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Note

Synthesis of biantennary β-D-(1→6) glucosamine oligosaccharides

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Abstract

Biantennary β-D-(1→6) glucosamine hexa-, octa-, and dodecaoligosaccharide derivatives were synthesized convergently using isopropyl thioglycosides as donors in NIS/TMSOTf-catalyzed glycosylation.

Keywords: Glycosylation; Oligosaccharides; Glucosamine

As part of an ongoing research effort on glucosamine oligosaccharides, we have synthesized the linear β-D-(1→6) glucosamine hexa- and nonasaccharides.[1–3] The one-pot sequential synthesis of the tetrasaccharide counterpart had been reported recently by Baasov's group.[4] More interestingly, we proved, in mice tests, that the β-D-glucosamine hexamer could significantly increase the number of white blood cells and marrow cells compared to the results from chemotherapy (CTX). Both the linear hexa- and nonaoligosaccharides showed mild anticancer activities against S_{180} and H_{22} tumors.[3] We found that simple elongation of the sugar chains did not appreciably increase the activity, therefore, we turned our attention to the preparation of glucosamine oligosaccharides having branched structures. Herein, we report the synthesis of biantennary β-D-(1→6) glucosamine hexa-, octa-, and dodecaoligosaccharide derivatives using isopropyl thioglycosides as donors in NIS/TMSOTf-catalyzed condensations.

Recently, much attention has been paid toward the multiple binding of synthetic glycoligands with protein receptors in relation to cell–cell interactions.[5] For examples, a dimeric Tn antigen glycolipid has been shown to be highly immunogenic,[6] and a divalent galabioside was a 100 times more efficient than the monomer in inhibiting hemagglutination by Gram-positive bacteria.[7] Since our linear structures showed only mild activities, we expected that the easily accessible biantennary β-D-(1→6) glucosamine oligosaccharides would increase the bioactivities compared to compounds reported previously. The convergent syntheses of the expected oligosaccharides are described in Scheme 1.

Regioselective coupling of isopropyl thioglycoside donor **1** with diol **3** in CH_2Cl_2 in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) at −20 °C gave a good yield of (1→4)-linked chitobiose derivative **4**.[8] Acetylation of **4** with acetic anhydride in pyridine (→**5**), followed by $BF_3{\cdot}Et_2O$-catalyzed desilylation, gave the 6,6′-diol **6** in a total yield of 61.7% from **3**. It should be noted that the TBAF-catalyzed desilylation caused acyl migration from C-4 to C-6 in this case,[9a] while trifluoroacetic acid (TFA) the promoted reaction might produce a C-6 acylated byproduct based on ^1H NMR and mass spectra analyses.[9b] Glycosylation of the disaccharide diol **6** with 2.5–3.0 equivalents of thioglycoside **1** afforded an excellent yield of tetrasaccharide **7**, which was desilylated with $BF_3{\cdot}Et_2O$ (→8) and condensed with 2.5–3 equivalents of isopropyl thioglycoside **9**, as described in the preparation of **7**, to give the fully *O*-acetylated hexaglucosamine derivative **10** in a total yield of 43% (from **6**). A major side product was characterized as 3,4-di-*O*-acetyl-1,6-anhydro-2-deoxy-2-phthalimido-β-D-glucopyranose (21% of isolated yield). Deprotection of **10** with ammonia in methanol gave free oligosaccharide **11** in good yield. When

