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Note

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

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Abstract—Various types of protein glycosylation have been identified from prokaryotes. Recent investigations have revealed the presence of N-linked glycoproteins in the pathogenic bacterium, *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-D-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-trideoxy-glucopyranose, 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-trideoxy-D-galactopyranose 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-glycosylations 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]

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

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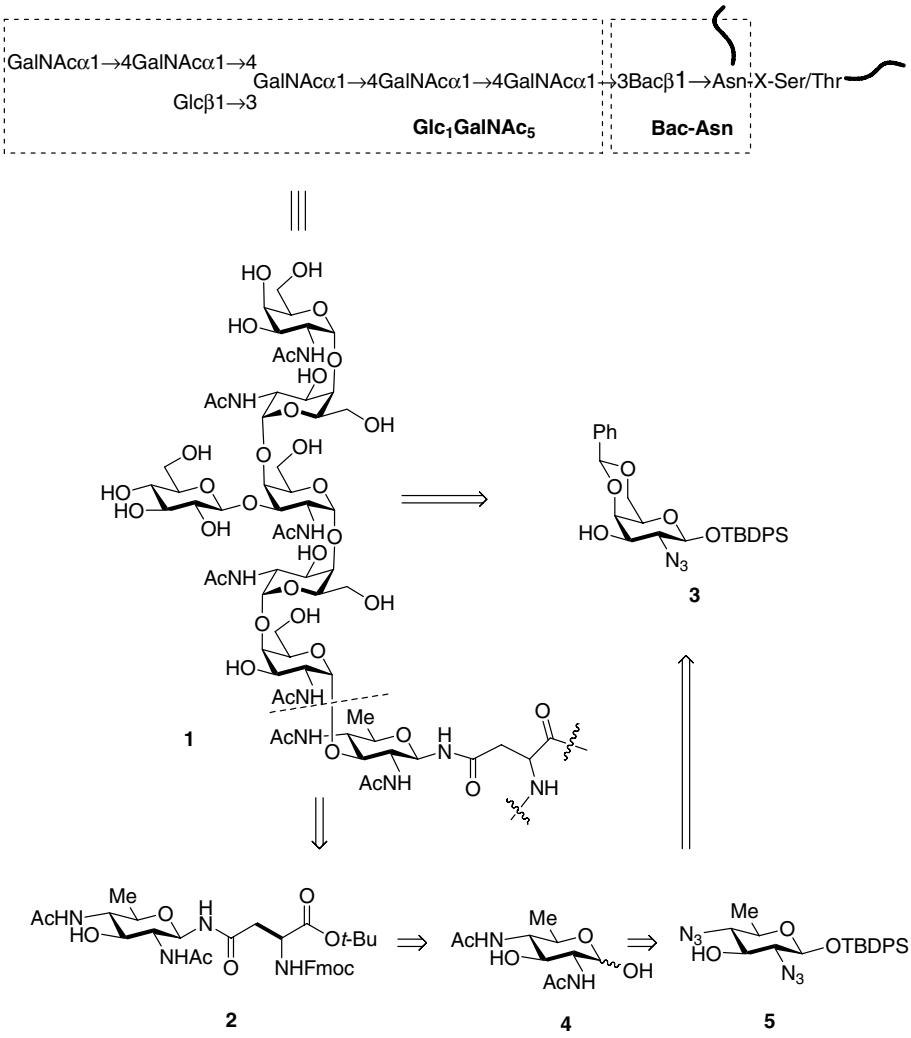
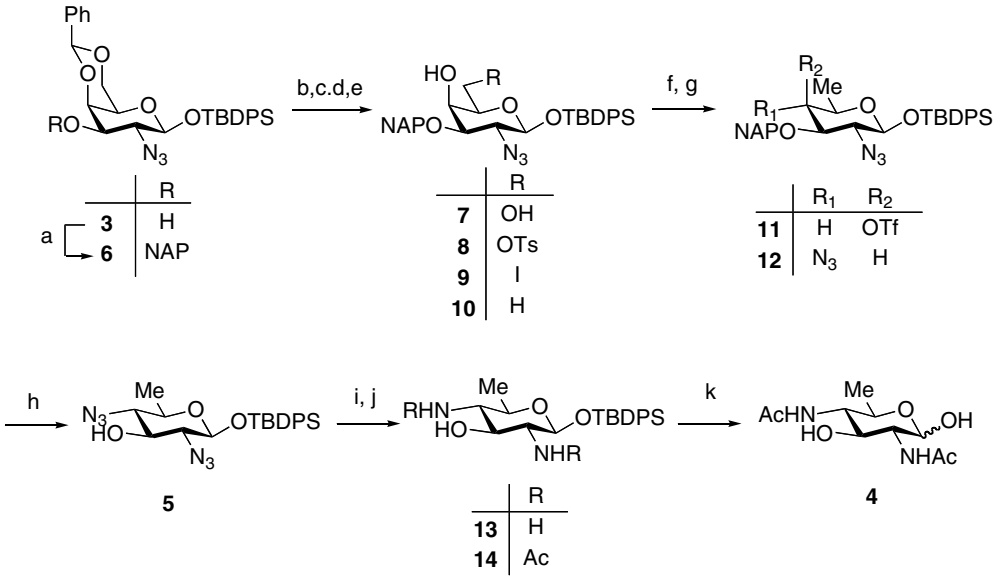


