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New amphiphilic aminosaccharide derivatives as chiral selectors in capillary electrophoresis

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Abstract

Two amphiphilic aminosaccharide derivatives were investigated as chiral selector additives in capillary electrophoresis. Each substance has a glucosamine backbone carrying three hydrocarbon chains as the hydrophobic region and three carboxylic groups as the hydrophilic region, which is an artificial biologically active compound. Using each compound as a chiral selector, the optical resolution of dansylated amino acids or new quinolone antibacterial agents (NQs) was observed. Increasing the concentration of the chiral selector or the ionic strength of running solution led to successful optical resolution. In consideration of the chemical structure of each selector and the migration behavior of the enantiomers, the resolution seemed to be based on micellar electrokinetic chromatography mode. Both selectors differed in their enantioselectivity for dansylated amino acids or NQs although the chemical structures were similar.

Keywords: Chiral selectors; Enantiomer separation; Background electrolyte composition; Amino acids, Dns derivatives; Aminosaccharides; Quinolones

1. Introduction

When biologically active compounds possess a chiral center in their structures, some of them exhibit different pharmacological, toxicological, or pharmacodynamics properties between enantiomers. Owing to this, in the pharmaceutical field, it has required that a new drug should be basically developed and provided as an eutomer. For this purpose, many chiral resolution techniques such as HPLC have been utilised. In addition, recently, capillary electrophoresis (CE) has been used. CE is a powerful and sophisticated resolution technique because of its high-resolution efficiency and the use of small volume of analytes, running solution and its additives, in comparison with other known resolution techniques.

Several approaches for optical resolution by CE have been attempted [1–3]. Mostly, optical resolution by CE is carried out by the use of a running solution containing chiral compound(s). Many chiral selector additives for CE have been investigated. Especially, natural or derivatized cyclodextrins are the most effective chiral selectors and widely used for optical resolution of many compounds [4–8].

Many naturally biologically active compounds are chiral, therefore, they would be expected to act as chiral selectors for CE and other separation techniques. Recently, natural-biologically active compounds have been studied as new chiral selectors in

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CE: Anionic polysaccharides such as heparin [9] and chondroitin sulfate [10] were applied for the optical resolution of basic compounds. Armstrong et al. reported that macrocyclic antibiotics such as rifamycin B [11], vanconlycin [12,13], ristocetin A [13] and teicoplanin [13] appear to be useful chiral selectors for a variety of racemic compounds.

On the other hand, we looked for a new chiral selector for CE from synthetic chiral drugs or new molecular entities. The focus of our study in the present paper is to investigate the usefulness of two aminosaccharide derivatives (**1** and **2**: Fig. 1) as chiral selectors in CE. Each is an artificial biologically active compound, which was firstly prepared as a biological response modifier. Structurally, both are amphiphilic compounds that commonly possess a glucosamine chiral head group carrying three carboxylic groups as the hydrophilic region and three long hydrocarbon chains as the hydrophobic region. Both are relatively low UV absorbers, which is an advantageous point for running solution additive for CE. When using a running solution containing each compound, successful optical resolution of dansylated (Dns) amino acids or new quinolone antibacterial agents (NQs) were observed. Optimization of the CE conditions was examined and the resolution mechanism was also discussed.

2. Experimental

2.1. Apparatus

CE studies were performed with a CE-800 system (JASCO, Tokyo, Japan) equipped with JASCO 875 UV detector. Untreated fused-silica capillaries [750 mm (effective length 500 mm)×50 μm I.D.] were purchased from JASCO. Electropherograms were recorded on a Shimadzu C-R4A chromatopac (Shimadzu, Kyoto, Japan).

2.2. Reagents

Aminosaccharide derivatives examined in this study, **1** (2-carboxy-1-(carboxymethyl)ethyl-6-*O*-[4-carboxy-2*R*-tetradecanoylaminobutanoyl]-2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoyl-amino-α-D-glucopyranoside), and **2** (2-carboxy-1-(carboxymethyl)ethyl-6-*O*-[3-carboxy-3*S*-tetradecanoyloxypropanoyl]-2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside) were synthesized at Daiichi Pharmaceutical (Tokyo, Japan).

Dns-Amino acids were purchased from Sigma (St. Louis, MO, USA). NQs such as ofloxacin (OFLX: (±)-(*R, S*)-9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2.3-dihydro-7H-pyrido(1, 2, 3-de)-1, 4-

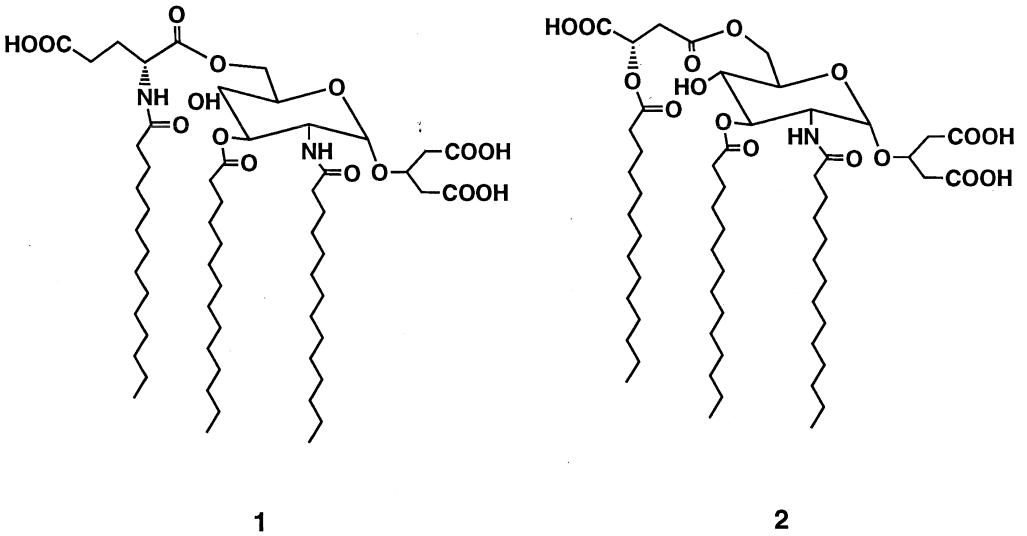

Fig. 1. Chemical structures of aminosaccharide derivatives examined as chiral selectors.

