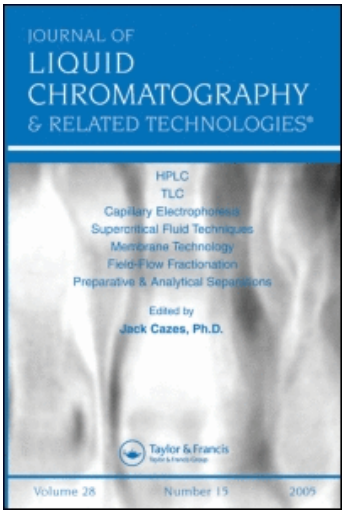

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC ENANTIOMERIC SEPARATION OF AROMATIC AMINES USING CROWN ETHER TETRACARBOXYLIC ACID

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Online publication date: 29 December 2009

Journal of Liquid Chromatography & Related Technologies, 33:153–166, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903439119

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HPLC ENANTIOMERIC SEPARATION OF AROMATIC AMINES USING CROWN ETHER TETRACARBOXYLIC ACID

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□ *Separation of an aromatic amine is presented using Chirosil RCA(+), which consists of a chemically bonded crown ether tetracarboxylic acid stationary phase. To separate this amine, we used as mobile phase aqueous-organic mobile phases consisting of 0.1% $HClO_4$ in water and acetonitrile or methanol. Plots of ln k′ vs. the volume fraction of the organic modifier showed a non-linear behavior which prompted us to investigate non-chromatographic techniques. In particular, we pursued vibrational circular dichroism in order to elucidate the interactions between the chiral amine and the crown ether tetracarboxylic acid. The enantiomeric separation of several other aromatic amino alcohols molecules was also performed. The relationship between the retention, enantioselectivity and the structure of the molecules is discussed.*

Keywords aromatic amine, chiral separation, crown ether tetracarboxylic acid, vibrational circular dichroism (VCD)

INTRODUCTION

Advances in asymmetric synthesis in the pharmaceutical industry drove the demands for the determination of optical purity by chromatographic enantiomeric separation. Due to the very polar nature of the amino groups, chromatographic separation of chiral amines is a very difficult task. It can be often performed through the chemical modification of the amino groups in order to achieve enantiomeric separation. Introduction of crown ether stationary phases for chromatographic separation of chiral amines represented a quantitative and qualitative leap in the field of enantiomeric separation of these compounds. They are cyclic compounds with repeating units of (-X-C_2H_4-), where X can be a heteroatom. The heteroatom has a

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pair of electrons, which can be involved in either electrostatic interaction with an inorganic cation or in hydrogen bonding with ammonium ions. Crown ethers which contain a molecular asymmetry, can be successfully utilized for the separation of chiral amines.

Cram was the first to use chiral crown ethers for the separation of amino acids and chiral primary amines.[1] His work demonstrated that the interaction is primarily based on inclusion and hydrogen bonding between the hydrogen of the ammonium group and the crown ether's oxygen lone-pair electrons. Cram also investigated structural features which enhance the ability of the host molecule to bind alkyl ammonium compounds. He discovered that the introduction of bulky groups such as binaphtyl groups on to the exterior of the crown ether could provide additional interaction and produce the separation with protonated amines. Later, Shimbo used a variant of Cram's crown ethers. The chiral phase was coated on a reversed phase stationary phase[2,3] and separated a variety of amino acids, chiral primary amines, amino alcohols, etc.[4,5]

A different type of crown ether stationary phase is the one derived from 18-crown-6 tetracarboxylic acid, covalently immobilized on silica gel via the reaction between 18-crown-6 tetracarboxylic acid and amino propyl silica gel. This crown ether is also able to resolve a large class of compounds containing primary and secondary amines.[4,6–12]

Choi et al. studied the influence of temperature on the separation of amino alcohols. The results showed that the interaction with the crown ether tetracarboxylic acid of the compounds which had the secondary amine linked directly to the stereogenic center was enthalpy driven, while the compound which had the hydroxy groups linked to the stereogenic center was entropically driven.[7]

Nagata et al. investigated the conformational changes of crown ether tetracarboxylic acid upon interacting with various amines and amino acids.[13] The authors showed that upon interaction with the enantiomeric analytes, crown ether undergoes guest dependent conformational changes.