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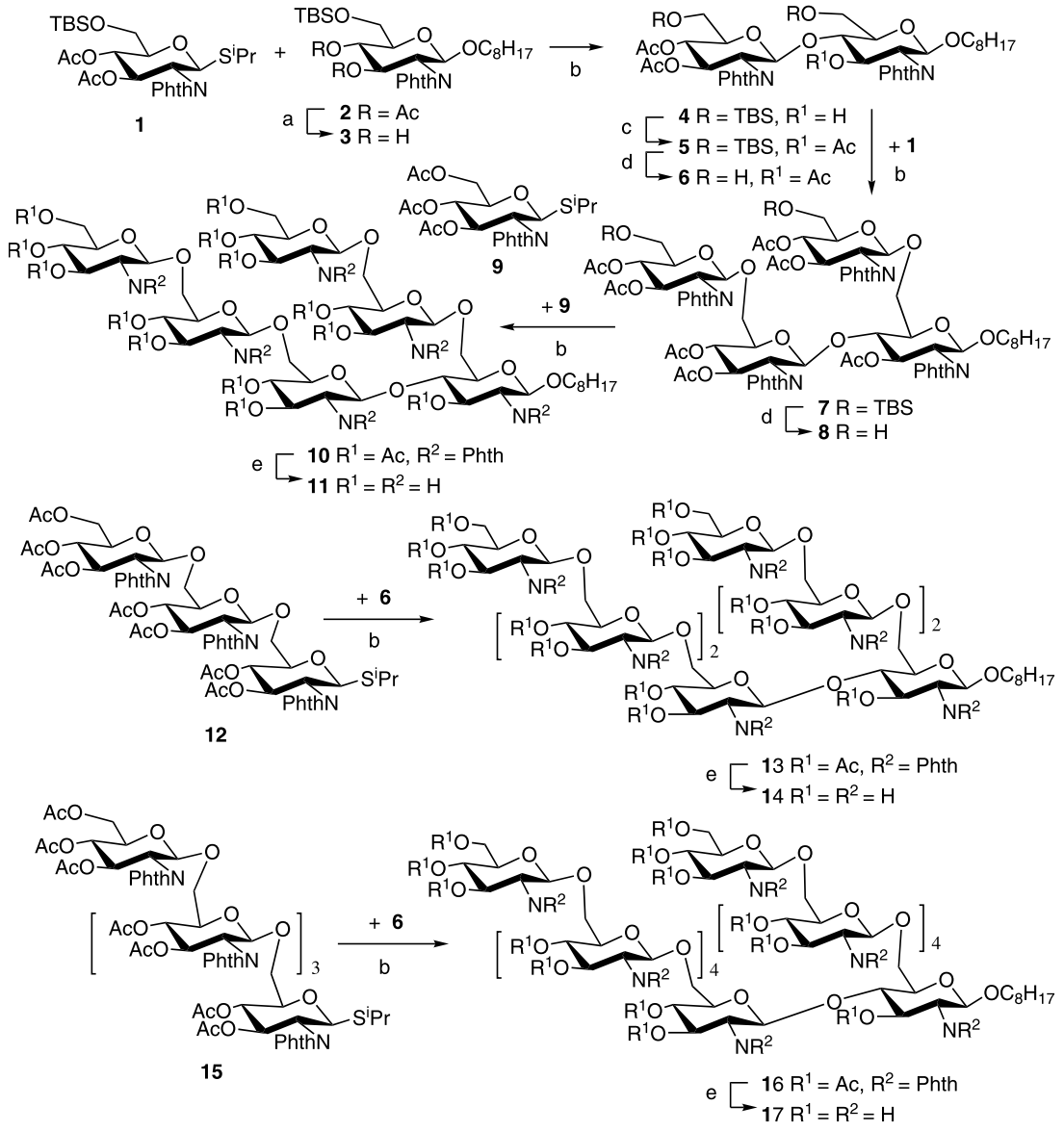

Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, 78%; (b) NIS, TMSOTf, CH_2Cl_2; 70% for **4**; 90% for **7**; 67% for **10**; 39% for **12**; 25% for **14**; (c) Ac_2O, Pyr; (d) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 89% for **6**; 71% for **8**; (e) NH_3, MeOH, 84% for **11**; 78% for **14**; 82% for **17**.

trisaccharide donor **12**[1] was coupled with diol **6**, octasaccharide **13** was observed as a major product on thin-layer chromatography (TLC) and was isolated in a yield of 39%. The major side products were deduced by MALDITOF-MS and ^{1}H NMR spectral analyses to be a mixture of monosubstituted **6** and pentasaccharides. Using a larger amount of donor **12** (up to 5 equivalents) did not appreciably increase the isolated yield, but did make the TLC more complicated. Furthermore, when the pentasaccharide donor **15**[3] was glycosylated with diol **6** at -20 °C, a 25% yield of pure dodecasaccharide **16** was obtained after purification. Both **13** and **16** were deprotected with ammonia in methanol to furnish free octa- and dodecasaccharides **14** and **17**.

In conclusion, we have synthesized biantennary β-D-(1→6) glucosamine hexa-, octa-, and dodecaoligosaccharide derivatives by reiteratively using isopropyl thioglycosides as donors in NIS/TMSOTf-catalyzed glycosylations. These compounds could be further used for the studies of structure–activity relationships among glucosamine oligosaccharides.

