CHAPTER III

EXPERIMENTAL

3.1 Procedures

All solvents for the synthesis were dried with molecular sieves UOP type 4A. The progress of the reactions was followed by thin layer chromatography (TLC) on TLC aluminum sheets, silica gel 60 F_{254} (Merck) and detected under ultraviolet light at 254 nm. The identities of the synthesized esters were confirmed by ^{1}H-NMR spectroscopy (Bruker ACF200 at 200 MHz) using deuterated chloroform ($CDCl_3$, 99.8 %D, Aldrich) as solvent.

All chromatographic separations were performed on a gas chromatograph (Agilent 6890) equipped with a split/splitless injector and a flame ionization detector (FID). Columns were prepared from 33 m x 0.25 mm deactivated fused-silica capillary tubing (J&W Scientific). Solutions of analytes were injected with 10 μL syringe (Hamilton).

3.2 Materials

Most chemicals and solvents were purchased from Aldrich, Fluka, and J.T. Baker and used without further purification. Some racemic mixtures of esters studied were synthesized from carboxylic acids as well as acid chlorides, and the others were obtained commercially from Aldrich and Fluka.

Chiral compounds used are:

- 2-chloro-2-phenylacetyl chloride 90% (Aldrich)
- ethyl-2-bromopropionate 99% (Aldrich)
- ethyl-2-chloropropionate 97% (Aldrich)
- ethyl mandelate 97% (Aldrich)
- α-methoxyphenylacetic acid 99% (Aldrich)
- α-methylhydrocinnamic acid 98% (Aldrich)
- methyl-α-bromophenylacetate 97% (Aldrich)
- methyl-2-bromobutyrate 97% (Aldrich)
- methyl-2-bromopropionate 98% (Aldrich)

- methyl-2-chloropropionate 97% (Fluka)
- (*R*)-(-)-methyl 3-hydroxybutyrate 99% (Aldrich)
- (*S*)-(+)-methyl 3-hydroxybutyrate 99% (Aldrich)
- (*R*)-(-)-methyl 3-hydroxy-2-methylpropionate 99% (Aldrich)
- (*S*)-(+)-methyl 3-hydroxy-2-methylpropionate 99% (Aldrich)
- (*R*)-(+)-methyl lactate 98% (Aldrich)
- (*S*)-(-)-methyl lactate 98% (Aldrich)
- methyl mandelate 97% (Aldrich)
- methyl 2-methylbutyrate 99% (Aldrich)
- 2-phenylbutyric acid 98% (Fluka)
- 3-phenylbutyric acid 97% (Fluka)
- 2-phenoxypropionic acid 98% (Aldrich)
- 2-phenylpropionic acid 97% (Aldrich)

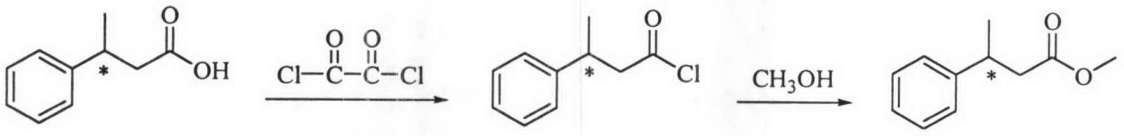
Polysiloxane OV-1701 (7% phenyl, 7% cyanopropyl, 86% methyl polysiloxane; Supelco) was used as a reference stationary phase and as a diluent for solid cyclodextrin derivatives. The cyclodextrin derivatives used in this study were received from Professor Gyula Vigh (Texas A&M University) and are as follows:

- heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (or BSiMe)
- heptakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (or BSiAc).

3.3 Syntheses of ester derivatives

The ester derivatives used in this study were prepared from either their corresponding carboxylic acids or acid chlorides. The general synthesis procedures are described below.

