



Unusual enantiomeric separation due to residual amines in chiral crown ether stationary phase linked by long alkyl chain

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ABSTRACT

A new chiral stationary phase (CSP) in which (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was linked to a silica gel surface through a long alkyl chain and which did not contain additional aminoundecyl groups was prepared. Generally, when enantiomers containing a primary amine group are optically resolved using a crown-ether-type CSP, a higher resolution is achieved if the surface of the CSP does not contain any residual amine. In this study, the chiral separation factor and resolution factor of a CSP with a long alkyl chain such as the aminoundecyl group were unusually low in the absence of the residual aminoundecyl groups.

In this study, a chiral column was prepared by introducing a chiral selector having a long alkyl chain on the surface of silica gel to separate enantiomers of α -amino acids. Furthermore, it was confirmed that the residual-amine-containing CSP, which was easier to synthesize, facilitated more effective enantiomeric separation than the CSP without residual amines.

1. Introduction

Amino acids are present in a variety of natural ingredients such as fruit juices. Because D-amino acids have different physiological effects, the analysis of optical isomers of amino acids has always drawn the attention of many researchers. Accurate measurement of the optical purity of amino acids, which are the basic constituents of peptides, is very important for the management of raw materials. In this regard, considerable efforts have been devoted toward the development and commercialization of various chiral columns for liquid chromatography. Many researchers have investigated the column characteristics by introducing various organic molecules on the surface of silica gel used as a column filler for liquid chromatography. These studies are important to broaden the application scope of silica gel because modifying the surface of silica gel significantly changes its properties depending on the degree of deformation. In an achiral column, the adsorption properties of *n*-octyl-modified mesoporous silica gel were determined from nitrogen sorption data, and it was concluded that the pore size of silica gel significantly affected its surface properties [1].

To improve the performance of a reverse-phase HPLC column, the correlation between the retention factor and the alkyl chain length was investigated [2]. In addition, the extent of silica gel surface coverage by

the alkyl functional groups was investigated by elemental analysis to determine the separation efficiency for acidic, basic, and neutral analytes. L-Lysine-based dendritic polymer-modified porous silica was synthesized from aminopropyl silica gel and characterized through X-ray photoelectron spectroscopy, conventional Fourier transform infrared (FT-IR) spectroscopy, elemental analysis, and ¹³C nuclear magnetic resonance (NMR) spectroscopy [3]. The result of this study was effectively utilized for the derivatization of organic matter on the silica gel surface.

A chiral stationary phase (CSP) was characterized by ¹H high resolution magic angle spinning (¹H-HR/MAS) NMR and ²⁹Si-cross-polarization magic angle spinning (²⁹Si-CP/MAS) NMR spectroscopies. Furthermore, molecular recognition of the chiral resolution was investigated, and a correlation between the retention time of the (R)- and (S)-isomers on HPLC columns was established [4]. Chitosan-based CSPs were modified using *N*-nicotinoyl-L-phenylalanine and 3,5-dimethylphenylisocyanate to render them suitable for the optical separation of α -amino acids [5]. This approach was combined with the identification of chiral selectors with high solubility, and the results were used to develop a more effective chiral separation column. Armstrong et al. conducted numerous studies using organic molecules derivatized with cyclodextrin, vancomycin, and cyclofructan as CSPs [6–9]. Their studies

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contributed significantly to the field of chiral separation. Recently, they used core-shell-particle-based-vancomycin CSPs for supercritical fluid chromatography and HPLC to rapidly and effectively separate enantiomers of novel psychoactive substances (NPS), a class of pharmaceutical compounds [10].

A CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid as a chiral selector, used in this study, has been investigated in many studies [11,12]. It was used for the chiral separation of molecules containing various types of amine groups. For example, after the first analysis of fluoroquinolone antibacterial agents [11], the CSP has been widely used to separate enantiomers with primary or secondary amino groups [12]. The amount of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid introduced as a chiral selector on the CSP was measured by elemental analysis based on the carbon and nitrogen contents [13]. It is typical to calculate the amount of chiral selector introduced into the unit area ($\mu\text{mol}/\text{m}^2$) instead of calculating the amount of carbon added to a certain weight of silica gel. Crown-ether-based CSPs are known to have high chiral resolution if the chiral selector does not encounter unnecessary interference from the silanol (Si-OH) or amine ($-\text{NH}_2$) groups on the silica gel surface [12].