1. Experimental

1.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY and HMQC spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or D_2O. Chemical shifts are given in

ppm downfield from internal Me_4Si. Mass spectra were measured using MALDITOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. TLC was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector.

1.2. Octyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3)

Compound **2** (890 mg, 1.438 mmol) in MeOH (20 mL) was treated with 1 M NaOMe at room temperature (rt) for 2 h (maintained pH at 9) and then neutralized with Amberlite IR-120 (H^+) resin. After filtration, the filtrate was concentrated and subjected to the column chromatography (1:1 EtOAc–petroleum ether) to give syrupy **3** (600 mg, 78%): $[\alpha]_D$ +22° (*c* 1, $CHCl_3$); ^1H NMR: 0.12, 0.13 (2 s, 6 H, $Si(CH_3)_2$), 0.81 (t, 3 H, *J* 7.2 Hz, CH_3), 0.92 (s, 9 H, *t*-Bu), 0.88–1.42 (m, 12 H, 6 CH_2), 3.14 (dt, 1 H, *J* 8.4, 6.2 Hz, $-OCH_aH_b-$), 3.54 (ddd, 1 H, $J_{4,5}$ 8.4, $J_{6a,5}$ 6.8, $J_{6b,5}$ 4.8 Hz, H-5), 3.63 (t, 1 H, *J* 8.4 Hz, H-4), 3.78 (dt, 1 H, *J* 8.4, 6.2 Hz, $-OCH_aH_b-$), 3.88 (dd, 1 H, $J_{6b,6a}$ 10.4, $J_{5,6a}$ 6.8 Hz, H-6a), 4.00 (dd, 1 H, $J_{6a,6b}$ 10.4, $J_{5,6b}$ 4.8 Hz, H-6b), 4.11 (dd, 1 H, $J_{2,3}$ 11.0, $J_{4,3}$ 8.4 Hz, H-3), 4.36 (dd, 1 H, $J_{3,2}$ 11.0, $J_{1,2}$ 8.4 Hz, H-2), 5.20 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1), 7.70–7.84 (m, 4 H, Phth). Anal. Calcd for $C_{28}H_{45}NO_7Si$: C, 62.77; H, 8.47. Found: C, 63.02; H, 8.40.

1.3. Octyl 3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4)

To a solution of compound **1** (631 mg, 1.116 mmol) and **3** (580 mg, 1.08 mmol) in anhyd CH_2Cl_2 (8 mL) at −20 °C was added NIS (388 mg, 1.5 mmol) and Me_3SiOTf (18 μL, 0.1 mmol), respectively, under N_2 protection. The mixture was stirred under these conditions for 60 min, neutralized with Et_3N and then concentrated. The residue was subjected to the silica gel column chromatography (2:1 EtOAc–petroleum ether) to give **4** (774 mg, 70%) as a syrup: $[\alpha]_D$ +15° (*c* 1, $CHCl_3$); ^1H NMR: −0.29, −0.20, −0.19, −0.15 (4 s, 12 H, 2 $Si(CH_3)_2$), 0.64, 0.71 (2 s, 18 H, 2 *t*-Bu), 0.77–1.50 (m, 15 H), 1.83, 2.01 (2 s, 6 H, 2 $COCH_3$), 3.27 (dd, 1 H, $J_{6b,6a}$ 11.2, $J_{5,6a}$ 5.0 Hz, H-6a), 3.33–3.38 (m, 2 H, $-OCH_aH_b-$, H-5), 3.45 (dd, 1 H, $J_{6a,6b}$ 11.2, $J_{5,6b}$ 1.4 Hz, H-6b), 3.56 (dd, 1 H, $J_{5,4}$ 9.6, $J_{3,4}$ 8.0 Hz, H-4), 3.59–3.62 (m, 2 H, H-6a′, H-6b′), 3.69 (dt, 1 H, *J* 10.0, 6.4 Hz, $-OCH_aH_b-$), 3.77–3.79 (m, 1 H, H-5′), 4.04 (dd, 1 H, $J_{3,2}$ 10.8, $J_{1,2}$ 8.4 Hz, H-2′), 4.31 (dd, 1 H, $J_{3,2}$ 10.8, $J_{1,2}$ 8.4 Hz, H-2), 4.39 (dd, 1 H, $J_{3,2}$ 10.8, $J_{4,3}$ 8.0 Hz, H-3), 4.94 (dd, 1 H, $J_{5,4}$ 10.0, $J_{3,4}$ 9.0 Hz, H-4′), 5.12 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1), 5.54 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1′), 5.76 (dd, 1 H, $J_{3,2}$ 10.8, $J_{4,3}$ 9.0 Hz, H-3′), 7.68–7.86 (m, 8 H, Phth). MALDITOF-MS: Calcd for $C_{52}H_{76}N_2O_{15}Si_2$: 1024.5 [M]. Found: 1047.3 $[M+Na]^+$.

