

## Chiral Separation with Polyurea Membrane Consisting of L-Lysinyl Residue: Proposal of Facile Method for Prediction of Permselectivity

Makoto Hatanaka, Yuki Nishioka, Masakazu Yoshikawa

Department of Biomolecular Engineering, Kyoto Institute of Technology, Matsugasaki, Kyoto 606-8585, Japan

Correspondence to: M. Yoshikawa (E-mail: masahiro@kit.ac.jp)

**ABSTRACT:** Novel chiral polyureas were prepared from L-lysine-4-nitroanilide and 2,4-toluene diisocyanate. The polyurea thus prepared gave a durable self-standing membrane that can be directly converted into molecular recognition membranes by applying an alternative molecular imprinting. The Z-D-glutamic acid (D-Glu) molecularly imprinted membrane adsorbed D-Glu in preference to L-glutamic acid (L-Glu) from racemic mixture of Glu and *vice versa*. Those two types of molecularly imprinted membrane showed optical resolution ability, adopting a concentration gradient as a driving force for membrane transport. A facile method to predict permselectivity of racemic mixture of charged permeant was proposed. From the observed membrane resistance, the permselectivity was predicted. The predicted permselectivity coincided with the observed one. © 2012 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 000: 000–000, 2012

**KEYWORDS:** chiral separation; lysinyl residue; molecular imprinting; optical resolution; permselectivity; polyurea; prediction of permselectivity

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### INTRODUCTION

Chirality plays an important role in biological processes.<sup>1,2</sup> One of familiar examples is a difference between a taste of D-glutamic acid and that of L-glutamic acid (L-Glu). L-Glu, which is usually used as a seasoning in Japan, is tasty, whereas the antipode, D-Glu, gives a different taste. A lot of optically active compounds show different physiological activities, depending on their mirror image isomers, like glutamic acids. To this end, optically pure enantiomers are required to be obtained in various industries, involving pharmaceuticals, agrochemicals, food additives, fragrances, and so forth. Asymmetric synthesis is thought to be an ultimate way to obtain enantiomerically pure compounds from a standpoint of green chemistry. However, at the moment, all of optically pure compounds are not always synthesized by an asymmetric synthesis. Chiral separation methods, such as crystallization resolution, kinetic resolution, chromatographic separation, membrane-based separation, and so forth, are still mighty and promising methods to obtain optically pure enantiomers. Among those chiral separation methods, membrane-based separation is thought to be ecologically and economically competitive to other chiral separation methods. However, membrane-based separation has not been practically applied in the production of enantiomerically pure compounds because of the fact that suitable membrane materials aimed

for chiral separation have not been developed. The study on membrane materials for chiral separation is an interesting and an indispensable subject.<sup>3–6</sup>

Membrane-based chiral separation is divided into a couple of membrane systems, such as liquid<sup>7–10</sup> and polymeric (solid) membranes.<sup>11–29</sup> A liquid membrane consisted of a carrier with chiral environment, which worked like a transporter in a biological membrane, and a liquid of a membrane matrix.<sup>7–10</sup> In a polymeric membrane system, chiral separation was mostly attained by a membrane with a chiral environment or that bearing a chiral selector.<sup>11–25</sup> Polymeric filtration membrane with no chiral environment also expressed optical resolution ability in cooperation with enantioselective binding agents, such as bovine serum albumin.<sup>26,27</sup> Enantioselective polymeric membrane with enzyme<sup>28</sup> or nonenantioselective one with enzyme<sup>29,30</sup> showed optical resolution ability, which was categorized as kinetic resolution. From results of chiral separation with synthetic membranes so far,<sup>7–25</sup> there can be found chiral recognition sites or chiral environment in those chiral separation systems, even though the membrane system of methodology for chiral separation was different.

Amino acid residues, except glycine one, are promising building blocks for membrane materials for chiral separation. Among 38 types of amino acid residue, D-glutamyl,<sup>25</sup> L-glutamyl,<sup>20,21,25</sup> D-

aspartyl,<sup>23</sup> or L-aspartyl<sup>23</sup> residue was adopted as a diacid component for chiral polyamides. Polyamides were prepared from those amino acid residues and their chiral separation ability was investigated. Lysine residue with a couple of amino moieties is another promising building block for membrane materials, which will be synthesized by a step-growth polymerization. From this, polyurea was prepared from L-lysine ethyl ester (L-Lys-OEt) and 1,4-phenylene diisocyanate (1,4-PDI), and their chiral separation ability was studied, adopting racemic glutamic acids as a model racemate.<sup>24</sup> In this study, polyurea was prepared from L-lysine-4-nitroanilide (L-Lys-4-NA), which is a building block introducing asymmetric center into polyurea, and 2,4-toluene diisocyanate (2,4-TDI) as a diisocyanate component, and their optical resolution ability was investigated. In the last part of this study, the authors proposed a facile way to predict permselectivity of racemic mixture of amino acids by a simple membrane resistance measurement.