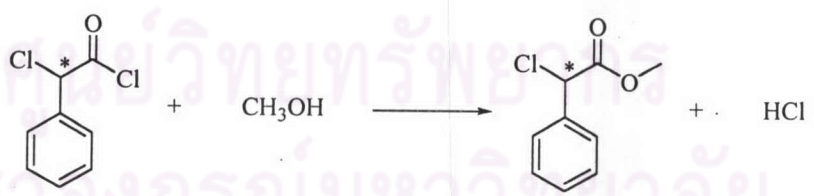
3.3.1 Synthesis of ester derivatives from carboxylic acids



P3 **P3-Me**

*Methyl 3-phenylbutyrate (**P3-Me**):* Oxalyl chloride ($(COCl)_2$, 0.2 mL, 2.36 mmol) was added into a solution of 3-phenylbutyric acid (**P3**, 0.2 g, 1.22 mmol) in dichloromethane (3 mL). One drop of dimethylformamide was added into the stirred solution, and the stirring was continued for 2-3 hours at room temperature. The progress of the reaction was followed by TLC (hexane-ethyl acetate, 10:1). Excess solvent and reagent were removed, and afterward methanol (6 mL) was added into the remaining acid chloride. The solution was then stirred at room temperature. After the reaction was complete, a solution of saturated sodium bicarbonate was added dropwise in order to destroy the remaining acid residue. The aqueous solution was extracted 3-4 times with dichloromethane. The organic layers were combined, dried with anhydrous sodium sulfate, and concentrated, affording methyl 3-phenylbutyrate; 98% yield; R_f = 0.54 (hexane-ethyl acetate 10:1); ^{1}H NMR ($CDCl_3$, 200MHz): δ 1.29 (3H, d, $CHC\underline{H}_3$), 2.57 (2H, m, $CHC\underline{H}_2$), 3.25 (1H, m, $C\underline{H}Ph$), 3.61 (3H, s, $OC\underline{H}_3$), 7.22 (5H, m, $Ar\underline{H}$).

3.3.2 Synthesis of ester derivatives from acid chlorides



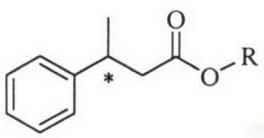
C8 **C8-Me**

*Methyl 2-chloro-2-phenylacetate (**C8-Me**):* An excess amount of methanol (6 mL) was rapidly added into 2-chloro-2-phenylacetyl chloride (**C8**, 0.6 mL) in a round bottom flask. The solution was stirred at room temperature for 3-4 hours. A solution of saturated sodium bicarbonate was added dropwise after the reaction was complete. The product was extracted 3-4 times with dichloromethane. The combined organic layers were dried with anhydrous sodium sulfate and

concentrated, affording methyl 2-chloro-2-phenylacetate in 66% yield; $R_f = 0.49$ (hexane-ethyl acetate 10:1); ^{1}H NMR ($CDCl_3$, 200MHz): δ 3.76 (3H, s, $OC\underline{H}_3$), 5.35 (1H, s, C<u>H</u>Ph), 7.39 (5H, m, Ar<u>H</u>).

Other esters were synthesized with the same procedure and obtained at least 90% yield, except for **C8-Me** and **C8-Et**. The formulas and abbreviations for all esters used in this study, classified as groups of structurally similar analytes, are shown in tables 3.1-3.5.

Table 3.1 Esters with different alkyl chain length (Series 1)



P3-R
alkyl esters of 3-phenylbutyric acid

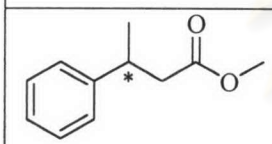
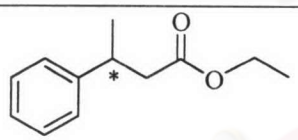
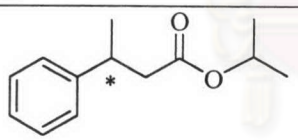
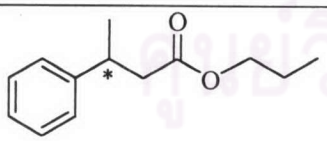
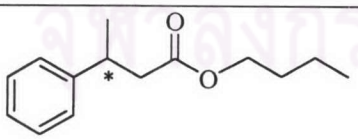
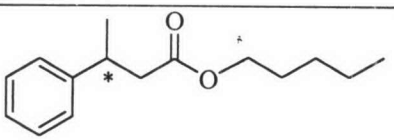
structure	abbreviation	compound
	**P3-Me**	methyl 3-phenylbutyrate
	**P3-Et**	ethyl 3-phenylbutyrate
	**P3-*i*Pr**	isopropyl 3-phenylbutyrate
	**P3-*n*Pr**	*n*-propyl 3-phenylbutyrate
	**P3-*n*Bu**	*n*-butyl 3-phenylbutyrate
	**P3-*n*Pen**	*n*-pentyl 3-phenylbutyrate

