Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 69 (2007) 436–444
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Synthesis of a new polylysine-dendritic oligosaccharide with alkyl spacer having peptide linkage

Shuqin Han [a], Takashi Yoshida [a,*], Toshiyuki Uryu [b]

[a] *Applied Chemistry Department, Kitami Institute of Technology, 165 Koen-cho, Kitami 090-8507, Japan*
[b] *Department of Environment and Material Engineering, Teikyo University of Science and Technology, 2525 Yatsusawa, Uenohara, Yamanashi 409-0193, Japan*

Received 8 December 2006; received in revised form 22 December 2006; accepted 23 December 2006
Available online 17 January 2007

Abstract

A new polylysine-dendritic cellobiose (**8**) as a model compound of glycodendrimers with biological activities was synthesized by a core lysine dendrimer generation 3 and cellobiose derivative. The oligosaccharide was connected to the lysine dendrimer G3 through the C12 spacer having a peptide bond. That is, acetylcellobiose was reacted with the benzoyloxycarbonyl (*Z*)-protected 6-amino-1-hexanol, followed by the deprotection of the *Z*-group to reproduce amino group. The amino group at the end of the C6 spacer was reacted with adipic acid to form acetylcellobiose with the C12 spacer having a carboxyl group at the end of the spacer. The carboxylic acid was condensed with the amino group of the polylysine dendrimer G3, which was obtained by the stepwise condensation of di-boc-lysine from tris(2-ethylamino)amine, to give the polylysine-dendritic cellobiose (**8**) after deacetylation. The structure was characterized by NMR, IR, GPC, and MALDI-TOF-MS measurements.The polylysine-dendritic cellobiose synthesized here has the following characteristics: (1) all linkages were connected by peptide bond without an ether bond between cellobiose and the C12 spacer, (2) cellobiose moiety was kept the disaccharide structure and increased the flexibility by the long spacer, and (3) based on the peptide linkage, vital affinities and the stabilization under both alkaline and weak acidic conditions are expected.

Keywords: Glycodendrimer; Peptide linkage; Long alkyl spacer; Cellobiose; Molecular weight

1. Introduction

There is an increasing interest, recently, in the synthesis of hyper-branched and dendritic polymers containing of mono or oligosaccharides in the terminal (glycodendrimers), because these dendritic structures are expected to be potent biological activities on sugar–protein interactions based on the multi-valence or cluster effects. In fact, there are many reports on the synthesis of glycodendrimers and their biological applications were reported (Bezouska, Kien, Kieburg, & Lindhorst, 1998; Röckendorf & Lindhorst, 2001; Röy, 2003; Vrasidas, Mol, Liskamp, & Pieters, 2001). The cluster effects based on the dendritic- or hyper-branched structure worked effectively for the higher sugar–protein recognitions. The mannose-modified poly(aminoamine) dendritic caged leucyl leucine methyl ester (LeuLeuOMe) released LeuLeuOMe derivative by the UV irradiation at 350 nm. The LeuLeuOMe was induced apoptosis in immunological natural killer (NK) cells and macrophages (Watanabe & Iwamura, 2003). The GlcNAc terminated poly(aminoamine) dendrimers were found to be improved the binding to the rat NK cells, NKR-P1, in many orders compared to the corresponding oligosaccharides (Krist et al., 2001). A dendritic L-lysine core with α-thiosialosides was synthesized by the solid-phase method for the purpose of the inhibitor of influenza A virus (Röy, Zanini, Meunier, & Romanowska, 1993). A polylysine dendrimer with cellobiose on the terminal was reacted with a tripeptide glycyl-prolyl-leucine and a cyclic

* Corresponding author. Tel./fax: +81 157 26 9388.
E-mail address: yoshida@chem.kitami-it.ac.jp (T. Yoshida).

0144-8617/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2006.12.027

oligopeptide from HIV to give the dendritic HIV vaccine (Baigude, Katsuraya, Okuyama, & Uryu, 2004, 2005).

We have synthesized stereoregular polysaccharides by ring-opening polymerization of anhydrosugar derivatives (Yoshida, 2001). After sulfation, we found that the synthetic and natural polysaccharides had specific biological activities such as anti-HIV and blood anticoagulant activities (Yoshida, Katayama, Inoue, & Uryu, 1992). For example, sulfated (1 → 5)-α-D-ribofuranan and (1 → 4)-β-D-ribopyranan gave both high anti-HIV and blood anticoagulant activities (Yoshida et al., 1994). Curdlan sulfate had high anti-HIV activity but low blood anticoagulant activity (Yoshida et al., 1990). We found that these specific activities might be affected by the cluster effects of the sulfate groups and the flexibility of the main chain from the results on polyacrylate having sulfated oligosaccharide side chain (Yoshida et al., 1999).

Recently, we reported the synthesis of the ester-linkage based spherical and hemispherical polylysine-oligosaccharide (Han, Baigude, Hattori, Yoshida, & Uryu, 2006). The purpose of this work is to synthesize a new dendritic oligosaccharide consisting of polylysine-dendritic scaffolding and cellobiose as a model oligosaccharide. All linkages were peptide bonds and the polylysine and cellobiose were connected with the C12 long alkyl spacer to improve the flexibility of the surface cellobiose. The structure of the polylysine-dendritic cellobiose was determined by NMR, IR, and MALDI-TOF-MS spectrometric analyses. The molecular weight of the dendrimer was also measured by the GPC, suggesting that the dendrimer had compact and spherical structures.

