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Syntheses of a series of lacto-*N*-neotetraose clusters using a carbosilane dendrimer scaffold

Akihiro Yamada,[a] Ken Hatano,[a] Tetsuo Koyama,[a] Koji Matsuoka,[a]
Yasuaki Esumi[b] and Daiyo Terunuma[a,*]

[a] *Department of Functional Materials Science, Faculty of Engineering, Saitama University, 255 Shimo-ohkubo, Sakura-ku, Saitama 338-8570, Japan*

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

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Abstract—4-Pentenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (**4**) was synthesized by regioselective glycosylation of 4-pentenyl (2,6,-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside and (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride. By conversion of the protecting groups followed by thioacetylation, **4** was transformed into the corresponding lacto-*N*-neotetraose derivative, 5-(acetylthio)pentenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (**6**). The lacto-*N*-neotetraose derivative **6** was introduced into carbosilane dendrimer cores of three shapes, and three kinds of new carbosilane dendrimers peripherally functionalized by lacto-*N*-neotetraose were obtained.

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1. Introduction

The surface of eukaryotic cells is covered with an array of glycoconjugates, such as glycoproteins and glycolipids. Carbohydrates in some of the glycoconjugates play a key role in the process of cell adhesion with proteins of pathogens such as bacteria, viruses, and toxins, that is, the early stage of cell adhesion involves carbohydrate-mediated specific recognition of pathogens.[1] Although most carbohydrate ligands bind only weakly to their protein receptors, clustering of carbohydrate ligands can not only efficiently compensate for the usual weak carbohydrate–protein affinity but greatly increases the overall avidity of carbohydrate ligands toward their receptors.[2]

Recently, we reported syntheses of some glycoclusters having β-cyclodextrin,[3] globotriaose,[4] sialyllactose,[5] mannose,[6] mannobiose,[6] and functional saccharides[7] in which carbosilane dendrimers were employed as the scaffolds of carbohydrate, and described the biological activities of some of these glycoclusters.[4,5] A glycocluster periphery functionalized with globotriaose (Galα1-4Galβ1-4Glcβ1-) neutralized Vero toxins producing *Escherichia coli* O157:H7 with high affinity in in vivo experiments using mice.[8] In our knowledge, carbosilane dendrimers are one of the most effective scaffolds for therapeutic usefulness, because such dendrimers have some advantages over other carbohydrate scaffolds commonly used, including the potential for tailor-made molecular design, great chemical stability, and charge neutrality in aqueous solution.

The dengue virus causes dengue and dengue hemorrhagic fevers. In recent years, dengue has become a major infectious disease. The prevalence of dengue has increased significantly in recent decades. Since there is still no effective treatment for dengue fever, the development of an anti-dengue virus drug is important.

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[*] Corresponding author. Fax: +81 48 858 3532; e-mail: teru@fms.saitama-u.ac.jp

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Recently, Suzuki et al. discovered that dengue virus preferentially adheres to lacto-*N*-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-) located on the surface of eukaryotic cells.[9] Lacto-*N*-neotetraose cluster compounds are thus expected to be candidates for an artificial dengue virus receptor. In this paper, we report the successful synthesis of a novel class of carbosilane dendrimer periphery-functionalized lacto-*N*-neotetraose.

2. Results and discussion

2.1. Synthesis of lacto-*N*-neotetraose derivative

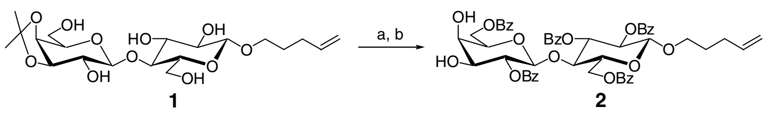
Some chemical syntheses of lacto-*N*-neotetraose have already been reported.[10,11] For the construction of the tetrasaccharide sequence, a final linkage of two disaccharides has been designed.[10] In most of these studies, 3′-hydroxylactose derivatives were used as glycosyl acceptors.[10a–k] Paulsen and Steiger utilized benzyl (2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (lactose derivative having a free 3′,4′-diol) as a glycosyl acceptor.[10l] In the present study, we also employed the 3′,4′-unprotected lactose derivative **2** as a glycosyl acceptor for the preparation of a lacto-*N*-neotetraose derivative. This strategy takes advantage of the ease of preparation of the diol compound.

Although, an initial attempt to prepare an acetyl-protected 3′,4′-diol lactose derivative, 4-pentenyl (2,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside, failed because of the low yields, the related benzoyl-protected 3′,4′-diol **2** was readily synthesized by benzoyl protection of the known 3′,4′-isopropylidene-protected glycoside **1**[12] followed by deacetonation, giving a 77% overall yield, as shown in Scheme 1. The structure of glycosyl acceptor **2** was confirmed by means of ^{1}H and ^{13}C NMR spectra, mass spectroscopy, and elemental analysis. Further evidence for **2** was obtained by acetylation of **2**, the NMR spectra of the product showed characteristic signals based on two acetyl groups (3′,4′-positions): ^{1}H NMR ($CDCl_3$): δ 2.03, 1.83 (each s, OAc), ^{13}C NMR ($CDCl_3$): δ 20.41, 20.32 (CH_3COO–) 169.87 (CH_3COO–, two carbonyl peaks were overlapped).