In the present paper we report on the enantiomeric separation of a chiral aromatic amine, using a crown ether tetracarboxylic acid stationary phase. We investigated the influence of organic modifiers on the retention and enantioselectivity of this amine. Vibrational circular dichroism (VCD) was applied in order to assess the behavior of crown ether as well as the intimate interaction between the chiral stationary phase and the enantiomeric analytes. To our knowledge, this is the first report on the study of the influence of the type and the concentration of organic modifiers on the enantioselective interactions using VCD.

EXPERIMENTAL

Solvents and Reagents

Methanol, water, and acetonitrile were HPLC grade and were purchased from EMD Chemicals (Gibbstown, NJ, USA). Perchloric acid was purchased from Sigma Aldrich (St. Louis, MO, USA).

HPLC Columns and Chromatographic Conditions

The chiral HPLC column used in this study included Chirosil RCA (+) was purchased from Regis Technologies, Inc. (Morton Grove, IL, USA). The chromatographic conditions used to elute the enantiomers on the crown ether columns consisted of a mixture of water containing 0.1% perchloric acid and acetonitrile or methanol. According to the conditions of the experiments, the amount of acetonitrile or methanol in the mobile phase was varied.

Reagents

Among the reagents used in the study, (S)-1-(4-bromophenyl)-ethylamine was purchased from BASF (Florham Park, NJ, USA), and (R)-1-(4-bromophenyl)-ethylamine was purchased from Fluka (St. Louis, MO, USA).

All other reagents used in the study, (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, 2-amino-1-phenylethanol (S)-2-amino-1-phenylethanol (R)-2-phenylglycinol (S)-2-phenylglycinol (R)-cis-amino-indanol (−)-cis-amino-indanol (+)-naphtyl ethyl amine (R)-naphtyl ethyl amine (S)-(R)-(+)-2-amino-3-phenyl-1-propanol, (S)-(−)-2-amino-3-phenyl-1-propanol, were purchased from Sigma Aldrich (St. Louis, MO, USA).

Sample Preparation

The sample solutions of all the analytes used in this study were made by dissolving each analyte in methanol or acetonitrile at a concentration of 0.1 mg/ml. One or two microliters of each solution were injected into the HPLC system. Each sample was injected three times and the average results were calculated.

Instrumentation

Chromatographic studies were performed on an Agilent 1200 rapid resolution HPLC instrument (Agilent, Palo Alto, CA, USA) equipped with

a vacuum degasser, a binary pump, an autosampler, a thermostated-column compartment, and a photo diode array detector. Chromatographic data were acquired with Agilent ChemStation Software.

Experimental VCD

The VCD spectra of the R enantiomer of 1-(4-bromophenyl)-ethylamine were measured in a DMSO-d_6 solution at a concentration of 40 mg/ml in a 200 μm liquid IR cell. The spectra were collected for 4 hours with 4 cm^{-1} resolution on a Chiral*IR* VCD spectrometer (BioTools, Jupiter, FL, USA) equipped with a dual-PEM bench. The final VCD spectra were corrected by subtracting the solvent (DMSO-d_6) spectra measured at the same conditions.

For measurements of the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, an ~100 mg/ml sample was dissolved in a water/acetonitrile or water/methanol solution at different concentrations of the organic modifier. The VCD spectra were collected using 6-μm biocell windows (BioTools) for 12 hours with an 8 cm^{-1} resolution. The dry film of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was prepared by adding an aliquot of 200 μL of a solution of crown ether (~10 mg/ml in methanol) on a 32 mm diameter BaF_2 window. The window was subsequently dried at 25°C in vacuum oven operating overnight. The VCD spectra of the film were measured using the same conditions used for the solution. The complexes were made by mixing (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid with each enantiomer of 1-(4-bromophenyl)-ethylamine at a 1:1 molar ratio in a deuterated dimethylsulfoxide (DMSO-d_6) solution. The spectra were collected with a resolution of 4 cm^{-1} during 8 hours in a 100 μm BaF_2 cell. The final spectra were corrected by subtracting the corresponding solvent's spectra.

