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Synthesis of new spherical and hemispherical oligosaccharides with polylysine core scaffold

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Abstract

A new spherical polylysine dendrimer generation 3 with acetyl cellobiose unit through a C6 spacer (TLACD3) was synthesized for the investigation of the structural effects on the specific biological activities such as anti-HIV and blood anticoagulant activities, which are our continuous research works. Tris(2-ethylamino)amine was used as an initiator of the core compound and was reacted with di-*t*-butoxycarbonyl lysine (di-boc-lysine) by the stepwise condensation according to the literature to give the polylysine dendrimer generation 3. Adipic acid monocellobiose ester as a model compound of oligosaccharide units was synthesized by the mono-esterification of adipic acid and 1-hydroxyl acetyl cellobiose. The adipic cellobiose was reacted with the deprotected polylysine dendrimer generation 3 to afford the spherical dendrimer with cellobiose unit (TLACD3) in the terminal. The hemispherical polylysine dendrimer generation 3 with acetylated cellobiose in the terminal through the C6 spacer (ALACD3) was also prepared from β-alanine methyl ester and di-boc-lysine by the same procedures as above. The spherical and hemispherical dendrimers have 24 and 8 terminal cellobiose units in each molecule, respectively, and the structure was characterized by NMR, IR, and MALDI TOF mass measurements. Although the hemispherical dendrimer ALACD3 had eight cellobiose units in a molecule, for the spherical dendrimer TLACD3, one cellobiose unit was eliminated partially from the molecule by the results of the MALDI TOF mass measurements.

Keywords: Spherical; Hemispherical; Dendrimer; Oligosaccharides; Polylysine; Cellobiose; Adipic acid

1. Introduction

As dendritic and hyper-branched oligosaccharides with polypeptide core scaffold (glycodendrimers) are expected to be a multivalent or cluster effect on sugar–protein interactions (Gillies & Fréchet, 2002; Kojima, Haba, Fukui, Kono, & Takagishi, 2003; Lis & Sharon, 1998; Newkowe, Moorefield, & Vögtle, 2003; Röckendorf & Lindhorst, 2001; Roy, 2003), influenza and AIDS vaccines with dendritic structures have been reported (Baigude, Katsuraya, Okuyama, & Uryu, 2004; Shao & Tam, 1995; Roy, Pon, Tropper, & Andersson, 1993; Röy, Zanini, Meunier, & Romanowska, 1993; Wang et al., 1991). We have investigated the synthesis of biological active polysaccharides having anti-tumor, anti-HIV, and blood anticoagulant activities and elucidated the relationship between the structures of polysaccharides and the biological activities (Hattori et al., 1998; Nakashima et al., 1987; Yoshida, Katayama, Iniue, & Uryu, 1992; Yoshida, Oda, & Uryu, 1994). Previously, we prepared curdlan sulfate by the sulfation of naturally occurring curdlan, which has a linear (1 → 3)-β-D-glucopyranosidic structure, and examined in vitro the biological activities, indicating that curdlan sulfate was found to be high anti-HIV and low blood anticoagulant activities (Kaneko et al., 1990; Yoshida et al., 1990; Yoshida et al., 1994). For sulfated oligosaccharides binding to polyacrylate main chain, (Yoshida et al., 1995) the distance between branched

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oligosaccharides in the side chain was important for high anti-HIV and blood anticoagulant activities as well as low cytotoxicity, suggesting that the multivalent or cluster effect should be improved for the biological activities of poly- and oligosaccharides, respectively. Therefore, the dendritic and hyper-branched structures play an important role for the biological activities (Yoshida et al., 1999).

The final purpose of our researches is to elucidate the structure–biological activity relationship on anti-HIV and blood anticoagulant activities of biomacromolecules. In this paper, we wish to report a synthesis of new types of the spherical and hemispherical polylysine dendrimers generation 3 with oligosaccharides through a C6 spacer. The spherical and hemispherical dendrimers were synthesized by the stepwise condensation from tris(2-ethylamino)amine and β-alanine cores, respectively, with di-*t*-butoxycarbonyl lysine (di-boc-lysine) according to the literature and then the glycodendrimers were prepared by binding of cellobiose unit as a model oligosaccharide to the terminal amino groups of the polylysine dendrimers through adipic acid as a C6 spacer. The structure of the spherical and hemispherical polylysine dendrimers was determined by NMR, IR, and MALDI TOF mass spectrometric analyses.

2. Experimental

2.1. General

NMR spectra were recorded at 40 °C in DMSO-d_6 solution on a JEOL ECM-400 spectrometer by using a phase-sensitive mode and a field gradient probe. Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal standard at 0 ppm for ^{1}H and 0.015 ppm for ^{13}C spectra. Infrared spectra were taken on a Shimazu FT–IR 8300 spectrometer by a KBr method. MALDI TOF mass spectra were measured by a Bruker Ultraflex II instrument with a 337 nm nitrogen laser. Methanol solution of a mixture of 2,5-dihydroxybenzoic acid and 5-methoxysalicilic acid was used as a matrix. The MALDI TOF mass measurements were carried out with a mixture of the sample and the matrix solution. For the MALDI TOF mass measurement of the hemispherical polylysine cellobiose dendrimer generation 3 (LACD3) (**5**), the methanol solution of sodium trifluoroacetic acid (NaTFA) was added to the mixture solution.

