Synthesis of asparagine-linked bacillosamine

Mohammed Nurul Amin,[a,b] Akihiro Ishiwata[a,c] and Yukishige Ito[a,b,c]*

[a]*RIKEN (The Institute of Physical and Chemical Research), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

[b]*Graduate School of Science and Engineering, Saitama University, Sakura-ku, Saitama 338-8570, Japan*

and

[c]*CREST, Japan Science and Technology Agency (JST), Kawaguchi, Saitama 332-1102, Japan*

---

*Corresponding author. Tel.: +81 48 467 9430; Fax: +81 48 462 4680; e-mail: yukito@riken.jp

Abstract:

Various types of protein glycosylation have been identified from prokaryotes. Recent investigations have revealed the presence of *N*-linked glycoproteins in pathogenic bacteria, *Campylobacter jejuni*. The structure of this glycan is unique, consisting of 5 GalNAc and 1 Glc, in addition to 2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (bacillosamine; Bac), which is *N*-glycosidically linked to the side chain of asparagine (Asn). We synthesized Bac from a 2-azido-2-deoxy-galactose derivative, which was further converted to the Asn-linked form.

Keywords: *N*-linked glycoprotein; bacillosamine; *Campylobacter jejuni*

Bacillosamine (Bac), 2,4-diacetamido-2,4,6-trideoxyglucopyranose, is the reducing terminal monosaccharide component of the glycoproteins derived from bacterial species.[1,2] Bac was first isolated in 1960 from the cell wall polysaccharides of *Bacillus licheniformis* ATCC9945.[3] It is located in polysaccharides including glycoproteins and lipopolysaccharides of various prokaryotes.[4] A number of syntheses of Bac[5] and related compounds such as 2,4-diacetamido-2,4,6-trideoxygalactopyranose derivatives[6,7] have been reported.

N-Glycosylation is widespread in eukaryotic proteins, where the attachment of glycan occurs at the Asn-Xaa-Ser/Thr motif. It is believed that only eukaryotes are able to produce the *N*-glycosylated proteins,[8,9] while several types of prokaryotic *O*-glycosylation have been reported, such as *O*-linked pseudaminic acid and bacillosamine.[10] Recent investigations revealed the presence of a novel non-flagellin glycoprotein in a pathogenic gram-negative bacterium, *Campylobacter jejuni*, which was found to be a major antigenic protein designated PEB3 or Cj0289c.[1] This glycoprotein is multiply *N*-glycosylated with a novel glycan at sites having the eukaryote-like consensus amino acid sequence of Asn-Xaa-Ser/Thr (Fig. 1).[2] The structure of this *N*-linked glycan, GalNAc-α(1→4)-GalNAc-α(1→4)-[Glc-β(1→3)-]GalNAc-α(1→4)-GalNAc-α(1→4)-GalNAc-α(1→3)-Bac-β (**1**) is distinct from those of eukaryotic origin.[10c,d] In the latter case, glycans that share a common pentasaccharide [Man-α(1→3)-]Man-α(1→6)-Man-β(1→4)-GlcNAc-β(1→4)-GlcNAc-β(1→Asn) are decorated by various sugar residues. However, their biosynthetic pathways are similar to each other, in a sense that preassembled lipid-linked oligosaccharide ($Glc_3Man_9GlcNAc_2$-PP-Dol or $Glc_1GalNAc_5Bac_1$-PP-Udp; Dol: dolichyl, Udp: undecaprenyl) is transferred to a nascent peptide either in the ER lumen (eukaryotes) or periplasm (*C. jejuni*) by oligosaccharyl transferase (OST).[9,11]

The *N*-linked glycoprotein from *C. jejuni* was found to be immunodominant and *N*-glycosylation is essential for their adhesion to host cells.[10c,d] Considering the potential utility for immunochemical studies aiming at the development of antibodies and vaccines against this pathogenic bacterium as

well as for understanding the mechanisms of protein *N*-glycosylation, *C. jejuni N*-glycan in Asn-linked form would be attractive as a synthetic target.[6]

We recently completed the synthesis of the hexasaccharide region ($Glc_1GalNAc_5$) of this glycan, using 2-azido galactose ($GalN_3$) derivative **3** as the common precursor of GalNAc components.[12] In order to target the whole structure **1**, we chose 2,4-diazido-2,4,6-trideoxy glucose **5** as the Bac equivalent. This diazide was selected as the acceptor for the oligosaccharide construction toward **1**, because it has been reported that the hydroxyl groups of partially protected *N*-acetylglucosamine derivatives are poor acceptors.[13] Now, we describe the synthesis of **5** and its conversion to free Bac (**4**) as well as to asparagine-linked Bac (Bac-Asn, **2**), also starting with **3** (Fig. 1).

[**Figure 1**]

Our synthesis commenced with *tert*-butyldiphenylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside **3**,[14] which is readily obtainable from D-galactal. Protection of the 3-hydroxyl group with a 3-*O*-naphtylmethyl (NAP) gave **6** (Scheme 1).[15] Protection with NAP proved to be highly suitable for our purpose, because of its stability under various reaction conditions, including acetal hydrolysis and reductive dehalogenation, and chemoselective removal under oxidative conditions. Removal of the benzylidene acetal of **6** in AcOH–H_2O–MeOH (4:1:1) gave diol **7** in 92% yield.[15c,16] Since attempted iodination of **7** with Ph_3P, imidazole and I_2[17] resulted in the reduction of the azide, the diol **7** was first tosylated regioselectively to give **8**, then converted to iodide **9**. Chemoselective reduction was conducted with $NaBH_3CN$[18] in diglyme to afford 6-deoxy derivative **10** without affecting the N_3 groups.[19] In a large scale preparation, this compound was obtained in 67% yield through one pot transformation from **8**.

An additional azide group was introduced at the 4-position with inversion of configuration. Thus, by treatment with Tf_2O and pyridine, compound **10** was converted to triflate **11**, which was subjected to nucleophilic substitution with NaN_3,[20] to give 4-azide **12** in 91% yield in two steps. The

gluco configuration was rigorously confirmed by the ^{1}H NMR spectrum, which revealed a triplet (J = 9.6 Hz) at 3.09 ppm, assignable as H-4. Removal of the NAP ether was achieved by DDQ oxidation[15c] to afford **5** in quantitative yield.

