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Synthesis and antitumor activities of glucan derivatives

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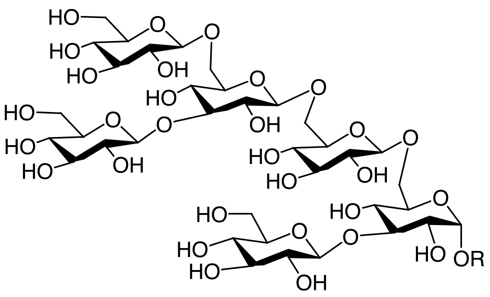
Abstract—A highly efficient and practical method for the preparation of β-D-Glc-(1→6)-[β-D-Glc-(1→3)]-β-D-Glc-(1→6)-β-D-Glc-(1→6)-[β-D-Glc-(1→3)]-D-Glc-OMe was described. A dendritic nonasaccharide was also synthesized. The antitumor activities of hexasaccharide, the dendrimer, their sulfated derivatives, together with the natural glucan–protein and the corresponding polysaccharide isolated from barmy mycelium of *Grifola frondosa*, were preliminarily investigated based on Sarcoma-180 studies in mice tests. Our results suggest that the sulfated branching oligosaccharide and natural glycoprotein have better antitumor activities comparing to the parent sugar residue (oligosaccharide or polysaccharide).

1. Introduction

A family of glucans containing a main chain of β-D-(1→3)-glucopyranosyl units, and a short β-D-glucopyranosyl side chains at O-6 have received considerable attention because of their antitumor activities (immunomodulating action).[1] Schizophyllan,[2] scleroglucan,[3] epiglucan[4] and lentinan[5] are the most well-known members of this group of polysaccharides. It is known that the immunopharmacological activities of soluble (1→3)-β-D-glucans are closely related to the organization of the (1→3)-β-linked backbone into a triple helix, the frequency and the complexity of side-branching, and their molecular weight.[6] However, Tsuzuki and co-workers[7] have also found that the conformation of β-glucans, either single or triple helix, is independent on the hematopoietic response. To investigate the structure–activity relationship, we have synthesized a series of β-D-glucosyl oligosaccharides to mimic the repeating units of natural β-glucan chains.[8] The mice tests revealed that our previously synthesized β-D-glucopyranosyl oligosaccharides showing weaker antitumor activities compared to the reported natural polysaccharides. A literature survey suggested that sulfation of the oligosaccharides could result an increasing anti-tumor and anti-HIV activities.[9] Here, we would like to report the synthesis of sulfated methyl β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-gluco pyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-α-D-glucopyranoside and a sulfated cluster compound containing three β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-α-D-glucopyranoside components. Interestingly, the hexa-β-D-glucoside, β-D-Glc-(1→6)-[β-D-Glc-(1→3)]-β-D-Glc-(1→6)-β-D-Glc-(1→6)-[β-D-Glc-(1→3)]-D-Glc, has been well characterized as an elicitor of plant phytoalexin accumulation.[10] Our research revealed that the sulfated hexa-β-D-glucoside may also be a potent antitumor agent based on Sarcoma-180 model studies of mice tests.

2. Results and discussion

Hexa-β-D-glucopyranosides (compounds **1** and **2**, Fig. 1) have been previously prepared by Takahashi[11] and Ogawa.[12] We here modified the synthesis based on our findings of highly efficient and practical synthesis of 3,6-branched oligosaccharides.[13] Thus, phenyl 2,4-di-*O*-acetyl-



1 R = Me
2 R = CH_2=CH-CH_2-

Figure 1. Structures of hexa-β-D-glucopyranosides **1** and **2**.

