5}$ 10.5 Hz, H-4$^{2'}$), 5.04 (m, 1 H, J_c 11.0 Hz, CH_2=), 4.94–4.81 (m, 4 H, H-Bn), 4.57 (d, 1 H, *J* 10.9 Hz, H-Bn), 4.50 (s, 1 H, H-1^2), 4.41 (d, 1 H, *J* 12.9 Hz, H-Bn), 4.32 (dd, 1 H, H-2$^{2'}$), 4.30–4.26 (m, 3 H, H-3^1, H-2^1, H-6$^{2'}$), 4.23 (m, 1 H, *J* 12.7, 5.4, 2.0 Hz, H$_\alpha$-All), 4.12 (m, 1 H, *J* 12.7, 4.9, 1.5 Hz, H$_\alpha$-All), 4.07 (dd, 1 H, $J_{5,6'}$ 2.4 Hz, $J_{6,6'}$ 12.7 Hz, H-6′$^{2'}$), 4.02–3.86 (m, 4 H, 2 H$_\alpha$-All, H-2^2, H-6^1), 3.83–3.75 (m, 3 H, H-6′^{1}, H-4^1, H-4^2), 3.74 (s, 3 H, OMe), 3.69 (dd, 1 H, $J_{5,6}$ 1.6 Hz, $J_{6,6'}$ 11.2 Hz, H-6^2), 3.63 (m, 1 H, H-5^1), 3.50 (dd, 1 H, $J_{5,6'}$ 5.6 Hz, H-6′^{2}), 3.43 (dd, 1 H, $J_{3,4}$ 9.8 Hz, $J_{2,3}$ 2.9 Hz, H-3^2), 3.40 (m, 1 H, H-5$^{2'}$), 3.29 (m, 1 H, *J* 9.8, 5.4, 1.5 Hz, H-5^2), 2.04, 2.02, 1.84 (3 s, 3 H each, 3 Ac); ^{13}C NMR: δ 101.03 (C-1^2), 97.40 (C-1$^{2'}$), 96.46 (C-1^1), 82.46 (C-3^2), 79.43 (C-4^1), 77.01 (C-3^1), 75.90 (C-5^2), 75.08 (C-2^2), 74.95 (C$_\alpha$-Bn), 74.56 (C-4^2), 74.43 (C$_\alpha$-Bn), 74.34 (C-5^1), 74.20 (C$_\alpha$-Bn), 72.24 (C$_\alpha$-All), 71.89 (C-5$^{2'}$), 70.75 (C-3$^{2'}$), 70.64 (C$_\alpha$-All), 69.40 (C-6^2), 68.50 (C-4$^{2'}$), 68.11 (C-6^1), 61.31 (C-6$^{2'}$), 55.35 (C-O*Me*), 55.34 (C-2^1), 54.47 (C-2$^{2'}$), 20.61, 20.38, 20.27 (3 *Me*C=O); FABMS: Calcd for $C_{74}H_{76}N_2O_{22}$: *m*/*z* 1344.5. Found: *m*/*z* 1367.6 $(M + Na)^+$. Anal. Calcd for $C_{74}H_{76}N_2O_{22}$: C, 66.07; H, 5.69; N, 2.08. Found: C, 65.98; H, 5.78; N, 1.75.

7α: R_f 0.47 (3:1 toluene–EtOAc,); ^{1}H NMR (600 MHz): δ 5.90 (m, 2 H, 2 CH_2=C*H*-), 5.64 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-11), 5.62 (dd, 1 H, $J_{3,4}$ 7.4 Hz, $J_{2,3}$ 10.4 Hz, H-3$^{2'}$), 5.54 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1$^{2'}$), 5.32–5.08 (m, 4 H, 2 CH_2=), 5.15 (s, 1 H, H-1^2), 3.29 (m, 1 H, *J* 9.8, 5.4, 1.5 Hz, H-5$^{2'}$), 2.08, 2.00, 1.84 (3 s, 3 H each, 3 Ac); ^{13}C NMR: δ 100.86 (C-1^2), 97.09 (C-1$^{2'}$), 95.49 (C-1^1), 80.38, 79.19, 75.35, 75.19, 74.86, 74.68, 74.66, 74.63, 72.96, 72.38, 72.18, 71.48, 71.07, 70.93, 69.31, 68.52, 68.23, 61.28, 55.31 (C-O*Me*), 55.29 (C-2^1), 54.52 (C-2$^{2'}$), 20.67, 20.36, 20.33 (3 *Me*C=O).

