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Synthesis of a C_3-symmetric (1→6)-*N*-acetyl-β-D-glucosamine octadecasaccharide using click chemistry

Qi Chen, Feng Yang and Yuguo Du*

The State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

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Abstract—A C_3-symmetric (1→6)-*N*-acetyl-β-D-glucosamine octadecasaccharide was convergently synthesized on the basis of a copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of azide and alkyne. The target octadecasaccharide showed good antitumor activity against H22 in the preliminary mice tests.

Keywords: Glycosylation; Click chemistry; Glucosamine; Antitumor activity; Thioglycosides

1. Introduction

The roles of the carbohydrate moieties of glycoproteins in a variety of biological systems have been extensively investigated.[1] Despite the scope and importance of carbohydrates in biology, the difficulties in studying carbohydrate–protein interactions have hindered the efforts to develop a mechanistic understanding of carbohydrate structure and function.[2] Among these difficulties, the availability of structurally complex carbohydrates and the weak binding affinities of carbohydrate–protein interaction with dissociation constants in the millimolar range are two major challenges.[3] Intense interest is thus being directed to the design and application of multivalency of oligosaccharides known as the 'glycoside-cluster effect'.[4] Several multivalent models have already been proposed for sialyl Le^X and globotriaosyl antigens, in which polymers, dendrimers, and starfish models are widely examined. Some of the carbohydrate clusters exhibited improved bioactivities.[5–7] For example, a dimeric Tn antigen glycolipid has been shown to be highly immunogenic,[6] and a divalent galabioside was 100 times more efficient than the monomer in inhibiting hemagglutination by Gram-positive bacteria.[7]

In preceding papers, we have reported the synthesis of linear β-D-(1→6)-glucosamine hexa- and nonasaccharides.[8] We found that, in addition to the ability to inhibit tumor growth in mice tests, the β-D-glucosamine hexamer could significantly increase the number of white blood cells and marrow cells compared to the results from chemotherapy (CPA). Both the linear hexa- and nonaoligosaccharides showed mild anticancer activities against the murine carcinoma 180 tumor (S180) and liver cancer (H22 hepatoma) tumors. However, a simple elongation of the sugar chains did not appreciably increase the activity,[9] therefore, we turned our attention to the preparation of *N*-acetyl-glucosamine oligosaccharides having cluster structures. Herein, we report the synthesis of a C_3-symmetric (1→6)-*N*-acetyl-β-D-glucosamine octadecasaccharide on the basis of the copper(I)-catalyzed 1,3-dipolar cycloaddition reaction of azide and alkyne.[10]

2. Results and discussion

The convergent synthesis of the hexasaccharide unit is described in Scheme 1. Glycosylation of 3,4-di-*O*-acet-

* Corresponding author. Tel.: +86 10 62914475; fax: +86 10 62923563; e-mail: duyuguo@mail.rcees.ac.cn