To complete the synthesis of Bac, azide groups were reduced under hydrogenolytic conditions to afford **13**, which was acetylated to give **14** in 98% yield. Bacillosamine **4** was obtained in 90% yield through deprotection of the anomeric TBDPS group by NH_4F in MeOH.[5a,21]

[**Scheme 1**]

Synthetic Bac (**4**) was converted to the glycosylamine, according to Kochetkov's procedure[22] (sat. ammonium bicarbonate), which was isolated as an allyl carbamate **15 (**Scheme 2). At this stage, the strereochemical homogeneity was confirmed by ^{1}H NMR, which revealed the H-1 signal at 4.73 ppm ($J_{H1\text{-}H2}$ = 10.0 Hz). It was subjected to coupling with aspartic acid fluoride in the presence of $Pd(PPh_3)_4$ and $PhSiH_3$[23] to provide **2** in 86% yield as a pure β-isomer (δ 4.86 ppm, J = 9.6 Hz).

[**Scheme 2**]

In conclusion, we developed a synthesis of *N*-asparagine-linked bacillosamine from D-galactose by employing: 1) NAP ether as a compatible protection group; 2) regioselective formation of iodide **9** from diol **7** and chemoselective reduction of **9** in the presence of NAP ether and azide; and 3) inversion at the 4-position of **10** via triflate **11** to efficiently introduce nitrogen functionality. Furthermore, *N*-asparagine-linked bacillosamine **2** was obtained through the synthesis of Alloc-protected glycosyl amine **15**, followed by one-pot Pd(0)-$PhSiH_3$-mediated removal of Alloc and coupling with aspartic acid fluoride. Synthetic studies for the novel *N*-glycan (**1**) from *C. jejuni* are now in progress.[12]

1. Experimental

1.1. General procedures. All reactions sensitive to air and/or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Column chromatography was performed on silica gel 60N, 100-210 mesh (Kanto Kagaku Co., Ltd.). Preparative TLC was performed on silica gel 60 F_{254}, 0.5 mm (E. Merck). Melting points were determined with a Büchi 510 melting point apparatus. Optical rotations were measured with a JASCO DIP 370 polarimeter. ^{1}H NMR spectra were recorded at 400 MHz on a JEOL JNM-AL 400 spectrometer and chemical shifts are referred to internal $CDCl_3$ (7.24 ppm), D_2O (4.65 ppm) or CD_3OD (3.30 ppm). ^{13}C NMR spectra were recorded at 100 MHz on the same instrument and chemical shifts are referred to internal $CDCl_3$ (77.00 ppm), CD_3OD (49.00 ppm) or dioxane (67.19 ppm) in D_2O. MALDI-TOF mass spectra were recorded on a SHIMADZU Kompact MALDI AXIMA-CFR spectrometer with 2,5-dihydroxybenzoic acid as the matrix. ESI-TOF mass spectra were recorded on a JEOL AccuTOF JMS-T700LCK with CF_3CO_2Na as the internal standard. Elemental analyses were performed with a Fisons EA1108 instrument.

1.2. *tert*-Butyldiphenylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-naphthylmethyl-β-D-galactopyranoside (6).

To a solution of *tert*-butyldiphenylsilyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (**3**) (9.00 g, 16.9 mmol) in dry tetrahydrofuran 2-(bromomethyl)naphthalene (3.93 g, 17.8 mmol) was added sodium hydride (570 mg, 23.8 mmol) followed by tetrabutylammonium iodide (500 mg, 1.35 mmol) under Ar atmosphere at room temperature. The reaction was stirred at ambient

temperature for 6 h. T. l. c. (hexane–ethyl acetate, 7:3) indicated a single faster moving product (R_f = 0.65) than the starting materials (R_f = 0.50). Ice chips were added to the mixture, which was extracted twice with ethyl acetate. The combined organic layers were washed with brine and dried over $MgSO_4$, concentrated *in vacuo* and subjected to silica gel chromatographic purification (using a gradient solvent system of hexane–ethyl acetate, 8:1 to 7:1 to 6:1) to afford the title compound as a white foamy amorphous solid (10.8 g, 95%); $[\alpha]^{25}_D$ +39.6° (*c* 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.12 (s, *t*-Bu, 9H), 2.83 (brs, H-5, 1H), 3.27 (dd, *J* = 3.6 Hz, 10.4 Hz, H-3, 1H), 3.75 (dd, *J* = 1.6, 12.0 Hz, H-6a, 1H), 3.89 (dd, *J* = 1.2, 12.4 Hz, H-6b, 1H), 3.91–4.11 (m, H-2, H-4, 2H), 4.37 (d, *J* = 8.0 Hz, H-1, 1H), 4.83 and 4.87 (2d, *J* = 12.8 Hz, ArCH_2, 1H each), 5.37 [s, PhC*H*$(O)_2$, 1H], 7.29–7.54 (m, Ar, 14H), 7.69–7.82 (m, Ar, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.29, 26.93, 64.73, 66.19, 68.79, 71.65, 72.39, 77.73, 96.75, 101.05, 125.60, 125.93, 126.09, 126.39, 126.44, 127.16, 127.39, 127.61, 127.78, 128.14, 128.17, 128.99, 129.50, 129.64, 132.96, 135.23, 135.78, 135.93; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{40}H_{41}N_3O_5SiNa$, 694.27, found 694.72; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{40}H_{41}N_3O_5SiNa$, 694.2729, found 694.2713.

Anal. Calcd. for $C_{40}H_{41}N_3O_5Si$: C, 71.51; H, 6.15; N, 6.25. Found: C, 71.26; H, 6.15; N, 6.10.

1.3. *tert*-Butyldiphenylsilyl 2-azido-2-deoxy-3-*O*-naphthylmethyl-β-D-galactopyranoside (7).

