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Thermodynamic study of *N*-trifluoroacetyl-*O*-alkyl nipecotic acid ester enantiomers on diluted permethylated β-cyclodextrin stationary phase

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Abstract

Thermodynamic studies were performed on 12 pairs of *N*-trifluoroacetyl-*O*-alkyl nipecotic acid ester enantiomers on diluted permethylated β-cyclodextrin stationary phase (CP Chirasil-Dex CB). The influence of ester alkyl group structure on interaction with permethylated β-cyclodextrin (Me-CD) and enantioselectivity was studied. The types of alkyl groups studied included *n*-alkyl (C_1–C_5) and groups containing branching at differing locations relative to the chiral center of the molecule. The results show that for a given molecular weight, the *n*-alkyl esters have stronger interactions with Me-CD than esters containing branched alkyl groups. However, although having weaker interactions with Me-CD, esters containing α-branched alkyl groups exhibit higher enantioselectivity than the corresponding *n*-alkyl or β-branched isobutyl esters. From the retention data, thermodynamic parameters were estimated using the retention increment method and enthalpy–entropy compensation plots ($\ln R'$ versus ΔH) were constructed. The results suggest that ester enantiomers with branching at the α-carbon of the ester alkyl group have additional and/or different types of enantioselective interactions with Me-CD than the C_1–C_5 *n*-alkyl esters or β-branched isobutyl ester. In order to obtain a qualitative sense of the interaction with Me-CD, structures of the diastereomeric complexes formed between Me-CD and some of the ester enantiomers were modeled using simulated annealing molecular dynamics.

Keywords: Nipecotic acid; Permethylated β-cyclodextrin; Gas chromatography; Thermodynamic parameters; Retention increment method

1. Introduction

Derivatized cyclodextrin phases are the most frequently used chiral stationary phases (CSP) for the direct separation of volatile enantiomers by gas chromatography (GC) [1,2]. They are prepared by substituting the hydroxyl groups at the 2, 3, and 6 positions of each glucose unit contained in native α, β, or γ cyclodextrin with different groups [3]. Through the appropriate choice of groups or combinations of groups, it is possible to prepare stationary phases that provide chiral recognition for a wide range of analytes containing different types of functional groups. This can be used along with other factors such as the size of the cyclodextrin, the polarity of the achiral solvent (i.e. the phase that the cyclodextrin is dissolved in) [4] or the concentration of the cyclodextrin to maximize enantioselectivity. Since a large number of theoretical plates are obtainable using capillary gas chromatography, it is often possible to obtain adequate separation of enantiomers having α values as low as 1.02.

Permethylated β-cyclodextrin (heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin) (Me-CD), the chiral selector used in these studies, was first employed in capillary columns for high resolution separations in the late 1980s [5]. Me-CD has a high melting point and, unlike several other types of derivatized cyclodextrins, is usually not used directly as

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a stationary phase in GC. The CSP is typically prepared by dissolving the Me-CD in bonded polysiloxanes of moderate polarity (e.g. Rtx-1701) [4] or, as first proposed by Schurig and Nowotny [6], by chemically attaching it to a bonded phase. CP Chirasil-Dex CB, the stationary phase used in these studies, consists of 10% (w/w) permethylated β-cyclodextrin bonded to dimethylpolysiloxane through an octamethylene spacer.

To date, the mechanism of chiral recognition by derivatized cyclodextrins (CDs) is still not fully understood. When these phases were first explored for use in chiral GC separations [7], separation of enantiomers was thought mostly to be the result of differing degrees of inclusion of the analyte or "guest" molecule into the cavity of the CD "host". Saying that the analyte is "included" does not necessarily mean that the entire molecule is located within the CD cavity. The accommodation of only part of the molecule into the CD cavity can also be considered as inclusion [8]. However, since the introduction of these CSPs, it has become more apparent that differing inclusion of enantiomers is not the only mechanism by which chiral recognition can take place. Interaction of the analyte with the outer sphere of the CD may, in some cases, also play an important role [3]. Overall, analyte molecules do not interact with the CD exclusively by one mechanism or another but through a combination of inclusion, hydrogen-bonding, nonpolar interactions, dipole–dipole interactions, and/or electrostatic interactions.

Chiral recognition of analytes is thought to occur mostly at the groups located at the 2 or 3 positions of the CD glucose units. Interaction with groups at the 6 position, at the narrower end of the CD, is thought to be less important for chiral recognition [9]. It is thought by some authors [9] that, if the analyte interacts at the surface of the CD, most of the interactions are with groups located at the 2-positions of the CD. These groups are located at the wider opening and are oriented away from the CD. When inclusion of the analyte into the CD cavity is important for chiral recognition, groups at the 3-positions, also located at the wider rim and oriented towards the inside of the CD are believed to play a larger role.

Since its introduction as a CSP for gas chromatography, numerous studies have been done in order to understand the mechanisms involved in the separation of enantiomers on Me-CD. Several of these studies involved study of the retention behavior of homologous series of chiral compounds. In this way, the effect of small variations in the structure of the alkyl substituents on enantioselectivity could be studied while keeping the structure of the remainder of the molecule constant. Early studies done by Mraz et al. [7] involving the interactions of various homologous series of alkanes, alcohols, and aromatic hydrocarbons with partially methylated α-CD and β-CD coated on Chromosorb W showed that the size and shape of the molecule were very important in determining the amount of interaction with the cyclodextrin. In many cases, when comparing compounds of the same molecular weight, compounds containing branching eluted earlier than the corresponding linear compound. These differences were attributed to steric effects present in the branched compounds that made inclusion into the Me-CD cavity less favorable. Studies done by Meierhenrich et al. [10] on the interaction of chiral aliphatic hydrocarbons with polysiloxane-anchored Me-CD showed that, generally, within a homologous series the resolution obtained decreased as the chain length was increased. This decrease was attributed to larger amount of nonspecific interactions with the exterior of the Me-CD as the chain length was increased. Spanik et al. [9] performed temperature studies on *N*-TFA-*O*-*n*-alkyl esters of alanine on Chirasil-Dex CB in which the chain length of the *n*-alkyl substitiuents was varied. As the chain length was increased, there was a decrease in selectivity between the enantiomers. Studies done by Benicka et al. [11] on selected *N*-TFA-*O*-alkyl amino acid esters showed a nonuniform dependence of selectivity on chain length that depended on the structure of the remainder of the molecule. For example, the methyl and ethyl esters of alanine and 2-aminobenzoic acid were better separated than the 1-propyl, 1-butyl, or 1-pentyl esters while, in the case of proline, alkyl chain length had little effect on enantioselectivity. They also studied the effect of introducing branched alkyl groups into the ester portion of the molecule. In this case, the effect on enantioselectivity also depended on the entire structure of the amino acid. For example, the isopropyl and isopentyl esters of leucine displayed higher enantioselectivity than the corresponding C_1–C_5 *n*-alkyl esters while the isopropyl ester of proline displayed less enantioselectivity.