2. Experimental section

2.1. General procedures

Molecular weights were determined by an organic phase GPC of $CHCl_3$ eluent (column: TOSOH TSK-gel, $G3000H_{XL}$, $G4000H_{XL}$, and $G5000H_{XL}$, 7.6 mm × 600 mm × 3) by use of polystyrene (Shodex standard SM-105) as a reference at 40 °C. Infrared spectra were taken on a Shimadzu FT-IR 8300 spectrometer by a KBr pellet method. Specific rotations were measured on a JASCO DIP-140 digital polarimeter in $CHCl_3$. NMR spectra were recorded at 27 °C or at 40 °C in $CDCl_3$ or D_2O solution on a JEOL ECM-400 spectrometer by using a phase sensitive mode and a field gradient probe. Tetramethylsilane (TMS) in $CDCl_3$ or 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) in D_2O was used as an internal standard. The assignment of the proton and carbon signals was carried out by the combination of the H–H and HMQC 2D NMR measurements. The MALDI-TOF-MS spectrum was 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.

Polylysine dendrimer generation 3 (PLDG3) was synthesized from tris(2-ethylamino)amine by the stepwise condensation with di-*tert*-butoxycarbonyl lysine (di-boc-lysine) according to the same procedure of our previous paper (Han et al., 2006). Peracetyl-α-D-cellobiose was prepared by the acetylation of cellulose with acetic anhydride in pyridine and the specific rotation was $[\alpha]_D^{25}$ +40.5° (*c*1, $CHCl_3$). The BOP (benzotriazol-1-yloxytris(dimethylamino)phosphornium hexafluorophosphate) reagent and DIEA (diisopropyl ethylamine) were purchased from Wako Pure Chemical Industry Co., Inc. and used for the condensation of carboxylic acid and amino group. Di-*tert*-butoxycarbonyl lysine (di-boc-lysine) was prepared by the protection of the two amino groups with *tert*-butoxycarbonyl chloride.

2.2. Preparation of benzyloxycarbonyl (*Z*) aminohexanol

Carbobenzoxy chloride (2.15 ml, 15 mmol) was added through syringe to the solution of 6-amino-1-hexanol (1.75 g, 15 mmol) in water at 0 °C. To the aqueous solution, 2 M $NaHCO_3$ in water (20 ml) was added and then the mixture was further stirred for 8 h at 0 °C. The water wed as solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed successively with water several times. The solution was dried over anhydrous Na_2SO_4. A white crystalline *Z*-protected aminohexanol (2.24 g) was obtained in a 60% yield after the evaporation and by vacuum drying.

2.3. Preparation of 6-(benzoyloxycarbonyl)aminohexyl-2,2′,3,3′,4′,6,6′-hepta-*O*-acetyl cellobioside (**4**)

Ag_2CO_3 (2 g, 7.3 mmol) and $CaSO_4$ (5.8 g, 4.3 mmol) was added to a solution of 1-bromo-2,2′,3,3′,4′,6,6′-hepta-*O*-acetyl-D-cellobioside (1.25 g, 5 mmol) and *Z*-protected aminohexanol (1.25 g, 5 mmol) in CH_2Cl_2. The mixture was stirred with shading at room temperature for 18 h. The mixture was filtered through a powdered Celite bed. Then the residue was chromatographed over silica gel with 2:1 (v/v) hexane and ethyl acetate as an eluent to give **4** (2.79 g) in a 64% yield. The specific rotation was $[\alpha]_D^{25}$ −11.0° (*c*1, $CHCl_3$).

2.4. Preparation of 6-aminohexyl-2,2′,3,3′,4′,6,6′-hepta-*O*-acetyl cellobioside (**5**)

To a solution of **4** (1.75 g, 2 mmol) in methanol (20 ml) was added a spatula of 10% palladium carbon and then the mixture was stirred for 20 h under hydrogen atmosphere at room temperature. After the mixture was filtered through a Celite bed, the acetyl cellobiose with a free amino group **5** was obtained after the evaporations of methanol. This compound was used immediately for the next reaction without purification.

2.5. Condensation of **5** with adipic acid

Adipic acid (1.5 g, 10 mmol) was added through a syringe to the mixed solution of **5** and DIEA (0.6 ml,

3.6 mmol) in anhydrous DMF (15 ml) under nitrogen atmosphere. After the solution was cooled to 0 °C, the BOP reagent (1.45 g, 3.3 mmol) was added, and then the mixture was stirred for 24 h at room temperature. The residue was dissolved in chloroform and washed successively with water several times. The chloroform layer was dried over anhydrous Na_2SO_4 and then evaporated. The residue was chromatographed over silica gel with a mixed eluent of methanol and chloroform in the proportion of 1:10 (v/v) to give **6** (1.0 g) in a 32% yield.

2.6. Preparation of polylysine-dendritic acetylcellobiose (7)

The polylysine dendrimer PLDG3 TFA salt (0.2 g, 0.043 mmol), the oligosaccharide moiety **6** (1.1 g, 1.2 mmol), and DIEA (0.2 ml, 1.2 mmol) were dissolved in anhydrous DMF (20 ml) under nitrogen atmosphere. After the solution was cooled to 0 °C, the BOP reagent (0.8 g, 1.8 mmol) was added, and then the mixture was stirred for 24 h at room temperature. After the residue was dissolved in chloroform and washed successively with water several times to give a white crystalline (**7**) (0.53 g) was obtained after vacuum drying in a 53% yield. The product (**7**) was purified by column chromatography over silica gel using a mixture of chloroform and methanol (6:1) as an eluent.

