Resolution of β-Amino Acids on a Chiral Stationary Phase Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxilic Acid without Extra Free Aminopropyl Groups on Silica Surface: the Effect of Ammonium Ion Mobile Phase Modifier on the Resolution Behaviors

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Received August 8, 2006

A liquid chromatographic chiral stationary phase (CSP) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxilic acid without extra free aminopropyl groups on silica surface has been demonstrated to be quite effective for the resolution of various β-amino acids. The retention factors (k_1) for the resolution of β-amino acids on the CSP were quite large and the large retention factors might be quite attractive along with the reasonable separation factors (α) for preparative scale enantioselective chromatography. The large retention factors on the CSP were found to be reduced effectively by adding ammonium ion to mobile phase without sacrificing the chiral recognition efficiency of the CSP. Consequently, the CSP is also quite applicable for use in analytical enantioselective chromatography.

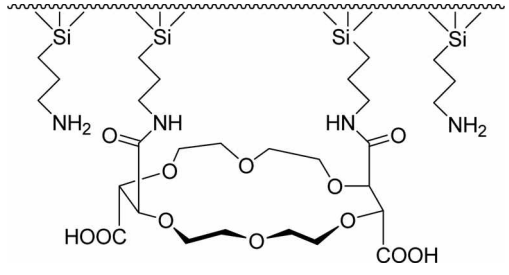
Key Words : β-Amino acids, Chiral stationary phase, Enantiomer separation, Liquid chromatography

Introduction

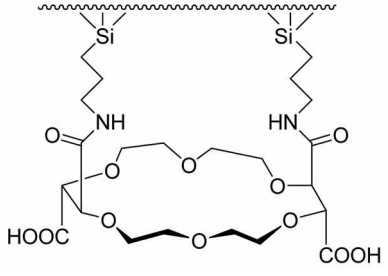
Liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) have been known very effective in the exact determination of enantiomeric composition of chiral compounds.[1] Especially CSPs based on chiral crown ethers have been known very effective for the liquid chromatographic chiral separation of racemic compounds containing a primary amino group.[2] For example, optically active (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 dynamically coated on octadecylsilica gel[3] or covalently bonded to silica gel[4] has been successfully utilized as CSPs for the resolution of racemic compounds containing a primary amino group. Another crown ether-based chiral stationary phase (CSP **1**, Figure 1) developed in our laboratory by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to 3-aminopropylsilica gel was very successful in the resolution of various racemic primary amino compounds including α-amino acids,[5] β-amino acids,[6] α-amino acid derivatives,[7] racemic amines,[8] racemic amino alcohols[8] and racemic fluoroquinolone antibacterials.[9]

However, CSP **1** intrinsically contains unreacted residual aminopropyl groups on the surface of silica gel because the process of bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic anhydride to aminopropylsilica gel cannot be complete. The unreacted residual aminopropyl groups of CSP **1** can be protonated under the acidic mobile phase condition and the resulting primary ammonium ions were expected to compete with the primary ammonium ions of analytes for the complexation inside the cavity of the crown ether ring of the CSP. Protection of the unreacted residual aminopropyl groups of CSP **1** with acetyl or butyryl group actually improved the retention (k) and the resolution factors (R_S) for the resolution of α-amino acids, but diminished the separation factors (α) slightly.[10] In order to avoid the problems related to the unreacted residual aminopropyl groups on CSP **1**, a new residual aminopropyl group-free CSP (CSP **2**, Figure 1) was prepared by bonding *N,N'*-triethoxysilylpropyl *syn*-diamide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to silica gel directly.[11]

CSP **2** has been successfully applied to the resolution of various α-amino acids, amines and amino alcohols.[11] The



CSP 1



CSP 2

Figure 1. Structures of CSP **1** and CSP **2**.

chiral recognition efficiency of CSP **2** was found generally superior to that of CSP **1** in terms of the separation and the resolution factors as expected. However, CSP **2** has not been applied to the resolution of β-amino acids yet while CSP **1** has been found useful for the resolution of β-amino acids.[6]

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] In this instance, analytical methods for the exact determination of the enantiomeric composition of β-amino acids are essential and the liquid chromatographic chiral separations on CSPs might be the desired analytical method.