Figure 1.

We recently completed the synthesis of the hexasaccharide region ($Glc_1GalNAc_5$) of this glycan using the 2-azido-2-deoxy-D-galactose ($GalN_3$) derivative **3** as the common precursor of GalNAc components.[12] In order to target the whole structure **1**, we chose the 2,4-diazido-2,4,6-trideoxy-D-glucose derivative **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).

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*-naphthylmethyl (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 4:1:1 AcOH–H_2O–MeOH 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, 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 a one-pot transformation from **8**.

An additional azido 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 D-gluco configuration was rigorously confirmed by the ^1H NMR spectrum, which



Scheme 1. Reagents and conditions: (a) 2-(bromomethyl)naphthalene, NaH, Bu_4NI, THF, 95%; (b) 4:1:1 AcOH–H_2O–MeOH, 80 °C, 92%; (c) TsCl, pyridine, DMAP, 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) DDQ, 10:1 CH_2Cl_2–H_2O, quant; (i) $Pd(OH)_2/C$, H_2, MeOH; (j) Ac_2O, MeOH, 98% in two steps; (k) NH_4F, MeOH, 90%.

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, the azido groups were reduced with hydrogen–$Pd(OH)_2/C$ 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]

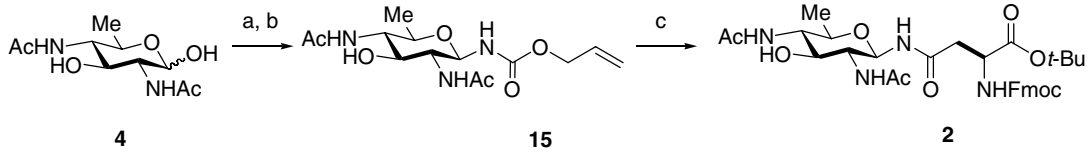
Synthetic Bac (**4**) was converted to the glycosylamine, according to Kochetkov's procedure[22] (satd ammonium bicarbonate), which was isolated as an allyl carbamate **15** (Scheme 2). At this stage, the stereochemical homogeneity was confirmed by ^1H NMR, which revealed the H-1 signal at 4.73 ppm (J_{H1-H2}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

In conclusion, we developed the 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 an aspartoyl fluoride. Synthetic studies of 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 plates (E. Merck). Melting points were determined with a Büchi 510 melting point apparatus. Optical rotations were measured with a JASCO DIP 370 polarimeter. ^1H NMR spectra were recorded at 400 MHz on a JEOL JNM-AL 400 spectrometer, and chemical shifts are referred to internal $CDCl_3$