This paper presents the results of thermodynamic studies performed on 12 pairs of *N*-TFA-*O*-alkyl nipecotic ester enantiomers on CP Chirasil-Dex CB. The structure of nipecotic acid (3-piperidinecarboxylic acid), a cyclic β-amino acid, is shown in Fig. 1. Initially, a separation method for the *t*-butyl ester enantiomers was developed. (*R*)-nipecotic acid *t*-butyl ester is an intermediate used in the synthesis of a drug candidate. CP Chirasil-Dex CB provided baseline resolution and elution of the undesired *S*-enantiomer before the desired *R*-enantiomer. Previous temperature studies performed on the *N*-TFA-*O*-alkyl esters of chiral α-amino acids on permethylated β-cyclodextrin have shown that the nature of the ester alkyl group can have a significant influence on retention behavior [9,11]. Since nipecotic acid is a cyclic β-amino acid, the influence of the ester alkyl group structure on the retention behavior of the enantiomers could differ from that seen with α-amino acids. In order to study the effect of the *O*-alkyl group structure on retention behavior and enantioselectivity, nipecotic acid esters having widely varying *O*-alkyl group structures were prepared. The structures of these esters are shown in Fig. 1. Structural variations included chain length, branching, location of branching relative to the chiral center, and the size and shape of the ester alkyl group. Temperature studies were performed and, because Chirasil-Dex CB is a diluted phase, thermodynamic parameters related to interactions with the Me-CD were estimated using the retention increment method [12]. In order

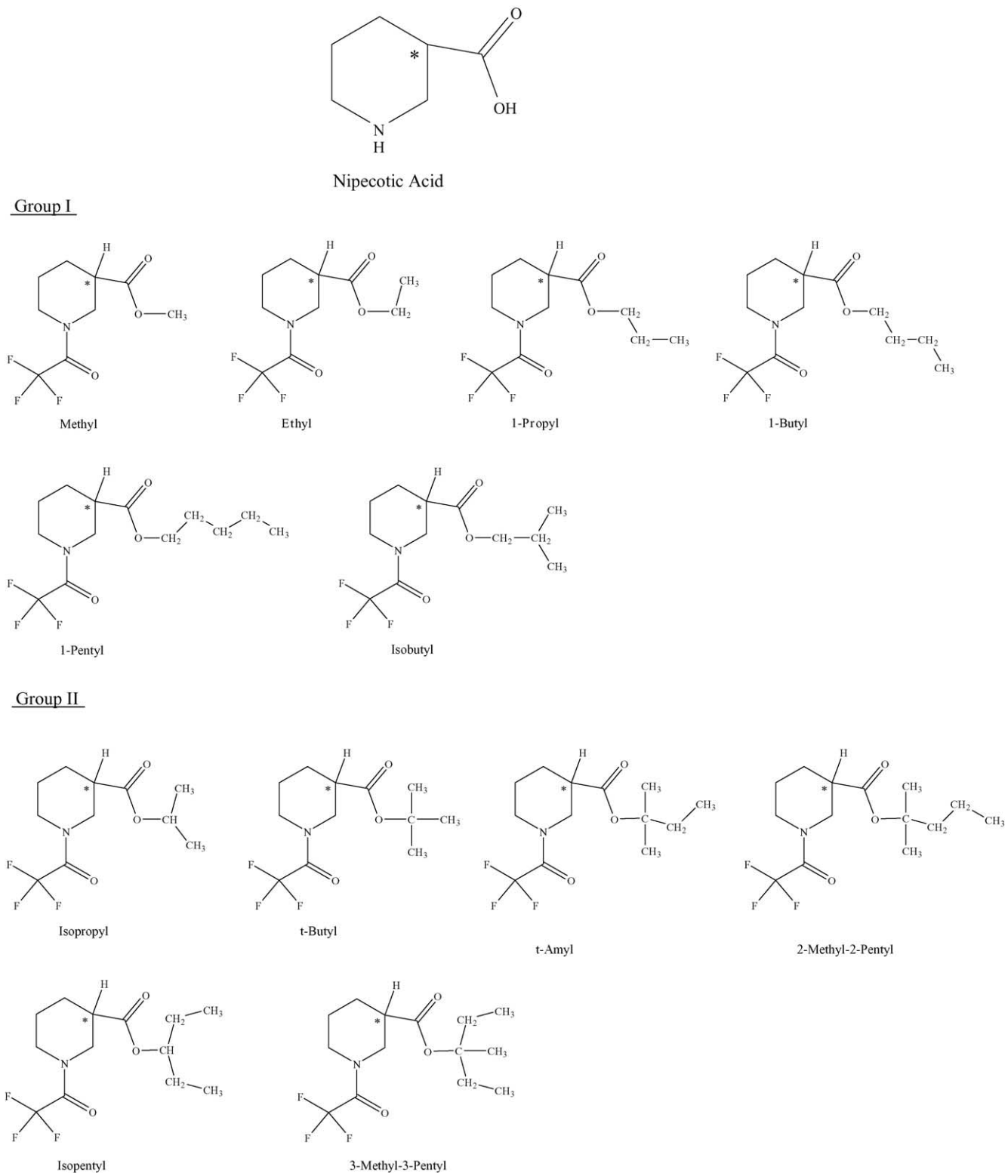


Fig. 1. Structures of nipecotic acid and *N*-TFA-*O*-alkyl ester derivatives used in temperature studies on Chirasil-Dex CB.

to determine whether there are differences in the mechanism of interaction with the Me-CD among the esters studied, the theoretical concept of enthalpy–entropy compensation was employed. Additionally, in order to obtain a qualitative understanding of the interaction of the ester enantiomers with Me-CD, some of the diastereomeric Me-CD–ester complex structures were modeled using simulated annealing molecular dynamics.

2. Experimental

2.1. Equipment

Studies were performed on an Agilent 6890 gas chromatograph with split injection and FID detection. Ultra high purity helium was used as the carrier gas at linear velocities of 25–65 cm/s. Injections (1 μL) were done at split ratios of 20:1–100:1. Data was collected and integrated using a PE Nelson Turbochrom system (Cupertino, CA). The enantioselective column was a CP Chirasil-Dex CB, 25 m × 0.32 mm, d_f = 0.25 μm (Varian Inc., Palo Alto, CA). The achiral reference column was a CP-Sil 5CB (100% dimethylpolysiloxane), 50 m × 0.32 mm, d_f = 0.25 μm (Varian Inc.). Molecular weight confirmations were performed on a Finnigan/Thermoquest TRACE GC/MS system in positive ion CI mode (San Jose, CA/Rodano, Italy).

2.2. Reagents

All alcohols for preparation of esters, racemic nipecotic acid, thionyl chloride, *n*-octane, *n*-nonane, and dichloromethane were purchased from Aldrich Chemical (Milwaukee, WI, USA). (*S*)-nipecotic acid hydrochloride was obtained from Yamakawa Chemical Industry Co., Ltd. (Tokyo, Japan). Trifluoroacetic anhydride (TFAA) was obtained from Halocarbon Products Corp. (River Edge, NJ, USA).

