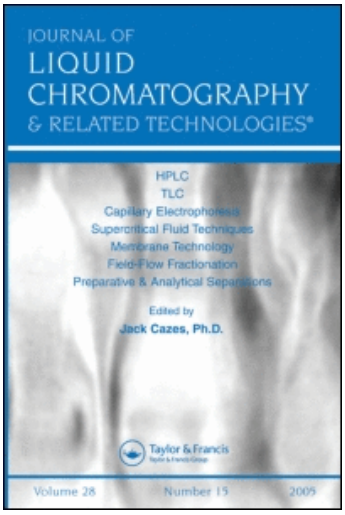

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Effect of the Tethering Groups of Chiral Stationary Phases Based on (+)-(18-Crown-6)-2,3,11,12-Tetracarboxylic Acid on the Liquid Chromatographic Resolution of β-Amino Acids

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ABSTRACT

Effect of the tethering groups of chiral stationary phases (CSPs) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid on the liquid chromatographic resolution of *β*-amino acids was investigated by resolving various *β*-amino acids on two CSPs having different tethering groups. In the resolution of *β*-amino acids with the mobile phase of 50% methanol in water, containing 10 mM acetic acid or 10 mM sulfuric acid, it was found that acetic acid is more effective as an acidic modifier than sulfuric acid on the CSP, which has an N—H amide tethering group, while sulfuric acid

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is more effective than acetic acid on the CSP which has an N—CH_3 amide tethering group. In addition, the elution orders for the resolution of 3-amino-3-phenylpropionic acid on the two CSPs were opposite to each other. From these chromatographic resolution results, it was concluded that the tethering groups of the CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid play some important role in the chiral recognition of β-amino acids, even though the exact role of the tethering groups is not clear at the present time. However, the chromatographic trends for the resolution of selected β-amino acids with the variation of the content of organic and acidic modifiers in aqueous mobile phase and the column temperature were generally consistent on the two CSPs.

Key Words: Chiral phase; Chiral separation; Crown ether; β-amino acids.

INTRODUCTION

Liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been known as the most convenient and accurate technique for the analysis of enantiomeric composition of chiral compounds and also for the preparative resolution of racemates.[1–3] Consequently, extensive efforts have been devoted to the development of effective CSPs for the liquid chromatographic separation of enantiomers, and hundreds of CSPs have been developed.[1–5] All of those CSPs have been classified into five or six types, according to their manner of chiral recognition.[4,5]

Among various CSPs, crown ether-based CSPs have been successfully utilized in the resolution of racemic compounds containing a primary amino group.[6] We have also been interested in the resolution of racemic compounds containing a primary amino group and have devoted our efforts to the development of crown ether-based CSPs. Finally, we were able to develop a very effective crown ether-based CSP (CSP **1**, Fig. 1) by covalently bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to amino-propylsilica gel. CSP **1** was very effective in the resolution of racemic α-amino acids,[7] amines,[8] amino alcohols,[8] and fluoroquinolone antibacterial agents.[9,10] More recently, CSP **1** was also applied in the resolution of racemic β-amino acids.[11]

Optically active β-amino acids have attracted considerable attention, due to their potent pharmacological activities and their usefulness as building blocks of many natural products.[12,13] Consequently, analytical methods of determining the enantiomeric composition of β-amino acids are essential and the liquid chromatographic resolution of enantiomers on CSPs might be the most valuable technique available. However, liquid chromatographic resolution of β-amino acids on CSPs is rarely reported. Only a limited number of β-amino acids were

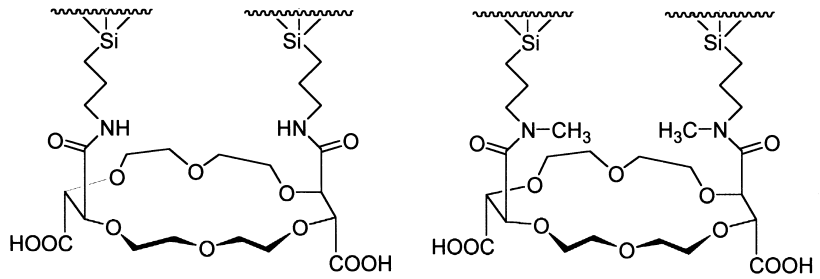

Figure 1. The structures of CSP **1** and CSP **2**.

reported to be resolved on a ligand exchange CSP[14] or a Pirkle-type CSP[15] and on several commercial CSPs.[16] In this instance, the successful resolution of various *β*-amino acids on CSPs might be very important.

