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Synthesis of a new amphiphilic glycodendrimer with antiviral functionality

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ABSTRACT

A new third generation amphiphilic glycodendrimer was synthesized from a stearylamide lysine dendrimer by condensation of the oligosaccharide moiety. By stepwise condensation and deprotection of di-boc lysine from a core of stearyl amide lysine, a third-generation stearylamide lysine dendrimer was constructed. Acetyl cellobiose and glucose units with the carboxylic acid at the end of alkyl chain attached to the reducing end of the sugar moiety was condensed with surface amino groups of the third generation lysine dendrimer, respectively, to give a new stearylamide acetylcellobiose and acetylglucose lysine dendrimers. The structural analysis was carried out using NMR, IR, and matrix-associated laser desorption/ionization time-of-flight (MALDI TOF) mass spectroscopies. After deacetylation to recover hydroxyl groups and subsequent sulfation, the third-generation sulfated cellobiose stearylamide lysine dendrimer was preliminarily found to have high anti-HIV activity at a 50% effective concentration (EC_{50}) as low as 6.4 μg/ml and low cytotoxicity at a 50% cytotoxic concentration (CC_{50}) as high as 1000 μg/ml, indicating that the dendrimer gave the enhancement of the functionality of oligosaccharides with low molecular weights. The glycodendrimer with a hydrophobic stearyl chain is immobilized on hydrophobic surfaces by hydrophobic interaction and is expected to provide a new biomedical material with the surface functionality of hydrophilic sulfated oligosaccharides.

1. Introduction

Ionic interaction between negatively charged sulfated polysaccharides and HIV, which has a positively charged surface glycoprotein originating from lysine and arginine residues, produces potent anti-HIV activity (Uryu et al., 1992). Curdlan sulfate with negatively charged sulfate groups, which was prepared by sulfation of curdlan, a naturally occurring polysaccharide with a linear (1 → 3)-β-glucopyranosidic structure, was found to completely inhibit the infection of MT-4 cells by HIV at concentrations low as 3.3 μg/ml (Yoshida et al., 1990). Other sulfated polysaccharides had also strong anti-HIV activity (Yoshida, 2001).

A dendrimer is a spherical or semi-spherical structure having regular hyper branches and many terminal units with functional groups that was first reported by Tomalia et al. (1985). Further, a glycodendrimer that contained carbohydrates on the surface of the dendrimer scaffold was first described by Roy, Zanini, Meunier, and Romanowska (1993), and several additional studies on its biological activities have appeared. The cluster or multivalent effect of the terminal functional groups is expected to enhance its biological activities. Among them, there are several reports on the synthesis of glycodendrimers with sialic acid at the surface. Sialic acid is a competitive inhibitor of influenza because influenza virus hemagglutinin, a surface glycoprotein, infects cells by binding to α-sialosides as a receptor. Roy synthesized a fourth generation lysine dendrimer from a β-alanine core, and sialic acid was introduced on the surface through a glycine spacer. The sialyl dendrimers of each generation exhibited potent inhibition of hemagglutination of erythrocytes at low concentrations (Roy, Pon, Tropper, & Andersson, 1993). The activity increased with increasing generations of dendrimers, suggesting that dendritic clusters or a multivalent effect was played an important role in the biological activity. Synthesis of hyperbranched lysine cores with *N*-acetylglucosamine was reported and sialyl LewisX was enzymatically transformed into the *N*-acetyl glucosamine for evaluation of selectin-sialyl LewisX interaction (Palec, Li, Zanini, Bhella, & Roy, 1998). Carbosilane dendrimers with lactose or sialyllactose moieties were synthesized by a one-pot coupling reaction of thioacetate at the terminal of spacer connected the sugar with bromo carbosilane dendrimer (Matsuoka, Oka, Koyama, Esumi, & Terunuma, 2001). Glycodendrimers containing 12 and 18 peripheral α-D-mannopyranosidic units were synthesized by a Cu(I)-catalyzed click reaction of 2-azidoethyl-2,3,4,6-*tetra*-*O*-acetyl-α-D-mannopyranoside and an aromatic octadecapropargylated dendritic scaffold (Chabre, Contino-Peĭpin, Placide, Shiao, & Roy, 2008). A glycodendrimer with mannosidic units attached to a glucoside core was constructed by using

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multiple glycosylation between a thioether mannose glycosyl donor, ethyl-2,3,4,6-*tetra*-*O*-benzyl-1-thio-D-mannopyranoside and a glycosyl acceptor, 3-hydroxypropyl-2,3,4,6-*tetra*-*O*-(3-hydroxypropyl)-β-D-glucopyranoside to study mannose-specific bacterial adhesions or some other viral infections (Wang, Sanders, & Baker, 2011). The in vitro activity of a dendrimeric heparin-binding peptide against human papillomavirused (HPVs) was evaluated and indicated that the peptide dendrimer was a potent inhibitor of genital HPVs at concentrations between 2.8 and 4.2 μg/ml with no cytotoxicity (Manuela et al., 2010).

