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# Steric effects on the enantiodiscrimination of diproline chiral stationary phases in the resolution of racemic compounds

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ABSTRACT

Eight diproline chiral stationary phases with different end-capping groups were prepared and evaluated for the enantio-selective resolution of 41 racemic analytes. The end-capping group on the N-terminal of the peptide proved to be important as the chiral separation efficiency was decreased significantly without it. In general, increasing steric bulkiness near the N-terminal of diproline increases the enantioselectivity. Electronic structures of the end-capping groups are also important. One stationary phase with an adamantanecarbonyl capping group was found to provide both higher average separation and resolution factors than our previous leader. Three other stationary phases with 2-methylpropanoyl, cyclopropanecarbonyl and cyclobutanecarbonyl end capping groups were found to provide comparable average separation factor but higher resolution factors than our previous leader.



## 1. Introduction

Because enzymes and other biological receptors often possess chiral structures, two enantiomers of a drug may have very different biological activities [1]. As a result, methods to analyze and to prepare enantiomerically pure compounds are becoming increasingly important. Among various technologies developed, high-performance liquid chromatography (HPLC) separation of enantiomers on chiral stationary phases (CSPs) is a convenient and accurate method for the determination of the enatiomeric purity of chiral compounds. This method is also capable of preparative separation of racemic mixtures [2,3]. In the past few decades the design and development of CSPs has attracted a significant amount of attention and over a hundred CSPs have been reported. Well-known examples include the Pirkle-type columns and columns based on polysaccharide derivatives, cyclodextrins derivatives, macrocyclic antibiotics, proteins, ligand exchange complexes, chiral crown ethers, cinchona alkaloid quinine, and other chiral selectors [4–6].

Our group and others are interested in peptide-based chiral stationary phases [7–13]. We discovered that oligoproline stationary phases with proper structures have broad enantioselectivity [14–16]. For 53 racemic analytes chosen based on availability, some of the columns approached the performance of commercial columns [16]. However, those oligoproline phases still lack the performance of market leader Chiralpak AD-H and Chiralcel OD-H columns. Therefore, further improvement of these stable and covalently bounded proline columns is necessary. In a previous publication [15], we demonstrated that end-capping groups impact the performance of our diproline columns. The stationary phase with a trimethylacetyl (Tma) end-capping group proved more effective than one with the fluorenylmethyloxycarbonyl (Fmoc) group. We suspected that the improvement of Tma group over other groups may be due to its steric hindrance. In this article, we performed a systematic study of the steric hindrance provided by the end-capping group, in order to further improve the performance of these proline columns and to further understand the steric effect on diproline chiral stationary phases.

## 2. Experimental

### 2.1. Abbreviations

HATU, O-(7-azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate; DIPEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; DCM, dichloromethane; IPA, 2-propanol; Fmoc, 9-fluorenylmethoxycarbonyl; Pro, proline; Fmoc-Pro-OH, 6-[(9H-fluoren-9-ylmethoxy)carbonyl]-L-proline; MAPS, 3-methylaminopropyl silica gel

### 2.2. General supplies and equipment

Amino acid derivatives were purchased from NovaBiochem (San Diego, CA, USA). All other chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Fluka (Ronkonkoma, NY, USA),

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or Fisher Scientific (Pittsburgh, PA, USA). Hexanes (from Fisher) are a mixture of hexanes (86.1% n-hexane, 9.7% methylcyclopentane, 4.2% various methylpentanes). HPLC grade Kromasil silica gel (particle size 5 μm, pore size 100 Å, and surface area 298 $m^2$/g) was purchased from Akzo Nobel (EKA Chemicals, Bohus, Sweden). A modular column system (50 mm × 4.6 mm) was purchased from Isolation Technologies (Hopedale, MA, USA). Solvents were purchased from Fisher (Springfield, NJ, USA), or Sigma Aldrich. An Agilent 1100 HPLC system (Agilent, Wilmington, DE, USA) consisting of a vacuum degasser, a quaternary pump, an autosampler, and a multiple wavelength detector was used to evaluate the columns. UV spectra for measuring Fmoc loading were obtained with a Shimadzu UV 2550 spectrometer using a 10 mm × 610 mm cell.

### 2.3. Preparation of 3-methylaminopropyl silica gel (MAPS)

MAPS was prepared from Kromasil silica gel and 3-(methylamino)propyltrimethoxysilane according to a procedure described for the preparation of 3-aminopropyl silica gel (APS) [17]. The surface methylamino concentration is 0.56 mmol/g, based on nitrogen elemental analysis (C, 3.10; H, 0.0.75; N, 0.79).

### 2.4. Preparation of Fmoc-Pro-Pro-MAPS

To 8.0 g of MAPS prepared above (the surface methylamino concentration was 0.56 mmol/g) were added Fmoc-Pro-OH (3 equiv., 4.5 g), HATU (3 equiv., 5.1 g), and DIPEA (3 equiv., 1.7 g) in 70 mL of DMF. After agitating for 24 h, the resulting silica was filtered and washed with DMF, methanol, and DCM. Then any unreacted free methylamine groups on the silica gel were end-capped by reacting with acetic anhydride and pyridine in DCM. The surface Pro concentration was determined to be 0.47 mmol/g based on the Fmoc cleavage method [15]. The Fmoc protecting group was then removed by treatment of the silica with 100 mL of 20% (v/v) piperidine in DMF for 3 h. The deprotected silica, H-Pro-MAPS, was collected by filtration and washed with DMF, methanol, and DCM. Then the next reagent, Fmoc-Pro-OH, was coupled to the resulting silica following an identical reaction sequence and yielded the Fmoc-Pro-Pro-MAPS. The surface Fmoc concentration was determined to be 0.45 mmol/g based on the Fmoc cleavage method.