2.2. Adipic monocellobiose ester (**11**)

To a mixed solution of 2,2′,3,3′,4′,6,6′-heptaacetylcellobiose (Wolform & Thompson, 1963) (1.75 g, 2.7 mmol), 4-dimethylaminopyridin (DMAP) (0.33 g, 2.7 mmol) and adipic acid (3.9 g, 27 mmol) in DMF (20 ml) were added gradually 1,3-dicyclohexylcarbodiimide (DCC) (0.56 g in 3 ml of pyridine) for 1 h at room temperature. The mixture was stirred for 24 h at room temperature (Wang, Sakairi, & Kuzuhara, 1991). After filtration, the filtrate was evaporated under reduced pressure and then chloroform was poured into the residue. The chloroform layer was washed with water several times to give adipic acid monocellobiose (**11**) (1.24 g) in 40% yield after evaporation of the solvent and purification by column chromatography on silica gel.

2.3. Tris amino lysine dendrimer generation 1 (TLD1) (**1**)

Tris(2-ethylamino)amine (0.15 ml, 1 mmol) was added through syringe to a mixed solution of di-boc-lysine (1.68 g, 3.2 mmol) and *N*,*N*′-diisopropylethylamine (DIEA) (0.6 ml, 3.3 mmol) in anhydrous DMF (15 ml) under nitrogen atmosphere. After the solution was cooled to 0 °C, benzotriazol-

Table 1
Synthesis of dendrimers[a]

	Starting material [g (mmol)]		Di-boc-lysine [g (mmol)]	BOP reagent [g (mmol)]	DIEA [mL (mmol)]	Yield [g (%)]
TLD1	TEA	0.15 (1)	1.7 (3.2)	1.6 (3.5)	1.5 (9.5)	0.7 (65)
TLD2	TLD1[b]	1.13 (1)	1.7 (3.2)	3.2 (7.2)	1.5 (9.5)	1.4 (58)
TLD3	TLD2[b]	0.31 (1)	0.8 (1.5)	1.3 (2.8)	0.9 (5.6)	0.6 (56)
ALD1	AME[b]	1.4 (10)	5.3 (10)	4.4 (10)	1.9 (11)	1.5 (69)
ALD2	ALD1[b]	1.9 (4)	4.5 (8.4)	3.7 (8.4)	1.9 (11)	2.5 (68)
ALD3	ALD2[b]	0.9 (1)	2.3 (4.2)	2.2 (4.8)	1.9 (11)	1.6 (89)

Abbreviations: DIEA, diisopropylethylamine; AME, β-alanine methylester; TEA, tris(2-ethylamino)amine; BOP, benzotriazol-1-yloxytris-(dimethylamino)-phosphonium-hexafluorophosphate.

[a] The reaction was carried out in DMF at room temperature for 24 h.

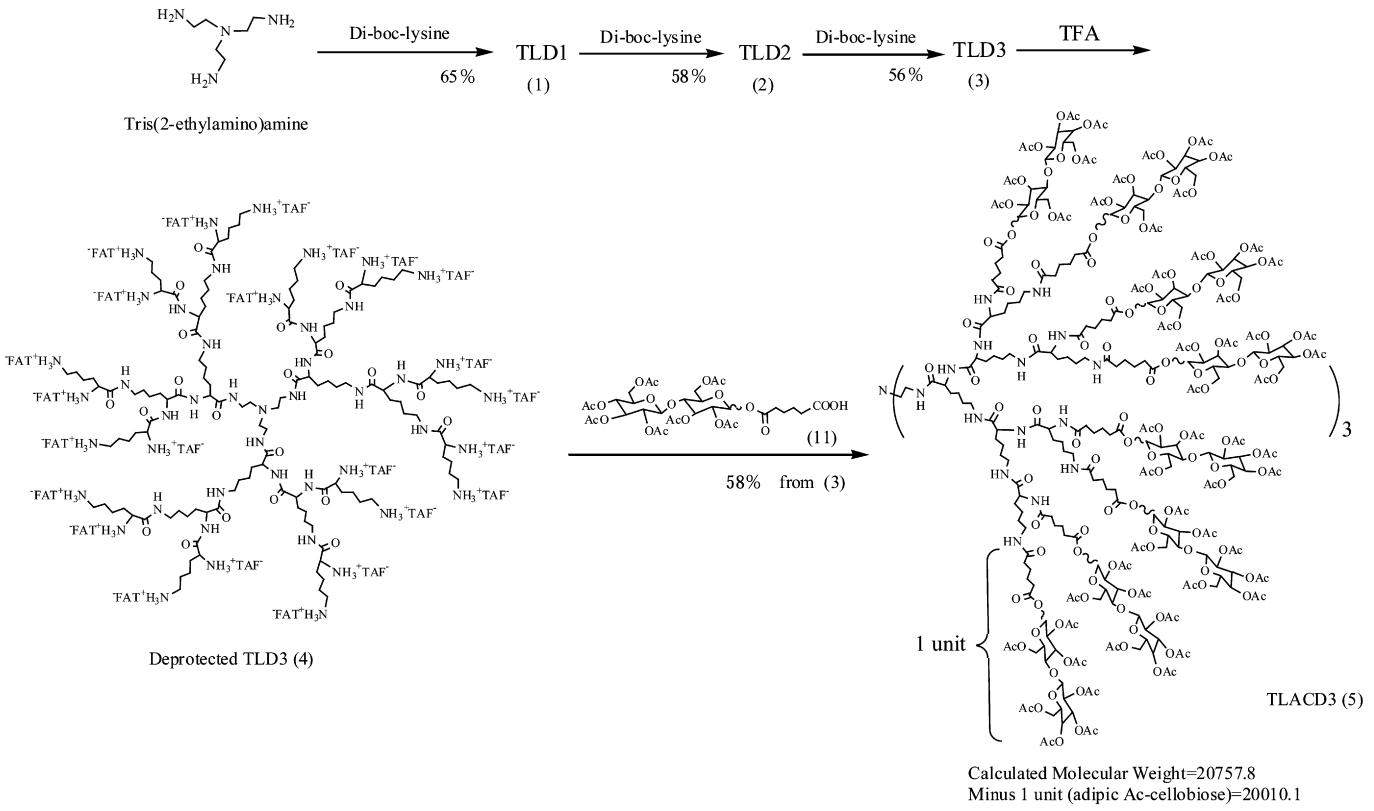
[b] TLDs and ALDs in the column of starting material were deprotected by TFA before the synthesis of the next generation of dendrimer.