For synthesis of the anti-HIV glycodendrimer, we recently prepared a third generation spherical polylysine dendrimer with sulfated oligosaccharides, and the dendrimer was found to have anti-HIV activity as high as that of ddC, a clinical HIV drug, and low cytotoxicity in concentrations higher than CC_{50} = 1000 μg/ml (Han et al., 2010). We also reported the development of a membrane filter with influenza A virus-adsorptive functionality. A long alkyl chain with 12 sugar residues was introduced into curdlan sulfate, and the resulting alkyl curdlan sulfate was coated on a hydrophobic membrane filter by hydrophobic interaction of the long alkyl chain. The alkyl curdlan sulfate-coated membrane filter was found to decrease the concentration of influenza A virus to below 1/32. These results suggest that influenza A viruses were removed by adsorption between the negatively charged sulfate groups and the positively charged envelope glycoprotein of the viruses, and this fact made it possible to develop a new biomaterial with a bio-functional surface from hydrophobic materials (Tegshi, Han, Kanamoto, Nakashima, & Yoshida, 2011).

In this paper, we report the synthesis of a third generation amphiphilic lysine dendrimer from lysine stearylamide as a core and with hydrophilic sulfated oligosaccharide units at the terminal of the dendrimer. The purpose of introducing a long hydrophobic alkyl chain (stearyl group) into a dendrimer is to immobilize the sulfated oligosaccharide cluster on a hydrophobic surface by hydrophobic interaction and to develop a new biomedical material. We also describe a preliminary result, indicating that the sulfated cellobiose oligosaccharide dendrimer with a stearyl chain had potent anti-HIV activity.

2. Experimental

2.1. Materials

Benzotriazol-1-yloxytris(dimethylamino)phosphornium hexafluorophosphate (BOP) and *N*,*N*-diisopropyl ethylamine (DIEA) were purchased from Wako Pure Chemical Industry Co., Inc. Di-*tert*-butoxycarbonyl lysine (di-boc lysine) was prepared by protection of the two amino groups with *tert*-butoxycarbonyl chloride. The acetylglucose **1** and acetylcellobiose **2** units were synthesized from glucose and cellobiose by stepwise condensation with Z-protected aminohexanol and adipic acid according to the same procedure described in our previous paper (Han, Yoshida, & Uryu, 2007).

2.2. Measurements

The ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM 400 spectrometer at 400 MHz and 100 MHz, respectively, at 40 °C in D2O or DMSO-d_6 as a solvent and 4,4′-dimethyl-4-silapentane-1-sulfonate (DSS) as an internal standard. Infrared spectra were recorded by a Perkin Elmer Spectrum One FT-IR spectrometer using a KBr pellet method. MALDI TOF mass spectra were measured by a Bruker Ultraflex III instrument with a 337 nm nitrogen laser. A methanol solution of a mixture of 2,5-dihydroxybenzoic acid (5 mg/100 μl MeOH) and 5-methoxysalicilic acid (5 mg/100 μl MeOH) was used as the matrix. The sample solution (1 μl, 0.5 mg/1 μl of 0.1 M TFA) and the matrix solution were applied to the 24 × 16 well ground steel MALDI probe. The sample was dried by air evaporation and then the nitrogen laser was irradiated to each sample to obtain the corresponding mass spectrum.

2.3. Preparation of lysine stearylamide

Stearylamine (0.27 g, 1 mmol) was injected through a syringe into a mixture of di-boc lysine (0.55 g, 1.1 mmol) and DIEA (2.0 ml, 1.2 mmol) in anhydrous DMF (30 ml) under a nitrogen atmosphere. After the mixture was cooled to 0 °C, BOP (0.53 g, 1.2 mmol) was added and then the mixture was stirred for a further 24 h at room temperature. After the DMF was removed under reduced pressure, the residue was dissolved in ethyl acetate and then washed successively with 15% NaCl solution, 5% aqueous citric acid solution, 5% $NaHCO_3$ solution, and water several times. The ethyl acetate layer was dried over anhydrous Na_2SO_4 and then evaporated to give lysine stearylamide in 96% yield as a white crystal after purification by silica gel column chromatography.

2.4. Preparation of stearylamide lysine dendrimers ***SLD1***, ***SLD2***, and ***SLD3***

Lysine stearylamide (0.57 g, 0.95 mmol) was stirred in a mixture solution of TFA (10 ml) and anhydrous dichloromethane (10 ml) at room temperature for 30 min. After the solvent was evaporated under reduced pressure, diethyl ether was added, and a precipitate appeared. The precipitate was collected by centrifugation and washed three times with anhydrous diethyl ether. The deprotected lysine stearylamide TFA salt was obtained after drying under reduced pressure and used immediately without purification for the next condensation. Condensation of the deprotected lysine stearylamide TFA salt and di-boc lysine with BOP and DIEA in DMF gave the first generation stearylamide lysine dendrimer **SLD1** in 89% yield after column chromatography purification. The second generation stearylamide lysine dendrimers **SLD2** and the third generation **SLD3** were obtained by the same procedures in 87% and 85% yields, respectively. The results are shown in Table 1.

2.5. Preparation of third generation acetylglucose and acetylcellobiose stearylamide lysine dendrimers ***AGSLD3*** and ***ACSLD3***

Acetycellobiose moiety (1.2 g, 1.45 mmol) and DIEA (5.0 ml, 3 mmol) were dissolved in anhydrous DMF (30 ml) under a nitrogen atmosphere and added to the deprotected **SLD3** TFA salt (0.7 g, 0.18 mmol). After the mixture was cooled to 0 °C, BOP (1.3 g, 3 mmol) was added and then the mixture was stirred for 24 h at room temperature. After the residue was extracted by chloroform, and the chloroform layer was washed successively with water several times and **ACSLD3** (1.7 g) was obtained after vacuum drying in a 60% yield after purification by reprecipitation using an ethyl acetate-hexane system several times, followed by centrifugation, and vacuum drying. The acetylglucose dendrimer **AGSLD3** was obtained in 52% yield from the acetylglucose moiety 1 and deprotected **SLD3** by the same procedures. The acetylcellobiose dendrimer **ACSLD3** was treated with 14% NaOMe in MeOH solution at room temperature to give deacetylated cellobiose dendrimer **CSLD3** in quantitative yield after dialysis and freeze-drying.