Keywords: Carbohydrates; Glycosylations; Antitumor agents; Glycodendrimers; Oligosaccharides.
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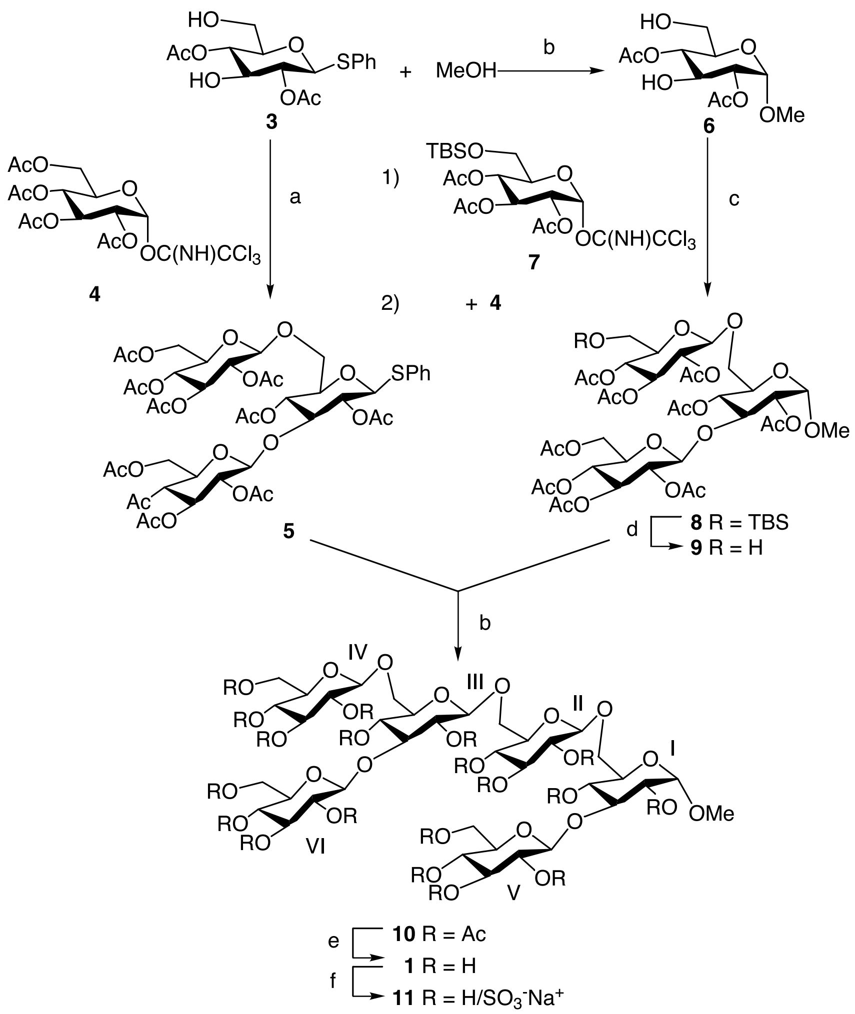
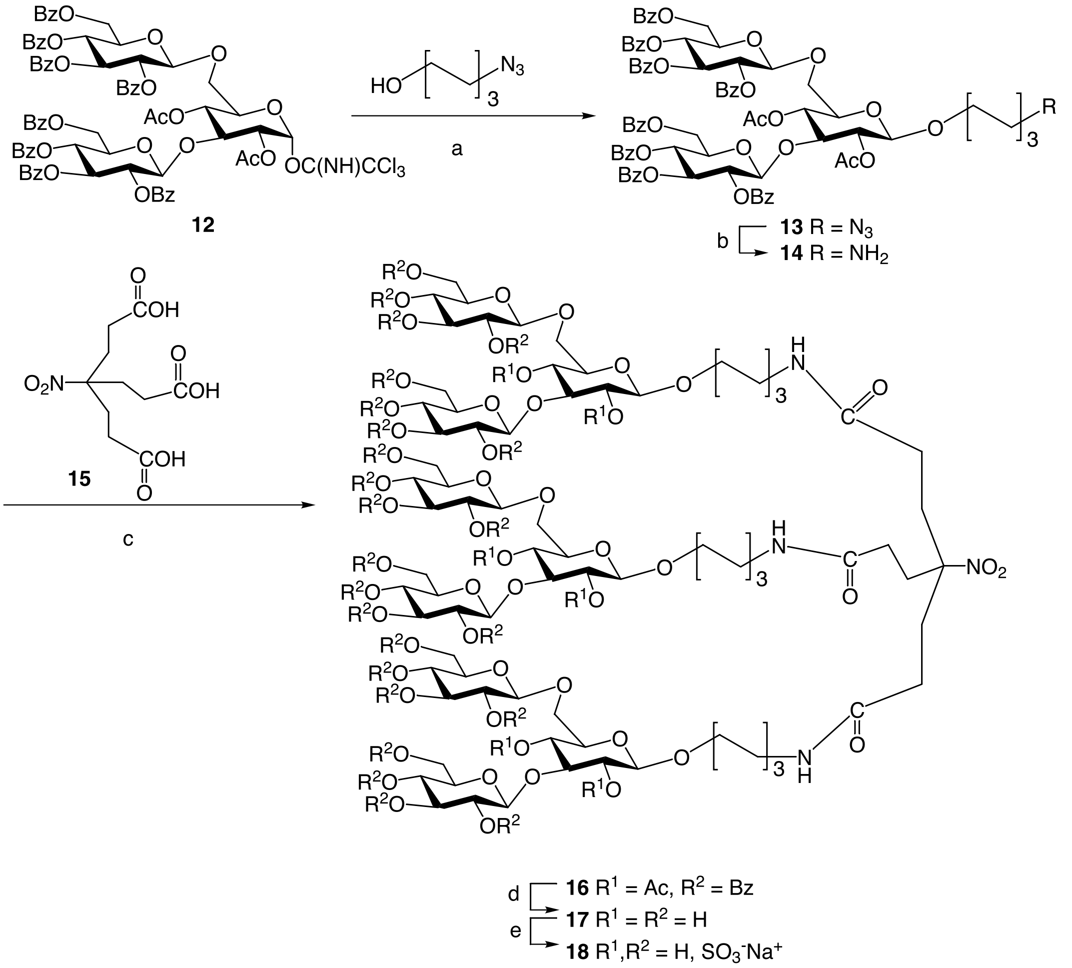


Scheme 1. Synthesis of hexa-β-D-glucopyranoside **1**. Reaction conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 82%; (b) NIS, TMSOTf, 63% for **6**; 86% for **10** (from **8**); (c) TMSOTf, CH_2Cl_2, −42 °C; then TMSOTf, 0 °C, 76% (two steps); (d) 95% TFA; (e) NaOMe, MeOH, 93%; (f) SO_3·Pyr, DMF.