benzoxazine-6-carboxylic acid) and its related substances, DU-6859a ((−)-7-[(7*S*)-amino-5-azaspiro[2, 5]heptan - 5 - yl] - 8 - chloro - 6 - fluoro - 1 - [(1*R*, 2*S*) - *cis*-2-fluoro-1-cyclopropyl]-1, 4-dihydro-4-oxo-quinoline-3-carboxylic acid sesquihydrate) and its enantiomer were obtained from Daiichi Pharmaceutical. The chemical structures of the enantiomers resolved with **1** or **2** are shown in Fig. 2. Sodium tetraborate decahydrate and sodium hydroxide (NaOH) were purchased from Kishida Chemical (Osaka, Japan). Sodium dodecyl sulfate, methanol and 2-propanol (IPA) were purchased from Kanto Chemical (Tokyo, Japan). All the chemicals were of analytical grade and used without further purification. Water was purified with a Milli RO-Milli-Q system (Millipore Japan, Tokyo, Japan).

The aminosaccharide derivatives were dissolved in sodium tetraborate adjusted pH with 0.1 *M* NaOH and those solutions were used as the running solutions. The analytes were dissolved in 0.1 *M* NaOH.

2.3. Procedure

All experiments were performed at ambient temperature. The applied voltage was constantly held at 10 kV. The peaks of methanol, Dns-amino acids and NQs were monitored by the UV detector at 215 nm, 260 nm and 295 nm, respectively. The sample solutions were injected by siphoning (20 cm height, 5 s). The capillary was pretreated 0.1 *M* NaOH followed by the running solution under pressure for 2 min prior to analysis.

2.4. Calculation

Effective electrophoretic mobility (μ_{cp}) of the

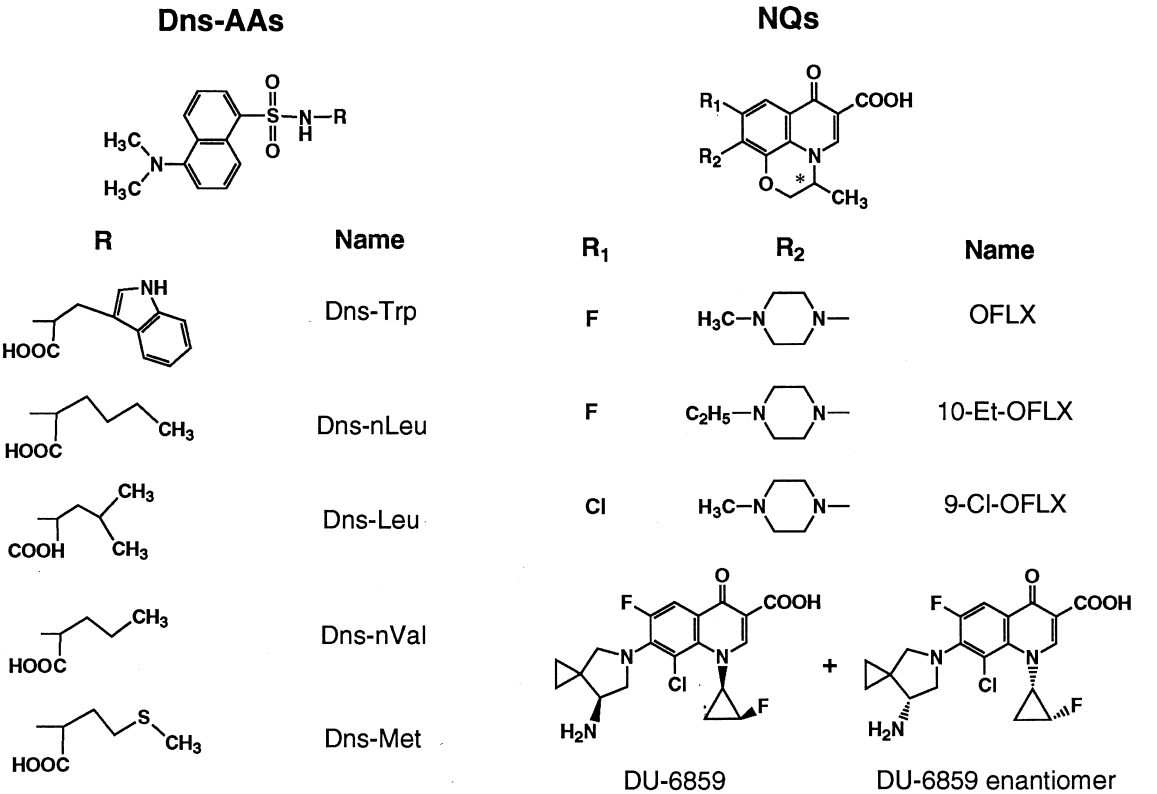

Fig. 2. Chemical structures of separable analytes with the aminosaccharide derivatives examined. AA = Amino acid; nLeu = norleucine (Nle); nVal = norvaline (Nva).

faster migrated peak in the enantiomeric pair resolved is given by

$$\mu_{cp} = lL/V(1/t_1 - 1/t_{eo}) \quad (mm^2\ V^{-1}\ s^{-1}) \tag{1}$$

where l, L, V, t_1 and t_{eo} are the effective length and total length of the fused-silica capillary, applied voltage, a migration time of the faster migrated peak in the enantiomeric pair resolved and the migration time of electroosmosis, respectively.