2.7. Deacetylation of 7

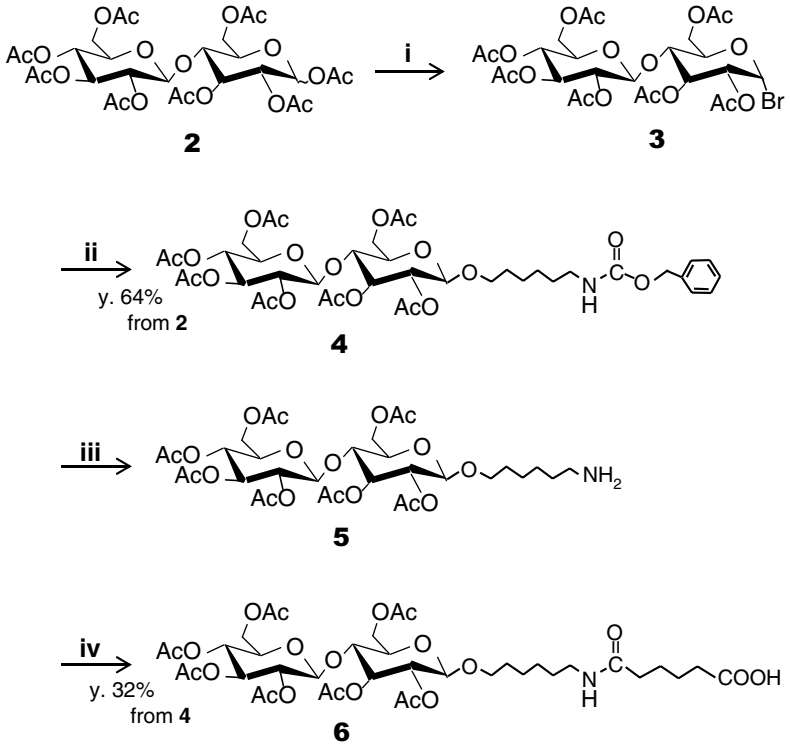
A solution of **7** (0.03 g, 0.0012 mmol) in methanol (5 ml) and NaOMe (0.07 g, 0.13 mmol) was stirred for 3 h at room temperature. The precipitation of the deacetylated polylysine-dendritic cellobiose appeared in the solution and then the solution was dialyzed against deionized water for 2 days. The dialyzate was freeze-dried to give the deacetylated polylysine-dendritic cellobiose **8** (0.011 g) in a 58% yield.

3. Results and discussion

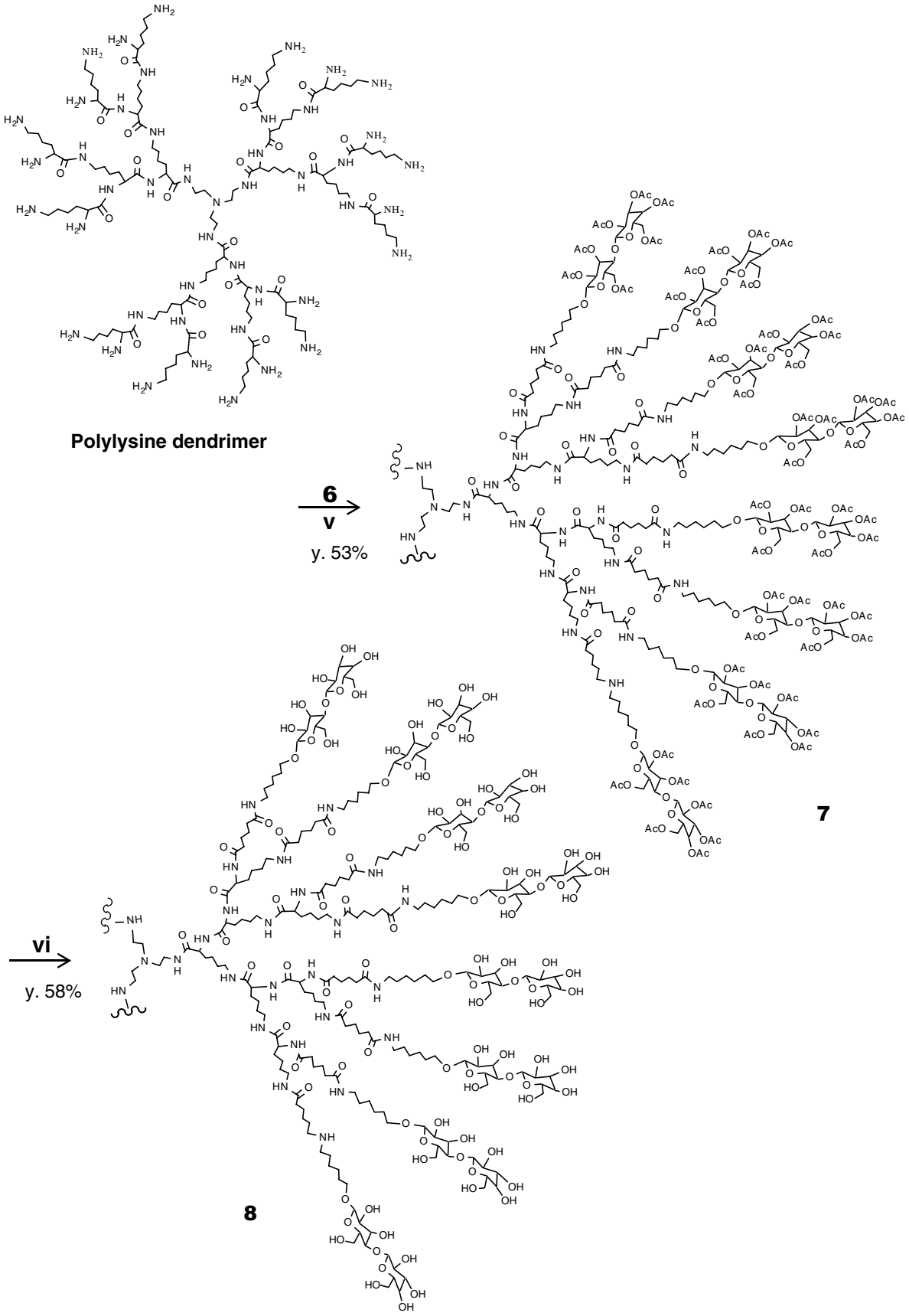
3.1. Synthesis of polylysine-dendritic cellobiose with alkyl spacer having peptide linkage

The dendritic compounds are expected for the biological activities based on the cluster effect because of having highly branched structure. In this work, we designed a new polylysine-dendritic oligosaccharide as a candidate of the glycodendrimers having specific biological activities such as high anti-HIV and blood anticoagulant activities. On the structure of the polylysine-dendritic oligosaccharide, the peptide bond was used for all linkages. Polylysine dendrimer and cellobiose moieties were connected by the long spacer, which gave the flexibility of the oligosaccharide moiety. The peptide linkage should increase the stability against both alkaline and weak acidic conditions, as well as vital affinity.