Table 2
Synthesis of spherical and hemispherical dendrimers with acetyl cellobiose (**5**) and (**10**)[a]

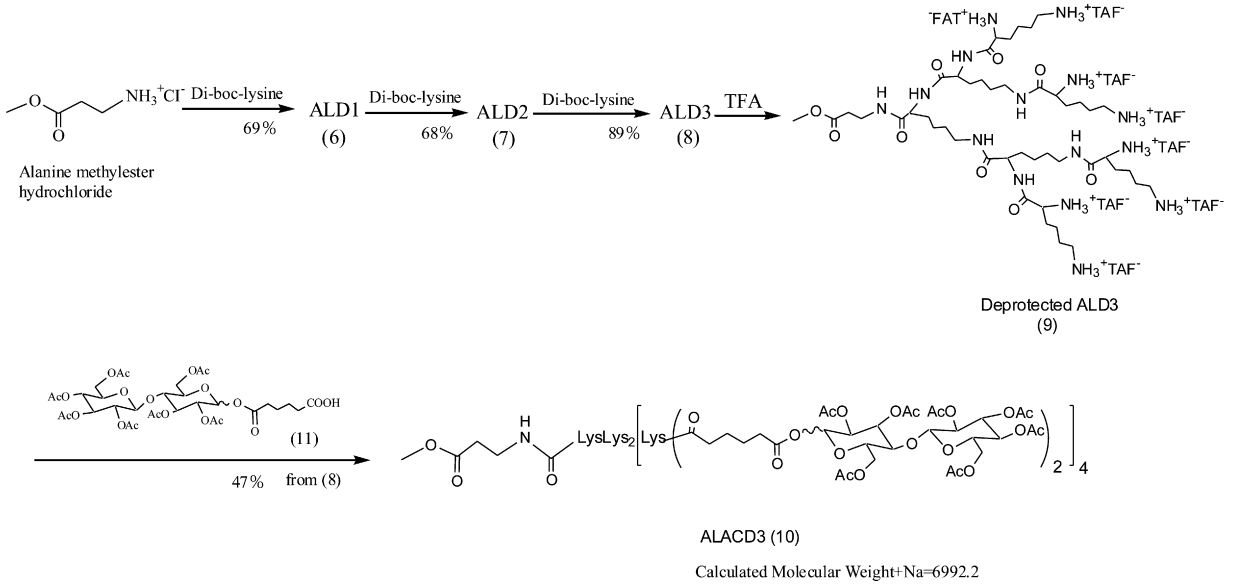
Starting material g (mmol)		AAC (**11**)	DIEA	BOP	Dendrimer yield	
					Product	g (%)
Deprotected TLD3 (**4**)	0.05 (0.25)	0.9 (1.2)	0.2 (1.6)	0.8 (1.8)	**5**	0.6 (58 from 3)
Deprotected ALD3 (**9**)	1.2 (1.6)	0.2 (1.6)	0.8 (1.8)	0.034 (0.2)	**10**	0.65 (47 from 8)

Abbreviations: AAC, adipic acetyl cellobiose; BOP, benzotriazol-1-yloxytris-(dimethylamino)-phosphonium-hexafluorophosphate; DIEA, diisopropylethylamine.

[a] The reaction was carried out in DMF (15 ml) for 24 h at room temperature. The BOP reagent was added at 0 °C.



Scheme 1. Synthesis of spherical cellobiose-polylysine dendrimer TLACD3 (**5**).



Scheme 2. Synthesis of hemispherical cellobiose-polylysine dendrimer ALACD3 (**10**).

1-yloxytris-(dimethylamino)-phosphonium-hexafluorophosphate (BOP) (1.45 g, 3.3 mmol) was added and then the solution was stirred at room temperature. The reaction was monitored by thin-layer chromatography using mixed solution of chloroform and methanol (5:1) as eluent. After 12 h, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed successively with 15% NaCl solution, 5% aqueous citric acid solution, 5% $NaHCO_3$ solution, and water. The solution was dried over anhydrous Na_2SO_4 and the product was purified by column chromatography over silica gel using the mixed eluent solution of ethyl acetate, hexane, and methanol in the proportion of 8:1:2. A white crystalline (0.74 g) (**1**) was obtained in 65% yield after drying under reduced pressure.