2.6. Sulfation of deacetylated cellobiose stearylamide lysine dendrimer ***CSLD3***

SO_3-pyridine complex (2.34 g, 14.9 mmol) was added to deacetylated dendrimer **CSLD3** (0.8 g, 0.16 mmol) solution in dry

Table 1
Synthesis of stearylamide lysine dendrimer each generations.[a]

Dendrimer	Starting material		Di-boc-lysine[b]	BOP reagent[c]	DIEA[d]	Yield[e]
	Type	(g, mmol)	mmol (g)	mmol (g)	ml (mmol)	g (%)
LS	Stearylamine	(0.27, 1)	0.55 (1.1)	0.53 (1.2)	0.2 (1.2)	0.57 (96)
SLD1	LS	(0.58, 0.97)	1.12 (2.1)	1.06 (2.4)	0.4 (2.4)	0.91 (89)
SLD2	**SLD1**	(0.91, 0.86)	1.90 (3.6)	1.60 (3.6)	0.6 (3.6)	0.68 (87)
SLD3	**SLD2**	(1.64, 0.83)	3.65 (6.9)	3.10 (7.0)	1.2 (7.2)	2.68 (85)

[a] The reaction was performed in DMF for 24 h at room temperature.
[b] *N*,*N*′-Bis(tert-butyloxycarbon-yl)-L-lysine dicyclohexylamine salt (Mw = 527.4).
[c] Benzotriazol-1-yloxytris(dimethylamino)phosphornium hexafluorophosphate (Mw = 442.28).
[d] Diisopropyl ethylamine (Mw = 129.24).
[e] Based on the starting one generation younger dendrimer.

DMSO (30 ml), and then the mixture was stirred at 38 °C for 3 h. After the mixture was cooled to room temperature, aqueous NaOH solution (10%) was added to neutralize the solution. The neutralized solution was dialyzed against deionized water for 1 d. The dialyzate was freeze-dried to give sulfated cellobiose stearylamide lysine dendrimer **SCSLD3** in 71% (1.1 g) yield.

2.7. Preliminary anti-HIV test

Anti-HIV activity of **SCSLD3** was examined by the method described previously (Han et al., 2010).

3. Results and discussion

3.1. Synthesis of third generation stearylamide polylysine dendrimer *SLD3*

A glycodendrimer with a long alkyl chain (amphiphilic glycodendrimer) is expected to exhibit biological activities originating from clustered or multivalent oligosaccharides. The long alkyl chain may be immobilized on the hydrophobic surface by hydrophobic interaction (Tegshi et al., 2011). In this work, we constructed a new amphiphilic lysine dendrimer with sugar units from stearylamide as a starting core. Lysine stearylamide was prepared by the condensation of stearylamine with carboxylic acid of di-boc lysine using BOP and DIEA reagents in DMF and subsequent deprotection of the boc groups. Using the same procedure described in our recent our paper (Han et al., 2007), a third generation stearylamide polylysine dendrimer was prepared from the core lysine stearylamide by the stepwise condensation using BOP and DIEA reagents and deprotection of boc groups of di-boc lysine moieties using 2 N TFA to recover the amino groups shown in Scheme 1 and Table 1. A third generation stearylamide lysine dendrimer **SLD3** was obtained in relatively good yield. Each generation of dendrimers was purified by precipitation.

Fig. 1 shows the ^{13}C NMR spectrum of the third generation stearyl amide lysine dendrimer **SLD3** with boc amino protective groups. A lysine carbonyl signal with several peaks appeared around 172 ppm, and a lysine α carbon signal produced two peaks at 53.5 and 52.5 ppm due to surface lysine and inner lysine residues, respectively, because the neighboring carbon signal of boc-protected amino groups shifted the higher magnetic field by 1 ppm. Boc-protected groups were observed at 154, 75, and 31 ppm due to signals of carbonyl, quaternary carbons, and three methyl groups, respectively. Stearyl and lysine side chain carbons appeared between 41 and 17 ppm as complex and overlapped signals, and the stearyl methyl signal appeared at 5 ppm. Fig. 2 shows the MALDI TOF mass profile of **SLD3**, Fig. 2 in which a signal due to (M+Na) at m/z = 3814.5 appeared, indicating that the fully substituted and monodispersed **SLD3** was successfully prepared.