1-thio-β-D-glucopyranoside (**3**)[13a] was condensed with glycosyl donor 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**4**)[14] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in CH_2Cl_2 to give trisaccharide **5** in one pot with 82% isolated yield. Three doublets at δ 4.43 ppm (J=7.9 Hz), 4.52 ppm (J=8.0 Hz) and 4.54 ppm (J=10.0 Hz) in ^{1}H NMR spectra of **5** clearly indicated all three β-configuration in this trisaccharide. Thioglycoside **5** was used as a latent glycosyl donor in the final assembly of the target hexasaccahride. Attempt to transfer the partially protected donor **3** into its methyl glycoside derivative using *N*-iodosuccinimide (NIS) and TMSOTf as catalysts resulted in however **6** as a major product (63%). The formation of α isomer can be rationalized by a S_N2 reaction of methanol with 1,6-anhydrosugar intermediate formed from intermolecular ring closure of **3** (Scheme 1).[15]

With 3,6-diol **6** in hand, we next applied a one-pot sequential glycosylation to the synthesis of trisaccharide acceptor **9**. To this end, 6-*O*-silylated trichloroacetimidate **7**[16] (1.1 equiv.) was regioselectively coupled with diol **6** using catalytic amount of TMSOTf (0.07 equiv.) at −42 °C in anhydrous methylene chloride. The second donor **4** (1.5 equiv.) was added into the above mixture at 0 °C 2 h later, affording trisaccharide **8** in 76% yield within another 2 h. It is noteworthy that an extra amount of TMSOTf (0.01 equiv.) was needed to complete the reaction after the addition of **4**. The treatment of **8** with 95% trifluoroacetic acid (TFA) for 1 h gave trisaccharide acceptor **9**. The resulting crude product was co-evaporated with toluene three times and then directly used for the next step without further purification. Coupling of **5** and **9** in CH_2Cl_2 at 0 °C under promotion of NIS and TMSOTf gave hexasaccharide **10** in 86% yield over two steps. ^{1}H–^{1}H COSY, TOCSY, HMBC and HMQC spectra analyses clearly indicated 6 H-1s [δ_H 4.29 (H-1^{III}), 4.49 (H-1^{II}), 4.51 (H-1^{IV}), 4.58 (H-1^{VI}), 4.61 (H-1^{V}), 4.77 (H-1^{I}) ppm] and 6 C-1s [δ_C 96.4 (C-1^{I}), 100.6 (C-1^{III}, C-1^{V}), 100.8 (C-1^{VI}, C-1^{IV}), 100.9 (C-1^{II}) ppm], confirming the correct linkages of **10**. Standard Zemplén deacetylation[17] of **10** furnished hexa-β-D-glucopyranoside **1** as an amorphous solid. Sulfation of **1** with SO_3·Pyr (10 equiv.) at 50 °C in *N*,*N*-dimethylformamide (DMF) for 3 days, followed by conversion to the sodium salt, removal of pyridine and purification on a Sephadex LH-20 column, furnished a mixture of sulfated **11**. The microanalysis for **11** was C 16.22%, H 1.73% and S 19.90%. This highly sulfated mixture was thus obtained in 5 steps at 38% overall yield starting from **3**, and was directly used for the following bioassay.



Scheme 2. Synthesis of nonasaccharide dendritic compound **17**. Reaction conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 84.7%; (b) $Pd(OH)_2/C$, H_2, EtOAc–EtOH, 93.4%; (c) HOBt, DCC, DMF, rt, 57.2%; (d) NaOMe, MeOH, 91.5%; (e) SO_3·Pyr, DMF.

Glycodendrimers have been prepared to give rise of new kinds of glycoconjugate derivatives and polysaccharide mimics.[18] Some of them have shown highly improved bioactivities compared to the monomers.[19] Encouraged by these results, we prepared a carbohydrate dendrimer based on a combination of 3,6-branched trisaccharides as dendritic components and noncarbohydrate units as trivalent cores (Scheme 2). Thus, the coupling of trisaccharide imidate **12**[8c] and 6-azido-1-hexanol under standard glycosylation conditions gave 6-azidohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)- [2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (**13**), a key component for our target synthesis, in high yield. $Pd(OH)_2$ catalyzed hydrogenation of **13** gave amine derivative **14**, which was further condensed with triacid **15**[20] in the presence of HOBt and DCC in DMF to give fully protected trimer **16** in 57.2% yield. The newly formed amide bond was characterized by CON*H* peaks appearing at δ 6.04 ppm (3H, J=5.5 Hz) in ^{1}H NMR spectrum, and further confirmed with a mass of 4804 $(M+Na)^+$ of MALDITOF-MS spectrum. Deacylation of **16** with 1 N NaOMe afforded free nonasaccharide dendritic compound **17**. Further sulfation of **17** with SO_3·Pyr (10 equiv.) in DMF as described in the preparation of **11**, furnished the desired dendrimer **18**. Sodium salt of **18**, after purification on LH-20 column, was directly used for the next bioassay.

Working for the same project to investigate possible antitumor β-glucan, we have also extracted and isolated a glucan protein (**19**) from barmy mycelium of *Grifola frondosa* (Maitake) with a molecular weight of 95 K. After removal of the protein (accounts for 24% of total molecular weight), a pure polysaccharide (**20**) was obtained. The structure of this polysaccharide is determined as a β-D-glucan with the following basic repeating unit (Fig. 2) by a $NaIO_4$ oxidation, methylation, acetolysis and 2D NMR spectra analysis.[21]