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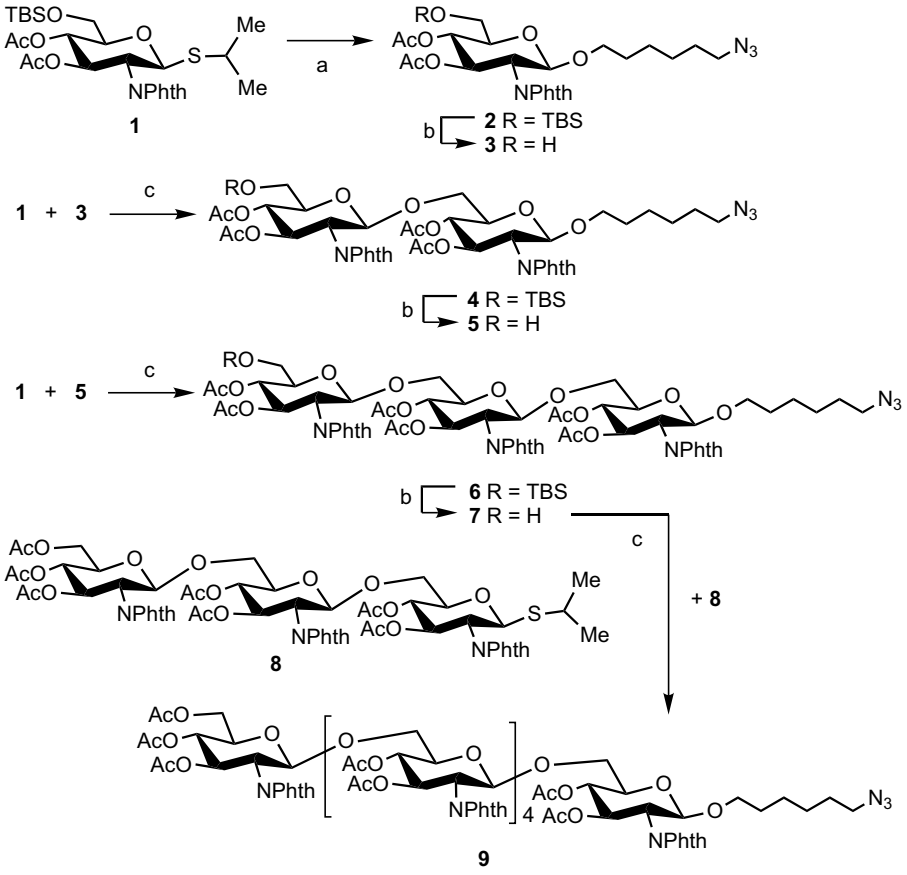
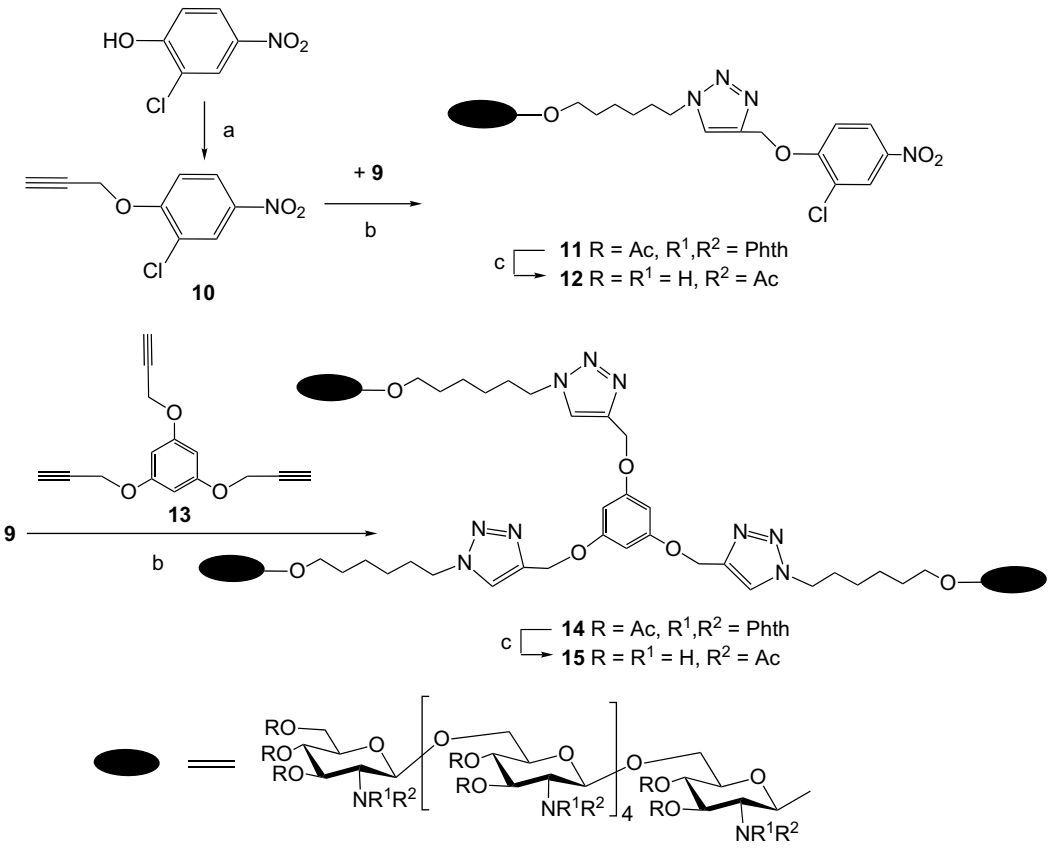


Scheme 1. Synthesis of glucosamine hexasaccharide derivative **9**. Reagents and conditions: (a) 6-azidohexyl-1-ol, NIS, TMSOTf, CH_2Cl_2, −20 °C, 95%; (b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 90% for **3**, 85% for **5**, 88% for **7**; (c) NIS, TMSOTf, CH_2Cl_2, −20 °C, 85% for **4**, 83% for **6**, 50% for **9**.

