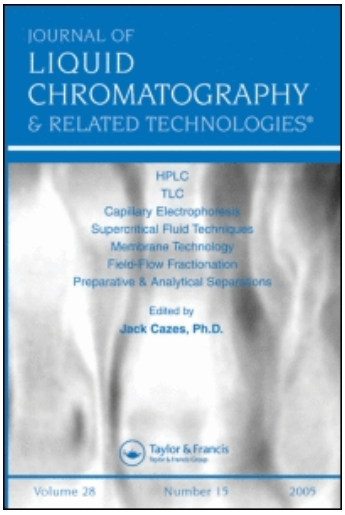

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Evaluation of A Chiral Crown Etherlc Column For the Separation of Racemic Amines

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EVALUATION OF A CHIRAL CROWN ETHER LC COLUMN FOR THE SEPARATION OF RACEMIC AMINES

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ABSTRACT

Enantiomeric resolution of more than fifty racemic primary amines can be achieved on a column that utilizes a crown ether as a chiral selector. The racemic solute is solubilized in an acidic solvent, forming an ammonium ion from the primary amine functional group. An interaction between the lone pair electrons on the oxygens of the crown ether and the positive charge of the ammonium group leads to the formation of an inclusion complex. Due to the chirality of the crown ether there is stereoselective interaction resulting in enantiomeric separation. Excellent resolution is possible for amino acids, amino alcohols, amino esters and amines. Compounds are separated that were poorly resolved by conventional ligand exchange columns and by other means.

INTRODUCTION

Considerable interest has been shown in the enantiomeric resolution of racemic amines, amino alcohols, amino esters and amino acids.[1,2] Reasons for this attention include topics such as different

reactivity of enantiomers in stereoselective synthesis,[3] geochronology, geothermometry[4,5] and varied biological responses to different enantiomers. Primary amine containing compounds are an important part of many pharmaceutical formulations as well as being essential to life in the form of amino acids.[6] Ligand exchange chromatography has been a prevalent method for the separation of amino acid racemates.[7-9] In this method an enantioselective complex is formed between a metal ion (Cu^{2+}) and the amino acid.[7-9] This method is generally limited to molecules that are bidentate ligands of the α-amino-carboxylic acid and α-hydroxy-carboxylic acid types. An alternative approach utilizes a chiral crown ether complexing agent. In this case no metal ions are needed and the only requirement for complexing is that a primary amine functional group be present.

Crown ethers are macrocyclic ring compounds containing several oxygens separated by ethylene moities as shown in Figure 1. The term "crown ether" was coined in 1967 by Charles J. Pedersen because of the appearance of the molecular model and the compound's ability to "crown" or surround cations. In these early studies the cyclic compounds containing from five to ten oxygens atoms were shown to form stable complexes or "crown" a variety of cations.[10,11] Cyclic polyethers had been reported previously in studies of the solubility of alkali metals in ethers and these types of macrocyclic compounds were included.[12,13] Pedersen, however, was the first to develop an organized feasible synthesis to produce them and he synthesized many new ones. While working with the compounds, Pedersen recognized that the ability to form complexes between the various crown ethers and specific cations was size dependent. Cram described the interaction of forming the complex as host-guest interactions[14]. Host-guest complexes are formed when the cavity of the host molecule and the size

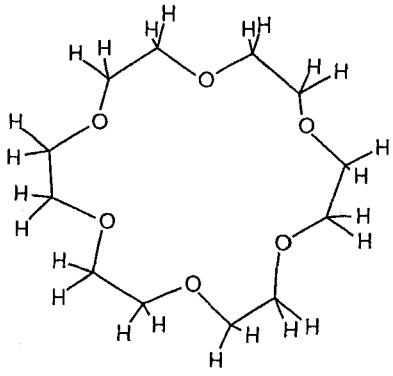


Figure 1: The structure of a nonchiral crown ether.

and shape of the guest molecule are complementary. A complex is composed of a host and guest held together in a definite stochiometric relationship.