The capacity factor (k'_1) of the faster migrated peak in the enantiomeric pair resolved is given by

$$k'_1 = (t_1 - t_{eo})/t_{eo} \tag{2}$$

where t_1 and t_{eo}, are same as in (1).

The optical resolution (R_s) of each enantiomer was evaluated by

$$R_s = 2(t_2 - t_1)/(W_1 + W_2) \tag{3}$$

where t_1, t_2, W_1 and W_2 are the migration times of faster and slower migrated peak, the peak bandwidths of the faster and slower migrated peaks, respectively.

3. Results and discussion

3.1. Profiles of the chiral selectors studied

Table 1 shows profiles of both compounds examined. The physico–chemical properties of both are very similar. Each compound shows a positive optical rotation value due to the glucosamine backbone. 10.5 mg (ca. 10 μmol) of each is soluble in 1 ml of alkaline solution and less soluble at neutral pH. Both glucosamine derivatives are soluble not only in a polar alcohol such as methanol or IPA but also in a less polar alcohol such as 1-butanol or 1-octanol. Amphiphilic compounds are known to be capable of forming self-organized assembly above critical micellar concentration (CMC) in various solutions [14,15]. When an amphiphilic compound forms aggregations in a running solution above its CMC, hydrophobic compounds can be solubilized in them. Fig. 3 shows effects of the concentrations of **1** or **2** on the migration behavior of methanol and naphthalene. Migration times of methanol are basically independent of the concentrations of **1** or **2**. In each system, whereas, naphthalene migrates slower than methanol above a certain concentration. These results suggest that the aminosaccharide derivatives studied form self-organized aggregations above their CMCs in the running solutions, and naphthalene is solubilized in the aggregations although the aggregations have basically no affinity for methanol. Therefore, methanol is applicable as the tracer of electroosmotic flow (EOF). The CMC value of **1** by this method was found to be approximately 0.1 m*M*, which is very similar to that of **2**. It should be noted that both aminosaccharide derivatives show enantioselectivity for Dns-Nle at the concentration of 5 m*M*, as shown below. Thus, the resolution mode examined is considered to be based on micellar electrokinetic chromatography (MEKC) [16]. The aggrega-

Table 1
Profiles of aminosaccharide derivatives examined

	Compound	
	1	**2**
Molecular formula	$C_{58}H_{104}N_2O_{15}$	$C_{57}H_{101}NO_{16}$
Molecular mass	1069.47	1056.43
Appearance	White Powder	
Optical Rotation	$[\alpha]_D^{25} = +41.3°$ (0.6) mg ml^{-1}, CH_3OH)	$[\alpha]_D^{20} = +27.8°$ (0.6 mg ml^{-1}, CH_3OH)
Solubility (10 μmol ml^{-1})		
0.5 *M* Sodium tetraborate	Soluble	
Water	Less soluble	
CH_3OH	Soluble	
$CH_3(CH_2)_3OH$	Soluble	
$CH_3(CH_2)_7OH$	Soluble	

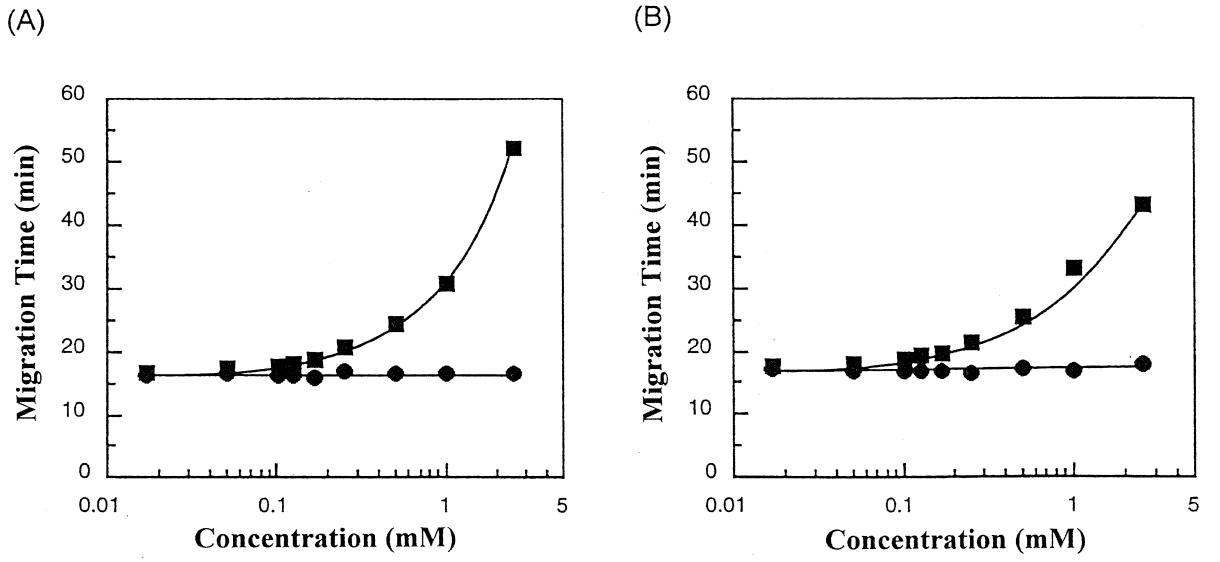

Fig. 3. Effect of the concentration of the aminosaccharide derivative examined on the migration times of methanol and naphthalene. Analytical conditions: capillary, 50 μm I.D.×750 mm (500 mm to the detector); running solution, (A) 0.0125 m*M* to 2.5 m*M* of **1** in 0.1 *M* sodium tetraborate and (B) 0.0125 m*M* to 2.5 m*M* of 2 in 0.1 *M* sodium tetraborate; temperature, ambient; applied voltage, 10 kV; detection, UV at 220 nm; injection, siphon method (20 cm, 5 s). Figure symbols (●) methanol, (■) naphthalene.

tions formed in the running solution can be regarded as a pseudo-stationary phase (PSP).

