การใช้เฮปตะคิส (2,3,6-ไทร-โอ-เมทิล)-บีตา-ไซโคลเดกซ์ทริน เพื่อเป็นเฟสคงที่ชนิดไครัลในเทคนิคแก๊สโครมาโทกราฟี และเพื่อเป็นตัวคัดในปฏิกิริยาการคัดเลือกอิแนนชิโอเมอร์

นาย นพพร สงค์อิ่ม

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THE USAGE OF HEPTAKIS (2,3,6-TRI-*O*-METHYL)-β-CYCLODEXTRIN AS A GAS CHROMATOGRAPHIC CHIRAL STATIONARY PHASE AND AS A DISCRIMINATOR IN AN ENANTIOSELECTIVE REACTION

Mr. Nopporn Song-im

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Thesis Title	The usage of heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin as a gas chromatographic chiral stationary phase and as a discriminator in an enantioselective reaction
By	Mr. Nopporn Song-im
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Thesis Advisor	Aroonsiri Shitangkoon, Ph. D.

Accepted by the Faculty of Science Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

..... Dean of Faculty of Science
(Associate Professor Wanchai Phothiphichitr, Ph.D.)

THESIS COMMITTEE

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(Professor Udom Kokpol, Ph.D.)

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..... Member
(Assistant Professor Kanyarat Kalampakorn, M.Sc.)

..... Member
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

นพพร สงค์อิ่ม : การใช้เฮปตะคิส (2,3,6-ไทร-โอ-เมทิล)-บีตา-ไซโคลเดกซ์ทริน เพื่อเป็นเฟสคงที่ชนิดไครัลในเทคนิคแก๊สโครมาโทกราฟี และเพื่อเป็นตัวคัดในปฏิกิริยาการคัดเลือกอิแนนชิโอเมอร์. (THE USAGE OF HEPTAKIS (2,3,6-TRI-*O*-METHYL)-β-CYCLODEXTRIN AS A GAS CHROMATOGRAPHIC CHIRAL STATIONARY PHASE AND AS A DISCRIMINATOR IN AN ENANTIOSELECTIVE REACTION) อ. ที่ปรึกษา : ดร.อรุณศิริ ชิตางกูร, 71 หน้า. ISBN 974-03-0352-8.

ได้สังเคราะห์เฮปตะคิส (2,3,6-ไทร-โอ-เมทิล)-บีตา-ไซโคลเดกซ์ทริน (TRIMEB) เพื่อใช้เป็นเฟสคงที่ชนิดไครัลในเทคนิคแก๊สโครมาโทกราฟีและเพื่อเป็นตัวคัดในปฏิกิริยาการเปิดวงของสไตรีนออกไซด์ด้วยโซเดียมโบโรไฮไดรด์ในน้ำ TRIMEB ที่ได้ส่วนหนึ่งจะผสมกับ OV-1701 แล้วนำไปเคลือบบนคอลัมน์แคพิลลารีด้วยวิธีสแตติก คอลัมน์ที่เตรียมขึ้นสามารถใช้แยกสารที่จะทำการวิเคราะห์ได้โดยมีลำดับของการถูกชะคือ สไตรีนออกไซด์ 1-ฟีนิลเอทานอลและ 2-ฟีนิลเอทานอล ในกรณีสารประกอบไครัลนั้นพบว่าอิแนนชิโอเมอร์ที่มีโครงแบบเอส (*S*) ของทั้งสไตรีนออกไซด์และ1-ฟีนิลเอทานอลถูกชะออกมาหลังจากอิแนนชิโอเมอร์ที่มีโครงแบบอาร์ (*R*)

TRIMEB อีกส่วนหนึ่งถูกใช้เป็นตัวคัดชนิดไครัลในปฏิกิริยาการเปิดวงแบบรีดักทีฟของสไตรีนออกไซด์ พบว่าผลิตภัณฑ์ที่เป็นสารไครัลไม่มีการคัดเลือกอิแนนชิโอเมอร์และผลิตภัณฑ์หลักในปฏิกิริยาคือ 2-ฟีนิลเอทานอล ผลที่ได้นี้เหมือนกับผลจากปฏิกิริยาที่ไม่มีการเติมตัวคัด เมื่อทำการเพิ่มปริมาณของ TRIMEB พบว่าผลการทดลองยังคงเหมือนเดิมอีกทั้งตรวจพบสไตรีนออกไซด์เหลืออยู่ในปริมาณที่มากขึ้นด้วย ไซโคลเดกซ์ทรินที่มีหมู่ไฮดรอกซิลสองชนิดได้แก่ ไซโคลเดกซ์ทรินที่ยังไม่ผ่านการดัดแปลงและไซโคลเดกซ์ทรินที่ทำการเติมหมู่เมทิลอย่างสุ่ม ถูกนำมาใช้ในปฏิกิริยาเช่นเดียวกับ TRIMEB พบว่า ในปฏิกิริยาที่มีตัวคัดเลือกทั้งสองได้ 1- ฟีนิลเอทานอลเป็นผลิตภัณฑ์หลักแต่การคัดเลือกอิแนนชิโอเมอร์เกิดขึ้นเฉพาะกรณีที่ใช้ไซโคลเดกซ์ทรินที่ยังไม่ได้ผ่านการดัดแปลง สิ่งที่น่าสนใจที่พบจากการศึกษาครั้งนี้ คือ จำนวนโมลของไซโคลเดกซ์ทรินที่ใช้ในปฏิกิริยามีบทบาทต่อการคัดเลือกชนิดผลิตภัณฑ์มากกว่าจำนวนสมมูลของตัวคัดเลือกที่ใช้

ภาควิชา.....เคมี..... ลายมือชื่อนิสิต.....นพพร สงค์อิ่ม.....

สาขาวิชา.....เคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....อรุณศิริ ชิตางกูร.....

ปีการศึกษา.....2544..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: CYCLODEXTRIN DERIVATIVE / ENANTIOSELECTIVITY /
HEPTAKIS (2,3,6-TRI-*O*-METHYL)-β-CYCLODEXTRIN /
CHIRAL DISCRIMINATOR / GAS CHROMATOGRAPHY / STYRENE OXIDE /
1-PHENYLETHANOL
NOPPORN SONG-IM : THE USAGE OF HEPTAKIS (2,3,6-TRI-*O*-METHYL)-β-CYCLODEXTRIN AS A GAS CHROMATOGRAPHIC CHIRAL STATIONARY PHASE AND AS A DISCRIMINATOR IN AN ENANTIOSELECTIVE REACTION. THESIS ADVISOR : AROONSIRI SHITANGKOON, Ph.D., 71 pp. ISBN 974-03-0352-8.

Heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) was synthesized to use as a chiral gas chromatographic stationary phase and as a discriminator in reductive ring-opening of styrene oxide with sodium borohydride in water. TRIMEB was mixed with OV-1701 and was coated onto a capillary column by static method. By using this column, the analyte could be separated in the order of styrene oxide, 1-phenylethanol and 2-phenylethanol. For the chiral compounds, the *S*-enantiomers of both styrene oxide and 1-phenylethanol were eluted after the *R*-isomers.

When TRIMEB was used as a chiral selector in reductive ring-opening of styrene oxide, no enantioselectivity on chiral product was observed and the main product was 2-phenylethanol similar to the reaction without chiral selector. When the amount of TRIMEB was increased, similar results were still obtained and more styrene oxide was recovered. The other two types of cyclodextrin which possess hydroxyl groups (underivatized and randomly methylated) were also used in the same reaction. Both selectors provided 1-phenylethanol as the predominant, however, only underivatized cyclodextrin induced enantioselectivity in chiral product. Another interesting point discovered from the study was that the number of moles of cyclodextrin in the reaction plays more important role in product selectivity than the equivalent of selector.

Department.....Chemistry..... Student's signature.....
Field of study.....Chemistry..... Advisor's signature.....
Academic year .....2001..... Co-advisor's signature.....

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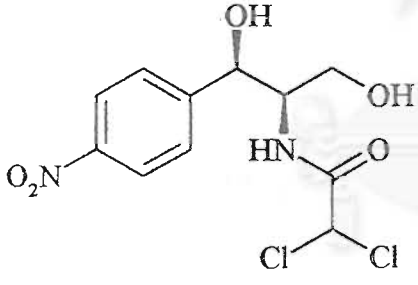
LIST OF ABBREVIATIONS AND SIGNS

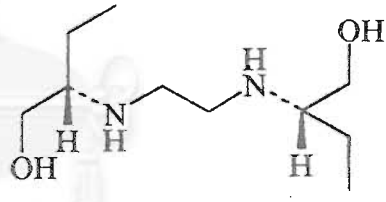
ee	=	enantiomeric excess
RAMEB	=	randomly methylated β-cyclodextrin
TRIMEB	=	hepatakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin
α	=	chiral selectivity factor
β	=	phase ratio
$\Delta_{R,S}(\Delta G°)$	=	difference of change in the Gibbs free energy of association for an enantiomeric pair
$\Delta_{R,S}(\Delta H°)$	=	difference of change in enthalpy of association for an enantiomeric pair
$\Delta H°$	=	change in enthalpy upon complexation of each enantiomer
k′	=	capacity factor
m	=	molality
R′	=	retention increment or chemical capacity factor
$\Delta_{R,S}(\Delta S°)$	=	difference of change in entropy of association for an enantiomeric pair
$\Delta S°$	=	change in entropy upon complexation of each enantiomer

CHAPTER 1
INTRODUCTION

Chirality is an important concept in various fields of chemistry. Only differences in the spatial arrangement of certain atoms or groups at asymmetrical centres or planes of bioactive substances can exhibit distinct biological, pharmacological or toxicological activities. The desired activity often associates with only one enantiomer, while the other may be inactive or produce an unwanted effect. For example, the antibacterial activity resides solely in the (*R*,*R*)-isomer of chloramphenicol. Another example is ethambutol, an antimycobacterial commonly used for HIV & AIDS patients. The (*S*,*S*)-isomer exhibits tuberculostatic property whereas the (*R*,*R*)-isomer causes blindness [1]. Thus the demand for efficient methods to obtain optically pure compounds is increasing and the studies on enantioselective synthesis and chromatographic enantioseparation have become a subject of interest.



(*R*,*R*) - chloramphenicol



(*S*,*S*) - ethambutol

Figure 1.1 Chemical structure of (*R*,*R*) – chloramphenicol and (*S*,*S*) – ethambutol

Among various approaches for obtaining highly enantioenriched compounds, the use of chiral additives in synthesis is one of the useful strategies. The advantages of this procedure are the readily commercial availability of additives at reasonable cost and its variety in the market with an increasing number of new additives each year. Furthermore, most of additives can be recovered after the reaction is complete.