Recently, as an effort to improve the chiral recognition efficiency of CSP **1**, we prepared a new CSP (CSP **2**, Fig. 1) by simply replacing the two N—H hydrogens of the connecting amide tethers of CSP **1** with a methyl group. The intramolecular hydrogen bonds between N—H hydrogens of the two connecting amide tethers of CSP **1** and the crown ether oxygens of the crown ether ring of the CSP, which was proposed previously in a similar chiral crown ether system,[17] was expected to hinder the effective complex formation between the crown ether ring and the analytes. Consequently, removal of the two N—H hydrogens of CSP **1** was expected to improve the chiral recognition ability of the CSP. Indeed, CSP **2** was superior to CSP **1** in the resolution of various racemic amines.[18]

In this study, we applied CSP **2** to the resolution of various *β*-amino acids. Even though CSP **1** and CSP **2** were expected to show similar resolution behaviors with somewhat improved chiral recognition on CSP **2**, they showed quite different chiral resolution behaviors. The different tethering groups of the two CSPs seem to be responsible for the different chiral recognition behaviors in the resolution of *β*-amino acids on the two CSPs. In this study, we wish to demonstrate how the tethering groups of the CSPs can affect the chiral recognition behaviors for the resolution of *β*-amino acids on CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid.

EXPERIMENTAL

Chromatography was performed with an HPLC system consisting of a Waters Model 515 pump, a Rheodyne Model 7725i injector with a 20 μL

sample loop, a Youngin M 720 Absorbance detector (variable wavelength), and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The chiral column, packed with the CSP **1** (150 mm × 4.6 mm I.D. stainless steel column) was available from a previous study.[11] The chiral column packed with CSP **2** (150 mm × 4.6 mm I.D. stainless steel column) was available from another previous study.[18] Column temperature was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. Both enantiomers of *β*-amino acids (**3**–**11**) shown in Fig. 2 were purchased from Peptech Corporation (Cambridge, MA). 3-Aminobutyric acid **12** and 3-aminoadipic acid **13**, shown in Fig. 2, were available in racemic form from Aldrich. Injection samples were prepared by dissolving each analyte (1 : 1 mixture of optically active analyte or racemic) in water at a concentration of 1.0 mg/mL and usually 2 μL was injected. The chromatographic parameters