### 2.5. Preparation of CSP **1–9**

The Fmoc group from 0.8 g of Fmoc-Pro-Pro-MAPS was removed by treatment with 10 mL of 20% (v/v) piperidine in DMF for 2 h to yield CSP **2**. CSP **1**, **3–9** were prepared by reacting CSP **2** (prepared from 0.8 g of Fmoc-Pro-Pro-MAPS) with the appropriate acyl chloride (0.60 g, 5 mmol) and DIPEA (0.65 g, 5 mmol) in 10 mL of dry DCM for 3 h. The desired chiral stationary phase was collected and washed with DMF, methanol, and DCM. For CSP **9**, the required camphanoyl chloride was obtained by the treatment of the corresponding acid with thionyl chloride.

### 2.6. Chromatographic measurements

All the chiral stationary phases were packed into a 50 mm × 4.6 mm HPLC column following the conventional high-pressure slurry packing method with ethanol as the slurring solvent as described in the literature [18]. A packing pump from Chrom Tech (Apple Valley, MN, USA) was employed. Retention factor ($k$) equals $(t_r - t_0)/t_0$, where $t_r$ is the retention time and $t_0$ is the dead time. The separation factor ($\alpha$) equals $k_2/k_1$, ratio of the retention factor of the two enantiomers. The resolution factor (Rs) was calculated using the equation $Rs = 2 \times (t_{r2} - t_{r1})/((w)_1 + (w)_2)$, where $(w)_1$ and $(w)_2$ are the peak widths. The dead time $t_0$ was measured with 1,3,5-tri-t-butylbenzene as the void volume marker. A flow rate of 1 mL/min was used. Detection was done using a UV diode array detector.

## 3. Results and discussion

The diproline chiral stationary phases were prepared by stepwise coupling of Fmoc-proline to 3-methylaminopropyl silica gel, which was synthesized by coupling the commercially available 3-(methylamino)propyltrimethysilane to the silica gel, by following a published protocol [14,17].

Forty one analytes were chosen to evaluate these diproline chiral stationary phases (Fig. 1). These analytes have been studied previously in our group. The chromatographic performance of eight new diproline CSPs (Fig. 2) was studied with these analytes (Table 1). For comparison, CSP **1** with a trimethylacetyl (Tma) end-capping group was re-synthesized. All the columns (including CSP **1**) were packed under identical conditions. The average separation factor and resolution factor for all 41 analytes on those CSPs are summarized in Table 2.

In order to determine the effect of an end-capping group on the diproline chiral stationary phase, CSP **2**, which is not end-capped, was prepared and evaluated in HPLC studies. CSP **1** resolved all 41 compounds with an average separation factor of 1.21, an average resolution factor of 1.39, and 17 of the analytes were baseline-resolved. In contrast, the uncapped CSP **2** only resolved 24 compounds with an average separation factor of 1.06, an average resolution factor of 0.47, and none of the test compounds were baseline-resolved. These results clearly indicate that the end-capping group is important for optimizing these proline-based CSPs. The data in Table 1 show that the retention time of almost all analytes are much longer on CSP **2** than on CSP **1** using the same mobile phase. CSP **2**, without a carbonyl electron withdrawing end capping group, has a terminal secondary amine functional group with a lone pair of electrons on the second proline unit. This group could result in strong hydrogen bonding with the analytes, leading to longer retention times. At this time, it is not clear why the chiral selectivity of CSP **2** is significantly lower.

CSP **3** with a t-butylsulfinyl end-capping group was studied next. CSP **3** also contains a bulky t-butyl group, similar to the trimethylacetyl group in CSP **1**. However, CSP **3** only resolves 13 of the compounds with an average separation factor of 1.03, an average resolution factor of 0.22 and none of the analytes were baseline-resolved (Table 2). In fact, the chiral selectivity of CSP **3** was even lower than that of CSP **2**, which has no end-capping group. The bond between the sulfur and oxygen atoms in the sulfinyl group differs from the conventional carbonyl carbon/oxygen double bond. The S–O bond is very polar, with more negative charge centered on oxygen than in the carbonyl function. Moreover, a lone pair of electrons resides on the sulfur atom, giving it a tetrahedral geometry. The sulfur is a chiral center with this tetrahedral geometry. This lone pair of electrons could also hydrogen bond with analytes. Since the preparation procedure for adding the end-capping group has no enantioselectivity, two diastereomers of CSP **3** should be present. The presence of two diastereomers and the hydrogen bonding ability both may have contributed to the low chiral selectivity of CSP **3**.

CSP **4** was studied next. In CSP **4**, the 3,3-dimethylbutyryl group is similar to the trimethylacetyl group in CSP **1**, except with an extra $CH_2$ between the carbonyl group and t-butyl group. CSP **4** resolved 29 compounds with an average separation factor of 1.14, an average resolution factor of 0.82, and 4 of these compounds were baseline-resolved (Table 2). This is a significantly poorer performance than that of CSP **1**. In order to understand the difference between these two CSPs, 3D molecular models of the chiral selectors in both CSP **1** and CSP **4** were built via a molecular dynamic