## EXPERIMENTAL

### Materials

L-Lys-4-NA dihydrobromide was purchased from BACHEM AG (Bubendorf, Switzerland) and used without purification. 2,4-TDI was purchased from nacalai tesque (Kyoto, Japan) and used as received. N- $\alpha$ -Benzyloxycarbonyl-D-Glu (Z-D-Glu) and N- $\alpha$ -benzyloxycarbonyl-L-Glu (Z-L-Glu) were purchased from Watanabe Chemical Industries (Hiroshima, Japan) and used without purification. Methanol, ethanol, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), D-Glu, and L-Glu were obtained from commercial sources and used as received. Dimethylacetamide (DMAc) and triethylamine were purified by the conventional methods.<sup>31</sup> Water purified with an ultrapure water system (Simpli Lab. Millipore S. A., Molsheim, France) was used.

### General Polyaddition Procedure

Requisite amounts of chemicals were placed in a reaction flask fitted with a condenser and a thermometer. The mixture was magnetically stirred at 0°C for 18 h. The resulting solution was poured into water under rapid stirring, and the precipitate product was washed with water and then with methanol and dried *in vacuo*.

### Characterization of the Polyurea

The viscosity number was determined with an Ubbelohde viscometer at a concentration of  $5.0 \times 10^{-3}$  g cm<sup>-3</sup> in DMAc/LiCl (5 wt %) at 25°C.

The IR spectrum was recorded by using a Perkin-Elmer Spectrum GX; 64 scans at a resolution of 4 cm<sup>-1</sup> were collected with a membrane, which was prepared from HFIP solution.

The <sup>1</sup>H NMR spectrum was recorded in dimethylformamide-d<sub>7</sub> (DMF-d<sub>7</sub>) using a BRUKER AV-300 with residual partially protonated solvent of DMF-d<sub>7</sub> as an internal standard ( $\delta = 2.91$ )<sup>32</sup>.

The thermal stability of the polyurea was evaluated on a Hi-Res Modulated TGA 2950 (TA Instruments) under nitrogen at heating rate of 10°C min<sup>-1</sup>.

Dynamic mechanical thermal analysis measurement was carried out with a dynamic mechanical analyzer, RSA III (TA Instruments) in tension mode. The experiment was performed at a

frequency of 1 Hz and a heating rate of 1.0°C min<sup>-1</sup> from 100 to 180°C. The dimension of sample was 20.00 × 8.06 × 0.095 mm<sup>3</sup>.

The specific rotations were obtained with Horiba SEPA-200 polarimeter at 589 nm at ambient temperature in DMF.

### Membrane Preparation

The control membrane was prepared as follows: 60.0 mg of polyurea was dissolved in 1.20 cm<sup>3</sup> of DMAc. The polymer solution thus prepared was poured into a flat laboratory dish (6.0 cm diameter), and the solvent was allowed to evaporate at 50°C for 2 days. The thickness of the membrane thus obtained was around 18.8  $\mu$ m.

Molecularly imprinted polyurea membranes were also prepared. In the preparation of molecularly imprinted polymeric membranes, it was requisite to know the amount of the lysinyl residue in the polyurea, which was adopted as a candidate material. It was necessary to know the amount of the lysinyl residue in the polyurea to determine the molecular imprinting ratio, which was the mole ratio of the amount of print molecule to that of lysinyl residue in polyurea. In the case of molecular imprinting by adopting chiral print molecules, the lysinyl residue in the polyurea was expected to show a dominant role to construct chiral (molecular) recognition sites. In this study, the polyurea was prepared by step-growth addition reaction, in other words, there was no by-product, which simplified the polymerization process, even though biuret group was also formed in addition to urea linkage during the polymerization reaction.<sup>33,34</sup> The amount of lysinyl residue in the candidate polyurea (Exp. No. 5 in Table I) was determined to be  $2.10 \times 10^{-3}$  mol Lys per g-polyurea, assuming that the polyaddition reaction was quantitatively proceeded, in other words, the composition of the obtained polyurea was exactly equal to the monomer composition. About 60.0 mg of ( $1.26 \times 10^{-4}$  mol of lysinyl residue) of polyurea (Exp. No. 5 in Table I) and 17.7 mg ( $6.29 \times 10^{-5}$  mol) of a print molecule (Z-D-Glu or Z-L-Glu) were dissolved in 1.20 cm<sup>3</sup> of DMAc. The imprinting ratio, the mole ratio of the amount of print molecule to that of lysinyl residue in polyurea, was fixed to be 0.50. The solution was poured into a flat laboratory dish (6.0 cm diameter) and dried at 50°C for 2 days to evaporate the solvent. After drying, the print molecule was extracted from the resultant membrane by a large amount of 50 vol % aqueous ethanol until the print molecule was hardly detectable in aqueous ethanol solution by UV analysis. In this study, most of added print molecule was leached from the membrane. Thickness of the membranes thus obtained was around 24.8  $\mu$ m for the Z-D-Glu molecularly imprinted membrane and 26.4  $\mu$ m for the Z-L-Glu one.