Table 3.2 Esters with different positions of substituent or chiral center (Series 2)

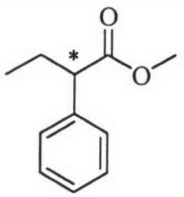
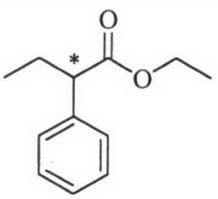
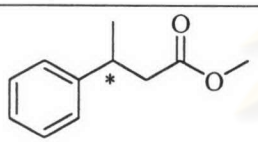
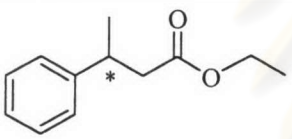
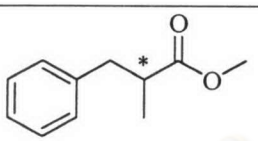
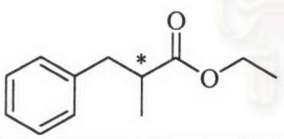
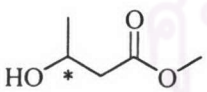
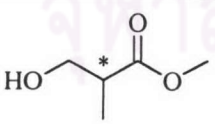
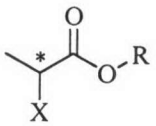
structure	abbreviation	Compound
	**P4-Me**	methyl 2-phenylbutyrate
	**P4-Et**	ethyl 2-phenylbutyrate
	**P3-Me**	methyl 3-phenylbutyrate
	**P3-Et**	ethyl 3-phenylbutyrate
	**M6-Me**	methyl α-methylhydrocinnamate
	**M6-Et**	ethyl α-methylhydrocinnamate
	**M15-Me**	methyl 3-hydroxybutyrate
	**M16-Me**	methyl 3-hydroxy-2-methyl propionate

Table 3.3 Esters with different types of substituents, alkyl esters of 2-X-propionic acid (Series 3)



alkyl esters of 2-X-propionic acid

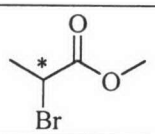
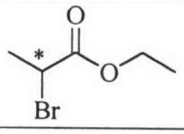
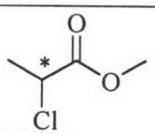
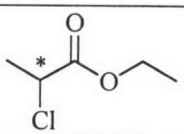
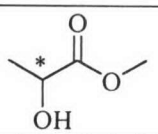
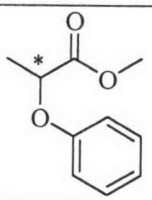
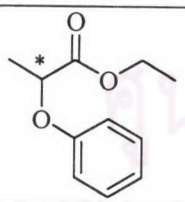
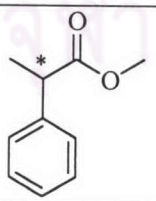
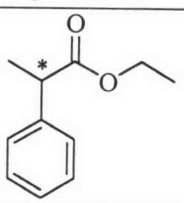
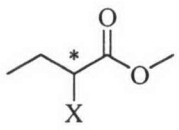
structure	abbreviation	Compound
	**M11-Me**	methyl 2-bromopropionate
	**M11-Et**	ethyl 2-bromopropionate
	**M12-Me**	methyl 2-chloropropionate
	**M12-Et**	ethyl 2-chloropropionate
	**M17-Me**	methyl lactate
	**P2-Me**	methyl 2-phenoxypropionate
	**P2-Et**	ethyl 2-phenoxypropionate
	**P13-Me**	methyl 2-phenylpropionate
	**P13-Et**	ethyl 2-phenylpropionate