Hyun and Cho compared the chiral resolution of two crown-ether-based chiral selectors linked to an aminopropyl functional group on the silica gel surface [14]. They prepared a chiral column with no residual aminopropyl groups on the silica gel surface. Its structure was same as that of a previously prepared CSP [15]. The absence of residual

amine groups was highly advantageous as the preferential complex formation of the analyte ammonium group in the cavity of the crown ether could proceed smoothly, without any competition for the chiral resolution of the analytes. When the enantiomers of α -amino acids were optically resolved in two columns, the CSP without residual aminopropyl groups was found to be more efficient. In addition, Hyun et al. prepared a CSP in which the alkyl chain was changed from propyl to dodecyl to lengthen the tether; this would eliminate the interference of the silanol (Si-OH) functional groups on the silica gel surface during the chiral resolution in the column [16]. In general, the column with a longer chain showed higher optical resolution than that with a shorter chain. Nevertheless, the long-chain column had residual aminoundecyl groups.

Thus, for the first time, we attempted to prepare a chiral column with a chiral selector connected to the silica gel surface through a long alkyl chain, without any residual amine groups. We used the new column and the column containing aminoundecyl groups, and compared the optical resolution of α -amino acids.

2. Experimental

2.1. Preparation of C_{11}NH_2 -CSP 1 and C_{11} -CSP 2

Chiral columns C_{11}NH_2 -CSP 1 and C_{11} -CSP 2 were prepared using the procedure shown in Fig. 1A and B.

