# Preparation and Chiral Recognition of a Mono[6$^A$-*N*-1-(2-hydroxy)-phenylethylimino-6$^A$-deoxy]-β-Cyclodextrin HPLC Stationary Phase

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## Abstract

**A novel chiral stationary phase (CSP) is obtained by linking the β-CD to a chiral block (commercially available phenylglycinol) by rigid C=N bond. This chiral stationary phase exhibites good enantioselectivity for several alkylaromatic alcohols and a variety of ferrocene derivatives under reversed-phase conditions, with the best Rs value achieve up to 6.19. The hydrogen bonding interaction may be the primary factor, also π–π interaction or dipole–dipole interaction has some effect on chiral separation. The dependence of the natural logarithms of retention and selectivity factors (lnk′ and lnα) on the inverse of temperature 1/T (van't Hoff plots) is used to determine thermodynamic data referring to the separation of the enantiomers. Calculated thermodynamic constants Δ(ΔH°), Δ (ΔS°), and Δ (ΔG°) are applied to help understand of the thermodynamic driving forces for retention and enantio-recognition for this chromatographic system. It can be concluded that the separations for all the investigated analytes on this CSP are enthalpically favored.**

## Introduction

Chromatographic enantioseparation on chiral stationary phases (CSPs) represents one of the most direct and facile approaches for the determination of enantiomeric purity with strong potentials for development into convenient preparative enantioseparation processes (1,2). It is not surprising that there has been tremendous research impetus focused on the development of efficacious CSPs over the past decades (3). A number of review articles and books deal with the methods and results of the direct enantioseparation of various compounds on chiral stationary phases (CSPs) and many attempts have been made to interpret how these CSPs operate with respect to molecular recognition (4–6).

Cyclodextrins (CD) are cyclic oligosaccharides containing six or more D-(+) glucopyranose units, which are bonded through α-(1,4)-linkages. The structures of CDs give rise to their remarkable ability in forming inclusion complexes with a variety of molecules (7–9) as well as ions (10,11). Meanwhile, the chirality of the CD moieties makes them amenable for application in enantioseparation processes. Accordingly, it is not surprising that CDs and their derivatives are extensively used as chiral selectors in enantioselective chromatography (12–15). But to date, literature reports have shown that the use of CD-based CSPs for chiral separation of ferrocenes is not so satisfactory (16).

As the application of the native CD-bonded stationary phases were not always satisfactory, it was necessary to design and prepare cyclodextrin derivatives to expand the range of compounds separable by CDs immobilized on silica gel. Armstrong et al. (12) synthesized a series of acetylated CD-bonded phases, which changed the hydrophilicity and the selectivity of the stationary phases. Fujimura (8) modified the CD-bonded phases with isocyanate groups and separated a variety of amino acids under suitable LC conditions. Nakamura et al. (13) prepared the perphenylated CD-bonded phases. As the hydroxyl groups were derivatized completely, the stereoselectivity also increased. Many amino acids were separated more satisfactorily than previously. Hattori et al. (14) and Chankvetadze et al. (15) synthesized amino- and aminomethyl-phenylcarbamate CD-bonded phases and achieved enantiomeric resolution.

It has been reported that rigid molecular structures afford better enantioseparation (17,18). Thus, by introducing the rigid imino group to the CD, it is expected that some interactions, including π–π, hydrogen-bonding, and polar–polar interactions

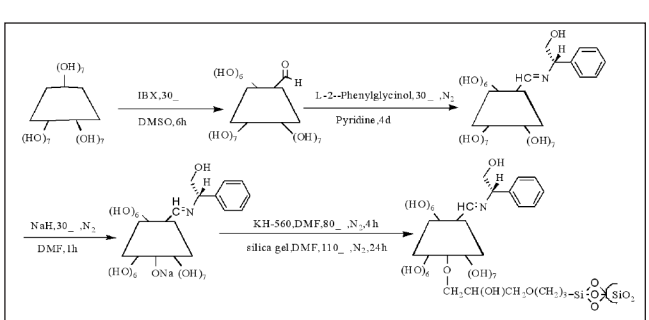


**Figure 1.** Scheme for preparation of L-PGCDs.

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would increase, leading to a change in the separation selectivity of the CD phase. Therefore, we have focused our attention on β-cyclodextrin derivatives with α-Schiff bases. We have previously reported a facile procedure into structurally well-defined chiral stationary phases (CSPs) based on cyclodextrin derivatives (19). These CSPs are stable and effective for the separation of positional isomers of substituted benzenes and for the chiral separation of racemates of amino acids, but they were unsuccessful when attempted for the chiral separation of ferrocenyl alcohols and amines.