The antitumor activities of compounds **1**, **11**, **17**, **18**, **19** and **20** were preliminarily studied according to the method described by Sasaki and co-workers.[5b] ICR mice weighing about 20 g were used for the bioassay. Seven-day-old Sarcoma-180 ascites (0.2 mL, about 5×10^6 cells) were transplanted into the right groins of mice. The test samples, dissolved in distilled water, were injected daily for 10 days starting 24 h after tumor implantation. At the end of the 12th day, the mice were killed, and the tumors were extirpated and weighted. The results (Table 1), compared to lentinan and cyclophosphamide (CTX) in the parallel test, suggest that compound **11** and **19** may be potent antitumor agents. Low tumor inhibition rates of **17** and **18** indicate that structurally highly branched oligosaccharides may not be helpful to their bioactivities. A main chain with β-(1→6)[22]

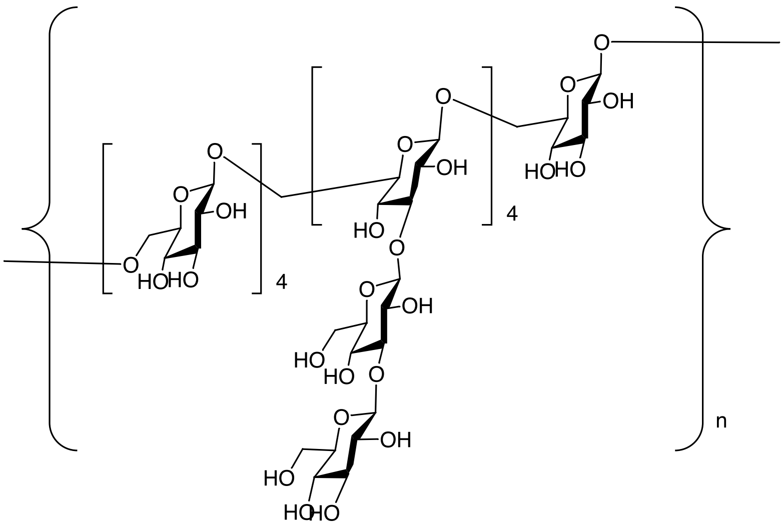


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Figure 2. Proposed structure of β-D-glucan isolated from barmy mycelium of *Grifola frondosa* (Maitake).

Table 1. Preliminary studies on antitumor activities of compounds **1**, **11** and **17**–**20**

Sample	Dose (mg/Kg)	ΔBody weight (g)	Weight of tumor (g)	Inhibition rate (%)
Control	0	10.4±2.3	1.42±0.45	0
CTX	30	8.7±1.5	0.35±0.09***	75
Lentinan	2.0	12.9±2.0	0.95±0.56**	33
1	5.0	9.6±1.8	1.15±0.41**	19
11	2.5	12.6±3.2	0.88±0.46*	38
11	5.0	11.0±1.1	0.74±0.23**	48
11	10.0	10.8±1.9	0.58±0.15***	59
17	2.0	6.9±1.3	1.11±0.60*	22
18	2.0	9.6±1.6	1.09±0.63*	23
19	1.0	12.7±1.8	0.44±0.13***	69
20	1.0	10.8±1.3	0.80±0.22**	43

t-test: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

or β-(1→3)[8b,9b] linkage can be important. More details about the action mechanism for **11** and **19** are currently under investigation by our collaborators.

3. Conclusions

A highly efficient and practical method was described for the preparation of 3,6-branched hexa-β-D-glucopyranosyl derivatives. A dendritic nonasaccharide was also synthesized. The antitumor activities of oligosaccharide **1**, dendrimer **17**, their sulfated derivatives **11** and **18**, together with a natural glucan–protein **19** and the corresponding glucan **20**, isolated from barmy mycelium of *Grifola frondosa* (Maitake), were preliminarily investigated in vivo based on Sarcoma-180 model studies. Our current research suggests that the sulfated branching oligosaccharide and natural glycoprotein have better antitumor activities comparing to the parent sugar residue alone (oligosaccharide or polysaccharide). Beside on this result, some structural closely related glycopeptides and BSA attached glycoconjugates are now under preparation in our lab.

4. Experimental

4.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H COSY, NOESY and ^{1}H–^{13}C COSY spectra were recorded with Bruker ARX 400 or 500 spectrometers in $CDCl_3$, CD_3OD or D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using MALDITOF-MS with 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 a UV detector. General column chromatography was conducted by elution of a column (8×200 mm, 15×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent, while the sulfated products were purified on Sephadex LH-20 column using water as eluent. Solutions were concentrated at <60 °C under reduced pressure.

4.1.1. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*- acetyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-α-D-glucopyranoside (5). To a cooled solution (0 °C) of **3** (1.1 g, 3.1 mmol) and **4** (3.2 g, 6.5 mmol) in anhydrous CH_2Cl_2 (20 mL) was added TMSOTf (50 μL, 0.28 mmol). The mixture was stirred at these conditions for 4 h and quenched with Et_3N. The solvents were evaporated in vacuo and the residue was purified on a silica gel column (petroleum ether–EtOAc, 1:1) to give latent trisaccharide donor **5** as a syrup (2.54 g, 82%); $[\alpha]_D^{25}=-11$ (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96, 1.97, 2.00, 2.01, 2.02, 2.03, 2.10, 2.17, 2.21, 2.23 (10 s, 10×3H, $COCH_3$), 3.57 (ddd, 1H, J=5.5, 11.9, 8.8 Hz), 3.60–3.66 (m, 3H), 3.78 (t, 1H, J=8.8 Hz), 4.02 (dd, 1H, J=5.5, 11.9 Hz), 4.11–4.17 (m, 2H), 4.43 (d, 1H, J=7.9 Hz, H-1^{II}), 4.52 (d, 1H, J=8.0 Hz, H-1^{III}), 4.54 (d, 1H, J=10.0 Hz, H-1^{I}), 4.57 (dd, 1H, J=2.0, 11.9 Hz), 4.62

(dd, 1H, J=3.3, 12.5 Hz), 4.72 (dd, 1H, J=3.3, 12.6 Hz), 4.96 (dd, 1H, J=10.0, 10.8 Hz, H-2^{I}), 5.00–5.05 (m, 2H), 5.11–5.21 (m, 4H), 7.25–7.50 (m, 4H, Ph). Anal. Calcd for $C_{44}H_{56}O_{25}S$: C, 51.97; H, 5.55. Found: C, 52.20; H, 5.48.