Initial interest in crown ethers grew rapidly. One reason was the discovery that their interaction with Hg^{2+} and Pb^{2+} ions showed promise for removal of poisonous heavy metal ions from an organism without disrupting the delicate balance of physiologically important ions.[15] In the cited study, treatment of lead poisoned rats with a derivative of a crown ether, called a cryptand, increased the concentration of lead excreted in the urine by a factor of 40. Size was an important factor in the proper cationic selectivity. Analogies have been made between the crown ethers ability to complex ions and the action of some naturally occurring antibiotic ionophores.[15,16] Biological transport of amino acids and their derivatives through membranes utilize the same mechanism.

Crown ethers and particularly [18]-crown-6-derivatives form complexes with ammonium ion and protonated alkyl amines. Crown ethers

selectively complex with cations and in this case the ammonium ion has the proper ionic radius needed to fit into the [18]-crown-6-cavity. This interaction had been utilized to separate positional isomers of disubstituted anilines in HPLC.[17] Additional applications of crown ethers include ion-selective electrodes, liquid-liquid extraction systems and phase transfer catalysts.[16,18]

The process of crown ether complexation with protonated amines is different than with metal ions because dipole-ion interactions are the only possible attractive forces in the metal ion case.[3,19] However, in the case of [18]-crown-6 polyethers different theories exist on the relative importance of the possible attractive forces between the host ether and guest ammonium ions. All six oxygens of the cyclic ether are interior to provide dipole to ion forces and hydrogen bonding as well as the interactions between the oxygens and nitrogen that may occur as visualized in Figure 2. By design, secondary interactions can occur between the ring and the various R group substituents of the amine molecule[10] causing even more specificity of interaction. These interactions can be steric, electrostatic, hydrophobic and/or charge transfer in nature.

The focus of this work is enantiomeric differentiation via chiral crown ether complexation. Resolution of enantiomers (non-superimposible, mirror-image isomers) can be achieved by an HPLC column with a chiral crown ether as the stationary phase . The initial work in this area was carried out by Cram and coworkers.[20-23] Figure 3 shows shows one of chiral crown ethers synthesized by Cram *et. al.* and used to separate enantiomeric primary amines[20]. The separation mechanism is attributed to the formation of a diastereomeric inclusion complex where the interactions are different for each enantiomer.[24] The host crown ether was thought to complex more tightly with one antipode than the other.

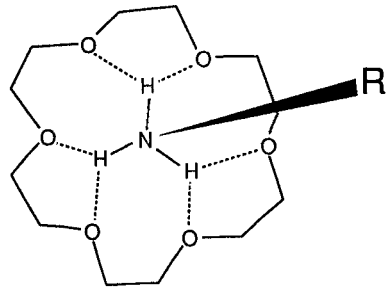


Figure 2: The interaction of an alkylamine with a crown ether. The size, charge and orientation of the alkyl group affects the interaction.

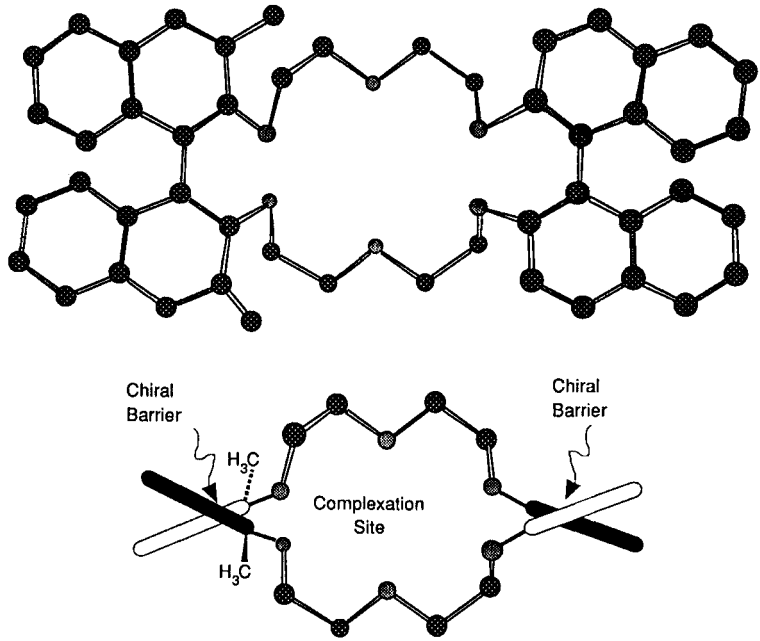


Figure 3: An example of a chiral crown ether as redrawn from reference 20. The chirality of the crown ether is due to the staggered position of the naphthyl rings.