Compound **6** (15.12 g, 22.53 mmol) was dissolved in tetrahydrofuran. A mixture of AcOH–H_2O–MeOH (4:1:1) was added to the solution and stirred at 80 °C for 22 h. The concentrated crude mixture was purified by silica gel column chromatography (hexane–ethyl acetate, 5:2) to provide the title compound as a white solid (12.06 g, 92%); mp 117–118 °C; $[\alpha]^{27}_D$ +9.09° (*c* 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.12 (s, *t*-Bu, 9H), 3.02 (m, H-5, 1H), 3.24 (dd, *J* = 3.2, 10.0 Hz, H-3, 1H), 3.44 (dd, *J* = 4.0, 12.0 Hz, H-6a, 1H), 3.66 (dd, *J* = 7.2, 12.0 Hz, H-6b, 1H), 3.74 (dd, *J* = 7.6, 10.4 Hz, H-2, 1H), 3.79 (d, *J* = 2.8 Hz, H-4, 1H), 4.44 (d, *J* = 7.6 Hz, H-1,

1H), 4.82 (s, ArCH_2, 2H), 7.34–7.50 (m, Ar, 9H), 7.69–7.85 (m, Ar, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.12, 26.82, 62.22, 65.37, 66.13, 72.27, 74.40, 78.91, 96.97, 125.56, 126.16, 126.27, 126.84, 127.37, 127.64, 127.82, 128.46, 129.80, 129.90, 132.53, 133.05, 133.56, 134.41, 135.65, 135.70; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{33}H_{37}N_3O_5SiNa$, 606.24, found 606.10; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{33}H_{37}N_3O_5SiNa$, 606.2424, found 606.2400.

Anal. Calcd for $C_{33}H_{37}N_3O_5Si$: C, 67.90; H, 6.39; N, 7.20. Found: C, 67.62; H, 6.28; N, 6.87.

1.4. *tert*-Butyldiphenylsilyl 2-azido-2-deoxy-3-*O*-naphthylmethyl-6-*O*-(*p*-toluenesulfonyl)-β-D-galactopyranoside (8).

A solution of compound **7** (5.20 g, 8.92 mmol) in dry pyridine was treated with TsCl (1.87 g, 9.81 mmol) in the presence of 4-dimethylaminopyridine (49.0 mg, 0.40 mmol) under Ar atmosphere at ambient temperature for 72 h. Monitoring of the reaction by t.l.c. (hexane–ethyl acetate, 5:2) showed a single product (R_f = 0.60). Ice chips were added in order to quench the excess reagent. The reaction mixture was extracted with ethyl acetate and the organic layer was washed with $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography (hexane–ethyl acetate 5:2) to afford the title compound as a white amorphous solid (6.18 g, 94%); $[\alpha]^{27}_D$ +17.19° (*c* 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.11 (s, *t*-Bu, 9H), 2.39 (s, CH_3Ar, 3H), 2.49 (br s, 4-O*H*, 1H), 3.17 (dd, *J* = 2.4, 10.0 Hz, H-3, 1H), 3.23 (br t, *J* = 6.4 Hz, H-5, 1H), 3.66 (dd, *J* = 8.0, 9.2 Hz, H-2, 1H), 3.77 (d, *J* = 3.2 Hz, H-4, 1H), 3.91 (dd, *J* = 7.2, 10.0 Hz, H-6a, 1H), 4.12 (dd, *J* = 7.2, 9.6 Hz, H-6b, 1H), 4.24 (d, *J* = 8.0 Hz, H-1, 1H), 4.74 (s, ArCH_2, 2H), 7.33–7.47 (m, Ar, 10H), 7.65–7.83 (m, Ar, 11H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.10, 21.59, 26.75, 60.33, 64.97, 67.93, 71.69, 72.23, 78.72, 96.45, 125.46, 126.13, 126.23, 126.77, 127.33, 127.47, 127.59, 127.76, 127.78, 128.39, 129.66, 129.71, 129.77, 132.18, 132.33, 132.61, 132.96, 132.97, 134.22, 135.69, 135.76, 144.75; MALDI-TOF: $[M+Na]^+$ calcd for

$C_{40}H_{43}N_3O_7SSiNa$, 760.24, found 760.41.

Anal. Calcd for $C_{40}H_{43}N_3O_7SSi$: C, 65.10; H, 5.87; N, 5.69; S, 4.35. Found: C, 65.09; H, 5.76; N, 5.57; S, 4.59.

1.5. *tert*-Butyldiphenylsilyl 2-azido-2,6-dideoxy-6-iodo-3-*O*-naphthylmethyl-β-D-galactopyranoside (9).

A mixture of compound **8** (2.50 g, 3.39 mmol) and sodium iodide (2.54 g, 17.0 mmol) in diethylene glycol dimethyl ether was stirred in a brown flask under Ar atmosphere at 120 °C for 10 h until t.l.c. (hexane–ethyl acetate, 5:2) showed the complete conversion of starting materials in to a faster moving (R_f = 0.70) product. The reaction mixture was co-evaporated with toluene and then purified by flash chromatography (hexane–ethyl acetate, 5:1) to afford the title compound as a white foamy-solid mass (2.14 g, 91%); $[\alpha]^{24}_D$ +4.96° (*c* 0.30, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.10 (s, *t*-Bu, 9H), 2.29 (br s, 4-O*H*), 2.99 (dd, *J* = 6.0, 10.0 Hz, H-6a, 1H), 3.19 (m, H-5, 1H), 3.23–3.29 (m, H-3, , H-6b, 2H), 3.66 (dd, *J* = 8.0, 10.0 Hz, H-2, 1H), 4.04 (br s, H-4, 1H), 4.28 (d, *J* = 8.0 Hz, H-1, 1H), 4.82 (br s, ArCH_2, 2H), 7.32–7.49 (m, Ar, 9H), 7.69–7.85 (m, Ar, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.27, 26.92, 53.46, 64.89, 65.69, 72.38, 74.76, 79.19, 96.4, 125.47, 126.09, 126.18,126.8, 127.24, 127.43, 127.58, 127.76, 128.4, 129.58, 129.74, 132.35, 132.73, 132.98, 134.2, 135.75, 135.86; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{33}H_{36}N_3O_4ISiNa$, 716.14, found 716.65; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{33}H_{36}N_3O_4ISiNa$, 716.1395, found 716.1417.

Anal. Calcd for $C_{33}H_{36}N_3O_4ISi$: C, 57.14; H, 5.23; N, 6.06; I, 18.30. Found: C, 57.09; H, 5.00; N, 5.98; I, 18.07.

1.6. *tert*-Butyldiphenylsilyl 2-azido-2,6-dideoxy-3-*O*-naphthylmethyl-β-D-galactopyranoside

(10).