Calculated VCD and Molecular Modeling

The structure of the R-enantiomer of 1-(4-bromophenyl)-ethylamine was built in HyperChem (Hypercube, Gainesville, FL, USA) and subsequently used for a conformer search at the molecular mechanic level to obtain all the conformers with the lowest energies. Two conformers were found to be the lowest energy conformers, within an energy range of 5 kcal/mol. The two conformers were further used for geometries optimization and vibrational frequencies calculation (IR and VCD spectra) using Gaussian 03 (Gaussian, Wallingford, CT, USA) at the density functional heory (DFT) level with a B3LYP functional and 6–31G(d) basis set. The final calculated spectra were composed by averaging the spectra of each conformer based on a Boltzmann distribution of corresponding energies.

The model and conformer search of the complexes of crown ether tetracarboxylic acid with each enantiomer were performed using HyperChem and a molecular mechanics force field, MM+ method. We choose the conformers with the lowest energies for DFT calculation using Gaussian with B2LYP/6-31G (d) basis set.

RESULTS AND DISCUSSION

The structures of the analytes, 1-(4-bromophenyl)-ethylamine and the stationary phase (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid are presented in Fig. 1. Enantiomeric separation was performed on a commercially available HPLC column made by chemically bonded (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid on silica gel, namely Chirosil RCA(+). Baseline separation of the two enantiomers was achieved at various concentrations of acetonitrile or methanol in water containing 0.1% $HClO_4$. A typical chromatogram of the enantiomeric separation is presented in Fig. 2.

Determination of the Absolute Configuration

In order to assess the order of elution during the chromatographic separation, the first step was to assess the absolute configuration of each enantiomer of this compound. We applied experimental and theoretical VCD calculations to determine the absolute configuration of the two enantiomers. The details about the measurements and calculations were described in the experimental section. A good agreement was obtained between the experimental VCD and the calculated VCD, as shown in Fig. 3. Based on the calculation the absolute configuration of the compound in Fig. 3 is R.

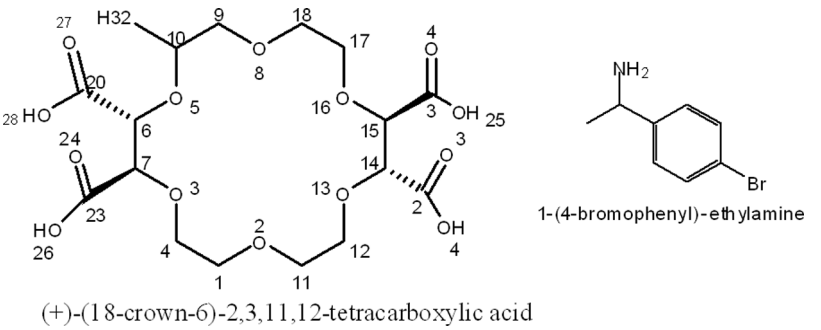


FIGURE 1 Structures of 1-(4-bromophenyl)-ethylamine and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid.

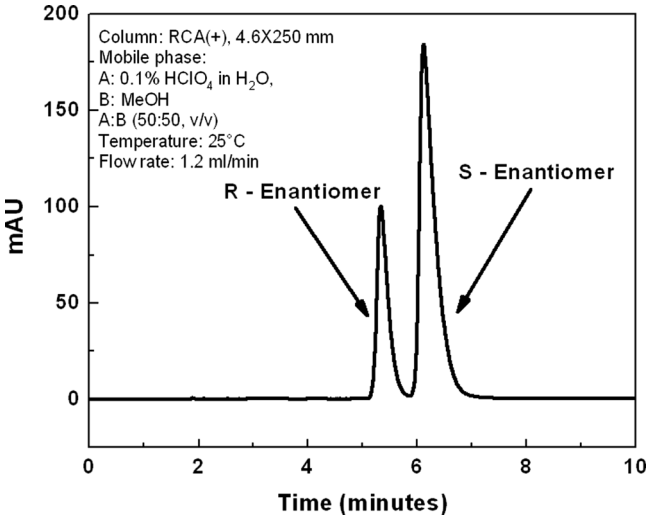


FIGURE 2 Chromatogram of the separation of 1-(4-bromophenyl)-ethylamine on Chirosil RCA(+) column: details of the separation were listed in the figure.