Cyclodextrins, a family of nonreducing cyclic (α-1,4)-linked oligosaccharides of α-D-glucopyranose, are one of the interesting compounds, which have a potential use as an effective chiral additive in syntheses. Due to their characteristic structure, which is composed of relatively hydrophobic cavity and highly hydrophilic outer surface owing to the presence of the glucose hydroxyl groups (see figure 1.2), cyclodextrins can act as host molecule to form inclusion complexes with a large variety of guest molecules ranging from organic or inorganic compounds of neutral or ionic nature to noble gases.

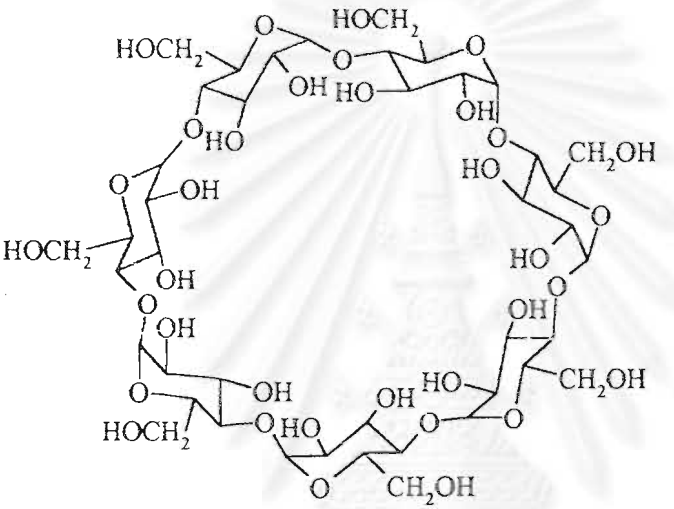


Figure 1.2 Chemical structure of β-cyclodextrin*.

As a result of complex formation, the physicochemical properties of the included guest, such as solubility, diffusion, spectral properties and reactivity will be modified. Furthermore, these macrocycles can discriminate stereochemical isomer, diastereomers as well as enantiomers, by forming complexes with different stability constants. Consequently, the use of cyclodextrins as microvessels to perform and induce selectivity in chemical reaction has attracted chemists and has been intensively studied.

When cyclodextrins are used as reaction vessels, the effect of cyclodextrins on the reactions can be classified into two types. The first involves the formation of a definite covalently bonded intermediate between cyclodextrins and the

---

* Use Greek letters to denote the number of glucopyranose units: α for 6, β for 7, γ for 8 and so on.

substrate. In this case, cyclodextrins behave as an artificial enzyme and accelerate reaction on bound substrates, hence this type was called "*covalent catalysis*" [2]. The other type does not involve a covalent bond at any stage of the reaction. Cyclodextrins create an "*extra reaction field*" and act as enantio-controlling media. Even though they do not function as true catalysts, the central cavity of cyclodextrin molecule gives the reactant an access to a new environment in which the reactivity was changed. Furthermore, the cavity also provides geometric restriction in which size, conformation and/or stereoselectivity can be induced with the combination of microenvironmental effect and conformational effect of cyclodextrin. Nevertheless, in some case the microenvironmental effect reduced rate of reaction and provided low chemical yield. This may be due to the blocking of some active sites of an included molecule against the reagent or other reactant. The phenomenon that guest molecule is relatively or completely blocked by the cyclodextrin molecular wall was called "*stabilizing effects*" or "*negative catalysis*" [3].

The pioneering use of cyclodextrins as an additive in synthesis was the work of Breslow and Campbell [4]. They reported the effect of α-cyclodextrin on the chlorination of anisole by using HOCl in water at room temperature. From the investigation, the *para* / *ortho* isomer ratio of product was increased when increasing the amount of cyclodextrin which indicated that aromatic substitution can be prepared selectively in the presence of cyclodextrin. This opened the way for the use of cyclodextrin as "*mediator*" in organic reaction. Besides substitution reaction, cyclodextrins were used in various classes of reaction such as reduction, oxidation, and isomerization. Moreover, organic reactions mediated by cyclodextrins can be carried out in the form of gas-solid, liquid-solid or solution because of its characteristic complexing properties, which can be observed either in aqueous solution or in solid state.

Due to the presence of a large number of hydroxyl groups, natural cyclodextrins can be chemically modified to improve the physicochemical properties such as water solubility and complexing behavior. The strategy for modification depends on the purpose of the final product. There are four common ways to modify cyclodextrin molecules [5]

(i) substitution of the hydrogen atom of the primary or secondary hydroxyl groups

(ii) substitution of one or more primary and/or secondary hydroxyl groups

(iii) elimination of the hydrogen atoms of the $-CH_2OH$ groups, e.g. by conversion to –COOH

(iv) splitting one or more C_2–C_3 bonds through a periodate oxidation.

The most common substituents are short alkyl groups, hydroxyalkyl groups, long alkyl chains with ionic end groups, and ionic substituents which connected directly on the rim of cavity or *via* a short alkyl chain. Moreover, the groups with certain specific binding or catalytic site can be used to prepare "*artificial enzyme*" for the study of molecular catalysis and biomimetic function. By 1997, the syntheses of more than 1500 derivatives have been published [6] but the majority involved the preparation for pharmaceutical applications and especially in analytical separation methods. There are not many publications and studies in the use of cyclodextrin derivatives in organic reactions, thus further information about the effect of the addition of cyclodextrin derivatives into an organic reaction is still needed.

This study is to investigate the possibility in using a cyclodextrin derivative, with chromatographic properties to separate chiral compounds, to create a chiral environment in organic reactions containing a racemate as a reactant. Numerous available chromatographic data were used to select the appropriate cyclodextrin derivative. One of the popular derivatives in analytical separation methods especially gas chromatographic technique is heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB). There are over 100 chiral compounds reported that could be separated by using this derivative as gas chromatographic stationary phase [7-15]. Moreover, this derivative is more water-soluble than unmodified cyclodextrin and it can be dissolved in many organic solvents which makes it very useful in organic reactions.

From the chromatographic data of TRIMEB, styrene oxide was chosen as a racemic starting material in this study. Because epoxides are versatile raw materials for a wide variety of fine chemicals such as glycols, alcohols, carbonyl compounds, alkanolamines, and polymers such as polyester, polyurethanes, and epoxy resins. Furthermore, epoxides, *via* enantioselective ring opening epoxides, can be converted to many optically active chiral compounds such as α-amino alcohols, arylalkylcarbinols, which are valuable key intermediates in the synthesis of pharmaceuticals, and biological active compounds.

Among many known methods of the ring opening of epoxides, reduction to alcohols by hydrides reagent is one of the interesting reaction due to the utilization of alcohol products as building block in organic synthesis. In this work, reduction with sodium borohydride ($NaBH_4$) was focused because this reagent is readily available and easy to handle when compare with other hydride reagents. However, reductive cleavage with $NaBH_4$ has not been considered as an applicable reaction due to the fact that this hydride reagent reduces epoxides sluggishly except for the epoxides with nitro or hydroxyl as neighboring functional groups. Consequently, no practical procedure for the reduction of general epoxides with $NaBH_4$ was published before 1982 [16].

Soai and colleagues [16] reported a procedure for the reduction of epoxides with $NaBH_4$ by using a mixed solvent of *tert*-butyl alcohol and methanol. Aliphatic, α-aryl-, mono- or di-substituted epoxides were open reductively by this method and the more substituted alcohols were predominant products. In their continued work, they reported the facile method for chemoselective and regioselective reduction of epoxides with $NaBH_4$ in single solvent such as ethanol and 2-propanol in 1988 [17]. Shaozu and their group [18] investigated the effect of solvent on the reaction of styrene oxide with $NaBH_4$. Only phenylethanal was formed by rearrangement when benzene or toluene was used as a solvent; however when tetrahydrofuran, ethanol or isopropanol was used as a solvent only alcohol products from reduction were detected.

Up to now, only a few publications about enantioselective reduction of epoxide to give the corresponding alcohol were reported. Cha and his colleagues applied the optically active (-)-diisopinocampheylborane – lithium chloride (1:0.1) system for enantioselective reduction of three-membered heterocyclic compounds [19]. With this reagent in tetrahydrofuran at 0° C, various racemic epoxides were reduced to give 79-86% yield of alcohol with 5.5-30.5% enantiomeric excess (ee). Moreover, this group also developed four more chiral reducing agents for enantioselective reduction of epoxides [20,21]. The first was B-Isopinocampheyl-9-borabicyclo[3.3.1]nonane – potassium hydride system which provided alcohol product in the range of 75-85% yield with 8-37% ee. The second was potassium B-Isopinocampheyl-9-boratabicyclo[3.3.1]nonane system which provided lower chemical and optical yield of alcohol product, 72-80% with 7-23% ee. The other was chiral 9-alkoxy-9-borabicyclo[3.3.1]nonane – potassium hydride system and chiral potassium 9-alkoxy-9-boratabicyclo[3.3.1]nonane which developed from two previous systems. Santosh Laxmi and Iyengar [22] reported the reductive cleavage of epoxide with zirconium tetrachloride – sodium borohydride in the presence of L-proline as a chiral auxiliary. The alcohols obtained were in the range of 45-80% yield with 4.5-44.2% ee.

(a) (b)

Figure 1.3 Chemical structure of B-Isopinocampheyl-9-borabicyclo[3.3.1]nonane (a) and potassium B-Isopinocampheyl-9-boratabicyclo[3.3.1]nonane (b).

Hu et al. [23] first applied cyclodextrin to ring opening of epoxide. Their communication reported the effect of addition of α, β, and γ-cyclodextrin in reaction of styrene oxide with $NaBH_4$ in aqueous media at room temperature. They found that the reaction was remarkably accelerated and the predominant product was 1-phenylethanol. When α-cyclodextrin was used, the alcohol product was obtained as a racemic mixture; while β- and γ-cyclodextrins provided (*S*)-(-)-1-phenylethanol as a

majority. The highest enantiomeric excess of 1-phenylethanol was 46 % at the mole ratio between starting epoxide and β-cyclodextrin of 1:2. In their detailed study [24], they confirmed that (*R*)-(+)-styrene oxide gave only (*S*)-(-)-1-phenylethanol and (*S*)-(-)-enantiomer of epoxide gave only (*R*)-(+)-enantiomer of alcohol product. They concluded that the origin of the formation of the chiral product is due to kinetic resolution.

Doussot and his colleagues [25,26] studied the influence of the nature of substituent in the ring opening of *ortho*- and *para*-substituted styrene oxide with borohydride reagent in the presence of β-cyclodextrin. They proposed the orientation of epoxide in the cavity of cyclodextrin and concluded that polar substituent with hydrogen-bonding acceptor character was favorable to an enantioselective reduction while hydrophobic and bulky substituent was not favorable to an enantioselective reduction.