yl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**1**) with 6-azido-1-hexanol[11] in CH_2Cl_2 in the presence of *N*-Iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) at −20 °C gave exclusively 6-azidohexyl 3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**). The chemical shift of H-1 at 5.31 ppm (*J* 8.5 Hz) in the ^1H NMR spectrum indicated complete β stereoselectivity. $BF_3{\cdot}Et_2O$-catalyzed desilylation of **2** gave the acceptor, 6-azidohexyl 3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**3**), in 90% yield. We found that the removal of the *tert*-butyldimethylsilyl group using tetrabutylammonium fluoride (TBAF) as catalyst caused significant acyl migration from C-4 to C-6.[12] The reaction of donor **1** with acceptor **3** afforded 85% yield of 6-azidohexyl 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-glucopyranoside (**4**). Compound **4** was desilylated with $BF_3{\cdot}Et_2O$ (→**5**), followed by coupling with **1** (→**6**) and removal of the 6-*O*-*tert*-butyldimethylsilyl group with $BF_3{\cdot}Et_2O$, to give the triglucosamine acceptor **7** in a total yield of 62% over three steps. The ^1H NMR data corresponding to the newly formed three glycosidic bonds (H-1: 5.17 ppm, *J* 8.5 Hz; H-1′: 5.35 ppm, *J* 8.5 Hz; and H-1″: 5.50 ppm, *J* 8.5 Hz) clearly indicated the desired structure **7**. Coupling of trisaccharide thioglycoside **8**, prepared according to our previous work,[8a] with trisaccharide acceptor **7**, as described in the preparation of **4** from **1** and **3**, furnished the (1→6)-linked hexaglucosamine derivative **9** in 50% yield. Doublets (*J* 8.4 Hz) in the ^1H NMR spectra at chemical shifts of 5.13, 5.25, 5.27 (2H), 5.31, and 5.50 ppm confirmed the correct structure of **9**.

With this hexasaccharide unit in hand, we next tried to incorporate it with a C_3-symmetric alkyne derivative to form a multivalent compound for further bioassay studies. To facilitate the comparison of bioactivities and the monitoring of products, a monoantennary hexasaccharide derivative containing a UV-sensitive group was also prepared (Scheme 2). Thus, 2-chloro-4-nitrophenol was reacted with propargyl bromide in refluxing acetone in the presence of anhydrous K_2CO_3 to generate the 2-chloro-4-nitrophenyl propargyl ether (**10**) in 95% yield. Copper(I)-catalyzed click chemistry[13] of azide **9** and alkyne **10** was carried out in the presence of $CuSO_4$ (2–5 mol %) and sodium ascorbate (5–10 mol %) in a 1:1 mixture of water and THF at 50–60 °C to generate the



Scheme 2. Synthesis of C_3-symmetric octadecasaccharide **15**. Reagents and conditions: (a) propargyl bromide, K_2CO_3, acetone, reflux, 95%; (b) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, 1:1 THF–H_2O, 50–60 °C, 60% for **11**, 62% for **14**; (c) (i) MeOH, NH_3; (ii) Ac_2O, Py; (iii) NaOMe , MeOH, 76% for **12** for three steps, 68% for **15** for three steps.

desired 1,2,3-triazole **11** in 60% yield. Global deacylation of **11** in NH_3 saturated methanol, N,O-acetylation with acetic anhydride in pyridine, followed by O-deacetylation with sodium methoxide in methanol, gave the monoantennary derivative **12** in a yield of 76% for three steps. Following the same procedure, condensation of the triacetylene core **13**[14] and hexasaccharyl azide **9** under copper(I)-promoted click chemistry afforded trivalent octadecasaccharide **14** in 62% yield. The MALDITOF-MS and ^{1}H NMR spectrum confirmed the structure of the symmetric trimer **14**. Deprotection and N-reacetylation of **14**, as described in the preparation of **12**, furnished C_3-symmetric oligosaccharide **15** in a yield of 68% for three steps.

The antitumor activities of hexasaccharide **12** and octadecasaccharide **15** were preliminarily studied according to the method described by Sasaki and Takasuka.[15] Kunmin mice weighing about 20 g and H22 (2.1×10^7 cells) were used for the bioassay. Lentinan[16] [a (1→3)-β-glucan for medical usage] and Cisplatin® (CDDP) were selected as the positive controls in parallel tests. The samples were injected daily for 12 days, while CDDP was given every other day. The tumor inhibition ratios for compounds **12**, **15**, lentinan, and CDDP are summarized in Table 1. In our experiment, when the dosage of **12** was increased to 10 mg/kg/mouse, 30% of the test mice showed anorectic and low-spirited effect. The test of this dosage was thus terminated. This preliminary in vivo bioassay presented a better tumor growth inhibition ratio for C_3-symmetric octadecasaccharide **15** compared to its monomer counterpart **12**.