Influence of Mobile Phase Composition on the Separation Parameters

To study the influence of organic modifiers on the chromatographic parameters, k′ and α, solutions of acetonitrile and methanol in water solutions containing 0.1% perchloric acid were prepared. The concentration of

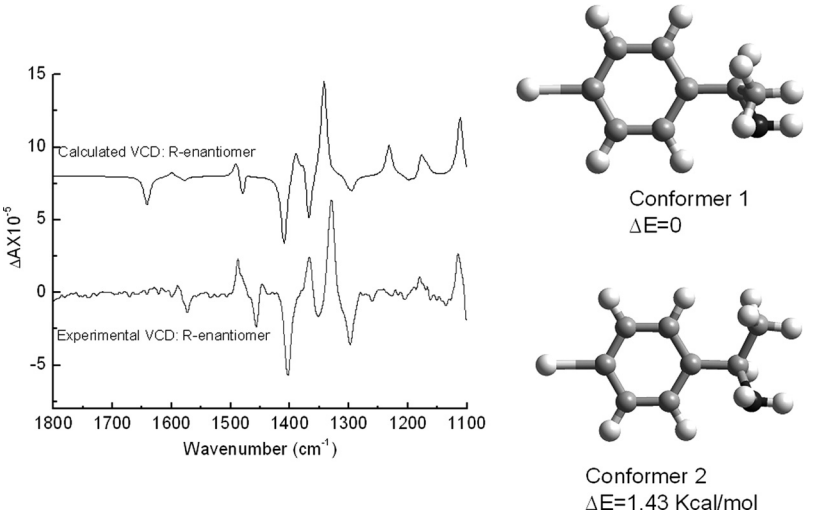


FIGURE 3 Comparison of experimental and calculated VCD spectra, and the optimized geometry of the two conformers of R enantiomer of 1-(4-bromophenyl)-ethylamine.

the two organic modifiers was varied between 0.01 and 0.8 volume fraction. The results are presented in Fig. 4a and b. Both graphs show a non-linear behavior of ln k′ vs. volume fractions of the two organic modifiers. While the behavior of the retention factor showed a constant decrease in values with the increase of acetonitrile concentration, the behavior of the retention factor vs. the volume fraction of methanol showed an almost constant behavior until a volume fraction of 0.5, increasing slightly beyond this value.

The influence of the organic modifiers on the selectivity, α, showed an increase in values with the increased concentration of acetonitrile. With methanol, the selectivity (α) showed almost no change with the increase in the volume fraction of this modifier (Fig. 5a and b).

In order to ascertain the reason for such behavior we pursued a VCD study on the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid film in the presence of solvent mixtures resembling the mobile phase used in the chromatographic experiments containing acetonitrile or methanol.

In the spectral region corresponding to C−O−C vibrations coupled with CH_2 deformation vibrations (Fig. 6a), one can observe significant changes comparing the dry film with the solution in the solvent mixtures resembling the mobile phases used in the chromatographic studies. Thus, in the dry film, the region corresponding to the C−O−C vibrations coupled with CH_2 deformation vibrations show two negative maxima, at 1231 cm^{-1} and 1205 cm^{-1}. The positive maxima at 1157 cm^{-1} with a shoulder at 1125 cm^{-1} corresponds to C−O−C antisymmetric stretching. Such band splitting is an indication of the existence of dissimilar C−O−C groups.