In this study, we wish to apply CSP **2** to the resolution of various β-amino acids and to compare the chromatographic resolution results with those on CSP **1**. Comparison of the chromatographic resolution results on CSP **2** with those on CSP **1** is expected to elucidate the characteristics of CSP **2** in the resolution of β-amino acids.

Experimental Section

The chromatography was performed on a liquid chromatography system equipped with a Waters model 510 pump, a Rheodyne model 7725i injector with a 20 μL sample loop, a Waters model 486 Absorbance detector (variable wavelength) and a Younglin Auto Data Module (Software: Younglin Autochro-Win 2.0 plus). The detection was set at 210 nm. Chiral column (150 mm × 4.6 mm I.D.) packed with CSP **2** was available from prior study.[11] Racemic and optically active β-amino acid samples were available from prior studies.[6b] The column temperature was controlled with a Julabo F30 heating-cooling circulator system. The flow rate was set at 0.5 mL/min.

Results and Discussion

CSP **2** was applied to the resolution various β-amino acids. The structures of β-amino acids **3-12** used in this study are shown in Figure 2. In our previous study, β-amino acids were found to be resolved best when 50% methyl alcohol in water containing 10 mM acetic acid was used as a mobile phase on CSP **1**.[6a,6b] The identical mobile phase condition was applied to the resolution of β-amino acids on CSP **2**.

Table 1. Comparison of the resolution of β-amino acids **3-12** on CSP **1** and CSP **2** with the use of 50% methanol in water containing 10 mM acetic acid as a mobile phase[a]

β-Amino acids	CSP **1**			CSP **2**		
	k_1'	α	R_S	k_1'	α	R_S
3	3.60 (R)	1.60	2.76	29.91 (R)	1.25	1.90
4	1.26 (S)	1.40	2.15	10.18 (S)	1.33	1.77
5	1.33 (S)	1.33	1.66	11.12 (S)	1.26	2.02
6	3.72 (S)	1.28	1.55	20.68 (S)	1.24	1.03
7	2.38 (S)	1.53	2.07	21.08 (S)	1.48	2.24
8	0.67 (S)	1.34	1.38	3.72 (S)	1.41	2.65
9	2.30 (S)	1.44	2.65	12.88 (S)	1.24	1.68
10	2.09 (S)	1.54	2.21	17.43 (S)	1.49	2.40
11	1.02 (S)	1.37	1.86	18.54 (S)	1.48	2.64
12	2.16	1.16	1.57	9.87	1.19	1.32

[a]The resolution data on CSP **1** are quoted from reference 6b. Flow rate: 0.5 mL/min, Detection: 210 nm UV, Temperature: 20 °C. k_1': Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α: Separation factor. R_S: Resolution factor.

The chromatographic results for the resolution of β-amino acids **3-12** on CSP **2** are summarized and compared to those on CSP **1** in Table 1. The elution orders shown in Table 1 were determined by injecting configurationally known samples. In the case of 3-aminobutyric acid (**12**), configurationally known sample was not available and consequently the elution order was not determined. The elution order for the resolution of 3-amino-3-phenylpropionic acid (**3**) is different from that for the resolution of other β-amino acids. The inconsistent elution order for the resolution of 3-amino-3-phenylpropionic acid (**3**) has been rationalized to stem from the priority inversion of the substituents at the chiral center of the analyte according to the Cahn-Ingold-Prelog sequence rule.[6b]

As shown in Table 1, both CSP **1** and CSP **2** are quite good for the resolution of β-amino acids **3-12**. In every case the clean baseline-resolution was observed. Based on the comparison of the resolution results on the two CSPs, CSP **2** was better than CSP **1** for the resolution of some β-amino