The polylysine dendrimer generation 3 (PLDG3) was prepared as a TFA salt from tris(2-ethylamino)amine as a trivalent core and the stepwise condensation of di-boc-lysine according to the same method of the recent paper (Han et al., 2006). The boc-protective group was



Scheme 1. Synthesis of acetylcellobiose with C12 alkyl spacer (**6**). (i) HBr–CH_3COOH, rt 5 h; (ii) Z–NH–$(CH_2)_6$–OH (**1**), Ag_2CO_3/CH_2Cl_2, rt 18 h; (iii) H_2, Pd/C, rt 20 h; (iv) adipic acid, DIEA, BOP, DMF, rt 24 h.



Scheme 2. Synthesis of polylysine-dendritic cellobiose with C12 alkyl spacer (**8**). (v) DIEA, BOP, DMF, rt, 24 h; (vi) NaOMe/MeOH, 3 h.

removed by 2 M TFA to reproduce amino group. The polylysine dendrimer (PLDG3) has 24 amino groups on the surface.

Scheme 1 shows the synthetic route of the sugar moiety and alkyl spacer having a peptide linkage (**6**). Acetylcellobiose ($[\alpha]_D^{25}$ +40.5°) was reacted with HBr-acetic acid solution to give α-1-bromo acetylcellobiose, which was used for the next reaction without purification. The half part of the spacer, 1-aminobenzyloxycarbonyl-6-hexanol (*Z*-protected aminohexanol) (**1**), was prepared by the protection of the amino group with benzyloxycarbonyl chloride in a 60% yield. The etherification of 1-bromoacetylcellobiose was carried out with the *Z*-protected aminohexanol (**1**) by Ag_2CO_3 to afford **4** in a 64% yield from peracetylated cellobiose (**2**). The specific rotation of **4** was changed from positive value to negative of $[\alpha]_D^{25}$ −11.0° (*c*1, $CHCl_3$), suggesting that the C1 anomeric proton had β-structure. The deprotection of the *Z*-group was performed by the catalytic reduction using Pd/C to give **5** having a free amino group, which was used immediately for the next reaction without purification because of unstable. The condensation of **5** with adipic acid gave the cellobiose moiety with the C12 carboxylic acid having a peptide linkage (**6**) in a 32% yield from **4**.

Polylysine dendrimer TFA salt was condensed with **6** to give the dendritic acetylcellobiose **7** in a 53% yield as shown in Scheme 2. Deacetylation of **7** with NaOMe in methanol gave the OH-free polylysine-dendritic cellobiose **8** in a 58% yield.

3.2. Structural analysis

Fig. 1 shows the ^{13}C NMR spectra of (A) acetylcellobiose, (B) acetylcellobiose with the C12 spacer (**6**), and (C) polylysine-dendritic acetylcellobiose (**7**), respectively. All signals were assigned with the combination of H–H COSY