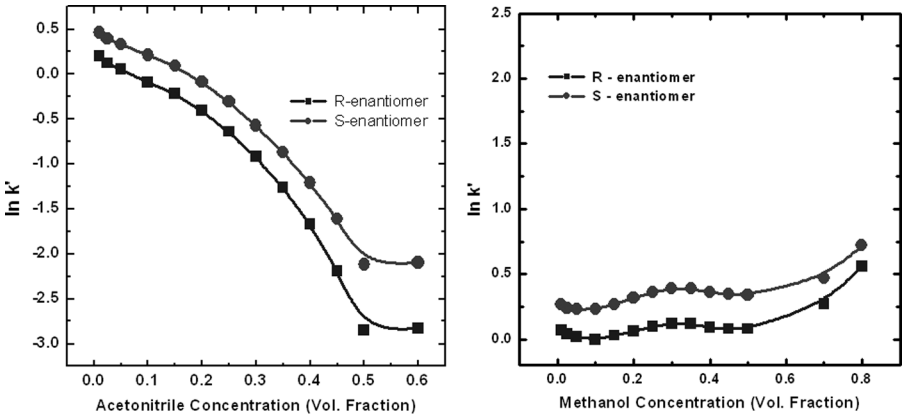


FIGURE 4 Influence of the organic modifier concentration on retention factor (k′) of the two enantiomers of 1-(4-bromophenyl)-ethylamine: (a) Acetonitrile (ACN); (b) Methanol.

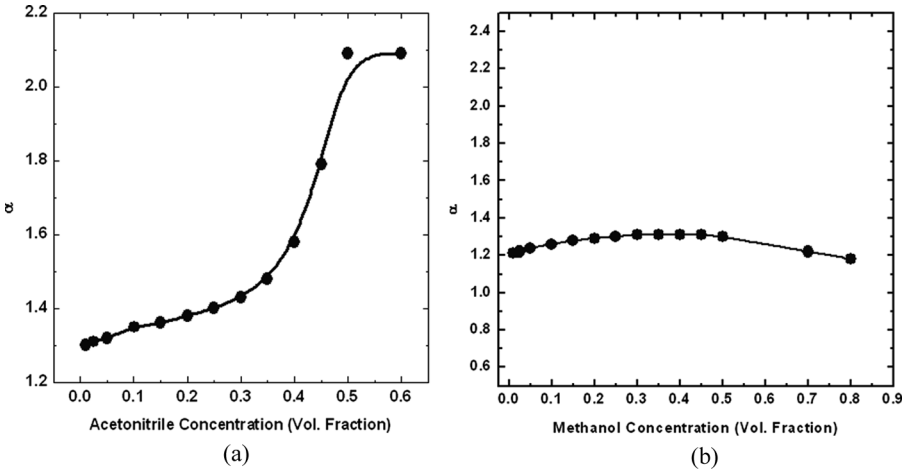


FIGURE 5 Influence of the organic modifier concentration on enantioselectivity factor (α) of 1-(4-bromophenyl)-ethylamine: (a) Acetonitrile; (b) Methanol.

With the addition of methanol in the solution, the maxima at 1205 cm^{-1} merges into the one at 1231 cm^{-1} with a band sharpening. There are no significant differences in the intensity of the bands at 1231 cm^{-1}, 1157 cm^{-1} and 1125 cm^{-1} with the addition of methanol into the solvent mixtures (Fig. 6a). With increasing the concentration of methanol in the solvent mixture, the region of the spectra corresponding to the C=O of the carboxyl functional group shows more changes compared to previous regions of the spectra (Fig. 6b). Thus, the maximum at 1753 cm^{-1} observed in the dry film presents a red shift towards a lower wave number upon increasing