Aimed to enhance the chiral recognition performance of the selector of CD CSP, we proposed to link the commercially available L-2-phenylglycinol to β-CD in a rigid way through a C=N bond, anticipating cooperation of the two chiral recognition sites, through the combination of β-CD inclusion effect, hydrogen bond and π–π stacking of phenyl group of L-phenylglycinol. To the best of our knowledge, we are among the first in such a design and preparation of this type of β-CD selector. Furthermore, by investigation on the effects of temperature on enantioselective separations, and calculation of thermodynamic constants from the van't Hoff plots (20–22), discussion on the mechanistic aspects of chiral recognition is reasonably presented.

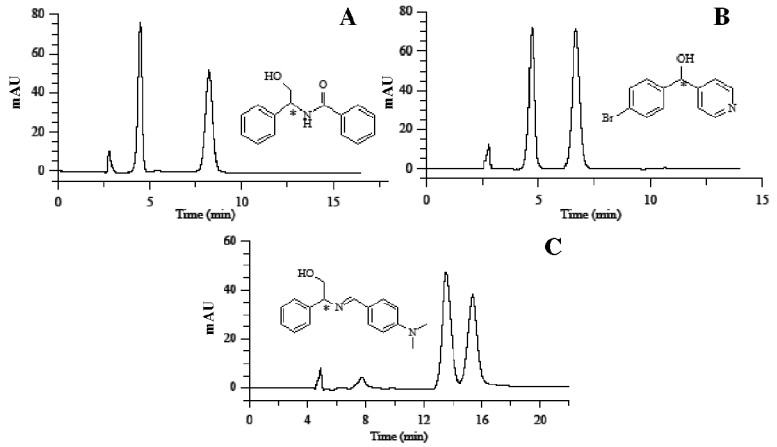
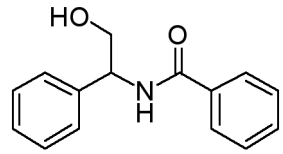
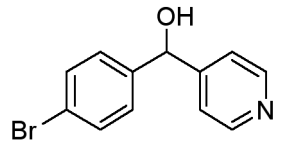
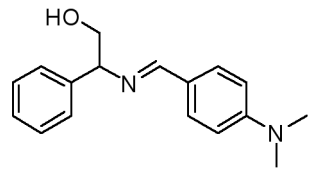



**Figure 2.** Representative chromatograms on the column packed with L-PGCD-CSP $H_2O$–MeOH (v/v) = 50/50; flow rate 0.6 mL/min; UV detector, 254 nm.

**Table I. Enantioseparation of Alkylaromatic Alcohols on L-PGCDs Under Reversed-Phase Conditions**

| S/N | compound | $H_2O$–MeOH | k' | α | Rs |
|---|---|---|---|---|---|
| 1 |  | 10:90 | 1.55 | 1.04 | 0.45 |
| | | 20:80 | 1.71 | 1.06 | 0.69 |
| | | 30:70 | 1.84 | 1.16 | 1.29 |
| | | 40:60 | 2.09 | 1.41 | 2.35 |
| | | 50:50 | 2.84 | 2.26 | 6.19 |
| 2 |  | 10:90 | 1.78 | 1.07 | 0.93 |
| | | 20:80 | 1.84 | 1.11 | 0.98 |
| | | 30:70 | 1.95 | 1.19 | 1.34 |
| | | 40:60 | 2.35 | 1.36 | 2.42 |
| | | 50:50 | 2.81 | 1.51 | 4.08 |
| 3 |  | 10:90 | 1.67 | 1.04 | 0.51 |
| | | 20:80 | 1.75 | 1.07 | 0.67 |
| | | 30:70 | 1.93 | 1.11 | 0.81 |
| | | 40:60 | 2.46 | 1.12 | 0.89 |
| | | 50:50 | 3.68 | 1.13 | 1.10 |