3.2. Synthesis of third generation acetylglucose and acetylcellobiose stearylamide lysine dendrimer *AGSLD3* and *ACSLD3*

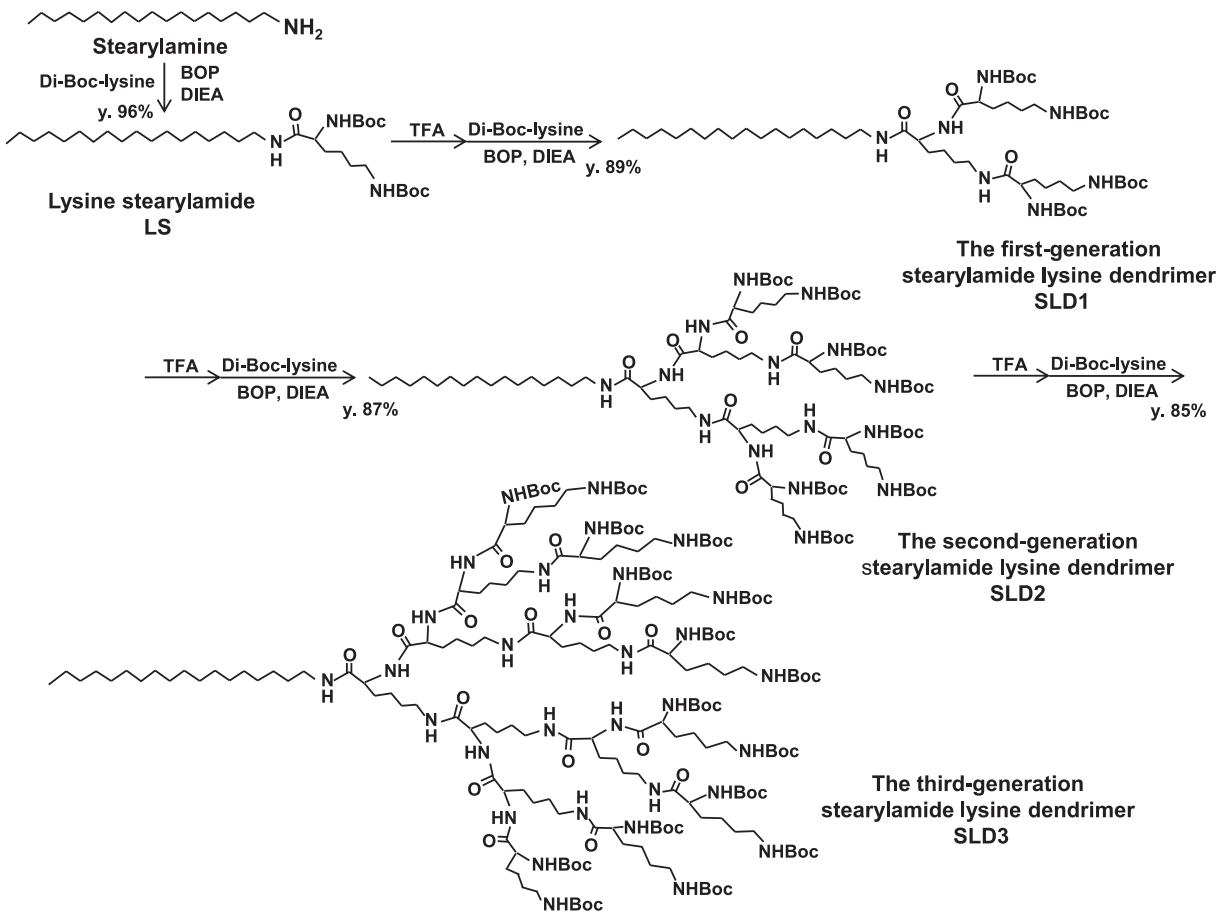
After removal of boc-protected groups from the third generation stearylamide polylysine dendrimer to recover amino groups, an acetylcellobiose unit with a C12 spacer at the sugar reducing end with carboxylic acid at the end of the spacer was condensed using BOP and DIEA in DMF to give a third generation acetylcellobiose stearylamide lysine dendrimer (**ACSLD3**) in 60% yield as expressed in Scheme 2.

By the same method, the third generation acetylglucose stearylamide lysine dendrimer **AGSLD3** was obtained in 52% yield.

The structures of the resulting acetylglucose and cellobiose dendrimers **AGSLD3** and **ACSLD3** were analyzed by ^{13}C NMR measurement as shown in Fig. 3, in which (A) acetylglucose **AGSLD3** and (B) acetylcellobiose dendrimers **ACSLD3** in DMFO-d_6 as the solvent at 40 °C, respectively, gave complex spectra. Acetyl and lysine carbonyl carbons appeared at 171 ppm as several peaks, and methyl signals of acetyl and stearyl groups were assigned to 22 and 15 ppm, respectively, in both spectra 2A and 2B. Carbon signals due to the sugar moiety appeared between 64 and 110 ppm, in which the C1′ and C1″ and C6′ and C6″ signals due to the cellobiose moiety (spectrum 2B) were observed at 110 ppm and 64 ppm as two singlet signals, respectively. The α-methylene carbon signal of lysine side chains was assigned to 54 ppm as broad and small peaks. Methylene signals due to stearyl, lysine side chain, and spacer moieties were observed between 41 and 22 ppm as overlapped signals. These spectra data indicate that acetylglucose and cellobiose dendrimers **AGSLD3** and **ACSLD3** had homogeneous structures because the C1 signals due to the sugar units in Fig. 3 appeared at 99 ppm as one singlet signal. Several impurity signals (x) appeared in Figs. 1 and 3A, probably due to spacer portions without sugar units that still contaminated after purification by column chromatography.

Fig. 4 shows the MALDI TOF mass profiles of glucose-connected **AGSLD3** and cellobiose-connected **ACSLD3**, respectively. Several signals appeared in the both spectra, probably because the ionization of sugar units in the glycodendrimers was difficult in the MALDI method. However, the signals near the calculated molecular weights appeared in the spectra. Table 2 shows the calculated and found molecular weights of **AGSLD3** and **ACSLD3** by measuring MALDI TOF mass, respectively.

