Published in final edited form as:

J Chromatogr A. 2008 May 16; 1191(1-2): 199–204.

Enantiomeric separation of alcohols and amines on a proline chiral stationary phase by gas chromatography

Min Li, Junmin Huang¹, and Tingyu Li*

Department of Chemistry, Box 9573, Mississippi State University, Mississippi State, MS 39762, USA

Abstract

A new chiral stationary phase for gas chromatography was prepared by covalently attaching a diproline chiral selector that has proven to be effective in liquid chromatography to a methylhydrosiloxane-dimethylsiloxane copolymer. With this new chiral stationary phase for GC, racemic aromatic alcohols could be resolved without derivatization. Racemic aromatic and aliphatic amines could also be resolved after derivatization of the amino groups with trifluoroacetic anhydride or isopropyl isocyanate. On this stationary phase, the isopropyl isocyanate derivatives of amines showed higher enantioselectivity than the trifluoroacetic anhydride derivatives. In both the enantiomeric separations of alcohols and derivatized amines, the aromatic racemic analytes showed higher enantioselectivities than their aliphatic analogs. Some of the α -amino and α -hydroxy aromatic acids could also be separated after derivatization to N-trifluoroacetyl methyl esters for amino acids or O-trifluoroacetyl methyl esters for hydroxy acids.

Keywords

Enantiomer separation; proline stationary phase; enantioselective gas chromatography

1. Introduction

Enantioselective gas chromatography (GC) has demonstrated the potential for a broad array of applications in diverse industries and it remains a dynamic area for analytical chiral separations. Examples of enantioselective GC include the direct enantiomeric separation of optically active components in natural products, asymmetric synthesis, environmental contaminants, and those important to space science, agricultural, food, flavor and fragrance industries.[1] In enantioselective GC, three major types of chiral stationary phases have been well studied. [2,3] They include one class based on chiral amino acid derivatives, one class based on chiral metal coordination compounds, and a third class based on cyclodextrin derivatives. The well-known and popular Chirasil-Val is made from the readily available amino acid valine and this stationary phase is commercially available in both enantiomeric forms. There has been a number of attempts to improve its performance.[4–6]

We have been interested in developing enantioselective columns using readily available amino acid and short oligopeptide derivatives.[7–10] This class of compounds has been studied as chiral selectors for enantioselective chromatography and capillary electrophoresis due to their

*Corresponding author. Tel.: +1 703 292 4949. Email address : li1640@gmail.com (T. Li).

¹Current address: Synta Pharmaceuticals Corp., 45 Hartwell Avenue, Lexington, MA 02421, USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ready availability. Examples include the Pirkle-type stationary phases [11] and the chiral diamide phases in chromatography [12] and the polymeric chiral surfactant research in capillary electrochromatography.[13] Recently, a series of enantioselective columns, with proline oligomers as chiral selectors, were developed in our laboratory.[8–10] With the right structural features, these columns demonstrated broad enantioselectivity in liquid chromatography. In our study of fifty three racemic analytes chosen by their availability, one oligoproline column was able to resolve over forty of these analytes. These proline chiral selectors compare very well with other chiral selectors that have been studied.

In this report, we investigated the application of these oligoproline chiral selectors for enantioselective Gas Chromatography. For this purpose, a diproline chiral selector was chemically bonded to the backbone of 5–7% methylhydrosiloxane-dimethylsiloxane copolymer to prepare a stable GC stationary phase. In this stationary phase, we maintained the key structural features such as the end-capping group and the linkage point to proline. The resolution of racemic alcohols, amines, amino acids, and hydroxyl acids on this stationary phase was investigated.

2. Experimental

2.1. 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), or Fisher Scientific (Pittsburgh, PA, USA). Untreated fused silica capillary column, 30 m × 0.25 mm FSOT undeactivated, was obtained from Alltech Associates Inc., Deerfield, IL, USA. A HP 5890 series II gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with an autoinjector (model HP6890) and a flame ionization detector (FID) was used. Helium served as the carrier gas.