4.1.2. Methyl 6-*O*-*tert*-butyldimethylsilyl-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl- (1→6)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-α-D-glucopyranoside (8). To a cold solution (−42 °C) of **6** (1.37 g, 4.91 mmol) and **7** (2.78 g, 4.93 mmol) in anhydrous CH_2Cl_2 (20 mL) was added TMSOTf (60 μL, 0.33 mmol). The mixture was stirred at this temperature (usually 2 h) until all starting materials were consumed according to TLC (petroleum ether/EtOAc 1/1), and then warmed to 0 °C. Compound **4** (2.42 g, 4.93 mmol) in dry CH_2Cl_2 (5 mL) was added into the above mixture dropwise at 0 °C, followed by the addition of extra TMSOTf (10 μL, 0.05 mmol), and the mixture was kept at these conditions for 2 h, then quenched with Et_3N. The solvents were evaporated in vacuo and the residue was purified by silica gel column chromatography (petroleum ether–EtOAc, 1:1) to give trisaccharide **8** as a syrup (3.77 g, 76%); $[\alpha]_D^{25}$=+41° (c 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.03, 0.04 (2 s, 6H, $(CH_3)_2Si$), 0.88 (s, 9H, t-Bu), 1.97, 1.98, 1.99, 2.01, 2.02, 2.03, 2.07, 2.18 (8 s, 27H, 9 CH_3CO), 3.38 (s, 3H, OCH_3), 3.45 (dd, 1H, J=6.8, 10.7 Hz, H-6a^{III}), 3.52 (ddd, 1H, J=2.9, 4.7, 9.9 Hz, H-5^{I}), 3.64 (ddd, 1H, J=2.2, 9.4, 4.6 Hz, H-5^{II}), 3.66–3.75 (m, 2H, H-6^{I}), 3.88 (ddd, 1H, J=1.8, 6.8, 9.5 Hz, H-5^{III}), 3.93 (dd, 1H, J=1.8, 10.7 Hz, H-6b^{III}), 4.04 (dd, 1H, J=2.2, 12.4 Hz, H-6a^{II}), 4.11 (t, 1H, J=9.3 Hz, H-3^{I}), 4.34 (dd, 1H, J=4.6, 12.4 Hz, H-6b^{II}), 4.49 (d, 1H, J=8.0 Hz, H-1^{III}), 4.65 (d, 1H, J=8.1 Hz, H-1^{II}), 4.80 (dd, 1H, J=9.3, 9.9 Hz, H-4^{I}), 4.81 (d, 1H, J=3.7 Hz, H-1^{I}), 4.83 (dd, 1H, J=3, 7, 9.3 Hz, H-2^{I}), 4.88 (dd, 1H, J=8.1, 9.3 Hz, H-2^{II}), 4.97 (dd, 1H, J=8.0, 9.5 Hz, H-2^{III}), 5.01 (t, 1H, J=9.5 Hz, H-4^{III}), 5.04 (t, 1H, J=9.4 Hz, H-4^{II}), 5.11 (t, 1H, J=9.4 Hz, H-3^{II}), 5.20 (t, 1H, J=9.5 Hz, H-3^{III}). MALDITOF-MS calcd for $C_{43}H_{66}O_{25}Si$: 1010 $[M]^+$. Found 1033 $[M+Na]^+$. Anal. Calcd for $C_{43}H_{66}O_{25}Si$: C, 51.08; H, 6.58. Found: C, 51.27; H, 6.52.

