# Effect of the Protection of the Residual Aminopropyl Groups of a Chiral Stationary Phase Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid on the Chiral Resolution Behaviors

**Myung Ho Hyun,* Young Hwa Kim, and Yoon Jae Cho**

*Department of Chemistry and Chemistry Institute for Functional Materials, Pusan National University, Busan 609-735, Korea*

*Received December 19, 2003*

**Key Words :** Chiral stationary phase, (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid, Enantiomer separation

(+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid **1** (Figure 1), which was first developed by Lehn and co-workers[1] has been successfully utilized as a very effective chiral selector for the enantioseparation by capillary electrophoresis.[2] The utilization of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** as a chiral selector of liquid chromatographic chiral stationary phases (CSPs) was also appeared in recent years.[3] For example, CSP **2** (Figure 1) developed in our laboratory by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** to 3-aminopropylsilica gel was very successful in resolving various racemic primary amino compounds including $\alpha$- and $\beta$-amino acids,[4] $\alpha$-amino acid derivatives,[4,5] racemic amines, racemic amino alcohols[6] and racemic fluoroquinolone antibacterials.[7]

CSP **2** intrinsically contains unreacted residual aminopropyl groups because the process of bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** to 3-aminopropylsilica gel can not be complete. The unreacted residual aminopropyl groups of CSP **2** can be protonated under the acidic mobile phase condition and the resulting primary ammonium ions are expected to compete with the primary ammonium ions of analytes for the complexation inside the cavity of the crown ether ring of the CSP. The complexation of the primary ammonium ion of a racemic analyte inside the cavity of the crown ether ring of the CSP has been known to be essential for the chiral recognition.[8] In this instance, the chiral recognition behaviors of CSP **2** might be affected by protecting the unreacted residual aminopropyl groups of the CSP. However, the effect of the protection of the unreacted aminopropyl groups of CSP **2** on the chiral recognition behaviors has not been reported so far.

In order to elucidate the effect of the protection of the unreacted residual aminopropyl groups of CSP **2**, in this study, we prepared new CSPs (CSPs **3**, **4** and **5** in Figure 1) in which the unreacted residual aminopropyl groups were protected by acetyl, butyryl or pivaloyl group, respectively and compared the chiral recognition behaviors on CSP **2** with those on CSPs **3**, **4** and **5**. The chromatographic results for the resolution of selected one amino alcohol, two amines and four $\alpha$-amino acids on CSPs **2**, **3**, **4** and **5** are summarized in Table 1. As an example, Figure 2 shows the representative chromatograms for the resolution of phenylalanine on CSP **2**, CSP **3**, CSP **4** and CSP **5**. All resolution results summarized in Table 1 and the chromatograms shown in Figure 2 were obtained with the use of 50% methanol in water containing 10 mM sulfuric acid as a mobile phase.

As shown in Table 1 and Figure 2, the retention factors ($k_1$) on CSP **3** and CSP **4** are generally greater than those on CSP **2** while the retention factors ($k_1$) on CSP **5** are generally worse than those on CSP **2**. Similarly, the resolution factors ($R_S$) on CSP **3** and CSP **4** are also generally greater than those on CSP **2** while the resolution factors ($R_S$) on CSP **5**

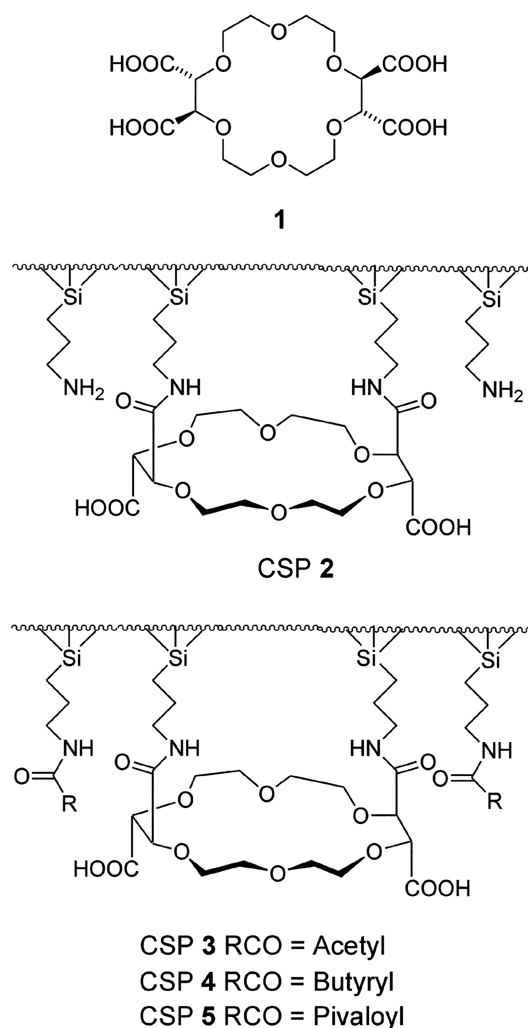


**Figure 1**. Structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1**, CSP **2**, CSP **3**, CSP **4** and CSP **5**.