2.2. Preparation of the stationary phase

The chiral selector was synthesized by following known procedures (Figure 1). First, methyl amine was acylated with 10-undecenoyl chloride and the resulting methyl amide was reduced with lithium aluminum hydride to yield the methyl amine linker.[14] Prolines were then coupled to the linker by following the procedure reported in our previous work.[9]

The chiral selector was then coupled to the copolymer by the following procedure.[15] A 2.0 g of 5–7% methylhydrosiloxane-dimethylsiloxane copolymer and 0.5 g of the selector were dissolved in 120 mL of dry toluene under an atmosphere of nitrogen. The solution was heated to 70°C and half of a solution of 2 mg of $\text{H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$ in 20 mL dry THF was added under nitrogen. After 2 h, the second half of the solution was added and the mixture was heated to reflux for 48 h. After evaporation of the toluene, the resulting dark yellow polymer was washed with three 150 mL portions of anhydrous methanol, which were removed by decantation. Residual methanol was evaporated in vacuo, yielding the chiral stationary phase, which was used for the coating procedure. The absence of olefinic carbon signals from DEPT-135 NMR indicates that there is no unbound chiral selector in the resulting stationary phase.

2.3. Column coating procedure

0.5 g of the chiral stationary phase was dissolved in 10 mL anhydrous dichloromethane, and the solution was added into the container of S.G.E. Solvent Rinse Kit bought from SUPELCO (Bellefonte, PA, Product#: 23626). A helium stream from the GC instrument pushed the solution of the dissolved stationary phase into a capillary column (30 m × 0.25 mm FSOT undeactivated, with Part#: 602630, Alltech Associates Inc.). When the column was filled up with the stationary phase solution, the continuous helium gas forced the excess liquid out of

the column and formed a film of the stationary phase on the wall of the capillary column. One end of the column was then connected into the GC instrument, and the column was conditioned at 40°C for 2 hours, 80°C for 2 hours, 120°C for 2 hours, 160°C for 2 hours, and 200°C for 4 hours. After these procedures, the other end of the column was connected into the FID detector and the column was evaluated.

2.4. Derivatization of amines, amino acids, and hydroxy acids

The alcohols were injected directly into GC without derivatization. The amines, amino acids, hydroxyl acids are derivatized first before analysis.

2.4.1. Derivatization of amines by trifluoroacetic anhydride [16] or by isopropyl isocyanate. [17]—1.5 mg of amine was allowed to stand at room temperature (30 min) in a mixture solution of 0.4 mL of dichloromethane and 0.2 mL of trifluoroacetic anhydride or isopropyl isocyanate. After the solvent and the excess reagent were removed by a stream of nitrogen, the residue was re-dissolved in dichloromethane for injection.

2.4.2. Derivatization of amino acids and hydroxy acids—The methylation of acids was achieved by reaction of acids with hydrogen chloride in methanol. The dichloromethane solution of acids was heated at 100 °C with 2N dry hydrogen chloride in methanol for 60 min in a sealed vial. After removing the solvent with a stream of nitrogen, trifluoroacetic anhydride (0.4 mL) was added to the residue and the mixture was allowed to stand at room temperature for 30 min.

2.5. Chromatographic measurements

The separation factor α was determined as follows, $\alpha = (t_2 - t_d)/(t_1 - t_d)$, where t_1 and t_2 are the retention times of the first and second eluted enantiomers, respectively, and t_d is the holdup time measured through co-injection of methane. The resolution factor was determined using the following expression, $R_s = 1.18(t_2 - t_1)/(w_1 + w_2)$, where w_1 and w_2 are the width of the peaks at the half-height of the first and the second eluted enantiomers, respectively. In all the measurements, the retention times were timed to be between 10 min to 50 min by adjusting the column temperature.