1.4. Octyl 3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (5)

Compound **4** (550 mg, 0.537 mmol) was dissolved in anhyd Py (3 mL), and Ac_2O (1 mL) was added in one portion. The mixture was stirred at rt for 6 h, then concentrated under a reduced pressure with the help of toluene. Column chromatography separation (1:2 EtOAc–petroleum ether) gave **5** (567 mg, 99%) as an amorphous solid: $[\alpha]_D$ +5° (*c* 1, $CHCl_3$); ^1H NMR: 0.07 (s, 12 H, 2 $Si(CH_3)_2$), 0.92, 0.93 (2 s, 18 H, 2 *t*-Bu), 1.83, 1.95, 2.00 (3 s, 9 H, 3 $COCH_3$), 0.78–1.4 (m, 15 H), 3.31–3.34 (m, 2 H, H-5, $-OCH_aH_b-$), 3.45 (dd, 1 H, *J* 9.2, 3.4 Hz, H-6a), 3.63–3.70 (m, 3 H, H-6b, H-5′, $-OCH_aH_b-$), 3.74 (dd, 1 H, *J* 10.8, 6.4 Hz, H-6a′), 3.81 (dd, 1 H, *J* 10.8, 2.4 Hz, H-6b′), 4.00 (t, 1 H, *J* 9.4 Hz, H-4), 4.10 (dd, 1 H, $J_{3,2}$ 10.8, $J_{1,2}$ 8.4 Hz, H-2′), 4.19 (dd, 1 H, $J_{3,2}$ 10.8, $J_{1,2}$ 8.4 Hz, H-2), 5.11 (t, 1 H, *J* 9.4 Hz, H-4′), 5.19 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1), 5.45 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1′), 5.68 (dd, 1 H, $J_{3,2}$ 10.8, $J_{4,3}$ 9.4 Hz, H-3), 5.78 (dd, 1 H, $J_{3,2}$ 10.8, $J_{4,3}$ 9.4 Hz, H-3′), 7.68–7.86 (m, 8 H, Phth). MALDITOF-MS: Calcd for $C_{54}H_{78}N_2O_{16}Si_2$: 1066.5 [M]. Found: 1089.3 $[M+Na]^+$.

1.5. Octyl 3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6)

Compound **5** (1.50 g, 1.406 mmol) was treated with $BF_3{\cdot}Et_2O$ (0.5 mL, 4.0 mmol) in CH_2Cl_2 (15 mL) for 30 min at rt, then poured into cold satd aq $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 30). The organic phase was concentrated and subjected to column chromatography (1:1 EtOAc–petroleum ether) to give syrupy **6** (1.05 g, 89%): $[\alpha]_D$ −3° (*c* 1, $CHCl_3$); ^1H NMR: 0.79 (t, 3 H, *J* 7.3 Hz, CH_3), 0.88–1.40 (m, 12 H), 1.83, 1.96, 2.03 (3 s, 9 H, 3 $COCH_3$), 3.34 (dt, 1 H, $-OCH_aH_b-$), 3.42 (dd, 1 H, *J* 11.0, 3.6 Hz, H-6a), 3.50 (dt, 1 H, $-OCH_aH_b-$), 3.56–3.75 (m, 5 H, H-5, H-5′,H-6b, H-6a′, H-6b′), 4.02 (t, 1 H, *J* 9.3 Hz, H-4), 4.12 (dd, 1 H, *J* 10.4, 8.4 Hz, H-2′), 4.22 (dd, 1 H, *J* 10.8, 8.4 Hz, H-2), 5.01 (t, 1 H, *J* 9.6 Hz, H-4′), 5.27 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1), 5.59 (d, 1 H, $J_{2,1}$ 8.4 Hz, H-1′), 5.78 (2 dd, 2 H, H-3, H-3′), 7.70–7.88 (m, 8 H, Phth). ^{13}C NMR (100 MHz, $CDCl_3$): 13.94, 20.31, 20.56, 20.74, 22.48, 25.67, 28.99, 29.99, 29.16, 31.53, 34.90, 55.04, 55.12, 60.80, 61.68, 69.19, 70.03, 70.43, 71.89, 71.36, 74.57, 74.63, 97.36, 97.94, 123.42, 123.50, 131.43, 134.15, 134.23, 167.77, 170.00, 170.05, 170.67. MALDITOF-MS: Calcd for $C_{42}H_{50}N_2O_{16}$: 838.3 [M]. Found: 861.16 $[M+Na]^+$. Anal. Calcd for