Table 1. Preliminary studies on antitumor activity of compounds **12** and **15**

Sample	Dose (mg/kg mouse)	Tumor growth inhibition (%)
Control	0	0
CDDP	3 (every other day)	73
12	1 (0.64 mmol)	38
12	5 (3.20 mmol)	45
15	1 (0.23 mmol)	41
15	5 (1.16 mmol)	59
Lentinan	1	35
Lentinan	5	46

In conclusion, we have synthesized a C_3-symmetric triantennary *N*-acetyl-(1→6)-β-D-glucosamine octadecaoligosaccharide derivative using 2-propyl thioglycosides as donors in NIS–TMSOTf-catalyzed glycosylations and Cu(I)-catalyzed 1,3-dipolar cycloaddition reactions of an azide and alkyne. The prepared compounds could

be further used for the studies of interactions among glucosamine oligosaccharides and proteins. The method described here should be valuable in the synthesis of other oligosaccharide clusters via click chemistry.

3. Experimental

3.1. General

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter, and $[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. Mps were determined with a 'Mel-Temp' apparatus. ^{1}H NMR, ^{13}C NMR, and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for the solutions in $CDCl_3$ or D_2O. The chemical shifts are given in parts per million downfield from internal Me_4Si. Mass spectra were measured using a MALDI-TOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix or recorded with a VG PLATFORM mass spectrometer using the ESI(−) technique to introduce the sample. Thin-layer chromatography (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 UV detector. Column chromatography was conducted by elution of a column of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. The solutions were concentrated at <60 °C under reduced pressure.

3.2. General procedure for NIS–TMSOTf-catalyzed glycosylation

To a solution of 2-propyl thioglycoside donor (1 mmol) and alcohol acceptor (0.98 mmol or as claimed specifically) in anhyd CH_2Cl_2 at −20 °C were added NIS (1.5 mmol) and TMSOTf (0.05 mmol), respectively, under N_2 protection. The mixture was stirred under these conditions for 60 min, neutralized with Et_3N, and then concentrated under diminished pressure. The residue was subjected to silica gel column chromatography (EtOAc–petroleum ether) to give the desired product.

3.3. 6-Azidohexyl 3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2)

Compound **1** (1.0 g, 1.77 mmol) was reacted with 6-azido-1-hexanol (0.38 g, 2.65 mmol) as described in the general procedure to give **2** (1.06 g, 95%) as a syrup: $[\alpha]_D^{25}$ +25 (*c*1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.06, 0.08 (2s, 6H, $Si(CH_3)_2$), 0.90 (s, 9H, *t*-Bu), 1.05–1.10 (m, 4H, CH_2CH_2), 1.22–1.38 (m, 4H, CH_2CH_2), 1.85, 2.01 (2s, 6H, 2 Ac), 3.03 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.42–3.45 (m, 1H, OCH_aH_b), 3.67–3.75 (m, 3H, H-5, H-6a, H-6b), 3.78–3.81 (m, 1H, OCH_aH_b), 4.52 (dd, 1H, *J* 10.7, 8.5 Hz, H-2), 5.10 (t, 1H, *J* 9.4 Hz, H-4), 5.31 (d, 1H, *J* 8.5 Hz, H-1), 5.80 (dd, 1H, *J* 10.7, 9.4 Hz, H-3), 7.72–7.85 (m, 4H, Ph). Anal. Calcd for $C_{30}H_{44}N_4O_9Si$: C, 56.94; H, 7.01. Found: C, 56.69; H, 6.93.

3.4. 6-Azidohexyl 3,4-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3)

Compound **2** (1.0 g, 1.58 mmol) was treated with BF_3·Et_2O (0.41 mL, 3.2 mmol) in CH_2Cl_2 (10 mL) for 15 min at rt, then poured into a cold satd aq $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phase was dried over anhyd Na_2SO_4 and concentrated. The crude product was then subjected to column chromatography (1:1 EtOAc–petroleum ether) to give syrup **3** (0.738 g, 90%): $[\alpha]_D^{25}$ +23 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.10–1.14 (m, 4H, CH_2CH_2), 1.25–1.40 (m, 4H, CH_2CH_2), 1.88, 2.07 (2s, 6H, 2 Ac), 3.05 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.42–3.45 (m, 1H, OCH_aH_b), 3.63–3.71 (m, 2H, H-6b, H-5), 3.79–3.86 (m, 2H, H-6a, OCH_aH_b), 4.29 (dd, 1H, *J* 10.7, 8.5 Hz, H-2), 5.12 (t, 1H, *J* 9.4 Hz, H-4), 5.38 (d, 1H, *J* 8.5 Hz, H-1), 5.83 (dd, 1H, *J* 10.7, 9.4 Hz, H-3), 7.75–7.88 (m, 4H, Ph). Anal. Calcd for $C_{24}H_{30}N_4O_9$: C, 55.59; H, 5.83. Found: C, 55.78; H, 5.91.