4.1.3. Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-α-D-glucopyranoside (10). Compound **8** (4.55 g, 4.5 mmol) was stirred in 95% TFA (30 mL) at rt for 1 h and then evaporated with toluene (3×50 mL) for 3 times to give the dried crude **9**. To a cooled solution (0 °C) of **5** (4.576 g, 4.5 mmol) and crude **9** (4.03 g, 4.5 mmol) in anhydrous CH_2Cl_2 (50 mL) was added TMSOTf (60 μL, 0.33 mmol). The mixture was stirred at this temperature for 2 h, and then quenched with Et_3N. The solvents were evaporated in vacuo and the residue was purified by silica gel column chromatography (petroleum ether–EtOAc, 1.5:1) to give hexasaccharide **10** as a syrup (6.98 g, 86%); $[\alpha]_D^{25}$=−3 (c 4, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.93, 1.94, 1.95, 1.96, 1.97, 1.98, 1.99, 2.00, 2.01, 2.03, 2.05, 2.06, 2.13, 2.15 (14 s, 57H, 19 CH_3CO), 3.35 (s, 3H, OCH_3), 3.42–3.48 (m, 2H, H-5^{I}, H-6a^{I}), 3.50 (dd, 1H, J=7.0, 10.5 Hz, H-6a^{II}), 3.54–3.60 (m, 2H, H-5^{II}, H-5^{III}), 3.62–3.70 (m, 3H, H-5^{IV}, H-5^{V}, H-5^{VI}), 3.79–3.88 (m, 4H, H-3^{III}, H-6b^{I}, H-6a^{III}, H-6b^{III}), 3.91 (dd, 1H, J=2.5, 10.5 Hz, H-6b^{II}), 4.01 (dd, 1H, J=2.5, 7.5 Hz, H-6a^{VI}), 4.03 (dd, 1H, J=2.0, 7.5 Hz, H-6a^{V}), 4.06–4.12 (m, 2H, H-6b^{V}, H-3^{I}), 4.24 (dd, 1H, J=4.5, 12.0 Hz, H-6a^{IV}), 4.29 (d, 1H, J=8.0 Hz, H-1^{III}), 4.30 (dd, 1H, J=3.0, 7.5 Hz, H-6b^{VI}), 4.34 (dd, 1H, J=4.0, 12.0 Hz, H-6b^{IV}), 4.49 (d, 1H, J=8.0 Hz, H-1^{II}), 4.51 (d, 1H, J=8.0 Hz, H-1^{IV}), 4.58 (d, 1H, J=8.0 Hz, H-1^{VI}), 4.61 (d, 1H, J=8.0 Hz, H-1^{V}), 4.71 (t, 1H, J=9.5 Hz, H-4^{III}), 4.77 (d, 1H, J=3.5 Hz, H-1^{I}), 4.78–4.93 (m, 7H), 4.94 (dd, 1H, J=8.0, 9.5 Hz, H-2^{IV}), 5.01 (t, 1H, J=9.0 Hz, H-4^{VI}), 5.02 (t, 1H, J=9.5 Hz, H-4^{V}), 5.03 (t, 1H, J=9.5 Hz, H-4^{IV}), 5.08 (t, 1H, J=9.5 Hz, H-3^{VI}), 5.10 (t, 1H, J=9.0 Hz, H-3^{V}), 5.15 (t, 1H, J=9.5 Hz, H-3^{II}), 5.18 (t, 1H, J=9.5 Hz, H-3^{IV}). δ_C (125 MHz, $CDCl_3$) 20.3, 20.5, 20.6, 20.7, 20.9, 61.7, 61.8, 67.4, 68.0, 68.1, 68.3, 68.4, 68.5, 68.6, 68.7, 68.9, 71.0, 71.1, 71.2, 71.6, 71.7, 71.9, 72.5, 72.6, 72.7, 73.0, 73.2, 76.0, 78.7, 96.4 (C-1^{I}), 100.6 (C-1^{III}, C-1^{V}), 100.8 (C-1^{VI}, C-1^{IV}), 100.9 (C-1^{II}), 168.9, 169.0, 169.3, 169.4, 169.6, 169.7, 169.8, 170.1, 170.3, 170.4, 170.5, 170.6. MALDITOF-MS calcd for $C_{75}H_{102}O_{50}$: 1802 $[M]^+$. Found 1825 $[M+Na]^+$. Anal. Calcd for $C_{75}H_{102}O_{50}$: C, 49.95; H, 5.70. Found: C, 50.21; H, 5.77.H-4H

4.1.4. Methyl β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D- glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-α-D-glucopyranoside (1). A solution of **10** (2.6 g, 1.44 mmol) in ammonia–saturated MeOH (300 mL) was stirred at rt for 7 days. The solvents were evaporated, and the residue was purified on a Sephadex LH-20 column with water as the eluent to give **1** as an amorphous solid after lyophilization (1.3 g, 90%); $[\alpha]_D^{25}$=+7 (c 1, H_2O); ^1H NMR (500 MHz, D_2O) δ 3.28 (t, 1H, J=9.1 Hz,), 3.30 (t, 1H, J=9.1 Hz), 3.34 (t, 1H, J=9.30 Hz), 3.37 (t, 1H, J=9.5 Hz), 3.38–3.48 (m, 13H), 3.51 (t, 2H, J=9.5 Hz), 3.58–3.93 (m 18H), 4.15–4.23 (m, 2H, H-3^{I}, H-3^{III}), 4.50 (d, 2H, J=7.9 Hz), 4.55 (d, 1H, J=8.0 Hz), 4.69 (d, 1H, J=8.0 Hz), 4.73 (d, 1H, J=8.0 Hz), 4.80 (d, 1H, J=3.7 Hz). δ_C (125 MHz, $CDCl_3$) 55.0, 60.5, 67.6, 67.7, 68.4, 68.6, 69.2, 69.3, 69.4, 70.2, 70.5, 72.6, 72.9, 73.2, 74.4, 74.6, 75.3, 75.4, 75.7, 75.8, 82.0, 84.0, 99.0, 102.5, 102.6, 102.7 (3C). ESI-MS calcd for $C_{37}H_{64}O_{31}$: 1004 $[M]^+$, found 1003 $[M-H]^+$.