A mixture of compound **8** (7.50 g, 10.17 mmol) and sodium iodide (6.10 g, 40.7 mmol) in diethylene glycol dimethyl ether was stirred at 120 °C in a brown flask under Ar atmosphere until t.l.c. (hexane–ethyl acetate, 5:2) indicated the complete conversion of starting materials (R_f = 0.60) into a faster moving product (R_f = 0.70, compound **9**). The mixture was cooled to room temperature and then sodium cyanoborohydride (7.67 g, 0.122 mol) was added. The mixture was stirred at 120 °C under argon atmosphere for 1 day. Monitoring of the reaction by t.l.c. (hexane–ethyl acetate, 5:2) revealed the absence of starting materials and two slower moving products at R_f 0.50 and R_f 0.20, which correspond to desired product and compound **7**, respectively. The reaction mixture was diluted with ethyl acetate and washed with water and brine, dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography (hexane–ethyl acetate, 5:2) to afford the title compound as a colorless pasty mass (3.86 g, 67%); $[\alpha]^{27}_D$ +11.03° (*c* 0.47, CH_2Cl_2); FTIR (KBr, thin film) ν (cm^{-1}): 2112 (N_3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.12 (s, *t*-Bu, 9H), 1.13 (d, *J* = 6.0 Hz, H-6, 3H), 2.32 (br s, 4-O*H*, 1H), 3.10 (m, H-5, 1H), 3.23 (dd, *J* = 3.6 Hz, 10.0 Hz, H-3, 1H), 3.62 (br s, H-4, 1H), 3.67 (dd, *J* = 8.0 Hz, 10.0 Hz, H-2, 1H), 4.29 (d, *J* = 8 Hz, H-1, 1H), 4.82 (br s, ArCH_2, 2H), 7.33–7.51 (m, Ar, 9H), 7.70–7.85 (m, Ar, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.03, 19.23, 26.87, 65.25, 68.21, 70.01, 72.01, 79.52, 96.55, 125.6, 126.08, 126.19, 126.77, 127.19, 127.39, 127.63, 127.82, 128.4, 129.55, 129.66, 132.82, 133.02, 133.04, 133.14, 134.58, 35.82, 135.93; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{33}H_{37}N_3O_4SiNa$, 590.24, found 590.58; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{33}H_{37}N_3O_4SiNa$, 590.2437, found 590.2451.

1.7. *tert*-Butyldiphenylsilyl 2,4-diazido-3-*O*-naphthylmethyl-2,4,6-trideoxy-β-D-glucopyranoside (12).

To a solution of compound **10** (1.33 g, 2.34 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C was added

anhydrous pyridine (470 μL, 5.86 mmol) and trifluoromethanesulfonic anhydride (590 μL, 3.51 mmol). The solution was stirred at the same temperature under Ar atmosphere for 1.5 h. The reaction was monitored by t.l.c. (hexane–ethyl acetate, 3:1) which showed a faster moving product (R_f = 0.75) and absence of starting materials. Ice chips were added and the mixture was extracted with dichloromethane. The organic layer was washed with H_2O, $NaHCO_3$ and brine followed by drying over Na_2SO_4. It was concentrated to give the crude triflate **11** as a light yellow oil, which was immediately dissolved in anhydrous *N*, *N*-dimethylformamide (10 mL) and treated with NaN_3 (0.763 g, 11.7 mmol) at ambient temperature under argon atmosphere. After 3 h, t.l.c. (hexane–ethyl acetate, 6:1) revealed a single faster moving product (R_f = 0.75). The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic phase was dried over Na_2SO_4, concentrated and purified by silica gel column chromatography (hexane–ethyl acetate, 8:1) to provide **12** as a pale yellow semi-solid (1.26 g, 91%); $[\alpha]^{24}_D$ +30.27° (*c* 0.30, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.09 (d, *J* = 6.0 Hz, H-6, 3H), 1.10 (s, *t*-Bu, 9H), 2.79 (m, H-5, 1H), 3.09 (t, *J* = 9.6 Hz, H-4, 1H), 3.17 (t, *J* = 9.6 Hz, H-3, 1H), 3.46 (dd, *J* = 8.0, 9.2 Hz, H-2, 1H), 4.33 (d, *J* = 7.6 Hz, H-1, 1H), 4.91 and 5.00 (2d, *J* = 11.0 Hz, ArCH_2, each 1H), 7.32–7.52 (m, Ar, 9H), 7.66–7.69 (m, Ar, 4H), 7.80–7.84 (m, Ar, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.20, 19.28, 26.93, 67.83, 69.09, 70.51, 75.41, 81.39, 96.47, 125.90, 125.95, 126.01, 127.12, 127.42, 127.54, 127.89, 128.09, 129.62, 129.77, 132.47, 132.95, 132.97, 133.11, 134.65, 135.68, 135.74; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{33}H_{36}N_6O_3SiNa$, 615.25, found 615.34; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{33}H_{36}N_6O_3SiNa$, 615.2518, found 615.2516.

1.8. *tert*-Butyldiphenylsilyl 2,4-diazido-2,4,6-trideoxy-β-D-glucopyranoside (5).

To a mixture of compound **12** (1.20 g, 2.03 mmol), dichloromethane (15 mL) and H_2O (1.5 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.552 g, 2.43 mmol) at room temperature

under Ar atmosphere. The mixture was stirred for 15 h. T.l.c. (hexane–ethyl acetate, 25:1) showed a slower moving spots (R_f 0.20). The reaction was quenched with ascorbic acid-citric acid buffer (1.5 g of L-ascorbic acid, 1.8 g of citric acid monohydrate and 1.38 g of NaOH in 150 mL of H_2O) and extracted with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ (aq.) and brine and dried over Na_2SO_4. The dried organic phase was concentrated and purified by silica gel column chromatography (hexane–ethyl acetate, 25:1) to afford compound **5** as a white solid (0.916 g, quant.); mp 76–78 °C; $[\alpha]^{25}_D$ +12.48° (*c* 1.00, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.08 (d, *J* = 6.0 Hz, H-6, 3H), 1.10 (s, *t*-Bu, 9H), 2.53 (d, *J* = 9.6 Hz, 3-O*H*, 1H), 2.81 (qd, *J* = 6.0, 10.0 Hz, H-5), 3.03 (t, *J* = 9.6 Hz, H-4, 1H), 3.25 (dt, *J* = 3.6, 9.4 Hz, H-3, 1H), 3.34 (dd, *J* = 7.6, 9.6 Hz, H-2, 1H), 4.34 (d, *J* = 7.6 Hz, H-1, 1H), 7.32–7.42 (m, Ar, 6H), 7.65–7.69 (m, Ar, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 18.00, 19.17, 26.86, 67.57, 69.14, 70.61, 74.13, 96.47, 127.27, 127.49, 129.71, 129.88, 132.49, 132.93, 135.74, 135.82; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{22}H_{28}N_6O_3SiNa$, 475.18, found 475.36.