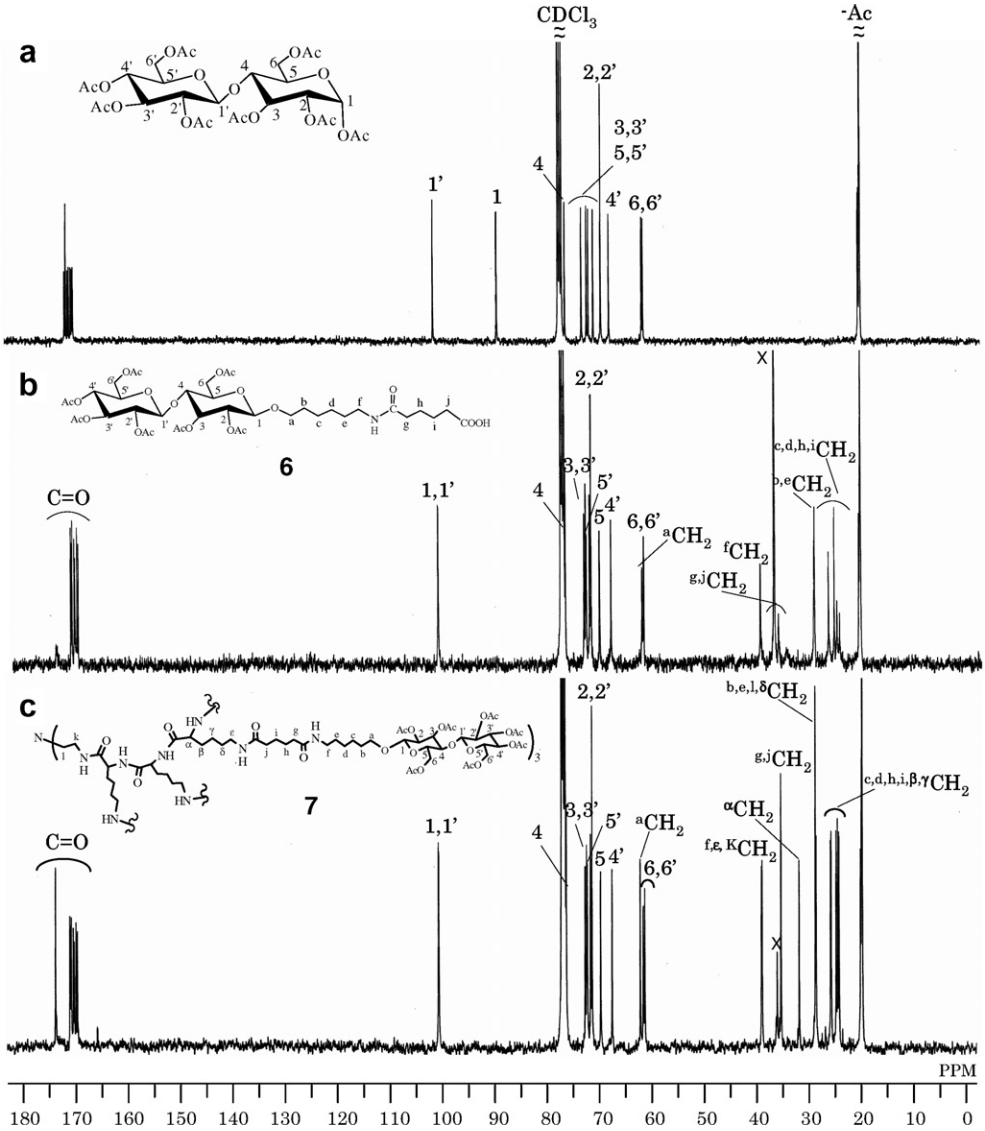


Fig. 1. One hundred megahertz ^{13}C NMR spectra of (a) acetylcellobiose, (b) acetylcellobiose with C12 alkyl spacer (**6**), and (c) polylysine-dendritic acetylcellobiose (**7**) ($CDCl_3$, 27 °C).

and HMQC 2D NMR measurements. The anomeric C1 signals due to acetylcellobiose in spectrum 1A were observed separately at 90 and 101 ppm, respectively, however, for the compounds **6** and **7**, the C1 signals appeared at the same position of 101 ppm (spectra 1B and 1C). In spectrum 1B, the acetyl methyl and carbonyl carbon signals appeared around 20 and 170 ppm, respectively, and the absorptions due to the methylene signals a–j in the side chain appeared between 23 and 63 ppm. The polylysine-dendritic acetylcellobiose (**7**) gave the complex spectrum (1C) and the carbon signals due to polylysine moiety appeared relatively narrow region between 24 and 32 ppm.

The molecular weight of **7** was measured by both an organic-phase GPC by using polystyrene standards and MALDI-TOF-MS measurements. GPC is a separation method for polymers by their molecular size and the molecular weights can be calculated by calibration. Branched polymers, in general, give a smaller size compared to the real one. Thus, the molecular weights by GPC afford smaller values. On the other hand, MALDI-TOF-MS gives the m/z signals by the ionization of polymers and the molecular ion peak shows the mass of the molecule. The molecular weight by GPC gave $\overline{M}_n = 4000$ and $\overline{M}_w = 4300$, which was smaller than the calculated one ($M_w = 23137.9$, the DS = 24), and the molecular weight distribution was very narrow, $\overline{M}_w/\overline{M}_n = 1.08$. Since the compound **7** had a hyper-branched structure, the molecular size should become small. Therefore, the MALDI-TOF-MS measurement was carried out. The MALDI-TOF-MS spectrum of **7** is shown in Fig. 2 and Table 1 exhibits the calculated and found molecular weights of **7**.

The dendrimer gave the $m/z = 19056.1$ as the main signal. From the results of the GPC and MALDI-TOF-MS measurements, the polylysine-dendritic acetylcellobiose (**7**) had very compact, spherical, and high homogeneous structures. However, several absorptions in the MALDI-TOF-MS profile were observed at the $m/z = 21407.3$, 20656.6, 19851.9, 19056.1, 18261.0, 17465.4, and 16677.4. The difference of the m/z between the absorptions were approximately $m/z = 800$, which value is approximately equal to the molecular weight of an acetylcellobiose unit **6**, $\overline{M}_w = 846.85$, suggesting that the polylysine-dendritic acetylcellobiose (**7**) synthesized here was the mixture having several DSs of **6**, the DS = 22, 21, 20, 19, 18, 17, 16, respectively. When the condensation of **6** with the polylysine dendrimer was carried out, the proportion of **6** to the amino group in the feed was 1.2. Therefore, the mole ratio of **6** increases, the substitution should increase.

Fig. 3 represents (a) ^{1}H and (b) ^{13}C NMR spectra of the OH-free polylysine-dendritic cellobiose (**8**) in D_2O solution, respectively. Although the ^{1}H NMR exhibited the complex spectrum, the signals of sugar moiety appeared as two regions around 3.5–4.0 and 4.5 ppm, respectively.