**Table 1**
Analytes and their resolution on CSP **1–9**.[a]

| Ana lyte | CSP **1** | | | CSP **2** | | | CSP **3** | | | CSP **4** | | | CSP **5** | | | CSP **6** | | | CSP **7** | | | CSP **8** | | | CSP **9** | | | Mobile phase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | $\alpha$ | Rs | $k_1$ | |
| 1 | 1.08 | 0.92 | 8.16 | 1 | 0 | 10.04 | 1 | 0 | 7.33 | 1 | 0 | 4.64 | 1.06 | 0.98 | 1.08 | 1.05 | 0.79 | 9.23 | 1.06 | 0.82 | 9.29 | 1.09 | 1.33 | 8.47 | 1 | 0 | 5.99 | 1%IH |
| 2 | 1.09 | 0.97 | 5.96 | 1 | 0 | 8.18 | 1 | 0 | 5.47 | 1 | 0 | 5.63 | 1.07 | 1.01 | 1.09 | 1.04 | 0.83 | 6.74 | 1.07 | 0.99 | 6.93 | 1.09 | 1.16 | 6.25 | 1 | 0 | 4.24 | 1%IH |
| 3 | 1.06 | 0.44 | 7.23 | 1 | 0 | 9.33 | 1 | 0 | 6.76 | 1 | 0 | 7.61 | 1.03 | 0.32 | 1.06 | 1.02 | 0.2 | 8.55 | 1.03 | 0.25 | 8.51 | 1.06 | 0.58 | 7.85 | 1 | 0 | 5.25 | 1%IH |
| 4 | 1.05 | 0.43 | 3.79 | 1 | 0 | 5.50 | 1 | 0 | 3.41 | 1 | 0 | 6.09 | 1.05 | 0.64 | 1.05 | 1.05 | 0.42 | 4.56 | 1.06 | 0.69 | 4.26 | 1.03 | 0.23 | 7.50 | 1 | 0 | 2.44 | 1%IH |
| 5 | 1.08 | 0.89 | 10.00 | 1 | 0 | 13.51 | 1 | 0 | 8.21 | 1 | 0 | 12.11 | 1.03 | 0.43 | 1.08 | 1.03 | 0.28 | 11.00 | 1.06 | 0.78 | 11.06 | 1.08 | 1.02 | 12.32 | 1 | 0 | 7.31 | 5%IH |
| 6 | 1.1 | 1.14 | 10.92 | 1 | 0 | 14.73 | 1 | 0 | 8.98 | 1 | 0 | 11.09 | 1.06 | 0.93 | 1.1 | 1.04 | 0.57 | 11.67 | 1.06 | 0.91 | 11.46 | 1.11 | 1.4 | 11.60 | 1 | 0 | 8.08 | 5%IH |
| 7 | 1.06 | 0.65 | 10.27 | 1 | 0 | 13.52 | 1 | 0 | 8.78 | 1 | 0 | 12.75 | 1.05 | 0.85 | 1.06 | 1.05 | 0.55 | 12.58 | 1.04 | 0.51 | 12.69 | 1.06 | 0.86 | 12.43 | 1 | 0 | 3.67 | 1%IH |
| 8 | 1.09 | 0.81 | 4.47 | 1 | 0 | 5.92 | 1 | 0 | 4.04 | 1 | 0 | 7.02 | 1.07 | 0.96 | 1.09 | 1.06 | 0.67 | 4.87 | 1.07 | 0.83 | 4.80 | 1.09 | 1.03 | 5.08 | 1 | 0 | 2.42 | 5%IH |
| 9 | 1.06 | 0.46 | 4.65 | 1.04 | 0.23 | 5.98 | 1 | 0 | 4.14 | 1 | 0 | 7.26 | 1.04 | 0.29 | 1.06 | 1.02 | 0.18 | 11.77 | 1.04 | 0.25 | 4.99 | 1.07 | 0.79 | 5.39 | 1 | 0 | 3.92 | 5%IH |
| 10 | 1.18 | 1.61 | 4.16 | 1 | 0 | 4.47 | 1 | 0 | 3.56 | 1.04 | 0.43 | 6.35 | 1.12 | 1.45 | 1.18 | 1.1 | 1.03 | 4.55 | 1.13 | 1.44 | 4.53 | 1.18 | 1.62 | 4.98 | 1.04 | 0.18 | 3.58 | 15%IH |
| 11 | 1.56 | 3.34 | 8.95 | 1.11 | 1.4 | 13.36 | 1.1 | 1.11 | 7.33 | 1.25 | 1.62 | 10.33 | 1.45 | 4.55 | 1.56 | 1.41 | 3.93 | 9.66 | 1.45 | 4.16 | 9.95 | 1.67 | 4.61 | 10.98 | 1.27 | 1.89 | 6.93 | 5%IH |
| 12 | 1.48 | 3.41 | 9.49 | 1.04 | 0.24 | 10.67 | 1.11 | 1.08 | 7.78 | 1.2 | 1.39 | 10.37 | 1.37 | 3.33 | 1.48 | 1.32 | 2.76 | 10.80 | 1.39 | 3.14 | 10.70 | 1.56 | 3.62 | 11.77 | 1.22 | 1.51 | 7.53 | 15%IH |
| 13 | 1.08 | 0.76 | 7.73 | 1 | 0 | 10.28 | 1 | 0 | 6.52 | 1 | 0 | 8.86 | 1.06 | 0.87 | 1.08 | 1.06 | 0.75 | 8.85 | 1.07 | 0.84 | 8.48 | 1.09 | 0.94 | 8.76 | 1 | 0 | 6.79 | 5%IH |