## Experimental

### Chemicals and equipments

β-CD was recrystallized twice and dried in vacuum over $P_2O_5$ at 100°C for 24 h before use. 3-Glycidoxypropyltriethoxysilane was purchased from the Chemical Plant of Wuhan University. It was redistilled under vacuum before use. Sodium hydride (80%), *N,N*-dimethylformamide (DMF) and pyridine were obtained from Chemical Reagent Factory No. 3 of Tianjing (Tianjing, China). DMF and pyridine were dried and redistilled before use. Silica gel (5 µm, 100 Å, 300 $m^2/g$, sphere) was purchased from Fuji (Japan). Other chemicals or reagents were analytical grade. The HPLC system used consisted of two Wellchrom HPLC pumps (K-501), a manual injection valve model (7725i), a Wellchrom spectrophotometer (K-2501), and a dynamic mixing chamber.

### Preparation of CSPs

The synthetic procedure to the new CSPs is depicted in Figure 1. As reported previously (19), in the first step, β-cyclodextrin monoaldehyde was obtained by oxidation of β-cyclodextrin using 2-iodoxybenzoic acid (IBX) as a mild and chemo-selective oxidizing agent. Condensation of β-cyclodextrin monoaldehyde with L-2-phenylglycinol gave the corresponding β-cyclodextrin derivative bearing a Schiff base group. The resulting mono[$6^A$-*N*-1-(2-hydroxy)-phenylethylimino-$6^A$-deoxy]-β-cyclodextrin, after treatment with NaH, reacted with 3-glycidoxypropyltriethoxysilane (KH-560), to immobilize the chiral selector onto the surface of silica gel via a long spacer. IR (KBr) ν: 3442, 2949, 1631, 1091, 804, 468 $cm^{-1}$ and the elemental analysis (C, 12.12%; H, 2.33%; N, 0.66%) of the CSP (L-PGCDs) showed that the cyclodextrin moieties had been successfully bonded onto the surface of the silica gel.

### Column evaluation

The performance of the stationary phase was evaluated in the reversed-phase mode using methanol and water (90:10, v/v) as the mobile phase. The column gave the efficiency

of 39260 P × $m^{-1}$, peak asymmetry (tailing factor) 0.93, using resorcinol as a probe test.

Reference separations were carried out periodically in order to control the long time reproducibility of the separations. This was done with the enantiomers of ferrocene derivatives. No significant shift of the separation factor (α) and resolution (*Rs*) values were detected over a 6-month period.

### Chromatographic conditions

The L-PGCDs were slurry-packed into 250 × 4.6 mm i.d. stainless-steel LC columns using methanol as the packing solvent. Mixtures of methanol or acetonitrile and water were used as the mobile phase to separate enatiomers of ferrocene derivatives and alkylaromatic alcohols. Triethylammonium acetate buffers (TEAA) of the desired pH were prepared by addition of glacial acetic acid to 1% aqueous triethylamine. Before use, the mobile phases were filtered through a membrane filter of 0.45-µm pore size and degassed under reduced pressure. The samples were dissolved in methanol or acetonitrile and filtered through a filter of 0.45-µm pore size. The wavelength used for detection was 254 nm. The flow rate of the mobile phase was set at 0.6 mL/min.

## Results and Discussion

### Separation of alkylaromatic alcohols

Table I depicts the enantioseparation results for several alkylaromatic alcohols on L-PGCDs under reversed-phase conditions. The volume ratio of $H_2O$–MeOH was varied from 10:90 to 50:50. Regarding the enantiomeric discrimination, it is remarkable that the best separation (α) and resolution (*Rs*) were achieved, with a 1:1 mixture of $H_2O$–MeOH. This indicates that suitable adjustment of the content of the organic modifier in the mobile phase can improve the chiral separation.