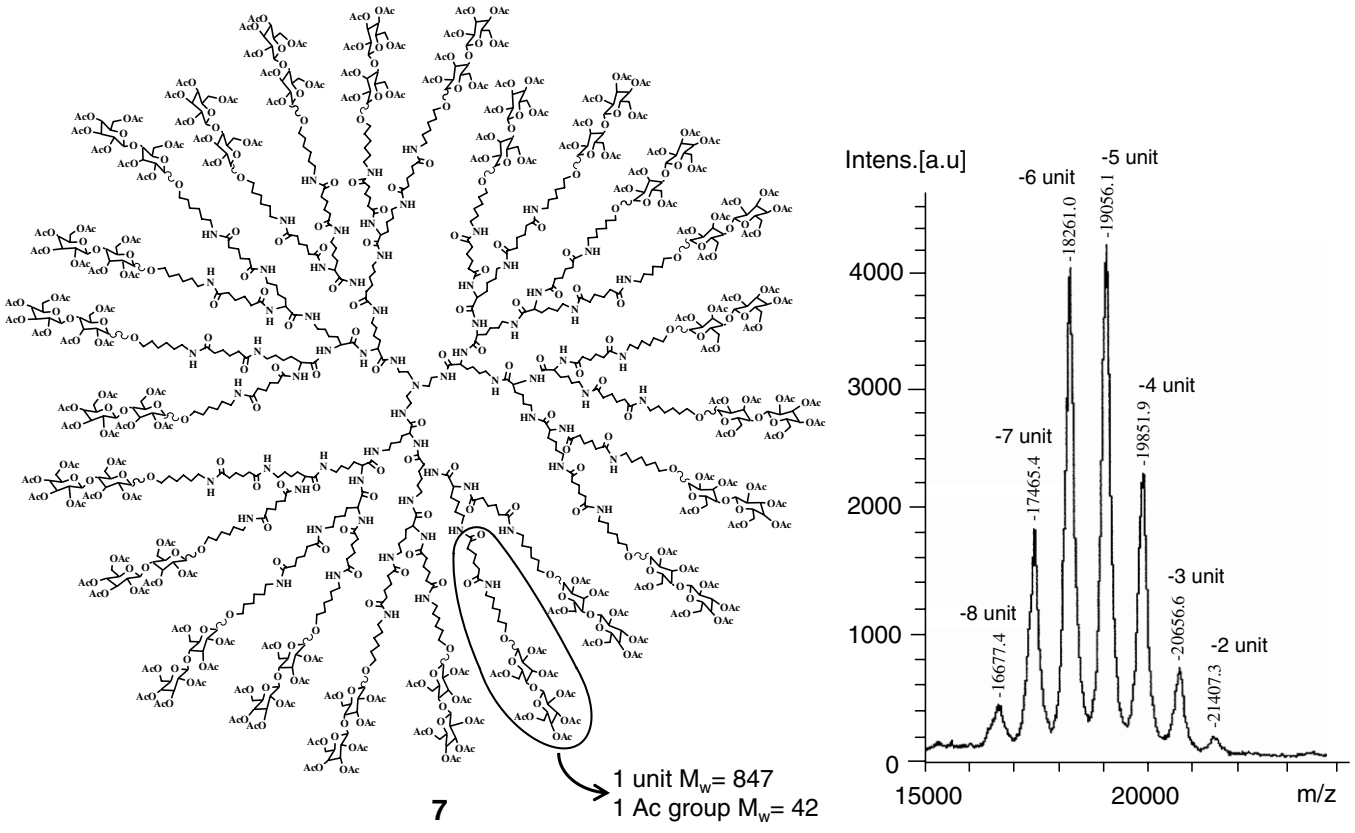


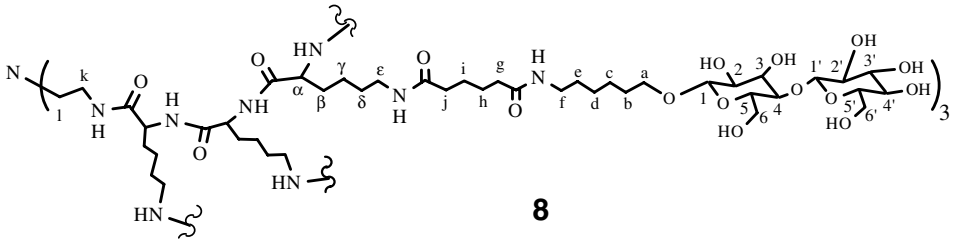
Fig. 2. Structure and MALDI-TOF-MS spectrum of polylysine-dendritic acetylcellobiose (**7**). The molecular weight of fully substituted dendrimer (**7**) is 23137.9.

Table 1
Calculated and found molecular weights of polylysine-dendritic acetylcellobiose (**7**)

Molecular weight		DS[a]
Calculated	Found	
16343.2	16677.4	16
17203.1	17465.4	17
18062.9	18261.0	18
18908.8	19056.1	19
19754.6	19851.9	20
20600.5	20656.6	21
21434.3	21407.3	22
22292.1		23
23137.9		24

The found molecular weight was determined by MALDI-TOF-MS measurement.
[a] Degree of substitution of acetylcellobiose unit (**6**).

The signals in the lower magnetic filed around 4.5 ppm were attributed to the H1 and H1′ absorptions due to cellobiose moiety. In the ^{13}C spectrum, the amido carbonyl carbon signals appeared around 178 ppm, and the C1 and C1′ signals of cellobiose 105 ppm. The cellobiose signals without the C1 and C1′ appeared between 63 and 82 as several absorptions. The methylene and methyne signals due to polylysine and spacer moieties were observed in the higher magnetic filed, more than 45 ppm. The results of the NMR measurements were agreed with the structure of polylysine-dendritic cellobiose with the C12 spacer (**8**). Fig. 4 exhibits the FT-IR spectra of the polylysine-dendritic acetyl cellobiose (**7**) and deacetylated one (**8**), respectively. The absorptions due to the peptide appeared clearly at 1641 and 1635 cm^{-1} in Figs. 4a and b, respectively. After deacetylation (Fig. 4b), the acetyl carbonyl signal at