| 14 | 1.16 | 1.22 | 8.37 | 1.02 | 0.19 | 8.89 | 1 | 0 | 6.57 | 1.06 | 0.29 | 8.57 | 1.11 | 1.4 | 1.16 | 1.09 | 1.1 | 9.23 | 1.12 | 1.29 | 9.19 | 1.14 | 1.21 | 10.54 | 1 | 0 | 6.94 | 15%IH |
| 15 | 1.15 | 1.55 | 8.33 | 1.02 | 0.18 | 8.93 | 1 | 0 | 9.76 | 1.07 | 0.43 | 8.91 | 1.11 | 1.39 | 1.15 | 1.09 | 1.1 | 9.20 | 1.12 | 1.3 | 9.20 | 1.14 | 1.4 | 10.50 | 1 | 0 | 6.86 | 15%IH |
| 16 | 1.14 | 1.37 | 12.24 | 1.09 | 1.02 | 13.89 | 1 | 0 | 8.13 | 1.08 | 0.32 | 8.80 | 1.14 | 1.58 | 1.14 | 1.12 | 1.31 | 11.47 | 1.16 | 1.71 | 11.26 | 1.23 | 1.87 | 12.10 | 1.07 | 0.37 | 8.26 | 15%IH |
| 17 | 1.05 | 0.52 | 11.94 | 1 | 0 | 20.97 | 1 | 0 | 13.52 | 1.02 | 0.26 | 11.27 | 1.05 | 0.98 | 1.05 | 1.05 | 0.52 | 14.29 | 1.05 | 0.56 | 13.67 | 1.06 | 0.51 | 12.49 | 1 | 0 | 11.20 | 1%IH |
| 18 | 1.11 | 0.75 | 6.12 | 1 | 0 | 7.14 | 1 | 0 | 6.46 | 1.11 | 0.73 | 6.76 | 1.14 | 1.2 | 1.11 | 1.11 | 1 | 7.09 | 1.13 | 1.32 | 6.77 | 1.15 | 0.89 | 7.36 | 1.08 | 0.42 | 5.13 | 15%IH |
| 19 | 1.1 | 1.07 | 3.68 | 1.1 | 0.85 | 4.79 | 1 | 0 | 4.82 | 1.09 | 0.64 | 5.15 | 1.18 | 1.72 | 1.1 | 1.18 | 1.83 | 4.76 | 1.16 | 1.51 | 4.58 | 1.19 | 1.25 | 4.11 | 1 | 0 | 3.67 | 15%IH |
| 20 | 1.13 | 0.65 | 8.26 | 1 | 0 | 9.37 | 1 | 0 | 8.55 | 1 | 0 | 7.26 | 1.15 | 1.08 | 1.13 | 1.11 | 0.88 | 9.64 | 1.15 | 1.2 | 8.74 | 1.2 | 0.78 | 9.17 | 1.11 | 0.5 | 7.00 | 15%IH |
| 21 | 1.23 | 1.36 | 7.47 | 1.09 | 0.71 | 7.82 | 1 | 0 | 8.01 | 1.14 | 0.83 | 6.40 | 1.2 | 1.53 | 1.23 | 1.16 | 1.28 | 8.44 | 1.23 | 1.74 | 8.09 | 1.29 | 1.72 | 9.31 | 1.07 | 0.44 | 6.17 | 15%IH |
| 22 | 1.31 | 1.73 | 11.34 | 1.09 | 0.85 | 15.42 | 1 | 0 | 7.25 | 1.14 | 1.03 | 7.62 | 1.26 | 2.21 | 1.31 | 1.23 | 1.9 | 12.92 | 1.28 | 2.47 | 11.85 | 1.39 | 1.77 | 11.47 | 1.12 | 1.1 | 19.87 | 5%IH |
| 23 | 1.28 | 2.03 | 7.61 | 1.1 | 0.91 | 11.17 | 1 | 0 | 7.40 | 1.14 | 0.9 | 6.48 | 1.27 | 2.31 | 1.28 | 1.26 | 2.11 | 8.63 | 1.3 | 2.47 | 7.81 | 1.4 | 2.31 | 7.66 | 1.14 | 0.71 | 6.73 | 5%IH |
| 24 | 1.41 | 1.21 | 2.33 | 1.48 | 0.84 | 3.16 | 1 | 0 | 2.77 | 1.37 | 0.83 | 2.63 | 1.37 | 1.32 | 1.41 | 1.32 | 1.31 | 2.85 | 1.36 | 1.69 | 2.72 | 1.54 | 1.51 | 2.55 | 1.17 | 0.51 | 1.69 | 60%IH |
| 25 | 1.32 | 1.79 | 7.14 | 1.12 | 1.21 | 9.41 | 1.07 | 0.44 | 10.37 | 1.19 | 1.3 | 8.44 | 1.31 | 2.53 | 1.32 | 1.33 | 2.91 | 8.12 | 1.35 | 2.86 | 7.86 | 1.41 | 2.32 | 8.42 | 1.13 | 0.77 | 6.67 | 15%IH |
| 26 | 1.27 | 1.85 | 7.5 | 1.09 | 0.92 | 9.67 | 1 | 0 | 10.99 | 1.14 | 0.94 | 8.31 | 1.27 | 2.53 | 1.27 | 1.27 | 2.55 | 8.58 | 1.28 | 2.95 | 8.33 | 1.34 | 1.9 | 8.83 | 1.09 | 0.61 | 6.93 | 15%IH |
| 27 | 1.35 | 2.02 | 17.08 | 1.1 | 0.95 | 9.58 | 1.09 | 0.75 | 26.50 | 1.2 | 1.3 | 13.20 | 1.32 | 2.25 | 1.35 | 1.32 | 2.45 | 20.42 | 1.35 | 3.06 | 19.45 | 1.46 | 2.97 | 20.00 | 1.1 | 0.83 | 9.07 | 15%IH |
