Branched Cationic Polyurethane Prepared by Polyaddition of Chloromethylated Five-Membered Cyclic Carbonate and Diethylenetriamine in Molten Salts

Bungo Ochiai,[1] Kazuhiro Koda,[1] Takeshi Endo[2]

[1]Department of Chemistry and Chemical Engineering, Faculty of Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan
[2]Molecular Engineering Institute, Kinki University, 11-6 Kayanomori, Iizuka, Fukuoka 820-8555, Japan
Correspondence to: T. Endo (E-mail: tendo@mol-eng.fuk.kindai.ac.jp)

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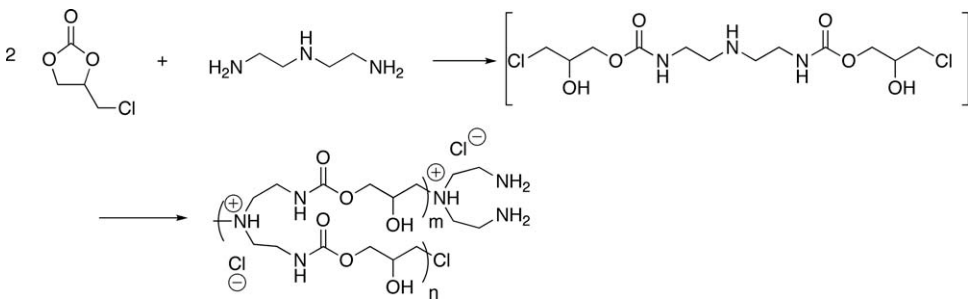
ABSTRACT: A branched cationic polymer was synthesized by polyaddition of 4-chloromethyl-1,3-dioxolan-2-one and diethylenetriamine in molten salts. The polyaddition proceeded through nucleophilic addition of the primary amino group to the cyclic carbonate structure and quarternization of the secondary amino group with the chloromethyl group. The resulting cationic polymer was a good and recyclable catalyst for the reaction of carbon dioxide and epoxides owing to the ammonium structure. The complexation with DNA was confirmed by dynamic light scattering and zeta potential measurements, and it suggests the potential application as a carrier for drug delivery system. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 50: 47–51, 2012

KEYWORDS: addition polymerization; biomaterials; branched; carbonate; catalysis; catalyst; cationic polymer; drug delivery systems; gene delivery; polyurethane

INTRODUCTION Ionic polymers have wide applications for carriers for drug delivery system,[1–12] biomaterials,[13] catalysts,[14–17] electrolytes,[18,19] and so forth.[20] For instance, poly(ethyleneimine) is a branched and cationic polymer, and it has been used as an efficient gene delivery carrier by ionic complexation.[1–9] However, its severe disadvantage is the toxicity, probably because of the permeation ability into cell menbranes.[21,22] A polymer with branched and cationic structures is a potential candidate of useful biomaterials, especially if it is less toxic. Cationic moieties play an important role in complexing with drug candidates, and branched structures are effective to higher loading of ligands inside. We thought that such branched and cationic polymers bearing biocompatible structures would have better properties. We focused on the reaction of five-membered cyclic carbonates with amines to produce hydroxyurethanes, which is very chemoselective.[23–31] The focused chemoselective reactions were (a) selective nucleophilic addition of the primary amino groups in diethylenetriamine (DETA) to the cyclic carbonate group remaining the secondary amine structure unreacted[29] and (b) the selective nucleophilic addition of primary amines to the cyclic carbonate structure in a five-membered cyclic carbonate bearing chloromethyl group (Cl5CC) without quarternization.[26] The low toxicity of DETA may also be mentioned (e.g., the patch testing of 0.05% aqueous solution did not cause stimulation, and the oral LD_{50} values for rats are 819–2600 mg/kg).[32] Our strategy for synthesizing branched and cationic polymers bearing biocompatible structures is the polyaddition of DETA and the cyclic carbonate bearing a halomethyl group, in which the nucleophilic addition of the primary amino group to the five-membered cyclic group produces a biocompatible urethane main chain, and the successive quarternization of the secondary amine structure with the halomethyl group produces cationic structure.[33–36] We examined this polyaddition under various conditions and the application of the resulting branched cationic polymer as a catalyst and a DNA capsule.