3. Results and discussion

3.1. Chiral stationary phase design and preparation

In our previous studies on liquid chromatography, we established the importance of the end capping group trimethylacetyl on the N-terminal of the proline dipeptide to improve enantioselectivity. The N-methyl group on the linker also proved important for enantioselectivity. [8–10] For example, a diproline stationary phase with the N-methyl group resolved 19 out of 53 analytes tested, while its closely related analog without the N-methyl group resolved only 2 analytes. For these reasons, the chiral selector we chose to study for gas chromatography (Figure 1) contains these two crucial features. In addition, a long apolar hydrocarbon C_{11} linker with a terminal olefin group was introduced in the chiral selector. Such a terminal olefin group allows the covalent immobilization of this chiral selector onto the copolymer to prepare the chiral stationary phases (Figure 2). This long apolar linker also renders the stationary phase less polar and helps to decrease its solidification temperature. [15,18,19] Embedding of apolar components into the stationary phase may even improve their enantioselective property (the squalane effect).[15] Detailed experimentation procedures for the preparation of the chiral selector and chiral stationary phases are described in the experimental section.

3.2. Separation of alcohols

In our tests, several aromatic alcohols can be baseline or partially resolved without derivatization using this stationary phase, as shown in Table 1. A trend is that the larger the aromatic ring is, the better the separation results. For instance, for α -methyl-9-anthracenemethanol (analyte **1** in table 1), the separation factor is 1.16 and the resolution factor is 4.33 (GC chromatogram was shown in Figure 3), while for α -methyl-1-naphthalenemethanol (analyte **2**), the separation factor declined to 1.09 and R_s value also dropped to 1.37. For α -methylbenzyl alcohol (analyte **3**), which contains only a single aromatic ring, the separation factor was lower at 1.03 and R_s was 0.79. When the methyl group of α -methylbenzyl alcohol was substituted with some other alkyl groups such as cyclopropyl, *t*-butyl, acetylene and ethyl, the di-proline column failed to resolve these compounds. However, when the methyl was substituted with a trifluoromethyl group (analyte **6**), both the separation factor and the resolution factor increased. It appears that the vicinal trifluoromethyl group of α -trifluoromethylbenzyl alcohol (analyte **6**) may increase the polarity of the hydroxyl group, which could enhance the hydrogen bonding interaction [20] with the selector. Two racemic aliphatic alcohols, 2-hydroxy heptane and 1,2-dihydroxy pentane, could not be separated by this stationary phase, which suggests that the aromatic rings of the aromatic alcohols contribute to their selective interaction with the diproline chiral selector.

3.3. Separation of aromatic and aliphatic amines

Since the direct injection of amines would result in heavily tailing peaks, they are derivatized first before GC analysis. Derivatization with trifluoroacetic anhydride was studied first. As shown in Table 2, the aromatic amines showed higher separation than the aliphatic amines did. For instance, α -methyl benzylamine (analyte **7** in table 2, GC chromatogram shown in Figure 4) and 1- α -methyl naphthylamine (analyte **10**) could be readily and baseline resolved with a high separation factor of 1.11 and 1.14, respectively. However, the aliphatic amines could only be partially separated. The result is consistent with our observation on the separation of racemic alcohols, which further demonstrated that the importance of aromatic rings in these separations.

For aliphatic amines, the separation appears correlated with the asymmetry and the steric hindrance around the amine stereogenic center. For example, in 2-aminopentane (analyte **14**), 2-aminoheptane (analyte **15**), and 2-aminooctane (analyte **16**), the separation increases with the size of the alkyl groups. However, no separation was observed for 2-ethyl-1-hexylamine (analyte **18**). These results are not difficult to understand. For the derivatized amines, a strong hydrogen bond should exist between the analytes and the chiral selector to form association to sustain a difference in the standard free energy of two enantiomers. When the bonds that form these associations are in the proximity of an asymmetric environment, a difference in the behavior of the enantiomers in the chiral stationary phase is possible [3].