| 28 | 1.31 | 1.71 | 9.75 | 1.1 | 1.03 | 9.88 | 1.1 | 0.72 | 13.00 | 1.2 | 1.01 | 9.48 | 1.34 | 2.36 | 1.31 | 1.32 | 2.21 | 11.00 | 1.34 | 2.33 | 10.25 | 1.4 | 2.33 | 11.67 | 1.11 | 0.81 | 9.46 | 15%IH |
| 29 | 1.22 | 1.67 | 5.67 | 1 | 0 | 8.82 | 1.12 | 0.83 | 7.17 | 1.11 | 0.54 | 6.34 | 1.25 | 1.82 | 1.22 | 1.27 | 1.85 | 6.22 | 1.25 | 2.27 | 5.58 | 1.26 | 1.72 | 6.00 | 1.08 | 0.41 | 6.08 | 15%IH |
| 30 | 1.37 | 1.52 | 7.83 | 1.07 | 0.31 | 12.58 | 1.09 | 0.29 | 8.42 | 1.4 | 1.72 | 6.89 | 1.41 | 1.74 | 1.37 | 1.38 | 1.96 | 9.83 | 1.31 | 1.81 | 10.33 | 1.59 | 2 | 9.83 | 1.18 | 0.59 | 5.89 | 60%IH |
| 31 | 1.3 | 1.25 | 6.83 | 1.12 | 0.93 | 9.79 | 1.08 | 0.40 | 8.75 | 1.14 | 1.06 | 6.95 | 1.35 | 2.23 | 1.3 | 1.34 | 1.91 | 7.88 | 1.39 | 1.97 | 7.46 | 1.36 | 1.54 | 8.58 | 1.11 | 0.51 | 7.03 | 30%IH |
| 32 | 1.26 | 1.80 | 11.42 | 1.1 | 0.95 | 14.67 | 1.08 | 0.66 | 15.00 | 1.11 | 0.96 | 11.10 | 1.3 | 2.5 | 1.26 | 1.29 | 2.37 | 12.75 | 1.32 | 2.56 | 12.00 | 1.34 | 2.23 | 14.17 | 1.13 | 0.94 | 11.33 | 15%IH |
| 33 | 1.07 | 0.41 | 6.88 | 1.06 | 0.6 | 9.85 | 1 | 0 | 5.77 | 1.03 | 0.28 | 6.39 | 1.11 | 0.98 | 1.07 | 1.09 | 0.84 | 8.33 | 1.1 | 1.01 | 8.11 | 1.13 | 1.07 | 9.11 | 1.08 | 0.41 | 5.08 | 30%IH |
| 34 | 1.17 | 1.41 | 3.15 | 1.1 | 0.61 | 3.20 | 1 | 0 | 3.28 | 1.01 | 0.15 | 6.51 | 1.12 | 1.37 | 1.17 | 1.11 | 1.04 | 3.76 | 1.1 | 1.35 | 3.71 | 1.19 | 1.84 | 3.68 | 1.08 | 0.59 | 2.91 | 15%IH |
| 35 | 1.13 | 1.47 | 9.26 | 1 | 0 | 12.25 | 1 | 0 | 8.91 | 1 | 0 | 15.84 | 1.11 | 1.75 | 1.13 | 1.11 | 1.6 | 10.41 | 1.13 | 1.72 | 10.77 | 1.15 | 1.76 | 11.42 | 1.08 | 0.81 | 8.04 | 1%IH |
| 36 | 1.13 | 0.97 | 15.50 | 1 | 0 | 18.62 | 1 | 0 | 11.12 | 1.03 | 0.38 | 14.19 | 1.25 | 1.21 | 1.13 | 1.18 | 1.59 | 16.66 | 1.13 | 1.29 | 16.03 | 1.24 | 2.74 | 22.59 | 1.06 | 0.46 | 13.82 | 15%IH |
| 37 | 1.61 | 2.02 | 9.13 | 1 | 0 | 15.27 | 1.19 | 0.85 | 9.24 | 1.45 | 1.37 | 7.34 | 1.51 | 1.79 | 1.61 | 1.51 | 2.06 | 11.18 | 1.61 | 3.01 | 10.68 | 2.03 | 2.57 | 9.36 | 1.35 | 1.36 | 7.85 | 5%IH |
| 38 | 1.29 | 1.9 | 3.52 | 1.08 | 0.8 | 4.80 | 1.09 | 0.67 | 2.82 | 1.36 | 2.07 | 2.66 | 1.35 | 2.62 | 1.29 | 1.28 | 2.35 | 5.86 | 1.38 | 2.59 | 6.33 | 1.32 | 2.12 | 4.42 | 1.04 | 0.25 | 5.31 | 5%ID |
| 39 | 1.28 | 2.65 | 12.51 | 1.09 | 0.9 | 13.80 | 1.08 | 0.66 | 7.27 | 1.44 | 2.37 | 6.80 | 1.39 | 3.29 | 1.28 | 1.29 | 2.33 | 19.98 | 1.53 | 3.3 | 14.92 | 1.27 | 3.01 | 17.76 | 1 | 0 | 9.18 | 5%ID |
| 40 | 1.18 | 1.45 | 10.24 | 1.15 | 1.42 | 10.37 | 1.08 | 0.74 | 6.92 | 1.13 | 0.93 | 10.87 | 1.21 | 1.9 | 1.18 | 1.25 | 2.03 | 12.37 | 1.29 | 2.37 | 13.19 | 1.12 | 0.95 | 9.17 | 1.11 | 0.62 | 6.42 | 15%IH |
| 41 | 1.31 | 1.83 | 10.02 | 1.11 | 1.06 | 14.61 | 1 | 0 | 14.10 | 1.02 | 0.38 | 7.59 | 1.28 | 2.96 | 1.31 | 1.25 | 2.54 | 11.47 | 1.3 | 2.53 | 10.69 | 1.42 | 2.35 | 10.24 | 1.15 | 1.08 | 8.62 | 5%IH |