### Adsorption Selectivity of Racemic Mixtures

Membranes were immersed in a racemic mixture of Glu solution, similar to the mixture studied in enantioselective membrane transport, that is, a 50 vol % aqueous ethanol solution of racemic Glu (each concentration,  $1.0 \times 10^{-3}$  mol dm<sup>-3</sup>), and the membranes were allowed to be equilibrated at 40°C. Aliquots of the solution at the initial stage and after equilibrium had been reached were used for quantitative estimation by liquid chromatography (LC) [Jasco PU-2080, equipped with a UV detector (Jasco UV-2075)], using a CHIRALPAK MA(+)

**Table I.** Polyaddition Reaction of L-Lys-4-NA/2HBr and 2,4-TDI<sup>a</sup>

Exp. no.	[L-Lys-4-NA]/[2,4-TDI]	L-Lys-4-NA/2HBr g (mol dm <sup>-3</sup> )	2,4-TDI g (mol dm <sup>-3</sup> )	DMAc (cm <sup>3</sup> )	TEA cm <sup>3</sup>	Yield g (%)	$\eta_i/C^b$ (g <sup>-1</sup> cm <sup>3</sup> )
1	1.00	0.6422 (2.00 × 10 <sup>-1</sup> )	0.2615 (2.00 × 10 <sup>-1</sup> )	7.05	0.45	0.5139 (77.74)	25.50
2	1.00	0.6422 (2.50 × 10 <sup>-1</sup> )	0.2613 (2.50 × 10 <sup>-1</sup> )	5.55	0.45	0.5567 (84.24)	25.75
3	1.00	0.6422 (3.00 × 10 <sup>-1</sup> )	0.2616 (3.00 × 10 <sup>-1</sup> )	4.55	0.45	0.5362 (81.11)	34.03
4	0.83	0.5352 (1.67 × 10 <sup>-1</sup> )	0.2605 (2.00 × 10 <sup>-1</sup> )	7.10	0.40	0.5092 (85.71)	69.32
5	0.83	0.5352 (2.08 × 10 <sup>-1</sup> )	0.2618 (2.51 × 10 <sup>-1</sup> )	5.60	0.40	0.5044 (84.82)	82.58
6	0.83	0.5352 (2.50 × 10 <sup>-1</sup> )	0.2614 (3.00 × 10 <sup>-1</sup> )	4.60	0.40	0.4848 (81.57)	79.61
7	0.67	0.4282 (1.33 × 10 <sup>-1</sup> )	0.2614 (2.00 × 10 <sup>-1</sup> )	7.15	0.35	0.4437 (82.73)	56.78
8	0.67	0.4282 (1.67 × 10 <sup>-1</sup> )	0.2614 (2.50 × 10 <sup>-1</sup> )	5.65	0.35	0.4319 (81.84)	64.95
9	0.67	0.4281 (2.00 × 10 <sup>-1</sup> )	0.2612 (3.00 × 10 <sup>-1</sup> )	4.65	0.35	0.4531 (85.89)	75.09

<sup>a</sup>Polymerization temp., 0°C; polymerization time, 18 h, <sup>b</sup>Measured at a concentration of 5.0 × 10<sup>-3</sup> g cm<sup>-3</sup> in DMAc/LiCl (5 wt.%) at 25°C.

column [50 × 4.6 mm (id)] (Daicel Chemicals Ind.) and aqueous copper sulfate solution as an eluent.

The amount of Glu in the supernatant subtracted from the initial amount in the solution gave the amount of Glu adsorbed by the membrane. The adsorption selectivity  $S_{A(i/j)}$  is defined as:

$$S_{A(i/j)} = ((i - \text{Glu})/(j - \text{Glu})) / ([i - \text{Glu}]/[j - \text{Glu}]) \quad (1)$$

where (*i*-Glu) and [*i*-Glu] are the amount of Glu adsorbed in the membrane and the concentration in the solution after equilibrium had been reached. Hereafter, subscripts of D and L mean the D- and the L-isomer, respectively.

### Enantioselective Transport

A membrane (3.0 cm<sup>2</sup>) was fixed tightly with Parafilm between two chambers of a permeation cell. The volume of each chamber was 40.0 cm<sup>3</sup>. A 50 vol % of aqueous ethanol solution of racemic Glu was placed in the left-side chamber (L-side) and a 50 vol % aqueous ethanol solution in the right-side chamber (R-side). Each concentration of racemic Glu was fixed to be 1.0 × 10<sup>-3</sup> mol dm<sup>-3</sup>. All experiments were carried out at 40°C with stirring. An aliquot was drawn from the permeate side (R-side) at each sampling time. The amounts of D- and L-isomers that transported through the membrane were determined by LC as described above.

The flux, *J* (mol cm<sup>-2</sup> h<sup>-1</sup>), is defined as:

$$J = Q/At \quad (2)$$

where *Q* (mol) is the amount of transported Glu, *A* (cm<sup>2</sup>) the effective membrane area, and *t* (h) is the time.