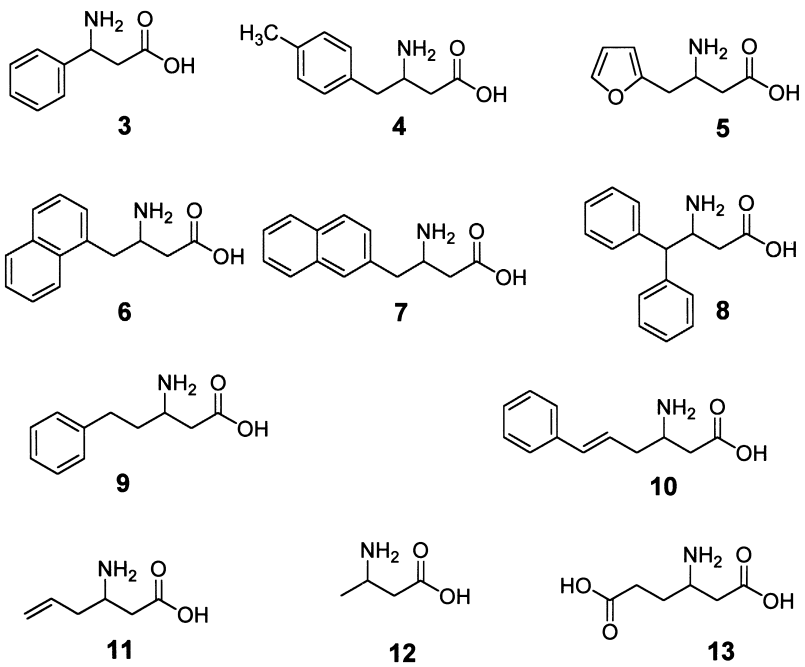

Figure 2. The structures of *β*-amino acids used in this study. 3-Amino-3-phenylpropionic acid **3**, 3-amino-4-(4-methylphenyl)butyric acid **4**, 3-amino-4-(2-furyl)butyric acid **5**, 3-amino-4-(1-naphthyl)butyric acid **6**, 3-amino-4-(2-naphthyl)butyric acid **7**, 3-amino-4,4-diphenylbutyric acid **8**, 3-amino-5-phenylpentanoic acid **9**, 3-amino-6-phenyl-5-hexenoic acid **10**, 3-amino-5-xehenoic acid **11**, 3-aminobutyric acid **12**, and 3-aminoadipic acid **13**.

were calculated based on the column void volume measured by injecting 2,6-lutidine.[11]

RESULTS AND DISCUSSION

In our previous study, CSP **1** was found to be quite effective in the resolution of β-amino acids.[11] The most effective acidic modifier in the mobile phase for the resolution of β-amino acids on CSP **1** was acetic acid. Complexation of primary ammonium ion ($R-NH_3^+$) inside the cavity of the chiral crown ether ring has been reported to be essential for the chiral recognition of racemic compounds containing a primary amino group by chiral crown ethers.[7] In this instance, acetic acid added to the mobile phase was believed to protonate the amino group of β-amino acids to produce ammonium ions and to improve their complexation inside the cavity of the chiral crown ether ring of CSP **1**. In the resolution of α-amino acids, amines, amino alcohols, and fluoroquinole antibacterial agents on CSP **1**, in general, sulfuric acid was most widely used as an effective acidic modifier in aqueous mobile phase.[7–10]

In order to see the effects of acetic and sulfuric acid as an acidic modifier in aqueous mobile phase on the resolution of β-amino acids on CSP **1**, we resolved the eleven β-amino acids shown in Fig. 2 with a mobile phase consisting of 50% methanol in water containing 10 mM acetic acid or with the mobile phase of 50% methanol in water containing 10 mM sulfuric acid, and we compared the resolution results in Table 1. As shown in Table 1, in every case, acetic acid is much better than sulfuric acid as an acidic modifier in the resolution of β-amino acids on CSP **1**.

CSP **2** was expected to show resolution behaviors similar to those on CSP **1** because the two CSPs are identical to each other in their chiral selector parts and are different from each other only in their tethering group parts. However, the effect of the acidic modifier in aqueous mobile phase on the resolution of β-amino acids on CSP **2** was quite opposite to that on CSP **1**. In Table 2, the resolution of β-amino acids on CSP **2** with the mobile phase of 50% methanol containing 10 mM acetic acid as an acidic modifier was compared with that for the mobile phase of 50% methanol containing 10 mM sulfuric acid. As shown in Table 2, the resolution of β-amino acids on CSP **2** is generally greater when sulfuric acid is used as an acidic modifier than when acetic acid is used. In addition, the retention time of the first eluted enantiomer (k_1') is always larger when acetic acid is used than when sulfuric acid is used as an acidic modifier in the resolution of β-amino acids on CSP **1**, as shown in Table 1. However, in the resolution of β-amino acids on CSP **2**, the retention time of the first eluted enantiomer (k_1') is larger

Table 1. Resolution of β-amino acids on CSP **1** with mobile phase of 50% methanol in water containing (A) 10 mM acetic acid or (B) 10 mM sulfuric acid as an acidic modifier.[a]

β-Amino acids	A (10 mM acetic acid)			B (10 mM sulfuric acid)		
	k_1'[b]	α[c]	R_S[d]	k_1'[b]	α[c]	R_S[d]
3	3.60 (R)	1.60	2.76	1.38 (R)	1.13	0.65
4	1.26 (S)	1.40	2.15	0.43 (S)	1.19	0.49
5	1.33 (S)	1.33	1.66	0.31	1.00	
6	3.72 (S)	1.28	1.55	0.90	1.00	
7	2.38 (S)	1.53	2.07	0.72 (S)	1.16	0.80
8	0.67 (S)	1.34	1.38	0.40 (S)	1.14	0.48
9	2.30 (S)	1.44	2.65	0.50 (S)	1.20	0.81
10	2.09 (S)	1.54	2.21	0.55 (S)	1.24	1.10
11	1.02 (S)	1.37	1.86	0.22 (S)	1.14	
12	2.16	1.16	1.57	0.34	1.00	
13	3.83	1.16	1.22	0.39	1.00	

[a]Underlined resolution data are quoted from Ref.[11] Flow rate: 0.5 mL/min, Detection: 210 nm UV, Temperature: 20°C.
[b]Retention factor of the first eluted enantiomer. The absolute configuration of the first eluted enantiomer was presented in the parenthesis.
[c]Separation factor.
[d]Resolution factor.

when sulfuric acid is used than when acetic acid is used as an acidic modifier, except for the resolution of β-amino acids **8** and **13**, as shown in Table 2.