2.3. Analytes

Primary and secondary nipecotic acid esters were prepared by dissolving approximately 50 mg of nipecotic acid in 1 mL of the respective alcohol in a vial, adding 200 μL of sulfuric acid, capping and then heating for 2–3 h at 100–120 °C. The mixture was then cooled on ice, neutralized with ammonium hydroxide, and then extracted with two 1 mL portions of toluene. The toluene extracts were then dried over sodium sulfate [13]. Trifluoroacetyl (TFA) derivatives were prepared by pipetting 200 μL of the toluene extract into a vial, evaporating to dryness with nitrogen, redissolving in 1 mL anhydrous dichloromethane, and then adding 500 μL of trifluoroacetic anhydride. The solution was allowed to stand at room temperature for 30 min, evaporated to dryness with nitrogen, and then reconstituted in acetonitrile for GC temperature studies [14]. Pure *R* and *S* *t*-butyl ester was obtained from the Process Research Dept., Merck Research Labs. The remaining tertiary esters were prepared by first dissolving 50 mg of nipecotic acid in 1 mL of dichloromethane in a vial, adding 500 uL of trifluoroacetic anhydride, allowing to stand at room temperature for 30 min and then evaporating to dryness with nitrogen [14]. The ester was then formed by heating with thionyl chloride, evaporating the excess reagent under nitrogen, and then reacting with the respective tertiary alcohol. The mixture was then dissolved in *n*-hexane, water was added, and the mixture was shaken to remove acidic components. The *n*-hexane was then separated and dried over sodium sulfate [11,13]. Aliquots of the *n*-hexane solutions were evaporated to dryness and reconstituted in acetonitrile for temperature studies. The pure *S*-enantiomers were prepared along with racemic mixtures of all esters in order to determine enantiomer elution orders. The molecular weights of all prepared esters were confirmed by GC–MS.

2.4. Temperature studies

All temperature studies were performed isothermally between 90 °C and 140 °C in steps of either 5 °C or 10 °C. Solutions of analytes were injected in duplicate or triplicate. The column void time (t_0, min) was determined by making an injection of dichloromethane before each set of experiments. The retention factor k' was calculated as $k' = (t_R - t_0)/t_0$, where t_R is the retention time (min) of the analyte. The separation factor, α, was calculated as $\alpha = k'_2/k'_1$ where k'_1 is the retention factor of the first eluted enantiomer and k'_2 is the retention factor of the second eluted enantiomer.

2.5. Molecular modeling

The program CERIUS2 was used for all molecular modeling studies. The structure of permethylated cyclodextrin was optimized by MOPAC at the AM1 level. Charges were computed by the charge equilibration method. Similar optimizations and charge computations were carried out for the analytes. The optimized structures for the complexes were then determined using Simulated Annealing Molecular Dynamics. The initial temperature was 300 K. The maximum temperature was set at 2000 K to ensure sufficient sampling of conformation space. The cyclodextrin was held rigid during the simulation. The analytes were docked inside the cavity and 100 cycles of simulated annealing were implemented. A preliminary study with 500 cycles produced the same global minimum structure; hence the abbreviated protocol was adopted without any compromise. Dynamics was carried out for 5000 steps with a time step of 1 fs. The model was minimized after each annealing cycle. Data were collected every 10 steps. The energy of the structure corresponding to the global minimum was used. The binding energies were calculated as follows:

$$E_{binding} = E_{complex} - E_{Me\text{-}CD} - E_{analyte}$$

2.6. Theoretical

In chiral gas chromatography, separation of enantiomers is based on the formation of transient diastereomeric complexes with a chiral selector. For the pair of enantiomers, *R* and *S*, separation results from a difference in the Gibbs free energy, $-\Delta_{R,S}(\Delta G)$, between the diastereomeric complexes that are formed upon interaction with the chiral selector. The chemical association equilibria are described by the thermodynamic association constants K_R and K_S. The separation can

be described by the Gibbs–Helmholtz Eq. (1):

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} \quad (1)$$

$$= -\Delta_{R,S}(\Delta H) + T\Delta_{R,S}(\Delta S) \quad (2)$$

where the *R*, *S* subscript arbitrarily refers to the second and first eluted enantiomers, respectively. Two different methods can be used to calculate the thermodynamic parameters $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$.

Method I: For undiluted phases, $\Delta_{R,S}(\Delta G)$ can be calculated directly from the separation factor, α:

$$-\Delta_{R,S}(\Delta G) = RT \ln \alpha \quad (3)$$

where $\alpha = K_R/K_S = k'_R/k'_S$. Eqs. (1–3) can be combined and rearranged to give the following:

$$\ln \alpha = \frac{-\Delta_{R,S}(\Delta H)}{RT} + \frac{\Delta_{R,S}(\Delta S)}{R} \quad (4)$$

From a plot of $\ln \alpha$ versus $1/T$, $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ can be determined from the slope and intercept, respectively.

Method II: For diluted phases, $\Delta_{R,S}(\Delta G)$ is dependent on the concentration of chiral selector [12]:

$$-\Delta_{R,S}(\Delta G) = RT \ln \frac{K_R}{K_S} = RT \ln \frac{R'_R}{R'_S} \quad (5)$$

with:

$$R' = \frac{t'_R - t'^0_R}{t'^0_R} = Km \quad (6)$$

where t'^0_R = adjusted retention time of the individual enantiomer measured with a reference column containing only solvent *S* and t'_R = adjusted retention time of the individual enantiomer measured with a column containing a chiral selector that is either dissolved in or chemically bonded to solvent *S* (reactor column). *K* is the association constant between the enantiomer and the chiral selector and m is the molality (mol/kg) of the chiral selector in the solvent.

The adjusted retention times are obtained by measuring the retention time of the enantiomer relative to a reference standard on both the reference and reactor columns. The reference standard should interact only with the solvent and not the chiral selector. This method is widely referred to as the retention increment method and should allow the measurement of thermodynamic quantities related to interactions with the chiral selector only. By plotting $\ln R'_R/R'_S$ versus $1/T$, $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ can be determined from the slope and intercept, respectively, analogous to Method I [12,15,16].

For a 1:1 complex between the enantiomer and the chiral selector, $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ have opposite effects on $\Delta_{R,S}(\Delta G)$. T_{iso}, the temperature at which the enthalpy and entropy effects compensate and the pair of enantiomers coelute, is described by:

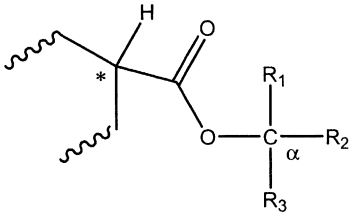
$$T_{iso} = \frac{\Delta_{R,S}(\Delta H)}{\Delta_{R,S}(\Delta S)} \quad (7)$$

At temperatures below T_{iso}, the separation is governed by $\Delta_{R,S}(\Delta H)$, the difference in enthalpy and at temperatures above T_{iso}, peak inversion occurs and the separation is governed by $\Delta_{R,S}(\Delta S)$ the difference in entropy [1].