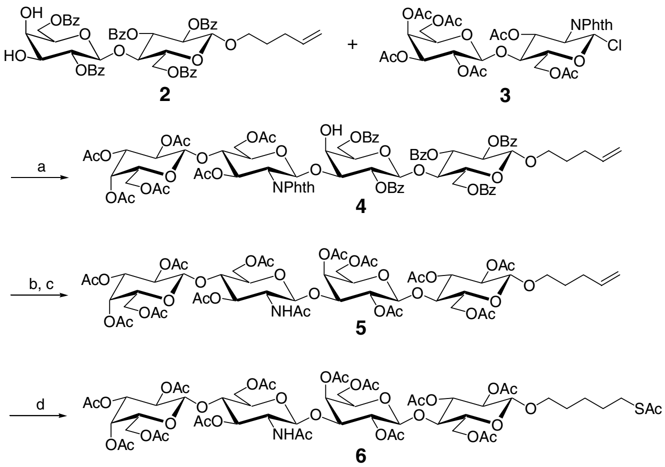
Silver triflate-promoted glycosylation of 3′,4′-diol **2** and lactosamine derivative **3**, which was readily obtained by Lemieux's procedure with slight modification,[13] provided the desired tetrasaccharide **4** but in low yield (36%). Several different conditions for the glycosylation were tested. A change in reaction temperature did not significantly improve this step. However, the yield of tetrasaccharide **4** improved with an increase in the amount of AgOTf used. The best conditions were as follows: Donor **3** was regio- and β-specifically reacted with acceptor **2** using 10.1 equiv of AgOTf and 4 Å molecular sieves powder as an acid scavenger, to afford tetrasaccharide **4** in 76% yield (Scheme 2). Its ^{13}C NMR signals showed the presence of four signals at 100.97, 100.87, 100.41, and 98.17 ppm corresponding to the four β-linked anomeric carbons. EI-MS also supported the tetrasaccharide structure of **4** (EI-MS Calcd for $[M+Na]^+$: 1685. Found: 1685). Thus, synthesis of lacto-*N*-neotetraose derivative **4** is efficiently achieved by condensation of benzoyl-protected 3′,4′-diol **2** and lactosamine derivative **3**. It is noteworthy that the 3′,4′-diol lactose derivative **2** is convenient and valuable as a novel glycosyl acceptor.

Deblocking of the phthaloyl (Phth) group from tetrasaccharide **4** with *n*-butylamine in refluxing methanol, followed by acetylation led to the formation of acetyl-protected lacto-*N*-neotetraose derivative **5** in 79% yield. The success of transformation was readily confirmed by the ^{1}H NMR spectrum, which exhibited disappearance of the aromatic proton signals of the phthaloyl group and the appearance of an NH signal at 5.39 ppm. Moreover, two doublet signals observed at 5.35 and 5.30 ppm with small coupling constants (J 2.6 and 2.7 Hz) are attributable to H-4 protons of the two galactose moieties in **5**. This means that a β linkage is undoubtedly formed at the 3′-position of **2** during glycosylation with lactosamine derivative **3**.

Next, the terminal alkene of lacto-*N*-neotetraose derivative **5** was functionalized for its introduction into a carbosilane dendrimer scaffold. The utility of thiolate anion on the sugar aglycon for coupling reactions with a ω-brominated carbosilane dendrimer was demonstrated in our previous investigation.[5] Since the thiolate anion can be readily generated via deacetylation of the acetylthio group upon treatment with NaOMe, thioacetylation of the double bond of the lacto-*N*-neotetraose derivative **5** was carried out. Reaction of the lacto-*N*-neotetraose derivative **5** with thioacetic acid in the presence of AIBN at 80 °C gave the anti-Markovnikov adduct **6**, ^{1}H NMR ($CDCl_3$): δ 2.83 (t, J 7.2 Hz, CH_2SAc), 2.31 (s, SAc), in 98% yield.



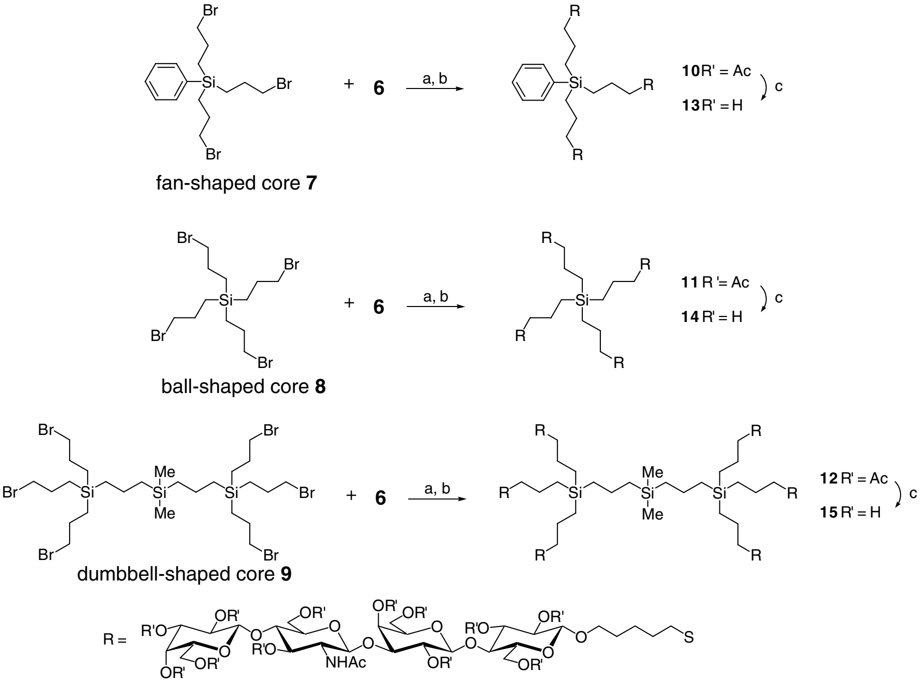
Scheme 1. Reagents and conditions: (a) BzCl, pyridine, rt; (b) 50% CF_3CO_2H aq, CH_2Cl_2, rt.