RESULTS AND DISCUSSION

Polyaddition of Cl5CC and DETA was conducted using 2:1 molar amounts of the monomers (Scheme 1 and Table 1). When the polymerizations were conducted in conventional solvents, the yields were better when polar solvents were used but the molecular weights were almost identical. The product obtained in the reaction in THF at room temperature was the 2:1 adduct resulting from the selective nucleophilic ring opening of the NH_2 group in DETA to the cyclic carbonate group in Cl5CC, indicating the first selective ring opening produced this intermediate prior to the reactions of the



SCHEME 1 Synthesis of branched cationic polyhydroxyurethane via polyaddition of Cl5CC with DETA.

secondary amine group or the chloromethyl group in a similar manner to the reported reactions [Fig. 1(a)].[26,29] Although typical nucleophilic addition of amines to cyclic carbonates gives two products with primary and secondary OH groups, this intermediate has only secondary hydroxyl groups owing to the electron-withdrawing character of the chloromethyl group. Then, we tried polymerizations in molten salts to conduct the polymerization under more polar environment.[37] The products obtained in molten salts, especially polar chloride salts, had higher molecular weight than the products obtained in conventional solvents. This result suggests the importance of polarity of the polymerization media. The lower amounts of molten salts were effective to obtain polymers in higher yields, probably because too high amounts of molten salts resulted in low concentration for efficient reactions. The structure of the polymer was elucidated by IR and nuclear magnetic resonance (NMR) [Fig. 1(b,c)] spectroscopic analyses. An strong IR absorption at 1695 cm^{-1} is assignable to the C=O moieties in the urethane group, and the wavenumber is the same value with that of typical polyhydroxyurethanes.[38] The polymers with very low molecular weight showed NMR spectra very similar with the nonionic intermediate produced via the nucleophilic addition of the primary amine group without quarternization, whereas the NMR signal of the methylene protons adjacent to the secondary amine moieties was very weak in the spectra of polymers with M_n higher than 3000. This result also supports that the nucleophilic addition proceeded prior to the quarternization. The signals observed at 2.5–3.0 ppm are attributable to the methylene groups in the terminal groups not quarternized, and the signals were disappeared by treatment with HCl aq (data not shown). This polymer may contain the cyclic urethane structure produced via the intramolecular cyclization of the chloroalkyl groups and the secondary amine groups, although its presence could not be confirmed due to the similarity in the structure. In a similar manner, we could not determine the degree of branching due to the overlap of the signals.

As applications of the resulting polymer, we evaluated the catalyst activity for the reaction of an epoxide and carbon dioxide[14,15,17,39] and the DNA complexing ability. First, the branched cationic polymer (run 14) was used as a catalyst for the reaction of epoxides with carbon dioxide in *N*-methylpyrrolidone (2 mol/L) (Scheme 2 and Table 2). The carbonation reactions of epichlorohydrin (ECH) and glycidyl phenyl ether (GPE) proceeded quantitatively, and Cl5CC and 4-phenoxymethyl-1,3-dioxolane-2-one were obtained in quantitative yields from the corresponding epoxides. The polymeric catalyst could be recycled twice keeping the good activity via precipitation of the polymer with acetone. Contrary to this, the reaction using a low-molecular-weight polymer (e.g., run 4) was unsuccessful (30% yield at the reaction of 4-phenoxymethyl-1,3-dioxolane-2-one for 24 h) due to the lower contents of the ammonium structure serving as the catalyst for this reaction.

Complexation with DNA was confirmed by dynamic light scattering (DLS) experiments (Fig. 2). Tris-HCl solutions of DNA (pH = 8.0 and 10 mg/mL) were mixed with an aqueous polymer solution (100 mg/mL, 10 mL). The mixtures were transparent when 1.0 or 0.5 wt % amounts of DNA were added. Clear scattering patterns indicated the formation of polyplexes with diameters of 171 and 148 nm, respectively.