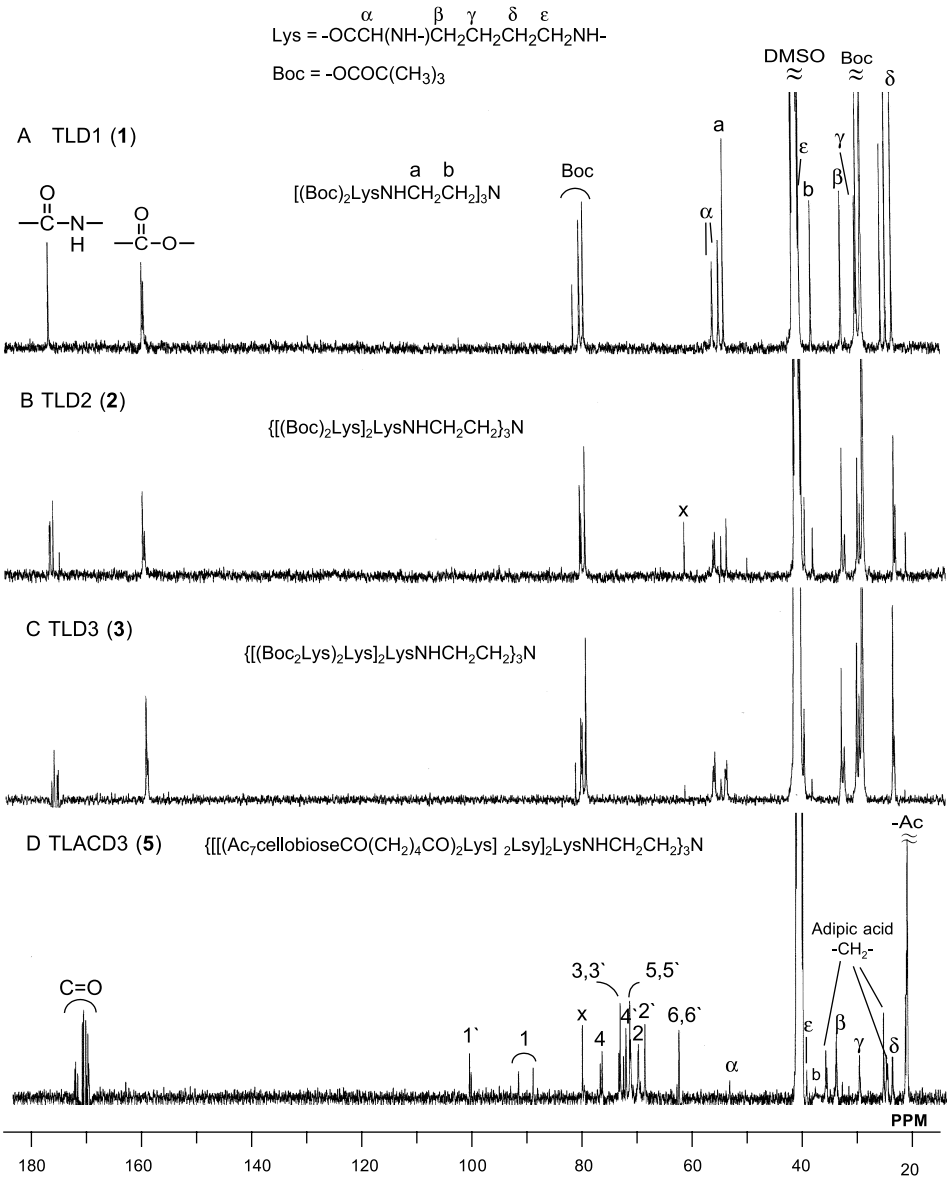


Fig. 1. 100 MHz ^{13}C-NMR spectra of spherical dendrimers: (A) TLD1 (**1**), (B) TLD2 (**2**), (C) TLD3 (**3**), and (D) spherical cellobiose-polylysine dendrimer TLACD3 (**5**) (DMSO-d_6, 40 °C).

*2.4. Deprotection of the boc group in TLD1 (**1**)*

TLD1 (**1**) (1.13 g, 1 mmol) was stirred in a mixed solution of TFA (20 ml) and anhydrous dichloromethane (20 ml) at room temperature for 30 min. After the solvent was evaporated under reduced pressure, diethyl ether was added and then a precipitate appeared. The precipitate was collected by centrifugation and washed three times with anhydrous diethyl ether. The deprotected TLD1 TFA salt was obtained after drying under reduced pressure and used immediately without purification for the next condensation.

*2.5. Preparation of TLD2 (**2**)*

The lysine dendrimer generation 2, TLD2 (**2**) was obtained in 58% yield from the deprotected TLD1 (**1**) obtained above and di-boc-lysine by the same procedure as described in "Tris amino lysine dendrimer generation 1 (TLD1) (**1**)."

*2.6. Preparation of TLD3 (**3**)*

After deprotection of the boc group in TLD2 (**2**) with TFA, TLD3 (**3**) was obtained in 56% yield from the deprotected TLD2 (**2**) TFA salt and di-boc-lysine by the same procedure as described in "Tris amino lysine dendrimer generation 1 (TLD1) (**1**)."

*2.7. Preparation of deprotected TLD3 (**4**)*

The deprotected TLD3 TFA salt (**4**) was obtained from TLD3 (**3**) (0.25 g, 0.05 mmol) treated with TFA (5 ml) in