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

(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. TLC (7:3 hexane–EtOAc) 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 EtOAc. 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 8:1 to 7:1 to 6:1 hexane–EtOAc) to afford the title compound as a white foamy amorphous solid (10.8 g, 95%): $[\alpha]_D^{25}$ +39.6 (*c* 1.00, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.12 (s, *t*-Bu, 9H), 2.83 (br s, 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 THF. A mixture of 4:1:1 AcOH–H_2O–MeOH was added to the solution, and the mixture was stirred at 80 °C for 22 h. The concentrated crude mixture was purified by silica gel column chromatography (5:2 hexane–EtOAc) to provide the title compound as a white solid (12.06 g, 92%): mp 117–118 °C; $[\alpha]_D^{27}$ +9.09 (*c* 1.00, CH_2Cl_2); ^1H 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 (DMAP, 49.0 mg, 0.40 mmol) under Ar atmosphere at ambient temperature for 72 h. Monitoring of the reaction by TLC (5:2 hexane–EtOAc) 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 EtOAc and the organic layer was washed with $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel column chromatography (5:2 hexane–EtOAc) to afford the title compound as a white amorphous solid (6.18 g, 94%): $[\alpha]_D^{27}$ +17.19 (*c* 1.00, CH_2Cl_2); ^1H 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 NaI (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 TLC (5:2 hexane–EtOAc) showed the complete conversion of starting materials into a faster moving (R_f 0.70) product. The reaction mixture was co-evaporated with toluene and then purified by flash chromatography (5:1 hexane–EtOAc) to afford the title compound as a white, foamy solid mass (2.14 g, 91%): $[\alpha]_D^{24}$ +4.96 (*c* 0.30, CH_2Cl_2); ^1H 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 $C_{33}H_{36}IN_3O_4SiNa$, 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 NaI (6.10 g, 40.7 mmol) in diethylene glycol dimethyl ether was stirred at 120 °C in a brown flask under Ar atmosphere until TLC (5:2 hexane–EtOAc) 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 $NaCNBH_3^-$ (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 TLC (5:2 hexane–EtOAc) revealed the absence of starting materials and two slower moving products at R_f 0.50 and R_f 0.20, which correspond to the desired product and compound **7**, respectively. The reaction mixture was diluted with EtOAc and washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel column chromatography (5:2 hexane–EtOAc) to afford the title compound as a colorless pasty mass (3.86 g, 67%): $[\alpha]_D^{27}$ +11.03 (*c* 0.47, CH_2Cl_2): FTIR (KBr, thin film) ν (cm^{-1}): 2112 (N_3); ^1H 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 $C_{33}H_{37}N_3O_4Si$-Na, 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-2,4,6-trideoxy-3-*O*-naphthylmethyl-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 TLC (3:1 hexane–EtOAc), which showed a faster moving product (R_f 0.75) and the absence of starting materials. Ice chips were added, and the mixture was extracted with CH_2Cl_2. 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, TLC (6:1 hexane–EtOAc) revealed a single faster moving product (R_f 0.75). The reaction mixture was diluted with EtOAc and washed with water and brine. The organic phase was dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography (8:1 hexane–EtOAc) to provide **12** as a pale-yellow semisolid product (1.26 g, 91%): $[\alpha]_D^{24}$ +30.27 (*c* 0.30, CH_2Cl_2); ^1H 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): δ18.20, 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), CH_2Cl_2 (15 mL), and H_2O (1.5 mL) was added 2,3-di-