The permselectivity  $\alpha_{i/j}$  is defined as the flux ratio  $J_i/J_j$  divided by the concentration ratio [*i*-Glu]/[*j*-Glu]

$$\alpha_{i/j} = (J_i/J_j) / ([i - \text{Glu}]/[j - \text{Glu}]) \quad (3)$$

### Membrane Conductance (Membrane Resistance)

Membrane conductance was estimated from membrane resistance. The resistances of each D-Glu or L-Glu solution with membrane and without membrane were measured by using

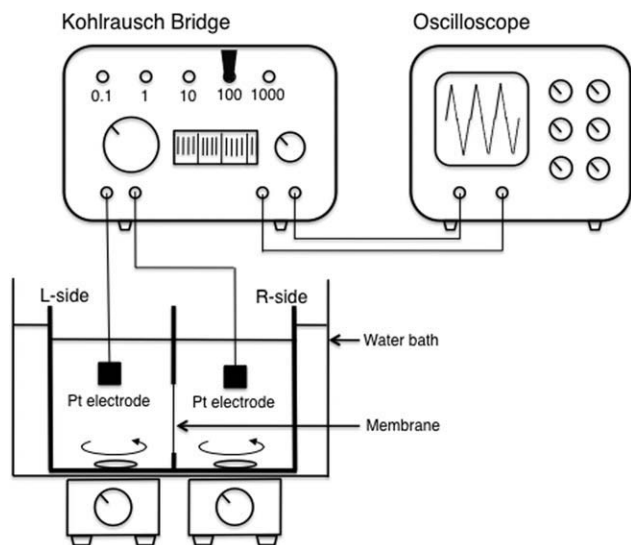
Portable Kohlrausch Bridge TYPE BF-62A (Shimadzu Rika Instruments Co.) and CO-1305 oscilloscope (KENWOOD), of which schematic diagram is shown in Figure 1. The area of each platinum electrode was 1.0 cm<sup>2</sup> and the distance between the electrodes was fixed to be 7.0 cm. A 50 vol % aqueous ethanol solution of optically pure D-Glu or L-Glu, of which concentration being 1.0 × 10<sup>-3</sup> mol dm<sup>-3</sup>, was poured into both chambers (D-side and L-side). The resistance was measured at a constant temperature of 40°C with stirring. The measurement of resistance was completed within a few minutes.

## RESULTS AND DISCUSSION

### Polymerization

The polymerization scheme of L-Lys-4-NA and 2,4-TDI is shown in Figure 2. Table I summarizes the results of polyaddition reaction of L-Lys-4-NA and 2,4-TDI. First, effect of monomer concentration on polyaddition reaction was studied adopting equimolar amount of each monomer (Exp. No. 1–3 in Table I). The viscosity number was increased with the increase in monomer concentration. The molecular weight (viscosity number) of polyureas (Exp. No. 1–3 in Table I) was too low to give a self-standing membrane. From the reaction conditions that the molar ratio of monomer, [L-Lys-4-NA]/[2,4-TDI], was unity, most linkage formed in the polyurea was thought to be a urea unit. The introduction of long side chains would both increase the molecular weight and enhance the entanglement of polymer chains. This would lead to the acquisition of durable self-standing membranes from the present polyurea. To this end, the introduction of biuret linkage into the polyurea was attempted.

The introduction of biuret linkage was attained by the addition of excess amount of diisocyanate monomer, 2,4-TDI, into the reaction mixture.<sup>33,34</sup> The polyaddition reaction with the monomer ratio, [L-Lys-4-NA]/[2,4-TDI], of 0.83 and 0.67 was studied in addition to that of unity. Those results are also summarized in Table I (Exp. No. 4–9) as well. Those results are visually shown in Figure 3 for convenience. Among those nine experiments, the polyurea obtained under the polyaddition reaction condition of the monomer ratio of 0.83 and the 2,4-TDI concentration of 2.51 × 10<sup>-1</sup> mol dm<sup>-3</sup> (Exp. No. 5 in Table I)



**Figure 1.** Schematic diagram of apparatus for resistance measurement.

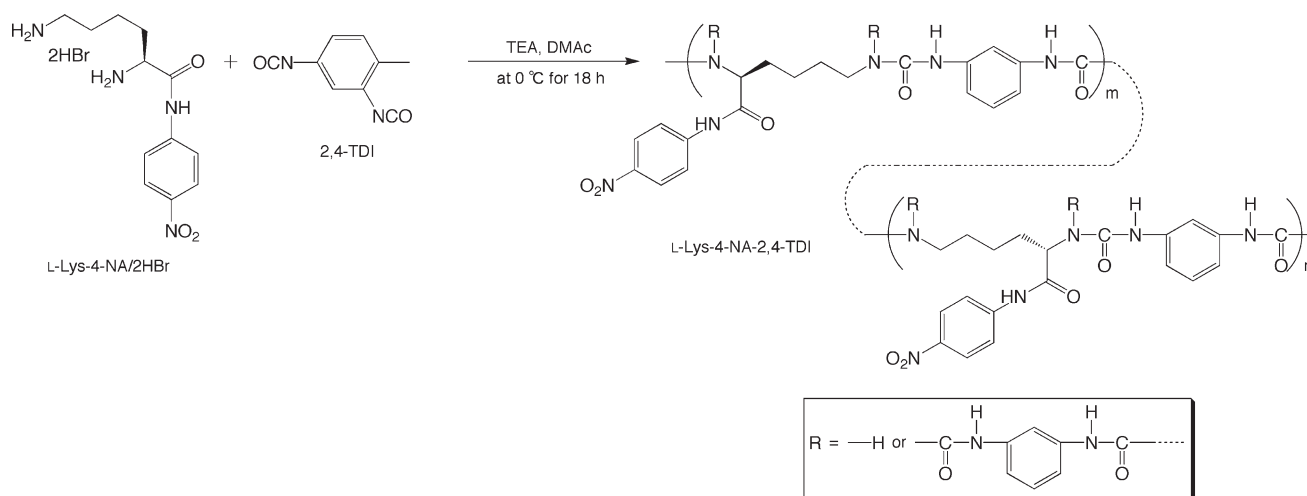
gave the highest viscosity number. In this study, hereafter, this polyurea was adopted as a membrane material.