Scheme 2. Reagents and conditions: (a) AgOTf, MS 4 Å powder, CH_3NO_2, 0 °C then rt; (b) n-$BuNH_2$, MeOH, reflux; (c) Ac_2O, pyridine; (d) AcSH, AIBN, 1,4-dioxane, 80 °C.

2.2. Synthesis of glycoclusters

Fundamental core structures of carbosilane dendrimers **7**–**9** (fan, ball, and dumbbell shapes) were used as scaffolds[14] for the syntheses of lacto-*N*-neotetraose cluster compounds. Coupling reactions between these carbosilane dendrimer scaffolds and peracetylated lacto-*N*-neotetraose **6** were effected by nucleophilic substitution of terminal bromide on the carbosilane dendrimer with the thiolate anion generated from **6** by treatment with NaOMe.[5] The thioacetic glycoside **6** reacted with the fan-shaped core **7** to give the acetylated fan-shaped cluster **10**, with the ball-shaped core **8** to yield the ball-shaped glycocluster **11**, and with the dumbbell-shaped core **9** to afford the corresponding dumbbell-shaped conjugate **12** in yields of 64%, 55%, and 44%, respectively (Scheme 3). Purification of the products was successfully performed by recycling preparative HPLC. All acetylated clusters were examined by means of ^1H and ^{13}C NMR spectra and mass spectra. The NMR spectra show a single signal pattern for all sugar units; the ratios of the integrals for these signals and for signals of the methylene protons of the core are consistent with each of the product as formulated. Further evidence of the glycoclusters was obtained by high-resolution mass spectroscopy. The electrospray-ionization mass



Scheme 3. Reagents and conditions: (a) NaOMe, MeOH, DMF, rt; (b) Ac_2O, pyridine, 30 °C; (c) NaOMe, MeOH, rt, then dil. NaOH aq, rt.

spectrum (ESI-MS) of acetylated fan-shaped **10** shows a peak at *m*/*z* 2107.1950 due to $[M+2Na]^{2+}$, and that of acetylated ball-shaped **11** shows a peak at *m*/*z* 2745.9112 due to $[M+2Na]^{2+}$. The molecular weight of the acetylated dumbbell-shaped **12** was too great to measure high-resolution mass spectra. The ESI-MS of **12** shows a peak at *m*/*z* 2799.56 due to $[M+3Na]^{3+}$. Successive O-deacetylation under Zemplén conditions and saponification (0.1 M aq NaOH) afforded glycoclusters of fan-shaped **13**, ball-shaped **14**, and dumbbell-shaped **15** in quantity, 68%, and 96%, yields, respectively (Scheme 3). The carbosilane dendrimers periphery-functionalized with lacto-*N*-neotetraose (**13**–**15**) obtained here were identified by ^{1}H and ^{13}C NMR spectra and by high-resolution mass spectra, and were soluble in an aqueous solution.

In conclusion, high regio- and stereo-selective synthesis lacto-*N*-neotetraose derivative **4** was accomplished by using the free 3′,4′-diol lactose derivative **2** and lactosamine derivative **3**. In addition, a novel class of multivalent glycoconjugates periphery-functionalized with lacto-*N*-neotetraose was efficiently synthesized using nucleophilic substitution of thiolate anion of the sugar aglycon on ω-brominated carbosilane dendrimers. These glycodendrimers **13**–**15** obtained here showed an inhibitory effect on cell adhesion of dengue virus in cell-culture assay. The detailed results of a biological assay will be reported elsewhere.

3. Experimental

3.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400, Bruker DRX 400, or Varian Gemini 2000 spectrometer at 400, 400, and 200 MHz for proton and at 100, 100, and 50 MHz for carbons, respectively. Proton chemical shifts are given in parts per million with use of tetramethylsilane (0 ppm) or residual solvent peaks as internal standard. NMR signals were assigned by ^{1}H, ^{13}C, HH, and HC COSY measurements. IR spectra were recorded on a Shimadzu IR-Prestage 21. Recycling preparative HPLC was performed with a LC-908 or LC-918W system (Japan Analytical Industry Co., Ltd.) connected to an RI detector RI-5. Optical rotations were recorded on a jasco DIP-1000 digital polarimeter.