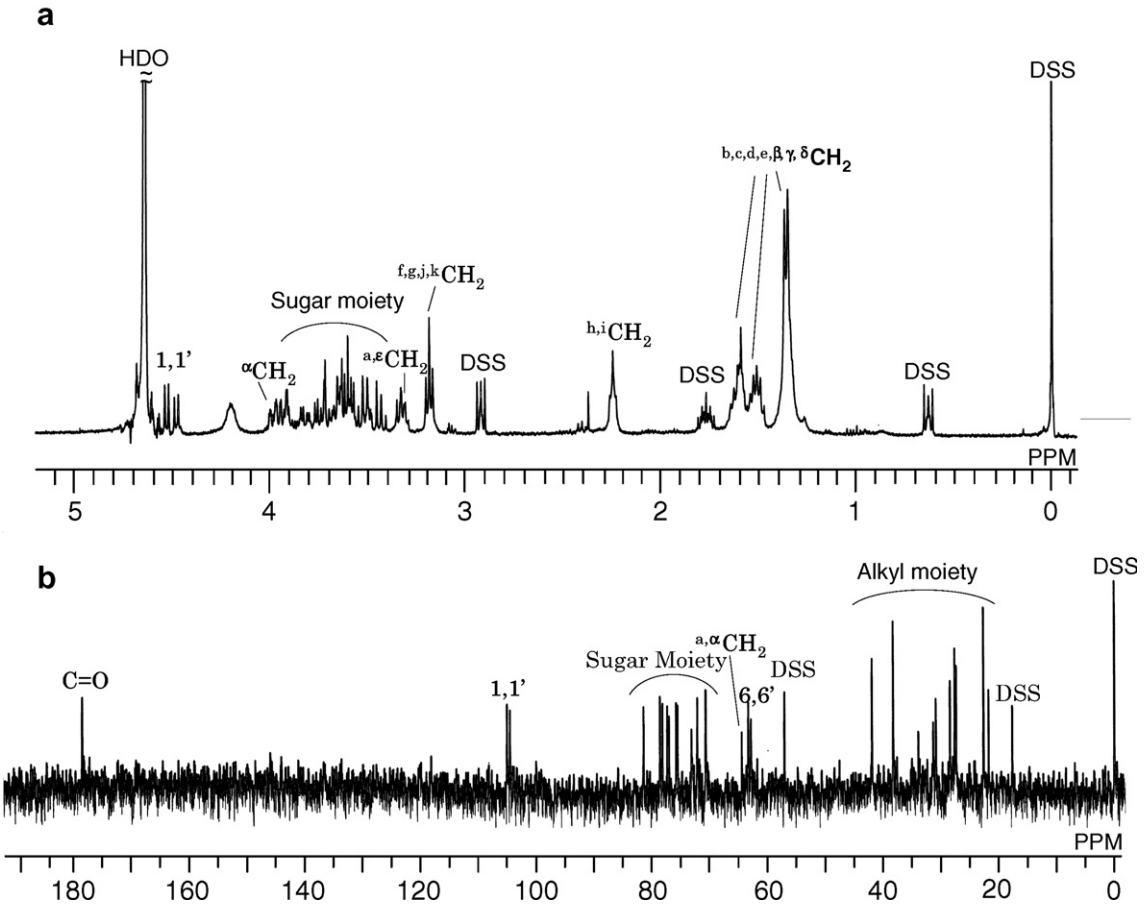


Fig. 3. ^{1}H (a) and ^{13}C NMR (b) spectra of polylysine-dendritic cellobiose (**8**) (D_2O, 40 °C).

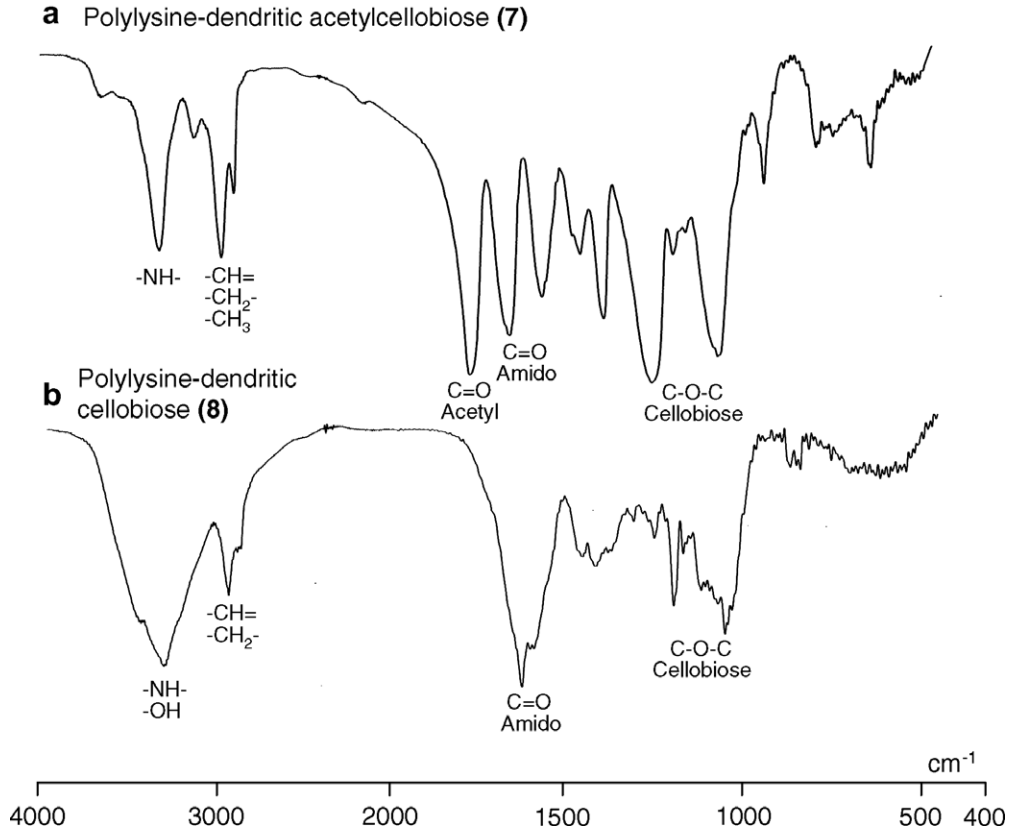


Fig. 4. FT-IR spectra of (a) polylysine-dendritic acetylcellobiose (**7**) and (b) polylysine-dendritic cellobiose (**8**).