Anal. Calcd for $C_{22}H_{28}N_6O_3Si$: C, 58.38; H, 6.24; N, 18.57. Found: C, 58.22; H, 6.12; N, 18.48.

1.9. *tert*-Butyldiphenylsilyl 2,4-diacetamido-2,4,6-trideoxy-β-D-glucopyranoside (14).

Compound **5** (75.0 mg, 0.166 mmol) was dissolved in dry methanol and $Pd(OH)_2$/C (20% wt) (6 mg) was added to the solution. The mixture was stirred under a hydrogen atmosphere for 4 h. Monitoring of the reaction mixture by t.l.c. (chloroform–methanol, 3:1) showed a polar spot (R_f = 0.05) which gave purple color with ninhydrin. The reaction mixture was filtrated and the filtrate was treated with acetic anhydride (0.30 mL, 3.1 mmol). After 10 minutes, t.l.c. (chloroform–methanol, 3:1) revealed a faster moving product (R_f = 0.65). The reaction mixture was concentrated and dried under vacuum overnight to afford the title compound as a white solid (79 mg, 98%); mp 204–205 °C; $[\alpha]^{27}_D$ –14.32° (*c* 1.00, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ 1.05

(d, J = 6.0 Hz, H-6, 3H), 1.07 (s, *t*-Bu, 9H), 1.93 (s, CH_3CO, 3H), 1.95 (s, CH_3CO, 3H), 3.06 (qd, J = 6.0, 10.0 Hz, H-5), 3.43 (t, H-4, J = 10.0 Hz, 1H), 3.50 (t, J = 10.0 Hz, H-3, 1H), 3.78 (dd, J = 8.4, 10.4 Hz, H-2, 1H), 4.55 (d, J = 8.4 Hz, H-1, 1H), 7.34–7.44 (m, Ar, 6H), 7.66–7.72 (m, Ar, 4H); ^{13}C NMR (CD_3OD, 100 MHz): δ 18.20, 20.04, 22.87, 23.20, 27.36, 59.09, 60.21, 72.01, 72.10, 97.23, 128.27, 128.48, 130.74, 130.78, 134.38, 134.41, 136.86, 137.05, 173.41, 173.49; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{26}H_{36}N_2O_5SiNa$, 507.23, found 507.28; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{26}H_{36}N_2O_5SiNa$, 507.2291, found 507.2292.

Anal. Calcd for $C_{26}H_{36}N_2O_5Si$: C, 64.43; H, 7.49; N, 5.78. Found: C, 64.74; H, 7.22; N, 5.66.

1.10. 2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (4).

To a solution of compound **14** (33.0 mg, 0.068 mmol) in dry methanol (5 mL) was added NH_4F (13 mg, 0.34 mmol) under Ar atmosphere at ambient temperature. The resultant mixture was stirred for 10 h at room temperature and then 4 h at 40 °C. T.l.c. (chloroform–methanol, 2:1) indicated the completeness of the reaction. Silica gel (300 mg) was added to the reaction mixture and solvent was removed under reduced pressure. The dried silica gel was transferred to a silica column and eluted with chloroform–methanol (3:1). Concentration of the proper fraction gave the title compound as a white solid (15.0 mg, 90%).

mp 259–261 °C, decomp. (lit.[4] mp 262–264 °C, decomp.); $[\alpha]^{26}_D$ –144.74° (*c* 1.00, H_2O); ^{1}H NMR (D_2O, 400 MHz): (α anomer, major), δ 1.03 (d, J = 6.4 Hz, H-6, 3H), 1.88 (s, CH_3CO, 3H), 1.89 (s, CH_3CO, 3H), 3.48 (t, J = 10.0 Hz, H-4, 1H), 3.61 (t, J = 10.8 Hz, H-3, 1H), 3.79 (dd, J = 3.6, 10.8 Hz, H-2, 1H), 3.85 (qd, J = 6.4, 10.0 Hz, H-5, 1H), 5.04 (d, J = 3.6 Hz, H-1, 1H); ^{13}C NMR (D_2O, 100 MHz): δ 17.65, 22.63, 22.86, 22.87, 22.90, 55.29, 57.68, 58.02, 67.25, 69.11, 71.67, 72.36, 91.28, 95.22, 174.85, 174.89, 174.97, 175.11; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{10}H_{18}N_2O_5Na$, 269.11, found 268.88; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{10}H_{18}N_2O_5Na$, 269.1105, found

269.1113.

^{1}H NMR (D_2O, 400 MHz): (β anomer, minor), δ 1.06 (d, J = 6.0 Hz, H-6, 3H), 1.96 (s, CH_3CO, 3H), 1.97 (s, CH_3CO, 3H), 3.40-3.60 (m, H-2, 3, 4, 5, 4H), 4.54 (d, J = 8.8 Hz, H-1, 1H).

1.11. *N*-Allyloxycarbonyl 1-amino-2,4-diacetamido-2,4,6-trideoxy-β-D-glucopyranose (15).