To our knowledge, there have been few presentations on the success of enantiomer resolution using mono- or di-saccharide as a chiral selector in CE [17]. In a preliminary study, we examined optical resolution of some enantiomers by CE with 2-amino-2-deoxy-D-glucopyranose. However, no enantioselectivity was observed. On the other hand, as mentioned below, **1** and **2**, possessing glucosamine backbone, exhibit enantioselectivity for some Dns-amino acids or NQs. This indicates that the amphiphilic structures play an all-important role in optical resolution. In last decade, many amphiphilic substances such as amino acid derivatives [18–21], steroids [22–26], saponins [27], natural and synthetic sugar derivatives [28–30] have been attempted on chiral selectors for CE. Recently, amphiphilic polymeric conjugated amino acids [31–33] are applied for the chiral selectors. All the chiral selectors are available above their CMCs.

*3.2. Optical resolution of enantiomers with **1***

Fig. 4A shows the results for the optical resolution of five Dns-amino acids using **1**. Thus, the aminosaccharide derivative examined has a potency of enantioselectivity in CE. In the absence of **1**, the migration time of the analytes was approximately 25 min with the migration order of Trp<Leu=Nle<Met=Nva. The net charge of Leu and nLeu is basically same because of the same migration time. On the other hand, in the presence of **1**, the migration order changed as can be seen in Fig. 4A (Met<Nva<Leu<Nle<Trp): The order depends on the hydrophobicity of each side chain. It is known that hydrophobicity of the straight chain isomer (Nle) is larger than that of the branching chain one (Leu). **1** shows the highest enantioselectivity for Trp with the migration order of D-Trp<L-Trp (data not shown), which indicates that the chiral PSP forms more stable complex with L-Trp than the corresponding enantiomer. To the contrary, **1** does not exhibit enantioselectivity for Dns-amino acids having (1) short hydrophobic side chain such as α-aminobutylic acid, (2) hydrophilic side chain such as Thr, (3) negatively charged side chain such as Glu (data not shown). Thus, the migration behavior and the enantioselectivity for Dns-amino acids in the present system are consistent with that in the octylglucoside-SDS

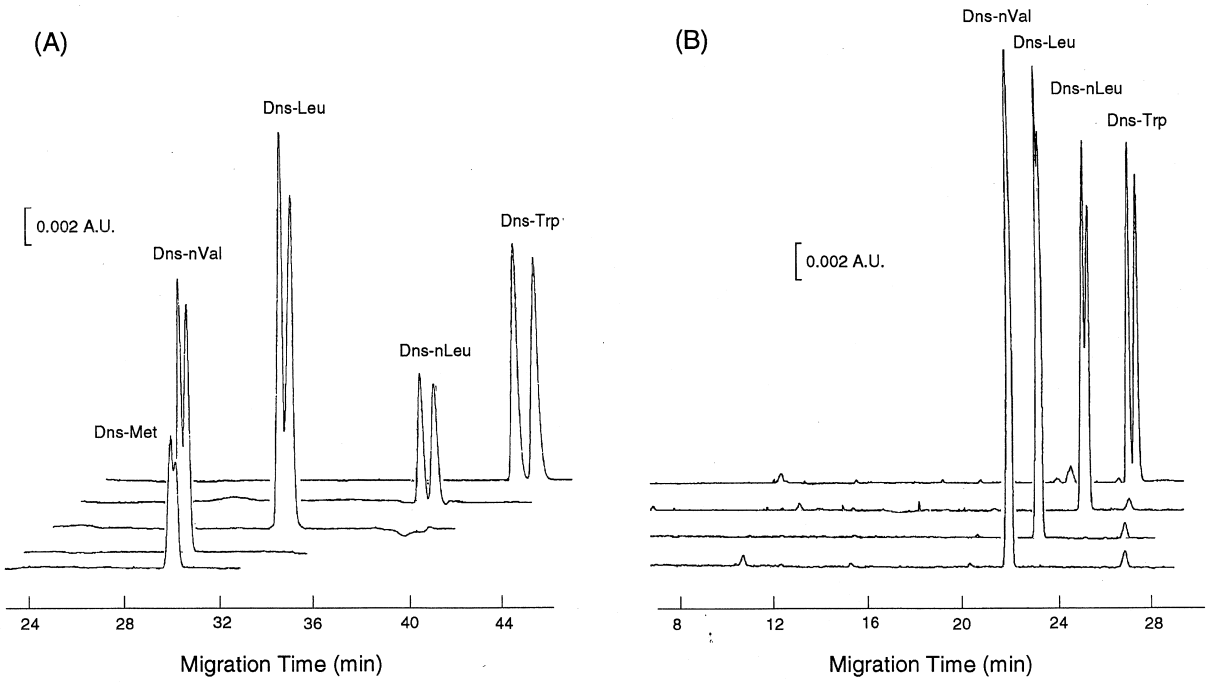

Fig. 4. Electropherograms of optical resolution of Dns-amino acids. Analytical conditions: capillary 50 μm I.D.×750 mm (500 mm to the detector running solution, (A) 20 m*M* of **1** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 9.5) and (B) 20 m*M* of **1** in 0.05 *M* sodium tetraborate–0.1 *M* NaOH (pH 9.5); temperature, ambient; applied voltage, 10 kV; detection, UV at 260 nm; injection, siphon method (20 cm, 5 s); sample, 1.0 mg ml^{-1}.

comicellar system [28] or the synthetic-glucopyranoside surfactant system [29].