Table 3.4 Esters with different types of substituents, methyl esters of 2-X-butyric acid (Series 4)



methyl esters of 2-X-butyric acid

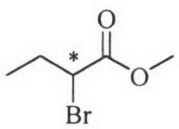
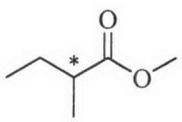
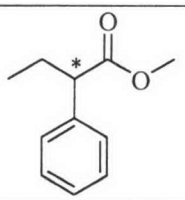
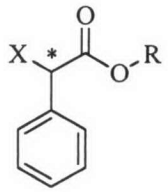
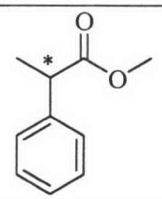
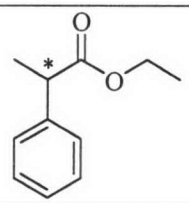
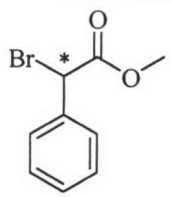
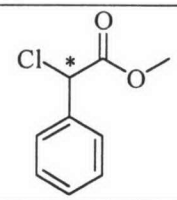
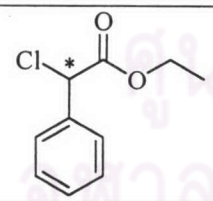
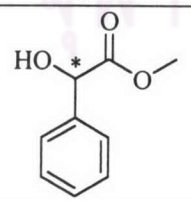
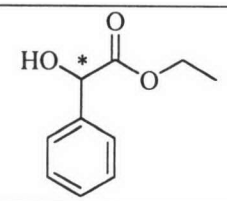
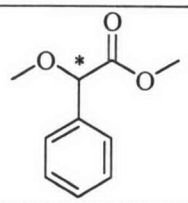
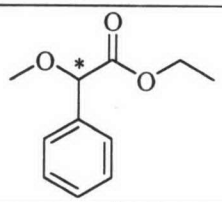
structure	abbreviation	Compound
	**M9-Me**	methyl 2-bromobutyrate
	**M18-Me**	methyl 2-methylbutyrate
	**P4-Me**	methyl 2-phenylbutyrate

Table 3.5 Esters with different types of substituents, alkyl esters of 2-X-2-phenylacetic acid (Series 5)



alkyl esters of 2-X-2-phenylacetic acid

structure	abbreviation	Compound
	**P13-Me**	methyl 2-phenylpropionate
	**P13-Et**	ethyl 2-phenylpropionate
	**M10-Me**	methyl α-bromophenylacetate
	**C8-Me**	methyl 2-chloro-2-phenylacetate
	**C8-Et**	ethyl 2-chloro-2-phenylacetate
	**M7-Me**	methyl mandelate
	**M7-Et**	ethyl mandelate

structure	abbreviation	Compound
	**M20-Me**	methyl α-methoxyphenylacetate
	**M20-Et**	ethyl α-methoxyphenylacetate

3.4 Capillary columns

Capillary gas chromatographic columns were prepared by statically coating [40] deactivated fused silica tubing (33 m × 0.25 mm i.d., J&W Scientific) with stationary phase solutions (0.4% w/v) to obtain a film of 0.25 μm thick. Two chiral columns were prepared and contained identical cyclodextrin concentration of 0.12 M in polysiloxane solvent. All columns were conditioned at 180-200 °C until a stable baseline was observed and overall column performance was determined by means of the Grob test [41-42]. Three capillary GC columns were used in this study:

- achiral reference column: 32.38 m long × 0.25 mm i.d., 0.25 μm film of OV-1701
- chiral BSiMe column: 31.80 m long × 0.25 mm i.d., 0.25 μm film of stationary phase (25.5% (w/w) of BSiMe in OV-1701)
- chiral BSiAc column: 30.24 m long × 0.25 mm i.d., 0.25 μm film of stationary phase (30.28% (w/w) of BSiAc in OV-1701).