3.5. 6-Azidohexyl 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-glucopyranoside (5)

Coupling of **1** (0.92 g, 1.62 mmol) and **3** (0.7 g, 1.35 mmol) as described in the general procedure to give **4** as a foamy solid (1.16 g, 85%): $[\alpha]_D^{25}$ +37 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.08, 0.10 (2s, 6H, $Si(CH_3)_2$), 0.92 (s, 9H, *t*-Bu), 0.86–0.88 (m, 4H, CH_2CH_2), 0.90–1.10 (m, 4H, CH_2CH_2), 1.78, 1.83, 1.92, 2.01 (4s, 12H, $4COCH_3$), 3.02 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.12–3.16 (m, 1H, OCH_aH_b), 3.42–3.46 (m, 1H, OCH_aH_b), 3.62 (dd, 1H, *J* 10.7, 6.8 Hz, H-6a), 3.65–3.85 (m, 4H, H-5, H-5′, H-6a′, H-6b), 3.91 (dd, 1H, *J* 10.7, 3.2 Hz, H-6b′), 4.15 (dd, 1H, *J* 10.7, 8.5 Hz, H-2), 4.30 (dd, 1H, *J* 10.7, 8.5 Hz, H-2′), 4.88 (t, 1H, *J* 9.2 Hz, H-4), 5.15 (t, 1H, *J* 9.2 Hz, H-4′), 5.18 (d, 1H, *J* 8.5 Hz, H-1), 5.44 (d, 1H, *J* 8.5 Hz, H-1′), 5.65 (dd, 1H, *J* 10.7, 9.2 Hz, H-3), 5.78 (dd, 1H, *J* 10.7, 9.2 Hz, H-3′), 7.72–7.85 (m, 8H, Ph). Compound **4** (1.14 g, 1.13 mmol) was desilylated as described in the preparation of **3** to give **5** as a foamy solid (0.86 g, 85%): $[\alpha]_D^{25}$ +37 (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.85–0.90 (m, 4H, CH_2CH_2), 0.92–1.12 (m, 4H, CH_2CH_2), 1.82, 1.87, 1.99, 2.07 (4s, 12H, $4COCH_3$), 3.03 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.20–3.24 (m, 1H, OCH_aH_b), 3.51–3.55 (m, 1H, OCH_aH_b), 3.63–3.74 (m, 4H, H-5, H-5′, H-6a′, H-6a), 3.82 (dd, 1H, *J* 12.0, 3.2 Hz, H-6b′), 3.90–3.93 (m, 1H, H-6b), 4.18 (dd, 1H, *J* 10.7, 8.5 Hz, H-2), 4.30 (dd, 1H, *J* 10.7, 8.5 Hz,

H-2′), 4.97 (t, 1H, *J* 9.4 Hz, H-4), 5.12 (t, 1H, *J* 9.4 Hz, H-4′), 5.20 (d, 1H, *J* 8.5 Hz, H-1), 5.49 (d, 1H, *J* 8.5 Hz, H-1′), 5.68 (dd, 1H, *J* 10.7, 9.4 Hz, H-3), 5.81 (dd, 1H, *J* 10.7, 9.4 Hz, H-3′), 7.73–7.87 (m, 8H, Ph). Anal. Calcd for $C_{42}H_{47}N_5O_{17}$: C, 56.44; H, 5.30. Found: C, 56.18; H, 5.39.

3.6. 6-Azidohexyl 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-glucopyranoside (7)