To a solution of 2,4-diacetamido-2,4,6-trideoxy-β-D-glucopyranose (**4**) (20.0 mg, 0.081 mmol) in H_2O was added enough amount of solid NH_4HCO_3 to make the solution saturated. It was stirred at 45 °C for 60 h. T.l.c. (chloroform–methanol, 1:1) revealed a single slower moving (R_f = 0.15) product. Water was added and evaporated *in vacuo* twice. The product was lyophilized, the white crude solid was dissolved in dioxane–water (10:1). To the solution $NaHCO_3$ (50.0 mg, 0.59 mmol) was added and the mixture was stirred at 0 °C for half an hour. Allyl chloroformate (65 μL, 0.61 mmol) was added dropwise. The reaction mixture was stirred for 13 h at ice-bath to room temperature. T.l.c. analysis (chloroform–methanol, 3:1) indicated the formation of a product (R_f = 0.55). Small pieces of ice were added and the mixture was concentrated under reduced pressure. Concentrated crude was purified by silica gel column chromatography (chloroform–methanol, 3:1) to give compound **15** as an off white solid (19.0 mg, 71%); mp 248–259 °C (decomp.); $[\alpha]_D^{25}$ –46.56° (*c* 0.50, H_2O); ^{1}H NMR (CD_3OD, 400 MHz): δ 1.17 (d, J = 6.0 Hz, H-6, 3H), 1.96 (s, CH_3CO, 3H), 1.97 (s, CH_3CO, 3H), 3.46–3.54 (m, H-3, H-4, H-5, 3H), 3.71 (dd, J = 10.0, 11.2 Hz, H-2, 1H), 4.54 (d, J = 5.2 Hz, CH_AH_B=CHCH_2-, 2H), 4.73 (d, J = 10.0 Hz, H-1, 1H), 5.18 (dd, J = 1.2, 6.4 Hz, CH_AH$_B$=CHCH$_2$-, 1H), 5.28 (dd, J = 1.2, 17.2 Hz, CH_AH_B=CHCH$_2$-, 1H), 5.91 (m, CH_AH_B=CHCH$_2$-, 1H); ^{13}C NMR (CD_3OD, 100 MHz): δ 18.45, 22.81, 22.94, 56.80, 58.88, 66.63, 73.79, 82.66, 117.55, 133.83, 157.88, 173.43, 174.10; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{14}H_{23}N_3O_6Na$, 352.14, found 352.39; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{14}H_{23}N_3O_6Na$, 352.1496, found 352.1485.

1.12. N^{α}-Fluoren-9-ylmethyloxycarbonyl-N^{γ}-(2,4-diacetamido-2,4,6-trideoxy-β-D-glucopyranosyl)-L-asparagine *t*-butyl ester (2).

A mixture of compound **15** (3.0 mg, 0.009 mmol), $NaHCO_3$ (8 mg, 0.1 mmol), FmocAsp(F)O^{t}Bu (6.0 mg, 0.15 mmol) and $PhSiH_3$ (10 μL, 0.081 mmol) in dioxane–water (10:1) was treated with $Pd(PPh_3)_4$ (1 mg, 0.9 μmol) at 0 °C. The stirring was continued at ambient temperature for 21 h. Monitoring of reaction by t.l.c. (chloroform–methanol, 4:1) indicated the formation of a UV active product (R_f = 0.60). The mixture was filtered and the filtrate was concentrated. The concentrated mass was subjected to preparative thin layer chromatography (chloroform–methanol, 6:1) to provide the compound **2** (5.0 mg, 86%) as a white solid; mp 233–234 °C (decomp.); $[\alpha]^{24}_{D}$ 14.0 ° (*c* 0.15, $CHCl_3$–CH_3OH, 1:1); ^{1}H NMR ($CDCl_3$–CD_3OD, 1:1, 400 MHz): δ 1.17 (d, *J* = 6.0 Hz, H-6, 3H), 1.42 (s, *t*-Bu, 9H), 1.92 (s, C*H*$_3$CO, 3H), 1.97 (s, C*H*$_3$CO, 3H), 2.70 (dd, *J* = 6.4, 6.8 Hz, Asn-C*H*$_2$, 2H), 3.40–3.47 (m, H-3, H-5, 2H), 3.55 (dd, *J* = 9.6, 10.0 Hz, H-4, 1H), 3.73 (dd, *J* = 9.6, 10.0 Hz, H-2, 1H), 4.19 (br t, *J* = 6.4 Hz, FmocC*H*, 1H), 4.28 (dd, *J* = 7.2, 10.4 Hz, FmocC*H*$_A$H$_B$, 1H), 4.41 (dd, *J* = 7.2, 10.4 Hz, FmocCH$_A$*H*$_B$, 1H), 4.46 (t, *J* = 5.6 Hz, Asn-αC*H*), 4.86 (d, *J* = 9.6 Hz, H-1, 1H), 7.26–7.38 (m, Fmoc, 4H), 7.60 (d, *J* = 7.2 Hz, Fmoc, 2H), 7.74 (d, *J* = 7.6 Hz, Fmoc, 2H); ^{13}C NMR ($CDCl_3$–CD_3OD, 1:1, 100 MHz): δ 18.29, 22.89, 22.92, 28.14, 38.16, 47.66, 51.77, 56.08, 58.00, 67.52, 73.05, 73.73.32, 78.21, 79.48, 82.69, 120.22, 125.37, 125.42, 127.38, 128.03, 141.59, 141.61, 141.61, 144.06, 144.15, 157.15, 170.77, 171.63, 172.84, 173.78; MALDI-TOF MS: $[M+Na]^+$ calcd for $C_{33}H_{42}N_4O_9Na$, 661.28, found 661.21; HRMS ESI-TOF: $[M+Na]^+$ calcd for $C_{33}H_{42}N_4O_9Na$, 661.2819, found 661.2850.

Acknowledgments

This work was partly supported by Grant-in-Aid for Creative Scientific Research from the Japan Society for the Promotion of Science (Grant No. 17GS0420) and the "Ecomolecular Science" and "Chemical Biology" programs of RIKEN. We also thank Ms. A. Takahashi for technical assistance.

References and Notes

1. Wacker, M.; Linton, D.; Hitchen, P. G.; Nita-Lazar, M.; Haslam, S. M.; North, S. J.; Panico, M.; Morris, H. R.; Dell, A.; Wren, B. W.; Aebi, M. *Science* **2002**, *298*, 1790-1793.

2. Young, N. M.; Brisson, J-R.; Kelly, J.; Watson, D. C.; Tessier, L.; Lanthier, P. H.; Jarrell, H. C.; Cadotte, N.; Michael, F. St.; Aberg, E.; Szymanski, C. M. *J. Biol. Chem.* **2002**, *277*, 42530-42539.

3. Schäffer, C.; Scherf, T.; Christian, R.; Kosma, P.; Zayni, S.; Messner, P.; Nathan, S. *Eur. J. Biochem.* **2001**, *268*, 857-864.