p-*Methoxyphenyl 2,4-di*-O-*benzyl*-β-D-*mannopyranosyl-(1→4)-[3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl-(1→6)]-3*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**8**).—A solution of $[Ir(COD)(PMePh_2)_2]PF_6$ (20 mg, 0.024 mmol) in THF (15 mL) was stirred under H_2 until the red solution became colorless. Then the H_2 atmosphere was replaced with N_2, and a solution of **7** (230 mg, 0.17 mmol) in THF (5 mL) was added. The mixture was stirred at room temperature for

1 h and concentrated in vacuo. The residue was dissolved in 90% aq acetone (20 mL) and treated with HgO (40 mg) and $HgCl_2$ (350 mg) for 1 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo. CC (5–3:1 toluene–EtOAc) of the residue gave compound **8** (181 mg, 84%) as a white solid. **8**: $[\alpha]_D$ +24.9° (*c* 1.1, $CHCl_3$); R_f 0.16 (2:1 toluene–EtOAc); ^{1}H NMR (600 MHz): δ 7.74–6.73 (m, 27 H, H-aromatic), 5.71 (dd, 1 H, $J_{3,4}$ 9.3 Hz, $J_{2,3}$ 10.7 Hz, H-3$^{2'}$), 5.61 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^1), 5.52 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1$^{2'}$), 5.16 (dd, 1 H, $J_{4,5}$ 10.2 Hz, H-4$^{2'}$), 5.00, 4.89, 4.83, 4.69 (4 d, 1 H each, *J* 11.6, 12.2, 11.2, 11.6 Hz, H-Bn), 4.58 (s, 1 H, H-1^2), 4.56, 4.39 (2 d, 1 H each, *J* 11.2, 12.2 Hz, H-Bn), 4.37 (dd, 1 H, H-2$^{2'}$), 4.33–4.30 (m, 2 H, H-3^1, H-2^1), 4.28 (dd, 1 H, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 12.5 Hz, H-6$^{2'}$), 4.16 (dd, 1 H, $J_{5,6'}$ 2.5 Hz, H-6′$^{2'}$), 4.04 (dd, 1 H, $J_{5,6'}$ 2.9 Hz, $J_{6,6'}$ 10.7 Hz, H-6^1), 3.87–3.79 (m, 3 H, H-2^2, H-4^1, H-6′^{1}), 3.75 (s, 3 H, OMe), 3.75–3.67 (m, 2 H, H-6^2, H-5^1), 3.64 (dd, 1 H, $J_{2,3}$ 3.9 Hz, $J_{3,4}$ 9.3 Hz, H-3^2), 3.56 (m, 1 H, H-5$^{2'}$), 3.46 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4^2), 3.42 (dd, 1 H, $J_{5,6}$ 5.4 Hz, $J_{6,6'}$ 12.2 Hz, H-6′^{2}), 3.8 (ddd, 1 H, *J* 9.3, 3.4, 6.8 Hz, H-5^2), 2.49 (d, 1 H, *J* 9.1 Hz, OH), 2.09, 2.04, 1.87 (3 s, 3 H each, 3 Ac); ^{13}C NMR: δ 101.69 (C-1^2), 97.79 (C-1$^{2'}$), 97.11 (C-1^1), 79.91 (C-4^1), 78.78 (C-2^2), 77.04 (C-3^1), 76.84 (C-4^2), 75.81 (C-5^2), 75.60 (C_α-Bn), 75.06 (C_α-Bn), 74.81 (C-51), 74.75 (C_α-Bn), 74.29 (C-32), 72.56 (C-5$^{2'}$), 71.21 (C-3$^{2'}$), 69.02 (C-4$^{2'}$), 67.89 (C-6^1), 62.66 (C-6^2), 61.94 (C-6$^{2'}$), 55.87 (C-O*Me*), 55.65 (C-2^1), 54.61 (C-2$^{2'}$), 21.12, 20.90, 20.79 (3 *Me*C=O); FABMS: Calcd for $C_{68}H_{68}N_2O_{22}$: *m*/*z* 1264.4. Found: *m*/*z* 1287.5 $(M + Na)^+$.