3.2. Synthesis

3.2.1. 4-Pentenyl (2,6,-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside (2). Benzoyl chloride (78.7 mL, 0.68 mol) was added dropwise to a solution of compound **1** (5.13 g, 11.39 mmol) in pyridine (55 mL) at 0 °C, and the mixture was stirred at rt for 3 h. After quenching with water, the mixture was extracted with $CHCl_3$. The organic solution was washed with 1 M HCl aq, satd $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$) and the filtrate concentrated under reduced pressure. Trifluoroacetic acid (25 mL) was added dropwise to a solution of the residue in CH_2Cl_2 (100 mL) and water (25 mL) at 0 °C, and then stirred at rt for 3 h. The solution was washed with water, satd $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on silica gel (1:2 EtOAc–hexane) to provide compound **2** (8.15 g, 77%). $[\alpha]_D^{13}$ +41.4 (*c* 1.1, $CHCl_3$); IR (KBr): *ν* 3437 (O–H), 1724 (CO, ester), 1273 (C–O, ester), 1068 cm^{-1} (C–O, ether); ^{1}H NMR ($CDCl_3$): *δ* 7.90–8.08 (m, 10H), 7.26–7.76 (m, 15H), 5.63 (t, 1H, *J* 9.6 Hz, H-3), 5.54–5.64 (m, 1H, $-CH{=}CH_2$), 5.41 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.37 (dd, 1H, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 9.8 Hz, H-2′), 4.75–4.80 (m, 2H, $-CH{=}CH_2$), 4.54–4.65 (m, 3H, H-1, H-1′, H-6a), 4.49 (dd, 1H, *J* 4.6 Hz, *J* 12.0 Hz, H-6b), 4.11 (t, 1H, *J* 9.4 Hz, H-4), 4.02 (dd, 1H, *J* 5.6 Hz, *J* 10.8 Hz, H-6′b), 3.84 (d, 1H, $J_{3',4'}$ 3.8 Hz, H-4′), 3.76–3.81 (m, 2H, H-5 and one of the $-OCH_2CH_2-$), 3.70 (dd, 1H, $J_{3',4'}$ 3.4 Hz, $J_{2',3'}$ 9.8 Hz, H-3′), 3.48–3.56 (m, 2H, H-5′ and H-6′b), 3.39–3.45 (m, 1H, one of the $-OCH_2CH_2-$), 2.99 (br s, 2H, OH), 1.84–1.97 (m, 2H, $-OCH_2CH_2CH_2-$), 1.47–1.64 (m, 2H, $-OCH_2CH_2CH_2-$); ^{13}C NMR ($CDCl_3$): *δ* 166.27, 166.03, 166.00, 165.93, 165.16, 137.65 ($-CH{=}CH_2$), 100.95 and 100.89 (anomeric carbons), 76.28 (C-4), 73.54 (C-2′), 73.67 (C-3), 72.92, 72.66, 72.55, 71.65 (C-2), 69.29 ($-OCH_2CH_2$), 68.65 (C-4′), 62.66 (C-6), 61.94 (C-6′), 29.67 ($-OCH_2CH_2CH_2-$), 28.42 ($-OCH_2CH_2CH_2-$). FAB-MS Anal. Calcd for $C_{52}H_{50}O_{16}$ $[M+Na]^+$: 953. Found: 953. Anal. Calcd for $C_{52}H_{50}O_{16}$: C, 67.09; H, 5.41. Found: C, 66.77; H, 5.36.

3.2.2. 4-Pentenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-benzoyl-β-D-glucopyranoside (4). A mixture of gycosyl donor **3** (1.66 g, 2.23 mmol), acceptor **2** (2.50 g, 2.68 mmol), and molecular sieves (4 Å powder, 5.0 g) in nitromethane was stirred at rt for 2.5 h under Ar. Silver triflate (2.87 g, 11.17 mmol) was added to the mixture at 0 °C under darkness and the mixture was stirred at the same temperature for 2.5 h. Additional silver triflate (2.87 g, 11.17 mmol) was added to the mixture. The mixture was allowed to stir over night. The insoluble solids were separated by filtration through a Celite bed and washed with EtOAc. The combined organic solution was washed with water, satd $NaHCO_3$ solution, and brine. The organic layer was dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (2:1 EtOAc–hexane) to yield tetrasaccharide **4**