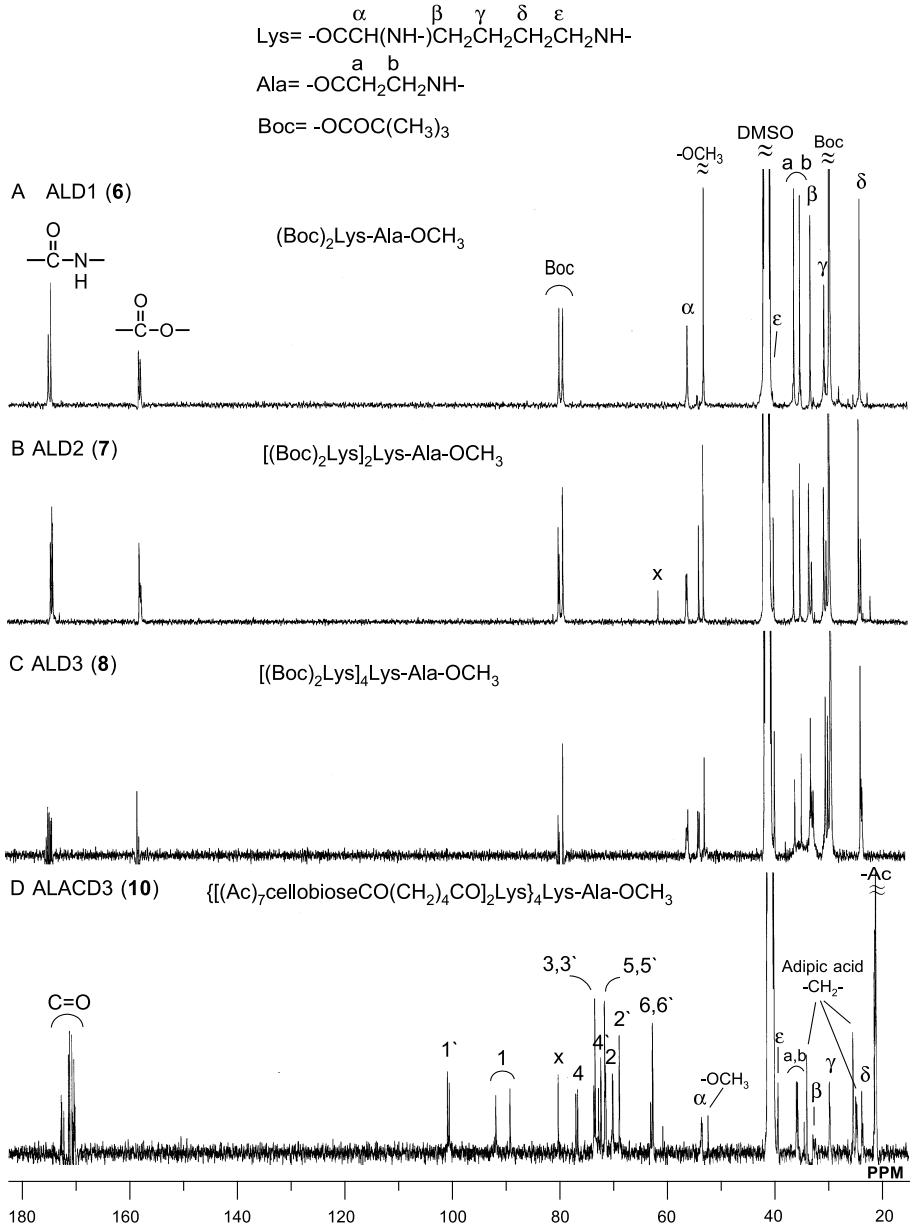


Fig. 2. 100 MHz ^{13}C-NMR spectra of hemispherical dendrimers: (A) ALD1 (**6**), (B) ALD2 (**7**), (C) ALD3 (**8**), and (D) hemispherical cellobiose-polylysine dendrimer ALACD2 (**10**) (DMSO-d_6, 40 °C).

anhydrous dichloromethane (5 ml) by the same procedure as described in "Deprotection of the boc group in TLD1 (**1**)" and then used immediately without purification for the next condensation.

*2.8. Preparation of the spherical polylysine dendrimer with cellobiose (TLACD3) (**5**)*

Deprotected TLD3 TFA salt (**4**) (0.05 g, 0.25 mmol), adipic monocellobiose ester (**11**) (0.9 g, 1.2 mmol), and DIEA (0.2 ml, 1.6 mmol) were dissolved in anhydrous DMF (15 ml) under nitrogen atmosphere. After the solution was cooled to 0 °C, BOP (0.8 g, 1.8 mmol) was added, followed by stirring for 24 h at room temperature. After work-up, the product was purified by column chromatography over silica gel using a mixture of ethyl acetate, hexane and methanol (5:1:0–5:1:1) as eluent. A white crystalline (0.6 g) TLACD3 (**5**) was obtained by vacuum drying in 58% yield from TLD3 (**3**).

The hemispherical polylysine dendrimer with cellobiose unit (**10**) was prepared from β-alanine methyl ester and di-boc-lysine (Baigude, Katsuraya, Okuyama, Tokunaga, & Uryu, 2003; Joonsig, Dongkyoon, Changhwan, Kwan, & Jongsang, 2000) by the same procedures as the synthesis of the spherical polylysine dendrimer TLACD3 (**5**).

The reaction conditions and yields of each step of the dendritic compounds are summarized in Tables 1 and 2.