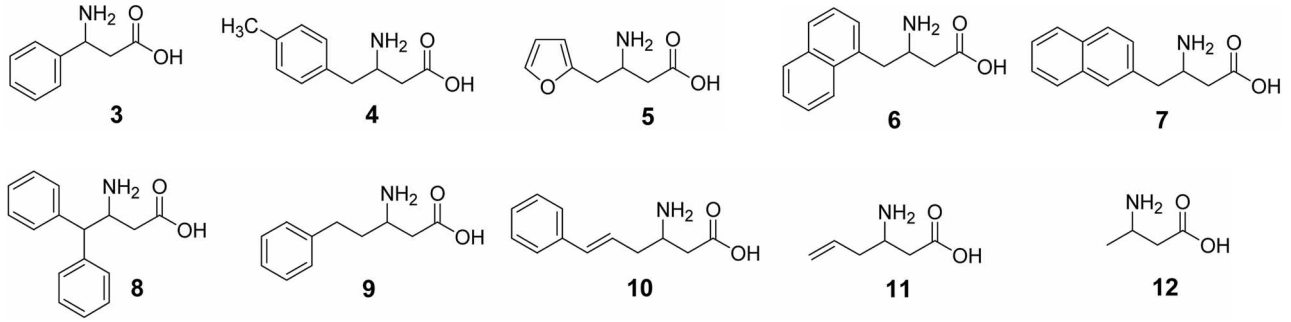


Figure 2. The structures of β-amino acids used in this study. 3-Amino-3-phenylpropionic acid (**3**), 3-amino-4-(4-methylphenyl)butyric acid (**4**), 3-amino-4-(2-furyl)butyric acid (**5**), 3-amino-4-(1-naphthyl)butyric acid (**6**), 3-amino-4-(2-naphthyl)butyric acid (**7**), 3-amino-4,4-diphenylbutyric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

acids, but CSP **2** was worse than or equal to CSP **1** for the resolution of some other β-amino acids. In the resolution of 3-amino-4,4-diphenylbutyric acid (**8**) and 3-amino-5-hexenoic acid (**11**), CSP **2** was better than CSP **1** in terms of both separation (α) and resolution factors (R_S). CSP **2** was also better than CSP **1** for the resolution of 3-amino-4-(2-furyl)butyric acid (**5**), 3-amino-4-(2-naphthyl)butyric acid (**7**) and 3-amino-6-phenyl-5-hexenoic acid (**10**) in terms of resolution factors (R_S). However, in other cases, CSP **2** was worse than or equal to CSP **1**.

The most interesting results for the resolution of β-amino acids on CSP **1** and CSP **2** are the retention factors. As shown in Table 1, the retention factors on CSP **2** are much greater than those on CSP **1**. Complexation of primary ammonium ions ($R\text{-}NH_3^+$) 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.[2] The amino groups of the residual aminopropyl groups of CSP **1** should be protonated to afford primary ammonium ions under acidic mobile phase condition and the resulting primary ammonium ions can compete with the primary ammonium ions of analytes for the complexation inside the cavity of the crown ether ring of the stationary phase. In this instance, the retention of analytes on the CSP should be reduced. However, the competition of the primary ammonium ions of the residual aminopropyl groups of CSP **1** with the primary ammonium ions of analytes for the complexation inside the cavity of the crown ether ring of the stationary phase was expected to be reduced by removing the residual aminopropyl groups of CSP **1**. In this instance, the retention factors on CSP **2**, which does not contain any residual aminopropyl groups on the stationary surface, are expected to be greater than those on CSP **1**.

The large retention factors on CSP **2** along with the reasonable separation factors might be quite attractive for the preparative scale enantioselective chromatography because substantial amount of substrate should be loaded on the column for preparative purposes.[13] However, for analytical purposes, the retention of the analyte should be adjustable so as to minimize the time required for analysis.[13] In this instance, the large retention factors on CSP **2** might cause a problem in the utilization of CSP **2** as an analytical tool even though CSP **2** was found to be quite good for the resolution of various β-amino acids. For example, more than three hours were needed to complete the resolution of 3-amino-3-phenylpropionic acid (**3**) on CSP **2**. In this instance, we tried to find the condition of reducing the retention times of the two enantiomers for the resolution of β-amino acids on CSP **2** without sacrificing the chiral recognition efficiency.

Previously, we reported that the retention times for the resolution of racemic primary amino compounds on a CSP based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 can be reduced by adding cationic modifier to aqueous mobile phase.[4] Competition of the cationic modifier in mobile phase with the primary ammonium ions ($R\text{-}NH_3^+$) of analytes for the complexation inside the cavity of the crown ether ring of the CSP can reduce the retention times of the enantiomers. In this study, to reduce the retention times for the resolution of β-amino acids on CSP **2**, ammonium ion (NH_4^+) was added to mobile phase as a cationic modifier. As ammonium ion source, we used ammonium acetate (NH_4OAc) or ammonium chloride (NH_4Cl). Both of ammonium acetate and ammonium chloride added to mobile phase were quite effective in reducing the retention times. However, the chromatographic peaks corresponding to the two enantiomers became broad in almost every case when ammonium chloride was used as a cationic modifier in aqueous mobile phase and consequently the resolution factors became quite worse compared to those obtained without the use of ammonium ion modifier. Consequently, we focused on the use of ammonium acetate as a cationic modifier.