$C_{42}H_{50}N_2O_{16}$: C, 60.14; H, 6.01. Found: C, 59.93; H, 6.08.

1.6. Octyl 3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7)

To a solution of compounds **6** (210 mg, 0.25 mmol) and **1** (354 mg, 0.626 mmol) in anhyd CH_2Cl_2 (5 mL) at −20 °C was added NIS (225 mg, 1 mmol) and Me_3SiOTf (15 μL, 0.08 mmol) under N_2 protection. The mixture was stirred under these conditions for 60 min, neutralized with Et_3N and then concentrated. The residue was subjected to silica gel column chromatography (2:1 EtOAc–petroleum ether) to **7** as a foamy solid (409 mg, 90%): $[\alpha]_D$ +11° (*c* 1, $CHCl_3$); ^1H NMR: 0.08, 0.09, 0.10, 0.11 (4 s, 12 H, 2 Si(CH_3)$_2$), 0.91, 0.92 (2 s, 18 H, 2 *t*-Bu), 0.77–1.23 (m, 15 H), 1.56, 1.82, 1.86, 1.87, 1.88, 2.01, 2.03 (7 s, 21 H, 7 COCH_3), 3.23 (dt, 1 H), 3.35–3.39 (m, 2 H), 3.54–3.68 (m, 6 H), 3.68–3.70 (m, 1 H), 3.75–3.78 (m, 3 H), 3.84 (dd, 1 H, *J* 11.4, 2.4 Hz), 3.95–4.03 (m, 2 H), 4.06–4.12 (m, 2 H), 4.20 (dd, 1 H, *J* 10.0, 8.4 Hz), 4.88 (t, 1 H, *J* 10 Hz), 5.11 (d, 1 H, *J* 8.4 Hz), 5.15 (d, 1 H, *J* 8.4 Hz), 5.17 (d, 1 H, *J* 8.4 Hz), 5.10–5.19 (m, 2 H), 5.24 (dd, 1 H, *J* 10.4, 8.8 Hz), 5.37 (d, 1 H, *J* 8.4 Hz), 5.56 (dd, 1 H, *J* 10.6, 8.8 Hz), 5.72 (dd, 1 H, *J* 10.6, 8.8 Hz), 5.73 (dd, 1 H, *J* 10.6, 8.8 Hz), 7.67–7.84 (m, 16 H, Phth). MALDITOF-MS: Calcd for $C_{90}H_{112}N_4O_{32}Si_2$: 1816.7 [M]. Found: 1839.5 [M+Na]$^+$.

