Account

Development and Application of Crown Ether-Based HPLC Chiral Stationary Phases

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Crown ether-based HPLC chiral stationary phases (CSPs) have been successfully utilized in the resolution of various racemic compounds containing a primary amino group. Especially, CSPs based on chiral crown ethers incorporating chiral binaphthyl unit or tartaric acid unit and based on phenolic pseudo chiral crown ethers have shown high chiral recognition efficiency. In this account paper, a review on the development of crown ether-based HPLC CSPs, their structural characteristics and applications to the resolution of racemic compounds including chiral drugs containing a primary or secondary amino group with the variation of the type and the content of mobile phase components and with the variation of the column temperature is presented.

Key Words : Chiral crown ether, Chiral stationary phase, Enantiomer separation, Liquid chromatography

Introduction

It is well known that only one enantiomer of chiral drugs often shows the desired pharmacological activity while the other enantiomer shows the undesired side effect such as toxicity.[1] Consequently, individual enantiomers of chiral drugs should be examined for their own pharmacological properties during the process of developing or marketing new chiral drugs according to the guidelines issued by the drug regulatory bodies.[2] For this purpose, the techniques of separating enantiomers and assaying the exact enantiomeric purity of individual enantiomers or the exact enantiomeric composition of chiral drugs are essential. There are various chiral separation methods, which can be applied for the separation of enantiomers and for the determination of the enantiomeric purity of individual enantiomers or the enantiomeric composition of chiral drugs.[3] However, the liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been proved to be the most accurate, convenient and economic means in separating the two enantiomers for both analytical and preparative purposes and in assaying the enantiomeric purity of individual enantiomers or the enantiomeric composition of chiral drugs.[4]

The success of liquid chromatographic chiral separations on CSPs depends mostly on the availability of effective CSPs. Consequently, various efforts have been devoted to the development of effective CSPs and as results various CSPs were developed. For example, CSPs based on proteins such as ovomucoids[5] or bovine serum albumin,[6] cellulose derivatives,[7] cyclodextrins,[8] macrocyclic antibiotics,[9] amino acid derivatives[10] and other low molecular weight optically active chiral molecules[11] have been successfully utilized in the resolution of various racemic compounds. Especially, for the separation of the two enantiomers of racemic compounds containing a primary amino group, crown ether-based CSPs have been most successfully utilized.[12]

Crown ethers, first introduced by Pederson in 1967,[13] are macrocyclic polyethers, which have a cavity of specific size. The ether oxygens, which are placed regularly around the inside wall of the cavity, can act as electron donor ligand atoms and consequently, metal or ammonium cations are incorporated into the cavity.[14] Chiral crown ethers have been developed by incorporating appropriate chiral units as chiral barrier(s) into crown ethers. For example, the bulky chiral aromatic rings such as binaphthyl[15] or biphenanthryl units,[16] helicene derivatives,[17] and suitable optically active natural compounds such as tartaric acid[18] or carbohydrates[19] have been successfully incorporated into crown ethers as chiral barrier(s) to produce chiral crown ethers. In addition, some chiral aza crown ethers,[20] chiral pyridino crown ethers[21] and phenolic pseudo chiral crown ethers[22] were also developed. However, the successful chiral crown ethers as chiral selectors of crown ether-based HPLC CSPs for the separation of enantiomers of racemic compounds containing a primary amino group are limited. Among others, chiral crown ethers incorporating chiral binaphthyl unit or tartaric acid unit and phenolic pseudo chiral crown ethers have been most

successfully utilized in the resolution of racemic compounds containing a primary amino functional group.

In this account paper, the discussion will be focused on the development and application of three different type crown ether-based HPLC CSPs, which are most widely applicable and commercially available. The first type is the CSPs based on chiral crown ethers incorporating a chiral 1,1'-binapthyl unit. The second type is the CSPs based on chiral crown ethers incorporating tartaric acid unit. The third type is the CSPs based on phenolic pseudo chiral crown ethers.
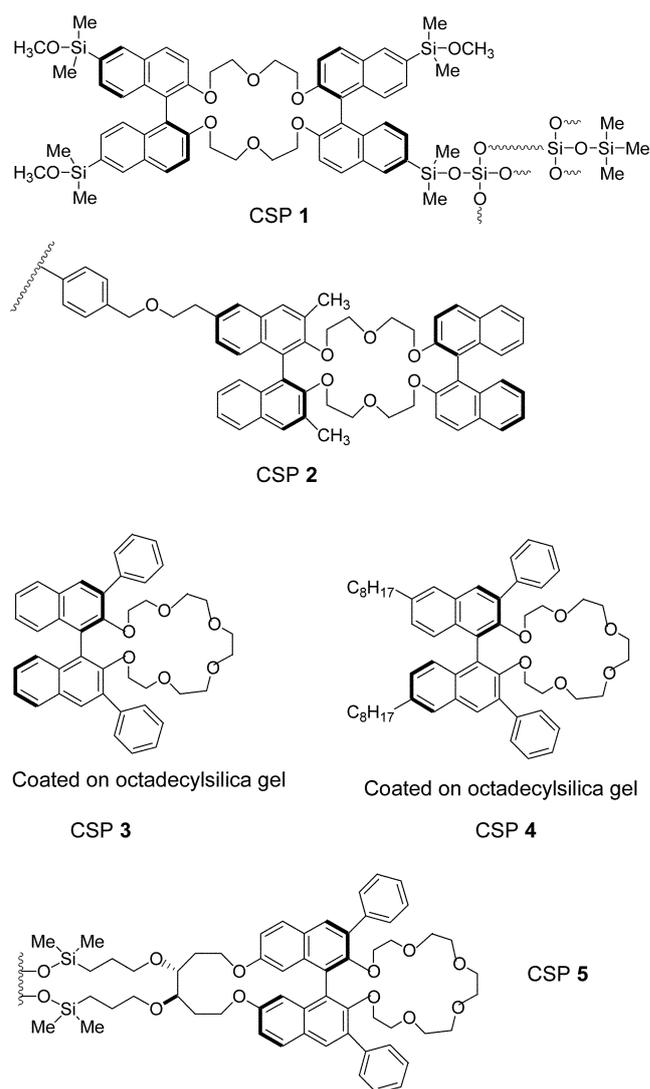
CSPs Based on Chiral Crown Ethes Incorporating Chiral 1,1'-Binaphthyl Unit

Chiral crown ethers incorporating 1,1'-binaphthyl unit were first developed by Cram.[15] Especially, optically active bis-(1,1'-binaphthyl)-22-crown-6 compounds were utilized as CSPs for the resolution of amines, amino acids and amino esters after the immobilization on silica gel (CSP **1**, Figure 1)[23] or polystyrene (CSP **2**, Figure 1)[24] in the late 1970s. However, the chiral resolution efficiency of the two CSPs based on optically active bis-(1,1'-binaphthyl)-22-crown-6 compounds was not good enough for the general use.