[a] $\alpha$ is the separation factor. $k_1$ is the retention factor of the first eluted enantiomer. Rs is the resolution factor. I for isopropanol, H for hexanes, D for dichloromethane, 1% IH for 1% isopropanol in hexanes. Column dimensions, 50 mm × 4.6 mm ID. Flow rate, 1.0 mL/min; UV DAD detection.

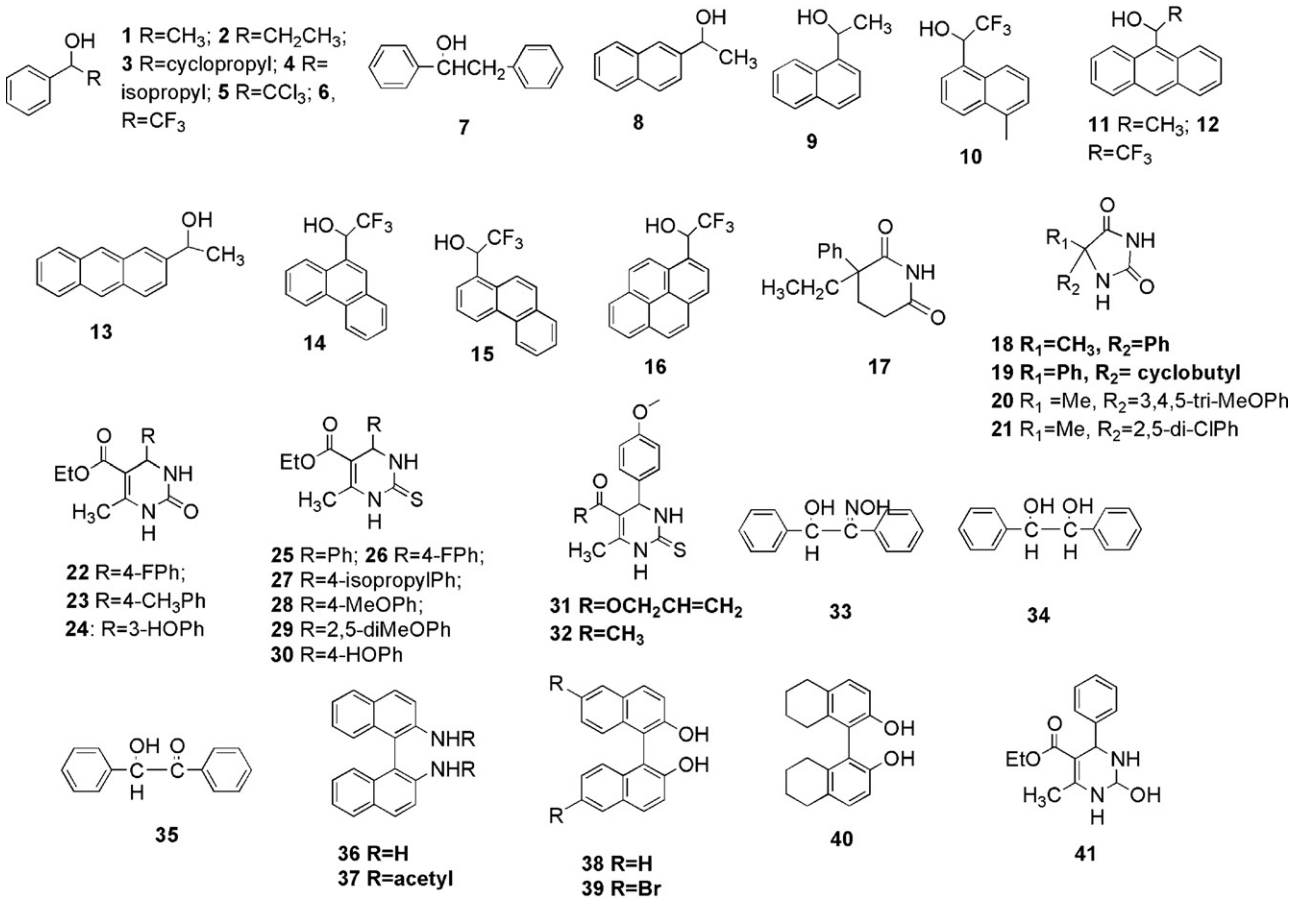



**Fig. 1.** Structures of analytes (**1–41**) used in this study.

MM2 optimization. In CSP **1**, the t-butyl group is attached directly to the carbonyl group. Thus, it is closer to the chiral centers in proline and other groups capable of having non-covalent interactions with the analytes. The t-butyl group of CSP **4** is further away from the carbonyl group, the chiral centers of proline, and the other groups capable of non-covalent interactions with the analytes. The t-butyl group in CSP**4** also has greater conformational flexibility. Since enantioselectivity is often enhanced when a sterically bulk group is near a chiral center, the poorer performance of CSP **4** is not surprising.

We then studied three stationary phases (CSP **5**, CSP **6**, CSP **7**) in which there are only two alkyl groups attached to the alpha carbon of the carbonyl end-capping group, in contrast to the three alkyl groups present in CSP **1**. Somewhat surprisingly, their chromatographic performance is almost equivalent to, or better, than that of CSP **1**, since the steric hindrance in these three CSPs is less