3. Results and discussion

3.1. Variation of ester alkyl group structure

The structure of the ester alkyl group can be represented by the following:



where R_1, R_2, and R_3 may represent either hydrogen atoms or different alkyl substituents bonded to the α-carbon. Since the structure of the remainder of the molecule is the same for all of the esters studied, it is possible to independently study the effect of varying the alkyl substituents on retention behavior and enantioselectivity. Based on the number of alkyl groups bonded to the α-carbon, the esters were placed into two groups. Group I includes the methyl ester along with esters that have only one alkyl substituent bonded to the α-carbon. The isobutyl ester, which has branching one carbon away from the α-carbon (i.e. R_1 and R_2 = H, R_3 = isopropyl) is also included in Group I. Group II includes esters that have at least two alkyl substituents bonded to the α-carbon with resultant branching that is in closer proximity to the chiral center of the molecule than that in the isobutyl ester.

3.2. Temperature studies and estimation of thermodynamic quantities

Plots of $\ln (k'_S/k'_R)$ versus $1/T$ for all of the esters are given in Fig. 2. α-Values obtained for each pair of enantiomers over the studied temperature range are listed in Table 1. From

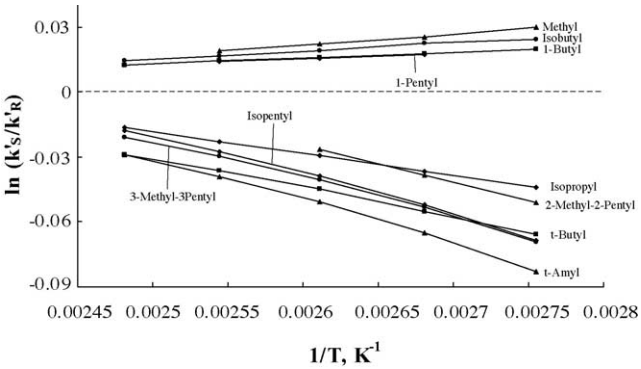


Fig. 2. Plots of $\ln (k'_S/k'_R)$ vs. $1/T$ for enantiomers of nipecotic acid *N*-TFA-*O*-alkyl ester derivatives on Chirasil-Dex CB.

Table 1
α-Values for enantiomers of nipecotic acid *N*-TFA-*O*-alkyl ester derivatives on Chirasil-Dex CB

Alkyl group	90 °C	100 °C	110 °C	120 °C	130 °C
Group I (elution order *R*, *S*)					
Methyl	1.030	1.026	1.022	1.019	1.014
Ethyl	1.0	1.0	1.0	1.0	1.0
1-Propyl	1.01	>1.0[a]	>1.0[a]	1.0	1.0
1-Butyl	1.020	1.018	1.016	1.014	1.013
1-Pentyl	1.015	1.017	1.016	1.014	–
Isobutyl	1.025	1.023	1.019	1.017	1.014
Group II (elution order *S*, *R*)					
Isopropyl	1.045	1.037	1.030	1.023	1.017
t-Butyl	1.068	1.057	1.046	1.037	1.029
t-Amyl	1.086	1.067	1.052	1.040	1.030
3-Methyl-3-pentyl	1.072	1.055	1.041	1.030	1.021
Isopentyl	1.071	1.054	1.040	1.028	1.018
2-Methyl-2-pentyl	1.053	1.039	1.027	1.019	–

[a] Enantiomers beginning to separate. Separation not adequate enough for accurate determination of k' values for individual enantiomers.

Group I, the methyl, 1-butyl, 1-pentyl, and isobutyl esters are shown and all have an elution order of *R*, *S*. Plots for the ethyl and 1-propyl esters are not shown. Over the temperature range studied, separation of the ethyl ester enantiomers was not observed while the 1-propyl ester began to show measurable separation at a column temperature of 90 °C where α was calculated to be 1.01. Spiking studies using the pure *S*-enantiomer confirmed that the elution order was *R*, *S* at this temperature. Table 1 shows that the α-values for the methyl, 1-butyl, and isobutyl esters increase slightly upon lowering the column temperature while the 1-pentyl ester displays a more random temperature dependence. The α-values observed for the methyl ester enantiomers tend to be slightly higher than those for the other *n*-alkyl esters. Upon going from methyl to ethyl, where the chain length is increased by one methylene unit, there is an initial loss in enantioselectivity. As the chain length is further increased, a small amount of enantioselectivity is once again obtained and initially increases upon further increases in chain length. However, increasing the chain length beyond 1-butyl does not provide a further increase in enantioselectivity.

A plot of $\ln k'$ versus methylene number for the later eluted enantiomers of the *n*-alkyl esters at 110 °C is given in Fig. 3a. The C_2 to C_5 esters show a linear homologous series type increase in retention ($R^2 = 0.999$) as consecutive methylene groups are added. However, based on the intercept calculated from linear regression analysis, the methyl ester (number of methylene groups = 0) would be predicted to have lower retention than that observed experimentally. The reason for this behavior becomes more apparent later when the retention increment method is applied to the data.

The ester enantiomers in Group II, which includes the isopropyl, *t*-butyl, *t*-amyl, 2-methyl-2-pentyl, isopentyl, and 3-methyl-3-pentyl esters, all have an elution order opposite to that of the Group I esters (i.e. *S*, *R*). Table 1 shows that for esters of any given molecular weight, the α-values of the Group II esters are higher at all of the studied temperatures

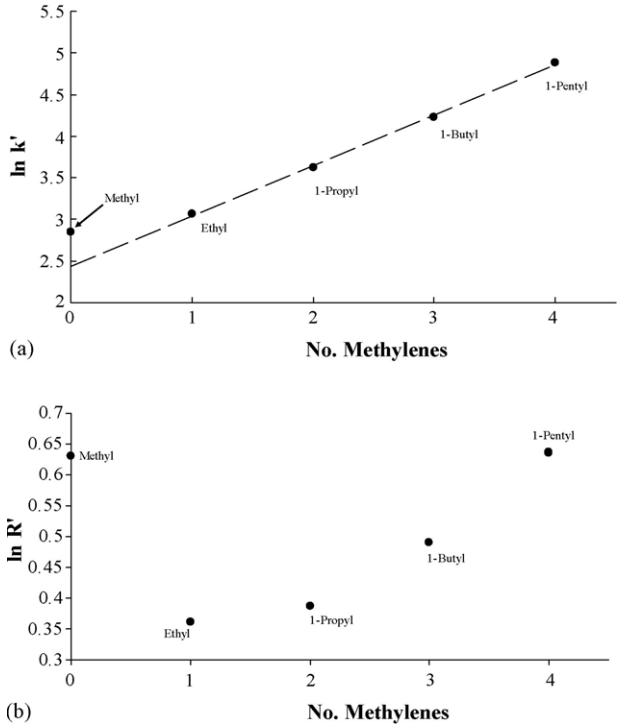


Fig. 3. Plots of (a) $\ln k'$ versus methylene number and (b) $\ln R'$ vs. methylene number for methyl and C_2 to C_5 *n*-alkyl nipecotic acid *N*-TFA-*O*-alkyl ester derivative second eluted enantiomers at 110 °C.

and increase faster with a decrease in column temperature than the corresponding Group I esters. The plots for some of the esters show curvature. This is due to the mixed retention mechanisms that take place with a two component stationary phase (Me-CD diluted in dimethylpolysiloxane). Thermodynamic parameters calculated from these plots can be inaccurate due to this nonlinearity and would also be a measure of the total interaction of the ester enantiomers with the stationary phase (i.e. the combined interaction with the Me-CD and dimethylpolysiloxane solvent). $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ values calculated from this data (Method I) are, however, presented in Table 3 for comparison, but will not be discussed in depth.