In IR spectrum, characteristic absorption bands at  $1641\text{ cm}^{-1}$  assigned to  $\text{C}=\text{O}$  stretching band of urea and amide I band, that at  $1552\text{ cm}^{-1}$  assigned to asymmetric stretching vibration of  $-\text{NO}_2$ , and that at  $1341\text{ cm}^{-1}$  assigned to symmetric stretching vibration of  $-\text{NO}_2$  were detected.

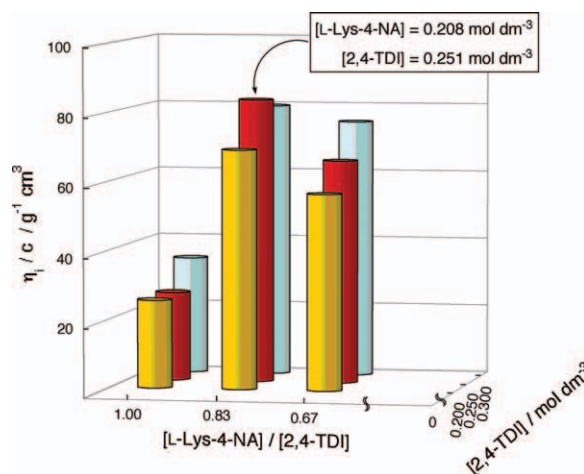
The  $^1\text{H}$  NMR spectrum of polyurea (Exp. No. 5 in Table I) is shown in Figure 4. The IR and  $^1\text{H}$  NMR spectra led to the conclusion that the expected polyurea was obtained from *L*-Lys-4-NA and 2,4-TDI. However, stereoregularity of the polyurea and the composition of urea and biuret linkages in the present polyurea were hardly determined.

### Characterization

Figure 5 shows thermogravimetric analysis thermograph of polyurea (Exp. No. 5 in Table I). From this, it cannot be concluded that thermal stability of the present polyurea was good, which



**Figure 2.** Synthetic scheme of chiral polyurea (*L*-Lys-4-NA-2,4-TDI).



**Figure 3.** Results of polyaddition reaction of *L*-Lys-4-NA and 2,4-TDI. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

might be due to the fact that the carboxyl group of lysinyl residue is protected by 4-NA moiety. Comparing the thermal stability of the previous chiral polyurea, *L*-Lys-OEt-1,4-PDI, from *L*-Lysine ethyl ester and 1,4-PDI, the present *L*-Lys-4-NA-2,4-TDI exhibited a higher degradation temperature of  $231.4^\circ\text{C}$  than the previous *L*-Lys-OEt-1,4-PDI ( $207.7^\circ\text{C}$ ).<sup>24</sup>

Figure 6 gives dependences of dynamic storage modulus  $E'$ , dynamic loss modulus  $E''$ , and  $\tan \delta$  on temperature for the polyurea (Exp. No. 5 in Table I). From degradation temperature of the present *L*-Lys-4-NA-2,4-TDI and previous *L*-Lys-OEt-1,4-PDI, the present polyurea would give higher glass transition temperature. However, the single peak on  $\tan \delta$ , assigned to the glass transition temperature, can be found at  $162.9^\circ\text{C}$ , which was lower than that of *L*-Lys-OEt-1,4-PDI ( $186.1^\circ\text{C}$ ).<sup>24</sup> The fluctuation of bulky protecting group of 2,4-NA moiety might lower the glass transition temperature of the present polyurea.<sup>33,35</sup>

The optical rotations ( $[\alpha]_D$ ) of the polyurea and the corresponding starting material of *L*-Lys-4-NA/2HBr are given in



**Figure 4.**  $^1\text{H}$  NMR spectrum of chiral polyurea (Exp. No. 5 in Table I). (300 MHz,  $\text{DMF-d}_7$ ). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

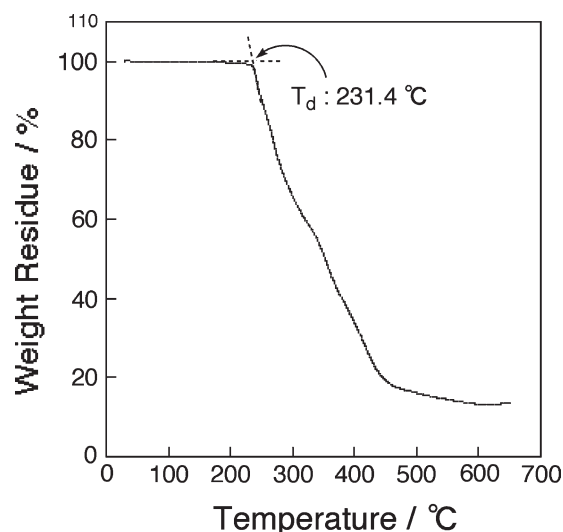
Table II. The determined optical rotations in Table II revealed that optically active polyurea was obtained. From this, the membranes from the present polyurea were expected to show optical resolution ability as chiral environment was found in the present polymers.