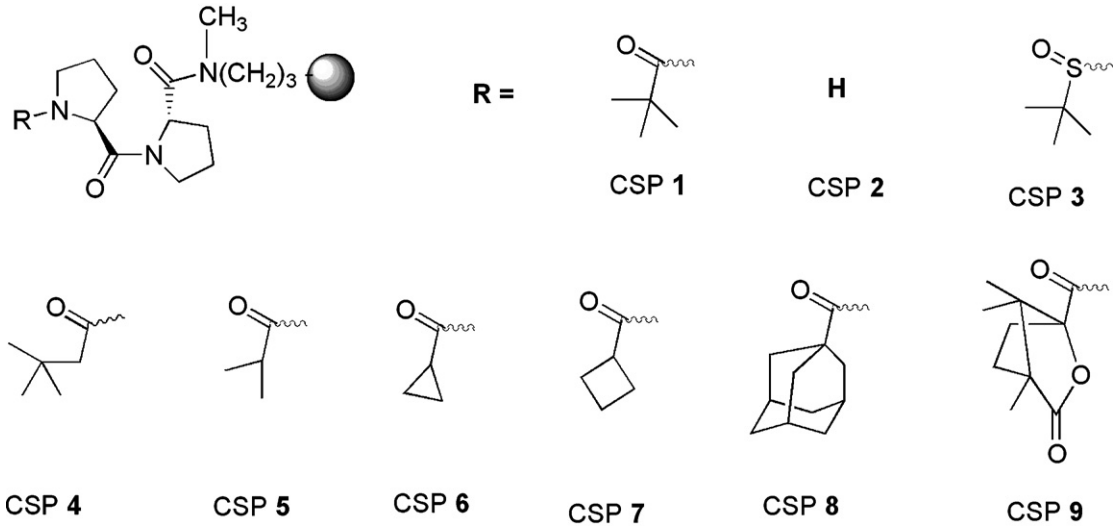


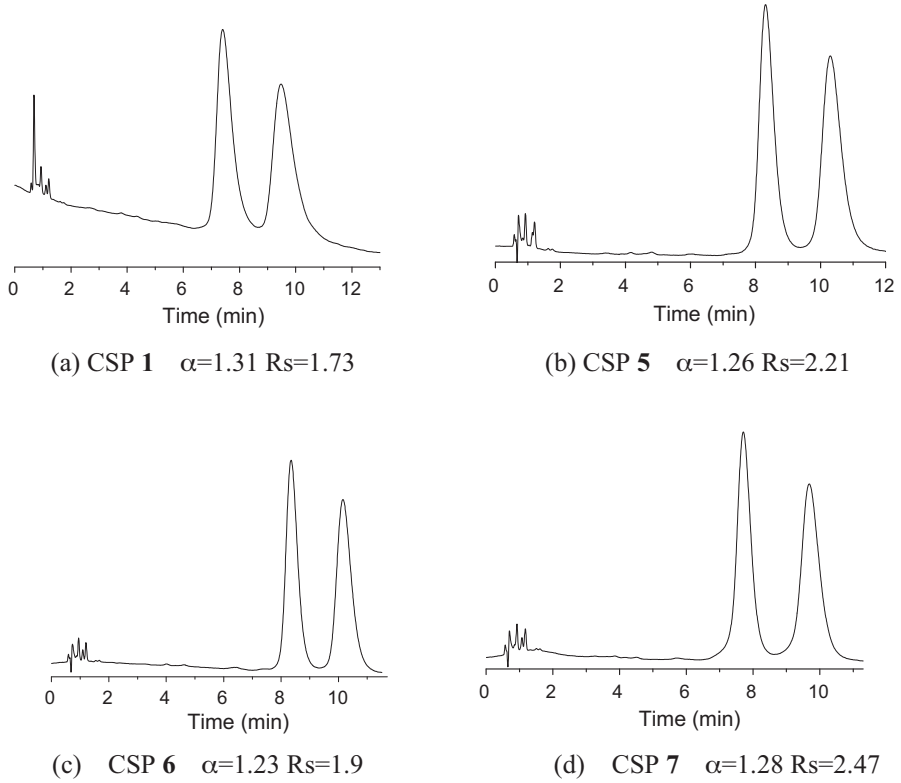

**Fig. 2.** Structures of the diproline stationary phases (CSP **1–9**) studied.

**Table 2**
Summary of the chromatographic behavior of the diproline stationary phases.

| | Analytesresolved | Analytesbaseline-resolved Rs > 1.5 | Average of all 41 analytes | | Average of the 18 analytes with one H-bond donor | | Average of the 20 analytes with two H-bond donors | | Average of the 3 analytes with three H-bond donors | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\alpha$ | Rs | $\alpha$ | Rs | $\alpha$ | Rs | $\alpha$ | Rs |
| CSP **1** | 41 | 17 | 1.21 | 1.39 | 1.14 | 1.22 | 1.25 | 1.52 | 1.36 | 1.52 |
| CSP **2** | 24 | 0 | 1.06 | 0.47 | 1.02 | 0.18 | 1.07 | 0.68 | 1.22 | 0.74 |
| CSP **3** | 13 | 0 | 1.03 | 0.22 | 1.01 | 0.12 | 1.05 | 0.34 | 1.03 | 0.1 |
| CSP **4** | 29 | 4 | 1.14 | 0.82 | 1.04 | 0.26 | 1.16 | 0.94 | 1.26 | 0.98 |
| CSP **5** | 41 | 21 | 1.20 | 1.69 | 1.11 | 1.60 | 1.26 | 1.97 | 1.35 | 2.01 |
| CSP **6** | 41 | 20 | 1.18 | 1.51 | 1.10 | 1.03 | 1.25 | 1.87 | 1.32 | 1.97 |
| CSP **7** | 41 | 22 | 1.21 | 1.75 | 1.12 | 1.23 | 1.28 | 2.18 | 1.32 | 2.01 |
| CSP **8** | 41 | 23 | 1.27 | 1.71 | 1.16 | 1.43 | 1.32 | 1.92 | 1.51 | 1.95 |
| CSP **9** | 26 | 2 | 1.08 | 0.46 | 1.04 | 0.26 | 1.10 | 0.59 | 1.17 | 0.73 |