TABLE 1 Polyaddition of Cl5CC with DETA[a]

Run	Solvent (mL)	Temp. (°C)	Yield (%)[b]	M_n (M_w/M_n)[c]
1	None	100	51	1,200 (1.59)
2	MeOH (1.0)	50	16	1,280 (1.24)
3	MeOH (1.0)	80	51	1,110 (1.27)
4	MeOH (1.0)	100	40	940 (1.29)
5	THF (1.0)	100	30	1,660 (1.39)
6	H_2O (1.0)	100	72	1,030 (1.42)
7	DMF (1.0)	100	44	1,050 (1.37)
8	DMSO (1.0)	100	51	1,000 (1.43)
9	$BMIBF_4$ (1.0)	100	15	2,090 (1.18)
10	$BMIBF_4$ (0.5)	100	86	1,530 (1.37)
11	$BMIPF_6$ (0.5)	100	90	1,680 (1.63)
12	$EMICF_3SO_3$ (0.5)	100	97	1,480 (1.32)
13	BMICl (0.5)	100	46	2,400 (1.20)
14	BMICl (0.05)	100	46	3,120 (1.81)

$BMIBF_4$, 1-*n*-butyl-3-methylimidazolium tetrafluoroborate; $BMIPF_6$, 1-*n*-butyl-3-methylimidazolium hexafluorophosphate; $EMICF_3SO_3$, 1-ethyl-3-methylimidazolium trifluoromethanesulfonate; BMICl, 1-*n*-butyl-3-methylimidazolium chloride.

[a] Conditions: Cl5CC (1.83 mmol), diethylenetriamine (0.915 mmol), 24 h, degassed sealed tube.

[b] Isolated yield after precipitation with acetone.

[c] Estimated by SEC [poly(ethylene glycol) standards, CH_3CN/0.5 N $NaNO_3$ aq. (v/v = 1/1)].

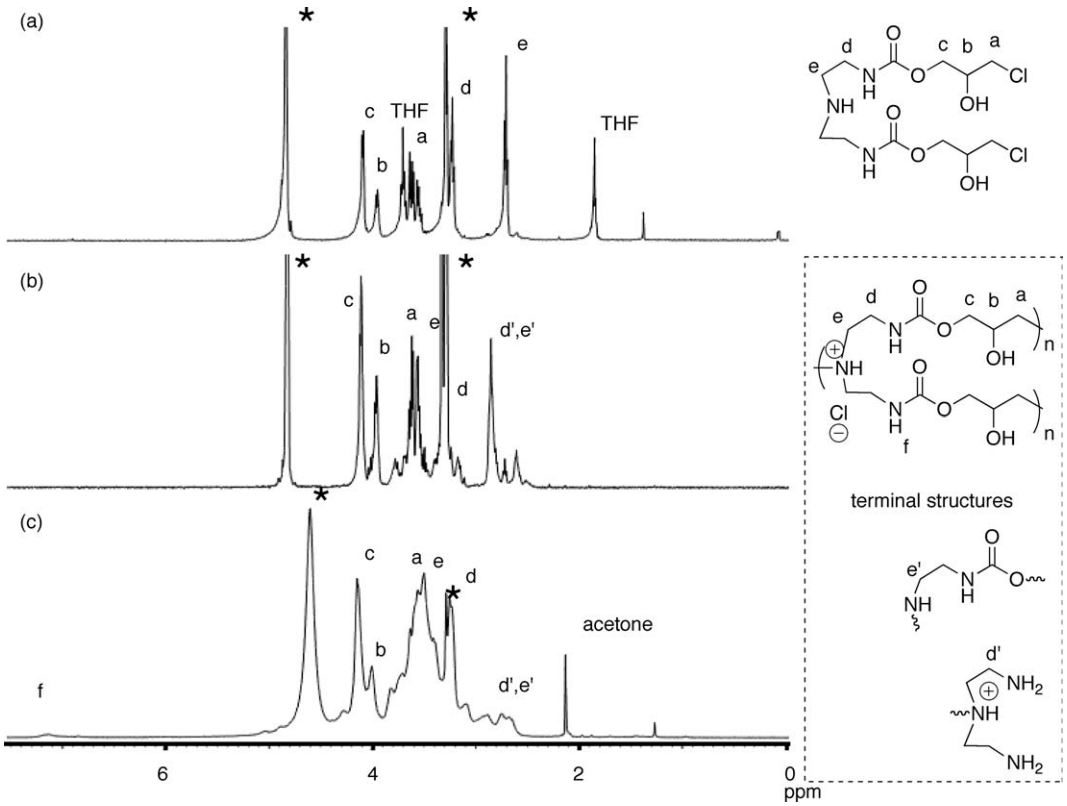
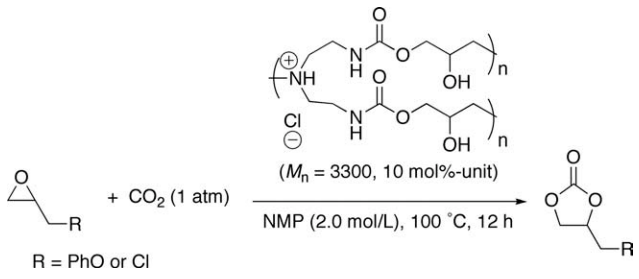


FIGURE 1 ^{1}H NMR spectra of (a) the intermediate, (b) oligomer obtained in run 4, and (c) polymer obtained in run 14.