#### Adsorption Selectivity

Adsorption selectivity of the present polyurea membrane toward racemic mixture of Glu was studied. The results are summarized in Table III. Against expectation, the polyurea membrane (control membrane) showed low adsorption selectivity of 1.04 toward the L-isomer, though it consisted of L-lysiny residue with an asymmetric center. In this study, the polyurea was constructed from 2,4-toluene residue and lysiny residue. The 2,4-toluene component would render the polyurea rigid, however, the latter lysiny residue would be flexible by four methylene units. The existence of the latter might be the dominant reason why the control membrane showed low-chiral recognition ability, though the lysiny residue is the origin of chirality for the present polyurea.

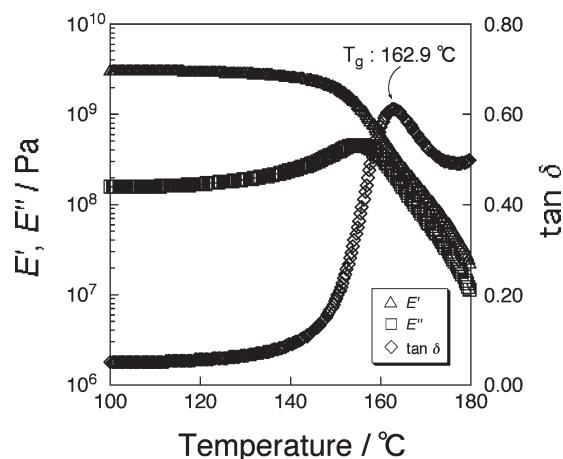
Molecular imprinting is a facile way to introduce molecular recognition sites into polymeric materials.<sup>36–41</sup> In this study, conventional molecular imprinting could not be applicable as polymeric materials, polyureas, were already prepared. In such a case, an alternative molecular imprinting is an effective and a suitable way to introduce molecular recognition sites into polymeric materials.<sup>14,16,22,24,42,43</sup> Adopting an alternative molecular imprinting, the present polyurea was directly converted into molecular recognition materials (membranes). To this end, molecularly imprinted polymeric membranes were prepared from the present polyurea and N- $\alpha$ -protected glutamic acid, such as Z-D-Glu or Z-L-Glu, as a print molecule.

The adsorption selectivities for those two types of molecularly imprinted polymeric membrane are also summarized in Table III together with that for the control nonimprinted membrane. As expected, the membrane imprinted by Z-D-Glu adsorbed D-Glu in preference to the corresponding L-isomer and *vice versa*. In other words, the Z-L-Glu imprinted membrane preferentially incorporated L-Glu into the membrane. In the previous study, molecularly imprinted polymeric membranes from



**Figure 5.** Thermogravimetric analysis curve of L-Lys-4-NA-2,4-TDI (Exp. No. 5 in Table I). (Heating rate,  $10^{\circ}\text{C min}^{-1}$ ).

synthetic polymers, such as polysulfone with carboxylic acid group<sup>44,45</sup> and aldehyde moiety,<sup>46</sup> and polyamide,<sup>47</sup> which were achiral polymeric materials, the print molecule with D-configuration and the antipode equally worked as a print molecule; in other words, the membrane imprinted by the D-isomer and that by the L-isomer showed nearly same selectivity value. Of course, the D-isomer imprinted polymeric membrane expressed adsorption selectivity toward the D-isomer and *vice versa*. Against those results, the L-isomer molecularly imprinted polymeric membrane gave a little bit higher adsorption selectivity than the membrane imprinted by the D-isomer. This is due to the fact that the present polyurea consisted of L-lysine residue as observed previously.<sup>24</sup> In the extreme case, as reported previously,<sup>16</sup> the formation of molecular recognition site was strictly dependent on the absolute configuration of candidate materials; for instance, a tetrapeptide derivative consisting of D-amino



**Figure 6.** Temperature dependence of  $E'$ ,  $E''$ , and  $\tan \delta$  for the chiral polyurea (Exp. No. 5 in Table I) at a frequency of 1 Hz and a heating rate of  $1.0^{\circ}\text{C min}^{-1}$ .