(2.78 g, 76%). $[\alpha]_D^{17}$ +44.5 (*c* 1.1, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 8.04–8.09 (m, 2H), 7.87–7.97 (m, 7H), 7.61–7.64 (m, 1H), 7.21–7.56 (m, 17H), 7.05 (t, 2H, *J* 7.4 Hz), 5.50–5.63 (m, 3H, H-3, H-3′ and –C*H*=CH$_2$), 5.47 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1″), 5.34 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.30 (d, 1H, $J_{3''',4'''}$ 3.1 Hz, H-4‴), 5.26 (dd, 1H, $J_{1',2'}$ 8.1 Hz, $J_{2',3'}$ 9.6 Hz, H-2′), 5.08 (dd, 1H, $J_{1''',2'''}$ 7.9 Hz, $J_{2''',3'''}$ 10.4 Hz, H-2‴), 4.94 (dd, 1H, $J_{2''',3'''}$ 10.4 Hz, $J_{3''',4'''}$ 3.4 Hz, H-3‴), 4.71–4.79 (m, 2H, –CH=C*H*$_2$), 4.46–4.55 (m, 4H, H-1, H-1′, H-1‴, and H-6″a), 4.35 (br d, 1H, *J* 10.6 Hz, H-6a), 4.14–4.25 (m, 3H, H-2″, H-6b and H-6′a), 3.92–4.09 (m, 5H, H-4, H-4′, H-6″b and H-6‴ab), 3.83 (t, 1H, *J* 7.0 Hz, H-5‴), 3.70–3.79 (m, 3H, H-4″, H-5″ and one of the OCH$_2$CH$_2$–), 3.66 (dd, 1H, $J_{2',3'}$ 9.8 Hz, $J_{3',4'}$ 3.2 Hz, H-3′), 3.56–3.62 (m, 2H, H-5 and H-6′b), 3.48–3.51 (m, 1H, H-5′), 3.26–3.40 (m, 1H, one of the OCH$_2$CH$_2$–), 2.10, 2.04, 2.02, 2.00, and 1.95 (each s, each 3H, OAc), 1.81–1.91 (m, 2H, OCH$_2$CH$_2$C*H*$_2$–), 1.79 (s, 3H, OAc), 1.43–1.58 (m, 2H, OCH$_2$C*H*$_2$CH$_2$–); ^{13}C NMR (CDCl$_3$): δ 170.26, 170.20, 170.02, 169.97, 169.45, 169.06, 165.96, 165.74, 165.44, 165.12, 163.97, 137.59, 133.77, 133.30, 133.10, 133.01, 132.80, 132.61, 129.88, 129.84, 129.71, 129.64, 129.54, 129.48, 129.40, 129.34, 129.21, 128.61, 128.50, 128.34, 128.23, 128.15, 128.03, 123.04, 114.71 (–CH=*C*H$_2$), 100.97, 100.87 100.39, 98.14 (C-1″), 81.31, 75.42, 72.79, 72.72, 72.61, 71.78, 70.87, 70.80, 70.52, 70.34, 69.20, 69.01, 67.59, 66.43, 62.87, 62.40, 61.68, 60.55, 54.37, 29.60, 28.36, 20.58, 20.50, 20.42, 20.28. EI-MS Anal. Calcd for C$_{84}$H$_{85}$NO$_{33}$ [M+Na]$^+$: 1658. Found: 1658. Anal. Calcd for C$_{84}$H$_{85}$NO$_{33}$: C, 61.65; H, 5.24; N, 0.86. Found: C, 61.38; H, 5.10; N, 0.83.

3.2.3. 4-Pentenyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (5). A mixture **4** (303.1 mg, 0.185 mmol), *n*-butylamine (5 mL) in MeOH (5 mL) was refluxed for 24 h under Ar. The solution was evaporated, the residue was suspended in a mixture of pyridine (5 mL) and Ac$_2$O (2.5 mL). The mixture was stirred at rt for 2 days. The resulting mixture was poured into ice water and extracted with EtOAc, subjected to the usual work-up process, and purified by flash chromatography on silica gel (9:1 EtOAc–hexane) to afford compound **8** (187.5 mg, 79%). $[\alpha]_D^{20}$ +5.9 (*c* 0.18, CHCl$_3$); IR (KBr), *v* (cm^{-1}): 1751 (CO ester), 1678 (CO amide I), 1543 (NH amide II); ^{1}H NMR (CDCl$_3$): δ 5.72–5.83 (m, 1H, –C*H*=CH$_2$), 5.39 (d, 1H, *J* 8.5 Hz, N*H*Ac), 5.35 (d, 1H, $J_{3''',4'''}$ 2.6 Hz, H-4‴), 5.30 (d, 1H, $J_{3',4'}$ 2.7 Hz, H-4′), 5.14–5.22 (m, 2H, H-3 and H-3″), 5.11 (dd, 1H, $J_{1''',2'''}$ 8.0 Hz, $J_{2''',3'''}$ 10.4 Hz, H-2‴), 4.96–5.03 (m, 4H, H-2′, H-3‴ and –CH=C*H*$_2$), 4.89 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.78 (br d, 1H, *J* 10.5 Hz, H-6″a), 4.83 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1″), 4.54 (d, 1H, $J_{1''',2'''}$ 7.9 Hz, H-1‴), 4.43–4.45 (m, 2H, H-1 and H-6a), 4.34 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.01–4.12 (m, 5H, H-6b, H-6′ab, and H-6‴ab), 3.97 (br d, 1H, *J* 9.2 Hz, H-6″b), 3.72–3.89 (m, 6H, H-4, H-3′, H-5′, H-4″, H-5‴ and one of the OC*H*$_2$CH$_2$–), 3.45–3.61 (m, 4H, H-5, H-2″, H-5″ and one of the OC*H*$_2$CH$_2$–), 1.91–2.15 (several s and overlapping m, 41H, Ac, and OCH$_2$CH$_2$C*H*$_2$), 1.61–1.73 (m, 2H, OCH$_2$C*H*$_2$CH$_2$); ^{13}C NMR (CDCl$_3$): δ 170.38, 170.30, 170.22, 170.12, 170.09, 169.89, 169.80, 169.63, 169.56, 169.33, 168.90, 168.74, 137.53 (–*C*H=CH$_2$), 114.82 (–CH=*C*H$_2$), 100.82 (C-1‴), 100.43 (C-1′), 100.33 (C-1), 99.99 (C-1″), 75.64, 75.59, 75.56, 72.39, 72.27, 71.74, 70.81, 70.67, 70.62, 70.43, 69.02, 68.19, 68.71, 66.43, 61.96, 61.34, 60.55, 60.19, 54.71, 29.54, 28.30, 22.84, 20.61, 20.55, 20.48, 20.43, 20.36, 20.25. EI-MS Anal. Calcd for C$_{55}$H$_{77}$NO$_{33}$ [M]$^+$: 1279. Found: 1279. Anal. Calcd for C$_{55}$H$_{77}$NO$_{33}$: C, 51.60; H, 6.06; N, 1.09. Found: C, 51.52; H, 6.10; N, 1.03.