In the present system, hydrophobicity of the side chain on Dns-amino acids contributes to the optical resolution. Since both surfaces of the PSP formed by **1** and Dns-amino acids are negatively charged under the investigated conditions, electrostatic repulsion occurs between them. Owing to this force, some Dns-amino acids, having lower hydrophobic side chains, have less interaction with the hydrophobic moiety of the PSP, which results in the lack of optical resolution. On the other hand, the PSP shows chiral discrimination for hydrophobic side chains. This may be due to as follows: (1) Increasing the hydrophobicity of the side chain on Dns-amino acid increases hydrophobic interaction between Dns-amino acids and inner part of the PSP, which overcome the electrostatic repulsion in both the hydrophilic moieties. (2) Owing to the greater hydrophobic interaction, pairs of enantiomers are capable of forming a more stable complex with the PSP through chiral barrier on the hydrophilic moiety. (3) As partitioning between the chiral PSP and the bulk aqueous phase, the anionic enantiomers migrate towards the detection site due to the EOF, resulting in the observation of optical resolution.

Optical resolution of Dns-amino acids is largely influenced by the concentration of the background electrolyte (BGE). Using 20 m*M* of **1** in 0.05 *M* sodium tetraborate –0.1 *M* NaOH (pH 9.5) as a running solution (Fig. 4B), the degree of optical resolution for Dns-amino acids is low in comparison with the same concentration of **1** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH at the same pH (Fig. 4A). Particularly, no enantioselectivity for Dns-Nva was observed. Furthermore, the migration times and retention of Dns-amino acids with the 0.05 *M* solution are lesser than those with the 0.1 *M* solution. Similar behavior is also observed in the CE system using **2** for the optical resolution of NQs. This finding will be discussed in below part.

Table 2 shows the effect of concentration of **1** on

Table 2
Effect of the concentration of **1** on migration behavior and optical resolution of Dns-amino acids[a]

	5 m*M*					10 m*M*					20 m*M*				
	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s
Dns-Trp	–	35.1	−2.33	1.31	0.80	–	41.3	−2.68	1.77	1.06	–	44.0	−3.04	2.14	1.51
Dns-Nle	–	33.3	−2.23	1.19	0.51	–	38.5	−2.57	1.58	1.03	–	40.2	−2.91	1.87	1.11
Dns-Leu	–	30.7	−2.08	1.02	0	–	33.4	−2.32	1.24	0.58	–	34.4	−2.65	1.46	0.87
Dns-Nva	–	29.3	−1.98	0.93	0	–	30.3	−2.13	1.03	0.45	–	30.3	−2.40	1.16	0.70
CH_3OH	15.2	–	4.11	–	–	14.9	–	4.19	–	–	14.0	–	4.46	–	–

[a] Analytical conditions: running solution, 5 to 20 m*M* of **1** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 9.5). Other conditions are as in Fig. 4.

the migration behavior and optical resolution of Dns-amino acids and EOF times, where the concentration of sodium tetraborate and pH of the running solution are fixed at 0.1 *M* and 9.5, respectively. Optical resolution of Nle and Trp is at least observed by 5 m*M* of **1**. As the concentration of **1** increases, each t_1 value increases and each μ_1 value become more negative although t_{eo} times and EOF values slightly decrease and increase, respectively. While, each k_1' value and R_s value of each pair of enantiomers dramatically increase with increasing the concentration of **1**, up to 20 m*M*.

The pH of the running solution can have an effect on the enantioselectivity when an ionizable chiral selector is used. Using 20 m*M* of **1** in 0.1 *M* sodium tetraborate 0.1–*M* NaOH, the R_s value for each pair of enantiomers was larger at pH 9.5 (Table 2) than at pH 10.0 (running solution A, Table 3) whereas EOF times and t_1 values under both conditions were almost constant. The reason for this finding may be due to that the change of pH provides different packing of the PSP and different affinity characteristic of tile solutes on the chiral PSP. It is well known that surrounding circumstances changes three-dimensional supramolecular structures formed by ionizable surfactants [14,15,34–36].

The addition of organic solvents as buffer modifiers has been reported to obtain optimum enantioselectivity in chiral CE systems [11–13,19,20,25,29,30]. To investigate the effect of an organic modifier on the migration behavior and optical resolution of Dns-amino acids, 10% IPA was added to the running solution A (=running solution B, Table 3). In the presence of IPA, each t_1 value increases, each μ_1 value becomes less negative, and EOF value decreases in comparison with **1** alone. While, all R_s values of each pair of enantiomer decrease although each k_1' value of the Dns-amino acid (except Dns-Trp) increases. Especially, no enantioselectivity for Dns-Nva was observed. In the

Table 3
Effect of the constituent of running solution containing **1** on migration behavior and optical resolution of Dns-amino acids[a]

	Running solution A					Running solution B					Running solution C				
	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s
Dns-Trp	–	43.8	−2.85	2.00	1.19	–	63.5	−1.78	1.81	0.90	–	46.4	−2.76	2.05	0
Dns-Nle	–	38.3	−2.65	1.62	0.99	–	63.6	−1.78	1.81	0.88	–	46.4	−2.76	2.05	0.78
Dns-Leu	–	34.3	−2.46	1.35	0.83	–	57.0	−1.67	1.52	0.42	–	41.7	−2.61	1.74	0
Dns-Nva	–	31.1	−2.27	1.13	0.69	–	51.8	−1.56	1.29	0	–	36.5	−2.40	1.40	0
CH_3OH	14.6	–	4.28	–	–	22.6	–	2.77	–	–	15.2	–	4.11	–	–