In 1987, Shinbo and coworkers developed a very successful CSP (CSP **3**, Figure 1) by dynamically coating (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 on octadecylsilica gel.[25] Chiral crown ether, (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6, first synthesized by Cram[15d] was dissolved in a mixed solvent of methanol-water and the resulting solution was eluted through octadecylsilica gel column (LiChrosorb RP-18, 125 × 4 mm I. D.) to afford CSP **3**. The lipophilic interaction between the octadecyl groups of silica gel and the chiral crown ether, which containing a highly lipophilic 3,3'-diphenyl-1,1'-binaphthyl group, is believed to be responsible for the dynamically coated nature of the CSP.

CSP **3** thus prepared was first applied for the resolution of various α-amino acids and 1-phenylethylamine with the use of 10^{-2} M perchloric acid as a mobile phase and the resolution results were found to be quite excellent.[25] The chiral column packed with CSP **3** has actually been commercialized as a brand name, CROWNPAK CR (Daicel Chemical Industries, Tokyo, Japan) and many users have used the commercial chiral column for the resolution of various racemic compounds containing a primary amino functional group. In addition to the resolution of α-amino acids and 1-phenylethylamine, CSP **3** was also successfully applied for the resolution of biologically important chiral primary amines,[26] chiral cyclic amines,[27] amino alcohols,[26b,28] and β-amino acids.[29] In addition, chiral drugs such as aminoglutethimide, baclofen (muscle relaxant), primaquine (antimalarial)[28] and gemifloxacin mesylate, a new fluoroquinolone antibacterial agent[30] were resolved on CSP **3**.

Even though CSP **3** has been successfully utilized in the resolution of various racemic compounds containing a primary amino group, it has a severe drawback in that it should be applied to the resolution racemic compounds with the use of a mobile phase containing less than 15% methanol

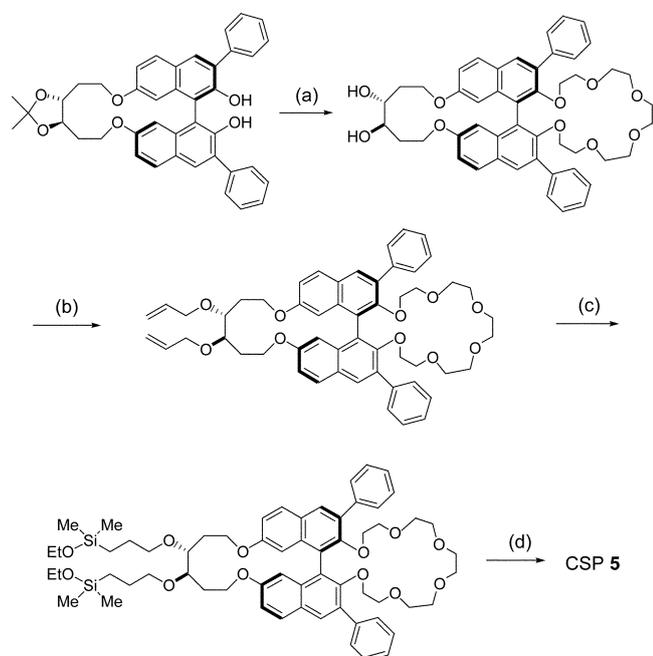**Figure 1.** Structures of CSPs **1-5** based on chiral crown ethers incorporating 1,1'-binaphthyl unit.

in water. When a mobile phase containing more than 15% methanol in water is used, CSP **3** is no more useful because the chiral selector material of the CSP leaches from the column. In order to improve the stability of the CSP, Shinbo and coworkers developed CSP **4** (Figure 1) by dynamically coating (6,6'-dioctyl-3,3'-diphenyl-1,1'-naphthyl)-20-crown-6 on octadecylsilica gel.[31] Two octyl groups attached to chiral crown ether are expected to improve the lipophilic interaction between the octadecyl group of the stationary phase and the selector, chiral crown ether. CSP **4** was quite successful for the resolution of α-amino acids, 1-phenylethylamine and 3-aminocaprolactam.[31] However, CSP **4** is also no more useful when a mobile phase containing more than 40% methanol in water is used because the chiral selector possibly leaches from the column under the mobile phase condition.

More recently, a new crown ether-based CSP (CSP **5**, Figure 1) was developed by covalently bonding the chiral crown ether selector material to silica gel as shown in Figure

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2.[32] As shown in Figure 2, a diphenyl substituted (*R*)-*cyclo*-BINOL, which was prepared through a diastereoselective biaryl oxidation with Koga's copper catalyst,[33] was transformed into a chiral crown ether having two hydroxy groups and then the resulting chiral crown ether was finally attached to silica gel through the *O*-allylation of the two hydroxy functional groups of the chiral crown ether, the hydrosilylation of the two terminal double bonds and the silica gel bonding. Because of the covalent nature of the chiral selector of the CSP, no limitation is expected in the use of mobile phase with CSP **5**.

CSP **5** thus prepared was reported to be very useful in the resolution of various *α*-amino acids,[32] racemic non-cyclic and cyclic amines,[34] amino alcohols,[34] various fluoroquinolone antibacterials,[35] tocainide (antiarrhythmic agent) and its analogues[36] and aryl *α*-amino ketones.[37] In general, the chiral recognition efficiency of CSP **5** was greater than that of CSP **3** or CSP **4**.[25,30,31,32,34,35] In addition, CSP **5** was reported to be practically very useful for the determination of enantiomeric composition or purity of optically active or enriched samples.[34,36,37] For example, the chromatograms for the resolution of racemic and (*R*)-tocainide prepared from racemic and (*R*)-alanine respectively are shown in Figure 3a and Figure 3b.[36] Based on the computer-generated peak areas corresponding to the two enantiomers shown in Figure 3b, the enantiomeric purity of (*R*)-tocainide was calculated to be 99.4% ee (*R*:*S*=99.7:0.3).[36]
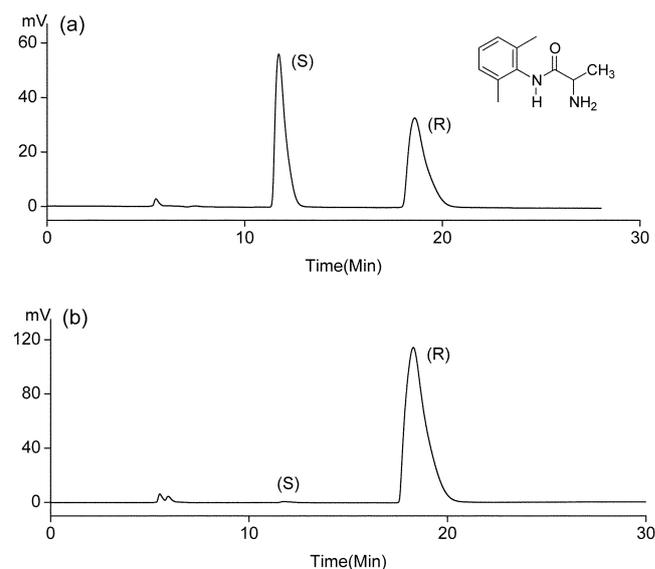
In the resolution of racemic primary amino compounds on crown ether-based CSPs, organic modifiers in aqueous

mobile phase are very important factor influencing the enantioseparation. Water or water-organic solvent mixture containing a small amount of acidic modifier has usually been used as a mobile phase. For the resolution of primary amino compounds on CSP **3**, **4** and **5**, water miscible solvent such as methanol, ethanol or acetonitrile has been used as an organic modifier.[26-32,34-37] When the content of an organic modifier in aqueous mobile phase increased, the retention factors (*k*) for the resolution of *α*-amino acids and amino alcohols on CSP **3** or CSP **4** diminished while the separation factors (*α*) improved.[25,28] These resolution trends with the variation of the content of organic modifier in aqueous mobile phase are exactly consistent with those for the resolution of *α*-amino acids,[32] amines,[34] amino alcohols,[34] fluoroquinolone antibacterials,[35] and tocainide (antiarrhythmic agent) and its analogues[36] on CSP **5**. However, in the resolution of aryl *α*-amino ketones on CSP **5**, both the retention (*k*) and the separation factors (*α*) diminished when the content of organic modifier in aqueous mobile phase increased.[37]