than that in CSP **1**. CSP **5**, which has a 2-methylpropanoyl end-capping group, resolves all 41 test compounds with an average separation factor of 1.20, an average resolution factor of 1.69, and 21 of these compounds were baseline-resolved. CSP **6**, which has a cyclopropanecarbonyl end capping group, also resolves all 41 test compounds with an average separation factor of 1.18, an average resolution factor of 1.51 and 20 analyte compounds were baseline-resolved. CSP **7**, which is capped with a cyclobutanecarbonyl group, resolves all 41 test compounds with an average separation factor of 1.21, an average resolution factor of 1.75 and 22 of these test compounds were baseline-resolved. The average separation factors of CSP **5**, CSP **6** and CSP **7** are slightly less than, or equivalent to, that of CSP **1**; however, the average resolution factors of CSP **5**, CSP **6** and CSP **7** are larger than that of CSP **1**. For example, the resolved chromatograms of analyte **22** on CSP **1**, CSP **5**, CSP **6** and CSP **7** are shown in Fig. 3. This figure clearly shows the improvement obtained when using these three CSPs over CSP **1**. These results indicate that the column efficiencies of CSP **5**, CSP **6** and CSP **7** are higher than that of CSP **1**. The end-capping reactions of dimethyl acetyl chloride, cyclopropanecarbonyl chloride and cyclobutanecarbonyl chloride may be more efficient than that using trimethylacetyl chloride because of their smaller steric hindrance during the acylation reactions.

Two end-capping groups with greater steric hindrance than the trimethyl acetyl group were then studied. CSP **8** contains the 1-adamantanecarbonyl group. CSP **8** resolves all 41 test compounds with an average separation factor of 1.27, an average resolution factor of 1.71 and 23 of these test compounds were baseline-resolved (Table 2). CSP **8**s average separation factor (1.27) is better than that for CSP **1** (1.21), its average resolution factor (1.71) is better than that for CSP **1** (1.39) and the number of test compounds that were baseline-resolved (23) is also greater than that for CSP **1** (17). A 3D molecular model of the chiral selector in CSP **8** was constructed by a molecular dynamics MM2 optimization, which shows that the cleft between the diproline structure and 1-adamantanecarbonyl group is more hindered than that between diproline and the t-butyl group of CSP **1**. The average separation factor of CSP **8** (1.27) is better




(a) CSP **1** α=1.31 Rs=1.73 (b) CSP **5** α=1.26 Rs=2.21

(c) CSP **6** α=1.23 Rs=1.9 (d) CSP **7** α=1.28 Rs=2.47

**Fig. 3.** Resolution of analyte **22** (mobile phase 5% isopropanol/hexanes) on CSP **1** (a), CSP **5** (b), CSP **6** (c), and CSP **7** (d). Column dimensions, 50 mm × 4.6 mm. Flow rate at 1 mL/min, UV detection at 254 nm.

than that for CSP **7** (1.21), but the average resolution factor (1.71) is slightly less than that for CSP **7** (1.75).

Another large bulky group, the camphanic carbonyl group, was chosen as the end-capping group in CSP **9**. CSP **9** resolves 26 of the test compounds with an average separation factor of 1.08, an average resolution factor of 0.46 and only 2 compounds were baseline-resolved, indicating the chromatographic performance test of CSP **9** is lower than those of CSPs-**5**, **6**, **7** and **8**. There is a lactone function in the camphanic carbonyl group. This lactone group can compete with other groups in the chiral selector to hydrogen bond with the analytes. In addition, the camphanic carbonyl group contains a chiral center. This hydrogen bonding capability and the chiral center may disrupt the enantioselective interaction between the analyte and proline chiral selector, leading to lower chromatographic resolving performance.

The structure of the analytes greatly affects the enantioseparations achieved on these diproline CSPs. In general, the number of H-bond donors (H–O or H–N groups) present in the analytes influences their enantioseparation on these stationary phases. This was implied in our previous paper [14] and demonstrated by another paper [10]. The data summarized in Table 2, show that more hydrogen bond donors often lead to higher enantioseparation performance. Interestingly, the average separation factor for the test compounds with three H-bond donors on CSP **8** (1.51) is significantly higher than that on CSP **1** (1.36). However, one should not draw too many conclusions from this observation because of the small number of analytes (3) with three hydrogen bond donors. The steric bulkiness of analytes may also influence their enantioseparation. For example, the chiral selectivity of analytes **1–6** with small substituents is generally less than that of the analytes **7–16** with larger substituents.

## 4. Conclusions

Eight new chiral stationary phases were prepared and evaluated in the normal phase mode in order to study steric effects in diproline chiral stationary phases and to improve their chromatographic performance. Several notable results were obtained. The end-capping group has major effects in diproline-based CSPs. In general, increasing the steric bulkiness near the N-terminal of diproline increases the enantioselectivity. This is demonstrated in the relative chromatographic performances of CSP **1**, CSP **4**, and CSP **8**. However, the specific placement of alkyl substituents on the functional group, rather than the absolute steric bulkiness, may be important, as demonstrated when comparing the performances of CSP **5**, CSP **6** and CSP **7**. The electronic structures of the end-capping groups are also important, as seen in both CSP **3** and CSP **9**. In CSP **3** and **9**, the presence of other heteroatoms leads to poorer performance. We found that one stationary phase, CSP **8**, provides both higher separation and higher resolution factors than our previous leader, CSP **1**. We also found three other stationary phases, CSPs **5**, **6**, **7**, provide comparable separation factors but higher resolution factors than our previous leader, CSP **1**. Further studies are underway for this class of promising chiral selectors.

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