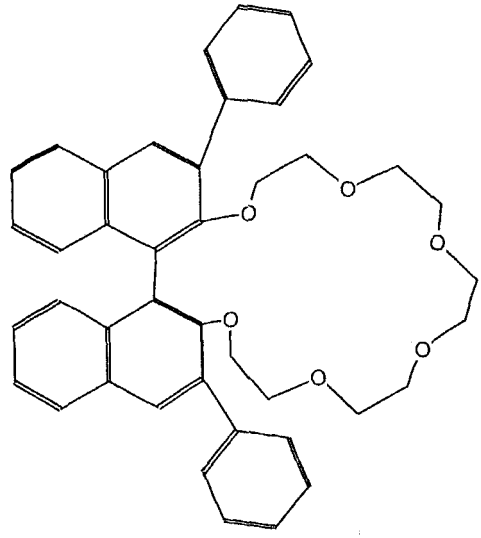


Figure 4: The crown ether in the columns used in the separations of this work as redrawn from references 25, 26 and 27.

Both attractive (binding) interactions and repulsive (steric) interactions contribute to the overall stability of the complex.[18]

Figure 4 shows the structure of the chiral crown ether used in this study.[25-27] This compound was first reported by Shinbo *et. al.* [27] who used it as part of a dynamically coated stationary phase. Most of the separations reported by Shinbo *et. al.*[27] were performed using the R form of the crown ether. This resulted in the L-enantiomer of the amino acids eluting first. When the S form was the coated stationary phase the D-antipode eluted first.[27]

EXPERIMENTAL

The high performance liquid chromatography was performed with a Waters (Milford, MA) model 590 solvent delivery module, a Shimadzu

(Kyoto, Japan) SPD-2AM ultraviolet spectrometric detector and a Shimadzu C-R6A chromatopac recorder. With the UV detectable separations, additional conformation of enantiomeric separation was made by shooting the compound again and varying the wavelength. If the compound was not detectable by UV a Waters series R401 differential refractometer was used for detection. The circular dicroism spectra were run on a Jasco J-600 spectropolarimeter (Tokyo, Japan) when there were any questions concerning the validity of an enantiomeric separation.

The mobile phase was a 0.01 *M* perchloric acid solution (pH =2) in HPLC grade water with zero to five percent methanol as a modifier. Specific conditions are given in Table 1. Solvents and chiral analytes were obtained from the following companies Aldrich (St. Louis, MO), Sigma (St. Louis, MO), Fisher (Pittsburgh, PA) and Fluka (Ronkonkoma, NY).

Flow rate varied from 0.1 to 1.5 milliliter per minute depending on the separation. The temperature variation was from 5 ^{o}C to 25 ^{o}C. When a difference in selectivity was seen with a temperature change, decreasing the temperature increased both the retention time and the enantioselectivity. The column was a Crownpak CR(+) supplied by Dr. Michael Henry of J.T. Baker Inc., (Phillipsburg, NJ).

RESULTS AND DISCUSSION

Figure 5 shows a chromatogram of typical separations obtained with the crown ether column. In order to assure protonation of the amine group, the solute must be dissolved in and separated with an acidic aqueous mobile phase. One disadvantage of this method is the possible solubility problem for very hydrophobic amines. One advantage of the perchloric acid mobile phase is that there is very little background when using UV detection. Stable baselines are easily obtained at 200 nm.