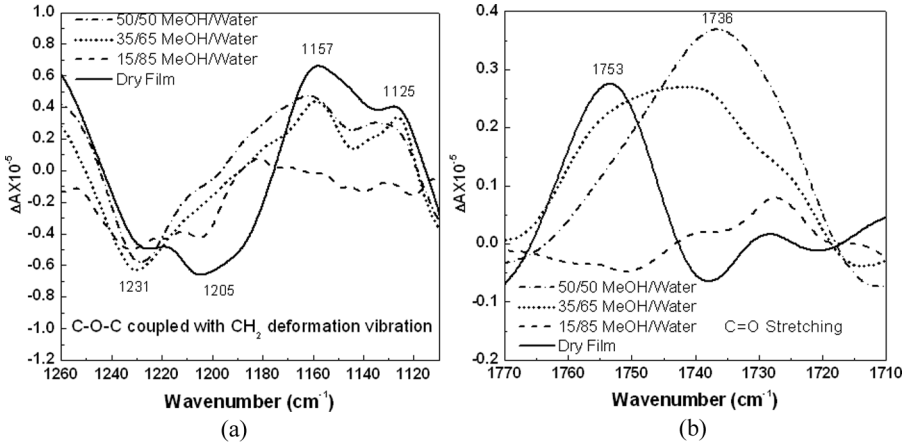


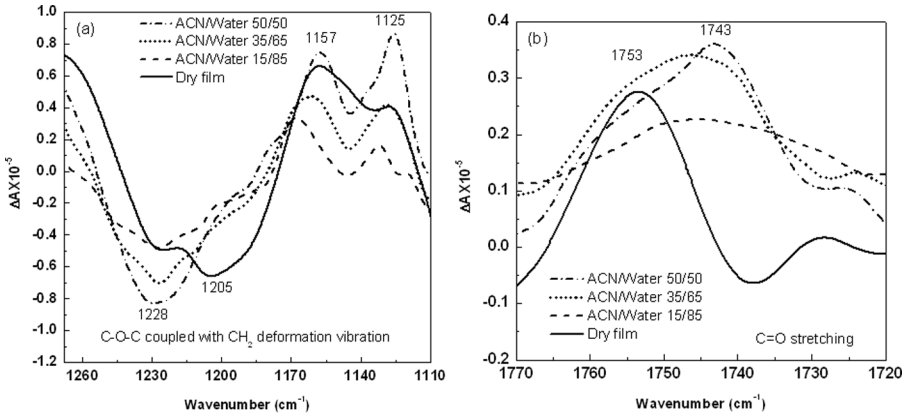
FIGURE 6 Influence of the methanol concentration on the VCD spectra of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid: (a) C–O–C region; (b) C=O region.

the methanol concentration. Such a shift is indicative of possible hydrogen bonded dimerized carboxyl, as well as interactions between methanol and the crown ether tetracarboxylic acid. These interactions occurred at the expense of the analyte interaction with crown ether tetracarboxylic acid, leading to no change in the chromatographic enantioselectivity.

Similar to methanol, the negative bands of dry film located at 1228 cm^{-1} and 1205 cm^{-1} also merged into a single band centered at 1228 cm^{-1} in the presence of acetonitrile (Fig. 7a). Their intensity, however, increases with the increase in acetonitrile concentration. At the same time the positive bands at 1157 cm^{-1} (which show a shoulder at 1125 cm^{-1} in the dry film), split into two distinct positive peaks upon increasing the concentration of acetonitrile. The intensity of the peaks increases with the increased concentration of acetonitrile. Such peak sharpening, along with increased intensity, indicates an enhanced molecular chirality of the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid upon increasing the acetonitrile concentration. The enhanced chirality is consistent with a change in conformation of the stationary phase.

A similar band shift for the C=O functional group was observed with acetonitrile as in the case of methanol (Fig. 7b). Comparing the two band shifts in this region (for methanol and acetonitrile), one can observe that the band shift is larger in methanol compared to acetonitrile (17 vs. 10 wave number, respectively). The larger shift in methanol can be attributed to the concerting interaction of the dimerized carboxyls, along with the H-bond interaction between the carboxylic groups of crown ether and methanol.

Such behavior correlates well with the chromatographic enantioselectivity for the two enantiomers. Thus, increasing acetonitrile concentration



(a) (b)

FIGURE 7 Influence of the acetonitrile concentration on the VCD spectra of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid: (a) C–O–C region; (b) C=O region.

prompts, a conformational change of crown ether, with enhanced intensities manifested especially in the C−O−C region of the VCD spectra. Such an increase in intensity is consistent with enhanced chirality of the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. This is manifested chromatographically by an increase in the enantioselectivity upon increasing the concentration of acetonitrile in the mobile phase (Fig. 5a). At the same time, the increase in the concentration of acetonitrile lowers the dielectric constant of the media; as a consequence it produces an enhanced strength of H-bonding between the enantiomeric analytes and the enantioselective sites of crown ether. In the case of methanol, due to the overwhelming interactions of the methanol with stationary phase, the interaction of the enantiomers is less affected by the organic modifier. As a consequence, there is no change in the enantioselectivity (Fig. 5b).