[a] Analytical conditions: running solution, running solution A: 20 m*M* of **1** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 10.0); running solution B: 20 m*M* of **1** in [0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 10.0)]–IPA (9:1, v/v); running solution C: 20 m*M* of **1**, [10 m*M* SDS in 0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 10.0)]–IPA (9:1, v/v). Other conditions are as in Fig. 4.

present system, increasing the retention of Dns-amino acids on the PSP does not necessarily lead to the success of optical resolution. Thus, the addition IPA to the **1** solution does not contribute to the increase of enantioselectivity for Dns-amino acids.

Also, SDS has been employed to change enantioselectivity or solubilize a selector in chiral MEKC systems [12,13,23,27,28]. In the present system, However, mixed micellar solution with SDS resulted in the reduction of chiral discrimination: When 10 m*M* SDS was added to the running solution B (=running solution C, Table 3), optical resolution of Dns-amino acids except Dns-Nle was omitted although the problem of less retention was improved. One of the reasons for the lower enantioselectivity may be due to the drastic change of the chiral barrier environment on the PSP. While, SDS may also be a large influence for not only the chiral selector but also the analytes. Armstrong et al., examined the effect of SDS concentration on the optical solution of Dns-amino acids in the vancomycin chiral selector system [12]. They reported the resolution of Dns-amino acids such as Dns-Trp, having high-affinity for SDS, decrease with increasing SDS concentration, and discussed the observation may be due to competition of SDS monomer with the analyte for the chiral selector.

*3.3. Optical resolution of enantiomers with **2***

Table 4 shows effect of the concentration of **2** on the migration behavior and optical resolution of Dns-amino acids, where the concentration of sodium tetraborate and pH of the running solution are fixed at 0.1 *M* and 9.5, respectively. As well as the **1** system, the k_1' value of each faster migrated solute increases with depending on the hydrophobicity of each side chain, and also optical resolution of Dns-Nle is at least observed at 5 m*M* of **2**. However, 15 m*M* of **2** cannot provide the enantioselectivity for Dns-Trp that allows the best enantioselectivity with **1**. As mentioned in Fig. 3, the CMC values of both selectors are found to be very similar (approximately 0.1 m*M*). Regarding the migration order of Dns-amino acids, the **2** system is consistent with the **1** system. The chemical structure of **2** is different from that of **1** except at C-6 position only. The difference in a part of monomer structure results in a large change of enantioselectivity, which may be attributed to the change of three-dimensional structure of the hydrophilic moiety of the self-organized assembly. Thus, in the present systems, strong retention of solutes on the PSP dose not necessarily lead to the success of optical resolution though it is considered to be one of the essential factors.

Each chiral selector exhibits different enantioselectivity for NQs as well as Dns-amino acids. When using **2** as a chiral selector, optical resolution of NQs is observed. Interestingly, no enantioselectivity for those compounds was observed using the **1** systems. Fig. 5A shows the electropherogram of optical resolution of OFLX and its related substances using 20 m*M* of **2** in 0.05 *M* sodium tetraborate–0.1 *M* NaOH solution (pH 9.5) as a running solution. The enantioselectivity of **2** largely depends on the substitution pattern of OFLX: Although OFLX can be

Table 4
Effect of the concentration of **2** on migration behavior and optical resolution of Dns-amino acids[a]

	5 m*M*					10 m*M*					20 m*M*				
	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s	t_{eo} (min)	t_1 (min)	$\mu_1\times10^{-2}$ (mm^2 s^{-1} V^{-1})	k_1'	R_s
Dns-Trp	–	NP	–	–	–	–	38.6	−2.55	1.57	0	–	40.9	−2.78	1.82	0
Dns-Nle	–	31.5	−2.10	1.06	0.51	–	36.1	−2.44	1.41	0.65	–	37.2	−2.63	1.57	0.78
Dns-Leu	–	29.7	−1.98	0.94	0	–	32.7	−2.26	1.18	0.34	–	33.6	−2.45	1.32	0.58
Dns-Nva	–	NP	–	–	–	–	NP	–	–	–	–	30.4	−2.25	1.10	0
CH_3OH	15.3	–	4.08	–	–	15.0	–	4.17	–	–	14.5	–	4.31	–	–

[a] Analytical conditions: running solution, 5 to 15 m*M* of **2** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 9.5). Other conditions are as in Fig. 4. NP: not performed.