In order to gain a better understanding of the interactions of the ester enantiomers with the Me-CD, thermodynamic parameters were estimated using the retention increment method (Method II). By using this method, it was possible to separate out the achiral or "physical"contribution of the dimethylpolysiloxane solvent from the chiral or "chemical" contribution of the Me-CD to retention. The retention increment method assumes that there are no interactions between the reference standard and the CD. There is, however, a small amount of interaction which may cause a systematic error in the calculated thermodynamic parameters. Also, this method is usually applied to chiral stationary phases in which the CD is physically dissolved in an achiral solvent. CP Chirasil-Dex CB, the stationary phase used in these studies, differs from this in that the CD is chemically bonded to the dimethylpolysiloxane. The quantities calculated using this

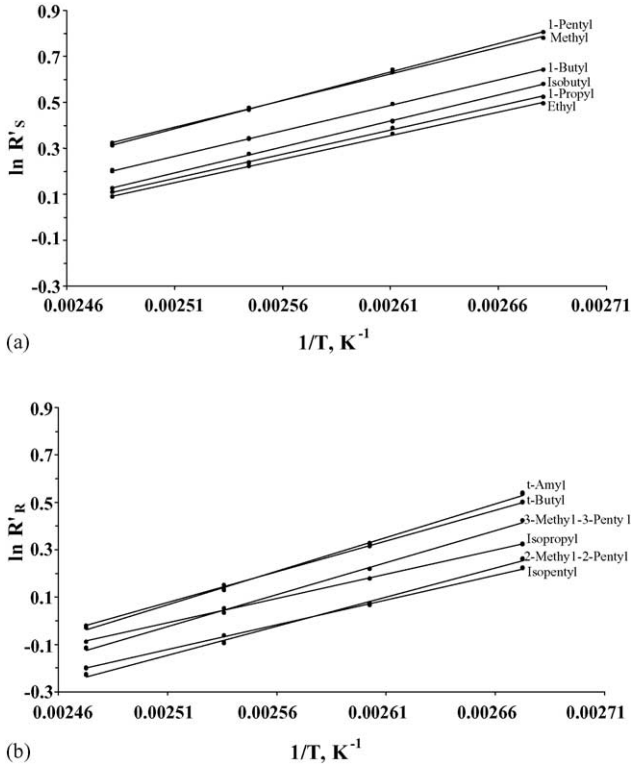


Fig. 4. Plots of $\ln R'$ vs. $1/T$ for second eluted enantiomers of nipecotic acid *N*-TFA-*O*-alkyl ester derivatives. (a) Group I *S*-enantiomers and (b) Group II *R*-enantiomers.

method can however be used for qualitative comparisons of the analytes. *n*-Octane (C_8) and *n*-nonane (C_9) hydrocarbon standards were used in these studies.

Fig. 4a and b show van't Hoff plots obtained using the C_8 data (similar plots were obtained for the C_9 data) for all of the second eluted enantiomers in Groups I and II. $\ln R'$ versus $1/T$ is plotted in order to compare interactions with the Me-CD only. A plot of $\ln k'$ versus $1/T$ would be a measure of the total interaction (i.e. the sum of achiral and chiral interactions with the stationary phase). From these figures it is seen that a linear relationship is obtained for all of the esters studied ($R^2 \geq 0.997$). This allowed for the calculation of ΔH, the enthalpy of interaction with the Me-CD. The ΔH values calculated using either C_8 or C_9 data are in good agreement and are listed in Table 2. Comparison of the plots in Fig. 4a and b for esters of equal molecular weight consistently shows that the Group I ester has stronger interactions with the Me-CD. Of all the esters studied, the methyl and 1-pentyl esters, both from Group I, have the strongest interactions with the Me-CD.

As discussed previously in Fig. 3a, the C_2 to C_5 *n*-alkyl esters display a homologous series type dependence when $\ln k'$ is plotted versus the number of methylene groups while the methyl ester deviates from this relationship. When $\ln R'$ is plotted instead of $\ln k'$, a much different relationship is observed. The plot given in Fig. 3b shows that, although the methyl ester has the lowest overall retention (i.e. combined

Table 2
Enthalpy values (ΔH) and regression parameters for second eluted nipecotic acid *N*-TFA-*O*-alkyl ester derivative enantiomers on Chirasil-Dex CB estimated using Method II

Alkyl group	$-\Delta H$(cal mol^{-1})[a]	R^2
Group I		
(*S*)-methyl		
C-8	4598	0.999
C-9	4711	0.999
(*R* + *S*)-ethyl		
C-8	4059	0.999
C-9	4189	0.999
(*R* + *S*)-1-propyl		
C-8	4186	0.999
C-9	4317	0.999
(*S*)-1-butyl		
C-8	4403	1.000
C-9	4527	1.000
(*S*)-1-pentyl		
C-8	4912	1.000
C-9	5032	1.000
(*S*)-isobutyl		
C-8	4488	1.000
C-9	4622	1.000
Group II		
(*R*)-isopropyl		
C-8	4075	0.999
C-9	4230	0.999
(*R*)-*t*-butyl		
C-8	5171	1.000
C-9	5346	1.000
(*R*)-*t*-amyl		
C-8	5667	0.999
C-9	5857	0.999
(*R*)-3-methyl-3-pentyl		
C-8	5393	0.998
C-9	5593	0.998
(*R*)-isopentyl		
C-8	4169	0.999
C-9	4344	0.999
(*R*)-2-methyl-2-pentyl		
C-8	4900	0.997
C-9	5109	0.997

[a] ΔH values estimated from retention data obtained between 100 °C and 130 °C at 10 °C intervals.

interaction with the Me-CD and dimethylpolysiloxane solvent), it has stronger interactions with the Me-CD than the ethyl, 1-propyl, or 1-butyl esters. Upon going from methyl to ethyl, there is an initial decrease in interaction, but as succesive methylene units are added, the interaction begins to increase until, upon reaching the 1-pentyl ester, the interaction with the Me-CD is comparable to that of the methyl ester.

The methyl ester is the smallest molecule of all the compounds studied so the increased interaction of this molecule might be attributed to a higher amount of inclusion into the Me-CD cavity. This is supported by the higher ΔH value cal-

culated for the methyl ester compared to the ethyl, 1-propyl, or 1-butyl esters which all have additional methylene groups (Table 2). If all of these esters were included to the same extent in the Me-CD cavity, the methyl ester would be expected to have a lower enthalpic component. The higher ΔH value suggests that the methyl ester gains additional interactions from other parts of the molecule due to greater inclusion.