Investigation of Enantiomeric Recognition Using VCD Spectroscopy

To ascertain the enantiomeric recognition, we again pursued VCD spectroscopy. Chromatographic experiments showed that using DMSO-d_6 as an organic modifier produced the same order of elution as in the case of methanol or acetonitrile. Therefore we pursued the VCD experiments in DMSO solutions. Thus, we used (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, along with each individual enantiomer of 1-(4-bromophenyl)-ethylamine dissolved in a DMSO solution. The VCD spectra were presented in Fig. 8. The red shift observed in the spectra indicates a stronger interaction in one complex relative to the other.

In the region of NH_3^+ deformation vibration (Fig. 8), the maximum corresponding to the complex of the S enantiomer with the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid shows a red shift relative to the R enantiomer (1520 cm^{-1} and 1528 cm^{-1}, respectively). Further examination of the spectra shows that the region in the spectra corresponding to the COOH shows two maxima at 1731 cm^{-1} and 1669 cm^{-1}. The maxima at 1731 cm^{-1} corresponds to the free carboxylic functional group of the crown ether, while the maxima at 1669 cm^{-1} corresponds to the interactive COOH either in the form of an inter or intramolecular dimer or interacting with the enantiomeric analytes. The complex corresponding to the interactive COOH at 1669 cm^{-1} presents a red shift for the S-complex relative to the R complex (1669 cm^{-1} and 1677 cm^{-1}, respectively). The C−O−C region of the spectra, centered at ~1226 cm^{-1}, (corresponding to the S complex) shows a slight red shift relative to R complex (1226 cm^{-1} versus 1233 cm^{-1}). This indicates a stronger complex between the S-enantiomer and the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, relative to the R enantiomer. Such results

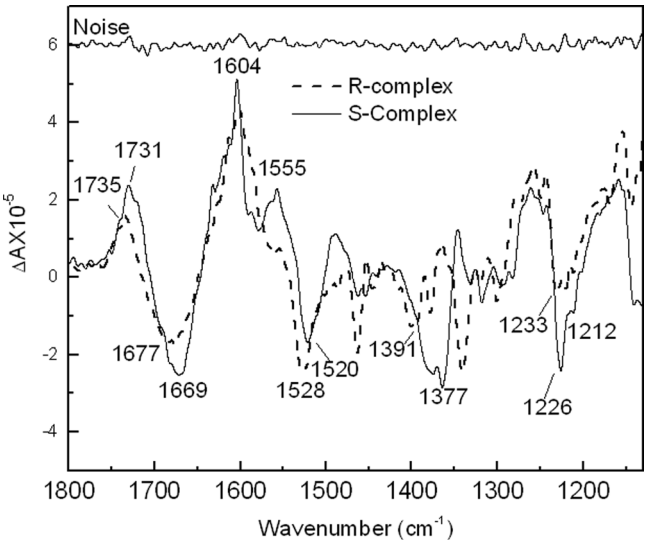


FIGURE 8 VCD spectra of the complexes of the two enantiomers of 1-(4-bromophenyl)-ethylamine with (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid in DMSO solution.

are very suggestive, indicating H-bond interactions between the crown ether's carbonyl group with the NH_3^+ group of each enantiomer. The VCD spectra also indicated a red shift of the complex of the S enantiomer relative to the R in the CH bending region (1377 cm^{-1} versus 1391 cm^{-1}), and C=C quadrant stretching of the phenyl ring, corresponding to the 1-(4-bromophenyl)-ethylamine (1604 cm^{-1} and 1555 cm^{-1}). Such a shift indicates a CH – π interaction between the stationary phase and the two enantiomers.[14]