The calculated molecular weights of **AGSLD3** $C_{524}H_{843}N_{47}O_{207}$ and **ACSLD3** $C_{716}H_{1099}N_{47}O_{335}$ were 11,113.49 and 15,725.50, respectively, and the degree of full substitutions is 16. Several peaks in the MALDI TOF mass profiles (Fig. 4) were observed due to each substitution of sugar units as shown in Table 2, suggesting that the number of sugar units of **AGSLD3** and **ACSLD3** was not very high because of the crowding of the surface amino groups due to the higher generations of the hyperbranched structure. The main degrees of substitutions of sugar units were 7–10 for **AGSLD3** and 6–8 for **ACSLD3**, respectively. The substitution should increase with



Scheme 1. Synthesis of the third generation stearylamide lysine dendrimer **SLD3** from lysine stearylamide as a core.

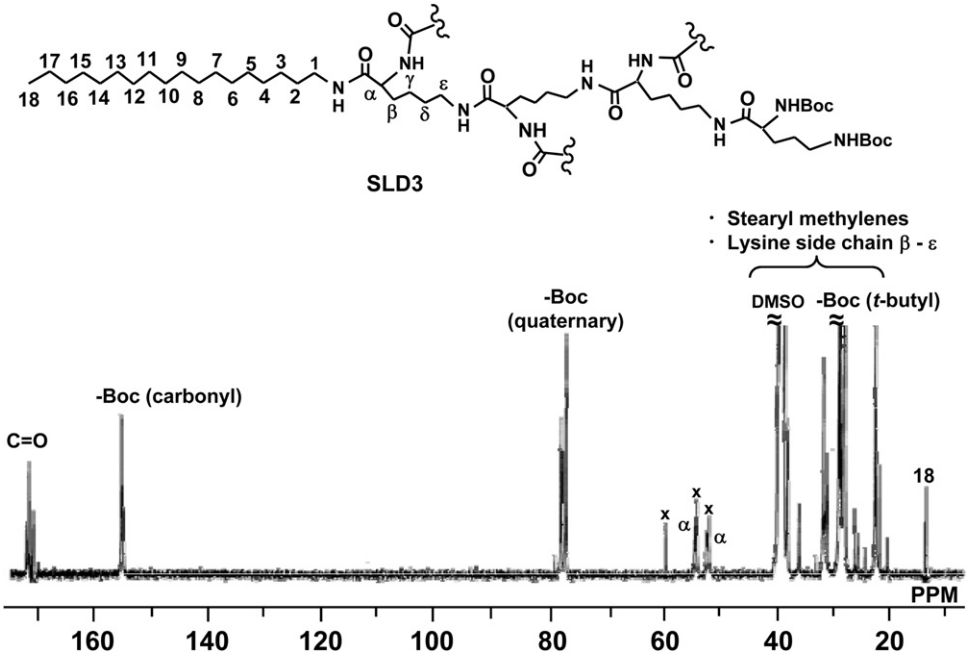
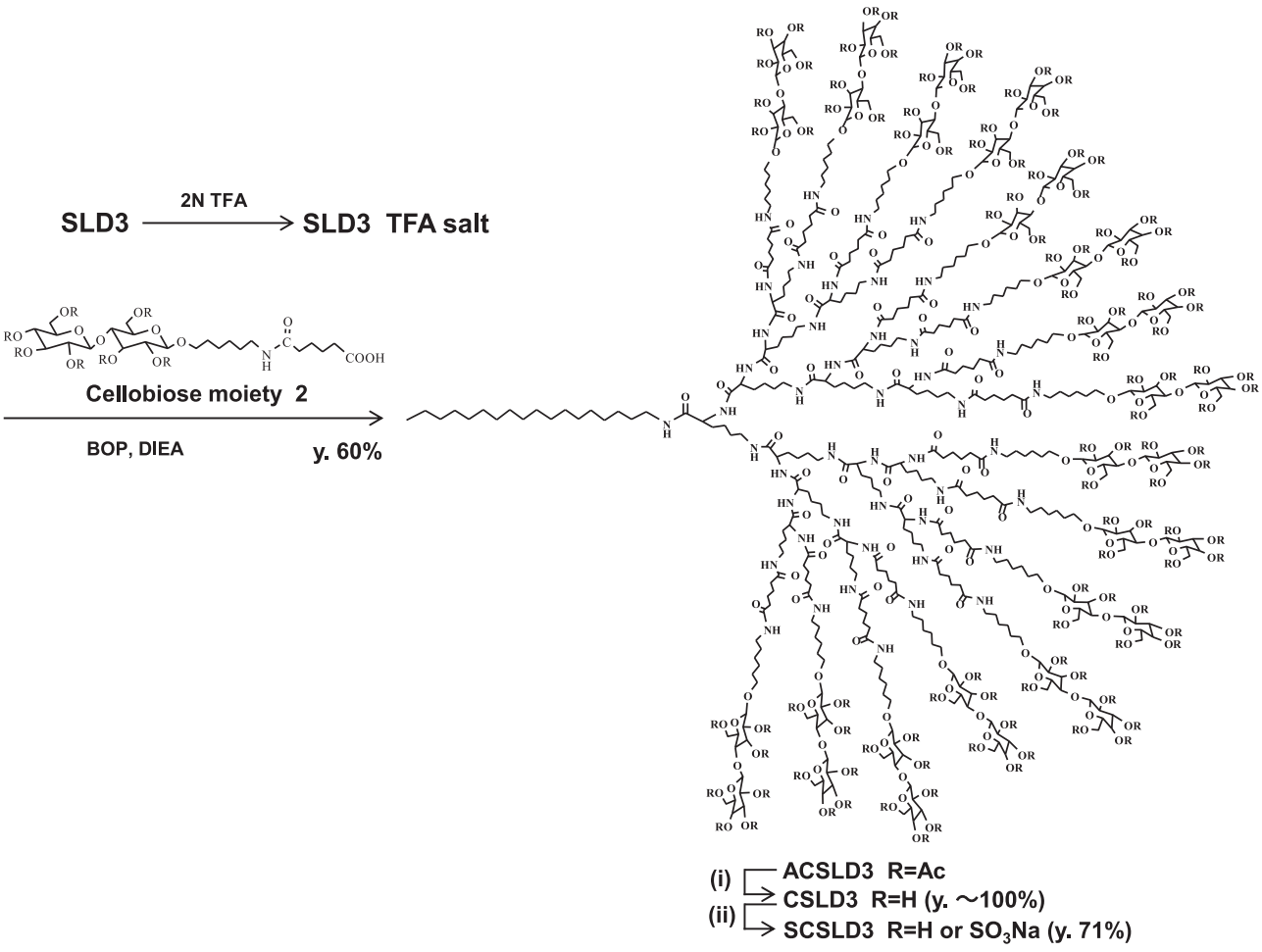