Under optimal separation conditions, it is evident that three alkylaromatic alcohol analogs exhibited excellent enantioseparations. The representative chromatograms are depicted in Figure 2.

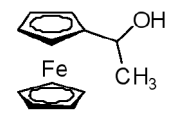
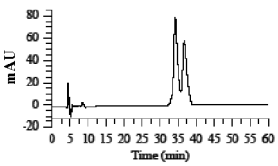
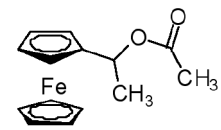
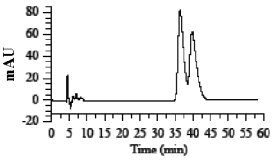
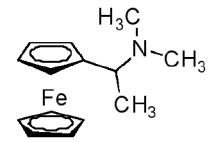
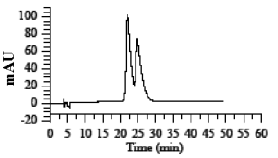
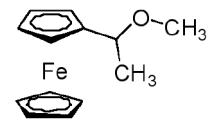
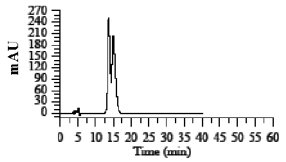
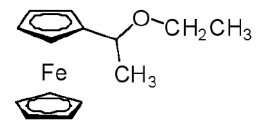
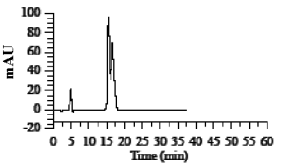
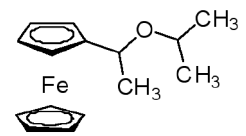
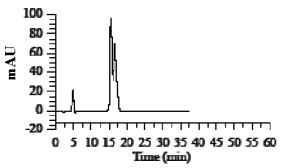
Primary alcohols 1 and 3 are similar in structure, but the resolution selectivity of compound 1 is much better than that of compound 3, this can be attributed to the fact that the hydrogen bonding interaction around the chiral center of compound 1 with the CSPs is stronger than that of compound 3. It seems that the tail group –$N(CH_3)_2$ of compound 3, capable of inducing hydrogen bonding interaction, only intensified the retention but did not improve the selectivity. The –OH group directly bonded to the chiral center, becomes pivotal for better chiral recognition of compound 2, although in this case, π–π interaction or dipole–dipole interaction on chiral separation can not be excluded.

### Separation of ferrocene derivatives

Table II lists 6 enantiomeric pairs of organometallic compounds and their corresponding chromatographic retention data, all enantiomeric pairs were baseline or nearly baseline resolved.

For chiral recognition in cyclodextrins in the reversed-phase mode, the chiral center of the analytes, that form an inclusion complex (23,24) must be near the rim of the cyclodextrin cavity, in order to generate at least one strong contact with the "mouth" of the cavity, such as a hydrogen bond (25). The molecular struc-

**Table II. Enantioseparation of Some Ferrocene Derivatives on L-PGCD-CSP Under Reversed Phase Conditions**

| S/N | Compound | Enantioseparation data: Separation results | Enantioseparation data: Chromatograms |
|---|---|---|---|
| 4 |  1-ferrocenylethanol | $k'_1$ = 16.09<br>$k'_2$ = 17.36<br>α = 1.08<br>*Rs* =1.29 |  |
| 5 |  1-ferrocenylethyl acetate | $k'_1$ = 17.18<br>$k'_2$ = 18.88<br>α = 1.10<br>*Rs* =1.15 |  |
| 6 |  *N,N*-dimethyl-*N*-(1-ferrocenyleth-yl)amine | $k'_1$ = 10.01<br>$k'_2$ = 11.38<br>α = 1.14<br>*Rs* =1.11 |  |
| 7 |  1-ferrocenyl-1-methoxyethane | $k'_1$ = 5.86<br>$k'_2$ = 6.47<br>α = 1.10<br>*Rs* = 0.83 |  |
| 8 |  1-ferrocenyl-1-ethoxyethane | $k'_1$ = 6.97<br>$k'_2$ = 7.48<br>α = 1.07<br>*Rs* = 0.77 |  |
| 9 |  1-ferrocenyl-1-(i-propoxy)ethane | $k'_1$ = 7.24<br>$k'_2$ = 7.90<br>α = 1.09<br>*Rs* = 0.75 |  |