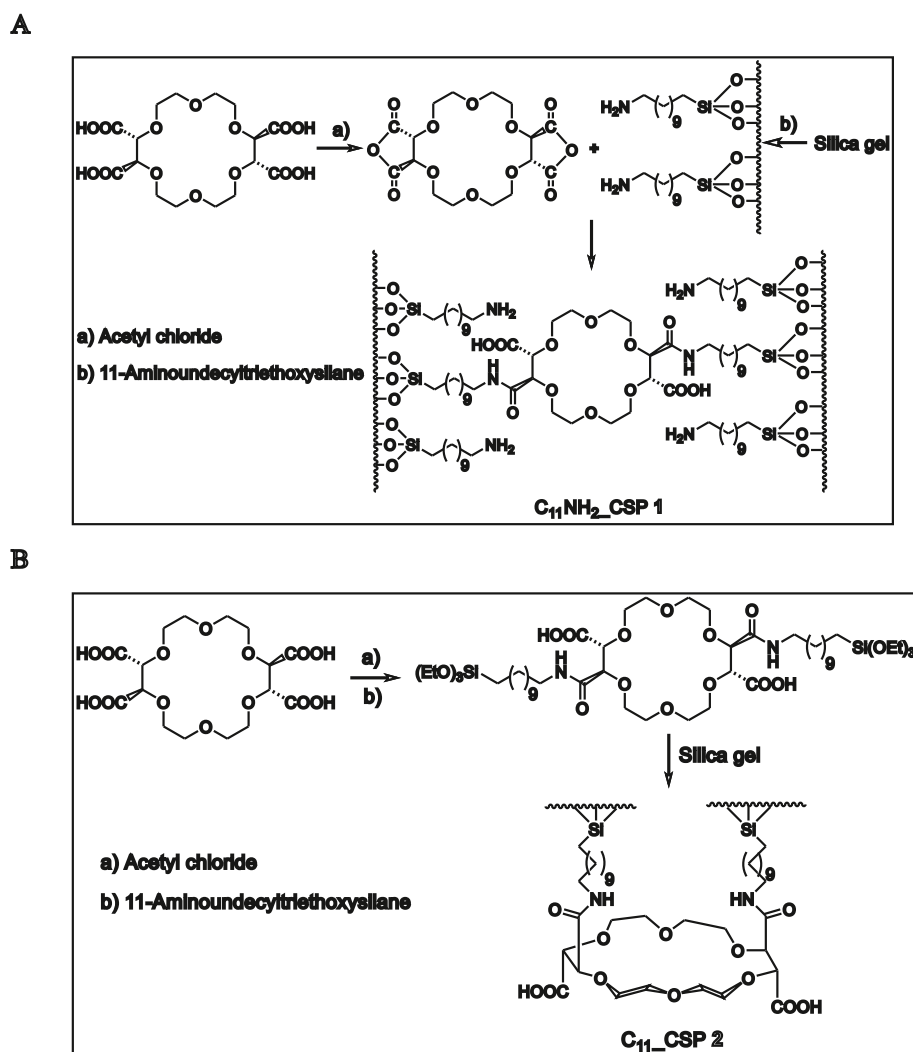


Fig. 1. Synthesis of C_{11}NH_2 -CSP 1 (A) and C_{11} -CSP 2 (B).

We further characterized C₁₁NH₂_CSP 1 and C₁₁_CSP 2. Researchers who prepared columns by synthesizing a silica-gel-based CSP generally calculated the amount of chiral selectors introduced through elemental analysis [13]. The results of elemental analysis are summarized in Table 1. The carbon content suggests that 11-aminoundecyl groups were additionally introduced into C₁₁NH₂_CSP 1, and similar amounts of the chiral selector were derivatized in both the phases.

Furthermore, this is the first study in which the chiral selector introduced on the silica gel surface was confirmed by time-of-flight secondary ion mass spectrometry (TOF-SIMS).

To confirm the successful preparation of the CSPs, bare silica gel, 11-aminoundecyl SP, C₁₁NH₂_CSP 1, and C₁₁_CSP 2 were analyzed by scanning electron microscopy (SEM; Fig. 1S) and FT-IR spectroscopy (Fig. 2S). SEM images of the silica gel were visualized during the preparation of the CSPs. Fig. 1S shows that the bare silica gel, which is the starting material, maintains a spherical shape with almost no cracks. However, the three silica gels derivatized with organic materials showed minor cracks. Therefore, stirring using a magnetic bar must be very slow during synthesis. FT-IR spectra (Fig. 2S) shows C–H stretching peaks, which were absent in the spectra of the bare silica gel, at 2936 and 2861 cm⁻¹ for the three derivatized silica gels. In addition, the intensity of the peak at 974 cm⁻¹, which corresponds to the Si–O bending mode of silanol, was lower for the derivatized silica gel than that for the pure silica gel, indicating that the silica gel surface was partially coated.

2.1.1. Synthesis of C₁₁NH₂_CSP 1 with 11-aminoundecyl residues on silica gel surface

Silica gel (6.21 g, Kromasil 5 μm, 100 Å, 320 m²/g) was taken in a 250 mL two-necked flask, and water was removed azeotropically using a Dean-Stark trap device with 150 mL of toluene. 11-aminoundecyltriethoxysilane (Gelest, 95.0%, 1.01 g, 2.87 mmol) was added and refluxed for 72 h. The synthesized 11-aminoundecyl silica gel was filtered, washed successively with methanol, ethyl acetate, methylene chloride, hexane, and diethyl ether to remove the remaining silane compound, and dried under high vacuum to completely remove the solvent. Elemental analysis revealed that the carbon and nitrogen contents were 6.27% and 0.64%, respectively. The loading of organics on the surface of the modified silica gel was also calculated using a previously reported equation [13], and the carbon content according to this equation was 1.62 μmol/m². Using a known method [11], C₁₁NH₂_CSP 1 was synthesized using (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (0.61 g, 1.35 mmol). Elemental analysis revealed the carbon and nitrogen contents of 9.41% and 0.58%, respectively (Table 1). The concentration of chiral selector, as calculated from the amount of modified silica gel per gram of silica gel, was 0.16 mmol, and the carbon content was 2.16 μmol/m². This is the increased carbon content as a result of the two processes of introducing chiral selectors into the 11-aminoundecyl stationary phases.