p-*Methoxyphenyl 2*-O-*acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl-(1→3)-[2*-O-*acetyl-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl-(1→6)]-2,4-di*-O-*benzyl*-β-D-*mannopyranosyl-(1→4)-[3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl-(1→6)]-3*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**10**).—To a stirred mixture of **8** (118 mg, 0.093 mmol), AgOTf (130 mg, 0.50 mmol) and 4A MS (1.0 g) in CH_2Cl_2 (8 mL) was added a solution of compound **9** (190 mg, 0.37 mmol) in CH_2Cl_2 (30 mL) at −40 °C. The mixture was gradually warmed to room temperature and stirred overnight. It was filtered through Celite and the filtrate was concentrated in vacuo. The residue so obtained was purified by silica gel CC (6–8:1 toluene–EtOAc) to gave **10** (173 mg, 84%) as a white solid. **10**: $[\alpha]_D$ +32.5° (*c* 1.5, $CHCl_3$); R_f 0.42 (3:1 toluene–EtOAc); ^{1}H NMR (600 MHz): δ 7.60–6.62 (m, 57 H, H-aromatic), 5.63 (dd, 1 H, $J_{3,4}$ 9.4 Hz, $J_{2,3}$ 10.7 Hz, H-3$^{2'}$), 5.61 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^1), 5.50 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1$^{2'}$), 5.48 (bd, 1 H, $J_{2,3}$ 1.0 Hz, H-2^3), 5.28 (bd, 1 H, $J_{2,3}$ 1.0 Hz, H-2$^{3'}$), 5.11 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4$^{2'}$), 5.09 (bs, 1 H, H-1^3), 4.94, 4.84 (2 d, 1 H each, *J* 11.7, 12.0 Hz, H-Bn), 4.83 (bs, 1 H, H-1$^{3'}$), 4.79, 4.77, 4.73, 4.67, 4.61, 4.59, 4.55 (7 d, 1 H each, *J* 13.2, 10.8, 11.7, 11.2, 11.2, 12.2, 11.7 Hz, H-Bn), 4.48–4.44 (m, 4 H, H-Bn), 4.42 (s, 1 H, H-1^2), 4.41–4.35 (m, 4 H, H-Bn), 4.33 (dd, 1 H, H-2$^{2'}$), 4.23 (dd, $J_{2,3}$ 10.7 Hz, H-2^1), 4.20–4.16 (m, 3 H, H-3^1, H-Bn, H-6$^{2'}$), 4.06 (dd, 1 H, $J_{5,6'}$ 2.0 Hz, $J_{6,6'}$ 12.3 Hz, H-6′$^{2'}$), 3.95 (dd, 1 H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 9.3 Hz, H-3^3), 3.93 (m, 1 H, H-5^3), 3.90 (bs, 1 H, H-2^3), 3.87–3.62 (m, 14 H, other protons of sugar rings), 3.73 (s, 3 H, OMe), 3.51 (bd, 1 H, $J_{6,6'}$ 10.0 Hz, H-6$^{3'}$), 3.48 (m, 1 H, H-5^1), 3.30 (m, 1 H, H-5$^{2'}$), 3.07 (m, 1 H, H-5^2), 2.11, 2.04, 2.00, 1.90, 1.82 (5 s, 3 H each, 5 Ac); ^{13}C NMR: δ 170.67, 170.04, 169.95, 169.76, 169.36 (5 C=O of Ac), 167.58, 167.51, 167.46, 167.40 (4 C=O of Phth), 101.82 (C-1^2), 99.55 (C-1^3), 98.22 (C-1$^{3'}$), 96.98 (C-1$^{2'}$), 96.30 (C-1^1), 69.17, 68.79, 66.60, 66.31 (4 C-6 of sugar 1, 2, 3, 3′), 61.53 (C-6$^{2'}$), 55.38 (C-O*Me*), 55.26 (C-2^1), 54.32 (C-2$^{2'}$), 20.94, 20.72, 20.69, 20.43, 20.31 (5 *Me*C=O); FABMS: Calcd for $C_{126}H_{128}N_2O_{34}$: *m*/*z* 2212.8. Found: *m*/*z* 2236.0 $(M + Na)^+$. Anal. Calcd for $C_{126}H_{128}N_2O_{34}$: C, 68.34; H, 5.83; N, 1.27. Found: C, 68.15; H, 5.80; N, 1.24.