* Separation conditions: flow rate 0.6 mL × $min^{-1}$; $H_2O$–MeCN (v/v) = 70:30 (compounds 4 and 5); $H_2O$–MeCN (v/v) = 60:40 (compounds 8, 9); $H_2O$–MeCN (v/v) = 55/45 (compounds 7); buffer (1% TEA aqueous adjusted with HOAc, pH = 7.11)–MeCN (v/v) = 60:40 (6); UV detector, 254 nm.

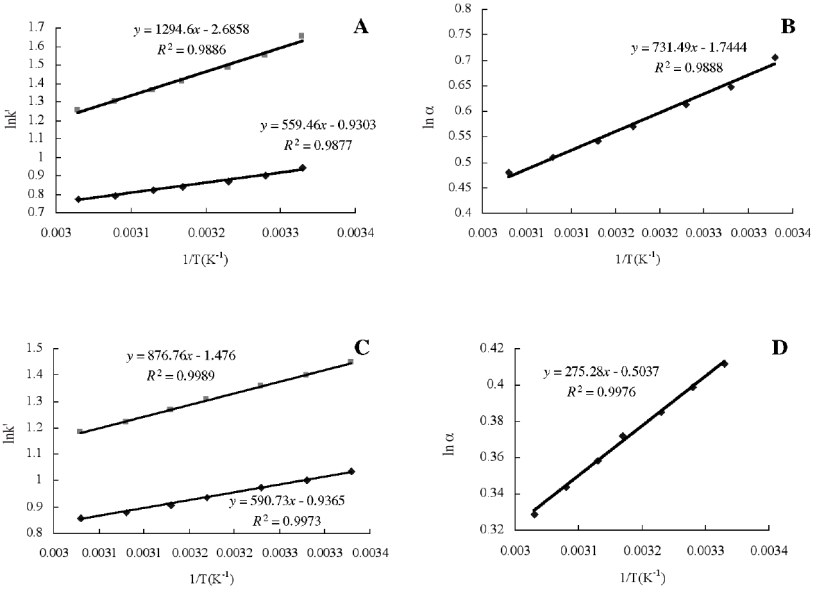
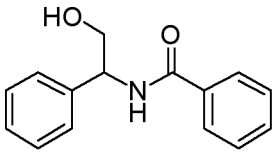
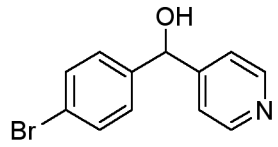



**Figure 3.** van't Hoff plot of compound 1 (A and B) and van't Hoff plot of compound 2 (C and D).

**Table III. Chromatographic Data of Compounds 1 and 2 at Different Temperatures**

| Compound | Chromatographic result | | | | | | |
|---|---|---|---|---|---|---|---|
| | $t_1$(min) | $t_2$(min) | $k'_1$ | $k'_2$ | α | *Rs* | Condition |
|  | 7.14 | 12.43 | 2.57 | 5.21 | 2.03 | 5.50 | 1 |
| | 6.91 | 11.39 | 2.46 | 4.70 | 1.91 | 4.80 | 2 |
| | 6.77 | 10.78 | 2.38 | 4.39 | 1.84 | 4.31 | 3 |
| | 6.63 | 10.20 | 2.32 | 4.10 | 1.77 | 4.07 | 4 |
| | 6.53 | 9.80 | 2.27 | 3.90 | 1.72 | 3.92 | 5 |
| | 6.43 | 9.36 | 2.21 | 3.68 | 1.67 | 3.39 | 6 |
| | 6.32 | 8.98 | 2.16 | 3.49 | 1.62 | 3.30 | 7 |
|  | 7.62 | 10.48 | 2.81 | 4.24 | 1.51 | 4.08 | 1 |
| | 7.44 | 10.10 | 2.72 | 4.05 | 1.49 | 3.72 | 2 |
| | 7.28 | 9.75 | 2.64 | 3.88 | 1.47 | 3.74 | 3 |
| | 7.11 | 9.38 | 2.55 | 3.69 | 1.45 | 3.67 | 4 |
| | 6.97 | 9.08 | 2.48 | 3.54 | 1.43 | 3.18 | 5 |
| | 6.82 | 8.77 | 2.41 | 3.39 | 1.41 | 2.95 | 6 |
| | 6.71 | 8.54 | 2.36 | 3.27 | 1.39 | 2.74 | 7 |