The diameters of the polyplexes were shorter than the diameter of the cationic polymer (478 nm). The longer diameter of the cationic polymer in this buffered solution indicates that the cationic polymer has self-aggregation character via dynamic intermolecular ion exchange. The smaller sizes of the polyplexes may be ascribed to the decreased dynamic character of the ion exchange originating from the multivalent character of the anionic moieties in DNA. Emulsification took place when more than 2 wt % amounts of DNA were added. The DLS measurements were difficult due to the very broad size distribution, and the diameters of the resulting complex exceeded 1000 nm. We presume that the emulsification originated from the insufficient amounts of the cationic polymer to cover DNA completely. The zeta potentials, which are the indexes of the total charges in the complexes, decreased as the amount of anionic DNA added. The shift to negative potential suggests that the neutralization between the cationic polymer and DNA took place.



SCHEME 2 Reaction of epoxides with carbon dioxide catalyzed by the branched cationic polymer.

EXPERIMENTAL

Materials

4-Chloromethyl-1,3-dioxolane-2-one (Cl5CC) was prepared by the reaction of ECH (Tokyo Kasei Kogyo) and CO_2 in *N*-methylpyrrolidinone (Kanto Chemical) catalyzed by LiBr (Kanto Chemical, Tokyo, Japan) and distilled under reduced pressure.[35] The spectroscopic data agreed with those in a literature.[36] DETA (Tokyo Kasei Kogyo) was dried with CaH_2 and distilled under reduced pressure. Low-molecular-weight DNA from salmon sperm (Fluka) was used for the DNA complexation experiment. The DNA was dissolved in a Tris-HCl solution (0.05 M, pH = 8.0), which is a solvent recommended by the supplier. Other reagents were used as received.

Measurements

NMR spectroscopy measurements were performed on a JEOL ECX-400 instrument using tetramethylsilane as an internal standard at ambient temperatures (400 MHz for ^{1}H, 100 MHz for ^{13}C, and 40 MHz for ^{15}N). Molecular weights were measured by size exclusion chromatography performed on a system consisting of a Tosoh DP-8020 pump, a Viscotek TDA Model 300 detector (refractive index detector was used), and tandem columns of Tosoh TSK gel G3000PWXL-CP (pore size: 7 μm) and TSK gel G5000PWXL-CP (pore size: 10 μm). A mixed solvent of acetonitrile and 0.1 mol/L $NaNO_3$

aqueous solution (v/v = 50/50) was used as an eluent at 40 °C. DLS and zeta potential measurements were performed using a Malvern Zetasizer nano-ZS instrument equipped with a 4-mW He–Ne laser (633 nm) in 12-mm-square glass cuvettes at 25 °C. Dip cell was used for zeta potential measurement.

Polyaddition of Cl5CC and DETA (Typical Procedure)

Cl5CC (0.250 g, 1.83 mmol), DETA (0.0945 g, 0.915 mmol), and solvents were stirred in a degassed sealed tube. The resulting mixture was diluted with water or methanol and poured into an excess amount of acetone. The precipitate was collected by centrifugation and drying under reduced pressure.

Synthesis of Intermediate from Cl5CC and DETA

Cl5CC (0.546 g, 4.00 mmol), DETA (0.206 g, 2.00 mmol), and THF (2.0 mL) were stirred at 25 °C for 24 h in a degassed sealed tube. The resulting mixture was poured into an excess amount of diethyl ether. The viscous product, bis(3-chloro-2-hydroxypropyl)carbamate of DETA was obtained by drying under reduced pressure (0.646 g, 1.72 mmol, 86%). The product contained a trace amount of THF to avoid self-quarternization spontaneously took place in the absence of THF.