p-*Methoxyphenyl 3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl-(1→3)-[3,4,6-tri*-O-*benzyl*-α-D-*mannopyranosyl-(1→6)]-2,4-di*-O-*benzyl*-β-D-*mannopyranosyl-(1→4)-[2-deoxy-2-acetamido*-β-D-*glucopyranosyl-(1→6)]-3*-O-*benzyl-2-deoxy-2-acetamido*-β-D-*glucopyranoside* (**11**).—A solution of **10** (20 mg, 9.0 mmol) and ethylenediamine (0.15 mL) in *n*-butanol (2 mL) was stirred at 90 °C for 2 days. After concentration in vacuo, the residue was dissolved in MeOH (10 mL), and to the solution was added acetic anhydride (5 mL) at 0 °C. The mixture then allowed to warm to room temperature. At the end of 4 h, the mixture was concentrated in vacuo, and the residue was first purified by LH-20 gel filtration and then by HPLC (Intersil Prep-Sil column, 10×250 mm; Det. 254 nm; Eluent: 8:92 EtOH–$CHCl_3$, 4 mL/min; t_R = 9.72 min) to afford **11** (12.4 mg, 74%) as a white solid. **11**: $[\alpha]_D$ +11.2° (*c* 0.4, $CHCl_3$); ^{1}H NMR (600 MHz): δ 7.34–6.14 (m, 45 H, H-aromatic), 6.90, 6.79 (2 d, 2 H each, *J* 8.8 Hz, H-aromatic^{Mp}), 5.90 (d, 1 H, *J* 6.8 Hz,

NH^1), 5.76 (bs, 1 H, $NH^{2'}$), 5.38 (d, 1 H, $J_{1,2}$ 6.0 Hz, H-1^1), 5.23 (s, 1 H, H-1^3), 4.97 (s, 1 H, H-$1^{3'}$), 4.87–4.76 (m, 5 H, H-Bn), 4.64, 4.61 (2 d, 1 H each, *J* 11.2, 12.2 Hz, H-Bn), 4.57 (s, 2 H, H-Bn), 4.55 (s, 1 H, H-1^2), 4.54–4.37 (m, 8 H, H-Bn), 4.38 (d, 1 H, *J* 12.2 Hz, H-Bn), 4.24 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-$1^{2'}$), 4.09 (dd, 1 H, *J* 6.3, 6.8 Hz, H-3^1), 3.99 (bs, 1 H, H-$2^{3'}$), 3.96–3.59 (m, 23 H, other sugar ring protons), 3.75 (s, 3 H, OMe), 3.47–3.44 (m, 2 H, H-$4^{2'}$, H-$2^{2'}$), 3.37–3.33 (m, 2 H, H-$3^{2'}$, H-5^1), 3.19 (m, 1 H, H-$5^{2'}$), 1.75, 1.61 (2 s, 3 H each, 2 Ac); FABMS: Calcd for $C_{104}H_{118}N_2O_{27}$: *m*/*z* 1826.8. Found: *m*/*z* 1850.0 $(M + Na)^+$. Anal. Calcd for $C_{104}H_{118}N_2O_{27}$: C, 68.33; H, 6.51; N, 1.53. Found: C, 67.88; H, 6.23; N, 1.72.