2.1.2. Synthesis of C₁₁_CSP 2 without 11-aminoundecyl residues on silica gel surface

C₁₁_CSP 2 was prepared as shown in Fig. 1B. (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid (0.61 g, 1.35 mmol) in 15 mL of acetyl chloride was refluxed for 24 h to afford its dianhydride, followed by drying under high vacuum to afford off-white solid product.

Table 1

Additional carbon and nitrogen contents on the silica gel surface determined by elemental analysis.

Stationary phases	N (%)	C (%)	Carbon content (μmol)/silica (m ²)	Chiral selector (mmol)/silica (g)
11-Aminoundecyl SP	0.64	6.27	1.62	–
C ₁₁ NH ₂ _CSP 1	0.58	9.41	2.16	0.16
C ₁₁ _CSP 2	0.50	8.47	0.67	0.19

The synthetic intermediate in Fig. 1B was identified through liquid ¹H NMR spectroscopy.

¹H NMR (200 MHz, CDCl₃, δ): 0.60–0.63 (t, 4H), 1.19–1.39 (m, 50H), 1.52–1.54 (m, 4H), 3.28–3.34 (m, 4H), 3.49–3.74 (m, 16H), 3.76–3.85 (m, 12H), 4.43–4.46 (m, 4H).

The dianhydride was dissolved in 20 mL of methylene chloride, and 2,6-lutidine (0.38 mL, 3.22 mmol) and 11-aminoundecyltriethoxysilane (Gelest, 95.0%, 0.94 g, 2.68 mmol) were added sequentially in a drop-wise manner. After allowing the reaction to proceed for a considerable amount time, the mixture was concentrated under vacuum to completely remove the solvent. Simultaneously, silica gel (6.18 g, Kromasil 5 μm, 100 Å, 320 m²/g) was added to a 250 mL two-necked flask, and water was removed azeotropically using a Dean-Stark trap device with 150 mL of toluene. After the complete removal of water, this concentrated product was dissolved in 10 mL of methylene chloride and refluxed for 72 h. The synthesized silica gel was filtered, washed successively with methanol, ethyl acetate, methylene chloride, hexane, and diethyl ether, and then dried under high vacuum. Elemental analysis revealed carbon and nitrogen contents of 8.47% and 0.50% in C₁₁_CSP 2, respectively. The concentration of the chiral selector, as calculated from the amount of modified silica gel per gram of silica gel, was 0.19 mmol, and the carbon content was 0.67 μmol/m².

2.2. Confirmation of derivatization on CSP via TOF-SIMS

To the best of our knowledge, the successful preparation of CSPs has never been confirmed through TOF-SIMS. In this study, the relative intensities of the NH₃⁺ ions obtained from TOF-SIMS for different silica gel samples were compared.

TOF-SIMS is a highly sensitive analytical technique for elucidating the chemical composition and has the advantage of directly analyzing the components present on the surface, without the need of pretreatment. For the analysis, the synthesized products were made into a tablet. TOF-SIMS was performed on a TOF-SIMS 5 (ION-TOF GmbH, Münster, Germany) instrument at the KBSI Busan center. Bi₃⁺ was used as a primary ion beam at an acceleration voltage of 30 keV, and the chemical images of the analyzed area were recorded with 128 × 128 pixel resolution during data acquisition. The mass scale of the spectrum obtained from TOF-SIMS was calibrated using H⁺, CH₃⁺, C₂H₅⁺, C₃H₇⁺, and C₄H₉⁺ in the positive mode.

TOF-SIMS was conducted to directly analyze the compounds introduced on the silica gel surface at each step. Fig. 2 shows the results of TOF-SIMS for the unreacted bare silica gel, 11-aminoundecyl SP, C₁₁NH₂_CSP 1, and C₁₁_CSP 2. It is evident that an 11-aminoundecyl group was introduced in the bare silica gel, resulting in a large NH₃⁺ peak. For C₁₁NH₂_CSP 1 and C₁₁_CSP 2, on which chiral selectors were introduced, the relative peak size was smaller than that for the 11-aminoundecyl SP. The NH₃⁺ peak of C₁₁_CSP 2 was smaller than that of C₁₁NH₂_CSP 1 because of the lack of residual amine groups in the former.