1.7. Octyl 3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8)

Compound **7** (323 mg, 0.178 mmol) was treated with $BF_3{\cdot}Et_2O$ (0.13 mL, 1 mmol) in CH_2Cl_2 (5 mL) for 30 min at rt, then poured into cold satd aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was concentrated and subjected to column chromatography (1:1 EtOAc–petroleum ether) to give syrupy **8** (200 mg, 71%): $[\alpha]_D$ +17° (*c* 1, $CHCl_3$); ^1H NMR: 0.79 (t, 3 H, *J* 7.2 Hz, –CH_2CH_3), 0.83–1.15 (m, 12 H), 1.71, 1.79, 1.85, 1.86, 1.87, 2.05, 2.08 (7 s, 21 H, 7 COCH_3), 3.05 (dt, 1 H, –OCH_aH$_b$–), 3.28 (dt, 1 H, –OCH$_a$$H_b$–), 3.38 (dd, 1 H, *J* 10.8, 6.0 Hz), 3.49–3.51 (m, 1 H), 3.62–3.85 (m, 11 H), 3.97 (t, 1 H, *J* 10.0 Hz), 3.98 (dd, 1 H, *J* 10.8, 8.4 Hz), 4.08 (dd, 1 H, *J* 10.8, 8.4 Hz), 4.16 (dd, 1 H, *J* 10.8, 8.4 Hz), 4.33 (dd, 1 H, *J* 10.8, 8.4 Hz), 4.97 (t, 1 H, *J* 10 Hz), 5.07 (t, 1 H, *J* 10 Hz), 5.09 (d, 1 H, *J* 8.4 Hz), 5.13 (t, 1 H, *J* 10 Hz), 5.29 (d, 1 H, *J* 8.4 Hz), 5.30 (d, 1 H, *J* 8.4 Hz), 5.43 (dd, 1 H, *J* 10.8, 9.0 Hz), 5.47 (d, 1 H, *J* 8.4 Hz), 5.6 (dd, 1 H, *J* 10.8, 9.0 Hz), 5.75 (dd, 1 H, *J* 10.8, 9.0 Hz), 5.80 (dd, 1 H, *J* 10.8, 9.0 Hz), 7.67–7.86 (m, 16 H, Phth). ^{13}C NMR (100 MHz, $CDCl_3$): 14.02, 20.33, 20.39, 20.45, 20.48, 20.70, 20.71, 20.76, 22.56, 25.76, 29.09, 29.09, 29.69, 31.61, 54.26, 54.54, 54.92, 54.92, 61.53, 62.03, 67.27, 68.38, 69.20, 69.3, 69.33, 69.72, 70.52, 70.71, 71.08, 71.08, 72.47, 73.66, 74.68, 74.74, 74.81, 96.55, 97.48, 97.51, 98.10, 123.45, 123.63, 123.77, 131.14, 131.56, 131.7, 134.33, 134.61, 167.55, 167.6, 167.7, 167.85, 169.06, 169.89, 169.92, 170.0, 170.087, 170.14, 170.19. MALDITOF-MS: Calcd for $C_{78}H_{84}N_4O_{32}$: 1588.5 [M]. Found: 1611.3 [M+Na]$^+$. Anal. Calcd for $C_{78}H_{84}N_4O_{32}$: C, 58.94; H, 5.33. Found: C, 59.23; H, 5.38.

1.8. Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10)

Coupling of **8** (140 mg, 0.088 mmol) and **9** (130 mg, 0.264 mmol) as described in the preparation of **7** gave **10** as a foamy solid (143 mg, 67%): $[\alpha]_D$ +11° (*c* 1, $CHCl_3$); ^1H NMR: 0.80 (t, 3 H, *J* 7.2 Hz, –CH_2CH_3), 0.82–1.43 (m, 12 H), 1.67, 1.75, 1.79, 1.82, 1.84, 1.86, 1.89, 1.90, 1.94, 2.01, 2.02, 2.07, 2.12 (13 s, 39 H, 13 COCH_3), 3.10 (dt, 1 H), 3.44–3.50 (m, 4 H), 3.56–3.59 (m, 1 H), 3.68–3.75 (m, 5 H), 3.87–4.01 (m, 9 H), 4.12–4.14 (m, 1 H), 4.20–4.23 (m, 2 H), 4.30–4.40 (m, 4 H), 4.69 (t, 1 H, *J* 9.2 Hz), 4.85 (t, 1 H, *J* 9.2 Hz), 4.93 (t, 1 H, *J* 9.2 Hz), 5.01 (d, 1 H, $J_{2,1}$ 8.4 Hz), 5.02 (d, 1 H, $J_{2,1}$ 8.2 Hz), 5.10 (d, 1 H, $J_{2,1}$ 8.4 Hz), 5.17 (t, 1 H, *J* 10 Hz), 5.20 (t, 1 H, *J* 10.4 Hz), 5.21 (t, 1 H, *J* 9.8 Hz), 5.23 (d, 1 H, *J* 8.4 Hz), 5.46 (dd, 1 H, *J* 10.8, 9.2 Hz), 5.46 (d, 1 H, $J_{2,1}$ 8.4 Hz), 5.48 (d, 1 H, $J_{2,1}$ 8.4 Hz), 5.61 (dd, 1 H, *J* 10.8, 9.2 Hz), 5.67 (dd, 1 H, *J* 10.8, 9.2 Hz), 5.61 (dd, 1 H, *J* 10.8, 9.2 Hz), 5.79 (dd, 1 H, *J* 10.8, 9.0 Hz), 5.81 (dd, 1 H, *J* 10.8, 9.0 Hz), 7.65–7.91 (m, 24 H, Phth). Selected ^{13}C NMR (100 MHz, $CDCl_3$): 14.02, 20.27 (2 C), 20.33, 20.38 (3 C), 20.45, 20.48, 20.49, 20.70, 20.71, 20.76, 22.56, 25.76, 29.09, 29.09, 29.69, 31.61, 54.23, 54.24, 54.29, 54.44, 54.91, 54.93, 61.53, 62.03 (2 C), 67.27, 68.38, 69.22 (2 C), 69.31, 69.33, 69.71, 70.55, 70.70, 71.08 (3 C), 72.47, 73.66, 74.68, 74.74 (>2 C), 74.81, 96.55, 97.48, 97.51, 97.55, 97.87, 98.10, 167.55, 167.62 (2 C), 167.73 (2 C), 167.85, 169.26 (2 C), 169.89, 169.98 (3 C), 170.00, 170.08 (2 C), 170.10, 170.14 (2 C), 170.19. MALDITOF-MS: Calcd for $C_{118}H_{122}N_6O_{50}$: 2422.72 [M]. Found: 2446.1 [M+Na]$^+$. Anal. Calcd for $C_{118}H_{122}N_6O_{50}$: C, 58.46; H, 5.07. Found: C, 58.68; H, 5.13.