p-*Methoxyphenyl* α-D-*mannopyranosyl-(1→3)-[*α-D-*mannopyranosyl-(1→6)]*-β-D-*mannopyranosyl-(1→4)-[2-deoxy-2-acetamido*-β-D-*glucopyranosyl-(1→6)]-2-deoxy-2-acetamido*-β-D-*glucopyranoside* (**2**).—Compound **11** (7.0 mg, 3.8 mmol) in water (2.0 mL) and MeOH (3.0 mL) was stirred with 20% $Pd(OH)_2$–C (20 mg) under a hydrogen atmosphere at room temperature for 2 days. After filtration and concentration in vacuo, the residue was dissolved in water and freeze-dried to get **2** (3.9 mg) as a white solid in quantitative yield. **2**: ^{1}H NMR (600 MHz, in D_2O, acetone as internal standard, 52 °C): δ 7.05, 7.00 (2 d, 2 H each, *J* 8.8 Hz, H-aromatic), 5.12 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^3), 5.07 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1^1), 4.92 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-$1^{3'}$), 4.73 (bs, 1 H, H-1^2), 4.55 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-$1^{2'}$), 4.24 (bd, 1 H, $J_{2,3}$ 2.0 Hz, H-2^2), 4.14 (dd, 1 H, $J_{5,6}$ 1. Hz, $J_{6,6'}$ 10.7 Hz H-6^1), 4.08 (dd, ^{1}H, $J_{2,3}$ 3.4 Hz, H-2^3), 3.98 (dd, 1 H, $J_{2,3}$ 2.4 Hz, H-$2^{3'}$), 3.97 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2^1), 3.94–3.65 (m, 22 H, other sugar protons), 3.81 (s, 3 H, OMe), 3.53 (dd, 1 H, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 9.5 Hz, H-$3^{2'}$), 3.45 (dd, 1 H, $J_{4,5}$ 10.0 Hz, H-$4^{2'}$), 3.40 (ddd, 1 H, $J_{5,6}$ 2.4 Hz, 5.9 Hz, H-$5^{2'}$), 2.04, 1.90 (2 s, 3 H each, 2 Ac); MOLDI-TOF-MS: Calcd for $C_{41}H_{64}N_2O_{27}$: *m*/*z* 1016.9. Found: *m*/*z* 1039.2 $(M + Na)^+$, 1055.2 $(M + K)^+$.

Acknowledgements

This work was financially supported by the Special Co-ordination Funds of the Science and Technology Agency of the Japanese Government and partly by CREST program of Japan Science and Technology Corporation. Z.-W.G. is grateful for a RIKEN Fellowship and thanks Dr. Yuko Nakahara for her generous helps. We thank Dr. P. Stanley for many helpful discussions, Dr. S. Kurono and Dr. N. Dohmae for MS measurements, Dr. J. Uzawa and his staff for NMR measurements and Ms. A. Takahashi for technical assistance.

References

[1] C.G. Gahmberg and M. Tolvanen, *TIBS.*, 21 (1996) 308–311.
[2] A. Varki, *Glycobiology*, 3 (1993) 97–130 and references cited therein.
[3] T.S. Raju, M.K. Ray, and P. Stanley, *J. Biol. Chem.*, 270 (1995) 30294–30302.
[4] (a) I. Matsuo, Y. Nakahara, Y. Ito, Y. Nukada, and T. Ogawa, *Bioorg. Med. Chem.*, 3 (1995) 1455–1463. (b) Z.-W. Guo, Y. Nakahara, and T. Ogawa, *Tetrahedron Lett.*, 38 (1997) 4799–4802.
[5] C.A.A. van Boeckel and T. Beetz, *Recl. Trav. Chim. Pays-Bas*, 106 (1987) 596–599.
[6] J.J. Oltvoort, C.A.A. van Boeckel, J.H. de Koning, and J.H. van Boom, *Synthesis*, (1981) 305–307.
[7] O. Kanie, S.C. Crawley, M.M. Palcic, and O. Hindsgaul, *Carbohydr. Res.*, 243 (1993) 139–144.