The initiation of using cyclodextrin derivatives in reductive ring opening of epoxide was the experiment by Ravichandran and Divakar [27] in 1999. In their work, styrene oxide was reduced with $NaBH_4$ at 50 °C for 8 hours in four different media: 0.1 M NaOH, 0.1 M Na_2CO_3, ethanol and 2-propanol. β-Cyclodextrin, β-cyclodextrin–epichlorohydrin (β-cyclodextrin polymer), and heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM) were used as selectors. In aqueous alkaline medium, the highest yield of 1-phenylethanol was obtained when using β-cyclodextrin (more than 96 % yield of chiral product with 22 % ee of *S*-enantiomer). While DM provided the best yield for reactions that carried out in organic solvent (more than 99 % with 16-19 % ee of *S*-enantiomer).

In this work, the effect of addition of heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) as selector in reductive ring opening of styrene oxide with $NaBH_4$ in aqueous medium at room temperature was studied.

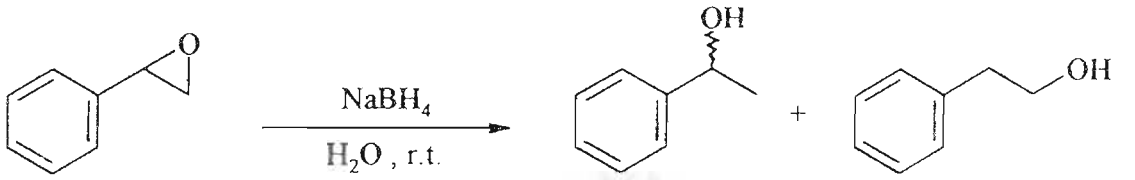


Figure 1.4 Schematic diagram of reductive ring-opening of styrene oxide with $NaBH_4$ in water at room temperature.

The product selectivity, yield and enantioselectivity of reaction were monitored by gas chromatography using the same cyclodextrin derivative, TRIMEB, as chiral stationary phase which offers simplicity, speed, high sensitivity and good reproducibility. Furthermore, The quantitative analysis of the composition in reaction mixture and enantiomeric excess of both reactant and products can be obtained simultaneously, whereas other techniques, e.g. polarimetry and nuclear magnetic resonance spectroscopy, can not deliver such information.

CHAPTER 2
THEORY

2.1 Gas chromatographic determination of thermodynamic parameters of enantiomer separation

The way to clarify and understand the mechanistic aspects of chiral discrimination of cyclodextrin is to determine the thermodynamic parameters which responsible for the complexation between enantiomers of chiral analyte and cyclodextrins. One of the general approaches is using enantioselective gas chromatography. The direct enantiomer separation is based on the formation of reversible diastereomeric associates or complexes that are created by intermolecular interactions of enantiomers with a chiral selector which dissolved in stationary phase. This formation process can be characterized by Gibbs-Helmholtz thermodynamic parameters ($\Delta G°$, $\Delta H°$, and $\Delta S°$) which are different for the *R* and *S* enantiomers and these parameters are usually determined and reported in the form of thermodynamic differences [$\Delta_{R,S}(\Delta G°)$, $\Delta_{R,S}(\Delta H°)$, and $\Delta_{R,S}(\Delta S°)$].

There are two known methods for the determination of thermodynamic parameters. The first (method A) is based on direct determination from the chiral separation factor (α) which is obtained by the separation of the enantiomer of analyte on single chiral column at different temperature. The other method (method B) relies on the determination of the relative retention of the enantiomers of analyte in respect to an inert reference standard (usually use $C_7 - C_{11}$ normal alkanes) on a reference column (containing only polysiloxane) and on a chiral column (containing cyclodextrin derivative dissolved in polysiloxane).

2.1.1 *Method A*

The difference in Gibb's free energy is calculated from the chiral selectivity, α, according to the following general equation.

$$\Delta_{R,S}(\Delta G^{\circ}) = -RT \ln \alpha = -RT \ln\left(\frac{k'_R}{k'_S}\right) \quad \ldots (1)$$

where k′ is the capacity factor of each enantiomer

R is the universal gas constant (1.987 cal/mol·K)

T is the absolute temperature

S, R refer arbitrarily to the first eluted and the second eluted enantiomers, respectively.

Applying the thermodynamic relationship ($\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$) to equation (1)

$$\Delta_{R,S}(\Delta H^{\circ}) - T\,\Delta_{R,S}(\Delta S^{\circ}) = -RT \ln \alpha$$

$$-\Delta_{R,S}(\Delta H^{\circ}) \cdot \frac{1}{RT} + \Delta_{R,S}(\Delta S^{\circ}) \cdot \frac{1}{R} = \ln \alpha \quad \ldots (2)$$

From equation (2), the thermodynamic parameters can be calculated from the slope and y-intercept of the plot between ln α and reciprocal of absolute temperature.

The thermodynamic parameter of individual enantiomer can be also obtained by employing the relationship between capacity factor and Gibb's free energy as follow.

$$k' = \frac{1}{\beta} \cdot e^{\left(-\frac{\Delta G}{RT}\right)} \; ; \; \beta = \text{phase ratio} \qquad \text{or}$$

$$\ln k' = -\ln \beta - \frac{\Delta G}{RT} \quad \ldots (3)$$

Using the relationship $\Delta G^{\circ} = \Delta H^{\circ} - T\Delta S^{\circ}$ and rewriting equation (3), the useful relationship is obtained.

$$\ln k' = -\frac{(\Delta H^{\circ})}{RT} + \frac{(\Delta S^{\circ})}{R} - \ln \beta \quad \ldots (4)$$

2.1.2 *Method B* or *Schurig's method* [28]

Volker Schurig introduced this method in 1991. It is based on a quantitative measure of the increase in the retention of chiral analyte caused by the addition of the cyclodextrin derivative to achiral solvent (polysiloxane) which is called "retention increment" or "chemical capacity factor", R′ and is defined as

$$R' = K \cdot m \qquad \ldots (5)$$

where K is the association constant between chiral analyte (selectand) and selector in stationary phase

m is molality of the selector in achiral solvent (polysiloxane).

The retention increment is experimentally accessed from relative adjusted retention data of the enantiomers and proper reference standards on a chiral column (r) and on a reference column which containing only polysiloxane (r_0).

$$R' = \frac{r - r_0}{r_0} \qquad \ldots (6)$$

and $$r = \frac{t'}{t'^*}$$ [chiral column (cyclodextrin in polysiloxane)]

$$r_0 = \frac{t'_0}{t'^*_0}$$ [reference column (only polysiloxane)]

where t' and t'^* are adjusted retention time of chiral analyte and reference standard respectively, on chiral column (cyclodextrin in polysiloxane)

t'_0 and t'^*_0 are adjusted retention time of chiral analyte and reference standard respectively, on reference column (only polysiloxane)

Thermodynamic parameters are then calculated from the following relationship.

$$\Delta_{R,S}(\Delta G^\circ) = -RT\ln\left(\frac{R'_R}{R'_S}\right) \quad \ldots (7)$$

$$\text{or } -\Delta_{R,S}(\Delta H^\circ)\cdot\frac{1}{RT} + \Delta_{R,S}(\Delta S^\circ)\cdot\frac{1}{R} = \ln\left(\frac{R'_R}{R'_S}\right) \quad \ldots (8)$$

From equation (8), the thermodynamic parameters can be calculated from the slope and y-intercept of the plot between $\ln\left(\frac{R'_R}{R'_S}\right)$ and reciprocal of absolute temperature.

Using the thermodynamic relationship ($\Delta G^\circ = -RT \ln K$) and equation (5), the thermodynamic parameter of individual enantiomers can be obtained.

$$\Delta G_i^\circ = -RT\ln K_i$$

$$\Delta G_i^\circ = -RT\ln\left(\frac{R'_i}{m}\right)$$

$$-\frac{\Delta H_i^\circ}{RT} + \frac{\Delta S_i^\circ}{R} + \ln m = \ln R'_i \quad \ldots (9)$$

The concept of method B allows the separation of the non-chiral contributions of the polysiloxane and the chiral contributions of the cyclodextrin selector from the overall retention. In method A, chiral selectivity is used as a criterion to express the enantioselectivity of the chiral stationary phase. Unfortunately, chiral selectivity is concentration dependent and the relationship between α and mole fraction of selector in stationary phase is non-linear whereas thermodynamic quantity should be strictly independent on the concentration. Thus, in many cases the plot from equation (2) is non-linear and cannot be used for the calculation of thermodynamic parameters. However, method A is still generally used as a first choice because of the simplicity and shorter analysis time.

CHAPTER 3
EXPERIMENTAL

3.1 Apparatus

- AVANCE™ DPX 300 NMR Spectrometer (Bruker)
- deactivated fused-silica capillary columns 30 m × 0.25 mm (J&W Scientific)
- dropping funnel
- gas chromatograph HP5890A equipped with a split injector, a flame ionization detector and an integrator model 3394A (Hewlett-Packard)
- 10 μl syringe (Hamilton)
- hot air oven (Memmert)
- laboratory stirrer/hotplate model PC-420 (Corning)
- low skin temperature vacuum oven model 273 800 (Hotpack Corporation) equipped with vacuum pump model 117 (Labconco)
- microslide TLC tank (Alltech Associates)
- pH – Fix 0-14 colour-fixed indicator sticks (Macherey Nagel)
- TLC aluminium sheets 20 × 20 cm Silica gel 60 F254 (Merck)

3.2 Chemicals

- ammonium hydroxide 28.0-30.0% Baker Analyzed® (J.T Baker)
- β-cyclodextrin purum (Fluka)
- boric acid RPG ACS (Carlo Erba)
- chloroform GR ISO (Merck)
- chloroform-d_3 purum (Fluka)
- deuterium oxide Uvasol® (Merck)
- diethyl ether GR ACS (Merck)
- dimethylformamide AR (Lab-Scan Analytical Sciences)
- dimethylsulfate for synthesis (Merck)
- ethanol absolute GR (Merck)