Table I SEPARATION DATA FOR RACEMATES ON THE CHIRAL CROWN ETHER COLUMN

COMPOUND	K_1'	K_2'	α	R_S	Moble Phase
DL-alanine	0.38	0.7	1.86	3.17	*
	0.24	0.48	2.02	1.44	+
DL-alanine benzyl ester	25.2	55.1	2.2	9.8	C
DL-alanylglycylglycine	0.5	0.6	1.3	2.1	A
DL-β-aminoadipic acid R.I. dectection	0.4	0.5	1.25	0.5	A
(±)-(1-aminoethyl)-4-hydroxybenzyl alcohol	2.8	3.9	1.4	4.2	C
DL-aminoglutethimide	0.1	0.2	2.0	0.8	B
DL-aminoisobutyric acid R.I. dectection	0.4	0.5	1.25	0.4	A
p-amino-DL-phenylalanine	7.0	9.6	1.3	1.5	A
DL-2-amino-2-thiopheneacetic acid	0.7	2.7	3.8	3.0	B
DL-arginine	0.65	1.43	2.2	5.2	•
DL-asparagine	0.53	0.90	1.69	3.15	* *
DL-aspartic acid	0.61	1.23	2.01	4.07	* *
	0.32	0.70	2.18	1.77	++

DL-aspartic acid methyl ester	3.5	4.2	1.2	1.0	C
DL-7-azatryptophan	2.3	2.6	1.1	1.0	C
DL-p-chloroamphetamine	32.3	35.5	1.1	0.9	A
DL-chlorophenylalanine	7.8	10.5	1.3	1.6	B
DL-p-chlorophenylalanine methyl ester	37	44.3	1.2	1.8	C
DL-4-chlorophenylalanine ethylester.HCl	33.0	44.0	1.3	2.8	B
DL-citrulline	0.43	0.94	2.18	3.97	•
R,S-cyclohexylethyl amine R.I. dect.	3.1	3.9	1.3	0.8	A
DL-cysteine	0.44	0.74	1.67	3.31	*
	0.24	0.43	1.81	0.88	+
R,S-1,3-dimethylbutylamine R.I. dect.	2.1	2.5	1.2	0.4	A
DL-dopa	2.88	3.67	1.28	2.47	*
DL-ethionine	2.43	4.68	1.93	6.03	*
p-fluoro-DL-phenylalanine	3.3	4.4	1.3	3.0	B
DL-fluorophenylglycine	1.2	3.9	3.3	3.7	B
o-fluoro-DL-tryptophan	45.5	56.2	1.2	2.8	C

(continued)

COMPOUND	K_1'	K_2'	α	R_S	Moble Phase
p-fluoro-DL-tryptophan	23.7	34.4	1.4	3.8	B
m-fluoro-DL-tyrosine	2.3	3.5	1.5	1.4	B
DL-glutamic acid	0.33	0.92	2.81	5.32	•
	0.32	1.18	3.73	4.94	+
DL-glutamine	0.25	0.53	2.13	3.11	•
glycyl-DL-norleucine	6.8	7.2	1.1	0.8	A
DL-histidine	0.90	1.64	1.82	5.28	•
	0.32	0.40	1.27	0.31	++
DL-β-hydroxyphenethylamine	7.3	8.2	1.1	1.4	C
5-hydroxy-DL-tryptophan	10.8	12.9	1.2	2.4	C
DL-isoleucine	1.76	2.79	1.58	4.29	* *
	2.82	3.7	1.31	2.48	++
DL-kynurenine	5.8	10.5	1.8	11.2	C
DL-leucine	1.44	2.39	1.67	3.73	•
	3.11	5.14	1.65	5.73	+
DL-*tert*-leucine	2.06	2.26	1.10	0.7	* *
DL-lysine	1.18	1.50	1.26	2.2	*
	0.51	0.63	1.22	0.61	+

DL-methionine	1.1	2.1	2.0	5.9	•
	1.77	3.65	2.06	6.66	+
DL-methionine methyl ester	3.2	5.9	1.8	4.8	C
(±)-α-methylbenzylamine	1.9	2.8	1.4	1.7	A
β-methyl-DL-phenylalanine	14	19.5	1.4	4.2	C
o-methyl-DL-tyrosine	5.5	7.5	2.0	1.3	B
5-methyl-DL-tryptophan	46	56.2	1.2	3.19	C
6-methyl-DL-tryptophan	52.2	63.0	1.2	6.5	C
7-methyl-DL-tryptophan	10.8	12.9	1.2	2.4	C
DL-3-(2-naphthyl)alanine	47.8	57.8	1.2	2.3	C
p-nitro-DL-phenylalanine	16.1	18.7	1.2	1.0	B
DL-norleucine	1.76	2.91	1.66	3.38	•
DL-norleucine methyl ester	3.2	5.0	1.6	3.8	C
DL-normetanephrine•HCl	6.5	7.1	1.1	0.5	A
DL-norphenylephrine	6.3	7.4	1.2	1.8	B
DL-ornithine	0.65	0.97	1.49	2.82	•
DL-α-phenethylamine	4.4	6.2	1.4	1.7	B