Coupling of **1** (0.64 g, 1.13 mmol) and **5** (0.84 g, 0.94 mmol) as described in the general procedure gave **6** as a foamy solid (1.08 g, 83%): $[\alpha]_D^{25}$ +50 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.10, 0.11 (2s, 6H, $Si(CH_3)_2$), 0.93 (s, 9H, *t*-Bu), 0.85–0.88 (m, 4H, CH_2CH_2), 0.90–1.11 (m, 4H, CH_2CH_2), 1.77, 1.80, 1.84, 1.90, 1.93, 2.02 (6s, 18H, $6COCH_3$), 3.02 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.20–3.22 (m, 1H), 3.47 (dd, 1H, *J* 10.7, 6.4 Hz), 3.49–3.53 (m, 2H), 3.65–3.83 (m, 6H), 3.92 (d, 1H), 4.15 (dd, 1H, *J* 10.7, 8.4 Hz), 4.20 (dd, 1H, *J* 10.7, 8.4 Hz), 4.31 (dd, 1H, *J* 10.7, 8.4 Hz), 4.80 (t, 1H, *J* 9.2 Hz), 4.92 (t, 1H, *J* 9.2 Hz), 5.12 (d, 1H, *J* 8.4 Hz), 5.16 (t, 1H, *J* 9.2 Hz), 5.30 (d, 1H, *J* 8.4 Hz), 5.47 (d, 1H, *J* 8.4 Hz), 5.63 (dd, 2H, *J* 10.7, 9.2 Hz), 5.77 (dd, 1H, *J* 10.8, 9.2 Hz), 7.75–7.93 (m, 12H). Compound **6** (1.0 g, 0.72 mmol) was desilylated as described in the preparation of **3** to give **7** as a foam (0.81 g, 88%): $[\alpha]_D^{25}$ +52 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.85–1.26 (m, 8H, $4CH_2$), 1.78, 1.81, 1.86, 1.92, 1.95, 2.06 (6s, 18H, $6COCH_3$), 3.03 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.22–3.25 (m, 1H, OCH_aH_b), 3.51–3.56 (m, 2H, OCH_aH_b, H-6a), 3.60–3.79 (m, 6H, H-5, H-6a′, H-5′, H-5″, H-6a″, H-6b), 3.82–3.85 (m, 1H, H-6b″), 3.96–3.98 (m, 1H, H-6b′), 4.16 (dd, 1H, *J* 10.7, 8.5 Hz, H-2), 4.22 (dd, 1H, *J* 10.7, 8.5 Hz, H-2′), 4.30 (dd, 1H, *J* 10.7, 8.5 Hz, H-2″), 4.83 (t, 1H, *J* 9.6 Hz, H-4), 5.03 (t, 1H, *J* 9.6 Hz, H-4′), 5.14 (t, 1H, *J* 10.0 Hz, H-4″), 5.17 (d, 1H, *J* 8.5 Hz, H-1), 5.35 (d, 1H, *J* 8.5 Hz, H-1′), 5.50 (d, 1H, *J* 8.5 Hz, H-1″), 5.64 (dd, 2H, *J* 10.6, 9.2 Hz, H-3, H-3′), 5.79 (dd, 1H, *J* 10.5, 9.2 Hz, H-3″), 7.73–7.91 (m, 12H, Ph). Anal. Calcd for $C_{60}H_{64}N_6O_{25}$: C, 56.78; H, 5.08. Found: C, 57.03; H, 5.13.

3.7. 6-Azidohexyl 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-glucopyranoside (9)

Coupling of **7** (0.54 g, 0.42 mmol) and **8** (0.75 g, 0.60 mmol) as described in the general procedure gave **9** as a foamy solid (0.51 g, 50%): $[\alpha]_D^{25}$ +30 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.83–1.10 (m, 8H, $4CH_2$), 1.76, 1.77, 1.78, 1.79, 1.80 (5s, 15H, $5COCH_3$), 1.87, 1.90, 1.92, 1.93, 1.94, 1.95, 2.05, 2.16 (8s, 24H, $8COCH_3$), 3.01 (t, 2H, *J* 6.9 Hz, CH_2N_3), 3.14–3.18 (m, 1H, OCH_aH_b), 3.39–3.58 (m, 9H), 3.60–3.78 (m, 6H), 3.90–3.93 (m, 2H, H-6b^V, H-5^{VI}), 4.10–4.25 (m, 6H), 4.35–4.40 (dd, 2H, H-6b^{VI}, H-2^{VI}), 4.75–4.84 (m, 4H, 4 H-4), 4.93 (t, 1H, *J* 9.5 Hz, H-4^V), 5.13 (d, 1H, *J* 8.4 Hz, H-1^I), 5.20 (t, 1H, *J* 9.5 Hz, H-4^{VI}), 5.25 (d, 1H, *J* 8.4 Hz, H-1^{II}), 5.27 (d, 2H, *J* 8.4 Hz, H-1^{III}, H-1^{IV}), 5.31 (d, 1H, *J* 8.4 Hz, H-1^V), 5.50 (d, 1H, *J* 8.4 Hz, H-1^{VI}), 5.54–5.69 (m, 5H), 5.79 (dd, 1H, *J* 10.5, 9.2 Hz, H-3^{VI}), 7.73–7.91 (m, 24H, Ph). Anal. Calcd for $C_{116}H_{117}N_9O_{50}$: C, 57.17; H, 4.84. Found: C, 57.41; H, 4.93. MALDITOF-MS: calcd for $C_{116}H_{117}N_9O_{50}$: 2435.7 [M]; found 2458.6 $[M+Na]^+$.