- hexane AR (Lab-Scan Analytical Sciences)
- iodomethane for synthesis (Merck)
- methanol GR ACS, ISO (Merck)
- methylene chloride ACS Reagent (J.T Baker)
- methylsulfoxide-d_6 puriss (Fluka)
- molecular sieve UOP Type 4A diameter 1.7-2.4mm (Fluka)
- poly (14% cyanopropyl 86% dimethylsiloxane) OV-1701, vinyl silicone (Supelco)
- naphthalene Laboratory reagent (BDH Chemicals)
- 1-naphthol GR ACS (Merck)
- performance evaluation sample for FID detector instruments (Hewlett-Packard)
- phenethyl alcohol purum (Fluka)
- (±)-1-phenylethanol purum (Fluka)
- (*R*)-(+)-1-phenylethanol ChiraSelect (Fluka)
- (*S*)-phenyl oxirane purum (Fluka)
- silica gel 60 particle size 0.063-0.200 mm for column chromatography (Merck)
- sodium borohydride purum (Fluka)
- sodium hydride pract. (Fluka)
- sodium hydroxide GR ISO (Merck)
- sodium sulfate anhydrous GR ACS, ISO (Merck)
- styrene oxide purum (Fluka)
- sulfuric acid 95-97 % GR ISO (Merck)
- toluene GR ACS, ISO (Merck)
- *n*-undecane Laboratory reagent (BDH Chemicals)

3.3 Synthesis of the single-isomer of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB)

β-Cyclodextrin was dried at 110 °C under vacuum over night prior to methylation. A 50-mL solution of dried cyclodextrin (3.0 g, 2.6 mmol) in dry dimethylformamide (DMF) was added dropwise to sodium hydride (washed with dry hexane prior to use, 3.5 g, 80.2 mmol) at 0 °C. After stirring the mixture for 1 hour

methyl iodide (6 mL, 96 mmol) was added dropwise. The reaction mixture was then stirred overnight at room temperature. The completion of reaction was checked by TLC (toluene–ethanol 4:1, v/v). After that, the mixture was poured into an ice-water mixture. The product was extracted with diethyl ether, washed with cold water and dried over anhydrous sodium sulfate. After removing the solvent, the white crystalline solid was obtained.

The purity of the product was checked by thin-layer chromatography using silica gel as stationary phase and toluene–ethanol (4:1, v/v) as mobile phase. Detection was performed by immersing the TLC plate into a developing reagent (1 g recrystallized 1-naphthol, 12 mL ethanol, 4 mL conc sulfuric acid and 2.4 mL distilled water) and placing the plate in a hot oven (120 °C) until spots developed. The structure of the product was confirmed by ^{1}H and ^{13}C NMR spectroscopy (see also figure B1 and B2 in appendix B).

2.28 g, 76% yield, R_F 0.55 (toluene–ethanol, 4:1). ^{1}H NMR (CD_3Cl) δ 5.13 (d, 7H, J = 3.4 Hz, H-1), 3.65, 3.51 and 3.39 (3s, each 21H, 21OCH_3), 3.19 (dd, 7H, J = 9.5, 3.4 Hz, H-2); ^{13}C δ 99.0 (C-1), 82.1,81.8 (C-2,3), 80.3 (C-4), 71.4 (C-6), 70.9 (C-5), 61.5 (OCH_3-3), 59.0 (OCH_3-6), 58.5 (OCH_3-2) [29]

3.4 Synthesis of randomly methylated β-cyclodextrin [30] (RAMEB)

β-Cyclodextrin (3.0 g, 2.6 mmol) was dissolved in 50 mL of 40% w/w aqueous sodium hydroxide solution. Then, dimethylsulfate (20 mL) was slowly added dropwise at 0 °C and the mixture was stirred for 17 hours. At the end of reaction, ammonium hydroxide (20 mL) was added to decompose unreacted dimethylsulfate and the reaction mixture was stirred for another 6 hours. The product was extracted into chloroform layer, washed with distilled water until neutral and dried over anhydrous sodium sulfate. After distilling off the solvent, the white crystalline solid was obtained. The product was used without further purification. Distribution of products was checked by TLC and degree of methylation was obtained from NMR spectroscopy (see also figure B3 in appendix B).

2.00 g, 67% yield, R_F 0.04, 0.11, 0.23, 0.49 and 0.73 (chloroform–methanol, 9:1). ^{1}H NMR (DMSO-d_6) δ 5.03 (m, 8H, OH-2,3), 4.79 (s, 7H, H-1), 3.25 (s, 14H, OCH_3-6); average degree of substitution: 1.7

3.5 Preparation of coated capillary columns

To obtain 0.25 μm film thickness of stationary phase containing 17% w/w of cyclodextrin in OV-1701 (0.18 molal), 0.0069 g TRIMEB and 0.0331 g OV-1701 were weighed and dissolved in methylene chloride in a 10 mL volumetric flask. The 30-m long, 0.25 mm i.d. deactivated fused-silica capillary column was coated with the stationary phase solution using static method.[31] For reference column, only 0.04 g OV-1701 was used to prepare stationary phase solution. The capillary columns were conditioned at 180 °C until a stable baseline was observed. Column efficiency was determined at 160 °C by injecting the evaluation sample mixture (0.03% v/v of each C_{14}-C_{15}-C_{16} normal paraffin hydrocarbon in hexane). Column adsorption was evaluated from the asymmetric factor of the alcohol and naphthalene peak.

3.6 Gas chromatographic condition

For all chromatographic analyses, helium was used as carrier gas at an average linear velocity of 35 cm/s, which measured by injecting methane as unretained compound. The injector and detector temperatures were set at 250 °C. The condition for separating the mixture of styrene oxide, 1-phenylethanol, 2-phenylethanol and naphthalene (internal standard) was as follows: initial temperature 110 °C, then at 2 °C/min to 120 °C, then at 5 °C/min to 155 °C, and finally isothermal for 1 min at 155 °C. The split ratio was set to 110 : 1 with the injection volume of 1 μL. All injected samples and standards were performed at least triplicate.

3.7 Gas chromatographic determination of thermodynamic parameters

A solution of styrene oxide, 1-phenylethanol, 2-phenylethanol and undecane (reference compound) in hexane was injected on two columns, cyclodextrin and reference column, at isothermal conditions in a temperature range of 115-160 °C with 5 °C intervals. In order to know elution order of chiral compound, enantiopure compounds were injected to compare the retention time. From the chromatograms obtained from the cyclodextrin-containing column, capacity factors and chiral selectivities were calculated and used to determine the thermodynamic parameters (method A). Relative retentions of each substances, which obtained from both

columns, were used to calculate the retention increment and the thermodynamic parameters were calculated by Schurig's method (method B).

3.8 Reductive ring-opening of styrene oxide with sodium borohydride

General procedure : Reduction was carried out by stirring styrene oxide in 3.0 mL of water at room temperature for 15 min, then $NaBH_4$ was added into the reaction tube. At specified reaction time, 7 mL of water was added and the reaction mixture was extracted with hexane, dried over anhydrous sodium sulfate and transferred to a 25 mL volumetric flask. One mL of 1.607 %w/v naphthalene (internal standard) in hexane solution was added. Hexane was finally added to adjust the volume and the solution was analyzed by gas chromatography with an injection volume of 0.4 μL and a split ratio of 200 : 1.

3.8.1 *Effect of reaction time*

Five mmol of styrene oxide was allowed to react with 0.5 equivalent of $NaBH_4$ at various reaction time ranging from 8 h to 48 h (the reactions were performed in duplicate). The effect of reaction time on the amount of reactant and products is shown in figure 3.1.

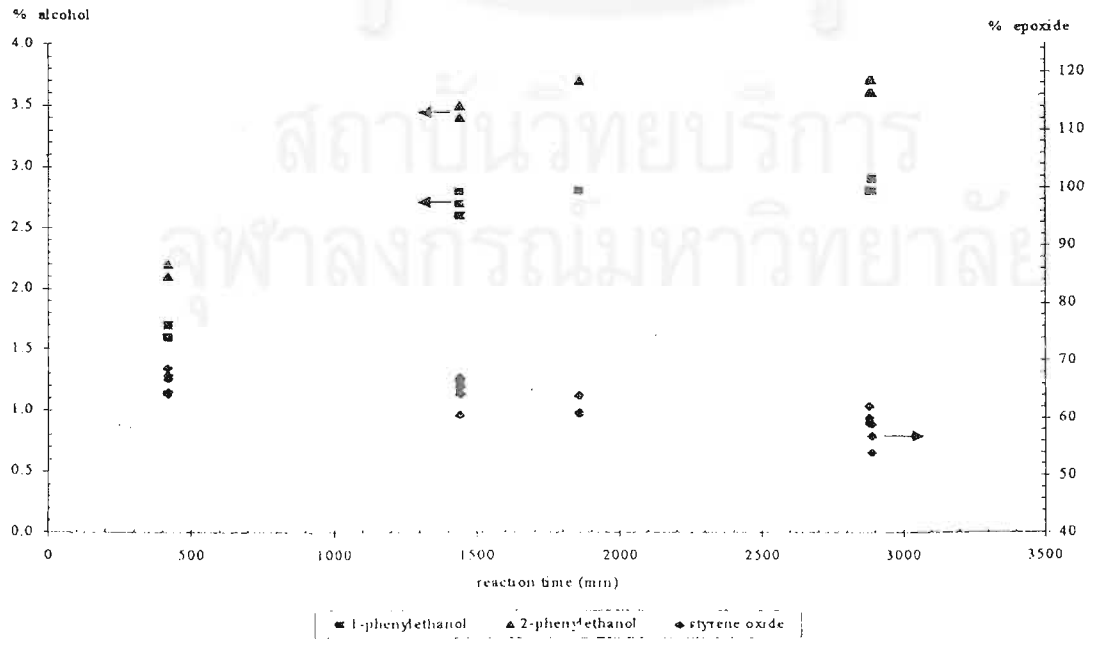


Figure 3.1 The reactant and product distribution at different reaction time intervals.

From the result above, the percentages of alcohol products were not changed significantly when the reaction time was longer than 24 hours (1440 minutes). For all subsequent experiments, the reaction time of 24 hours will be used.

3.8.2 *Effect of the amount of sodium borohydride*

Five mmol of starting epoxide was reacted with 2.5, 5.0, 7.5, 10.0 and 20.0 mmol of borohydride for 24 hours at room temperature. The effect of the amount of reagent on the amount of products is shown in figure 3.2.