2.3. Chromatography and chiral column preparation

The two CSPs were prepared with the Alltech slurry packer (Alltech Associations, Inc. Deerfield, IL, USA) in methanol according to the well-known slurry packing method [16] on a 150 × 4.6 mm stainless steel empty HPLC column.

Liquid chromatography was performed on a Waters Acquity Ultra Performance liquid chromatography system (Milford, MA, USA) equipped with a quaternary gradient pump, an auto-sampler, a column temperature controller, and a photodiode array UV detector. The data were acquired, calibrated, and quantified by the MassLynx software (Waters). The flow rate was 0.5 mL/min. The analytes used in this study were either available from a previous study [14] or were purchased from Aldrich. Injection samples were prepared by dissolving each analyte in methanol or water at a concentration of 1.0 mg/mL. The elution order

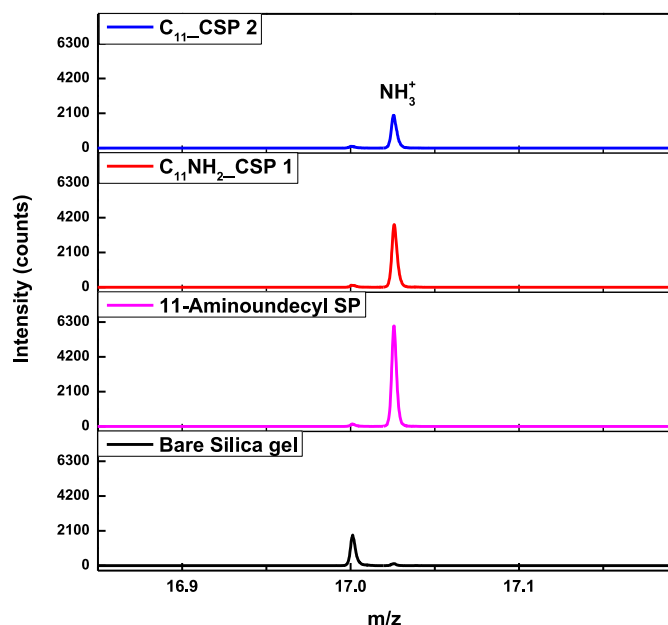


Fig. 2. NH_3^+ intensities obtained from TOF-SIMS for bare silica gel, 11-amino-undecyl SP, $\text{C}_{11}\text{NH}_2\text{-CSP 1}$, and $\text{C}_{11}\text{-CSP 2}$.

was determined by injecting samples of known configuration.

The chiral column exhibited excellent stability and reproducibility. When using the analytes in Table 2, the relative standard deviation (RSD) values of the retention times ($n = 6$) of the first eluted L-enantiomer were lower than 0.20% ($\text{C}_{11}\text{NH}_2\text{-CSP 1}$) and 0.22% ($\text{C}_{11}\text{-CSP 2}$). The separation factor (α) was found to be identical for the CSPs. Therefore, the chiral selector was found to be stably bound to the silica gel surface.

3. Results and discussion

3.1. Resolving capacity of two chiral columns as a function of temperature

Using 10-undecenoylchloride as a starting material, a chiral column was prepared by introducing an amine group, protecting *t*-Boc, silylation to double bond, and introducing a long alkyl chain into the silica gel surface. The chiral column prepared in this manner was more effective than the CSP linked by aminopropyl groups; this was a manifestation of the spacer length [16]. Although racemic α -amino acids were

enantioseparated with $\text{C}_{11}\text{NH}_2\text{-CSP 1}$, alanine, methionine, phenylalanine, and tyrosine were also optically resolved at the same temperature with $\text{C}_{11}\text{-CSP 2}$ to compare the efficiency of the two columns.