The van't Hoff plot in Fig. 4a shows that over the studied temperature range the isobutyl ester has weaker interactions with the Me-CD than the 1-butyl ester despite having the same molecular weight. The ΔH values calculated for the isobutyl and 1-butyl esters are similar (-4488 and -4403 cal mol^{-1}, respectively, Table 2) so the weaker interaction observed for the isobutyl ester is be due to a less favorable entropy of interaction. This might be explained by the presence of branching in the isobutyl ester alkyl group. Rekharsky et al. [17] have found that the stability of cyclodextrin complexes can be greatly influenced by the conformational degrees of freedom of the guest molecule. Guest molecules with more degrees of freedom have a more favorable complexation entropy since there are more possible conformations of the molecule that can interact with the cyclodextrin. For example, they found that the equilibrium constants for complexation of trans-3-hexenoate and 6-heptenoate were almost half of those for hexanoate and heptanoate [18]. Comparison of ΔH and ΔS for the complexes showed the differences to be entropic in origin. The complexation entropy of the unsaturated compounds is less favorable due to the presence of a double bond which reduces the conformational degrees of freedom of these molecules. In the present study, branching in the isobutyl group may introduce steric hindrance that limits the conformational degrees of freedom of this molecule thus making the entropy of interaction with the Me-CD less favorable.

For a given molecular weight, the Group II esters have weaker interactions with the Me-CD than the Group I esters. From Table 2 it can be seen that, in some cases, this is despite having a higher enthalpy of interaction with the Me-CD. As with the isobutyl ester, the presence of sterically hindered branched alkyl groups may lower the conformational degrees of freedom of these molecules thus resulting in a less favorable entropy of interaction with the Me-CD. Upon comparing esters of equal molecular weight within Group II only, further differences in interaction with Me-CD are observed. Comparison of the *t*-amyl and isopentyl esters shows that the isopentyl ester has weaker interactions with the Me-CD. The same sort of comparison can be made between the 2-methyl-2-pentyl and 3-methyl-3-pentyl esters, the former having weaker interaction in this case. The major difference between these esters is in the overall shape of the ester alkyl group.

The isopentyl (two ethyl groups bonded to the α-carbon) and 3-methyl-3-pentyl (two ethyl groups and one methyl group bonded to the α-carbon) have significantly different. ΔH values. Introduction of a single methyl group increases ΔH from 4169 to 5393 cal mol^{-1}. Similar behavior is observed for the isopropyl (two methyl groups bonded to the α-carbon) and *t*-butyl (three methyl groups bonded to the α-carbon) esters. In this case, ΔH increases from 4075 to 5171 cal mol^{-1} upon addition of a methyl group. In both cases, addition of a methyl group not only increases ΔH but also the amount of interaction with the Me-CD (i.e. there is an increase in R').

From the R' values calculated for each pair of enantiomers, plots of $\ln R'_R/R'_S$ (where R and S refer to the second and first eluted enantiomers, respectively) versus $1/T$ were constructed. All of the plots (C_8 and C_9 data) were linear with R^2 values ranging from 0.978 to 0.999. Estimated values of $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$, are given in Table 3. $\Delta_{R,S}(\Delta G)$ values at 110 °C are also listed in order to compare the enantioselectivities obtained for the two groups of ester enantiomers. The thermodynamic parameters estimated using the C_8 and C_9 data were in very good agreement. The quantities calculated using Method II, however, were significantly higher than those calculated using Method I. Similar differences between the two methods have been seen by other authors [15,19].

Schurig and co-workers [16,20,21] have noted that the interpretation of thermodynamic data obtained from enantiomers having separation factors of less than 1.1 should be treated with caution. With separation factors this low, the precision of the thermodynamic parameters calculated from plots of $\ln R'_R/R'_S$ versus $1/T$ is not adequate for a rigorous mechanistic discussion of the enantioselectivity obtained. In the present study, the standard deviations obtained from the linear regression analyses are given along with the $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ values listed in Table 3 in order to show that there are significant differences between Groups I and II. Also, the standard deviations of the $\Delta_{R,S}(\Delta G)$ values obtained for all enantiomer pairs at all studied temperatures were calculated using the C-8 and C-9 retention data. The largest standard deviation was 2.3 cal mol^{-1}. Using this worst case precision as the precision for all of the measurements, the $\Delta_{R,S}(\Delta G)$ values measured in these studies can be used to provide some general discussion of the differences in enantioselectivity seen for the two groups of esters.

The data listed in Table 3 shows that the enantioselective thermodynamic parameters calculated for Groups I and II differ significantly. Table 1 shows that the separation factors for all of the ester enantiomers decrease with an increase in temperature. This implies that the temperature ranges studied are below T_{iso} and hence the separations are enthalpy-controlled. The Group II ester enantiomers have enthalpic differences, $\Delta_{R,S}(\Delta H)$, that are, in some cases, almost threefold higher than the esters in Group I. These higher $\Delta_{R,S}(\Delta H)$ values suggest that the Group II ester enantiomers undergo increased chiral discrimination due to additional and/or different types of interactions with the Me-CD than those in Group I.

Errors in the determination of $\Delta_{R,S}(\Delta H)$ and $\Delta_{R,S}(\Delta S)$ can translate into very large errors in calculated T_{iso} values. Therefore, theoretical T_{iso} values were not calculated for the

Table 3
Estimated thermodynamic quantities and linear regression parameters for separation of enantiomers of nipecotic acid *N*-TFA-*O*-alkyl ester derivatives on Chirasil-Dex CB

Alkyl group	Temperature range (°C)[a]	$-\Delta\Delta H$ (cal mol^{-1})	$-\Delta\Delta S$ (cal mol^{-1} K^{-1})	$-\Delta\Delta G$ @ 110 °C (cal mol^{-1})	R^2
Group I					
Methyl					
C-8	115–135	137 ± 5	0.28 ± 0.01	29.0 ± 0.2	0.988
C-9		134 ± 5	0.27 ± 0.01	29.9 ± 0.2	0.988
Method 1		110	0.24	14	0.997
1-Butyl					
C-8	120–140	120 ± 5	0.24 ± 0.01	23.7 ± 1.8	0.978
C-9		121 ± 5	0.25 ± 0.02	24.3 ± 1.8	0.982
Method 1		90	0.19	13	0.993
1-Pentyl[b]					
C-8	–	–	–	23.8 ± 1.7	-
C-9				24.3 ± 1.7	-
Method 1		–	–	16	-
Isobutyl					
C-8	100–130	136 ± 5	0.27 ± 0.01	30.8 ± 0.2	0.990
C-9		135 ± 5	0.27 ± 0.02	31.9 ± 0.2	0.989
Method 1		120	0.26	16	0.998
Group II					
Isopropyl					
C-8	100–130	234 ± 5	0.51 ± 0.01	37.8 ± 0.1	0.997
C-9		236 ± 5	0.51 ± 0.01	39.5 ± 0.1	0.997
Method 1		170	0.38	16	0.998
t-Butyl					
C-8	100–140	369 ± 4	0.79 ± 0.01	65.8 ± 0.4	0.999
C-9		369 ± 5	0.79 ± 0.01	68.5 ± 0.4	0.999
Method 1		280	0.64	30	0.996
t-Amyl					
C-8	100–140	447 ± 8	0.99 ± 0.02	69.4 ± 0.1	0.997
C-9		448 ± 8	0.98 ± 0.02	72.3 ± 0.1	0.997
Method 1		340	0.79	34	0.993
3-Methyl-3-pentyl					
C-8	100–140	445 ± 6	1.01 ± 0.02	57.2 ± 0.6	0.998
C-9		451 ± 6	1.02 ± 0.02	59.8 ± 0.7	0.998
Method 1		310	0.72	30	0.990
Isopentyl					
C-8	110–140	457 ± 11	1.05 ± 0.03	53.5 ± 1.8	0.996
C-9		472 ± 11	1.09 ± 0.03	56.3 ± 1.9	0.996
Method 1		260	0.60	24	0.993
2-Methyl-2-pentyl					
C-8	100–130	437 ± 12	1.03 ± 0.03	41.6 ± 2.1	0.995
C-9		446 ± 13	1.05 ± 0.03	43.6 ± 2.2	0.995
Method 1		280	0.66	19	0.994