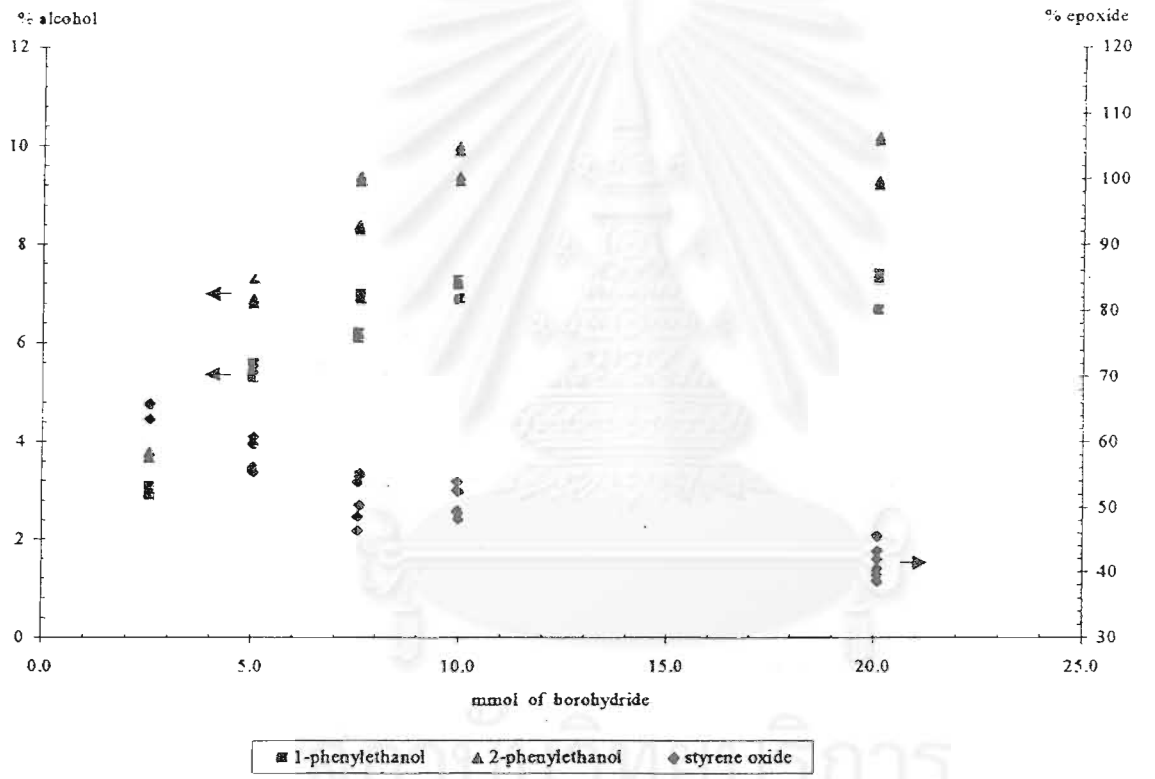


Figure 3.2 The reactant and product distribution at different amount of sodium borohydride.

From the result shown in figure 3.2, the maximum percentage of both products was obtained when the amount of added borohydride reached 10 mmol and the percentage was constant up to 20 mmol of reagent. Due to the high percentage of unreacted epoxide, which is from the very low water solubility of epoxide, the amount of starting epoxide was the next parameter being studied.

3.8.3 *Effect of the amount of styrene oxide*

When the amount of starting epoxide was decreased from 5 mmol to 0.8 mmol at a fixed amount of reagent (1.6 mmole), the yield of alcohol products did not change significantly. Next, the amount of reagent was fixed at 10 mmol and amount of epoxide was varied from 5 mmol to 0.5 mmol. The results are shown in figure 3.3.

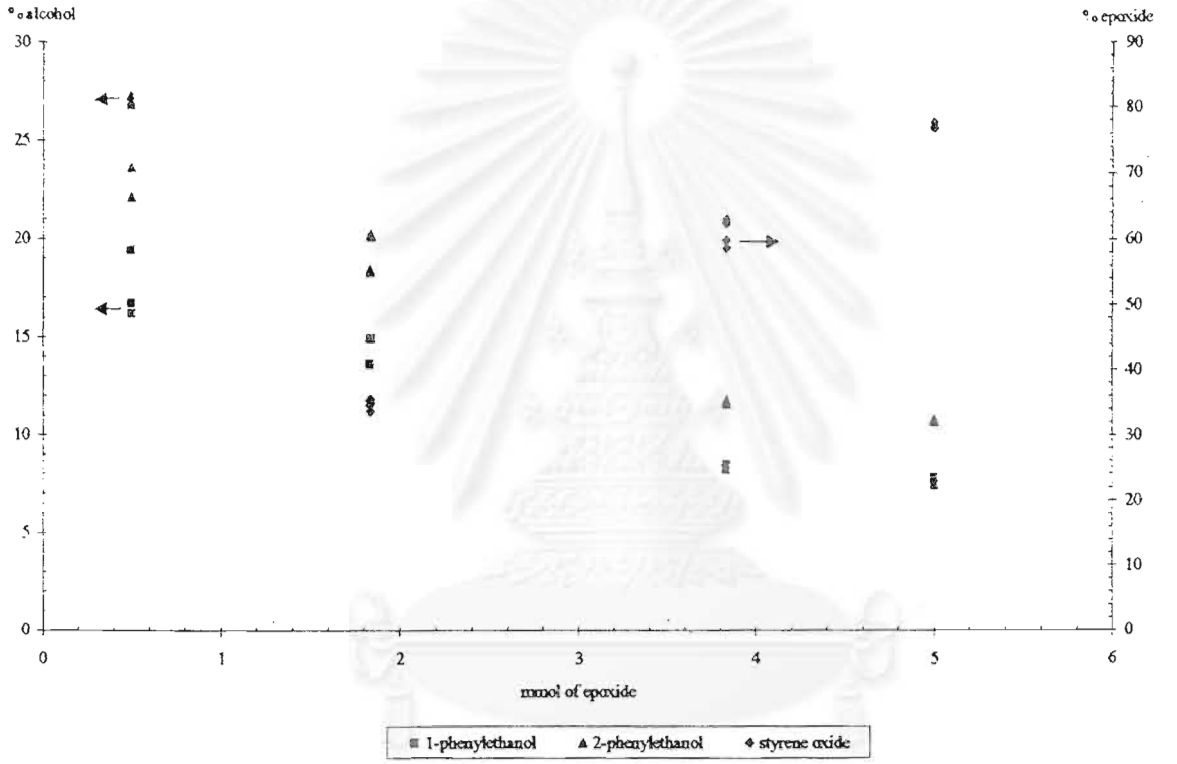


Figure 3.3 The reactant and product distribution at different amount of styrene oxide.

According to the above figure, the percentage of alocohol products increased as the amount of starting epoxide decreased while keeping the amount of reagent constant. The increased yield of product at lower amount of epoxide was possibly due to better dispersion of epoxide in water. The reaction condition obtained in this section will be used in the reductive ring-opening reaction of styrene oxide in the presence of cyclodextrin and its derivatives.

3.8.4 *Extraction efficiency in sample preparation step*

Since the total amount of products and unreacted epoxide was not equal to 100 %, the extraction efficiency of each compound had to be determined. The procedure was given as follow. Four different mixtures of the known amount of styrene oxide, 1-phenylethanol and 2-phenylethanol were stirred with 3.0 mL solution of $NaOH/H_3BO_3$, pH 11 at room temperature for 2 hours. Then, 7 mL of water was added to each mixture and the extraction was followed as described in general procedure. The experiments were performed in duplicate.

3.8.5 *Reductive ring-opening reaction in the presence of cyclodextrin and its derivatives*

The condition for the ring-opening reaction of styrene oxide in the presence of chiral selector, cyclodextrin or its derivatives, was given as follows. For the reaction that the amount of cyclodextrin was less than or equal to 0.18 mmol, certain amount of styrene oxide and 3 mL of cyclodextrin solution were mixed and stirred for 15 min at room temperature. Then, $NaBH_4$ (10 mmol) was added. After stirring the reaction for 24 h, 7 mL of water was added and the reaction mixture was extracted with hexane, dried over anhydrous sodium sulfate. Hexane was added to the solution to adjust volume in a 25 mL volumetric flask. Then, 2.0 mL of hexane solution was passed through a silica gel column and eluted with hexane-diethyl ether (2:3, v/v). The eluent from silica gel column was collected and transfered to a 10 mL volumetric flask. One mL of 0.076 %w/v naphthalene solution in hexane was added and the volume was adjusted with hexane. The solution was then analyzed by gas chromatcgraphy.

In the case that the amount of selector used was more than 0.18 mmol, certain amount of epoxide in 3.0 mL water was stirred for 10 min at room temperature, cyclodextrin (or its derivatives) was added and continued stirring for another 15 min. Then, $NaBH_4$ (10 mmol) was added. After stirring the reaction for 24 h, 7 mL of water was added and the reaction mixture was filtered under vacuum. The filtrate was extracted with hexane, dried over anhydrous sodium sulfate. Hexane was added to the solution to adjust volume in a 25 mL volumetric flask. The rest of the procedure was carried out similar to the procedure outlined above. However, when the starting epoxide of 5 mmol was used, only 1.0 mL of hexane solution was purified by column chromatography.

3.8.5.1 *Extraction efficiency in sample preparation step*

Two different mixtures of the known amount of styrene oxide, 1-phenylethanol and 2-phenylethanol were stirred in 3.0 mL solution of 0.04 M TRIMEB in $NaOH/H_3BO_3$, pH 11 at room temperature for 2 hours. Then, 7 mL of water was added and extraction was performed followed the procedure described for the reaction that used cyclodextrin less than or equal to 0.18 mmol. The experiments were performed in duplicate.

3.8.5.2 *Effect of TRIMEB in reductive ring-opening of styrene oxide*

In this study, the starting amount of epoxide of 0.6 mmol was used and TRIMEB was added into the reaction from 0.1 to 0.3 equivalent. The percentage yield and enantiomeric excess were calculated and compared to the control reaction (where there was no cyclodextrin added). The amount of TRIMEB that provided the highest yield of alcohol products would be used in the next study.

3.8.5.3 *Effect of the structure of selector*

The effect of the presence of hydroxyl groups in the structure of cyclodextrin on the yield and enantiomeric excess was studied by using three types of cyclodextrin: underivatized, RAMEB and TRIMEB. The amount of starting epoxide was kept constant at 0.6 mmol and the equivalent of cyclodextrin used was from the previous study.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characteristic of the coated capillary columns

The column efficiency of the reference and cyclodextrin columns was determined at 160°C with performance evaluation sample. The number of theoretical plates was calculated from haxadecane peak (with capacity factor, k' of 3 - 5) to be greater than 3000 plates/m on both columns. Adsorptive characteristic of column was evaluated from asymmetric factor of alcohols and naphthalene peak and it was found to be insignificant. The separation of the mixture of styrene oxide, 1-phenylethanol, 2-phenylethanol and naphthalene (internal standard) in hexane on cyclodextrin-containing stationary phase was shown in figure 4.1 (for detail see also figure B6 in appendix B).