4. Sharon, N.; Jeanloz, R. W. *J. Biol. Chem.* **1960**, *235*, 1-5.

5. (a) Liav, A.; Hildesheim, J.; Zehavi, U.; Sharon, N. *Carbohydr. Res.* **1974**, *33*, 217-227; (b) Bundle D.; and Josephson, S. *Can. J. Chem.* **1980**, *58*, 2679-2685.

6. Chemoenzymatic syntheses of undecaprenylpyrophosphate-linked glycans have been reported. (a) Weerapana, E.; Glover, K. J.; Chen, M. M.; Imperiali, B. *J. Am. Chem. Soc.* **2005**, *127*, 13766-13767; (b) Glover, K. J.; Weerapana, E.; Imperiali, B. *Proc. Nat. Acad. Sci.* **2005**, *102*, 14255-14259.

7. (a) Medgeyes, A.; Farkas, E.; Lipták, A.; Pozsgay, V. *Tetrahedron.* **1997**, *53*, 4159-4178; (b) Hermans, J. P. G.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1987**, *6*, 451-462.

8. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683-720.

9. Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. ed. *Essentials in Glyocbiology*, Cold Spring Harbor Laboratory Press, New York, **1999**.

10. (a) Benz, I.; Schmidt, M. A. *Mol. Microbiol.* **2002**, *45*, 267-276; (b) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1985**, *54*, 631-664; (c) Szymanski, C. M.; Yao, R.; Ewing, C. P.; Trust, T. J.; Guerry, P. *Mol. Microbiol.* **1999**, *32*, 1022-1030; (d) Linton, D.; Allan, E.; Karlyshev, A. V.; Cronshaw, A. D.; Wren, B. W. *Mol. Microbiol.* **2002**, *43*, 497-508; (e) Schmidt, M. A.; Riley, L. W.; Benz, I. *TRENDS in Microbiol.* **2003,** *11*, 554-561.

11. Szymanski, C. M.; Logan, S. M.; Linton, D.; Wren, B. W. *Trends Microbiol.* **2003,** *11*, 233-238.

12. Chemical synthesis of $Glc_1GalNAc_5$ has been completed very recently. Ishiwata, A.; Ohta, S.; Ito, Y. *Carbohydr. Res.* accepted.

13. Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001,** *123*, 6189-6825.

14. Nakahara, Y.; Iijima, H.; Shibayama, S.; Ogawa, T. *Carbohydr. Res.* **1991**, *216*, 211-225.

15. (a) Gaunt, M. J.; Yu, J.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172-4173; (b) Gaunt, M. J.; Boschetti, C. E.; Yu, J.; Spencer, J. B. *Tetrahedron Lett.* **1999**, *40*, 1803-1806; (c) Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169-173; (d) Liao, W.; Locke, R. D.; Matta, K. L. *Chem. Commun.* **2000**, 369-370; (e) Lipták, A.; Borbás, A.; Jánossy, L.; Szilágyi, L. *Tetrahedron Lett.* **2000**, *41*, 4949-4953; (f) Csávás, M.; Borbás, A.; Szilágyi, L.; Lipták, A. *Synlett* **2002**, 887-890.

16. Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am.Chem. Soc.* **1962,** *84*, 430-440.

17. Millar, J. G.; Underhill, E. W. *J. Org. Chem.* **1986**, *51*, 4726-4728.

18. Hutchins, R. O.; Kandasamy, D.; Maryanoff, A.; Masilamani, D., Maryanoff, B. E. *J. Org. Chem*. **1977**, *42*, 82-91.

19. Kuzuhara, H.; Sato, K.; Emoto, S. *Carbohydr. Res*. **1975**, *43*, 211-225.

20. Bruce, I.; Fleet, G. W. J.; G., A.; Haraldsson, M.; Peach, J. M.; Watkin, D. J. *Tetrahedron*. **1990**, *46*, 19-32.

21. Zhang, W.; Robins, M. J. *Tetrahedron Lett*. **1992**, *33*, 1177-1180.

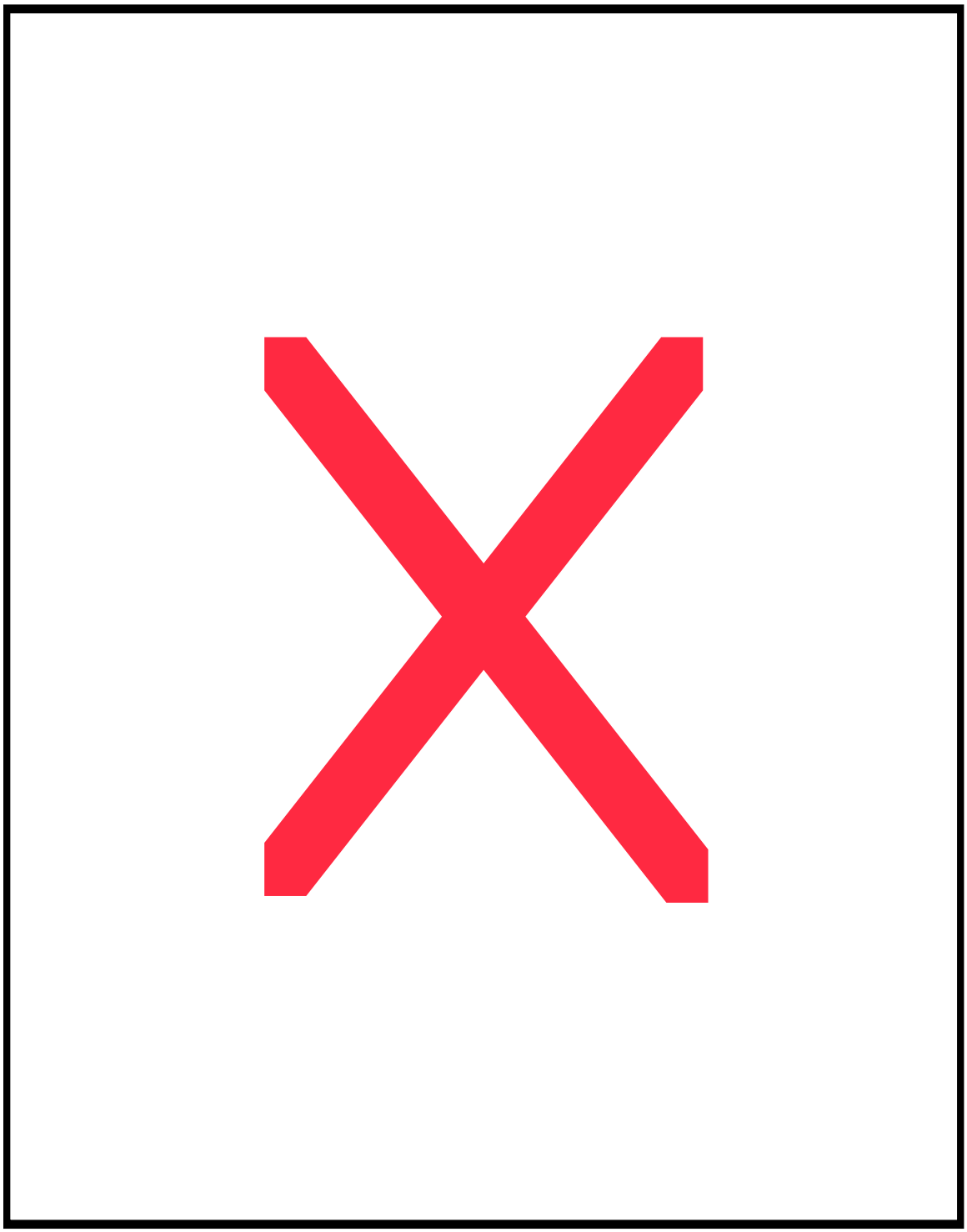
22. (a) Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res*. **1986**, *146*, c1-c5; (b) Lubineau, A.; Augé, J.; Droillat, B. *Carbohydr. Res*. **1995**, *266*, 211-219.