* Conditions: flow rate 0.6 mL × min$^{-1}$; MeOH–$H_2O$ (50:50): 1, 300K; 2, 305K; 3, 310K; 4, 315K; 5, 320K; 6, 325K; 7, 330K.

ture of compound 4 seems suitable to be near the cyclodextrin cavity, exhibiting the best resolution. Compared with compound 5, the selectivity value (α) of compound 6 was better, but the *Rs* was poorer. This may due to a little difference in hydrogen bond interactions, although molecular volumes of 5 and 6 are similar.

Enantiomeric separation of ferrocene ether derivatives 7–9, compared with that of compounds 4–6, showed poor resolution, the weaker hydrogen interaction of the CSP with these compounds apparently resulting in less chiral discrimination effect. On the other side, the relative importance of hydrogen bonding may well be dependent on factors such as steric repulsions and connected with the structure of the molecule. Thus, ether 7 having the least steric hindrance, with a methoxy group attached to the chiral center, performs in the best way, while ether 9 having the largest steric hindrance, with an iso-propoxy group attached to the chiral center, exhibits the poorest resolution among compounds 7–9.

### Effects of temperature and thermodynamic parameters

The dependence of the retention of an analyte on the temperature can be expressed by the van't Hoff equation (26), which may be interpreted in terms of mechanistic aspects of chiral recognition:

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi \qquad \text{Eq. 1}$$

where k' is the capacity factor.

This equation reveals that a plot of ln k' versus 1/T is linear, with a slope of $-\Delta H^\circ/R$ and an intercept of $\Delta S^\circ/R + \ln\phi$, if $\Delta H^\circ$ is invariant with temperature. The calculation of $\Delta S^\circ$ from the intercept requires the knowledge of the phase ratio (ϕ). Because the value of ϕ is often not known, the $\Delta S^{\circ\#}$ values [$\Delta S^{\circ\#} = \Delta S^\circ + R \ln\phi$] calculated from the intercepts of the plots via Equation 1 are generally used. Any uncertainty in the phase ratio affects the $\Delta S^{\circ\#}$ values virtually equally, and the trends in $\Delta S^{\circ\#}$ as a function of

**Table IV. Thermodynamic Parameters of First and Second-Eluting Enantiomers and Correlation Coefficients ($r^2$) of lnk' vs. 1/T Curves for Analytes 1,2**

| Compound | $\Delta H^0_1$ (kJmol$^{-1}$) | $\Delta H^0_2$ (kJmol$^{-1}$) | $\Delta H^0_1$ (kJmol$^{-1}$K$^{-1}$) | $\Delta H^0_1$ (kJmol$^{-1}$K$^{-1}$) | Correlation coefficient | Correlation coefficient | $\Delta G^0_{1;300K}$ (kJmol$^{-1}$) | $\Delta G^0_{2;300K}$ (kJmol$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | -4.65 | –10.76 | –7.73 | –22.33 | 0.9877 | 0.9886 | –2.33 | –4.06 |
| 2 | -4.91 | –7.29 | –7.79 | –12.27 | 0.9973 | 0.9989 | –2.57 | –3.61 |

**Table V. Thermodynamic Parameters Δ(ΔH°), Δ(ΔS°), and Δ(ΔG°) of Enantiomers and Correlation Coefficients ($r^2$) of lnα vs. 1/T Curves for Analytes 1,2**

| Compound | Δ(ΔH°) (kJmol$^{-1}$) | Δ(ΔS°) (kJmol$^{-1}$K$^{-1}$) | Correlation coefficient ($r^2$) | $\Delta(\Delta G^\circ)_{300k}$ (kJmol$^{-1}$) |
|---|---|---|---|---|
| 1 | –6.08 | –14.50 | 0.9888 | –1.73 |
| 2 | –2.29 | –4.19 | 0.9976 | –1.03 |

molecular structure are therefore largely unaffected.