4.1.5. 6-Azidohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (13). To a solution of 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (**12**, 586 mg, 0.4 mmol) and 6-azido-1-hexanol (52 mg, 0.4 mmol) in anhydrous dichloromethane (3 mL) was added TMSOTf (10 μL, 0.06 mmol) at 0 °C under N_2 protection. The reaction mixture was stirred for 2 h, at the end of which time TLC indicated the completion of the reaction. The mixture was neutralized with Et_3N, concentrated and purified by flash chromatography using 2:1 petroleum ether–EtOAc as the eluent to give syrupy **13** (476 mg, 84.7%); $[\alpha]_D^{20}$=−101 (c 0.5, $CHCl_3$); ^1H NMR (400 Hz, $CDCl_3$) δ 1.06–1.15 (m, 4H, $-CH_2CH_2-$), 1.19–1.26 (m, 2H, $-CH_2CH_2-$), 1.45–1.52 (m, 2H, $-CH_2CH_2-$), 1.86 (s, 3H, CH_3CO), 1.91 (s, 3H, CH_3CO), 2.95 (m, 1H, OCH_2), 3.21 (t, 2H, CH_2N_3), 3.34–3.38 (m, 1H, OCH_2), 3.49–3.51 (m, 1H, H-5^{I}), 3.62 (dd, 1H, $J_{6a,6b}$=11.2 Hz,

$J_{6a,5}$=5.7 Hz, H-6a^{I}), 3.78–4.92 (m, 2H, H-3^{I}, H-6b^{I}), 4.04–4.16 (m, 3H, H-1^{I}, H-5^{II}, H-5^{III}), 4.41–4.50 (m, 2H, 2H-6), 4.58–4.66 (m, 2H, 2H-6), 4.69–4.81 (m, 2H, H-2^{I} and H-4^{I}), 4.89 (d, 1H, $J_{1,2}$=7.6 Hz, H-1), 4.91 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 5.36 (dd, 1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=7.8 Hz, H-2), 5.49 (dd, 1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=7.6 Hz, H-2), 5.60–5.68 (m, 2H, H-4^{II} and H-4^{III}), 5.83–5.90 (m, 2H, H-3^{II} and H-3^{III}), 7.28–8.04 (m, 40H, *Ph*). MALDITOF-MS calcd for $C_{84}H_{79}N_3O_{26}$: 1545 $[M]^+$, found 1568 $[M+Na]^+$. Anal. Calcd for $C_{84}H_{79}N_3O_{26}$: C, 65.24; H, 5.15. Found: C, 65.05; H, 5.07.

4.1.6. 6-Aminohexyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranoside (14). Compound **13** (431 mg, 0.278 mmol) was dissolved in EtOAc–EtOH (1:1, 10 mL) containing $Pd(OH)_2$/C (20%, 40 mg) at rt. H_2 was bubbled into the mixture at the flow rate of 100 mL/min while stirring at atmospheric pressure for 4 h. The mixture was then filtered over Celite and the filtrate was concentrated under reduced pressure. Purification of the residue by flash chromatography (CH_2Cl_2/MeOH: 5/1) afforded **14** (394 mg, 93.4%) as a syrup; $[\alpha]_D^{20}=-4$ (*c* 1, $CHCl_3$); ^{1}H NMR (400 Hz, $CDCl_3$) δ 1.03–1.14 (m, 4H, $-CH_2CH_2-$), 1.17–1.24 (m, 2H, $-CH_2CH_2-$), 1.61–1.68 (m, 2H, $-CH_2CH_2-$), 1.88 (s, 3H, CH_3CO), 1.89 (s, 3H, CH_3CO), 2.86–2.95 (m, 3H, CH_2N_3 and one proton of OCH_2), 3.34–3.37 (m, 1H, OCH_2), 3.48–3.51 (m, 1H, H-5^{I}), 3.59 (dd, 1H, $J_{6a,6b}$=11.6 Hz, $J_{6a,5}$=5.7 Hz, H-6a^{I}), 3.85–4.92 (m, 2H, H-3^{I}, H-6b^{I}), 4.06 (d, 1H, $J_{1,2}$=7.8 Hz, H-1^{I}), 4.07–4.16 (m, 2H, H-5^{II} and H-5^{III}), 4.44–4.48 (m, 2H, H-6), 4.58–4.65 (m, 2H, H-6), 4.71–4.76 (m, 2H, H-2^{I} and H-4^{I}), 4.91 (d, 1H, $J_{1,2}$=7.8 Hz, H-1), 4.92 (d, 1H, $J_{1,2}$=7.6 Hz, H-1), 5.36 (dd, 1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=7.8 Hz, H-2), 5.50 (dd, 1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=7.6 Hz, H-2), 5.62–5.69 (m, 2H, H-4^{II} and H-4^{III}), 5.85–5.91 (m, 2H, H-3^{II} and H-3^{III}), 7.25–8.02 (m, 40H, *Ph*). MALDITOF-MS calcd for $C_{84}H_{81}NO_{26}$: 1519 $[M]^+$, found 1542 $[M+Na]^+$. Anal. Calcd for $C_{84}H_{81}NO_{26}$: C, 66.35; H, 5.37. Found: C, 66.21; H, 5.45.