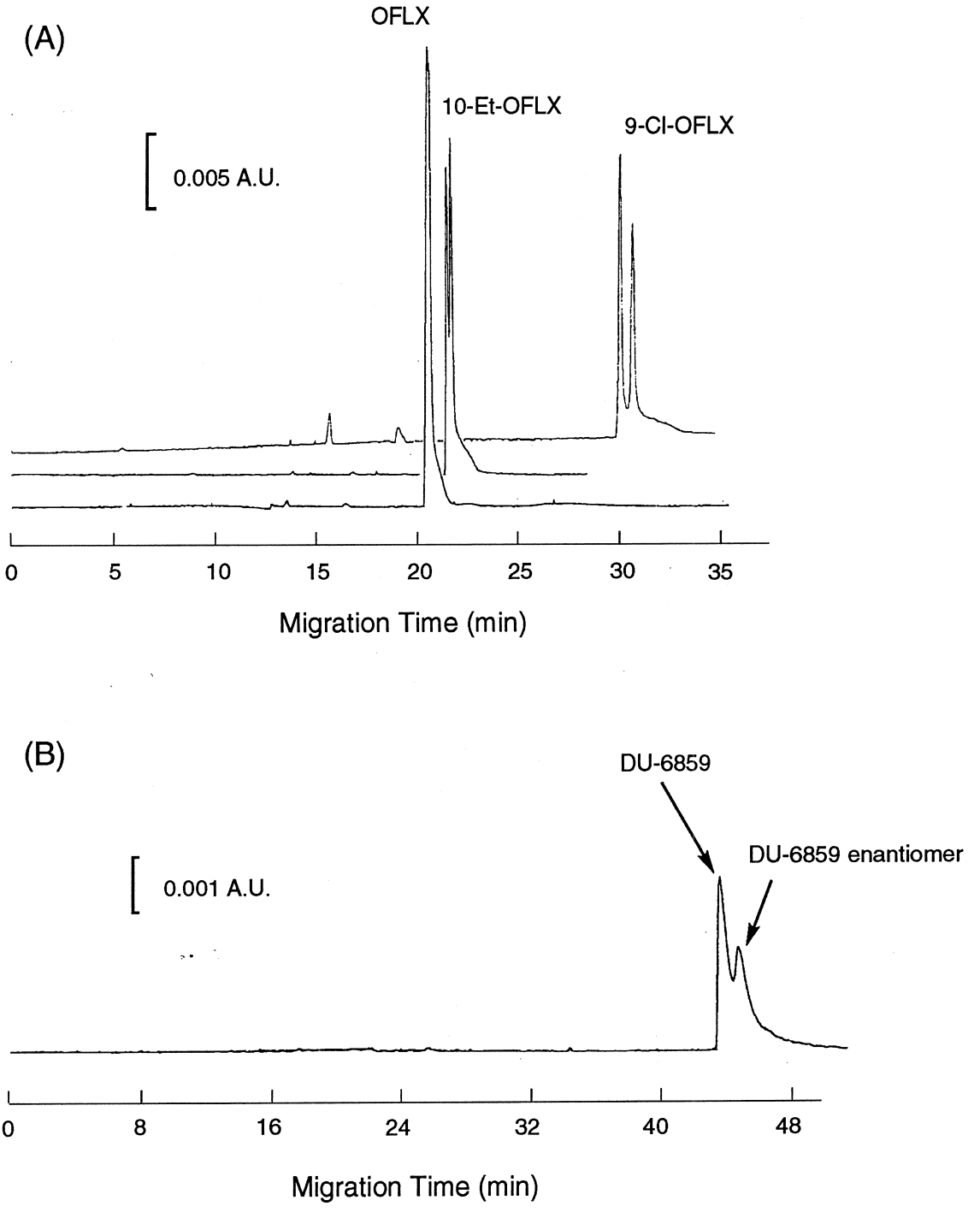

Fig. 5. Electropherograms of optical resolution of NQs with **2**. Analytical conditions: running solution, (A) 20 m*M* of **2** in 0.05 *M* sodium tetraborate–0.1 *M* NaOH (pH 9.5) and (B) 15 m*M* of **2** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH (pH 9.5); detection, UV at 295 nm; sample, OFLX, 10-Et-OFLX and 9-CI-OFLX, 1.0 mg ml^{-1}; DU-6859 enantiomers, 1.0 mg ml^{-1} DU-6859–1.0 mg ml^{-1} DU-6858 (7:3, v/v). Other conditions are as in Fig. 4.

poorly resolved under the investigated condition, for ethyl piperazino substituted OFLX at C-10 position (10-Et-OFLX) or Cl substituted OFLX at C-9 position (9-Cl-OFLX), the enantioselectivity is better than for OFLX. Thus, chemical structure of solute is sensitive to induce the enantioselectivity of **2**. Under the same analytical conditions, DU-6859a, a newly developed quinolone antibacterial agent, and its enantiomer cannot be resolved. Contrary to this, Fig. 5B shows the optical resolution of DU-6859 enantiomers using 15 m*M* of **2** in 0.1 *M* sodium tetraborate–0.1 *M* NaOH solution (pH 9.5) as a running solution. When using a greater concentration of the BGF even if at lower **2** concentration, DU-6859 enantiomers could be resolved with the migration order of DU-6859<the corresponding enantiomer.

Increasing the concentration of the BGE increases Joule heating, which generally causes a decrease of the separation efficiency in CE. However, in the present system, increasing the concentration of the BGE actually lead to an increase of enantioselectivity of **2**. As already shown in Fig. 4, similar behavior can be observed in the **1** system. These results are related to the general characteristic of ionic surfactants: Increasing the concentration of the surrounding circumstances is known to decrease the CMC value and increase of the aggregation number of ionic surfactants [14,15]. From these findings, the resolution mechanism in the present systems with higher concentration of the BGE is considered to be as follows: (1) the decrease of the electrostatic repulsion and the increase of the hydrophobic interaction occur among neighboring negatively charged polar head with increasing the concentration of the BGE, which leads to the formation of greater PSP and to the change of configuration of the chiral barrier on the PSP. (2) At the same time, a decrease of the electrostatic repulsion and an increase of the hydrophobic interaction also occur between the solute and the PSP, which leads to an increase of retention of anionic solute on the PSP. (3) The anionic charged enantiomers are migrated towards the cathode by EOF with partitioning though the chiral barrier between the bulk aqueous phase and the PSP. As a consequence of this, optical resolution of Dns-amino acids or NQs can be observed in the present system with higher concentration of the BGE.

4. Conclusion

We have shown that two new amphiphilic aminosaccharide derivatives prepared as new molecular entities for the modification of biological response offer new possibilities as chiral selectors in CE. The present investigation leads to the following conclusions.