23. Ishiwata, A.; Takatani, M.; Nakahara, Y.; Ito, Y. *Synlett* **2002**, 634-636.

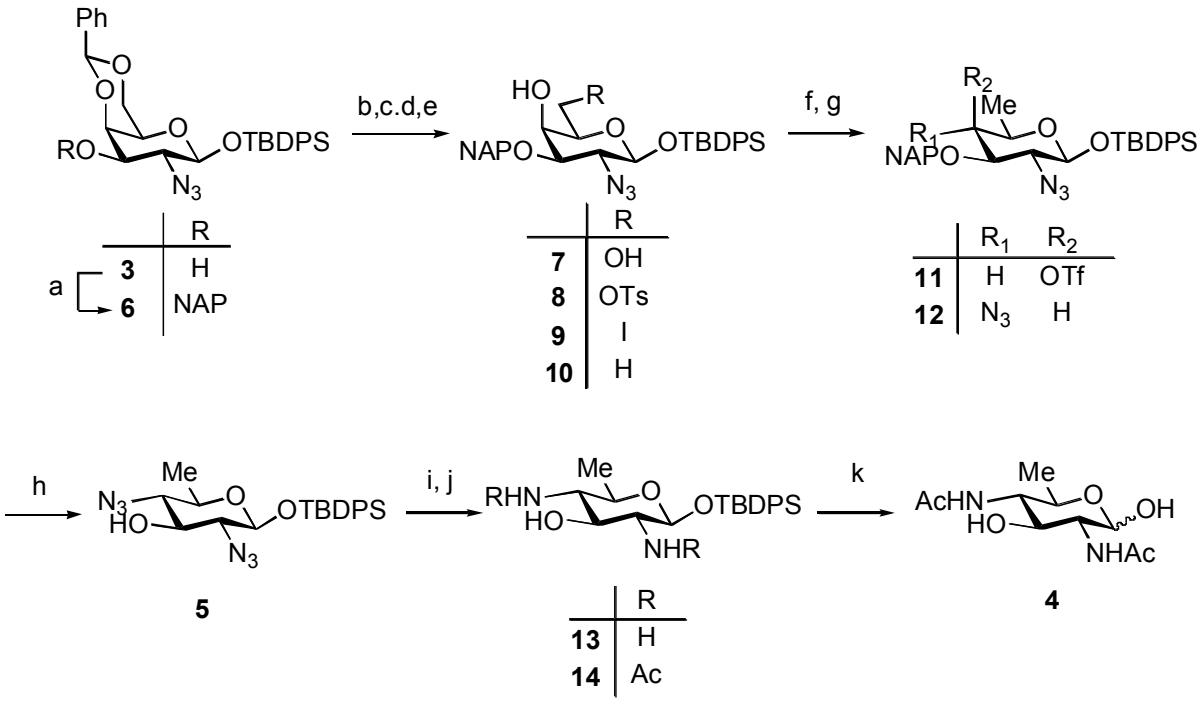
Captions

Scheme 1. *Reagents and conditions*: (a) 2-(Bromomethyl)naphthalene, NaH, Bu_4NI, THF, 95%; (b) AcOH–H_2O–MeOH (4:1:1), 80 °C, 92%; (c) TsCl, pyridine, 4-dimethylaminopyridine, 94%; (d) NaI, diglyme, 120 °C, 91%; (e) $NaCNBH_3$, diglyme, 120 °C, 70%; (f) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; (g) NaN_3, DMF, 91% in two steps; (h) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, CH_2Cl_2–H_2O (10:1), quant; (i) $Pd(OH)_2/C$, H_2, MeOH; (j) Ac_2O, MeOH, 98% in two steps; (k) NH_4F, MeOH, 90%.

Scheme 2. *Reagents and conditions*: (a) NH_4HCO_3, H_2O, 45 °C; (b) $NaHCO_3$, AllocCl, dioxane–H_2O (10:1), 71% in two steps; (c) $NaHCO_3$, FmocAsp(F)O*t*-Bu, $PhSiH_3$, $Pd(PPh_3)_4$, dioxane–H_2O (10:1), 86%.



Scheme 1


Ph
RO
OTBDPS
N3
b,c.d,e
HO
R
NAPO
OTBDPS
N3
f, g
R2
Me
R1
NAPO
OTBDPS
N3
a
3 H
6 NAP
7 OH
8 OTs
9 I
10 H
11 H OTf
12 N3 H
h
N3
HO
Me
OTBDPS
N3
5
i, j
RHN
HO
Me
OTBDPS
NHR
13 H
14 Ac
k
AcHN
HO
Me
OH
NHAc
4