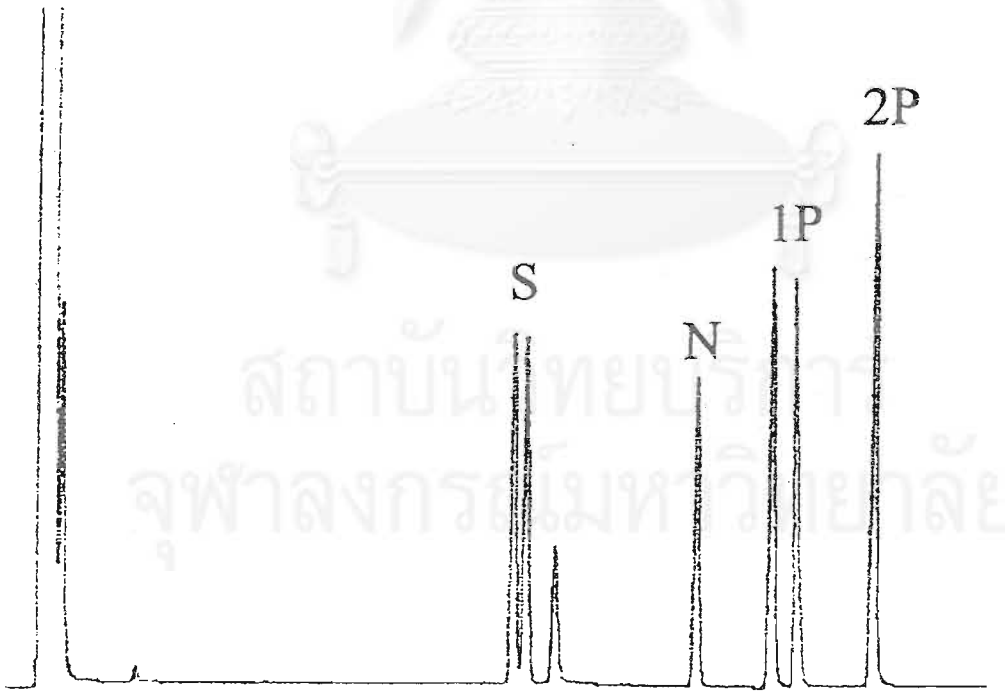


Figure 4.1 The separation of styrene oxide (S), 1-phenylethanol (1P), 2-phenylethanol (2P) and naphthalene (N) on cyclodextrin column using temperature program as described in section 3.6

4.2 Gas chromatographic determination of thermodynamic parameters

The thermodynamic parameters responsible for the separation of enantiomers of epoxide and alcohol with TRIMEB containing stationary phase were calculated using two different methods.

Method A, the relationship between ln k′ vs 1/T of the epoxide and alcohols was shown in figure 4.2. The relationships are all linear with the correlation coefficients greater than 0.999 (see table 4.1) and the calculated $\Delta H°$, $\Delta S°$ of each compound were reported in table 4.2.

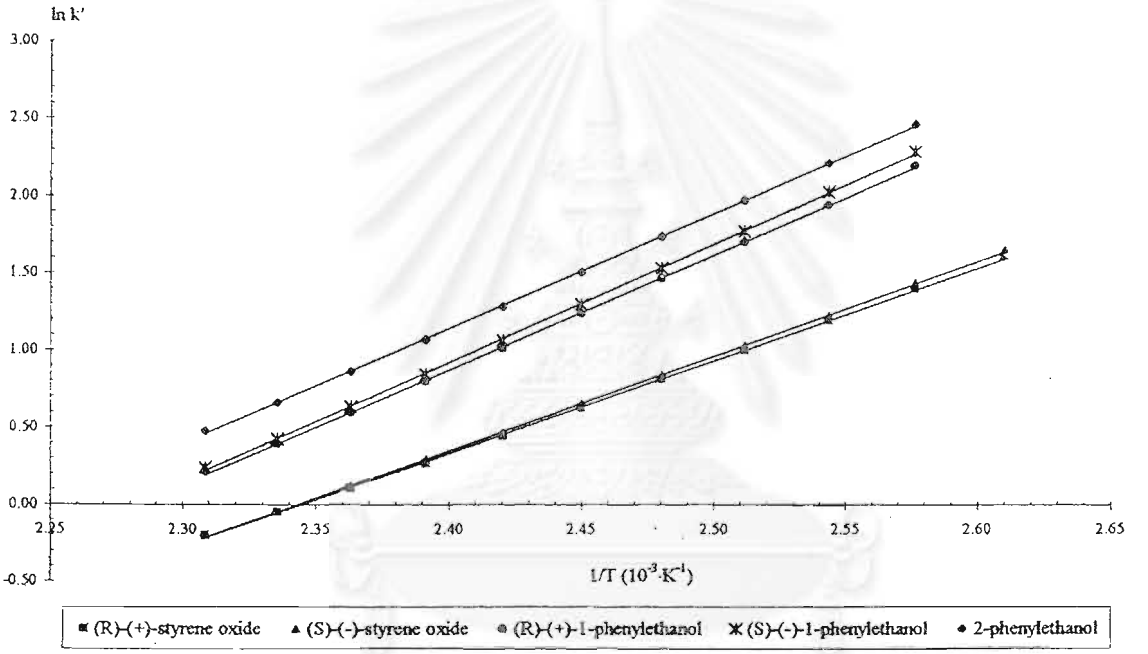


Figure 4.2 Plots of ln (capacity factor) versus reciprocal of temperature of epoxide and alcohols obtained from TRIMEB containing stationary phase.

Table 4.1 Equation and correlation coefficient for the relationships in figure 4.2

compounds	equation	correlation coefficient (R^2)
undecane	ln k′ = 6.053(1/T) – 14.94	0.9996
(*R*)-(+)-styrene oxide	ln k′ = 5.985(1/T) – 14.04	0.9997
(*S*)-(-)-styrene oxide	ln k′ = 6.135(1/T) – 14.38	0.9998
(*R*)-(+)-1-phenylethanol	ln k′ = 7.412(1/T) – 16.92	0.9998
(*S*)-(-)-1-phenylethanol	ln k′ = 7.643(1/T) – 17.43	0.9997
2-phenylethanol	ln k′ = 7.423(1/T) – 16.68	0.9998

Table 4.2 Thermodynamic parameters of epoxide and alcohols calculated from the plot shown in figure 4.2

compounds	ΔH° (cal/mol)	ΔS° (cal/mol·K)
undecane	-12.03	-40.66
(*R*)-(+)-styrene oxide	-11.89	-38.87
(*S*)-(-)-styrene oxide	-12.19	-39.54
(*R*)-(+)-1-phenylethanol	-14.73	-44.59
(*S*)-(-)-1-phenylethanol	-15.19	-45.60
2-phenylethanol	-14.75	-44.11

From figure 4.2, thermodynamic parameters that correspond to the separation of the enantiomers of epoxide and alcohols on the TRIMEB containing phase could be calculated from the plots of ln (chiral selectivity) versus the reciprocal of temperature (figure 4.3). The resulted Δ(ΔH°) of styrene oxide and 1-phenylethanol were – 0.23 and – 0.46 cal/mol and Δ(ΔS°) were – 0.50 and – 1.01 cal/mol·K, respectively.

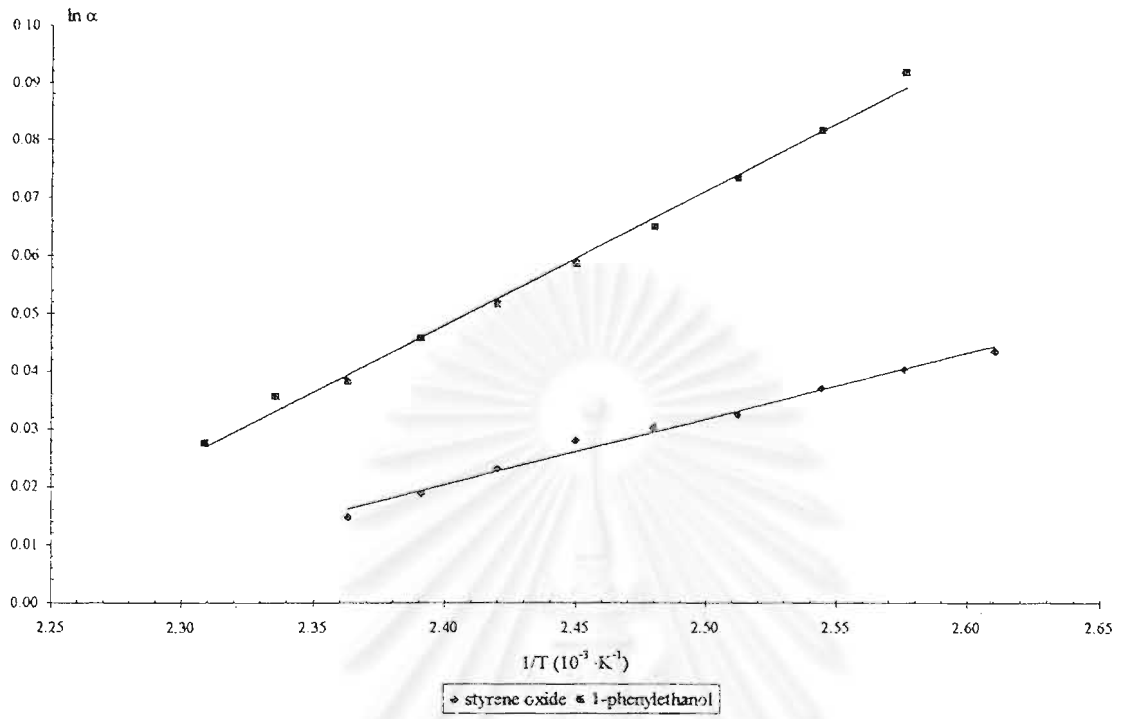


Figure 4.3 Plots of ln (chiral selectivity) versus reciprocal of temperature of styrene oxide and 1-phenylethanol obtained from the TRIMEB containing stationary phase.

Table 4.3 Equation and correlation coefficient for the relationships in figure 4.3

compounds	equation	correlation coefficient (R^2)
styrene oxide	$\ln \alpha = 0.114(1/T) - 0.25$	0.9894
1-phenylethanol	$\ln \alpha = 0.233(1/T) - 0.51$	0.9949

Method B, thermodynamic parameters were calculated from the plot of ln (retention increment) versus the reciprocal of temperature. The results were shown in figure 4.4 and tables 4.4 and 4.5.