(continued)

COMPOUND	K_1'	K_2'	α	R_S	Moble Phase
o-phospho-DL-tyrosine	2.8	4.0	1.4	0.5	B
DL-phenylalanine	3.88	4.93	1.27	2.80	*
	9.25	11.50	1.24	2.95	+
DL-phenylalanine hydroxamate	8.9	10.5	1.2	1.9	C
DL-phenylalaninamide	4.2	5.6	1.4	3.0	C
DL-phenylalanine methyl ester	13.4	16.2	1.2	2.3	C
DL-phenylglycine	1.06	2.49	2.35	7.14	• •
	2.00	9.17	4.58	13.66	+
DL-serine	0.48	0.85	1.75	3.04	• •
	0.16	0.28	1.71	0.65	++
DL-serine methyl ester	2.3	2.6	1.1	1.0	C
DL-threonine	0.39	1.00	2.58	4.20	* •
	0.22	0.50	2.26	1.67	++
DL-4-thiaisoleucine	0.6	1.0	1.6	1.0	A
DL-tryptophane	18.45	21.94	1.19	2.22	•
DL-tryptophan ethyl ester	16.4	19.8	1.2	3.4	C
DL-tryptophan butyl ester	17.7	20.6	1.2	2.2	C

DL-tryptophan octyl ester	19.5	23.8	1.2	4.2	C
DL-tryptophan methyl ester	12.7	16.3	1.3	2.8	C
DL-tryptophan hydroxamate	13.7	17.3	1.3	2.4	C
DL-tyrosine	2.88	3.67	1.28	2.47	*
	7.11	8.61	1.21	2.64	+
DL-tyrosine methyl ester	8.3	10.8	1.3	2.4	C
DL-threonine methyl ester	8.4	10.9	1.3	3.2	C
3-(2-thiazoyl)-DL-alanine hydrate	0.6	1.2	2	1.1	A
DL-valine	1.09	1.64	1.51	3.47	*
	1.06	1.31	1.24	1.38	++

* Separated and reported by Daicel @ 25° C[25]
** Separated and reported by Daicel @ 0° C [25]
+Published Separations by Shinbo *et. al.* @ 18° C [26]
++ Published Separations by Shinbo *et. al.* @ 2° C [26]
Mobile phase compositions A: 0.01 *M* perchloric acid
B: 0.01 *M* perchloric acid + 1% methanol
C: 0.01 *M* perchloric acid + 5% methanol