1757 cm^{-1} disappeared and the large hydroxyl signal around 3300 cm^{-1} appeared, suggesting that the deacetylation proceeded successfully to give the OH-free polylysine-dendritic cellobiose (**8**).

In conclusion, the polylysine-dendritic cellobiose synthesized here was designed as based on the peptide linkage and the core polylysine dendrimer and cellobiose moieties were connected by the C12 spacer. The synthetic method should provide a general procedure of glycodendrimers with long alkyl spacer. Instead of cellobiose, a variety of oligosaccharides can be used. The long alkyl spacer increases the flexibility and avoids the crowding of the surface oligosaccharide residues. Polylysine was used as the core scaffolding in this work, because the construction of the dendritic structure is easy and is expected to high vital affinities (Veprek & Jez, 1999a, 1999b). The molecular weights by measured GPC gave $\overline{M}_n = 4000$ and $\overline{M}_w = 4300$ and the MALDI-TOF-MS afforded the $m/z = 19056.1$ as the main signal. The difference between the molecular weights by measuring GPC and MALDI-TOF-MS was very large, suggesting that the dendritic acetylcellobiose (**7**) was compact and spherical structures. In addition, several signals between the $m/z = 21407.3$ and 16677.4 appeared as shown in Fig. 4, suggesting that some cellobiose units **6** were off the fully substituted dendritic compound **7** (Table 1).

After sulfation of **8**, to elucidate the relationship between the cluster effects based on the dendritic structure and the specific biological activities such as anti-HIV and blood anticoagulant activities are in progress.

Acknowledgment

We thank Mr. Daisuke Higo of Bruker Daltonics Co. for the MALDI-TOF-MS measurements.

References

Baigude, H., Katsuraya, K., Okuyama, K., & Uryu, T. (2004). Synthesis of structurally-controlled AIDS vaccine model with glycol-peptide dendrimer scaffolds. *Macromolecular Chemistry and Physics, 205*, 684–691.

Baigude, H., Katsuraya, K., Tokunaga, S., Fujiwara, N., Satoyam, M., Magome, T., et al. (2005). Synthesis of an oligosaccharide-polylysine dendrimer with reducing sugar terminals leading to acquired immunodeficiency syndrome vaccine preparation. *Journal of Polymer Science Part A: Polymer Chemistry, 43*, 2195–2206.

Bezouska, Z., Kien, V., Kieburg, C., & Lindhorst, T. K. (1998). GlcNAc-terminated glycodendrimers from defined precipitates with the soluble dimeric receptor of rat natural killer cells, sNKR-P1A. *FEBS Letters, 426*, 243–247.

Han, S., Baigude, H., Hattori, K., Yoshida, T., & Uryu, T. (2006). Synthesis of new spherical and hemispherical oligosaccharides with polylysine core scaffold. Carbohydrate Polymers 57, in press.

Krist, P., H-Rajnochova, E., Rauvolfova, J., Semenuk, T., Vavruskova, P., Pavlicek, J., et al. (2001). Toward an optimal oligosaccharide ligand for rat natural killer cell activation receptor NKR-P1. *Biochemical and Biophysical Research Communications, 287*, 11–20.

Röckendorf, N., & Lindhorst, T. K. (2001). Glycodendrimers. *Topics Current Chemistry, 217*, 201–238.

Röy, R. (2003). A decade of glycodendrimer chemistry. *Trends in Glycoscience and Glycotechnology, 15*, 291–310.

Röy, R., Zanini, D., Meunier, S. J., & Romanowska, A. (1993). Solid-phase synthesis of dendritic sialoside inhibitors of influenza a virus haemagglutinin. *Journal of the Chemical Society-Chemical Communication, 24*, 1869–1872.

Veprek, P., & Jez, J. (1999a). Peptide and glycopeptide dendrimers part I. *Journal of Peptide Science, 5*, 5–23.

Veprek, P., & Jez, J. (1999b). Peptide and glycopeptide dendrimers. part II. *Journal of Peptide Science, 5*, 203–220.

Vrasidas, I., Mol, N. J., Liskamp, R. M. J., & Pieters, R. J. (2001). Synthesis of lactose dendrimers and multivalency effects in binding to the cholera toxin B subunit. *European Journal of Organic Chemistry*, 4685–4692.

Watanabe, S., & Iwamura, M. (2003). Dendritic caged compounds. *Journal of Photochemistry and Photobiology A: Chemistry, 155*, 57–62.

Yoshida, T. (2001). Synthesis of polysaccharides having specific biological activities. *Progress in Polymer Science, 26*, 379–441.

Yoshida, T., Akasaka, T., Choi, Y., Hattori, K., Yu, B., Mimura, T., et al. (1999). Synthesis and specific biological activities of polymethacrylate derivatives having sulfonated oligosaccharide side chains. *Journal of Polymer Science Part A: Polymer Chemistry, 37*, 789–800.

Yoshida, T., Hatanaka, K., Uryu, T., Kaneko, Y., Yasuda, N., Mimura, T., et al. (1990). Synthesis and structural analysis of curdlan sulfate with potent anti-AIDS virus activity. *Macromolecules, 23*, 3717–3722.

Yoshida, T., Katayama, Y., Inoue, S., & Uryu, T. (1992). Synthesis of branched ribofuranans and their sulfates with strong anti-AIDS virus activity by selective ring-opening copolymerization of 1,4-anhydro-α-D-ribopyranose derivatives. *Macromolecules, 25*, 4051.

Yoshida, T., Wu, C., Song, L., Uryu, T., Kaneko, Y., Mimura, T., et al. (1994). Synthesis of cellulose-type copolymers and their branched ribopyranan sulfates with anti-AIDS virus activity by selective ring-opening copolymerization of 1,4-anhydro-α-D-ribopyranose derivatives. *Macromolecules, 27*, 4422–4428.