The chromatographic results for the resolution of β-amino acids **3-12** on CSP **2** with the variation of the content of ammonium acetate in aqueous mobile phase are summarized in Table 2. The trends of the retention factors with the variation of the content of ammonium acetate in aqueous mobile are graphically illustrated in Figure 3. The trends of

Table 2. Resolution of β-amino acids **3-12** on CSP **2** with the variation of the content of ammonium acetate in aqueous mobile phase[a]

β-Amino acids	50% CH_3OH + 10 mM AcOH			50% CH_3OH + 10 mM AcOH + 0.1 mM NH_4OAc			50% CH_3OH + 10 mM AcOH + 0.5 mM NH_4OAc		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
3	29.91 (R)	1.25	1.90	11.69 (R)	1.61	2.10	7. 91 (R)	1.42	2.56
4	10.18 (R)	1.33	1.77	6.20 (S)	1.41	2.18	4.03 (S)	1.34	2.16
5	11.12 (S)	1.26	2.02	5.74 (S)	1.29	2.05	3.95 (S)	1.25	2.09
6	20.68 (S)	1.24	1.03	11.43 (S)	1.37	1.25	7.59 (S)	1.37	1.71
7	21.08 (S)	1.48	2.24	11.48 (S)	1.60	2.26	7.56 (S)	1.59	2.40
8	3.72 (S)	1.41	2.65	2.54 (S)	1.30	1.71	2.35 (S)	1.16	1.25
9	12.88 (S)	1.24	1.68	7.07 (S)	1.27	1.61	5.03 (S)	1.25	1.56
10	17.43 (S)	1.49	2.40	9.61 (S)	1.56	2.36	6.15 (S)	1.51	2.42
11	18.54 (S)	1.48	2.64	4.10 (S)	1.25	2.22	1.87 (S)	1.20	2.04
12	9.87	1.19	1.32	5.44	1.19	1.60	3.88	1.18	1.88

[a]Flow rate: 0.5 mL/min. Detection: 210 nm UV. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α: Separation factor. R_S: Resolution factor.

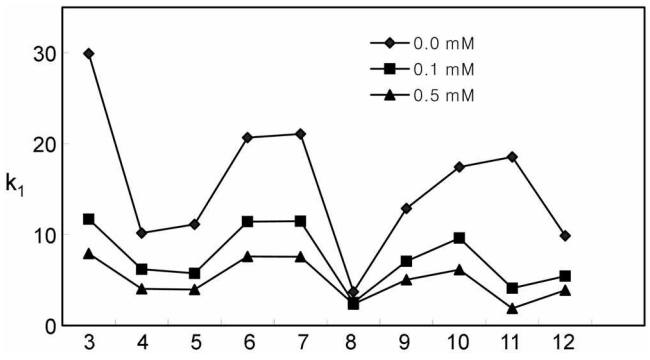


Figure 3. The trends of the retention factors (k_1) for the resolution of β-amino acids **3-12** on CSP **2** with the variation of the ammonium acetate (NH_4OAc) concentration (0.0 mM, 0.1 mM and 0.5 mM) in aqueous mobile phase.