Both the chiral columns had the same long alkyl group attached to them. However, to compare the optical resolution efficiency based on the presence or absence of residual amine groups, four types of α -amino acids were optically resolved. The results are summarized in Table 2. It is very important to measure the dead time (solvent front) to compare the capacity (k) and separation factor (α) of the enantiomers. In this experiment, chloroquine, which was also used in a previous study [17], was injected to set the t_0 value; the results are summarized in Table 2. As expected, the t_0 value decreased with increasing temperature and was slightly higher for $\text{C}_{11}\text{-CSP 2}$ than for $\text{C}_{11}\text{NH}_2\text{-CSP 1}$. As the temperature increased from 20 to 40 °C, α and resolution factor (R_S) decreased. The optical resolving capacity improved with decreasing column temperature. The retention time of the first eluted enantiomer was shorter in $\text{C}_{11}\text{NH}_2\text{-CSP 1}$ than in $\text{C}_{11}\text{-CSP 2}$. It is presumed that unnecessary amine groups on the silica gel surface of $\text{C}_{11}\text{NH}_2\text{-CSP 1}$ are competitively coordinated to the 18-crown-6 cavity through the amine group of the analyte. It is known that when a silica gel surface is linked to a chiral selector with an aminopropyl group, the CSP with unnecessary amine groups confers low optical resolution than a chiral column without amine groups [14]. However, in this study, the chiral column linked with an amino-undecyl group, which is a longer alkyl chain, showed the opposite trend—when separating the α -amino acid enantiomers, $\text{C}_{11}\text{NH}_2\text{-CSP 1}$ exhibited a higher optical resolution than $\text{C}_{11}\text{-CSP 2}$. We inferred that the optical resolving ability of the chiral column with the relatively short aminopropyl group was weak because the amine groups in the stationary phase and the amine functional group in the analyte competed for complexation with the 18-crown-6 ether cavity of the chiral selector.

When the chiral selector was linked to a silica gel surface with an amino-undecyl group, the capacity factor (k_1) was found to be higher for $\text{C}_{11}\text{-CSP 2}$, which had no residual amine groups, than for $\text{C}_{11}\text{NH}_2\text{-CSP 1}$, which contained residual amine groups. For a chiral column with a short alkyl group [14], k_1 of the first eluted L-enantiomer did not show any specific trend. Among alanine, methionine, phenylalanine, and tyrosine, the k_1 values of the first two amino acids were slightly higher in the column with residual amine groups, while those for the latter two amino acids were higher in the column without the amine groups. However, in the two columns with a long alkyl group, the k_1 values were lower for $\text{C}_{11}\text{NH}_2\text{-CSP 1}$. Similar to the chiral column with aminopropyl groups, it is considered that the presence of amine groups in $\text{C}_{11}\text{NH}_2\text{-CSP 1}$ results in a competitive complex mechanism because of the hydrophilic nature of this functional group.

Nevertheless, the enantiomer resolving capacity of $\text{C}_{11}\text{-CSP 2}$ was

Table 2

Separation of α -amino acid enantiomers on $\text{C}_{11}\text{NH}_2\text{-CSP 1}$ and $\text{C}_{11}\text{-CSP 2}$ at various temperatures.

$\text{C}_{11}\text{NH}_2\text{-CSP 1}$	20 °C			30 °C			40 °C		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
Alanine	0.26	2.62	3.87	0.32	2.25	3.81	0.38	1.94	3.14
Methionine	0.58	3.31	7.54	0.57	2.82	6.83	0.58	2.34	6.53
Phenylalanine	0.82	3.29	8.97	0.76	2.92	8.50	0.72	2.47	8.48
Tyrosine	0.50	3.03	6.85	0.48	2.65	6.28	0.48	2.20	5.40
$\text{C}_{11}\text{-CSP 2}$	20 °C			30 °C			40 °C		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
Alanine	0.36	2.29	2.64	0.35	2.08	2.08	0.36	1.79	1.75
Methionine	0.78	3.03	4.80	0.66	2.71	3.47	0.60	2.29	2.86
Phenylalanine	1.05	2.99	4.79	0.85	2.76	4.70	0.74	2.40	4.31
Tyrosine	0.68	2.81	4.26	0.55	2.54	3.86	0.48	2.17	2.95

Chromatographic conditions: Mobile phase, 50% methanol in water + sulfuric acid (1.0 mM); flow rate, 0.5 mL/min; detector, UV 210 nm k_1 : Retention factor of the first eluted enantiomer, α : Separation factor, R_S : Resolution factor.