**Table II.** Specific Rotations of Diamine Monomer and Chiral Polyurea

	$[\alpha]_D$ deg $\text{cm}^2 \text{g}^{-1}$
L-Lys-4-NA/2HBr <sup>a</sup>	45.0
L-Lys-4-NA-2,4-TDI <sup>b</sup>	-4.8

<sup>a</sup>In DMF,  $c = 5.00 \text{ g dL}^{-1}$ ,  $L = 10 \text{ cm}$ , <sup>b</sup>In DMF,  $c = 1.00 \text{ g dL}^{-1}$ ,  $L = 10 \text{ cm}$ .

acid residue was converted into the D-isomer recognition materials by adopting D-isomer of print molecule and *vice versa*, and the print molecule, of which absolute configuration was opposite to that of tetrapeptide derivative, hardly worked as a print molecule. However, in this study, the present polyurea was flexible enough to construct molecular recognition sites toward D-isomer by adopting a print molecule of D-isomer. From the adsorption selectivities summarized in Table III, the polyurea consisting of L-amino acid residue was easier to construct L-isomer recognition sites than D-isomer recognition sites. The flexibility of the present polyurea was advantageous to construct D-isomer or L-isomer recognition membrane depending on the selection of print molecule, but unfavorable to give higher adsorption selectivity. The results given in Table III suggested that those membranes would show optical resolution ability. In the coming section, selective transport of racemic mixtures of Glu was studied.

#### Enantioselective Transport

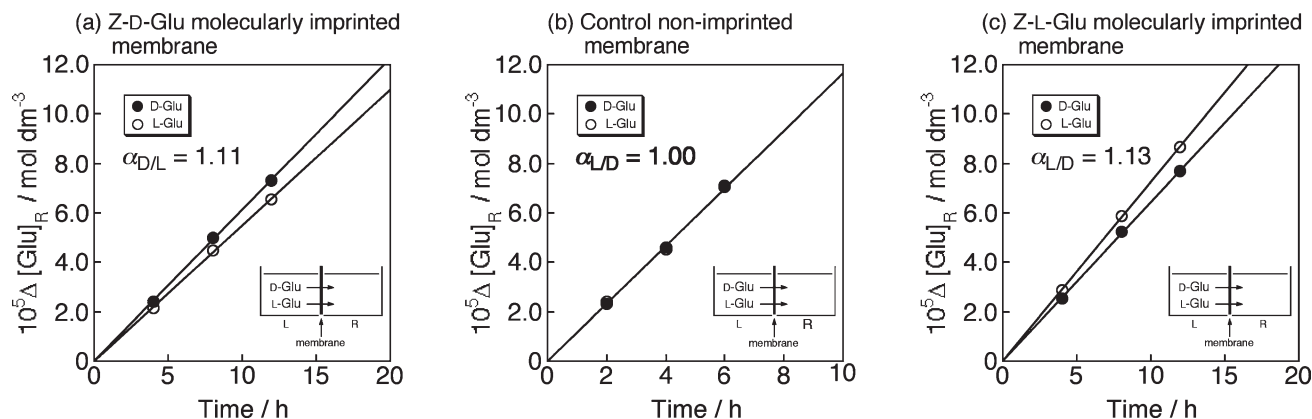
Time-transport curves of racemic mixture of Glu through three types of membrane, such as Z-D-Glu molecularly imprinted, control nonimprinted, and Z-L-Glu molecularly imprinted membranes, are shown in Figure 7. In this study, concentration gradient was adopted as a driving force for membrane transport. As anticipated from adsorption selectivity summarized in Table III, the control nonimprinted membrane hardly showed permselectivity, whereas molecularly imprinted membranes showed permselectivity. The Z-D-Glu molecularly imprinted membrane transported D-Glu in preference to L-Glu and *vice versa*, though the value of permselectivity was not so high. The permselectivity toward the D-isomer for the Z-D-Glu molecularly imprinted membrane was determined to be 1.11 and that for the Z-L-Glu molecularly imprinted one to be 1.13. In this study, the enantiomer preferentially incorporated into the membrane was selectively transported.

#### Facile method to Predict Permselectivity

As shown in Figure 7, in general, it will take a lot of time to study membrane transport phenomena, such as permselectivity and so forth. To this end, in this section, a facile method to predict permselectivity of optical resolution of a given membrane will be proposed. In this study, racemic mixture of Glu was adopted as a model racemate to investigate chiral separation ability of the present polyurea membranes. D-Glu and L-Glu give exactly same properties of electrolyte under same conditions of concentration. Assuming that a given membrane does not show permselectivity toward racemic mixture of Glu, an aqueous ethanol D-Glu or L-Glu solution with same concentration in both chambers (L-side and R-side) will show same

**Table III.** Adsorption Selectivity of Three Types of Membrane Toward Racemic Mixture of Glu in H<sub>2</sub>O/EtOH

Membrane	D-Glu		L-Glu		$S_{A(D/L)}$	$S_{A(L/D)}$
	$10^4(\text{mol g}^{-1}\text{-mem.})$	$10(\text{mol mol}^{-1} \text{ (Lys-residue)})$	$10^4(\text{mol g}^{-1}\text{mem.})$	$10(\text{mol mol}^{-1} \text{ (Lys-residue)})$		
Z-D-Glu imprinted mem	3.25	1.54	2.98	1.42	1.11	0.90
Control mem	5.85	2.78	6.02	2.86	0.96	1.04
Z-L-Glu imprinted mem	2.98	1.42	3.33	1.58	0.87	1.14