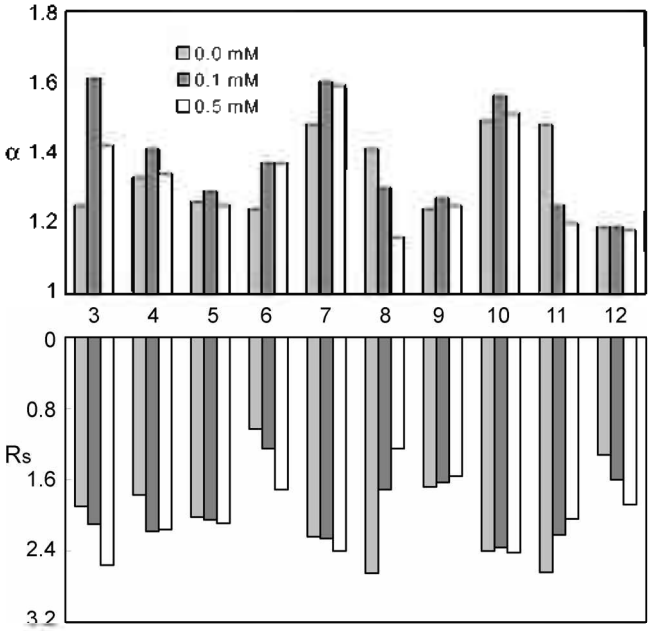


Figure 4. The trends of the separation (a) and the resolution factors (R_S) for the resolution of β-amino acids **3-12** on CSP **2** with the variation of the ammonium acetate (NH_4OAc) concentration (0.0 mM, 0.1 mM and 0.5 mM) in aqueous mobile phase.

the separation and the resolution factors with the variation of the content of ammonium acetate in aqueous mobile are also graphically illustrated in Figure 4 and the representative chromatograms for the resolution of 3-amino-4-(4-methylphenyl)butyric acid (**4**) with the variation of the content of ammonium acetate in aqueous mobile phase are presented in Figure 5. As shown in Table 2, Figure 3 and Figure 5, the retention factors for the resolution of β-amino acids on CSP **2** were reduced quite much when ammonium acetate was added to mobile phase. In contrast, the separation and/or the resolution factors for the resolution of β-amino acids on CSP **2** were generally improved by adding ammonium acetate (NH_4OAc) to aqueous mobile phase except for the resolution of 3-amino-3,4-diphenylbutyric acid (**8**) and 3-amino-5-hexenoic acid (**11**) as shown in Table 2, Figure 4 and Figure 5. In the resolution of 3-amino-3,4-diphenylbutyric acid (**8**)

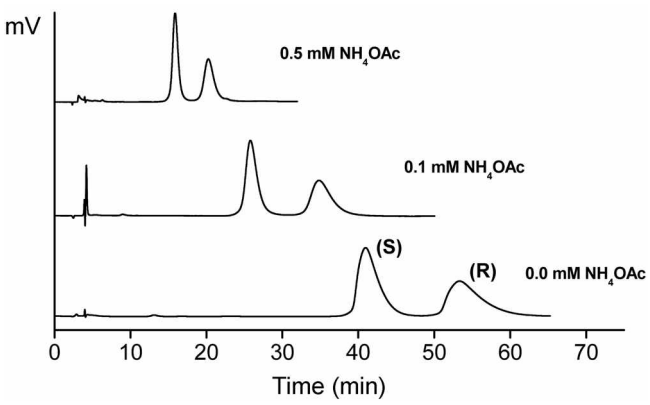


Figure 5. The representative chromatograms for the resolution of 3-amino-4-(4-methylphenyl)butyric acid (**4**) on CSP **2** with the variation of the ammonium acetate (NH_4OAc) concentration (0.0 mM, 0.1 mM and 0.5 mM) in aqueous mobile phase. For the chromatographic condition, see the foot note to Table 2.

and 3-amino-5-hexenoic acid (**11**) on CSP **2**, both the separation and the resolution factors were reduced by adding ammonium acetate to mobile phase. However, both the separation and the resolution factors for the resolution of 3-amino-3,4-diphenylbutyric acid (**8**) and 3-amino-5-hexenoic acid (**11**) on CSP **2** are good enough for the determination of the enantiomeric composition of the two β-amino acids. The reason for the inversed effect of the ammonium ion mobile phase modifier on the separation and the resolution factors for the resolution of 3-amino-3,4-diphenylbutyric acid (**8**) and 3-amino-5-hexenoic acid (**11**) on CSP **2** is not clear yet.

In conclusion, CSP **2** was found to be quite good for the resolution of various β-amino acids. The retention factors for the resolution of β-amino acids on CSP **2** were quite large and the large retention factors might be quite attractive along with the reasonable separation factors for preparative scale enantioselective chromatography. The somewhat large retention factors have been effectively controlled by adding ammonium acetate to aqueous mobile phase without sacrificing the chiral recognition efficiency of the CSP. Consequently, CSP **2** is concluded to be quite effective for use in both preparative and analytical enantioselective chromatography.

Acknowledgements. This work was supported for two years by Pusan National University Research Grant.

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