The reason for the trends of the separation factors (*α*) on CSP **3**, **4** and **5** with the variation of the content of organic modifier in aqueous mobile phase is not clear. However, the trends of the retention factors (*k*) on CSP **3**, **4** and **5** with the variation of the content of organic modifier in aqueous mobile phase was suggested to stem from the balance between the lipophilic interaction of analytes with a CSP and the hydrophilic interaction of analytes with mobile phase.[12,37] In reverse phase chromatography, the lipophilic interaction between the stationary phase and analytes is an important factor for the retention of analytes. With an increase in the content of organic modifier in aqueous

Figure 2. Scheme for the preparation of CSP **5**. (a) (1) KOH, pentaethyleneglycol ditosylate, tetrahydrofuran (THF), reflux for 72h. (2) 1 M HCl solution, methanol. (b) (1) NaH, THF, reflux for 30 min. (2) Allyl bromide, THF, reflux for 4h. (c) (1) (CH₃)₂ClSiH, H₂PtCl₆·6H₂O, methylene chloride, reflux for 5h. (2) Ethanol-triethylamine (1 : 1, v/v), methylene chloride. (d) 5 *µ*m silica gel, Dean-Stark trap, toluene, reflux for 72h.


Figure 3. (a) Chromatogram for the resolution of racemic tocainide on CSP **5**. (b) Chromatogram for the resolution of (R)-enriched tocainide prepared from (R)-alanine on CSP **5**. Chromatograms were obtained with the mobile phase of 80% acetonitrile in water containing sulfuric acid (10 mM) and ammonium acetate (1 mM). Flow rate, 0.5 mL/min; detection, 210 nm UV; temperature, 20 °C.

mobile phase, the polarity of the mobile phase decreases and consequently the lipophilic interaction between the stationary phase and analytes decreases. In this instance, the retention factors (k) should decrease and the decreasing trends of the retention factors (k) should be more significant with more lipophilic analytes.[37]

Even though the exact chiral recognition mechanism for the resolution of racemic primary amino compounds on crown ether-based CSPs is not clear yet and still controversial, the tripodal complexation of the protonated primary amino group ($R\text{-}NH_3^+$) inside the cavity of 18-crown-6 ring via three $^+N\text{-}H\cdots O$ hydrogen bonds has been proposed to be essential for the chiral recognition.[38] In this instance, acidic modifier added to aqueous mobile phase is believed to be used to protonate the primary amino groups of analytes. As an acidic modifier, any of perchloric acid, sulfuric acid, acetic acid, nitric acid, hydrochloric acid, methanesulfonic acid and phosphoric acid can be used.[12] In general, perchloric acid has been most widely and successfully used as an acidic modifier for the resolution of primary amino compounds on CSP **3** and CSP **4**.[25-29,31] However, in the resolution of a fluoroquinolone antibacterial agents on CSP **3**, sulfuric acid was found most effective as an acidic modifier.[30] For the resolution of primary amino compounds on CSP **5**, perchloric acid, trifluoroacetic acid, acetic acid and sulfuric acid were found to be equally effective.[32,34-37] However, sulfuric acid was most widely used as an acidic modifier for the resolution of primary amino compounds on CSP **5**.

The content of acidic modifier in aqueous mobile phase is another important factor which can influence the chromatographic behaviors for the resolution of primary amino compounds on crown ether-based CSPs. For the resolution of α-amino acids on CSP **3**, an increase in the content of acidic modifier in aqueous mobile phase was reported to improve the retention factors (k) and the separation factors (α).[17,39-41] To rationalize the chromatographic behaviors for the resolution of α-amino acids on CSP **3**, Shinbo and coworkers proposed a mechanism of interaction between the protonated amino acids and the chiral crown ether-coated stationary phase.[17] Under acidic condition, amino acids are converted to protonated forms (A^+) and distributed between the mobile phase and the stationary phase by the following equations.

$$A^+ + X^- \rightleftharpoons \overline{AX}$$

$$A^+ + \overline{C} + X^- \rightleftharpoons \overline{ACX}$$
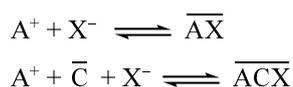
In the above equations, X^- is the acid anion present in mobile phase, and C, AX and ACX are the crown ether, the ion pair between A^+ and X^- and the ternary complex formed from A^+, C and X^-, respectively. The bars above the letters denote the stationary phase. An increase in the content of acidic modifier in mobile phase increases the concentration of acid anion, X^-, in mobile phase and shifts the equilibrium shown in the above two equations to the right. In this instance, the retention of the two enantiomers should

increase.

According to the above two equations, the lipophilicity of the acid anion, X^-, was also expected to influence the retention of the two enantiomers.[12] The ion pair, AX, formed from a protonated amino acid, A^+, and a lipophilic anion, X^-, is expected to approach to the stationary phase more effectively with a more lipophilic acid anion, X^-, and consequently the retention of the two enantiomers should increases with a more lipophilic anion. Actually, the resolution of phenylglycine and methionine on CSP **3** with a mobile phase containing perchloric acid, nitric acid or hydrochloric acid of identical concentration as an acidic modifier showed that the retention factors (k) increase as the lipophilicity of the acid anion increases as follows: $Cl^- < NO_3^- < ClO_4^-$.[25] In the resolution of aminoindanol on CSP **3**, the retention factors were also found to increase as the lipophilicity of the acid anion increases as follows: $H_2PO_4^- < NO_3^- < CF_3CO_2^- < ClO_4^-$.[42]