chloro-5,6-dicyano-1,4-benzoquinone (DDQ, 0.552 g, 2.43 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 15 h. TLC (25:1 hexane–EtOAc) showed a slower moving spot (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 EtOAc. The combined organic layers were washed with satd aq $NaHCO_3$ and brine and dried over Na_2SO_4. The dried organic phase was concentrated and purified by silica gel column chromatography (25:1 hexane–EtOAc) to afford compound **5** as a white solid (0.916 g, quant): mp 76–78 °C; $[\alpha]_D^{25}$ +12.48 (*c* 1.00, CH_2Cl_2); ^1H 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 MeOH and $Pd(OH)_2/C$ (20 wt %) (6 mg) was added to the solution (Caution! Extreme fire hazard!). The mixture was stirred under a hydrogen atmosphere for 4 h. Monitoring of the reaction mixture by TLC (3:1 $CHCl_3$–MeOH) showed a polar spot (R_f 0.05) that gave a purple color with ninhydrin. The reaction mixture was filtered and the filtrate was treated with AC_2O (0.30 mL, 3.1 mmol). After 10 min, TLC (3:1 $CHCl_3$–MeOH) 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; $[\alpha]_D^{27}$ −14.32 (*c* 1.00, CH_3OH); ^1H 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, C*H*$_3$CO, 3H), 1.95 (s, C*H*$_3$CO, 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 MeOH (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. TLC (2:1 $CHCl_3$–MeOH) indicated the completeness of the reaction. Silica gel (300 mg) was added to the reaction mixture, and the solvent was removed under reduced pressure. The dried silica gel was transferred to a silica column and eluted with 3:1 $CHCl_3$–MeOH. Concentration of the proper fraction gave the title compound as a white solid (15.0 mg, 90%): mp 259–261 °C, dec (lit.[4] mp 262–264 °C, dec); $[\alpha]_D^{26}$ −144.74 (*c* 1.00, H_2O); ^1H NMR (D_2O, 400 MHz): (α anomer, major), δ 1.03 (d, *J* 6.4 Hz, H-6, 3H), 1.88 (s, C*H*$_3$CO, 3H), 1.89 (s, C*H*$_3$CO, 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. ^1H NMR (D_2O, 400 MHz): (β anomer, minor), δ 1.06 (d, *J* 6.0 Hz, H-6, 3H), 1.96 (s, C*H*$_3$CO, 3H), 1.97 (s, C*H*$_3$CO, 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 satd. It was stirred at 45 °C for 60 h. TLC (1:1 $CHCl_3$–MeOH) 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 10:1 dioxane–water. To the solution $NaHCO_3$ (50.0 mg, 0.59 mmol) was added, and the mixture was stirred at 0 °C for 0.5 h. Allyl chloroformate (65 μL, 0.61 mmol) was then added dropwise. The reaction mixture was stirred for 13 h at ice-bath to room temperature. TLC analysis (3:1 $CHCl_3$–MeOH) 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 (3:1 $CHCl_3$–MeOH) to give compound **15** as an off-white solid (19.0 mg, 71%): mp 248–259 °C (dec); $[\alpha]_D^{25}$ −46.56 (*c*

0.50, H_2O); ^1H 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, $CH_AH_BCHCH_2$–, 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 10:1 dioxane–water 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 the reaction by TLC (4:1 $CHCl_3$–MeOH) 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 (6:1 $CHCl_3$–MeOH) to provide compound **2** (5.0 mg, 86%) as a white solid: mp 233–234 °C (dec); $[\alpha]_D^{24}$ 14.0 (c 0.15, $CHCl_3$–CH_3OH, 1:1); ^1H 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, CH_3CO, 3H), 1.97 (s, CH_3CO, 3H), 2.70 (dd, J 6.4, 6.8 Hz, Asn-CH_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, FmocCH, 1H), 4.28 (dd, J 7.2, 10.4 Hz, FmocCH_AH_B, 1H), 4.41 (dd, J 7.2, 10.4 Hz, FmocCH_AH_B, 1H), 4.46 (t, J 5.6 Hz, Asn-αCH), 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, 73.32, 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.

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