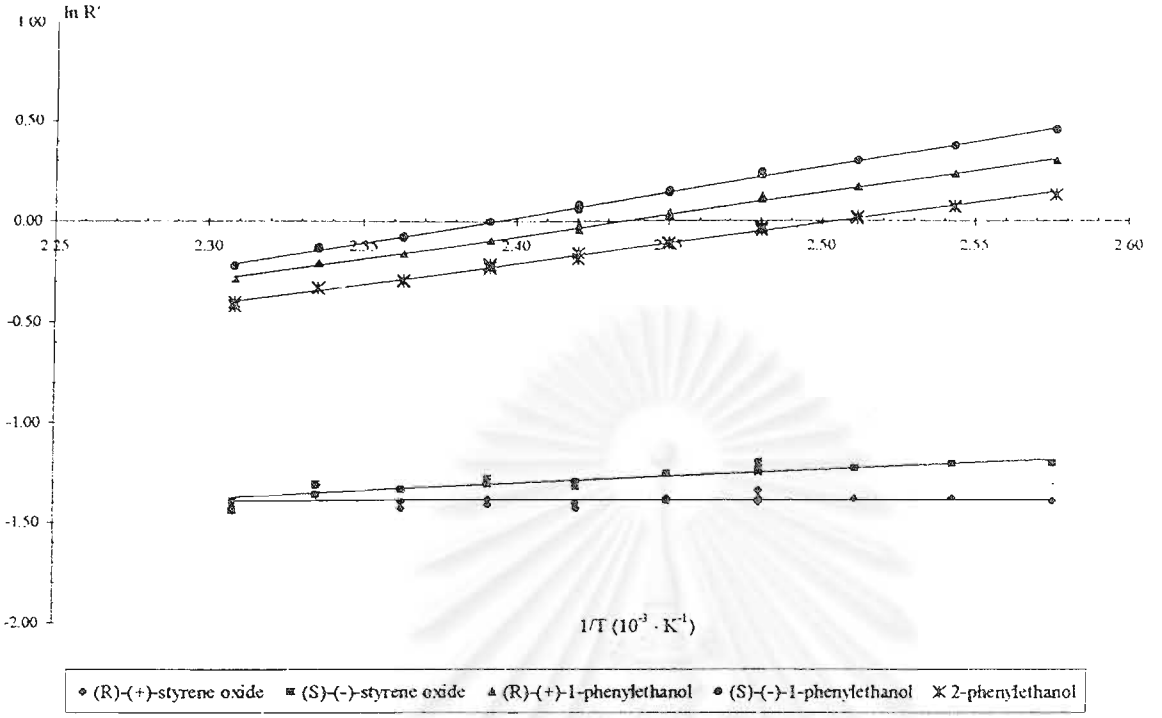


Figure 4.4 Plots of ln (retention increment) versus reciprocal of temperature of epoxide and alcohols obtained from TRIMEB containing stationary phase.

Table 4.4 Equation and correlation coefficient for the relationships in figure 4.4

compounds	equation	correlation coefficient (R^2)
(*R*)-(+)-styrene oxide	ln R′ = – 0.010(1/T) – 1.37	0.0006
(*S*)-(-)-styrene oxide	ln R′ = 0.702(1/T) – 2.99	0.8599
(*R*)-(+)-1-phenylethanol	ln R′ = 2.188(1/T) – 5.33	0.9977
(*S*)-(-)-1-phenylethanol	ln R′ = 2.515(1/T) – 6.02	0.9980
2-phenylethanol	ln R′ = 2.020(1/T) – 5.06	0.9950

Table 4.5 Thermodynamic parameters of epoxide and alcohols calculated from the plot shown in figure 4.4

compounds	ΔH° (cal/mol)	ΔS° (cal/mol·K)
(*R*)-(+)-styrene oxide	0.02	0.69
(*S*)-(-)-styrene oxide	-1.39	-2.53
(*R*)-(+)-1-phenylethanol	-4.35	-7.18
(*S*)-(-)-1-phenylethanol	-5.00	-8.55
2-phenylethanol	-4.01	-6.65

In method B, thermodynamic parameters that correspond to the separation of the enantiomer of styrene oxide and 1-phenylethanol were determined from the plots of ln (ratio of retention increment of the pair of enantiomer) versus reciprocal of temperature as shown in figure 4.5 with their corresponding equation in table 4.6.

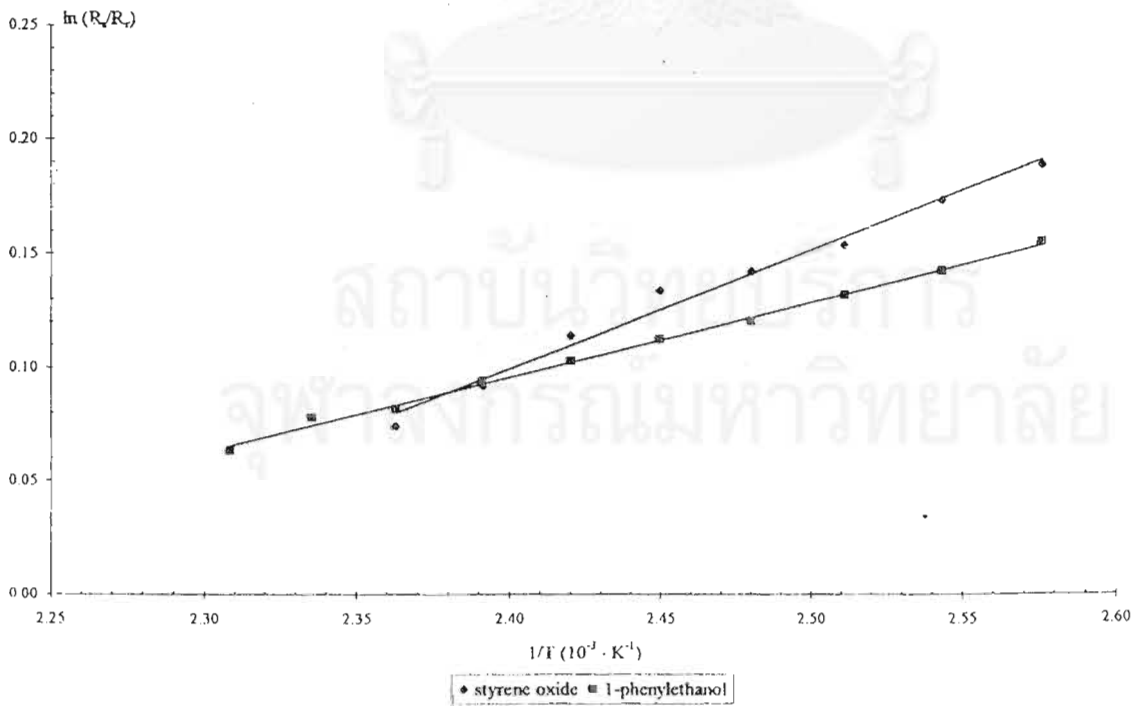


Figure 4.5 Plots of ln (ratio of retention increment of the pair of enantiomer) versus reciprocal of temperature of styrene oxide and 1-phenylethanol.

Table 4.6 Equation and correlation coefficient for the relationships in figure 4.5

compounds	equation	correlation coefficient (R^2)
styrene oxide	$\ln\left(\frac{R'_S}{R'_R}\right) = \frac{0.521}{T} - 1.15$	0.9851
1-phenylethanol	$\ln\left(\frac{R'_S}{R'_R}\right) = \frac{0.328}{T} - 0.69$	0.9963

From the slope and y-intercept of the plots in figure 4.5, the difference in enthalpy values, $\Delta(\Delta H°)$, for two enantiomers of styrene oxide and 1-phenylethanol were – 1.03 and – 0.65 cal/mol and the difference in entropy values, $\Delta(\Delta S°)$, for two enantiomers of styrene oxide and 1-phenylethanol were – 2.29 and – 1.37 cal/mol·K, respectively.

Table 4.7 Comparison of thermodynamic parameters of styrene oxide and phenylethanols obtained from two methods.

thermodynamic parameters	method A			method B		
	styrene oxide	1-phenyl ethanol	2-phenyl ethanol	styrene oxide	1-phenyl ethanol	2-phenyl ethanol
$\Delta H°$ (cal/mol)	-11.89 *R* -12.19 *S*	-14.73 *R* -15.19 *S*	-14.75	+0.02 *R* -1.39 *S*	-4.35 *R* -5.00 *S*	-4.01
$\Delta S°$ (cal/mol·K)	-38.87 *R* -39.54 *S*	-44.59 *R* -45.60 *S*	-44.11	+0.69 *R* -2.53 *S*	-7.18 *R* -8.55 *S*	-6.65
$\Delta(\Delta H°)$ (cal/mol)	-0.23	-0.46		-1.03	-0.65	
$\Delta(\Delta S°)$ (cal/mol·K)	-0.50	-1.02		-2.29	-1.37	

According to the calculated thermodynamic parameters (see table 4.7), it can be concluded that the interaction between the S-enantiomer of styrene oxide and stationary phase was stronger than the interaction between the R-enantiomer and stationary phase (larger negative ΔH° and ΔS° values). When the effect of polysiloxane was eliminated, as in method B, more information on the interaction between styrene oxide and TRIMEB in the stationary phase was revealed. The positive sign of enthalpy and entropy indicated that the interaction between the R-enantiomer and cyclodextrin was extremely weak (not favorable), while the interaction between the S-enantiomer and cyclodextrin was stronger (negative enthalpy and entropy values). Therefore, the enantiomers of styrene oxide could be separated with the R-enantiomer eluted before the S-enantiomer. In the case of 1-phenylethanol, both enantiomers showed stronger interaction with cyclodextrin than enantiomers of styrene oxide (larger negative values). Moreover, entropy values obtained from method B indicated the higher probability of multiple interactions between 1-phenylethanol and cyclodextrin than those between styrene oxide and cyclodextrin. The differences in enthalpy and entropy between two enantiomers [$\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ values] of both styrene oxide and 1-phenylethanol obtained from method A were lower than those from method B. This indicates the additional interaction contributed from nonchiral polysiloxane (in method A) which is the same for both R- and S-isomers, but has no influence on chiral separation.

4.3 Reductive ring-opening of styrene oxide with sodium borohydride in the presence of cyclodextrin and its derivatives

4.3.1 *Extraction efficiency in sample preparation step*

The extraction efficiency of each compound in the presence and in the absence of cyclodextrin was shown in table 4.8. These values will be used in all following sections.

Table 4.8 Extraction efficiency of epoxide and alcohols in systems without cyclodextrin and with 0.04 M TRIMEB.

compounds	extraction efficiency (%)	
	without CD	with 0.04 M TRIMEB
styrene oxide	85.3	84.5
1-phenylethanol	50.4	45.9
2-phenylethanol	43.6	34.4

The extraction efficiencies of epoxide and alcohols are similar in both systems. However, the amount of recovered styrene oxide is much higher than those of two alcohols due to lower water solubility of styrene oxide.