The trends of the retention factors (k) for the resolution of primary amino compounds on CSP **5** were not consistent with those on CSP **3**. CSP **5** is less lipophilic than CSP **3** and consequently the equilibriums expressed by the above two equations seems not to move to the right so effectively when the content of acidic modifier in aqueous mobile phase increases. In the resolution of α-amino acids on CSP **5**, the retention factors (k) showed a maximum at a certain concentration of acidic modifier.[32] However, in the resolution of amines, amino alcohols and other related primary amino compounds on CSP **5**, the retention factors (k) were diminished significantly as the content of acidic modifier in aqueous mobile phase increases.[34] In addition, for the resolution of fluoroquinolone antibacterials,[35] tocainide and its analogues,[36] and aryl α-aminoketones[37] on CSP **5**, the retention factor (k) also decreased significantly as the content of acidic modifier in aqueous mobile phase increased. As the content of acidic modifier in aqueous mobile phase increases, the ionic strength of the mobile phase is expected to increase. In this instance, on a less lipophilic CSP, the protonated analytes are expected to be distributed to the mobile phase more significantly than to the stationary phase because of the more significant hydration of the ionic analytes by aqueous mobile phase and consequently, are eluted faster as the content of acidic modifier in aqueous mobile phase increases.[12]

In order to reduce the retention of the two enantiomers for the resolution of hydrophobic amino compounds such as alanine-β-naphthylamide and 1-(1-naphthyl)ethylamine on CSP **3**, Machida and coworkers added some cations to mobile phase and found that the retention of amino compounds decreased with the addition of cation in the order: Li^+, Na^+, NH_4^+, K^+.[43] Competition between a cation added to mobile phase and a protonated primary ammonium ion ($R\text{-}NH_3^+$) of analytes for the complexation inside the cavity of the crown ether ring of the CSP is expected to reduce the retention of analytes. The stability of the complex between crown ether and cation significantly depends on the fitness of the size of the cavity to that of the cation (Li^+, 1.36

Å; Na^+, 1.94 Å; K^+, 2.66 Å; NH_4^+, 2.84 Å). The diameter of 18-crown-6 ether ring is estimated as 2.6 Å and consequently, K^+ was presumed to best fit the cavity size of the crown ether.[43] For the resolution of racemic amino compounds on CSP **5**, the retention time of the two enantiomers has been successfully controlled by adding ammonium ion (NH_4^+) to mobile phase.[32,34-37] For the resolution of racemic amines, amino alcohols, related primary amino compounds and fluoroquinolone compounds on CSP **5**, K^+ was also utilized to reduce the retention time of analytes and was found more significant in reducing retention time of analytes than NH_4^+.[34,35]

Column temperature is also important factor influencing the chromatographic behaviors for the resolution of racemic primary amino compounds on crown ether-based CSPs. For the resolution of racemic primary amino compounds on CSP **3**, **4** and **5**, the retention (k) and separation factors (α) were found to improve always as the column temperature was lowered.[25,28,31-32,34,35,37,39-41] At lower temperature, the diastereomeric complexes formed between the individual enantiomers of an analyte and the chiral crown ether selector of the CSP are expected to become energetically more favorable and this is more significant with the more stable diastereomeric complex. Consequently, the retention (k) and the separation factors (α) should increase as the column temperature decreases.[12]

CSPs Based on Chiral Crown Ethes Incorporating Tartaric Acid Unit

Among chiral crown ethers incorporating tartaric acid unit, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **6** (Figure 4), which was first developed by Lehn,[18] has been widely utilized since early 1990s as a chiral selector for the resolution of racemic primary amino compounds by capillary electrophoresis.[27,44-50] However, application of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **6** as a chiral selector of liquid chromatographic CSPs was reported first in 1998 by Machida[51] and Hyun.[52,53]

Machida and coworkers prepared CSP **7** (Figure 4) by treating (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **6** with aminopropylsilica gel in the presence of a coupling agent EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline).[51] In the preparation of CSP **7**, the mode of connecting chiral selector to aminopropylsilica gel in the presence of a coupling agent allows a structural variety in the linkage and consequently, the exact structure of CSP **7** is ambiguous even though the structure of CSP **7** was reported as shown in Figure 4. In contrast, a structurally well defined CSP (CSP **8**, Figure 4) was prepared by simple two step procedure as shown in Figure 5.[53] (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid **6** was converted into dian-hydride by treating with acetyl chloride and then (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride was treated with aminopropylsilica gel in the presence of triethylamine to afford CSP **8**. The structure of CSP **8** shown in Figure 4 is believed to be *syn*-diamide form, based on the study concerning the stereoselective *syn*-opening of the dianhydride by primary amino compounds in the presence of triethyl-amine.[53]

CSP **7** was applied to the resolution of α-amino acids, amino alcohols and other primary amino compounds including afloqualone (muscle relaxant), primaquine (anti malarial), α-methyltryptamine and 1-(1-naphthyl)ethylamine.[51] CSP **8** was applied more widely than CSP **7**. CSP **8** was very successful in the resolution of α-amino acids, α-amino amides and α-amino esters.[53] Among α-amino acids tested for their resolution, asparagine, aspartic acid, isoleucine, threonine and valine were not resolved on CSP **7**.[51] However, all natural and unnatural α-amino acids including
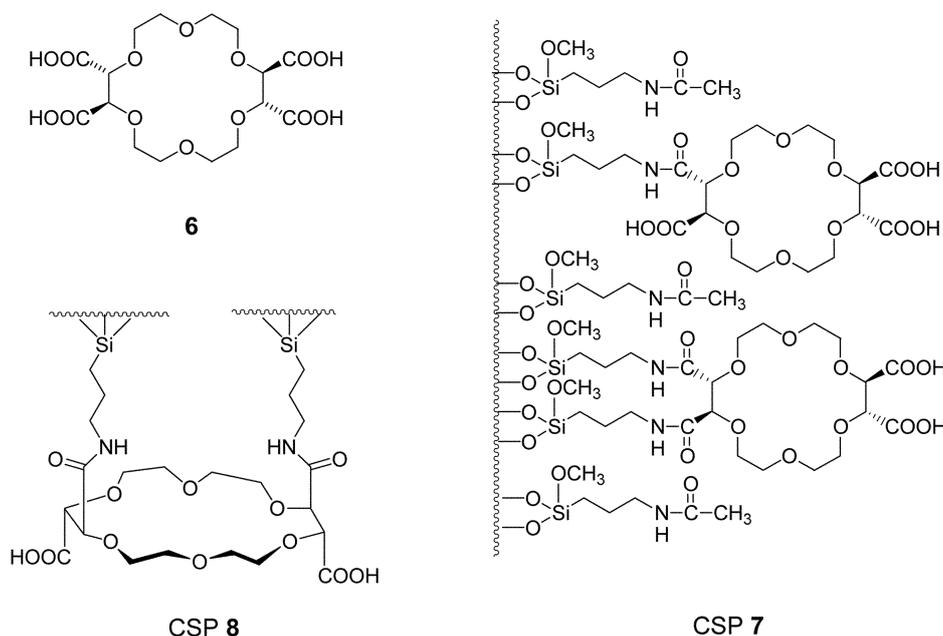