1.9. Octyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-[2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-amino-2-deoxy-β-D-glucopyranoside (11)

To NH_3-saturated MeOH (150 mL) was added **10** (230 mg, 0.095 mmol). The mixture was stirred at rt for 7 days, then concentrated. The residue was dissolved in H_2O (1 mL) and then passed through a Bio-Gel P-2 column using H_2O as eluent to give **11** (87 mg, 84%) as an amorphous solid after freeze drying: $[\alpha]_D$ −10° (*c* 1, H_2O); Selected ^{13}C NMR (100 MHz, D_2O): 102.50, 102.71, 102.86, 103.03, 103.47, 103.62. MALDITOF-MS: Calcd for $C_{44}H_{84}N_6O_{25}$: 1096.55 [M]. Found 1119.2 $[M+Na]^+$.

1.10. Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (13)

Coupling of **6** (180 mg, 0.215 mmol) and **12** (668 mg, 0.537 mmol) as described in the preparation of **7** gave **13** as a foamy solid (265 mg, 39%): $[\alpha]_D$ +18° (*c* 1, $CHCl_3$); ^1H NMR: 0.81 (t, 3 H, *J* 7.2 Hz, $-CH_2CH_3$), 0.82–1.45 (m, 12 H), 1.70, 1.76, 1.77, 1.85, 1.86, 1.88, 1.89, 2.03, 2.04, 2.05, 2.13, 2.18 (17 s, 51 H, 17 $COCH_3$, some overlaps), 3.07–3.12 (m, 2 H), 3.24–3.26 (m, 1 H), 3.41–3.47 (m, 5 H), 3.59–3.62 (m, 2 H), 3.66–3.71 (m, 4 H), 3.80–3.85 (m, 4 H), 3.93–4.02 (m, 7 H), 4.19–4.23 (m, 4 H), 4.38–4.45 (m, 4 H), 4.77–4.83 (m, 2 H), 4.90–5.15 (m, 6 H), 4.20–4.26 (m, 2 H), 5.16–5.35 (m, 5 H), 5.40–5.60 (m, 5 H), 5.72 (dd, 2 H, *J* 10.8, 9.2 Hz), 5.79 (dd, 1 H, *J* 10.8, 9.2 Hz), 5.84 (dd, 1 H, *J* 10.8, 9.2 Hz), 7.72–7.89 (m, 32 H, Phth). Selected ^{13}C NMR (100 MHz, $CDCl_3$): 97.48, 97.52, 97.59 (3 C), 97.63 (2 C), 97.87 (8 C-1 in total). MALDITOF-MS: Calcd for $C_{154}H_{156}N_8O_{66}$: 3172.9 [M]. Found 3195.9 $[M+Na]^+$.