3.8. 2-Chloro-4-nitrophenyl propargyl ether (10)

To a solution of 2-chloro-4-nitrophenol (0.2 g, 1.15 mmol) and propargyl bromide (0.165 g, 1.38 mmol) in acetone (10 mL) were added anhyd K_2CO_3 (0.19 g, 1.38 mmol) and Bu_4NBr (37 mg, 0.115 mmol), and the mixture was refluxed overnight. After removal of acetone, water (15 mL) was added, and the product was extracted with EtOAc (3 × 20 mL). The combined organic layers were dried over anhyd Na_2SO_4, and concentrated, and the crude product was recrystallized from EtOAc–petroleum ether to afford **10** as white crystals (0.23 g, 95%): mp 123–124 °C; ^1H NMR ($CDCl_3$): δ 2.62 (s, 1H, ≡C*H*), 4.90 (s, 2H, OCH_2), 7.18 (d, 1H, Ph), 8.17–8.20 (m, 1H, Ph), 8.31–8.32 (m, 1H, Ph). Anal. Calcd for $C_9H_6ClNO_3$: C, 51.08; H, 2.86. Found: C, 51.19; H, 2.75.

3.9. {4-[(2-Chloro-4-nitrophenyl)oxymethyl]-1*H*-1,2,3-triazol-1-yl}hexyl 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-glucopyranoside (11)

To a mixture of **9** (0.18 g, 0.074 mmol) and **10** (0.017 g, 0.08 mmol) in 1:1 H_2O–THF (10 mL) were added freshly prepared M aq sodium ascorbate (15 μL, 0.015 mmol) and 7.5% aq $CuSO_4$ (25 μL, 0.0075 mmol). The heterogeneous mixture was stirred vigorously in a dark room at 50–60 °C until the complete consumption of the reactants was indicated by TLC analyses. After removal of THF under reduced pressure, water (10 mL) was added, and the product was extracted with

EtOAc (3 × 15 mL). The combined organic layers were dried over anhyd Na_2SO_4 and evaporated in vacuo. The crude product was subjected to column chromatography (3:1 EtOAc–petroleum ether) to give **11** as a foamy solid (0.11 g, 60%): $[\alpha]_D^{25}$ +35 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.84–1.10 (m, 8H, 4CH_2), 1.76, 1.77, 1.79 (3s, 15H, 5COCH_3), 1.87, 1.91, 1.92, 1.94, 2.05, 2.16 (6s, 24H, 8COCH_3), 3.19–3.22 (m, 1H, OCH_aH$_b$), 3.40–3.56 (m, 9H), 3.63–3.79 (m, 6H), 3.90–3.94 (m, 2H, H-6b^V, H-5^{VI}), 4.10–4.25 (m, 8H), 4.36–4.41 (m, 2H, H-6b^{VI}, H-2^{VI}), 4.75–4.84 (m, 4H, 4 H-4), 4.93 (t, 1H, *J* 9.6 Hz, H-4^V), 5.13 (d, 1H, *J* 8.4 Hz, H-1^I), 5.20 (t, 1H, *J* 9.7 Hz, H-4^{VI}), 5.24–5.27 (m, 3H, H-1^{II}, H-1^{III}, H-1^{IV}), 5.31 (d, 1H, *J* 8.4 Hz, H-1^V), 5.42 (s, 2H, OCH_2), 5.50 (d, 1H, *J* 8.4 Hz, H-1^{VI}), 5.55–5.68 (m, 5H), 5.79 (dd, 1H, *J* 10.5, *J* 9.2 Hz, H-3^{VI}), 7.30 (d, 1H, Ph), 7.68–7.91 (m, 25H, Ph), 8.16–8.18 (m, 1H, Ph), 8.28–8.29 (m, 1H, Ph); ^{13}C NMR: δ 14.04, 14.13, 20.32, 20.36, 20.46, 20.46, 20.59, 20.75, 20.97, 22.61, 25.14, 25.86, 28.74, 29.28, 29.58, 29.61, 29.91, 31.85, 50.22, 54.37, 54.58, 61.93, 63.45, 67.33, 67.58, 67.68, 68.19, 68.90, 69.43, 69.52, 69.61, 70.52, 70.56, 70.59, 70.73, 71.99, 72.58, 72.64, 72.77, 72.93, 97.52 (C-1), 97.54 (2C, C-1), 97.65 (C-1), 97.69 (C-1), 98.03 (C-1), 112.79, 123.45, 123.64, 123.67, 123.96, 126.01, 131.28, 131.30, 131.44, 134.13, 134.29, 134.34, 134.41, 141.51, 169.32, 169.36, 169.40, 169.46, 169.89, 169.92, 169.98, 170.01, 170.69, 171.06. Anal. Calcd for $C_{125}H_{123}ClN_{10}O_{53}$: C, 56.68; H, 4.68. Found: C, 57.07; H, 4.61. MALDITOF-MS: calcd for $C_{125}H_{123}ClN_{10}O_{53}$: 2646.7 [M]; found 2669.64 $[M+Na]^+$.