6

CSP 8

CSP 7

Figure 4. Structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **6**, CSP **7** and CSP **8**.
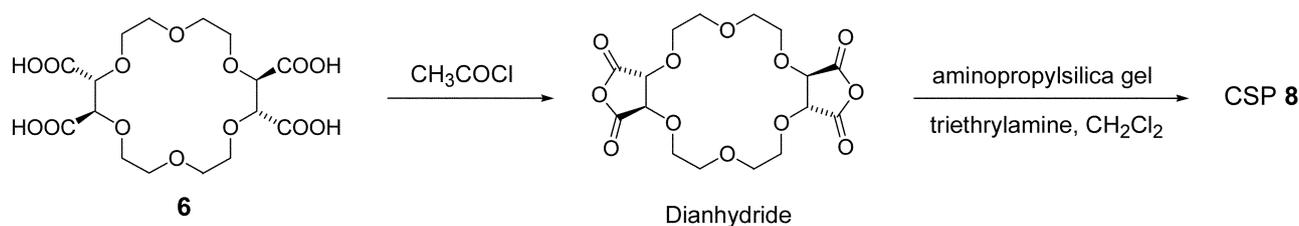
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Figure 5. Scheme for the preparation of CSP **8**.

thyroxine were resolved on CSP **8** with reasonable separation factors (a) except for proline, which does not contain a primary amino group.[53,55] In addition, the resolution factors (R_S) for most α-amino acids on CSP **8** were more than 1.00, except for asparagine, cysteine and isoleucine.[53] CSP **8** is now commercially available as a brand name, Chirosil RCA (+) and Chirosil SCA(−) (RS Tech, Daejeon, Korea). CSP **8** was also very successful in the resolution of various racemic amines,[56] amino alcohols,[56] fluoroquinolone antibacterial agents including gemifloxacin,[52,57,58] tocainide and its analogues,[55,59] β-amino acids,[60,61] and aryl α-amino ketones.[62] As examples, the representative chromatograms for the resolution of phenylglycine and gemifloxacin on CSP **8** are shown in Figure 6.

In addition to the primary amino compounds, five racemic secondary amino compounds including β-blockers were reported to be resolved on CSP **8** with the use of non-aqueous mobile phase.[63] Resolution of racemic secondary amino compounds on CSP **8** is very interesting in that tripodal complexation of the protonated primary amino group ($R-NH_3^+$) inside the cavity of 18-crown-6 ring via three $^+N-H\cdots O$ hydrogen bonds has been proposed to be essential for the chiral recognition of chiral crown ethers as descried above.[38] In the resolution of racemic primary amino compounds on CSPs based on chiral crown ethers incorporating chiral binaphthyl unit, in addition to the tripodal complexation, stereoselective steric interaction between the chiral barrier provided by the chiral binaphthyl unit of the CSPs and the three different substituents at the chiral center of analytes has been proposed to be responsible for the chiral recognition.[23,24] However, in the chiral recognition of primary amino compounds on CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, the two free carboxylic acid groups of the CSPs seem to act as chiral barriers, enantioselective hydrogen bonding sites or ionic interaction sites based on the NMR studies[64,65] and X-ray crystallographic study.[66] In this instance, the enantioselective hydrogen bonding interaction or ionic interaction between the carboxylate ion of CSP **8** and analytes in addition to the two $^+N-H\cdots O$ hydrogen bonds of the protonated secondary ammonium ions of analytes with the crown ether ring oxygens of the CSP might be responsible for the resolution of secondary amino compounds on CSP **8**.

In the resolution of primary amino compounds on CSP **7** and CSP **8**, a mixed solvent of water and water miscible organic solvent was usually used as a mobile phase.[51,53,55-62] As a water miscible organic modifier, ethanol, methanol,
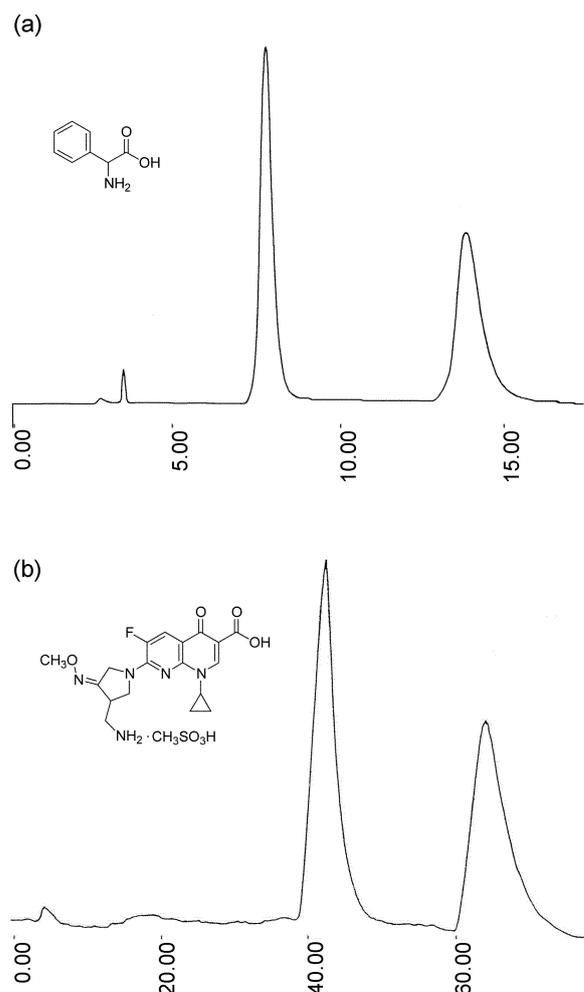

Figure 6. (a) Chromatogram for the resolution of phenylglycine on CSP **8**. Mobile phase: 80% methanol in water + sulfuric acid (10 mM), Flow rate: 0.5 mL/min, Detection: 210 nm UV, Temperature: 20 °C. (b) Chromatogram for the resolution of gemifloxacin on CSP **8**. Mobile phase: 80% methanol in water + sulfuric acid (10 mM), Flow rate: 0.8 mL/min, Detection: 254 nm UV, Temperature: 20 °C.

acetonitrile, 2-propanol or tetrahydrofuran can be used.[51,53,56] However, in general, methanol or acetonitrile has been most widely utilized as an organic modifier. For the resolution of α-amino acids, amines, amino alcohols, fluoroquinolone antibacterial agents, tocainide and its analogues, β-amino acids, and aryl α-amino ketones on CSP **8**, the retention factors (k), separation factors (α) and resolution factors (R_S) generally improved as the content of organic modifier in

aqueous mobile phase increased.[53,56-62] These trends of the retention factors (k) on CSP **8** are exactly opposite to those on CSP **3**, CSP **4** and CSP **5**. However, the retention factor (k) for the resolution of highly lipophilic analyte such as 1-(6,7-dimethyl-1-naphthyl)ethylamine on CSP **8** decreased as the methanol content in aqueous mobile phase increased.[56] In addition, the retention factors (k) for the resolution of relatively highly lipophilic analytes such as alanine-β-naphthylamide and 1-(1-naphthyl)ethylamine on CSP **7** also decreased as the acetonitrile content in aqueous mobile phase increased.[41]