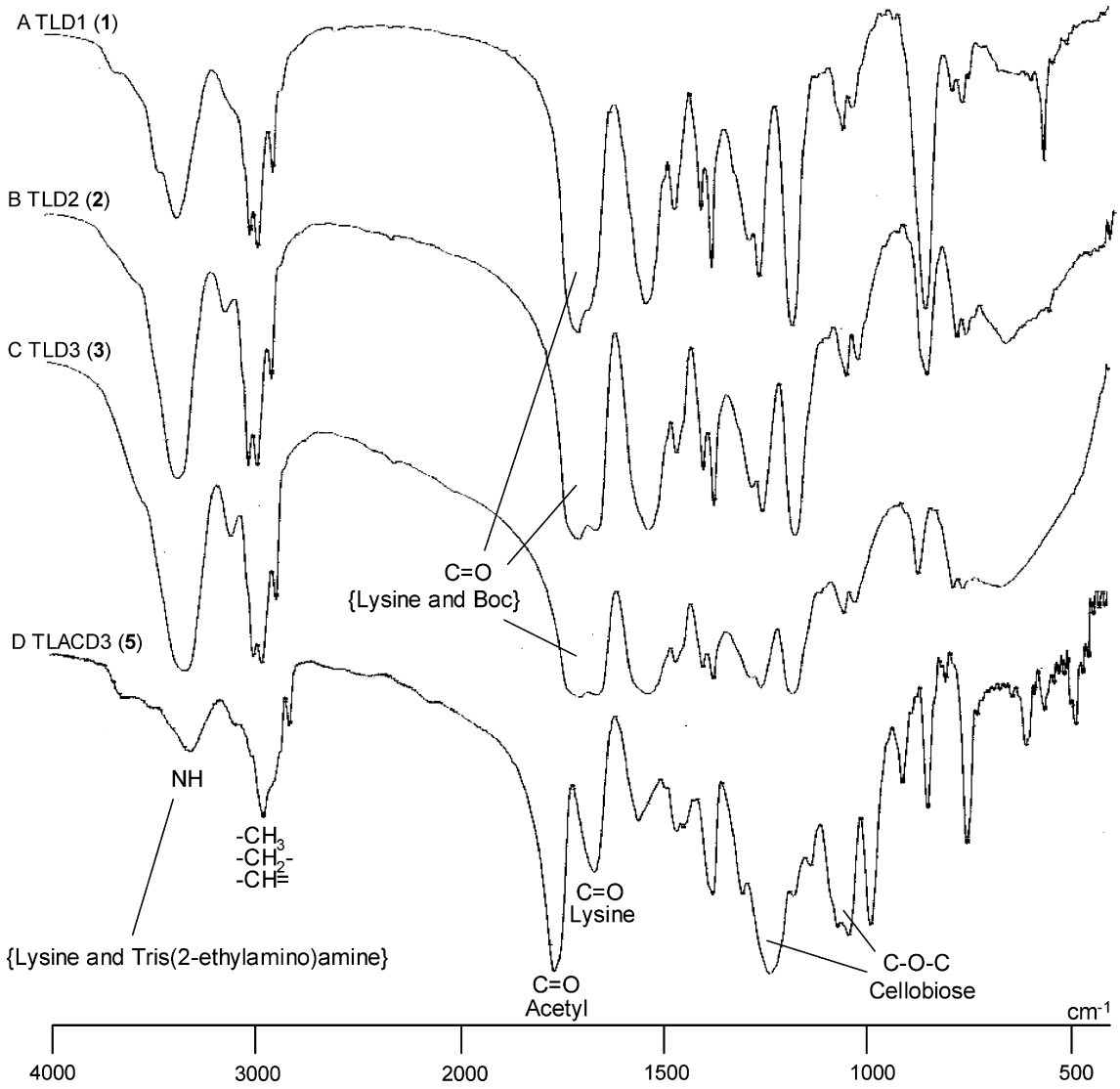


Fig. 3. FT–IR spectra of spherical dendrimers: (A) TLD1 (**1**), (B) TLD2 (**2**), (C) TLD3 (**3**), and (D) spherical cellobiose-polylysine dendrimer TLACD3 (**5**).

3. Results and discussion

*3.1. Synthesis of spherical and hemispherical dendrimers with cellobiose unit (**5**) and (**10**)*

For the increase of biological activities such as anti-tumor, anti-HIV, and blood anticoagulant activities of sulfated poly- and oligosaccharides, a cluster or multivalent effect of dendritic structures are expected (Yoshida, 2001). A new type of the spherical and hemispherical dendrimers with the cellobiose unit in the terminal was synthesized. In Scheme 1, the spherical polylysine dendrimer TLD3 (**3**) was prepared from tris(2-ethylamino)amine by the repeated condensation of di-boc-lysine according to the method of literature. After deprotection of the boc group in the protected TLD3 (**3**) by TFA, the deprotected TLD3 (**4**) was obtained and then used immediately for the next condensation with adipic monocellobiose ester (**11**), which was prepared by the mono-esterification of adipic acid with 1-hydroxyl acetylcellobiose in DMF by DCC (1,3-dicyclohexylcarbodiimide) in 40% yield after purification by column chromatography. The polylysine dendrimer generation 3 with trivalent core structure TLACD3 (**5**) was synthesized by the condensation of TLD3 (**4**) with adipic monocellobiose ester (**11**) using diisopropylamine (DIEA) and BOP reagent in 51% yields.

The deprotected hemispherical dendrimer (**9**) was also reacted with (**11**) by DIEA and BOP reagent to give the glycodendrimer ALACD3 (**10**) in 47% yield (Scheme 2). The hemispherical lysine dendrimer ALD3 (**8**) was obtained from β-alanine methyl ester with stepwise condensation and deprotection of the NH_2 protective group in di-boc-lysine. Table 1 summarizes the results of the stepwise condensation of di-boc-lysine and the reaction conditions for the spherical and hemispherical polylysine TLDs (**1**)–(**3**) and ALDs (**6**)–(**8**). The yield of each step was relatively high. Table 2 shows the results of the condensation of the deprotected TLD3 (**4**) and ALD3 (**9**)