Fig. 1. ^{13}C NMR spectrum of the third generation stearylamide lysine dendrimer **SLD3** in DMSO-d_6 at 40 °C. The small and broad signals at 53.5 and 52.5 ppm were assigned to carbons of neighboring to Bocgroup and of inner lysine, respectively.



Scheme 2. Synthesis of the third generation sulfated cellobiose stearylamide lysine dendrimer **SCSLD3**. Reaction conditions: (i) 14% NaOMe in MeOH solution, (ii) SO_3-Py in DMSO, 38 °C.

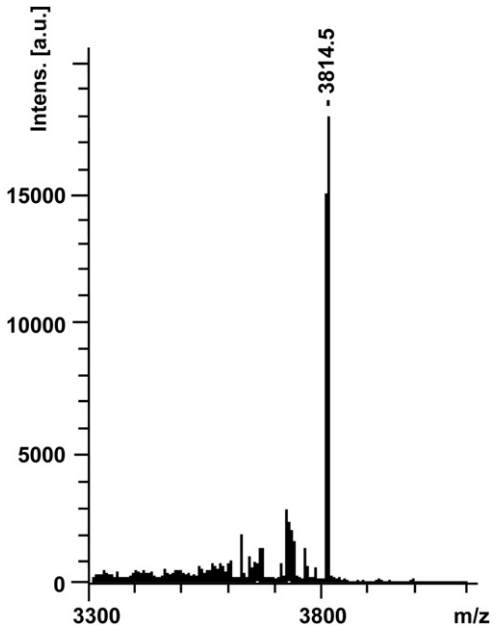


Fig. 2. MALDI TOF mass profile of the third generation stearylamide lysine dendrimer **SLD3**, which molecular weight was completely identified (Mw = 3791 + Na = 3814.5) with the calculated one.

Table 2
Calculated and MALDI TOF mass found molecular weights of acetylated glucose and cellobiose dendrimers **ACSLD3** and **AGSLD3**.

Sample	Molecular weight		
	Calculated[a]	Found[b]	DS[c]
ACSLD3	11,496	11,475	11
	10,650	10,655	10
	9808	9808	9
	8958	8966	8
	8112	8120	7
	7266	7275	6
	6420	6570	5
AGSLD3	8885	8876	12
	8328	8358	11
	7771	7841	10
	7210	7282	9
	6752	6725	8
	6197	6167	7
	5631	5651	6

[a] The calculated molecular weight by Chem Draw Ultra Software.
[b] The found molecular weight was determined by MALDI TOF mass measurement.
[c] Degree of substitution of acetylated sugar unit.