3.2.4. 5-Acetylthiopentyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (6). A mixture of **5** (896.3 mg, 0.70 mmol), thioacetic acid (0.99 mL, 14.0 mmol), and AIBN (229.8 mg, 1.40 mmol) in 1,4-dioxane (0.5 mL) was mildly heated to 80 °C under Ar. The reaction mixture was stirred at 80 °C for 2 h. and then cooled to room temperature. Cyclohexene (142 μL, 1.40 mmol) was added with stirring for a few minutes. The resulting solution was passed through a short column of silica gel. The residue was purified by recycling preparative HPLC (column, JAIGEL-1H and 2H; solvent, CHCl$_3$) to afford **6** (931.0 mg, 98%). $[\alpha]_D^{20}$ +2.9 (*c* 0.5, CHCl$_3$); ^{1}H NMR (CDCl$_3$): δ 5.47 (d, 1H, *J* 8.6 Hz, N*H*Ac), 5.33 (d, 1H, $J_{3''',4'''}$ 2.9 Hz, H-4‴), 5.29 (s, 1H, H-4′), 5.15–5.20 (m, 2H, H-3 and H-3″), 5.10 (dd, 1H, $J_{1'''2'''}$ 7.9 Hz, $J_{2'''3'''}$ 9.5 Hz, H-2‴), 4.95–4.98 (m, 2H, H-2′ and H-3‴), 4.86 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.5 Hz, H-2), 4.76 (br d, 1H, *J* 11.2 Hz, H-6″a), 4.66 (br d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.53 (d, 1H, $J_{1''',2'''}$ 7.9 Hz, H-1‴), 4.42–4.44 (m, 2H, H-1 and H-6a), 4.33 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.00–4.10 (m, 5H, H-6b, H-6′ab, and H-6‴ab), 3.96 (br d, 1H, *J* 10.8 Hz, H-6″b), 3.70–3.88 (m, 6H, H-4, H-3′, H-5′, H-4″, H-5‴ and one of the OC*H*$_2$CH$_2$–), 3.43–3.55 (m, 4H, H-5, H-2″, H-5″ and one of the OC*H*$_2$CH$_2$–), 2.83 (t, 2H, *J* 7.2 Hz, C*H*$_2$SAc), 1.89–2.14 (several s, 39H, Ac), 1.51–1.57 (m, 4H, –OCH$_2$C*H*$_2$CH$_2$C*H*$_2$CH$_2$SAc), 1.36–1.37 (m, 2H, C*H*$_2$-CH$_2$CH$_2$SAc); ^{13}C NMR (CDCl$_3$): δ 195.90 (SC=O), 170.63, 170.56, 170.47, 170.36, 170.12, 170.07, 169.87, 169.83, 169.58, 169.12, 168.95, 101.04 (C-1‴), 100.67 (C-1′), 100.53 (C-1), 100.23 (C-1″), 77.21, 75.75, 75.69, 72.66, 72.59, 71.86, 71.56, 71.06, 70.84, 70.68, 69.73, 69.15, 68.89, 66.61, 62.16, 61.53, 60.75, 60.33, 54.98, 30.60, 29.14, 28.88, 24.99, 23.14, 20.85, 20.79, 20.73, 20.67, 20.62, 20.48. FAB-MS Anal. Calcd for $C_{57}H_{81}NO_{34}S$ $[M+H]^+$: 1356. Found: 1356. Anal. Calcd for $C_{57}H_{81}NO_{34}S$: C, 50.48; H, 6.02; N, 1.03. Found: C, 50.31; H, 5.96; N, 1.03.