The elution orders for the resolution of 3-amino-3-phenylpropionic acid **3** on CSP **1** and CSP **2** are also noteworthy. As shown in Table 1, the elution order for the resolution of 3-amino-3-phenylpropionic acid **3** on CSP **1** is different from those for the resolution of other β-amino acids; these inconsistent elution orders were rationalized to stem from the priority inversion of the substituents at the chiral center of 3-amino-3-phenylpropionic acid **3**, according to the Cahn-Ingold-Prelog sequence rule.[19] However, the elution order for the resolution of 3-amino-3-phenylpropionic acid **3** on CSP **2** is consistent with others, the (S)-enantiomers being eluted first.

From the chromatographic results for the resolution of β-amino acids on CSP **1** and CSP **2** summarized in Tables 1 and 2, it is concluded that the tethering groups of the CSPs seem to play some important role in chiral recognition. However, at the present time, the exact role of the tethering groups

Table 2. Resolution of β-amino acids on CSP **2** with mobile phase of 50% methanol in water containing (A) 10 mM acetic acid or (B) 10 mM sulfuric acid as an acidic modifier.[a]

β-Amino acids	A (10 mM acetic acid)			B (10 mM sulfuric acid)		
	k_1'[b]	α[c]	R_S[d]	k_1'[b]	α[c]	R_S[d]
3	2.92	1.00		8.98 (S)	1.87	6.37
4	0.80	1.00		1.63 (S)	1.19	1.57
5	0.82	1.00		1.82	1.00	
6	1.04 (S)	1.06	0.21	3.19	1.00	
7	1.07 (S)	1.07	0.42	2.89 (S)	1.13	1.23
8	0.66	1.00		0.21 (S)	2.65	3.90
9	0.90 (S)	1.08	0.43	1.92 (S)	1.23	1.90
10	1.00 (S)	1.08	0.42	2.71 (S)	1.15	1.47
11	0.86	1.00		1.01 (S)	1.12	0.75
12	1.48	1.00		1.51	1.10	0.50
13	12.66	1.00		1.42	1.20	1.37

[a]Flow rate: 0.5 mL/min, Detection: 210 nm UV, Temperature: 20°C.
[b]Retention factor of the first eluted enantiomer. The absolute configuration of the first eluted enantiomer was presented in the parenthesis.
[c]Separation factor.
[d]Resolution factor.

of the CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid in the chiral recognition is not clear.

In our previous study, we investigated that the content of organic and acidic modifiers in aqueous mobile phase; the column temperature can affect the chromatographic trends for the resolution of β-amino acids on CSP **1**.[11] In order to see the effect of the tethering groups of the CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid on the chromatographic trends for the resolution of β-amino acids, we also investigated the resolution of selected β-amino acids on CSP **2** with the variation of the content of organic and acidic modifier in aqueous mobile phase, as well as the column temperature. Comparison of the chromatographic resolution results for the resolution of β-amino acids on CSP **1** with those on CSP **2** shows that the chiral recognition on CSP **1** is generally better than that on CSP **2**. However, in the resolution of 3-amino-3-phenylpropionic acid **3** and 3-amino-4,4-diphenylbutyric acid **8**, CSP **2** is much better than CSP **1**, as shown in Tables 1 and 2. In this instance, we selected the two β-amino acids (**3** and **8**) and resolved them on CSP **2** with the variation of the content of organic and acidic

modifiers in aqueous mobile phase and the column temperature. Resolution of 3-amino-3-phenylpropionic acid **3** and 3-amino-4,4-diphenylbutyric acid **8** on CSP **2**, with the variation of the content of organic and acidic modifiers in aqueous mobile phase and the column temperature is summarized in Table 3 and, as an example, the chromatographic trends for the resolution of 3-amino-4,4-diphenylbutyric acid **8** on CSP **2** are illustrated in Fig. 3.