1. The amphiphilic structures play an important role in the optical resolution: The resolution mode is considered to be based on MEKC.
2. Running solution constituents does affect their enantioselectivity: Especially, increasing of the selector concentration or the concentration of the BGE leads to an increase in their enantioselectivity. Whereas, the addition of methanol or SDS to the running solution results in the decrease of enantioselectivity.
3. Negatively charged chiral PSPs can chirally discriminate for anionic compounds such as Dns-amino acids or NQs: The decrease of electrostatic repulsion and the increase of hydrophobic interaction between the PSPs and the analyte are an important driving force for the chiral discrimination.
4. Both the selectors differed in the enantioselectivity for Dns-amino acids or NQs although the chemical structures of the monomers were similar.

We also investigated other aminosaccharide derivatives, lipid A analogs, as chiral selectors in CE, which will be described elsewhere in the future.

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References

[1] T.J. Ward, Anal. Chem. 66 (1994) 632A.
[2] M. Novotny, H. Soini, M. Stefansson, Anal. Chem. 66 (1994) 646A.

[3] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[4] H. Nishi, T. Fukuyama, S. Terabe, J. Chromatogr. 553 (1991) 503.
[5] A.M. Stalcup, K.H. Gahm, Anal. Chem. 68 (1996) 1360.
[6] Y. Mechref, Z. El Rassi, Anal. Chem. 68 (1996) 1771.
[7] T. Horimai, M. Ohara, M. Ichinose, J. Chromatogr. A 760 (1997) 235.
[8] F. O'Keeffe, S.A. Shamsi, R. Darcy, P. Schwinte, I.M. Warnner, Anal. Chem. 69 (1997) 4773.
[9] A.M. Stalcup, N.M. Agyei, Anal Chem. 66 (1994) 3054.
[10] H. Nishi, K. Nakamura, H. Nakai, T. Sato, S. Terabe, Electrophoresis 15 (1994) 1335.
[11] D.W. Armstrong, K. Rundlett, G.L. Reid III, Anal. Chem. 66 (1994) 1690.
[12] K. Rundlett, D.W. Armstrong, Anal. Chem. 67 (1995) 2088.
[13] M.P. Gasper, A. Berthod, U.B. Nair, D.W. Armstrong, Anal. Chem. 68 (1996) 2501.
[14] J.N. Israelachvili (Ed.), Bunshikan-ryoku to Hyomen-choiyoku, Asakura Shoten, Tokyo, 1995, T. Kondo and H. Oshima (Translators).
[15] The Chemical Society of Japan (Editor), Colloid-Kagaku II. Kaigo-Colloid to Hakumku, Tokyo Kagaku Dojin, Tokyo, 1995.
[16] S. Terabe, K. Otsuka, T. Ando, Anal. Chem. 57 (1985) 834.
[17] M. Saito, B. Chankvetadze, C. Yamamoto, E. Yashima, Y. Okamoto, in: Proceedings of the 9th International Symposium on Chiral Discrimination, Nagoya, 27–30 October, 1997, p. 77.
[18] A. Dobashi, T. Ono, S. Hara, J. Yamaguchi, Anal. Chem. 61 (1989) 1984.
[19] A. Dobashi, T. Ono, S. Hara, J. Yamaguchi, J. Chromatogr. 480 (1989) 413.
[20] K. Otsuka, J. Kawahara, K. Tatekawa, S. Terabe, J. Chromatogr. 559 (1991) 209.
[21] K. Otsuka, M. Kashihara, Y. Kawaguchi, R. Koike, T. Hisamitsu, S. Terabe, J. Chromatogr. A 652 (1993) 253.
[22] S. Terabe, M. Shibata, Y. Miyashita, J. Chromatogr. 480 (1989) 403.
[23] K. Otsuka, S. Terabe, J. Chromatogr. 515 (1990) 221.
[24] K. Otsuka, M. Kashihara, Y. Kawaguchi, R. Koike, T. Hisamitsu, S. Terabe, J. Chromatogr. A 652 (1993) 253.
[25] Y. Mechref, Z. El Rassi, J. Chromatogr. A 724 (1996) 285.
[26] A. Amini, I. Beijersen, C. Pettersson, D. Westerlund, J. Chromatogr. A 737 (1996) 301.
[27] Y. Ishihama, S. Terabe, J. Liq. Chromatogr. 16 (1993) 933.
[28] N. Nimura, C. Mitsuno, H. Itoh, T. Kinoshita, in: Proceeding of the 10th Symposium on Analytical Chemistry of Biological Substances, Kyoto, 24–25 September, 1992, p. 133.
[29] D.C. Tickle, G.N. Okafo, P. Camilleri, R.F.D. Jones, A.J. Kirby, Anal. Chem. 66 (1994) 4121.
[30] Y. Mechref, Z. El Rassi, Chirality 8 (1996) 518.
[31] A. Dobashi, M. Hamada, Y. Dobashi, J. Yamaguchi, Anal. Chem. 67 (1995) 3011.
[32] K.A. Agnew-Heard, M.S. Pena, S.A. Shamsi, I.M. Warner, Anal. Chem. 69 (1997) 958.
[33] S.A. Shamsi, J. Macossay, I.M. Warner, Anal. Chem. 69 (1997) 298.
[34] U. Seydel, K. Brandenburg, M.H.J. Koch, E.T. Rietschel, Eur. J. Biochem. 186 (1989) 325.
[35] K. Brandenburg, U. Seydel, Eur. J. Biochem. 191 (1989) 229.
[36] U. Seydel, H. Labischinski, M. Kastowsky, K. Brandenburg, Immunobiology 187 (1993) 191.