3.2.5. Fan-shaped dendrimer-OAc (10). Carbosilane dendrimer **7** (25.0 mg, 0.053 mmol) and lacto-*N*-neotetraose derivative **6** (301.2 mg, 0.222 mmol) dissolved in a mixture of DMF (0.3 mL) and MeOH (0.3 mL) was treated with NaOMe (224 µL, 1 M MeOH solution) at rt under Ar for 11 h. After HOAc (0.2 mL) had been added, the mixture was evaporated. The residue was dissolved in pyridine (4 mL) then Ac_2O (2 mL) was added. The mixture was stirred at 30 °C for 3 h. The resulting solution was poured into ice water and extracted with EtOAc, subjected to the usual work-up process, and purified on recycling preparative HPLC (column, JAI-GEL-2.5H and 3H; solvent, $CHCl_3$) to give **10** (142.0 mg, 64%). $[\alpha]_D^{23}$ +3.3 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.43–7.46 (m, 2H), 7.29–7.36 (m, 3H), 5.33–5.36 (m, 6H, H-4‴ and N*H*Ac), 5.30 (s, 3H, H-4′), 5.16–5.22 (m, 6H, H-3 and H-3″), 5.11 (dd, 1H, $J_{1'''2'''}$ 8.0 Hz, $J_{2'''3'''}$ 10.4 Hz, H-2‴), 4.96–5.00 (m, 6H, H-2′ and H-3‴), 4.84 (dd, 3H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.78 (br d, 3H, *J* 11.2 Hz, H-6″a), 4.68 (br d, 3H, $J_{1'',2''}$ 7.6 Hz, H-1″), 4.54 (d, 3H, $J_{1''',2'''}$ 7.6 Hz, H-1‴), 4.43–4.46 (m, 6H, H-1 and H-6a), 4.34 (d, 3H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.01–4.13 (m, 15H, H-6b, H-6′ab and H-6‴ab), 3.97 (br d, 3H, *J* 10.4 Hz, H-6″b), 3.72–3.89 (m, 18H, H-4, H-3′, H-5′, H-4″, H-5‴ and one of the OC*H*$_2$CH$_2$–), 3.41–3.62 (m, 12H, H-5, H-2″, H-5″ and one of the OC*H*$_2$CH$_2$–), 2.49 (t, 6H, *J* 7.2 Hz, CH_2S), 2.43 (t, 6H, *J* 7.2 Hz, CH_2S), 1.91–2.15 (several s, 117H, Ac), 1.50–1.59 (m, 18H, –OCH$_2$C*H*$_2$C*H*$_2$-C*H*$_2$CH$_2$S), 1.34–1.42 (m, 6H, SiCH$_2$C*H*$_2$CH$_2$S), 0.89–0.92 (m, 6H, SiC*H*$_2$CH$_2$); ^{13}C NMR ($CDCl_3$): δ 170.41, 170.31, 170.25, 170.15, 169.91, 169.84, 169.63, 169.57, 169.35, 169.11, 168.93, 168.73, 136.07, 133.79, 128.94, 127.68, 100.89 (C-1‴), 100.50 (C-1′), 100.37 (C-1), 100.04 (C-1″), 77.20, 75.67, 75.59, 72.47, 72.42, 72.15, 71.75, 71.39, 70.87, 70.77, 70.66, 70.50, 69.68, 69.53, 68.95, 68.72, 66.45, 61.99, 61.35, 60.99, 60.59, 60.20, 54.80, 35.67, 33.79, 31.77, 29.14, 28.83, 24.92, 23.84, 22.98, 20.71, 20.67, 20.61, 20.55, 20.51, 20.49, 20.46, 20.42, 20.31, 11.67. ESI-MS Anal. Calcd for $C_{180}H_{257}N_3O_{99}S_3Si$ [M+Na]+:4191.40 Found: 4193.47. Anal. Calcd for $C_{180}H_{257}N_3O_{99}S_3Si$ $[M+2Na]^{2+}/2$: 2107.1948 Found: 2107.1950.

3.2.6. Ball-shaped dendrimer-OAc (11). The reaction conditions and workup were as described in the synthesis of Fan-shaped-OAc **10**. The title compound **11** (121.3 mg, 55%) was obtained. $[\alpha]_D^{24}$ +4.3 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.47–2.49 (m, 16H, SCH_2), 1.91–2.15 (m, 156H, Ac), 1.57–1.64 (m, 24H, –OCH$_2$C*H*$_2$-C*H*$_2$C*H*$_2$CH$_2$S), 1.40 (br s, 8H, SiCH$_2$C*H*$_2$CH$_2$S), 0.60–0.62 (m, 8H, SiCH$_2$–); ^{13}C NMR ($CDCl_3$): δ 170.37, 170.29, 170.22, 170.12, 169.88, 169.81, 169.60, 169.54, 169.33, 168.89, 168.72, 100.87 (C-1‴), 100.53 (C-1′), 100.37 (C-1), 100.13 (C-1″), 77.20, 75.57, 72.42, 71.80, 71.37, 71.09, 70,95, 70.74, 70.64, 70.48, 69.69, 68.92, 68.72, 66.42, 62.04, 61.32, 60.57, 60.27, 54.78, 35.80, 31.86, 29.15, 28.84, 24.91, 23.99, 23.10, 20.69, 20.56, 20.50, 20.46, 20.40, 20.28, 11.72. ESI-MS Anal. Calcd for $C_{232}H_{336}N_4O_{132}S_4Si$ $[M+2Na]^{2+}/2$: 2745.9075 Found: 2745.9112.

3.2.7. Dumbbell-shaped dendrimer-OAc (12). The reaction conditions and workup were as described in the synthesis of Fan-shaped-OAc **10**. The title compound **12** (110.0 mg, 44%) was obtained. $[\alpha]_D^{24}$ +4.3 (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.47–2.49 (m, 24H, SC*H*$_2$), 1.90–2.17 (m, 234H, Ac), 1.57–1.61 (m, 36H, –OCH$_2$C*H*$_2$C*H*$_2$C*H*$_2$CH$_2$S), 1.40 (br s, 12H, SiCH$_2$-C*H*$_2$CH$_2$S), 1.24–1.28 (m, 4H, SiCH$_2$C*H*$_2$CH$_2$Si), 0.50–0.61 (m, 20H, SiC*H*$_2$–), −0.06 (s, 6H, $SiCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.42, 170.33, 170.27, 170.17, 169.93, 169.87, 169.66, 169.59, 169.38, 168.94, 168.76, 100.93 (C-1‴), 100.57 (C-1′), 100.43 (C-1), 100.13 (C-1″), 77.20, 75.59, 72.47, 71.81, 71.43, 70.97, 70.81, 70.69, 70.53, 69.75, 68.97, 68.76, 66.46, 62.06, 61.36, 60.60, 60.26, 54.84, 45.63, 35.91, 31.92, 29.22, 28.91, 24.98, 24.12, 23.19, 20.73, 20.69, 20.61, 20.56, 20.50, 20.45, 20.33, 20.21, 18.16, 16.91, 11.92. ESI-MS Anal. Calcd for $C_{356}H_{522}N_6O_{198}S_6Si_3$ $[M+3Na]^{3+}/3$: 2799.84 Found: 2799.56.