**Figure 7.** Time-transport curves of racemic mixture of Glu's through Z-D-Glu molecularly imprinted (a), control nonimprinted (b), and Z-L-Glu molecularly imprinted (c) membranes at 40°C in aqueous ethanol solution.

membrane conductance (membrane resistance). Contrary to this, in the case that a given membrane shows permselectivity toward racemic mixture of Glu, the membrane will give different membrane conductance between D-Glu and L-Glu; suppose that the membrane selectively transports *i*-isomer of Glu, a ratio of conductance  $G_i/G_j$  will agree with the permselectivity toward *i*-Glu,  $\alpha_{ij}$ . Hereafter,  $G_i$  and  $G_j$  mean membrane conductance for the membrane, which was in contact with *i*-Glu or *j*-Glu, respectively.

To this end, the membrane resistance was measured adopting apparatuses schematically shown in Figure 1. In the present measurement, the platinum electrode was in contact with aqueous ethanol solution of D-Glu or L-Glu with same concentration. Two types of solution measured in this study show identical chemical and physical properties excepting that they show opposite specific rotations, opposite optical activities. From this, a salt bridge was not requisite to be applied for the determination of membrane resistance. The observed total resistance ( $R_{\text{obs},i}$ ) con-

sists of the resistance for solution ( $R_{s,i}$ ) and that for membrane ( $R_{m,i}$ ). The membrane resistances for each Glu for three types of membrane were determined by the following equation:

$$R_{m,i} = R_{\text{obs},i} - R_{s,i} \quad (4)$$

The determined membrane resistance for each enantiomer and each membrane is summarized in Table IV. The reciprocal of the membrane resistance is referred to as the membrane conductance. Aqueous ethanol solution contained a single enantiomer of D-Glu or L-Glu in both chambers in Figure 1. The ratio of the obtained membrane conductance for each enantiomer reflects permselectivity. The predicted permselectivity can be determined by the following equation:

$$\alpha_{ij} = G_{m,i}/G_{m,j} = (1/R_{m,i})/(1/R_{m,j}) = R_{m,j}/R_{m,i} \quad (5)$$

where  $G_{m,i}$  means the membrane conductance for the enantiomer *i*-Glu and  $R_{m,i}$  is the membrane resistance for *i*-Glu. The

**Table IV.** Conductance and Membrane Performance

Membrane	$10^{-4}R_{m,D}/\Omega$	$10^{-4}R_{m,L}/\Omega$	$G_{m,D}/G_{m,L}^a$	$G_{m,L}/G_{m,D}^b$	$\alpha_{D/L}^c$	$\alpha_{L/D}^c$
Z-D-Glu imprinted mem	8.66	9.68	1.12	0.89	1.11	0.90
Control mem	7.13	7.13	1.00	1.00	1.00	1.00
Z-L-Glu imprinted mem	8.66	7.64	0.88	1.13	0.88	1.13

<sup>a</sup> $G_{m,D}/G_{m,L} = R_{m,L}/R_{m,D}$ , <sup>b</sup> $G_{m,L}/G_{m,D} = R_{m,D}/R_{m,L}$ , <sup>c</sup>Observed permselectivity.

conductance ratios thus obtained and observed permselectivities are also given in Table IV together with membrane resistances. From Table IV, it was revealed that the measurement of membrane resistance could be adopted as a facile method to predict chiral separation ability.

In this study, the membrane resistance was measured at one fixed concentration of  $1.0 \times 10^{-3}$  mol dm<sup>-3</sup>. If the concentrations of enantiomer, which was in contact with membrane, had been changed, the transport mechanism, in some case, would have been deduced. However, in this study, the observed permselectivity was not so high. Therefore, it will be faced difficulty to study the concentration dependence of permselectivity. From this, such a study has not been tried in the present study. In our future work, the concentration dependence of permselectivity should be investigated in the study on the membrane resistance so that the transport mechanism for chiral separation may be qualitatively or quantitatively elucidated.

## CONCLUSIONS

Novel chiral polyureas were synthesized from L-Lys-4-NA and 2,4-TDI. The optimum polyaddition condition was determined as follows: [L-Lys-4-NA] =  $2.08 \times 10^{-1}$  mol dm<sup>-3</sup> and [2,4-TDI] =  $2.51 \times 10^{-1}$  mol dm<sup>-3</sup> ([L-Lys-4-NA]/[2,4-TDI] = 0.83). The polyurea thus prepared gave a durable self-standing membrane and was directly converted into molecular recognition membranes by applying an alternative molecular imprinting. The Z-D-Glu molecularly imprinted membrane adsorbed D-Glu in preference to L-Glu from racemic mixture of Glu and *vice versa*. In this study, both print molecules, such as Z-D-Glu and Z-L-Glu, worked as print molecules though the chiral polyurea consisted of L-lysiny residue. Those two types of molecularly imprinted membrane showed optical resolution ability adopting a concentration gradient as a driving force for membrane transport. A facile method to predict permselectivity of racemic mixture of permeant with charge was proposed. From the observed membrane resistance, the permselectivity was predicted. The predicted permselectivity coincided with the observed one.

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