As shown in Table 3, an increase in the content of methanol in aqueous mobile phase (entry a) increases the retention, as denoted by the retention factors (k'_1). Especially, the increasing trends of the retention factors are much more significant with 3-amino-3-phenylpropionic acid **3**. As the content of methanol in aqueous mobile phase increases, the polarity of the mobile phase is expected to decrease and, consequently, the polar interaction between the mobile phase and polar β-amino acids seems to decrease. In this instance, the retention factors increase as the content of methanol in aqueous mobile phase increases. These trends are consistent with those on CSP **1**.[11] In the resolution of 3-amino-4,4-diphenylbutyric acid **8** on CSP **2**, the separation (α) and the resolution factor (R_S) also increase as the content of methanol in aqueous mobile phase increases, as shown in Table 3. However, in the resolution of 3-amino-3-phenylpropionic acid **3** on CSP **2**, the separation factor (α) decreases while the resolution factor (R_S) does not show any significant trend as the content of methanol in aqueous mobile phase increases.

An increase in the content of sulfuric acid in mobile phase decreases the retention denoted by the retention factor (k'_1), as shown in Table 3 (entry b). These trends are consistent with those for the resolution of some β-amino acids on CSP **1** with the variation of the content of acetic acid in aqueous mobile phase.[11] As the content of acidic modifier in aqueous mobile phase increases, the ionic strength of mobile phase is expected to increase. In this instance, the interaction between the mobile phase and the solute molecule increases and, consequently, the solute molecule is expected to elute faster. On the other hand, the separation (α) and the resolution factor (R_S) do not show any significant changes as the content of sulfuric acid in aqueous mobile phase increases, as shown in Table 3 (entry b).

As the column temperature decreases, the retention (k'_1) and the separation factor (α) increase, as shown in Table 3 (entry c). At lower temperature, the formation of the two diastereomeric complexes with the two enantiomers of β-amino acids inside the chiral cavity of the crown ether ring of the CSP is expected to improve. The formation of the more stable diastereomeric complex is expected to become more favorable than that of the less stable one at lower temperature. In this instance, the improved retention (k'_1) and the improved separation factor (α) are observed at lower temperature; these trends are consistent with those on CSP **1**.[11] The resolution factor (R_S) also improved at lower temperature for the resolution of 3-amino-4,4-diphenylbutyric acid **8**

Table 3. Resolution of 3-amino-3-phenylpropionic acid (**3**) and 3-amino-4,4-diphenylbutyric acid (**8**) on CSP **2** with the variation of the content of organic and acidic modifiers in aqueous mobile phase and the column temperature.[a]

		3			**8**		
Entry	Chromatographic condition	k_1'[b]	α[c]	R_S[d]	k_1'[b]	α[c]	R_S[d]
a	30% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 20°C	3.93	1.97	5.96	0.20	1.89	2.09
	50% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 20°C	8.98	1.87	6.37	0.21	2.65	3.90
	80% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 20°C	19.96	1.67	6.28	0.27	3.54	6.60
b	50% CH_3OH in $H_2O + 1\,mM\ H_2SO_4$, 20°C	15.45	1.84	5.24	0.35	2.51	4.65
	50% CH_3OH in $H_2O + 5\,mM\ H_2SO_4$, 20°C	11.19	1.89	6.06	0.26	2.59	4.24
	50% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 20°C	8.98	1.87	6.37	0.21	2.65	3.90
	50% CH_3OH in $H_2O + 20\,mM\ H_2SO_4$, 20°C	6.91	1.80	6.10	0.18	2.53	3.30
c	50% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 5°C	19.97	2.00	6.12	0.39	3.06	5.71
	50% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 10°C	15.04	1.96	6.05	0.32	2.92	5.06
	50% CH_3OH in $H_2O + 10\,mM\ H_2SO_4$, 20°C	8.98	1.87	6.37	0.21	2.65	3.90

[a]In every case, the (S)-enantiomer was eluted first. Flow rate: 0.5 mL/min, Detection: 210 nm UV, Temperature: 20°C.
[b]Retention factor of the first eluted enantiomer.
[c]Separation factor.
[d]Resolution.