The corresponding Δ(ΔH°) and Δ(ΔS°) values for the separated enantiomers, can be determined from a modification of Equation 1:

$$\ln\alpha = -\frac{\Delta(\Delta H^\circ)}{RT} + \frac{\Delta(\Delta S^\circ)}{R} \qquad \text{Eq. 2}$$

where α is the selectivity factor ($\alpha = k'_2/k'_1$).

For the investigation of temperature effects, compounds 1 and 2 were separated at a mobile phase composition of $H_2O$–MeOH = 50/50 (v/v), at seven different temperatures (300, 305, 310, 315, 320, 325, and 330 K). Comparison of the retention factors in Table III demonstrates that all of the recorded values decreased with increasing temperature.

In order to investigate the effect of temperature on enantioseparation, an extensive study dealing with thermodynamics of enantiomer separation was carried out. For this purpose, the van't Hoff plots were evaluated. All the plots of ln k' versus 1/T could be fitted by straight lines with good correlation coefficients (Figure 3, Table IV). The ΔH° and $\Delta S^{\circ\#}$ values calculated from the slopes and intercepts of the plots of Equation 1 for all enantiomers were negative (Table IV). Further, the ΔH° and $\Delta S^{\circ\#}$ values for the first-eluting enantiomer were always less negative than those for the second-eluting enantiomer. Since the second-eluting enantiomers have more negative $\Delta S^{\circ\#}$ values, they probably have fewer degrees of freedom on the CSP (i.e., they are held at more points or are less able to move or rotate). Simultaneous interactions between the chiral analyte and the CSP appear somewhat more likely for the second-eluting enantiomers than for the first-eluting enantiomers (21).

The data on the changes in Δ(ΔH°), Δ(ΔS°) and Δ(ΔG°) are depicted in Table V. It seems that on this CSP the intermolecular interactions between the analytes and the selector were generally exothermic (27), and the corresponding entropy change was also negative. It seems that the π-π interactions play the most important role in the enantiorecognition. The π-π interactions, as well as dipole-dipole, hydrophobic and steric interactions, may contribute to both the retention and the enantiorecognition. The rigidity, bulkiness, and aromaticity and the side-chains may direct the complexation of the stereoisomers on the selector.

In summary, the actual complex formation via multiple intermolecular interactions was generally exothermic, and the corresponding entropic contribution was also negative. The final balance is, as evidenced by the Δ(ΔG°) data listed in Table V, that the separations for all the investigated analytes on this CSP were enthalpically favored.

## Conclusion

Mono(6$^A$-*N*-1-(2-hydroxy)-phenylethylimino-6$^A$-deoxy)-β-cyclodextrin, has been successfully generated by direct condensation of β-cyclodextrin monoaldehyde with L-2-phenylglycinol. Accordingly, this afforded a convenient synthesis of a promising chiral selector. Thereafter, immobilization of the chiral selector onto the surface of silica gel via a long spacer afforded a facile entry into a structurally well-defined chiral stationary phase L-PGCD-CSP for HPLC. This chiral stationary phase exhibited good enantioselectivity for several alkylaromatic alcohols and a variety of ferrocene derivatives under reversed-phase conditions. This means that linking the commercially available phenylglycinol block to the β-CD can enhance the chiral recognition performance of the CD CSP selector; moreover such combination could produce cooperative functioning by the two chiral recognition sites (i.e., the assembly of inclusion of β-CD, hydrogen bond and π-π stacking of phenyl group of L-phenylglycinol). Investigation on the effects of temperature on enantioselective separations and theoretical study on the thermodynamic constants Δ(ΔH°), Δ(ΔS°), and Δ(ΔG°) reveal that separations for the studied analytes on this CD CSP were enthalpically favored.

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