*Retention time (t_0) of chloroquine.

- $\text{C}_{11}\text{NH}_2\text{-CSP 1}$: 3.33 min (20 °C), 3.12 min (30 °C), 2.88 min (40 °C).

- $\text{C}_{11}\text{-CSP 2}$: 3.37 min (20 °C), 3.21 min (30 °C), 2.98 min (40 °C).

lower than that of C₁₁NH₂_CSP 1. Separation factor (α) was higher for the column with a short alkyl group without residual amine groups, while it was higher for the column with a long alkyl group for C₁₁NH₂_CSP 1, which contained residual amine groups. There was no significant difference in R_s of the two columns for the short alkyl chain column. For the long alkyl chain column, R_s was significantly higher for C₁₁NH₂_CSP 1. This is also evident from the representative chromatograms (Fig. 3) obtained after chiral resolution using the two columns.

It is considered that the chiral selector is positioned relatively far from the silica gel surface upon the introduction of a long alkyl group. Owing to this, high separation efficiency is achieved in the presence of the hydrophilic amine groups in the stationary phase in the 50% water–methanol solvent system. Moreover, a chiral selector linked to a long alkyl chain will be more flexible than that linked to the silica gel surface with an aminopropyl group.

Therefore, C₁₁NH₂_CSP 1 with unreacted aminoundecyl groups would be less flexible than C₁₁_CSP 2. This is similar to the case in which the chiral selector has a stable doubly tethered structure; such a structure confers more effective enantioseparation [12]. The residual aminoundecyl group on C₁₁NH₂_CSP 1, as shown in Fig. 1A, acts as a support for reducing the flexibility of the chiral selector linked to the long alkyl chain, thus increasing the stability with increasing rigidity compared to those of the chiral selector of C₁₁_CSP 2. To confirm this, an additional experiment to compare the enantiomeric resolution of the α -amino acids based on the length of alkyl groups was needed. When two chiral columns were used to separate the enantiomers of various racemic flecainide molecules, the α -amino acids showed opposite chiral recognition ability. This study will be published in due course.

3.2. Enantiomeric separation of α -amino acids on two chiral columns C₁₁NH₂_CSP 1 and C₁₁_CSP 2

The chromatographic resolution data of C₁₁NH₂_CSP 1 and C₁₁_CSP 2 shown in Table 3. Table 3 shows that the k_1 of all α -amino acids was higher in C₁₁_CSP 2 than in C₁₁NH₂_CSP 1; consequently, the retention time was longer in the former. This is because the chiral selector of C₁₁_CSP 2 was loaded with more than 0.02 mmol/g of silica gel or the C₁₁NH₂_CSP 1 column with residual amine groups in the stationary phase behaved similar to a chiral column linked with aminopropyl silane [14].

Nevertheless, except for serine, α and R_s of all α -amino acids were higher on C₁₁NH₂_CSP 1 than on C₁₁_CSP 2. Overall, the aminoundecyl group with a long alkyl chain connecting the chiral selector and the silica gel surface conferred a higher optical resolution than the relatively short aminopropyl group. In the enantiomeric separation of α -amino acids, the chromatographic resolution ability was weaker in the absence of residual amine groups with a long alkyl chain on silica gel surface. Because the diamide intermediate produced during the preparation of

Table 3

Resolution of α -amino acid enantiomers on C₁₁NH₂_CSP 1 and C₁₁_CSP 2.