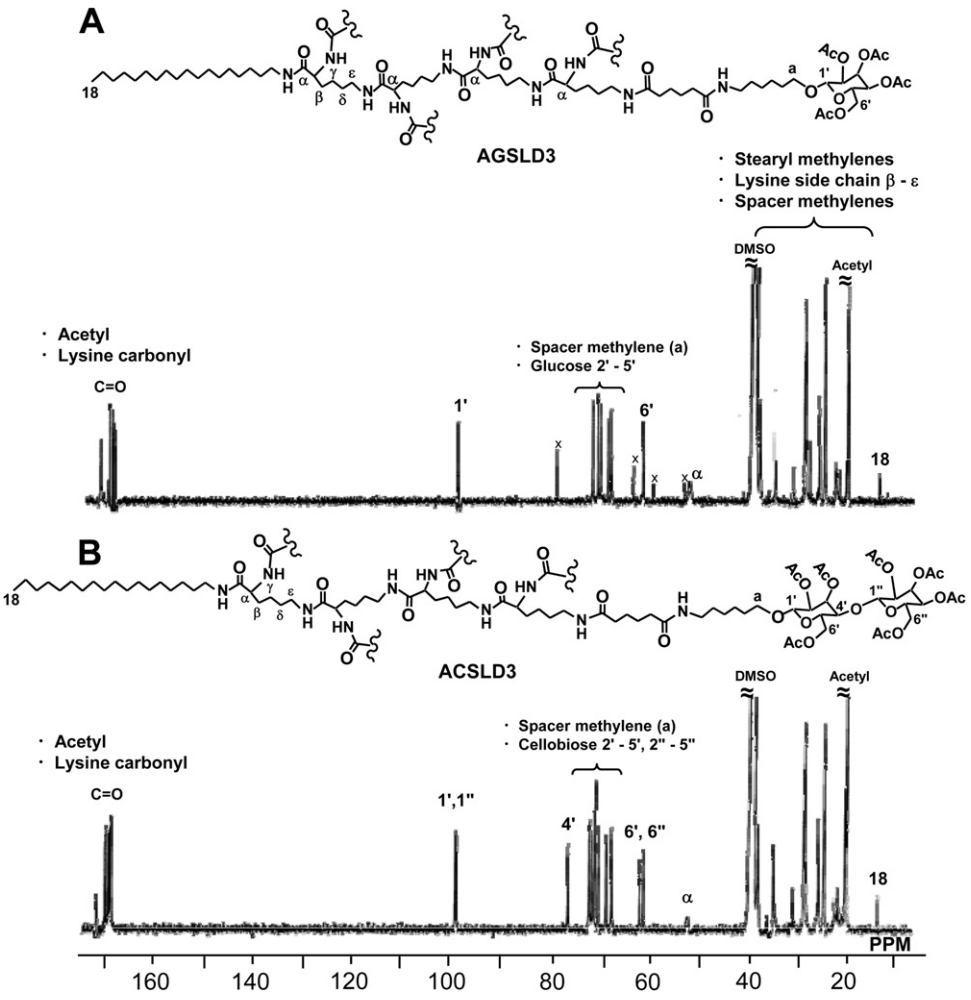


Fig. 3. ^{13}C NMR spectra of the third generation (A) acetylglucose and (B) acetylcellobiose stearylamide lysine dendrimers, **AGSLD3** and **ACSLD3**, in DMSO-d_6 at 40 °C.

increasing molar ratios of the sugar moieties as reported by us (Han et al., 2010).

3.3. *Preliminary sulfation and anti-HIV activity of cellobiose dendrimer **CSLD3***

After deacetylation of acetylcellobiose dendrimer **ACSLD3** with NaOMe to recover hydroxyl groups, OH-free cellobiose dendrimer **CSLD3** was sulfated with sulfur trioxide–pyridine complex to give the sulfated cellobiose dendrimer **SCSLD3** with a sulfur concentration of 15.11% in 71% yield. Fig. 5 shows the FT-IR spectra of (A) acetylcellobiose dendrimer **ACSLD3**, (B) deacetylated cellobiose dendrimer **CSLD3**, and sulfated cellobiose dendrimer **SCSLD3**, respectively. After deacetylation, the acetyl carbonyl signal at 1757 cm^{-1} disappeared and broad absorption due to the hydroxyl groups appeared around 3300 cm^{-1} in spectrum 4B. In the spectrum 4C of the sulfated cellobiose dendrimer **SCSLD3**, large adsorption due to an SO_3 group was observed at 1245 cm^{-1} and the shape of the hydroxyl group around 3300 cm^{-1} was changed by sulfation of the hydroxyl groups. Absorption of stearyl long alkyl chain was still observed clearly at 2940 cm^{-1} as a large signal.

Table 3 shows the preliminary examination of anti-HIV activity of the sulfated cellobiose dendrimer **SCSLD3** compared to that of standard sulfated polysaccharides and HIV drugs.

Table 3
Anti-HIV activity of sulfated cellobiose dendrimer **SCSLD3**.

	Sample[a]	$\bar{M}_n$[b] ($\times 10^3$)	$[\alpha]_D^{25}$[c] (deg)	Elemental analysis			EC_{50}[d] (μg/ml)	CC_{50}[e] (μg/ml)
				C	H	S		
1	**SCSLD3**			41.83	6.61	15.11	6.7	>1000
2	Dextran sulfate	8.5	+92.1			18.4	1.10	315
3	Curdlan sulfate	79.0	+3.0			14.1	0.34	606
4	ddC (μM)						3.27	1890
5	AZT (μM)						0.016	255

[a] Standard dextran (H-39) and curdlan sulfates were used. ddC, dideoxycytidine; AZT, azidothymidine.
[b] Determined by GPC.
[c] Measured in H2O (c1).
[d] Anti-HIV activity denoted by 50% inhibitory concentration of virus replication.
[e] 50% cytotoxic concentration on MT-4 cell.

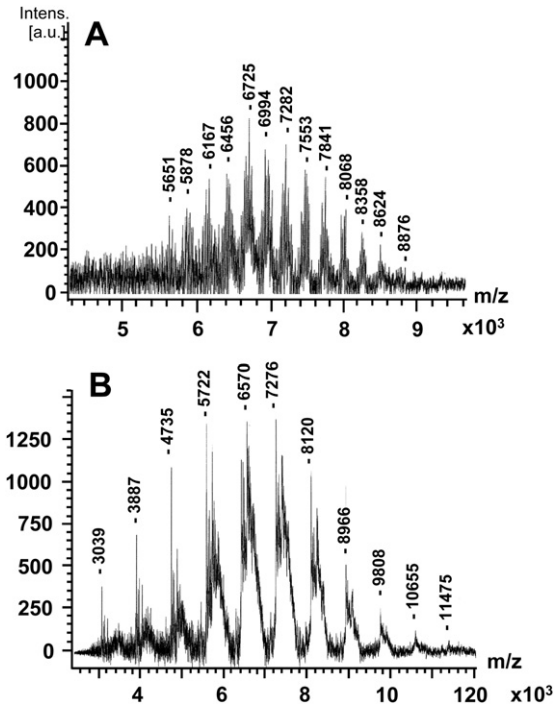


Fig. 4. MALDI TOF mass profiles of the third generation (A) acetylglucose and (B) acetylcellobiose stearylamide lysine dendrimers, **ACSLD3** and **AGSLD3**.