1.11. Octyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-[2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-amino-2-deoxy-β-D-glucopyranoside (14)

To NH_3-saturated 4:1 MeOH–CH_2Cl_2 (200 mL) was added **13** (210 mg, 0.066 mmol). The mixture was stirred at rt for 9 days, then concentrated. The residue was dissolved in H_2O (1 mL) and then passed through a Bio-Gel P-2 column using water as eluent to give foamy **14** (73 mg, 78%) after lyophilization: $[\alpha]_D$ −45° (*c* 1, H_2O); Selected ^{13}C NMR (100 MHz, D_2O): 102.76, 103.30, 103.56-103.66 (br s, 5 C), 103.70 (8 C-1). MALDITOF-MS: Calcd for $C_{56}H_{106}N_8O_{33}$: 1418.69 [M]. Found: 1441.5 $[M+Na]^+$.

1.12. Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)-3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16)

Coupling of disaccharide **6** (48 mg, 0.058 mmol) and pentasaccharide **15** (349 mg, 0.175 mmol) was carried out as described in the preparation of **7**. The reaction mixture was concentrated, then purified on silica gel columns (7:2 EtOAc–petroleum ether for the first column; 3:1 EtOAc–toluene for the second column) to give **16** as a foamy solid (67 mg, 25%): $[\alpha]_D$ +31° (*c* 1, $CHCl_3$); ^1H NMR: 0.78 (t, 3 H, *J* 7.2 Hz, $-CH_2CH_3$), 0.80–1.31 (m, 12 H), 1.63, 1.74, 1.76, 1.78, 1.86, 1.93, 2.04, 2.16 (8 s, 75 H, 25 $COCH_3$, some overlaps), 3.44–3.57 (m, 16 H), 3.71–3.75 (m, 12 H), 3.91 (m, 7 H), 4.10–4.30 (m, 11 H), 4.35–4.45 (m, 4 H), 4.70–4.85 (m, 6 H), 5.19 (m, 5 H), 5.25–5.35 (m, 5 H), 5.37–5.46 (m, 6 H), 5.49 (d, 4 H, *J* 8.4 Hz), 5.60–5.70 (m, 8 H), 5.79 (dd, 1 H, *J* 10.8, 9.2 Hz), 5.80 (dd, 1 H, *J* 10.8, 9.2 Hz), 7.69–7.90 (m, 48 H, Phth). MALDITOF-MS: Calcd for $C_{226}H_{224}N_{12}O_{98}$: 4673.3 [M]. Found: 4695.2 $[M+Na]^+$.

1.13. Octyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-[2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)-2-amino-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-amino-2-deoxy-β-D-glucopyranoside (17)

To NH_3-saturated 4:1 MeOH–CH_2Cl_2 (200 mL) was added **16** (150 mg, 0.032 mmol). The mixture was stirred

at rt for 9 days, then concentrated. The residue was dissolved in H_2O (1 mL) and then passed through a Bio-Gel P-2 column with H_2O as the eluent to give foamy **17** (54 mg, 82%) after freeze drying: $[\alpha]_D$ −61° (*c* 0.5, H_2O); Selected ^{13}C NMR (100 MHz, D_2O): 103.10–103.75 (br s with some overlapped shoulders, C-1). MALDITOF-MS: Calcd for $C_{80}H_{150}N_{12}O_{49}$: 2062.96 [M]. Found: 2085.6 $[M+Na]^+$.

1.14. 3,4-Di-*O*-acetyl-1,6-anhydro-2-deoxy-2-phthalimido-β-D-glucopyranose

This compound was isolated in a yield of 21% in the preparation of **10** as described above: $[\alpha]$ +43° (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): 1.92, 2.13 (2 s, 6 H, 2 COCH_3), 3.88 (dd, 1 H, $J_{6b,6a}$ 7.9, $J_{5,6a}$ 5.6 Hz, H-6a), 4.08 (dd, 1 H, $J_{6a,6b}$ 7.9, $J_{5,6b}$ 1.0 Hz, H-6b), 4.23 (d, 1 H, $J_{3,2}$ 9.2 Hz, H-2), 4.61 (dd, 1 H, $J_{6a,5}$ 5.6, $J_{6b,5}$ 1.0 Hz, H-5), 4.78 (d, 1 H, $J_{3,4}$ 7.6 Hz, H-4), 5.60 (dd, 1 H, $J_{2,3}$ 9.2, $J_{4,3}$ 7.6 Hz, H-3), 5.64 (s, 1 H, H-1), 7.74–7.87 (m, 4 H, Phth). MALDITOF-MS: Calcd for $C_{18}H_{17}NO_8$: 375.1 [M]. Found: 398.28 $[M+Na]^+$.

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