Amino acids	C ₁₁ NH ₂ _CSP 1			C ₁₁ _CSP 2		
	k_1	α	R_s	k_1	α	R_s
Alanine	0.26	2.62	3.78	0.36	2.29	2.64
Arginine	0.08	4.54	3.06	0.13	3.58	2.57
Asparagine	0.10	1.53	0.87	0.16	1.24	0.08
Aspartic acid	0.28	1.51	1.93	0.35	1.48	1.08
Cysteine	0.53	1.42	2.02	0.67	1.28	0.76
Glutamic acid	0.28	2.65	4.97	0.39	2.50	2.96
Leucine	0.35	4.03	7.48	0.49	3.45	4.50
Methionine	0.58	3.31	7.33	0.78	3.03	4.80
Phenylalanine	0.82	3.29	8.97	1.05	2.99	4.79
Phenylglycine	0.63	3.33	8.03	0.90	3.18	5.01
Serine	0.19	1.60	1.24	0.23	1.90	1.59
Threonine	0.03	1.00	0.00	0.05	1.00	0.00
Tyrosine	0.50	3.03	6.85	0.68	2.81	4.26
Valine	0.10	2.68	2.49	0.15	2.19	1.41
Aminobutylic acid	0.20	3.15	4.31	0.30	2.63	2.83
Norleucine	0.54	3.36	7.42	0.74	2.99	4.33
Lysine	0.47	1.00	0.00	0.44	1.00	0.00

Mobile phase: 50% methanol in water + sulfuric acid (1.0 mM). Flow rate: 0.5 mL/min. Detection: 210 nm UV. Temperature: 20 °C.

C₁₁_CSP 2 (Fig. 1B) is relatively unstable, C₁₁NH₂_CSP 1 can be prepared by directly combining aminoundecyl silica gel and dianhydride chiral crown ether. The method shown in Fig. 1A is thus more effective for the synthesis.

4. Conclusion

In the enantiomeric separation of α -amino acids using a CSP linked with aminoundecyl groups and 18-crown-6 tetracarboxylic acid, the retention time of the optical isomer was shorter in C₁₁NH₂_CSP 1 containing residual aminoundecyl groups on the silica gel surface than in C₁₁_CSP 2, which had no residual aminoundecyl groups. In addition, separation factor α and resolution factor R_s were higher in C₁₁NH₂_CSP 1 with residual amine groups. The chiral recognition ability was stronger than the conventional chiral columns containing the relatively short aminopropyl group. Therefore, during the preparation of chiral columns with long alkyl groups, which render the chiral selectors unaffected by the silica gel surface, it is recommended to prepare columns with residual aminoalkyl groups such as the aminoundecyl group.

Credit author statement

Ji Yeong Sung: Synthesis, Methodology, Validation, Formal analysis, Investigation, Writing. Sun-Mi Jin: Derivatization, SEM analysis. Sumin Lee: Formal analysis, Validation. Sung-Young An: Packing Columns, Validation. Jong Sung Jin: Writing - review & editing.

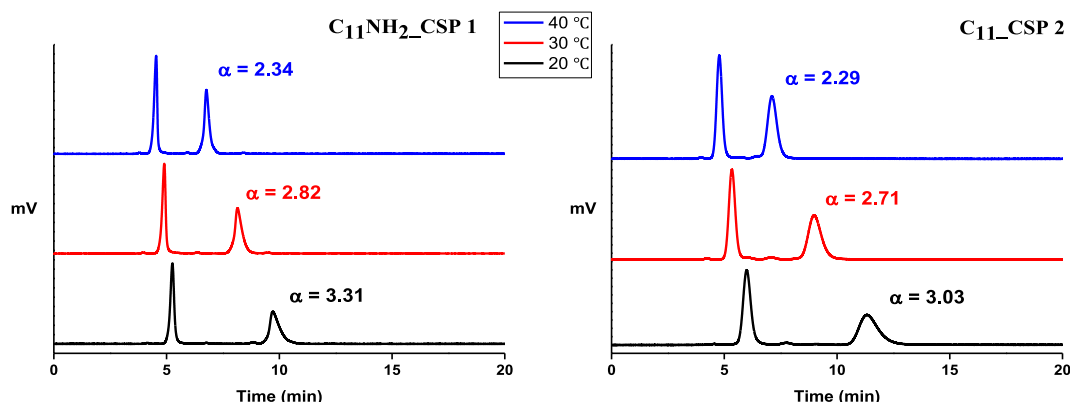


Fig. 3. Typical chromatograms for the resolution of methionine on C₁₁NH₂_CSP 1 and C₁₁_CSP 2 at different temperatures.

Conceptualization, Data Curation, Formal analysis, Visualization, Supervision. All authors have seen and approved the final version of the manuscript being submitted. We warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial or personal conflict of interest that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2021.122739>.

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