Sulfated cellobiose dendrimer **SCSLD3** was found to have anti-HIV activity at a 50% effective concentration (EC_{50}) of 6.7 μg/ml and low cytotoxicity at a 50% cytotoxic concentration of more than CC_{50} = 1000 μg/ml. This preliminary result suggests that the sulfated cellobiose dendrimer **SCSLD3** gave relatively high anti-HIV activity due to the cluster or multivalent effect because low molecular weight oligosaccharides have little or no anti-HIV activity (Lundquist & Toone, 2002). In addition, it was reported that the role of the long alkyl chain at the reducing end of sulfated oligosaccharides is to interact with the HIV lipid bilayer and then to destroy it similarly to surface active reagents having a hydrophilic functional group and hydrophobic long alkyl chain (Katsuraya et al., 1994).

Previously, we synthesized a spherical type sulfated glycodendrimer, which anti-HIV activity was almost the same as that of the hyper-branched type sulfated glycodendrimer synthesized this work. Both sulfated glycodendrimers had low cytotoxicity (CC_{50} > 1000 μg/ml). These results suggest that the cluster effect and compact structure were important factor in the high biological activities and low cytotoxicity. In addition, the amphiphilic glycodendrimer **SCSLD3** synthesized here should make micelle in water and the micelle formation is possible to give high anti-HIV activity and low cytotoxicity. The effects of micelle formation of the sulfated glycodendrimers on the biological activities will be investigated.

In conclusion, a stearylamide polylysine dendrimer with sulfated cellobiose was synthesized with lysine stearylamide as a core by stepwise condensation and deprotection of di-boc lysine, followed by connection of sugar moieties. The structures of the resulting acetyl sugar dendrimer were analyzed by ^{13}C NMR and MALDI TOF mass measurements, indicating that the dendrimers had homogeneous structures; however, the degree of substitution of sugar units ranged from 7 to 10 due to the crowding of amino acids on the dendrimer surface. Preliminary examinations of anti-HIV activity gave high activity below EC_{50} = 6.4 μg/ml and low cytotoxicity more than CC_{50} = 1000 μg/ml. Synthesis of fully oligosaccharide-substituted alkyl dendrimers of each generation and the relationship between the length of oligosaccharides and biological activities will be elucidated by NMR and SPR. In addition, development of biomedical materials, for example, a membrane filter is under investigated by immobilizing the long alkyl chain of sulfated glycodendrimers to a hydrophobic surface, and by the appearance of functionality originating from oligosaccharides is under investigation.

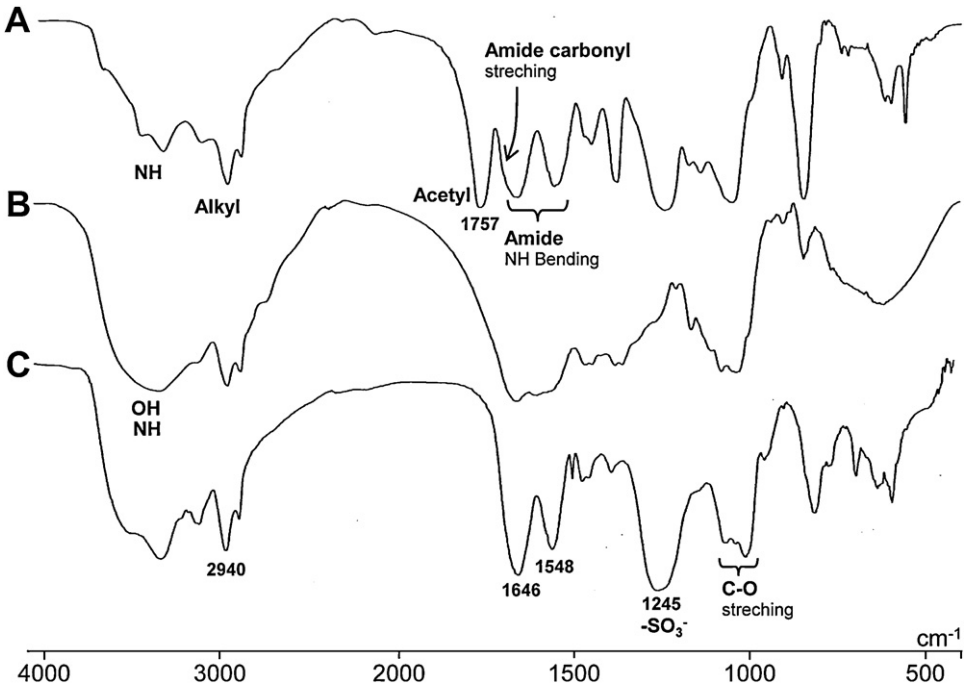


Fig. 5. FT-IR spectra of the third generation (A) acetylcellobiose stearylamide lysine dendrimer **ACSLD3**, (B) cellobiose stearylamide lysine dendrimer **CSLD3**, and (C) sulfated cellobiose stearylamide lysine dendrimer **SCSLD3**.

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