Separation of amines with isopropyl isocyanate as the derivatizing reagent was also studied, as isopropyl isocyanate is known to form urethanes with amino compounds. The reaction of amines with isopropyl isocyanate affords a urethane which contains two amide groups. The additional amide groups could improve the hydrogen bonding interactions with the proline chiral stationary phases. As shown in Table 3, improvement in both the separation factors and resolution factors were observed for the chiral amines studied. For example, the α value for 2-aminooctane was 1.04 and R_s was 1.02 (in Table 2) after it was derivatized by trifluoroacetic anhydride, however, its α and R_s values were increased to 1.05 and 1.58, respectively when it was derivatized by isopropyl isocyanate. In this case, a baseline separation of the two enantiomers was obtained.

3.4. Separation of amino acids and hydroxy acids

The amino acids are analyzed as the N-trifluoroacetylated amino acid methyl esters, while the hydroxy acids are analyzed as the O-trifluoroacetylated hydroxy acid methyl esters.

As shown in Table 4, modest chiral separations of the amino acid derivatives are achieved. For amino acids without polar side chain functional groups, such as valine, leucine, and phenylalanine, partial separations were obtained. For amino acids with polar side chain functional groups, such as lysine and aspartic acid, no separation was observed. The diproline column does not resolve racemic hydroxy acids well. While the aromatic mandelic acid (analyte **23**) exhibited a nearly baseline separation, other aliphatic α -hydroxy acids such as lactic acid displayed no separation.

The inadequate separation of the derivatives of racemic hydroxyl acids could be that such analytes possess no hydrogen bond donors to form hydrogen bonds with the diproline chiral selector, which does not contain hydrogen bond donors to form hydrogen bonds with the analytes. However, the significant enantiomeric separation of hydroxyl-acid mandelic acid by this diproline chiral stationary phase substantiates König's assertion that hydrogen bonding association cannot be solely responsible for the separation of enantiomers [11]. Of note is that in our HPLC studies, the diproline chiral stationary phase could resolve racemic Tröger's base, which cannot form hydrogen bonds with the chiral selector. These results indicate that weak polar interactions (dipole/dipole, dipole/induced dipole, induced dipole/induced dipole) alone are capable of providing adequate enantioselective recognition in this proline system [9].

4. Conclusion

Inspired by the successful application of the diproline chiral selectors in the separation of racemic compounds in HPLC, we chemically bonded the diproline chiral selector to a methylhydrosiloxane-dimethylsiloxane copolymer to prepare a chiral stationary phase for gas chromatography. This chiral stationary phase could resolve racemic aromatic alcohols without derivatization. Racemic aromatic and aliphatic amines could also be resolved after derivatization of the amino groups with trifluoroacetic anhydride or isopropyl isocyanate. In both the enantiomeric separations of alcohols and derivatized amines, the aromatic racemic analytes showed higher enantioselectivities than their aliphatic analogs. Some of the α -amino and α -hydroxy aromatic acids could also be separated after a two-step derivatization of the acids.

Acknowledgement

The financial support from NIH (GM 63812) is greatly appreciated.

References

1. He L, Beesley TE. J. Liq. Chromatogr. Relat. Technol 2005;28:1075.
2. Schurig V. J. Chromatogr 2001;A 906:275.
3. Juvancz Z, Petersson P. J. Microcolumn 1996 Sep;8:99.
4. Lohmiller K, Bayer E, Koppenhoefer B. J. Chromatogr 1993;634:65. [PubMed: 8486745]
5. Oi N, Kitahara H, Matsushita Y. J. High Resolut. Chromatogr 1990;13:720.
6. Oi N, Kitahara H, Matsushita Y, Kisu N. J. Chromatogr 1996;A 722:229.
7. Bluhm L, Huang J, Li T. Anal. Bioanal. Chem 2005;382:592. [PubMed: 15883787]
8. Huang J, Zhang P, Chen H, Li T. Anal. Chem 2005;77:3301. [PubMed: 15889922]
9. Huang J, Chen H, Li T. J. Chromatogr 2006;A 1113:109.
10. Huang J, Chen H, Zhang P, Li T. J. Chromatogr 2006;A 1109:307.
11. Welch CJ. J. Chromatogr 1994;A 666:3.