The reason for the trends of the separation (a) and resolution factors (R_S) with the variation of the content of organic modifier in aqueous mobile phase is not clear yet. However, the trends of the retention factors (k) with the variation of the content of organic modifier in aqueous mobile phase were also suggested to stem from the balance between the lipophilic interaction of analytes with a CSP and the hydrophilic interaction of analytes with mobile phase based on the study concerning the effect of analyte lipophilicity on the resolution of various α-amino acids containing lipophilically different alkyl group at the chiral center.[12,67] Compared to the chiral selector of CSP **3**, CSP **4** or CSP **5**, the chiral selector of CSP **8** is less lipophilic. In addition, CSP **8** does not contain octadecyl groups at the silica surface. Consequently, CSP **8** is less lipophilic than CSP **3**, CSP **4** or CSP **5**. In this instance, the lipophilic interaction of analytes with CSP **8** is less significant in aqueous mobile phase compared to that with CSP **3**, CSP **4**

or CSP **5**. As the organic modifier in aqueous mobile phase increases, the mobile phase becomes less polar and more hydrophobic. Under this condition, the hydration or dissolution of polar-protonated analytes becomes less favorable and consequently, polar-protonated analytes are eluted slower and slower as the organic modifier content in aqueous mobile phase increases.[12] However, for the resolution of highly lipophilic analytes, the lipophilic interaction of analytes with CSP **7** or CSP **8** seems to be more significant than the hydrophilic interaction of analytes with mobile phase. In this event, the retention factors (k) should decrease as the content of organic modifier in aqueous mobile phase increases as evidenced by the retention behaviors for the resolution of alanine-β-naphthylamide and 1-(1-naphthyl)ethylamine on CSP **7**[51] and for the resolution of 1-(6,7-dimethyl-1-naphthyl)ethylamine on CSP **8**.[56] These retention trends for the resolution of primary amino compounds on CSP **8** with the variation of the organic modifier content in aqueous mobile phase were demonstrated to change to those on highly lipophilic CSPs such as CSP **3**, CSP **4** or CSP **5** by increasing the lipophilicity of the CSP. A highly lipophilic version (CSP **9**, Figure 7a) of CSP **8** was prepared by dynamically coating *N*-dodecyl diamde of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to octadecylsilica gel.[68] The retention factors (k) for the resolution of primary amino compounds on CSP **9** decreased as the organic modifier content in aqueous mobile phase increased.[68] The octadecyl groups of CSP **9** are believed to provide additional lipophilic sites like CSP **3** or
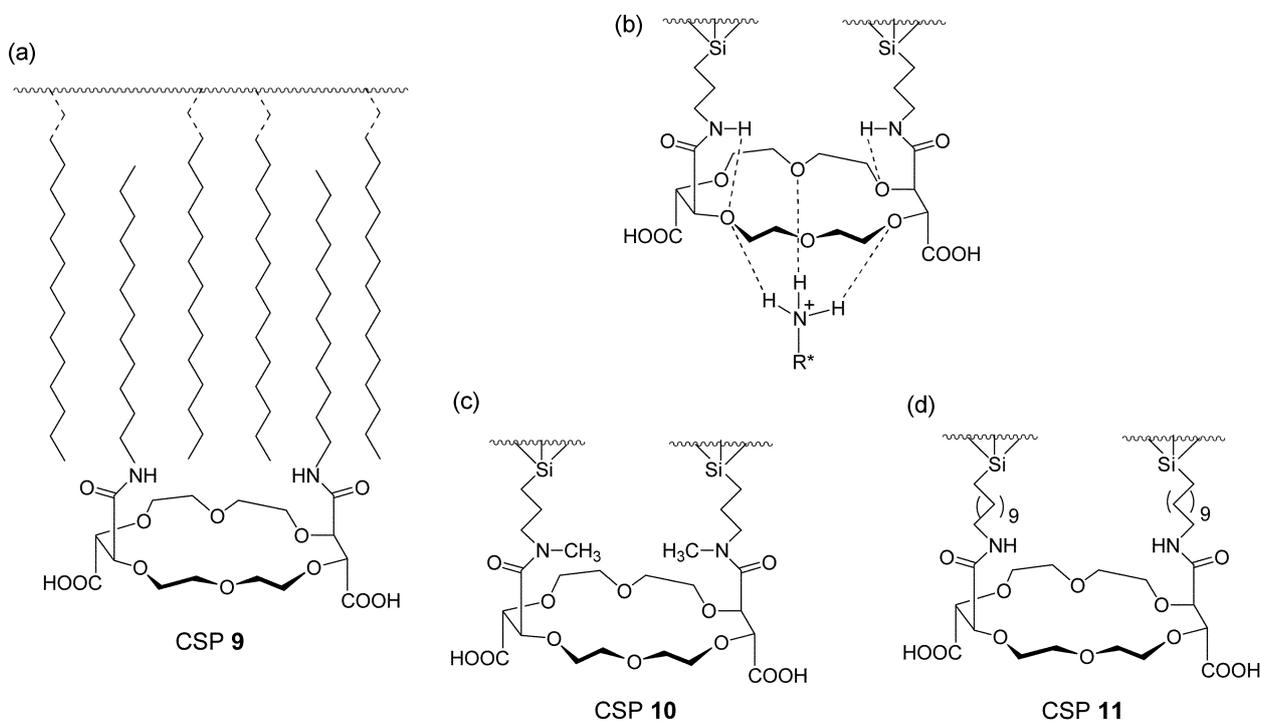
Figure 7. (a) Structure of CSP **9**. (b) Two intramolecular hydrogen bonds between the amide N-H hydrogen of the two connecting tethers of CSP **8** and the ether oxygens of the crown ether moiety of the CSP. These two intramolecular hydrogen bonds are expected to hinder the complex formation of the protonated analytes (R-NH₃⁺) with the crown ether ring. (c) Structure of CSP **10**. (d) Structure of CSP **11** containing two eleven methylene tethering groups.

CSP **4** and consequently the lipophilic interaction of analytes with CSP **9** should be quite significant. In this instance, the retention factors (k) for the resolution of primary amino compounds on CSP **9** decrease as the organic modifier content increases.

As an acidic modifier in aqueous mobile phase, which was required for the formation of primary ammonium ions (R-NH_3^+), perchloric acid was most successfully utilized in the resolution of racemic primary amino compounds on CSP **7**.[51] However, sulfuric acid was most widely and successfully utilized for the resolution of various racemic primary amino compounds except for β-amino acids on CSP **8** even though perchloric acid was still useful.[52,53,56-59, 61,62] In the resolution of β-amino acids on CSP **8**, acetic acid was much more effective as an acidic modifier than perchloric acid or sulfuric acid in terms of both enantioselectivity (α) and resolution (R_S).[60]

As the content of acidic modifier in aqueous mobile phase increased, the retention factors (k) for the resolution of alanine-β-naphthylamide and 1-(1-naphthyl)ethylamine on CSP **7** decreased.[51] These retention behaviors were rationalized in terms of the electrostatic interaction, which varies with the acidic modifier content in mobile phase, between the cationic analyte and the dissociated carboxylic groups of the crown ether chiral selector of CSP **7**.[51] For the resolution of phenyl glycine,[53] fluoroquinolone antibacterial agents,[57,58] β-amino acids[60] and tocainide analogues[59] on CSP **8**, the retention factors (k) generally decrease as the content of acidic modifier in aqueous mobile phase increases. As the content of acidic modifier in aqueous mobile phase increases, the ionic strength of mobile phase increases and consequently, the hydration or the dissolution of polar-protonated analytes by mobile phase is expected to increase. In this instance, polar-protonated analytes are eluted faster and faster as the acidic modifier content increases. However, the separation (α) and the resolution factors (R_S) did not show significant trends on CSP **8** with the variation of the acidic modifier content in aqueous mobile phase.[53,57-60]