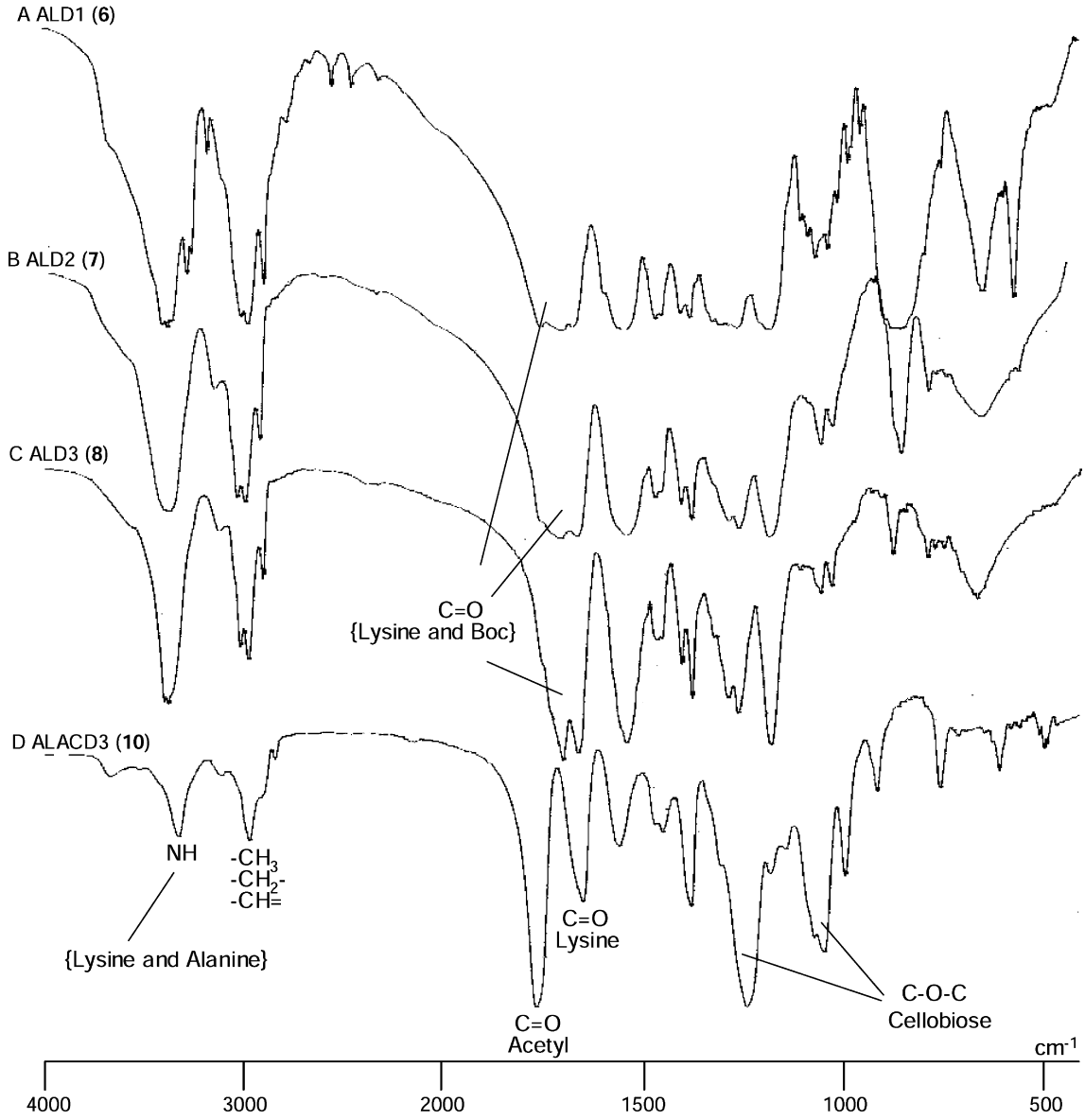


Fig. 4. FT–IR spectra of hemispherical dendrimers: (A) ALD1 (**6**), (B) ALD2 (**7**), (C) ALD3 (**8**), and (D) hemispherical cellobiose-polylysine dendrimer ALACD3 (**10**).

with the cellobiose unit (**11**), respectively. The spherical and hemispherical dendritic cellobiose (**5**) and (**10**) were obtained in moderate yields. The structural elucidation of (**5**) and (**10**) was examined by the MALDI TOF mass measurements. Their structures and molecular weights are described in the later part.

3.2. NMR and IR measurements

Fig. 1 shows the ^{13}C-NMR spectra of each generation of the spherical polylysines TLDs (**1**)–(**3**) and the spherical dendrimer with cellobiose unit TLACD3 (**5**), respectively. In Figs. 1A–C, the absorption of methylene signal in the lysine residues appeared in the range from 22 to 53 ppm. The amide and carbonyl groups due to the peptide bond and the boc protective groups were observed around 171 and 155 ppm, respectively. After deprotection of the boc group and the condensation of adipic cellobiose (**11**), the spherical cellobiose TLACD3 (**5**) were obtained and the ^{13}C-NMR is presented in Fig. 1D, in which the cellobiose residues were observed between 62 and 101 ppm, and the amide carbonyl and methylene signals due to lysine residues appeared around 172 and in the range of 23 and 53 ppm, respectively. The signals due to the protective boc carbonyl groups disappeared.

Fig. 2 shows the ^{13}C-NMR spectra of each generation of the hemispherical polylysines ALDs (**6**)–(**8**) and the hemispherical cellobiose ALACD3 (**10**). In Fig. 2D, the α-CH_2 signal due to lysine side chains also appeared at 53 ppm and the carbon signals due to cellobiose and polylysine were observed as several absorptions in the ranges 62–101 and 20–40 ppm, respectively.