**Table 1**. Resolution of racemic compounds containing a primary amino functional group on CSP **2**, CSP **3**, CSP **4** and CSP **5**[a]

| analytes | CSP **2** | | | CSP **3** | | | CSP **4** | | | CSP **5** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k_1$[b] | α[c] | Rs[d] | $k_1$[b] | α[c] | Rs[d] | $k_1$[b] | α[c] | Rs[d] | $k_1$[b] | α[c] | Rs[d] |
| 1-Aminoindan | 0.47 (R) | 1.91 | 2.64 | 0.74 (R) | 1.87 | 3.63 | 0.78 (R) | 1.90 | 3.75 | 0.48 (R) | 1.71 | 2.33 |
| 1-Naphthylethylamine | 3.05 (S) | 1.33 | 2.21 | 4.39 (S) | 1.28 | 2.25 | 4.96 (S) | 1.30 | 2.30 | 2.39 (S) | 1.25 | 1.58 |
| Phenylglycinol | 0.59 (S) | 1.34 | 1.26 | 0.85 (S) | 1.32 | 1.79 | 0.86 (S) | 1.34 | 1.86 | 0.54 (S) | 1.39 | 1.59 |
| Leucine | 0.57 (S) | 1.67 | 1.84 | 0.70 (S) | 1.46 | 2.09 | 0.70 (S) | 1.51 | 2.51 | 0.44 (S) | 1.49 | 1.54 |
| Phenylalanine | 0.47 (S) | 1.77 | 1.85 | 0.61 (S) | 1.60 | 2.25 | 0.64 (S) | 1.69 | 2.42 | 0.45 (S) | 1.65 | 1.91 |
| Phenylglycine | 1.90 (S) | 2.26 | 4.35 | 2.08 (S) | 2.19 | 6.10 | 2.15 (S) | 2.21 | 6.00 | 1.37 (S) | 2.28 | 5.40 |
| Tyrosine | 0.78 (S) | 1.66 | 2.03 | 0.85 (S) | 1.51 | 2.28 | 0.86 (S) | 1.58 | 2.52 | 0.60 (S) | 1.54 | 1.86 |

[a]Mobile phase: 50% Methanol in water containing sulfuric acid (10 mM). Flow rate: 0.5 mL/min. Detection: 210 nm UV. Column temperature: 20 °C. [b]Capacity factor of the first eluted enantiomer. Absolute configuration of the first eluted enantiomer is given in the parenthesis. [c]Separation factor. [d]Resolution factor.

are generally worse than those on CSP **2**. In contrast, the separation factors (α) are usually worse on all of the three residual amino group-protected CSPs compared to those on CSP **2**.

When the competition of the primary ammonium ions of the unreacted residual aminopropyl groups of CSP **2** with the primary ammonium ions of analytes for the complexation inside the cavity of the crown ether ring of the CSP is diminished by protecting the unreacted residual aminopropyl groups of the CSP, the retention factors are expected to increase on the residual amino group-protected CSPs. These expectations are indeed true on CSP **3** and CSP **4** as shown in Table 1. However, when the protecting acyl group of the CSP is relatively bulky as in CSP **5**, the retention factors even decrease. At the present time, we do not know why the retention factors on CSP **5** decrease compared to the retention factors on CSP **2**. The protecting bulky acyl group (pivaloyl group in this case) of CSP **5** might hinder the effective formation of the diastereomeric complex of the primary ammonium ions of analytes inside the cavity of the crown ether ring of the CSP. In this instance, the retention factors should decrease.

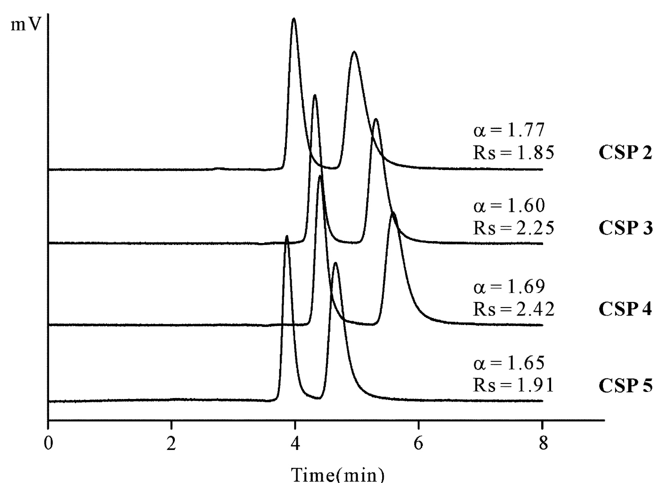


**Figure 2**. Chromatograms for the resolution of phenylalanine on CSPs **2**, **3**, **4** and **5**. Mobile phase: 50% methanol in water containing sulfuric acid (10 mM). Flow rate: 0.5 mL/min. Detection: 210 nm UV. Column temperature: 20 °C.