3.10. {4-[(2-Chloro-4-nitrophenyl)oxymethyl]-1*H*-1,2,3-triazol-1-yl}hexyl 2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranoside (12)

To NH_3–satd MeOH (50 mL) was added **11** (100 mg, 0.038 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 Sephadex LH-20 column to give the fully deprotected oligosaccharide. Acetylation of the crude product was carried out in pyridine (3 mL) with Ac_2O (1 mL). The mixture was then concentrated, and the crude product was purified on a silica gel column to give the fully acetylated intermediate. O-Deacetylation with NaOMe in MeOH for 6 h at rt, followed by purification on a Bio-Gel P-2 column, afforded **12** as a white solid (45 mg, 75.6%): $[\alpha]_D^{25}$ −10 (*c* 0.5, H_2O); Selected ^{13}C NMR (D_2O): δ 99.70, 100.10, 100.70 (2C), 101.60 (2C). ESI(−)-MS: calcd for $C_{63}H_{97}ClN_{10}O_{34}$: 1572.6 [M]; found 1572 $[M]^+$.

3.11. Tri-{{1-[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-glucopyranosyloxyhexyl]-1*H*-1,2,3-triazol-4-yl}methyl} phloroglucinolyl ether (14)

Cycloaddition of **9** (0.25 g, 0.10 mmol) and tripropargyl phloroglucinolyl ether **13** (0.007 g, 0.03 mmol) as described in the preparation of **11** gave **14** as a foamy solid (0.14 g, 62%): $[\alpha]_D^{25}$ +15 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) gave a symmetrical structure spectrum: δ 0.85–1.14 (m, 4H, 2CH_2), 1.18–1.22 (m, 2H, CH_2), 1.50–1.52 (m, 2H, CH_2), 1.74, 1.75, 1.76, 1.77, 1.78 (5s, 15H, 5COCH_3), 1.85, 1.91, 1.92, 2.03, 2.15 (5s, 24H, 8COCH_3), 3.14–3.17 (m, 1H, OCH_aH$_b$), 3.37–3.56 (m, 9H), 3.58–3.76 (m, 6H), 3.88–3.92 (m, 2H, H-6b^V, H-5^{VI}), 4.08–4.25 (m, 8H), 4.36–4.39 (m, 2H, H-6b^{VI}, H-2^{VI}), 4.73–4.83 (m, 4H, 4 H-4), 4.92 (t, 1H, *J* 9.5 Hz, H-4^V), 5.10–5.12 (m, 3H, OCH_2, H-1^I), 5.19 (t, 1H, *J* 9.6 Hz, H-4^{VI}), 5.23–5.26 (m, 3H, H-1^{II}, H-1^{III}, H-1^{IV}), 5.30 (d, 1H, *J* 8.5 Hz, H-1^V), 5.49 (d, 1H, *J* 8.5 Hz, H-1^{VI}), 5.54–5.67 (m, 5H), 5.77 (dd, 1H, *J* 10.3, 9.3 Hz, H-3^{VI}), 7.30 (s, 1H, Ph), 7.56 (s, 1H, C=C*H*), 7.60–7.90 (m, 24H, Ph). Anal. Calcd for $C_{363}H_{363}N_{27}O_{153}$: C, 57.73; H, 4.84. Found: C, 58.01; H, 4.79. MALDITOF-MS: calcd for $C_{363}H_{363}N_{27}O_{153}$: 7552 [M]; found 7575.75 $[M+Na]^+$.

3.12. Tri-{{1-[2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2- acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→6)-2-deoxy-2-acetamido-β-D-glucopyranosyloxyhexyl]-1*H*-1,2,3-triazol-4-yl}methyl} phloroglucinolyl ether (15)

Full deacylation and N-acetylation of **14** (130 mg, 0.017 mmol), using the same procedures as described in the preparation of **12** from **11**, gave **15** as a white solid (50 mg, 68%): $[\alpha]_D^{25}$ +10 (*c* 0.5, H_2O); Selected ^{13}C NMR (D_2O) for C-1s: δ 99.10, 100.50, 101.30 (2C), 101.82 (2C). MALDITOF-MS: calcd for $C_{177}H_{285}N_{27}O_{96}$: 4327 $[M]^+$; found 4350 $[M+Na]^+$, 4516 $[M+CCA]^+$, 4705 $[M+2CCA]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2005.08.013.

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