3.2.8. Fan-shaped dendrimer (13). Acetylated compound **10** (109.5 mg, 0.026 mmol) was dissolved in MeOH (1.0 mL) and NaOMe (5.5 mg, 0.10 mmol) was added under Ar. The reaction mixture was stirred for 30 min. When white solids separated out, 0.1 M aq NaOH (5.0 mL) was added. Progress of the reaction was monitored by TLC. When the reaction was complete, the mixture was treated with Amberlite IR120B (H^+) resin to neutralize the base. The resin was filtered off and the filtrate was concentrated, and purified by gel-permeation chromatography (Sephadex G50, 5% aq HOAc eluent) to give fan-shaped dendrimer **13** (71.2 mg, quant.). $[\alpha]_D^{17}$ −4.1 (*c* 0.17, H_2O); ^1H NMR (D_2O): δ 7.31, 7.15 (each br s, 5H, Si*Ph*), 4.26–4.34 (m), 4.01 (br s), 3.75–3.82 (m), 3.58–3.72 (m), 3.37–3.54 (m), 3.17 (br m), 2.32 (br s, 12H, –C*H*$_2$S), 1.90 (s, 9H, NH *Ac*), 1.41 (br s), 1.25 (br s), 0.49 (br s, 6H, SiCH$_2$–); ^{13}C NMR (D_2O): δ 181.09, 134.03, 128.05, 102.93 (two anomeric peaks were overlapped), 102.73, 102.39, 82.24, 78.48, 78.27, 75.34, 74.89, 74.56, 72.90, 72.53, 72.22, 70.99, 70.21, 69.94, 68.58, 68.29, 61.05, 60.27, 60.11, 59.89, 55.25, 35.50, 31.68, 29.39, 28.95, 25.00, 23.92, 22.45, 11.72. ESI-MS Anal. Calcd for $C_{108}H_{185}N_3O_{63}S_3Si$ $[M+Na]^+$: 2679.0194 Found: 2679.0096.

3.2.9. Ball-shaped dendrimer (14). The reaction conditions and workup were as described in the synthesis of Fan-shaped **13**, starting with acetylated ball-shaped **11** (91.3 mg, 0.017 mmol). The crude product was purified by recycling preparative HPLC (column, JAIGEL GS-320 and JAIGEL GS-220; solvent, 5% aq HOAc) to give the tetramer **14** (39.1 mg, 68%). $[\alpha]_D^{21}$ +1.43 (*c* 0.53, H_2O); ^{1}H NMR (D_2O): δ 4.28–4.34 (m), 4.00 (br s), 3.76–3.81 (m), 3.36–3.70 (m), 3.16 (br s), 2.42 (br s, 16H, $-CH_2S$), 1.89 (s, 12H, NH *Ac*), 1.49 (br s), 1.32 (br s), 0.57 (br s, 8H, $SiCH_2-$); ^{13}C NMR (D_2O): δ 181.24, 102.67, 102.58, 102.40, 102.01, 81.84, 78.17, 77.90, 75.03, 74.57, 74.44, 74.24, 72.54, 72.21, 71.89, 70.67, 69.95, 69.64, 68.24, 68.01, 35.30, 31.36, 29.67, 29.47, 29.04, 28.58, 24.67, 23.80, 22.0411.38. ESI-MS Anal. Calcd for $C_{136}H_{240}N_4O_{84}S_4Si$ $[M+2Na]^{2+}/2$: 1737.6540 Found: 1737.6544.

3.2.10. Dumbbell-shaped dendrimer (15). The reaction conditions and workup were as described in the synthesis of Fan-shaped **13**, starting with acetylated dumbbell-shaped **12** (69.6 mg, 0.008 mmol). The crude product was purified by recycling preparative HPLC (column, JAIGEL GS-320 and JAIGEL GS-220; solvent, 5% aq HOAc) to give hexamer **15** (42.4 mg, 96%). $[\alpha]_D^{20}$ −5.4 (*c* 0.12, H_2O); ^{1}H NMR (D_2O): δ 4.33–4.35 (br m), 4.02 (br s), 3.79–3.83 (br m), 3.59–3.70 (br m), 3.39–3.52 (br m), 3.20 (br m), 2.44 (br s, 24H, $-CH_2S$), 1.92 (s, 18H, NH *Ac*), 1.77 (br s), 1.51 (br s), 1.35 (br s), 0.49–0.57 (br m, 10H, $SiCH_2-$), −0.14 (br s, 6H, $SiCH_3$); ^{13}C NMR (D_2O): δ 177.089, 102.94, 102.74, 102.40, 99.98, 82.20, 78.28, 75.38, 74.91, 74.77, 74.60, 72.89, 72.78, 72.56, 72.25, 71.02, 70.41, 70.26, 69.98, 68.59, 68.35, 61.07, 60.85, 59.95, 55.28, 29.54, 29.05, 25.13, 24.26, 22.43, 20.68, −0.25. ESI-MS Anal. Calcd for $C_{212}H_{378}N_6O_{126}S_6Si_3$ $[M+3Na]^{3+}/3$: 1790.6916 Found: 1790.6891.

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