12. Dobashi A, Dobashi Y, Kinoshita K, Hara S. *Anal. Chem* 1988;60:1985.
13. Billiot E, Warner IM. *Anal. Chem* 2000;72:1740. [PubMed: 10784136]
14. Paetzold E, Jovel I, Oehme G. *J. Mol. Catal. A: Chem* 2004;214:241.
15. Levkin PA, Levkina A, Schurig V. *Anal. Chem* 2006;78:5143. [PubMed: 16841940]
16. Blau, K.; Halket, JM., editors. *Handbook of Derivatives for Chromatography*. Second Edition. 1993.
17. Koenig WA, Benecke I, Lucht N, Schmidt E, Schulze J, Sievers S. *J. Chromatogr* 1983;279:555.
18. Frank H, Nicholson GJ, Bayer EJ. *J. Chromatograph. Sci* 1977;15:174.
19. Frank H, Nicholson GJ, Bayer EJ. *Angew. Chem. Int. Ed* 1978;17:363.
20. Schurig V. *Angew. Chem. Int. Ed. Engl* 1984;23:747.

Figure 2.
Synthesis of the diproline chiral stationary phase

Figure 3.
Chromatogram of the α -methyl-9-anthracenemethanol (analyte **1** in table 1). T=190 °C.

Figure 4.
Chromatogram of the N-TFA α -methyl benzylamine (analyte **7** in table 2). T=110 °C.

Table 1

Separation of aromatic alcohols

#	Structure	T	k ₂ '	α	R _s	#	Structure	T	k ₂ '	α	R _s
1		190	14.2	1.16	4.33	4		100	9.04	1.04	0.92
2		150	5.9	1.09	1.37	5		145	5.13	1.03	0.78
3		80	7.6	1.03	0.79	6		100	7.5	1.06	1.15

Carrier gas, helium; flow rate, 0.8 ml/min.

T = column temperature (°C); k₂' = retention factor of the late eluting enantiomer; α = separation factor; R_s = resolution factor;

Table 2
Separation of amines derivatized by trifluoroacetic anhydride

#	Structure	T	k ₂ '	α	Rs	#	Structure	T	k ₂ '	α	Rs
7		110	8.7	1.11	3.27	14		70	3.2	1.02	0.59
8		110	6.7	1.13	2.77	15		90	3.8	1.03	0.79
9		110	8.3	1.00	0	16		90	5.1	1.04	1.02
10		160	5.5	1.14	4.13	17		90	2.9	1.05	1.21
11		90	8.2	1.06	1.13	18		90	8.3	1.00	0

#	Structure	T	k ₂ '	α	Rs	#	Structure	T	k ₂ '	α	Rs
12		145	7.3	1.03	0.78	19		60	3.4	1.07	1.86
13		140	7.1	1.00	0						

Separation conditions are the same as in Table 1.

Table 3
Separation of alkyl amines derivatized by isopropyl isocyanate

#	Structure	T	k ₂ '	α	Rs	#	Structure	T	k ₂ '	α	Rs
14		120	11.9	1.03	0.99	17		130	13.3	1.04	1.13
15		130	16.3	1.04	1.07	18		140	16.2	1.00	0
16		140	14.2	1.05	1.58	19		130	7.0	1.07	1.10

Separation conditions are the same as in Table 1.

Table 4

Separation of amino acids and hydroxy acids

#	Structure	T	k ₂ '	α	Rs	#	Structure	T	k ₂ '	α	Rs
20		80	5.5	1.02	0.87	23		90	5.6	1.09	1.62
21		90	7.2	1.02	0.56	24		50	4.7	1.00	0
22		120	5.7	1.03	0.69	25		90	8.3	1.00	0
						26		80	4.5	1.00	0

Separation conditions are the same as in Table 1