The effect of the column temperature on the chromatographic behaviors for the resolution of racemic primary amino compounds on CSP **7** or CSP **8** was identical to that on CSP **3**, CSP **4** or CSP **5**. Namely, the retention (k) and separation factors (α) always improved as the column temperature was lowered.[51,53,56-58,60,62] Data for the resolution of racemic amines on CSP **8** with the variation of the column temperature allowed the calculation of $\Delta\Delta H$ and $\Delta\Delta S$ value, the differential enthalpy and entropy of absorption for the two enantiomers, from the van't Hoff plots.[56] In this case, both $\Delta\Delta H$ and $\Delta\Delta S$ value were negative and consequently, negative $\Delta\Delta G$ is entirely dependent on $\Delta\Delta H$. In this event, the enantioselectivity was reported to be enthalpy controlled. However, in the resolution of β-blockers (secondary amino alcohols) on CSP **8** with the use of the mixture of trifluoroacetic acid-triethylamine-ethanol-acetonitrile (0.1/0.5/20/80, v/v/v/v) as a mobile phase, the separation factors (α) increased as the column temperature increased.[69] From the

van't Hoff plots, both $\Delta\Delta H$ and $\Delta\Delta S$ value were found positive and consequently negative $\Delta\Delta G$ is entirely dependent on $\Delta\Delta S$. Consequently, resolution of β-blockers on CSP **8** is entropy controlled.

In order to improve the chiral recognition efficiency or the stability of CSP **8**, various efforts have been devoted to the development of improved CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. In CSP **8**, the possibility of the intramolecular hydrogen bonds between N-H hydrogens of the two connecting amide tethers of the CSP and the ether oxygens of the crown ether ring as shown in Figure 7b was noted[70] from previous study.[71] The two hydrogen bonds shown in Figure 7b were expected to hinder the tripodal complexation between the crown ether ring of the CSP and the ammonium ion (R-NH_3^+) of analytes. Consequently, removal of the two hydrogen bonds shown in Figure 7b might improve the chiral recognition ability of CSP **8**. Based on this rationale, a new modified CSP (CSP **10**, Figure 7c) was prepared by treating (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride with 3-(*N*-methylamino)propylsilica gel.[70] CSP **10** thus prepared was applied to the resolution of α-amino acids, amines, amino alcohols,[70] tocainide analogues,[59] β-amino acids[61] and aryl α-amino ketones.[62] In the resolution of α-amino acids and amino alcohols, CSP **8** and CSP **10** were found to be complementary with each other in terms of their efficiency for the chiral recognition.[70] However, CSP **10** was always superior to CSP **8** for the resolution of racemic primary amines.[70] In the resolution of tocainide analogues, CSP **10** was better than CSP **8**.[59] However, in the resolution of aryl α-amino ketones, CSP **8** and CSP **10** were equally effective.[62] In the resolution of β-amino acids, CSP **8** was better than CSP **10** when acetic acid was used as an acidic modifier in aqueous mobile phase while CSP **10** was better than CSP **8** when sulfuric acid was used as an acidic modifier.[61]

The chiral recognition efficiency for the resolution of primary amino compounds on CSP **8** containing relatively short spacer of three methylene unit might be changed by increasing the spacer length because purely lipophilic and flexible long spacer might improve the mobility of the residual aminoalkyl groups and the chiral selector moiety of the CSP.[72] Actually, CSP **11** (Figure 7d) containing relatively long spacer of eleven methylene unit was prepared by treating (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride with 11-aminoundecylsilica gel.[72] CSP **11** was reported to be superior to CSP **8** in the resolution of α-amino acids, β-amino acids, amines and amino alcohols in terms of both the separation (α) and the resolution factors (R_S).[72] In the resolution of α-amino acids on CSP **11**, the retention factors (k_1) were quite small compared to those on CSP **8**. However, in the resolution of relatively more lipophilic β-amino acids, amines and amino alcohols, the retention factors (k_1) were generally greater on CSP **11** than on CSP **3**. All of these resolution behaviors were rationalized by the effective competition of the ammonium ions (R-NH_3^+) generated by the residual undecylamino groups of CSP **11** under acidic mobile phase condition with the ammonium

ions (R-NH$_3$$^+$) of analytes for the complexation inside the cavity of the crown ether ring of the CSP and the effective lipophilic interaction between the CSP and the relatively more lipophilic analytes.[72]

CSP **8** intrinsically contains unreacted residual amino-propyl groups on the surface of the stationary phase because the process of bonding (+)-(18-crown-6)-2,3,11,12-tetra-carboxylic acid **6** to 3-aminopropylsilica gel cannot be complete. The unreacted residual aminopropyl groups of CSP **8** can be protonated under the acidic mobile phase condition and the resulting primary ammonium ions were expected to compete with the primary ammonium ions (R-NH$_3$$^+$) of analytes for the complexation inside the cavity of the crown ether ring of the CSP. By protecting the unreacted residual aminopropylsilica gel of CSP **8**, the chiral recognition efficiency of the CSP might be improved. Protection of the unreacted residual aminopropyl groups of CSP **8** with acetyl or butyryl group was found to improve the retention (*k*) and the resolution factors (R$_S$).[73] However, the separation factors (*α*) were found to decrease slightly when the unreacted residual aminopropyl groups of the CSP were protected.

As an alternative to the protection of the unreacted residual aminopropyl groups of CSP **8**, a new CSP (CSP **12**) without extra free (unreacted residual) aminopropyl groups on silica gel surface was prepared via the three step procedure shown in Figure 8.[74] CSP **12** intrinsically does not contain unreacted residual aminopropyl groups because the CSP was prepared by bonding *N,N'*-triethoxysilylpropyl syn-di-amide of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to silica gel. CSP **12** was applied to the resolution of *α*-amino acids, amines and amino alcohols.[74] The chiral recognition efficiency of CSP **12** was generally superior to CSP **8** in terms of the separation (a) and the resolution

factors (R$_S$).[74]

CSP **8** or CSP **10** have been used usually under highly acidic condition with an aqueous mobile phase containing sulfuric acid. In this instance, CSP **8** or CSP **10** cannot be ensured to be stable enough for the use of long time. As an effort to improve the stability of the CSP, a new doubly tethered CSP (CSP **13**, Figure 8) by adding a second point of attachment through the nitrogen atom of the amide tethering group of the singly tethered CSP.[75] CSP **13** thus prepared was intrinsically the type of CSP **10** in that both CSPs contain a tertiary amide linkage. Indeed, CSP **13** was found to show the chromatographic resolution behaviors consisting exactly with those on CSP **10**. CSP **13** was found quite effective in the resolution of *α*-amino acids, *β*-amino acids, amino alcohols and the stability of CSP **13** was actually greater than that of CSP **10**. However, the chiral recognition efficiency on CSP **13** was worse than that on CSP **10**.[75]