[a] Retention data measured at 10 °C intervals with the exception of the methyl and 1-butyl esters which were measured at 5 °C intervals.
[b] $\Delta\Delta H$ and $\Delta\Delta S$ not calculated due to immeasurable change in α-values over studied temperature range.

ester enantiomer pairs. However, peak coalescence was observed experimentally for the 2-methyl-2-pentyl ester enantiomers at 140 °C. At this temperature there was still measurable separation of all other ester enantiomers from Groups I and II. A reversal in enantiomer elution order was not observed for this ester upon further increasing the temperature. This is usually the case at high temperatures since separation of enantiomers is only possible if the enantioselectivity is high enough to compensate for decreasing retention factors [20].

Branching at the α-carbon in the Group II esters has a significant influence on $\Delta_{R,S}(\Delta G)$. Table 3 shows that for any given molecular weight, the Group II ester enantiomers have higher $\Delta_{R,S}(\Delta G)$ values than those in Group I. In some cases, for example the *t*-butyl versus the 1-butyl ester or the *t*-amyl versus the 1-pentyl ester, there is almost a threefold increase in $\Delta_{R,S}(\Delta G)$ for the Group II ester. This is despite the fact that the Group II ester enantiomers have less interaction with the Me-CD than the corresponding Group I ester enantiomers.

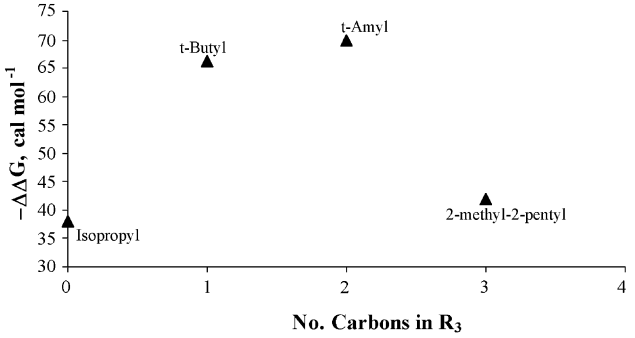


Fig. 5. Dependence of $-\Delta\Delta G$ on number of carbon atoms in third alkyl substituent for α-dimethyl nipecotic acid *N*-TFA-*O*-alkyl ester derivatives. $-\Delta\Delta G$ values calculated from C-8 retention data.

The isopropyl, *t*-butyl, *t*- amyl, and 2-methyl-2-pentyl esters all have two methyl groups (R_1 and R_2 = methyl) bonded to the α-carbon. Fig. 5 shows a plot $\Delta_{R,S}(\Delta G)$ versus the number of carbons in the third alkyl substituent R_3 at 110 °C. Upon going from the isopropyl ester (R_3 = H) to the *t*-butyl and *t*-amyl esters (R_3 = methyl and ethyl, respectively), $\Delta_{R,S}(\Delta G)$ increases significantly. Upon going to the 2-methyl-2-pentyl ester, where the length of R_3 is further increased to a 1-propyl group, there is a subsequent decrease in $\Delta_{R,S}(\Delta G)$.

As discussed earlier, the isopropyl and isopentyl esters both gain increased interaction with the Me-CD upon addition of a methyl group. However, as shown by the $\Delta_{R,S}(\Delta G)$ values for the isopentyl and 3-methyl-3-pentyl ester enantiomers listed in Table 3, addition of a methyl group does not produce a very significant change in the enantioselectivity. Comparison of the $\Delta_{R,S}(\Delta G)$ values obtained for the isopropyl and *t*-butyl ester enantiomers, however, shows that the addition of a methyl group produces a significant increase in the enantioselectivity obtained.

3.3. Enthalpy–entropy compensation

Testing for the existence of enthalpy–entropy compensation behavior in chromatographic systems is very often done by plotting ΔH versus ΔS. Linearity of the plot is usually taken as being indicative of a compensation effect. Krug et al. [22] have shown that compensation may often arise from statistical effects due to errors involved in determining ΔH. The same group demonstrated a procedure for testing for enthalpy–entropy compensation that minimizes statistical effects and is more of a measure of actual physico-chemical compensation [23]. This method, first used by Horvath in the study of separation mechanisms in reversed phase HPLC [24] and later used by Berthod et al. to study differences in enantioselective retention mechanisms on modified cyclodextrin stationary phases [20,25], is based on the free energy of partition, ΔG, of an analyte between two phases i.e. the stationary phase and the gaseous mobile phase. If a group of analytes have similar interactions with the stationary phase, they will have the same free energy change ΔG_β and hence retention at compensation temperature β. This relationship is described by the following equation:

$$\Delta H = \beta \Delta S + \Delta G_\beta \tag{8}$$

where ΔH and ΔS are the standard enthalpy and entropy. The following equation which describes the relationship between $\ln k'$ and ΔH can be derived [23]:

$$\ln k' = -\left(\frac{\Delta H}{R}\right)\left(\frac{1}{T} - \frac{1}{\beta}\right) + \frac{\Delta G_\beta}{R\beta} + \text{constant} \tag{9}$$

A linear plot of $\ln k'$ versus ΔH for a group of compounds would indicate that there is compensation. From *m*, the slope of the regression line, it is possible to calculate the compensation temperature β. If a group of analytes have similar β-values it could provide evidence that they are retained by a similar mechanism.

When studies are done on enantioselective retention mechanisms using this method, the $\ln k'$ and ΔH values for the second eluted enantiomers are typically used [20,25]. The approach used in the present study, however, differs slightly from that described above. $\ln R'$ is plotted instead of $\ln k'$ in order to determine differences in interaction with the Me-CD alone. ΔH values for all of the second eluted enantiomers were determined from plots of $\ln R'$ versus $1/T$ (Fig. 4) and are listed in Table 2. A plot of $\ln R'$ versus ΔH at 110 °C for all of the esters is given in Fig. 6. Similar plots were obtained at the other temperatures studied. Plots of the first eluted enantiomers were also done and did not differ significantly from those of the second eluted enantiomers.