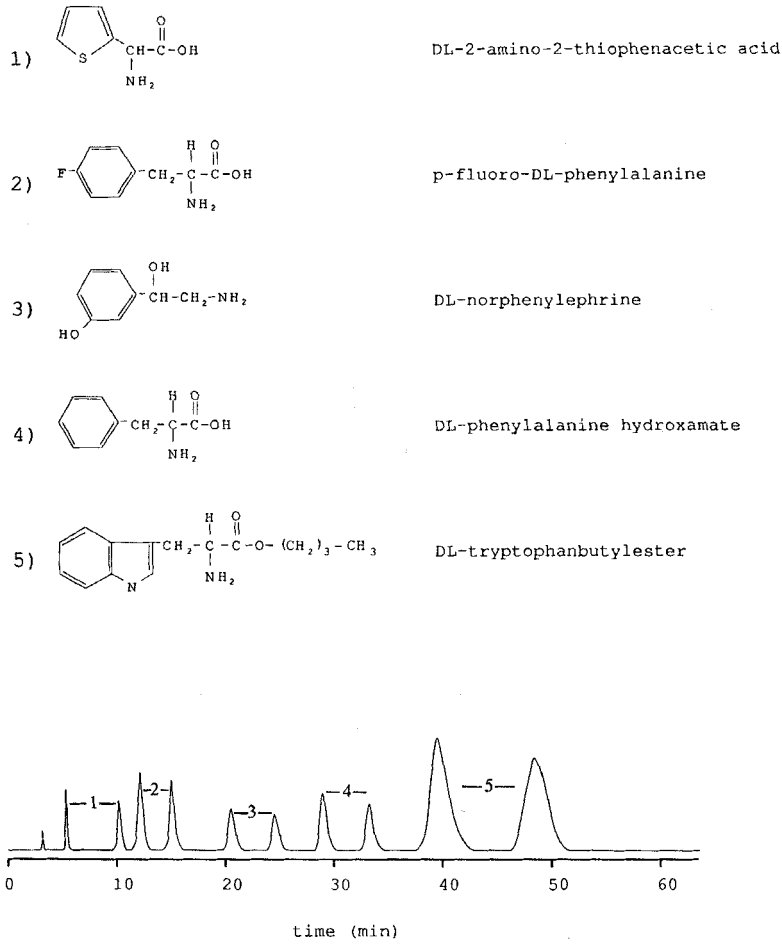


Figure 5: A chromatogram of five representative separations. The results are typical of the separations obtained.

Methanol was evaluated as a mobile phase modifier. The elution of the solutes was faster when some methanol was present in the mobile phase. A representative example of the effect of organic modifier may be seen with 5-hydroxy-DL-tryptophan. Without methanol in the 0.01 *M* perchloric mobile phase the values for k'_d and k'_l are 14.2 and 17.0 respectively. With five percent methanol in the mobile phase the values for k'_d and k'_l are 10.8 and 12.9. The separation factor (α) and resolution (R_s) were essentially the same in each separation. However, if the concentration of organic modifier is higher than 15 percent it can irreversibly damage the stationary phase. As shown in Table 1, over fifty racemates were resolved on the Crownpak column.

To obtain circular dicroism (CD) information, two approaches were used. To obtain a complete CD spectrum the separated enantiomers were collected as they eluted from the column and subsequently placed in the CD spectrophotometer. Another option was to have the CD connected directly to the column via a flow through cell. In this way the analytes were detected as they were eluted. An example of a chromatogram obtained with CD detection is shown in Figure 6.

The chiral crown ether utilized in this study does not contain a stereogenic center but exists as enantiomers because of the staggered position of the naphthyl rings which produce an "axis of chirality". The interaction between the lone pairs of electrons of the crown oxygens and the hydrogens of the protonated amines lead to the formation of an inclusion complex. Size, in addition to the coulombic attraction, is a crucial factor in any inclusion complex[24]. Attempts were made to separate several secondary chiral amines, in which the hydrogen was replaced with a methyl group, with no success.

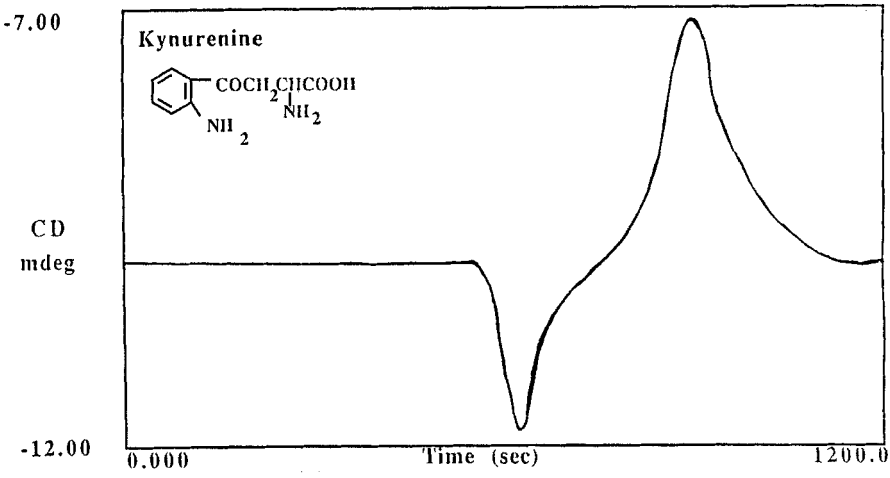
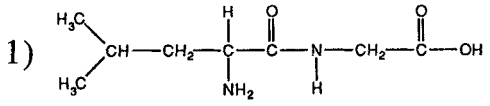
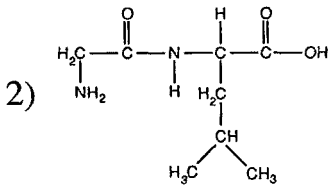


Figure 6: A typical circular dicroism chromatogram as the solute was detected as it was eluted. The approximate symmetry of the opposite peaks further substantiates the enantiomeric separation.