When the unreacted residual aminopropyl groups of the CSP are protected by acyl group, the lipophilicity of the CSP increases and consequently the lipophilic interaction between the CSP and analytes is also expected to increase in aqueous mobile phase. The increased lipophilic interaction between the CSP and analytes might also be responsible for the enhanced retention factors on the residual amino group-protected CSPs. The lipophilic interaction between the residual amino group-protected CSPs and analytes is expected to become more significant with longer or larger and consequently more lipophilic N-acyl protecting group of the CSP. In this instance, the lipophilic interaction between CSP **4** or CSP **5** and analytes should be greater than that between CSP **3** and anaytes and consequently the retention factors on CSP **4** and CSP **5** should be greater than on CSP **3**. However, the chromatographic resolution results are not consistent with these expectations. In this instance, it is concluded that the increased lipophilicity of the CSP resulting from the protection of the unreacted residual aminopropyl groups of the CSP is not significant for the enhanced retention factors.

The retention behaviors of the two enantiomers for the resolution of 1-aminoindan are graphically illustrated in Figure 3 as an example. As shown in Figure 3, both retention factors ($k_1$ and $k_2$) increase on CSP **3** and CSP **4**. However, the ratio of the two retention factors ($k_2/k_1$) does not increase, but even decrease. Consequently, the separation factor (α) decreases on CSP **3** and CSP **4**. In the case of CSP **5**, both retention factors decrease and the ratio of the two retention factors also decreases quite much, the separation factor decreasing. However, as clearly shown in Figure 3, the difference between the retention times denoted by the retention factors of the two enantiomers increases on CSP **3** and CSP **4** without peak broadening and consequently the resolution factor ($R_S$) increases on CSP **3** and CSP **4**. On the contrary, the difference between the retention times of the two enantiomers decreases quite much on CSP **5** and the resolution factor decreases.

In summary, the protection of the unreacted residual aminopropyl groups of CSP **2** was found to improve the retention and the resolution factors for the resolution of racemic compounds containing a primary amino group. However, the size of the protecting acyl group should not be

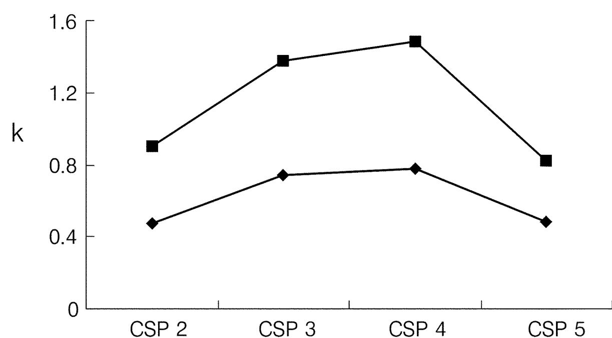


**Figure 3**. Variation of the retention factors $k_1$ ( ◆ ) and $k_2$ ( ■ ) for the resolution of 1-aminoindan on CSP **2**, CSP **3**, CSP **4** and CSP **5**. Mobile phase: 50% methanol in water containing sulfuric acid (10 mM). Flow rate: 0.5 mL/min. Detection: 210 nm UV. Column temperature: 20 °C.

too bulky. As a protecting acyl group, acetyl or butyryl group seems to be good enough for the improved retention and the improved resolution. However, the separation factors were found to decrease slightly when the unreacted residual aminopropyl groups of the CSP are protected by an acyl group.

## Experimental Section

**Preparation of CSPs.** CSP **2** was prepared by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** (1.2 g, 2.7 mmole) to aminopropylsilica gel (10.8 g, Kromasil 5 μm, 100 Å) *via* the same procedure as that reported previously except with the use of 2,6-lutidine instead of triethylamine.[4a] Based on the elemental analysis of aminopropylsilica gel (C, 5.45%; H, 1.47%; N, 1.74%) and CSP **2** (C, 9.60%; H, 1.14%; N, 1.57%), the surface concentration of the chiral selector calculated according to the equation reported[9] was 0.70 mmol/m$^2$.