^{1}H NMR (400 MHz, δ in ppm, CD_3OD): 2.70 (t, J = 12.2 Hz, 4H, —NHCH_2CH$_2$NHCOO—), 3.23 (t, J = 12.2 Hz, 4H, —NHCH$_2$CH_2NHCOO—), 3.50–3.73 (m, 4H, ClCH_2—), 3.92–4.00 (m, 2H, —CH—), 4.07–4.13 (m, 4H, —OCH_2—). ^{13}C NMR (100 MHz, δ in ppm, CD_3OD): 40.04 (—NH*C*H$_2$CH$_2$NHCOO—), 45.55 (Cl*C*H$_2$—), 48.31 (—NHCH$_2$*C*H$_2$NHCOO—), 65.89 (—OCH_2—), 69.31 (—CH—), 157.47 (*C*=O). ^{15}N NMR (40 MHz, δ in ppm, CD_3OD): 25 (—*N*HCH$_2$CH$_2$NHCOO—), 75 (—NHCH$_2$CH$_2$*N*HCOO—).

Reaction of Epoxides and CO_2 Catalyzed by the Branched Cationic Polymer

Typical procedure: GPE (0.45 g, 3.0 mmol) or ECH (0.28 g, 3.0 mmol) and the polymer (M_n = 970 or 3330, 0.30 mmol unit) were dissolved in NMP (1.5 mL). The mixture was stirred for 12 h at 100 °C with bubbling CO_2. The composition in the mixture was confirmed by ^{1}H NMR analysis. The product from GPE was isolated by precipitation with water and drying under reduced pressure. The product from ECH was isolated by Kugelrohr distillation after collecting the acetone-soluble parts. The polymer was isolated as the acetone-insoluble part and used as a recycled catalyst.

TABLE 2 Reaction of Epoxides with Carbon Dioxide Catalyzed by the Branched Cationic Polymer

		Yield (%)	
Epoxide		GPE[a]	ECH[b]
Virgin polymer		99	99
Recycled	First	96	99
	Second	86	90
	Third	85	51

[a] Isolated yield after precipitation with water and drying under reduced pressure.
[b] Isolated yield after Kugelrohr distillation of the acetone-soluble parts.

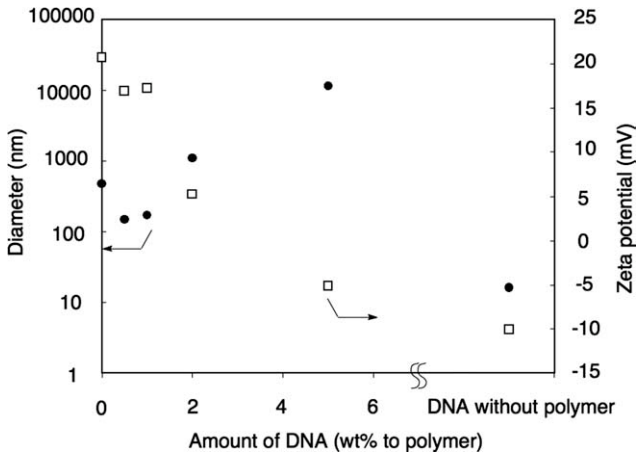


FIGURE 2 Size (solid circle) and zeta potential (open square) of polymer–DNA complexes under various DNA–polymer ratios.

Complexation of the Branched Ionic Polymer and DNA

An aqueous solution of the polymer (M_n = 3330, 20 mg/mL, 1 mL) was mixed with various amounts of Tris-HCl (0.05 M, pH = 8.0) aqueous solution of DNA (10 mg/mL, 0–5 wt % to polymer) at room temperature. The solution was adjusted to 2 mL by addition of Tris-HCl aq (0.05 M, pH = 8.0). Then, the mixture was sonicated for 1 min and filtered with a membrane filter (Whatman GMF-150, 2 μm). The filtered solution was analyzed by DLS and zeta potential measurements.

CONCLUSIONS

Branched cationic polyurethane was obtained by polyaddition of a five-membered cyclic carbonate bearing chloromethyl group and DETA. Molten salts were good media for this polyaddition by the high polarity. Owing to the ammonium structure, the polymer could serve as a catalyst and a capsule for DNA. The potential application of the branched cationic polymer is a recyclable catalyst and a gene delivery carrier. Specifically, we believe that the application to gene delivery system is promising, considering the biocompatible polyurethane backbone.

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