3.5 Gas chromatographic analyses

A split injector and a flame ionization detector were maintained at 250 °C. Hydrogen was used as a carrier gas at the average linear velocity of 50 cm/s. Each ester derivative was dissolved in dichloromethane to obtain a concentration of 3-6 mg/mL, and 0.2-0.6 μL of solution was injected with a split ratio of 100:1. Column efficiency was checked regularly at 180 °C with *n*-alkanes (retention factor, k′ >5; efficiency > 3000 plates/m).

Thermodynamic measurements were performed isothermally in a temperature range of 70-180°C with 10-20°C intervals. Each sample solution was

injected at least in duplicate on three columns, a reference column and two derivatized cyclodextrin columns. Retention factors and enantioselectivities of all analytes were calculated from the obtained chromatograms and used to determine the thermodynamic parameters by means of *van't Hoff approach*. Relative retentions of each compound, calculated from reference and cyclodextrin columns, were calculated to determine the retention increments and thermodynamic parameters by means of *Schurig approach*. However, the precise retention value of *n*-heptane (C_7), a reference standard for *Schurig approach*, could not be obtained directly at most of operating temperatures. For this reason, the Kovats plots of log t′ vs. n (n = number of carbon atoms) for a homologous series of *n*-alkanes were constructed at each temperature investigated. The retention values of *n*-heptane at different temperatures were then extrapolated from the log t′ vs. n plots. An example of these plots is shown in figure 3.1.

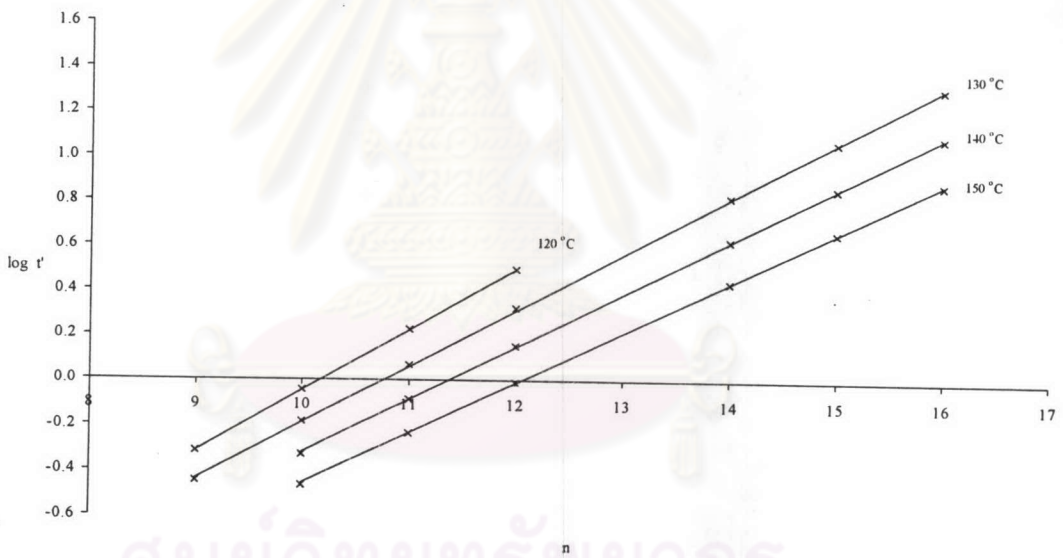


Figure 3.1 Kovats plots of log t′ vs. n (n = number of carbon atoms) for a homologous series of *n*-alkanes on a BSiMe column

Thermodynamic data obtained by both methods were compared. These data were used as a tool in explaining the strength of interaction and the enantioselectivity of esters studied on two types of derivatized cyclodextrins. The differences and/or similarities in the thermodynamic parameters were discussed in terms of types of substituents, positions of substituents, and alkyl chain length of analytes.