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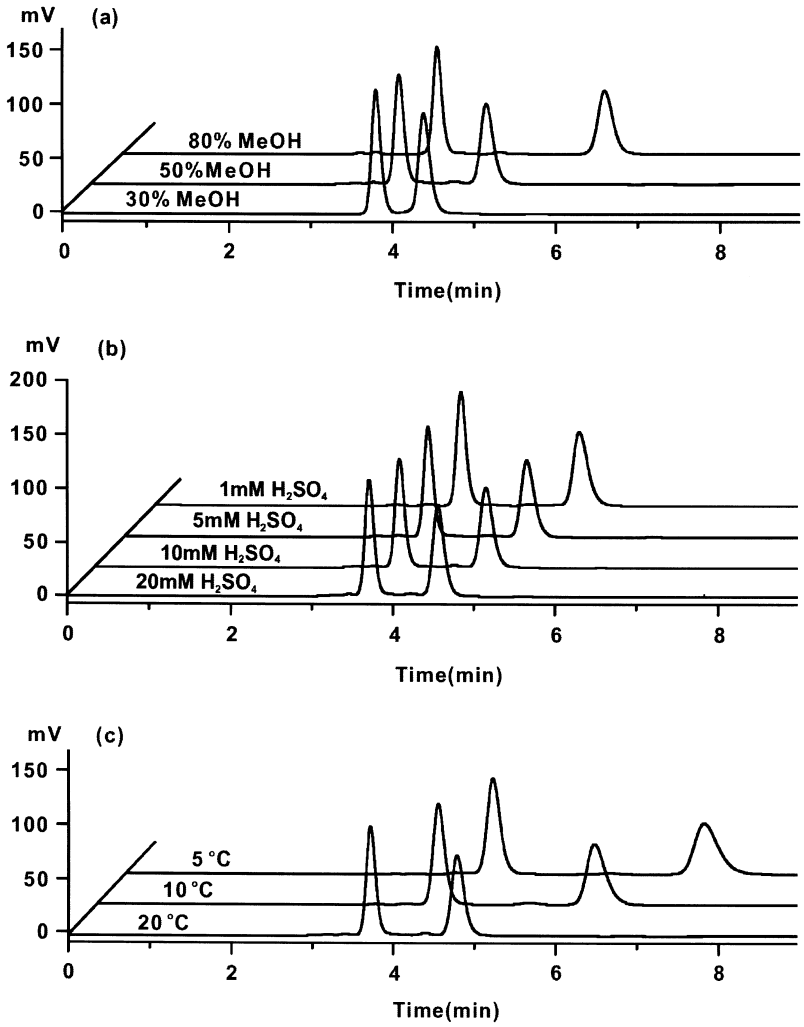

Figure 3. The chromatographic trends for the resolution of 3-amino-4,4-diphenylbutyric acid **8** on CSP **2** (a) with the variation of the content of methanol in aqueous mobile phase containing 10 mM sulfuric acid at 20°C; (b) with the variation of the content of sulfuric acid in aqueous mobile phase of 50% methanol in water at 20°C; and (c) with the variation of the column temperature in aqueous mobile phase of 50% methanol in water containing 10 mM sulfuric acid. Flow rate: 0.5 mL/min, Detection: 210 nm UV.

on CSP **2**. However, the resolution factor (R_S) for the resolution of 3-amino-3-phenylpropionic acid **3** on CSP **2** was almost invariant with the variation of the column temperature.

In summary, resolution of β-amino acids on CSP **1** and CSP **2** with the mobile phase of 50% methanol in water containing 10 mM acetic acid and with the mobile phase of 50% methanol in water containing 10 mM sulfuric acid demonstrated that acetic acid is more effective as an acidic modifier than sulfuric acid on CSP **1** while sulfuric acid is more effective than acetic acid on CSP **2**. In addition, the elution orders for the resolution of 3-amino-3-phenylpropionic acid **3** on CSP **1** and CSP **2** are opposite to each other. From these chromatographic resolution results, it was concluded that the tethering groups of the CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid affect the chiral recognition of β-amino acids, even though the exact role of the tethering groups is not clear at the present time. However, the chromatographic trends for the resolution of selected β-amino acids on CSP **2** with the variation of the content of organic and acidic modifiers in aqueous mobile phase and the column temperature were generally consistent with those on CSP **1**.

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