CSP **2** thus prepared was divided into four portions. One portion was reserved for column packing. Each (2.6 g) of the other three portions of CSP **2** was suspended in benzene (60 mL) in 150-mL two-neck round bottom flask equipped with a Dean-Stark trap, a condenser and a magnetic stirrer. The heterogeneous mixture was heated to reflux to remove water azeotropically. After the complete azeotropic removal of water, benzene was removed by rotary evaporation. Dried CSP **2** thus obtained was suspended in dry methylene chloride (50 mL) and then 2,6-lutidine (1.66 mL, 14.3 mmole) was added. To the stirred heterogeneous solution was slowly added a solution of acetyl chloride (0.92 mL, 12.9mmole), butyryl chloride (1.34 mL, 12.9 mmole) or pivaloyl chloride (1.59 mL, 12.9 mmol) in dry methylene chloride (10 mL), respectively at 0 °C. The whole mixture was stirred for 2 h at 0 °C and then for 24 h at room temperature. The modified slica gel (CSP **3**, CSP **4** or CSP **5**) was washed successively with methanol, acetone, ethyl acetate, methylene chloride, hexane and ether and then dried under high vacuum. Based on the elemental analysis of CSP **2** and CSP **3** (C, 10.54%; H, 1.17%; N, 1.50%), the surface concentration of the acetyl group of CSP **3** was calculated to be 1.17 mmol/m$^2$. Similarly, based on the elemental analysis of CSP **2**, CSP **4** (C, 10.90%; H, 0.97%; N, 1.50%) and CSP **5** (C, 10.87%; H, 1.16%; N, 1.54%), the surface concentration of the butyryl group of CSP **4** and the pivaloyl group of CSP **5** was calculated to be 0.81 μmol/m$^2$ and 0.63 μmol/m$^2$, respectively. Each of four CSPs thus prepared (CSPs **2**, **3**, **4** and **5**) was slurried in methanol and packed into 150 × 4.6 mm I.D. stainless steel HPLC colmn by using a conventional slurry packing method with an Alltech slurry packer.

**Chromatography.** Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20 μL sample loop, a Waters 2487 UV Dual λ Absorbance Detector and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus, Seoul, Korea). The temperature of the chiral column was controlled by using a JEIO TECH VTRC-620 Circulator (Seoul, Korea).

Racemic or optically active analytes used in this study were available from previous studies.[4,6] Elution orders were determined by injecting configurationally known samples. Column void volume was measured by injecting 2,6-lutidine, which was known as an unretained analyte.[4b]

**Acknowledgements.** This research work was supported by a grant from KISTEP, Korea (NRL program: M1-0318-00-0005).

## References

1. Behr, J. P.; Girodeau, J. M.; Heyward, R. C.; Lehn, J. M.; Sauvage, J. P. *Hel. Chim. Acta* **1980**, *63*, 2096.
2. (a) Lin, J. M.; Nakagama, T.; Hobo, T. *Chromatographia* **1996**, *42*, 559. (b) Mori, Y.; Ueno, K.; Umeda, T. *J. Chromatogr. A* **1997**, *757*, 328. (c) Kuhn, R. *Electrophoresis* **1999**, *20*, 2605. (d) Cho, S. I.; Lee, K.-N.; Kim, Y.-K.; Jang, J.; Chung, D. S. *Electrophoresis* **2002**, *23*, 972.
3. Hyun, M. H. *J. Sep. Sci.* **2003**, *26*, 242.
4. (a) Hyun, M. H.; Jin, J. S.; Lee, W. *J. Chromatogr. A* **1998**, *822*, 155. (b) Hyun, M. H.; Cho, Y. J.; Jin, J. S. *J. Sep. Sci.* **2002**, *25*, 648. (c) Lee, W.; Baek, C.-S.; Lee, K. *Bull. Korean Chem. Soc.* **2002**, *23*, 1677. (d) Hyun, M. H.; Cho, Y. J.; Kim, J. A.; Jin, J. S. *J. Liq. Chromatogr. Rel. Tech.* **2003**, *26*, 1083.
5. Hyun, M. H., Min, H. J.; Cho, Y. J. *Bull. Korean Chem. Soc.* **2003**, *24*, 911.
6. Hyun, M. H.; Jin, J. S.; Koo, H. J.; Lee, W. *J. Chromatogr. A* **1999**, *837*, 75.
7. (a) Hyun, M. H.; Jin, J. S.; Lee, W. *Bull. Korean Chem. Soc.* **1998**, *19*, 819. (b) Hyun, M. H.; Han, S. C.; Jin, J. S.; Lee, W. *Chromatographia* **2000**, *52*, 473. (c) Hyun, M. H.; Han, S. C.; Cho, Y. J.; Jin, J. S.; Lee, W. *Biomed. Chromatogr.* **2002**, *16*, 356.
8. Lehn, J.-M. *J. Inc. Phenom.* **1988**, *6*, 351.
9. Stalcup, A. M.; Williams, K. L. *J. Liq. Chromatogr.* **1992**, *15*, 29.