4.1.7. Fully protected dendrimer 16. A mixture of compound **14** (375 mg, 0.2 mmol), the triacid **15** (22 mg, 0.08 mmol) and HOBT (33 mg, 0.2 mmol) in dry DMF (3 mL) was stirred at 0 °C for 0.5 h. Then DCC (51 mg, 0.248 mmol) was added and the reaction mixture was stirred at 0 °C for 0.5 h, then at rt for 30 h. The mixture was filtered and the filtrate was concentrated. The resulting crude product was diluted in EtOAc (30 mL) and subsequently washed successively with 5% HCl, saturated aqueous $NaHCO_3$ and water. The organic phase was concentrated and purified by flash chromatography (petroleum ether–EtOAc, 1:5) to complete **16** (219 mg, 57.2%) as a syrup; $[\alpha]_D^{20}=-6$ (*c* 1, $CHCl_3$); ^{1}H NMR (400 Hz, $CDCl_3$) δ 1.04–1.18 (m, 3×4H, $-CH_2CH_2-$), 1.29–1.38 (m, 3×2H, $-CH_2CH_2-$), 1.58–1.72 (m, 3×2H, $-CH_2CH_2-$), 1.86 (s, 3×3H, CH_3CO), 1.89 (s, 3×3H, CH_3CO), 2.04–2.08 (m, 3×2H, CH_2), 2.18–2.23 (m, 3×2H, CH_2), 2.91–2.98 (m, 3×1H, OCH_2), 3.06–3.13 (m, 3×2H, CH_2NHCO), 3.34–3.39 (m, 3×1H, OCH_2), 3.47–3.51 (m, 3×1H, H-5^{I}), 3.63 (dd, 3×1H, $J_{6a,6b}$=11.7 Hz, $J_{6a,5}$=6.8 Hz, H-6a^{I}), 3.81–4.90 (m, 3×2H, H-3^{I} and H-6b^{I}), 4.05–4.15 (m, 3×3H, H-1^{I}, H-5^{II} and H-5^{III}), 4.42–4.49 (m, 3×2H, H-6), 4.59–4.65 (m, 3×2H, H-6), 4.70–4.79 (m, 3×2H, H-2^{I} and H-4^{I}), 4.90 (d, 3×1H, $J_{1,2}$=7.8 Hz, H-1), 4.92 (d, 3×1H, $J_{1,2}$=7.6 Hz, H-1), 5.36 (dd, 3×1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=7.8 Hz, H-2), 5.50 (dd, 3×1H, $J_{2,3}$=9.6 Hz, $J_{1,2}$=7.6 Hz, H-2), 5.60–5.68 (m, 3×2H, H-4^{II} and H-4^{III}), 5.84–5.91 (m, 3×2H, H-3^{II} and H-3^{III}), 6.04 (br t, 3×1H, *J*=5.5 Hz, N*H*), 7.25–8.02 (m, 3×40H, *Ph*). MALDITOF-MS calcd for $C_{262}H_{252}N_4O_{83}$: 4781 $[M]^+$, found: 4804 $[M+Na]^+$.

4.1.8. Free dendrimer 17. To a solution of **16** (196 mg, 0.04 mmol) in MeOH (5 mL) was added NaOMe until the pH reached 10. The mixture was stirred at rt for 2 days, neutralized with Amberlite IR-120 (H^+). The solvents were filtered, and the filtrate was concentrated to dryness under reduced pressure. The residue was subjected to chromatography on a Sephadex LH-20 column with MeOH as the eluent to give **17** as a white solid (76 mg, 91.5%); $[\alpha]_D^{20}=-5$ (*c* 1, CH_3OH); ^{1}H NMR (400 Hz, CD_3OD) δ 1.19–1.36 (m, 3×4H, $-CH_2CH_2-$), 1.48–1.56 (m, 3×2H, $-CH_2CH_2-$), 1.58–1.66 (m, 3×2H, $-CH_2CH_2-$), 2.06–2.18 (m, 3×4H, $-CH_2CH_2-$), 3.07 (t, 3×2H, CH_2NHCO), 3.12 (dd, 3×1H, $J_{2,3}$=9.6 Hz, $J_{3,4}$=10.2 Hz, H-3^{I}), 3.14–3.60 (m, 3×16H), 3.71 (dd, 3×1H, $J_{6a,6b}$=11.5 Hz, $J_{6a,5}$=5.5 Hz, H-6), 3.76–3.83 (m, 3×2H), 4.04–4.09 (m, 3×1H), 4.23 (d, 3×1H, $J_{1,2}$=7.8 Hz, H-1), 4.31 (d, 3×1H, $J_{1,2}$=7.6 Hz, H-1), 4.47 (d, 3×1H, $J_{1,2}$=7.6 Hz, H-1); δ_C (100 Hz, CD_3OD) 26.7, 30.2, 30.6, 31.2, 40.5 (CH$_2$NHCO), 62.6, 62.8, 69.8, 70.0, 71.0, 71.60, 74.4, 75.1, 75.5, 76.8, 77.8, 78.0, 78.2, 94.4 (CNO$_2$), 103.9 (C-1), 105.0 (C-1), 105.2 (C-1), 174.0 (NHCO). MALDITOF-MS Calcd for $C_{82}H_{144}N_4O_{53}$: 2032 $[M]^+$, found: 2055 $[M+Na]^+$; 2071 $[M+K]^+$.

4.1.9. General procedure for sulfation of compounds 1 and 17. A mixture of oligosaccharide (**1** or **17**) and SO_3·Pyr (10 equiv.) in DMF was stirred at 50 °C for 3 days. Before 3 N NaOH was added, pyridine was removed in vacuo, and the residue was purified on a Sephadex LH-20 column using water as eluent furnished a mixture of sulfated compound **11** or **18** which were used for the next bioassay after freeze-drying. Microanalysis data for compound **11**: C 16.22%, H 1.73%, S 19.90%. Microanalysis for compound **18**: C 21.01%, H 2.54%, S 17.79%.

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