Enantiomeric differentiation is aided by the stereogenic center of the analyte being in close proximity to the protonated amine. For example, under the same conditions glycyl-DL-leucine and DL-leucylglycine gave very different results. At 5 °C, at a flow rate of 0.5 ml/min the separation factor was 1.10 for glycyl-DL-leucine. For DL-leucylglycine the value was 3.80. Figure 7 shows the chromatogram and structures of the two compounds. The difference is attributable to the location of the primary amine functional group relative to the stereogenic center. In the DL-leucylglycine it is adjacent to the stereogenic center. Although a partial separation was obtained with the glycyl-DL-leucine the amine group is four atoms away from the stereogenic center.





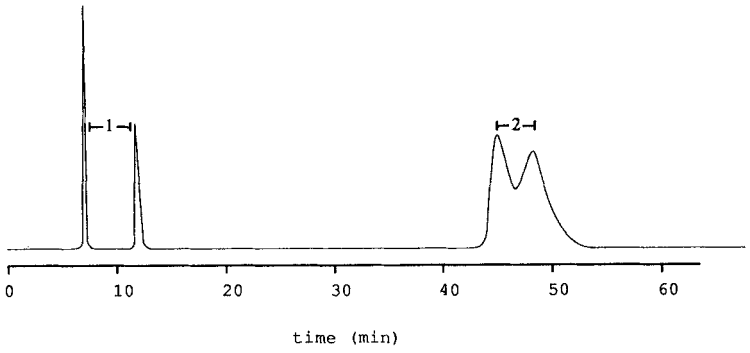


Figure 7: Under the same conditions glycyl-DL-leucine and DL-leucylglycine gave very differenf results. (5 °C, flow rate 0.4 ml/min, mobile phase 0.01 *M* perchloric acid)

Although a primary amine is the only functional group requirement of the analyte, the results indicate there are other factors that enhance enantiomeric resolution. For instance, analytes with aromatic groups or bulky substituents possessing any double bonds appeared to have greater separation factors. Some of the lowest resolutions were obtained for compounds that do not possess aromatic groups. Examples include DL-β-aminoadipic acid, DL-aminoisobutyric acid, R,S-cyclohexylethylamine,

R,S-1,3-dimethyl-butylamine, and DL-serine. Two examples in this catagory of compounds not listed in Table 1that were attempted but not separated include R,S-2-amino-1-propanol and R,S-3-aminoheptane. For compounds without aromatic moities, the presence of more than one primary amine functional group often enhanced the separation. Examples include DL-arginine, DL-asparagine and (±)-citruline. Additionally, the presence of an acid functionality versus an ester functionality enhanced resolution in both aromatic containing compounds and non-aromatic containing compounds. An example of this trend can be seen with DL-aspartic acid verses DL-aspartic acid methyl ester. Although DL-aspartic acid was less retained the resolution was better. The increased hydrophobicity of the ester resulted in an expected increase in retention behavior. However, as previously noted, this increased retention did not necessarily produce a better separation. If a compound did not have an aromatic group, two primary amines or two acid functionalities yet possess a sulfur containing moity, chiral separation with good resolution was still achieved as in DL-cysteine and DL-methionine.

Whenever possible, the elution order was determined by comparison against the pure enantiomer. The elution order of the amino acids using the CR (+) column was consistent with results reported by Daicel with the D-enantiomer eluting before the L. (Table 1) According to the work of Shinbo *et. al.*[27] the S enantiomer of the crown ether was the antipode utilized as the chiral selective stationary phase. If the R form would have been the stationary phase, the L-enantiomer would have eluted first.

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