From the plot in Fig. 6 it can be seen that the ethyl, 1-propyl, 1-butyl, and 1-pentyl esters, all from Group I, show a linear relationship between $\ln R'$ and ΔH (R^2 = 0.989) and hence should have a similar compensation temperatures β. From the slope *m*, β was calculated to be 240 °C. This was not confirmed experimentally since it is beyond the upper temperature limit of the stationary phase. Similar values of β suggests that these esters interact with the Me-CD by similar mechanisms. The methyl and isobutyl esters, also from Group I, lie slightly above and below the regression line,

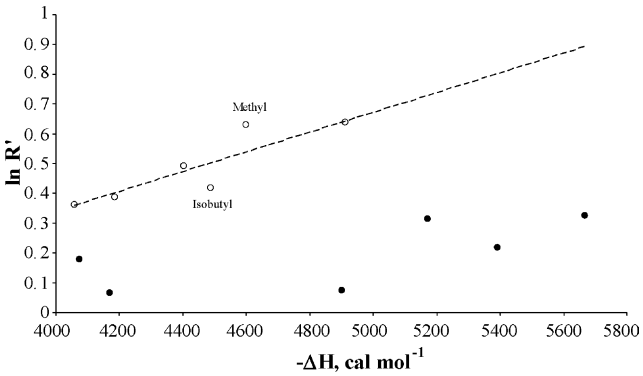


Fig. 6. Enthalpy–entropy compensation plot obtained for second eluted enantiomers of nipecotic acid *N*-TFA-*O*-alkyl ester derivatives on Chirasil-Dex CB at 110 °C. Group I esters are represented by (◯) and Group II by (●). ΔH and $\ln R'$ values were determined from C-8 retention data.

respectively. Possible explanations for these differences in interaction with the Me-CD were given earlier in terms of the smaller size of the methyl ester and the steric hindrance present in the isobutyl ester. However, since there may be cumulative errors involved in determining ΔH, it is not clear whether these deviations are due to actual differences in interaction with the Me-CD.

The Group II esters show a much more random relationship between $\ln R'$ and ΔH and lie well below the regression line. These esters have a lower $\ln R'$ for a given ΔH value than those in Group I. This is indicative that the Group II esters have a less favorable entropy of interaction with the Me-CD. A possible reason for this involving steric hindrance and lowered conformational degrees of freedom was discussed earlier. Due to the randomness of the relationship obtained between $\ln R'$ and ΔH, it is not possible to discern whether all of the Group II esters interact with the Me-CD by similar mechanisms. The enthalpy–entropy plots do suggest, however, that the esters in Group II interact with the Me-CD by different mechanisms than those in Group I.

3.4. Modeling of diastereomeric Me-CD–ester complex structures using simulated annealing molecular dynamics

In order to obtain a qualitative sense of the interaction with Me-CD, modeling of the diastereomeric complexes formed between Me-CD and the C_1–C_5 *n*-alkyl and isobutyl esters (Group I) and the isopropyl and *t*-butyl esters (Group II) was done. Modeling was done assuming simple 1:1 complexes between the Me-CD and the ester enantiomers. Ideally, this model would also include the dimethylpolysiloxane solvent and additional Me-CD molecules in order to more accurately simulate the random interactions that the analyte molecules would have with the CSP [26]. However, this type of simulation was not possible using available computer hardware. Simulations of the diastereomeric complex structures were done based only on electronic interactions between the analytes and the Me-CD (i.e. electrostatic and van der Waals interactions).

Annealing studies performed with only the TFA group of the ester molecule docked inside the Me-CD cavity at the wider rim gave the strongest binding energies for all of the complexes. Simulated structures of the diastereomeric complexes formed between Me-CD and the second eluted (*S*)-methyl and (*R*)-*t*-butyl ester enantiomers are given in Fig. 7a and b, respectively. Similar structures were obtained for the other complexes studied. These structures suggest that there may be only partial inclusion of the analyte molecules in the cavity along with interaction of the ester alkyl groups at the wider rim of the Me-CD. However, further spectroscopic studies (e.g. static NMR measurements) would be needed to provide more evidence for this.

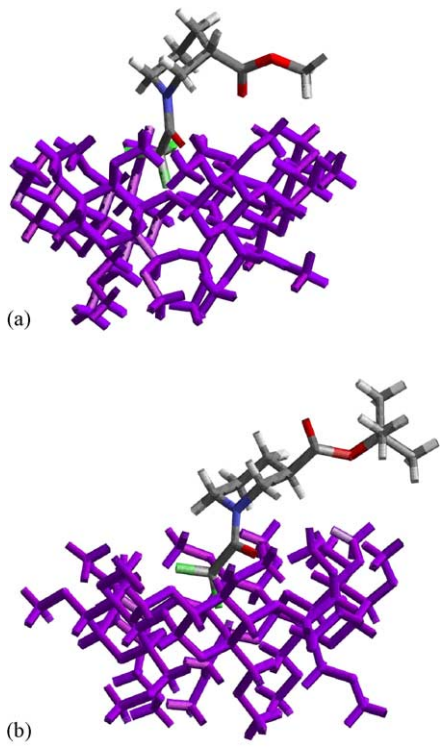


Fig. 7. Simulated structures of diastereomeric complexes formed between permethylated β-cyclodextrin and second eluted (*S*)-methyl and (*R*)-*t*-butyl esters. (a) (*S*)-methyl ester, (b) (*R*)-*t*-butyl ester.

4. Conclusions

Thermodynamic studies were performed on twelve pairs of *N*-TFA-*O*-alkyl nipecotic acid ester enantiomers on CP Chirasil-Dex CB. Of all the esters studied, the methyl and *n*-pentyl esters have the strongest interaction with the Me-CD. The stronger interaction of the methyl ester is attributed to increased inclusion due to the smaller size of this molecule. It also displays slightly higher enantioselectivity than the other *n*-alkyl esters. For the C_2 to C_5 *n*-alkyl esters, increasing the chain length increases interaction with the Me-CD, but results in only a small increase in enantioselectivity that levels off as the chain length is increased beyond an *n*-butyl group.

For a given molecular weight, esters containing branching in the *O*-alkyl group have weaker interactions with Me-CD than their *n*-alkyl analogs. This might be explained by a less favorable entropy of interaction due to steric hindrance and decreased conformational degrees of freedom in the branched ester alkyl groups. However, ester enantiomers that have branching at the α-carbon, although having weaker interactions with Me-CD, display higher enantioselectivity. The amount of interaction and the enantioselectivity obtained is influenced by the size and shape of the α-branched ester alkyl groups. Higher enantioselective thermodynamic parameters determined for the α-branched ester enantiomers, along with differing enthalpy–entropy compensation behavior compared to the *n*-alkyl or β-branched isobutyl esters, suggests that the α-branched esters undergo increased chiral discrimination

due to additional and/or differing types of interactions with the Me-CD.

Modeling of the diastereomeric complexes formed between Me-CD and the C_1–C_5 *n*-alkyl, isobutyl, isopropyl, and *t*-butyl ester enantiomers was performed using simulated annealing molecular dynamics. In all cases, annealing studies performed with only the TFA group docked inside the Me-CD cavity gave the strongest binding energies. The simulated complex structures suggest that interaction of these esters with Me-CD may involve only partial inclusion into the cavity along with interaction of the ester alkyl groups at the wider rim of the Me-CD. However, further spectroscopic studies would be needed to provide more evidence for this.

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