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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.