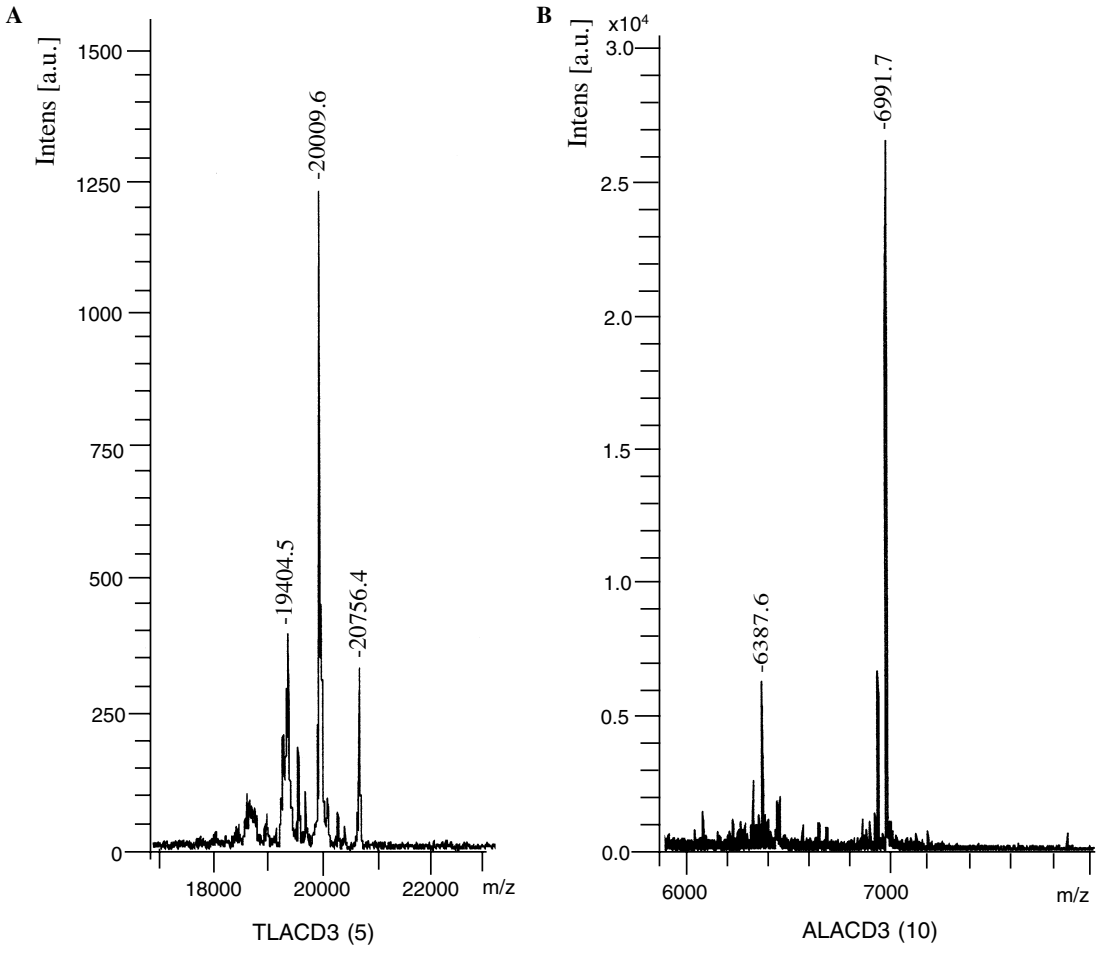


Fig. 5. MALDI-TOF mass spectra of (A) spherical cellobiose-polylysine dendrimer TLACD3 (**5**) and (B) hemispherical cellobiose-polylysine dendrimer ALACD3 (**10**).

The assignments of the ^{13}C signals were determined by the measurements of the filed gradient DQF COSY, HMQC, and HMBC spectra. From the results of the integral values of the ^{1}H signals (data does not shown) and the assignments of the ^{13}C signals, the cellobiose unit was substituted completely and the purity of the spherical and hemispherical cellobiose (**5**) and (**10**) was high.

Figs. 3 and 4 show the IR spectra of the spherical polylysines (**1**)–(**3**), (**5**) and the hemispherical polylysines (**6**)–(**8**), (**10**), which are corresponded to the NMR spectra in Figs. 1 and 2, respectively. In Figs. 3D and 4D, the stretching vibration of NH and amido carbonyl absorptions appeared around 3250 and 1650 cm^{-1}, respectively. The carbonyl groups due to ester and acetyl groups were absorbed around 1760 cm^{-1}. The hydroxyl group and ether signals due to the stretching vibration in the sugar units were observed as several absorptions between 1000 and 1300 cm^{-1}. The structures of the spherical and hemispherical polylysines with cellobiose unit (**5**) and (**10**) were supported by the results of NMR and IR measurements. However, as described in the next section, it was proved the disconnection of one cellobiose unit in the spherical cellobiose TLACD3 (**5**) by the measurement of the MALDI TOF mass spectrometry.

3.3. MALDI TOF mass measurements

Figs. 5A and B shows the MALDI TOF mass spectra of the spherical TLACD3 (**5**) and the hemispherical ALACD3 (**10**), respectively. In Fig. 5A, the signal due to the molecular weight at $m/z = 20756.4$ appeared.

The calculated molecular weight of (**5**) is 20757.8. The observed molecular weight agreed with the calculated one. However, the signal at $m/z = 20009.6$ had the highest intensity. The reason of the mass difference from 20757.8 might be attributed to remove one adipic acetylcellobiose unit (MW = 747.67) from (**5**). In Fig. 5B, for the measurements of the MALDI TOF mass spectrum of the hemispherical ALACD3 (**10**), NaTFA was added to the matrix solution. In the spectrum, the molecular weight of (**10**) increased 23 Da due to sodium. The hemispherical ALACD3 (**10**) gave the sharp and single signal at the molecular weight of $m/z = 6991.7$ (calculated M + Na = 6992.5), suggesting that the hemispherical (**10**) was successfully prepared with a fully substituted cellobiose unit.

From the results of the MALDI TOF mass spectra, the spherical TLACD3 (**5**) and the hemispherical ALACD3 (**10**) were found to be constituted of 24 and 8 sugar units in

the terminal. However, the spherical TLACD3 (**5**) with 23 sugar unit in the terminal was the main product.

As mentioned above, the monodispersed spherical and hemispherical polylysine dendrimers with cellobiose unit (**5**) and (**10**) were prepared from tris(2-ethylamino)amine and β-alanine methyl ester as core compounds, respectively, by the stepwise condensation of di-boc-lysine and then adipinic cellobiose unit. The structure of the spherical and hemispherical cellobioses was determined by NMR, IR, and MALDI TOF mass measurements. For the spherical cellobiose (**5**), it became apparent the partial elimination of one cellobiose unit by the determination of the MALDI TOF mass measurements. Sulfation of the spherical TLACD3 (**5**) and hemispherical ALACD3 (**10**) and their biological activities such as anti-HIV and blood anticoagulant activities are currently undergoing.

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