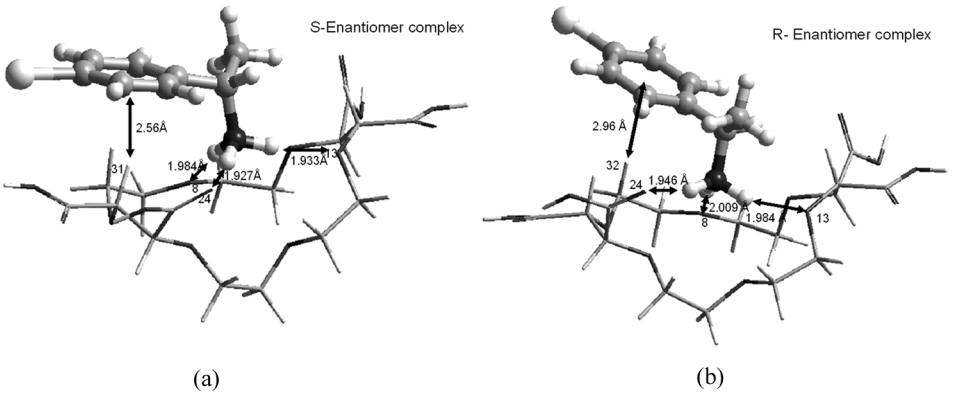


FIGURE 9 Structures of the complexes of the two enantiomer of 1-(4-bromophenyl)-ethylamine with (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid: (a) S-enantiomer complex; (b) R-enantiomer complex.

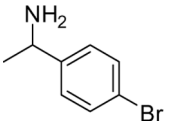
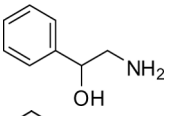
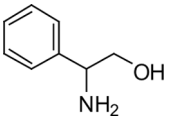
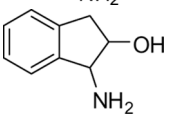
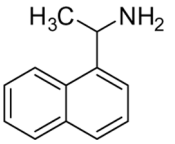
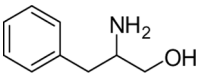
The DFT calculation shows agreement with the VCD experiments. In particular, the complex with the S enantiomer exhibits shorter H-bonding distances between the NH_3^+ group and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid, relative to the R complex (Fig. 9 a and 9b). At the same time the CH-π distances for the S complex also showed shorter distances relative to the R complex (Fig. 9a and b).

Influence of Structural Variation on the Chromatographic Parameters

To further explore the enantiomeric interactions with crown ether, several enantiomeric amine analytes with different structural features were studied. The results are presented in Table 1.

The separation factor and the retention are the highest in the case of 2-amino-1-phenylethanol, where the amino group is located at the end of the alkyl side chain. This is followed by the separation factor of

TABLE 1 Structure, Retention Factor and Enantioselectivity Factor of Chiral Amine Molecules by using Chiralsil RCA(+) Column and 5% ACN as Mobile Phase

Compound		k_1'	k_2'	α
l-(4-Bromophenyl)-ethylamine		4.73	5.33	1.27
2-Amino-l-Phenylethanol		6.78	8.68	1.28
2- Phenylglycinol		2.62	3.06	1.17
l-Amino-2-Indanol		2.04	2.04	1.00
Naphtyl Ethyl Amine		11.34	12.59	1.11
2-Amino-3-Phenyl-l-Propanol		2.40	2.48	1.03

1-(4-bromophenyl)-ethylamine, where the amino group is located at the stereogenic center, in the α position to the aromatic group. As the amino group moves closer to the phenyl ring, the separation and the retention time of the two enantiomers decreases. For 1-amino-2-indanol, the amino group is linked to a five member ring. There is no separation of the two enantiomers, indicating that the rest of the molecule produces steric hindrance, which is a factor affecting the enantiomeric separation. For naphtyl ethyl-amine, the retention time for the two enantiomers on the column is the highest; however, the selectivity factor is smaller than that of 2-amino-1-phenylethanol. This indicates that for the amino alcohols 2-phenylglycinol and 2-amino-1-phenylethanol the –OH group is also involved in the enantioselectivity. As the side chain increases, and since the amino group is located in the middle of the side chain, the selectivity and the retention decrease.

CONCLUSIONS

This is the first report of a VCD study to elucidate the intimate interactions between the enantiomers of a chiral amine and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. The experiments indicated that crown ether undergoes a conformational change induced by the solvent composition. DFT Calculations confirmed the experimental results obtained with the VCD spectroscopy.

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