CSPs Based on Phenolic Pseudo Chiral Crown Ethes

Phenolic pseudo chiral crown ethers have been reported to exhibit not only excellent enantioselectivity toward primary amine compounds but also show a distinct difference in the color developed upon complexation with each guest enantio-mer.[76] Application of phenolic pseudo chiral crown ethers bonded to silica gel as CSPs (CSP **14** and CSP **15**, Figure 9) for the resolution of racemic primary amino compounds was appeared quite recently.[77,78] Originally, in the resolution of 1-phenylethylamine, 1-(1-naphthyl)ethylamine and phenyl-glycinol, CSP **14** containing OH group on the phenyl ring inside the crown ether ring was reported better than CSP **15** containing methoxy group on the phenyl ring inside the crown ether ring.[77] Especially, normal mobile phase (hexane-ethanol mixture) was utilized for the resolution of
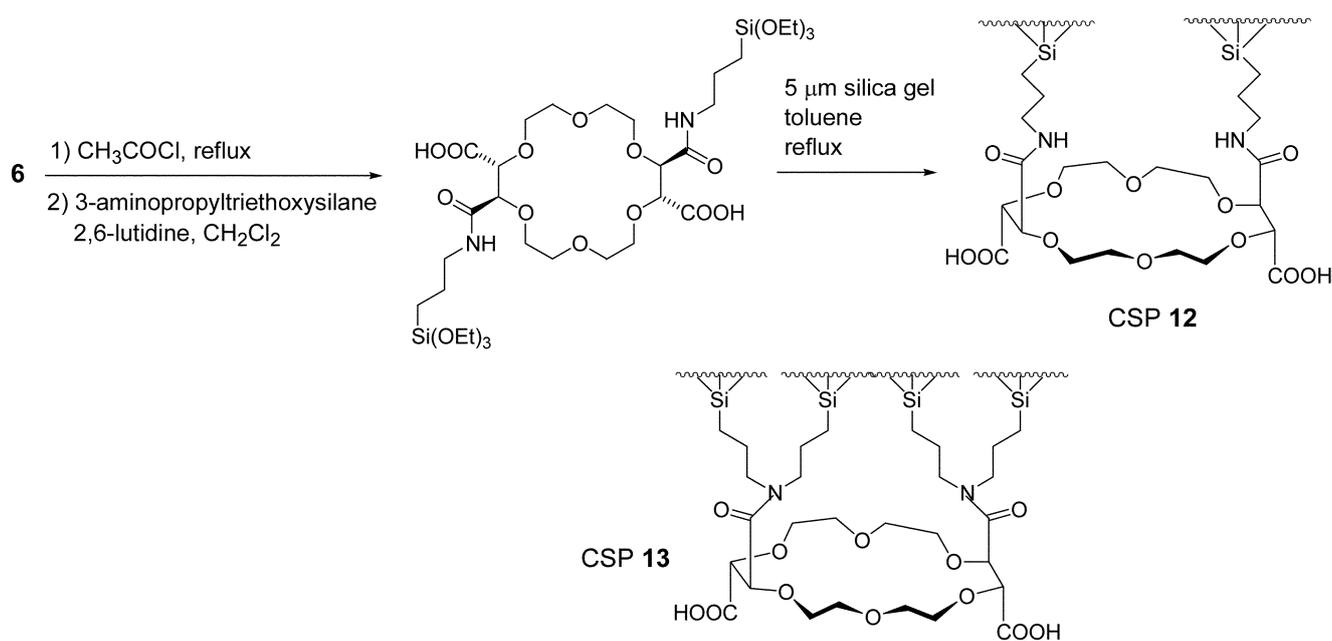
Figure 8. Scheme for the preparation of CSP **12**, which does not contain any extra free amino propyl group on silica gel surface and structure of doubly tethered CSP **13**.
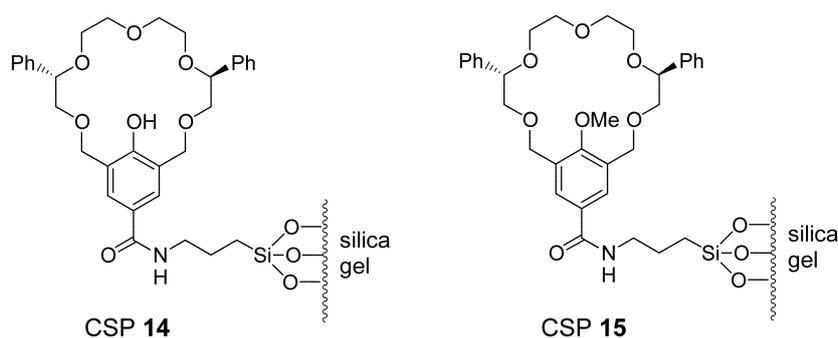
Figure 9. Structures of CSP **14** and CSP **15** based on phenolic pseudo chiral crown ethers.

primary amino compounds on CSP **14** or CSP **15**. In addition, triethylamine was added for the resolution of racemic primary amino compounds on CSP **14** while trifluoroacetic acid was added to the mobile phase for the protonation of the amino group of analytes for the resolution of primary amino compounds on CSP **15**.[77] When CSP **15** was applied to the resolution of α-amino acids, baseline separation with reasonable separation factors (α) were obtained with the use of hexane/ethanol/trifluoroacetic acid/ water (85 : 15 : 0.5 : 0.2) as a mobile phase.[78] However, in the resolution of amino alcohols on CSP **15**, the baseline resolutions were not obtained.[78]

Conclusion

Chiral crown ether-based HPLC CSPs have been known very useful for the separation of the two enantiomers of various racemic primary amino compounds. Among others, CSPs based on chiral crown ethers incorporating a chiral 1,1'-binapthyl unit or tartaric acid unit and based on phenolic pseudo chiral crown ethers have been most successfully utilized. In this account paper, these three different-type CSPs were reviewed for their structural characteristics and their applications to the resolution of mostly racemic primary amino compounds and some racemic secondary amino compounds. The chromatographic resolution behaviors for the resolution of primary amino compounds on CSPs based on chiral crown ethers incorporating a chiral 1,1'-binapthyl unit or tartaric acid unit were quite dependent of the content and type of organic and acidic modifiers in aqueous mobile phase and the column temperature. In contrast to the use of aqueous mobile phase, the resolution of some secondary amino compounds on a CSP based on a chiral crown ether incorporating tartaric acid unit and the resolution of primary amino compounds on CSPs based on phenolic pseudo chiral crown ethers was performed with the use of normal mobile phase. Even though the use of crown ether-based CSPs have been limited to the resolution of racemic primary or secondary amino compounds so far, efforts to expand the use of crown ether-based CSPs further for the resolution of other racemic compounds are undergoing in this field. In the near future, we hope that crown ether-based CSP will be utilized for the resolution of racemic compounds containing functional groups other than

primary or secondary amino groups without any limitation in the use of mobile phase.

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