4.3.2 *Effect of TRIMEB in reductive ring-opening of styrene oxide*

The effect of the addition of TRIMEB in reaction was the first parameter being studied. Styrene oxide (0.6 mmol) was allowed to react with sodium borohydride (10 mmol) in 3 mL of water with different amount of TRIMEB. The result was shown in table 4.9.

Table 4.9 Reactant and product distribution at different amount of TRIMEB

amount of TRIMEB (eq)	% recovered styrene oxide	% extracted 1-phenylethanol	% extracted 2-phenylethanol	% total extractable amount
0.0	1.4	33.9	52.7	88.0
0.1	2.0	30.9	52.7	85.6
0.2	7.3	39.0	67.5	113.8
0.3	1.3	3.9	8.1	13.3

From table 4.9, the main product obtained from the controlled reaction was 2-phenylethanol (52.7%) with 1-phenylethanol as minor product (33.9%). As TRIMEB wad added into the reaction, there was no significant change in yield or product distribution. The enantioselectivity of 1-phenylethanol could not be

attained even with the presence of chiral selector. As the amount of added TRIMEB reached 0.3 equivalent, a big white solid was observed at the end of reaction. It was suspected to be an inclusion complex of styrene oxide or alcohol and cyclodextrin. This was supported by the decrease in total amount recovered of only 13.3%. Therefore, 0.2 equivalent of selector will be used for further study.

4.3.3 *Effect of the structure of selector*

According to results from previous articles [23-27] that cyclodextrin and derivatives were used in the reductive ring opening of epoxide, the enantioselectivity of chiral alcohol product could be obtained. The difference between previous work and this study was the structure of cyclodextrin. In this study the structure of cyclodextrin derivative used was fully derivatized; while in the other previous work, cyclodextrin or derivatives possessing some hydroxyl groups in the structure were used. Therefore, the effect of hydroxyl groups in the structure of selector was next studied by using three types of cyclodextrin: underivatized, randomly methylated (RAMEB) and fully methylated cyclodextrins (TRIMEB). Styrene oxide (0.6 mmol) was allowed to react with sodium borohydride (10 mmol) in 3 mL of cyclodextrin solution (0.2 equivalent of selector), the result was shown in table 4.10.

Table 4.10 Reactant and product distribution when using various types of cyclodextrin.[0.6 mmol of starting epoxide and 0.1 mmol (0.2 eq) of selector]

type of cyclodextrin	% recovered styrene oxide	% extracted 1-phenylethanol	% extracted 2-phenylethanol	% total extractable amount
-	n.d	26.4	39.4	65.8
underivatized	n.d	19.4	25.0	44.4
RAMEB	1.9	41.2	46.6	89.7
TRIMEB	2.0	8.3	13.7	24.0

n.d. = not detectable

From table 4.10, non-chiral product, 2-phenylethanol, was still the main product and the obtained 1-phenylethanol was still racemic similar to those from controlled reaction. No enantioselectivity was observed in all reactions containing cyclodextrin-based selectors. In order to get better understanding of this result, the experiment by Hu et al.[24] was modified. The reason why Hu's experiment was chosen because reaction conditions such as volume of water, equivalent of cyclodextrin and temperature were almost identical to parameters used in this study. Next, the starting epoxide was increased to 5 mmol while keeping the reducing agent at 10 mmol and the selector at 0.2 equivalent, and the reactions were repeated. The effect of higher amount of epoxide and selector was shown in table 4.11.

Table 4.11 Reactant and product distribution when using various types of cyclodextrin. [5 mmol of starting epoxide and 1.0 mmol (0.2 eq) of selector]

type of cyclodextrin	% recovered styrene oxide (% ee)	% extracted 1-phenylethanol (% ee)	% extracted 2-phenylethanol	% total extractable amount
-	81.4	11.5	17.4	110.3
underivatized	45.2 (20.6, *S*)	30.9 (20.3, *S*)	15.4	91.5
RAMEB	25.7	9.4	4.4	39.5
TRIMEB	40.8	3.7	4.9	49.4

The result shown in table 4.11 for the underivatized cyclodextrin was similar to the result reported by Hu et al.[24]. With underivatized cyclodextrin as a selector, the main product was 1-phenylethanol with 20.3% enantiomeric excess of S-enantiomer while 2-phenylethanol was the main product in the absence of selector (see figure 4.6). In the case of RAMEB, the main product was also the chiral 1-phenylethanol, but it was still racemic. From the result in tables 4.10 and 4.11, the interesting point was that the change in product selectivity could be realized when only the starting epoxide was increased while keeping the equivalent of selector, reagent and volume of media constant. The result suggests that the number of mole of

added cyclodextrin have more influence to product selectivity than the number of equivalent of selector.

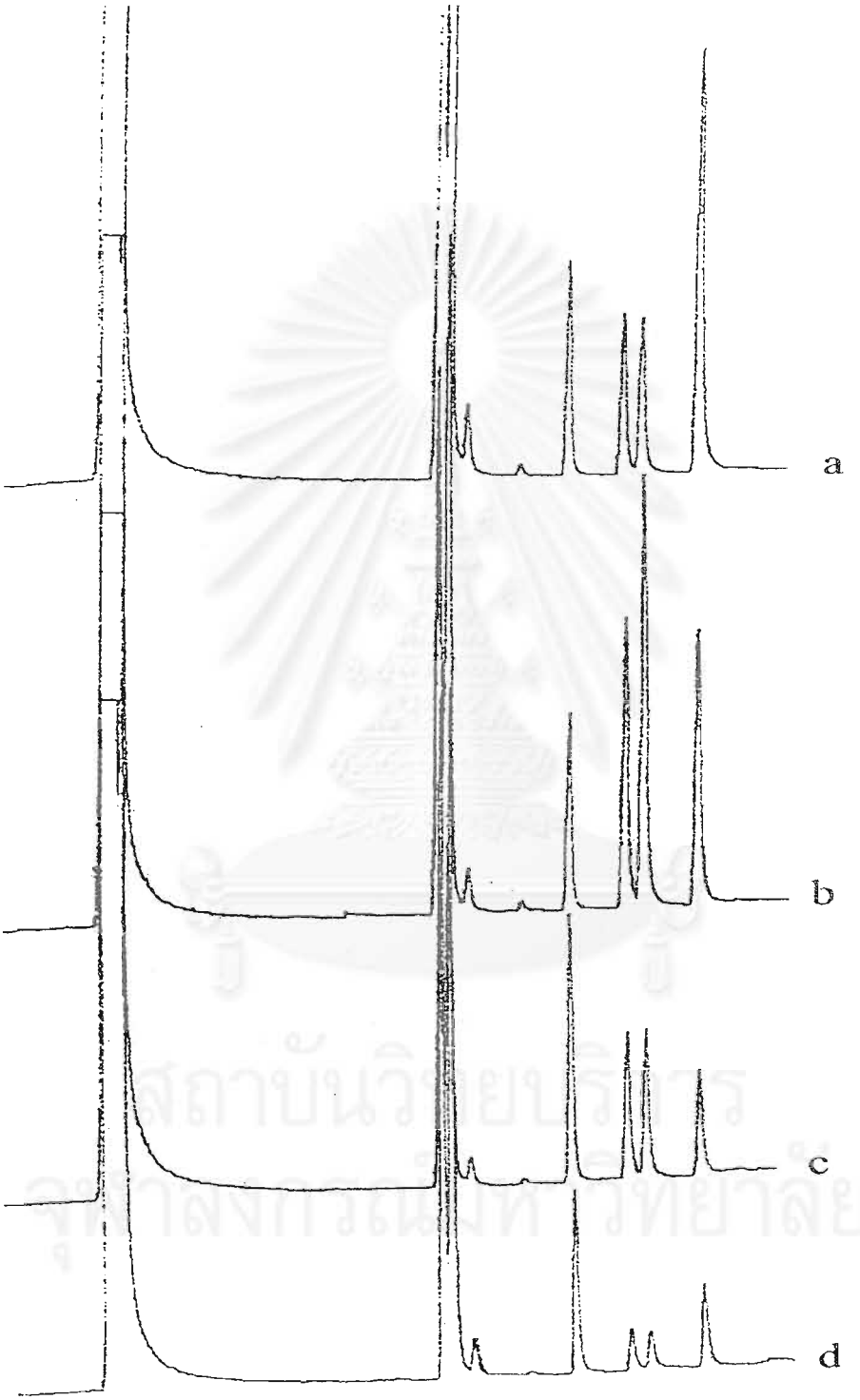


Figure 4.6 Separation of purified mixture from the reductive ring-opening of styrene oxide: (a) in the absence of cyclodextrin ; and in the presence of (b) underivatized cyclodextrin; (c) RAMEB ; and (d) TRIMEB. [5 mmol of starting epoxide and 1.0 mmol (0.2 eq) of selector]

To confirm this hypothesis, a large amount of cyclodextrin was added into the reaction to study the effect on selectivity of product. Amount of starting epoxide was again reduced to 0.6 mmol and it was allowed to react with 10 mmol of reagent in the presence of 0.7 and 1.0 equivalent (0.4 and 0.6 mmol) of three types of cyclodextrin. The result was reported as in tables 4.12 and 4.13.

Table 4.12 Reactant and product distribution when using various types of cyclodextrin.[0.6 mmol of starting epoxide and 0.4 mmol (0.7 eq) of selector]

type of cyclodextrin	% recovered styrene oxide	% extracted 1-phenylethanol (% ee)	% extracted 2-phenylethanol	% total extractable amount
-	n.d	20.6	32.1	52.7
underivatized	n.d	36.2 (11.0, *S*)	14.8	51.0
RAMEB	3.3	24.4	11.9	39.6
TRIMEB	8.3	8.9	18.9	36.1

n.d. = not detectable

Table 4.13 Reactant and product distribution when using various types of cyclodextrin. [0.6 mmol of starting epoxide and 0.6 mmol (1.0 eq) of selector]

type of cyclodextrin	% recovered styrene oxide	% extracted 1-phenylethanol (% ee)	% extracted 2-phenylethanol	% total extractable amount
underivatized	n.d	17.2 (10.4, *S*)	7.0	24.2
RAMEB	7.3	23.3	8.1	38.7
TRIMEB	40.0	3.9	7.6	51.5

n.d. = not detectable

Comparing the results from tables 4.12 and 4.13 with table 4.10, when the amount of cyclodextrin was changed from 0.12 mmol to 0.4 and 0.6 mmol with other parameters being constant, the main product was changed to chiral product, 1-phenylethanol, with 11.0 % and 10.4 % enantiomeric excess of S-enantiomer when

using underivatized cyclodextrin as a selector. In the case of RAMEB, the main product was also 1-phenylethanol but without enantioselectivity (see figure 4.7). These results support the idea that the number of mole of selector presented in the reaction has more influence to product selectivity than the number of equivalent of selector.

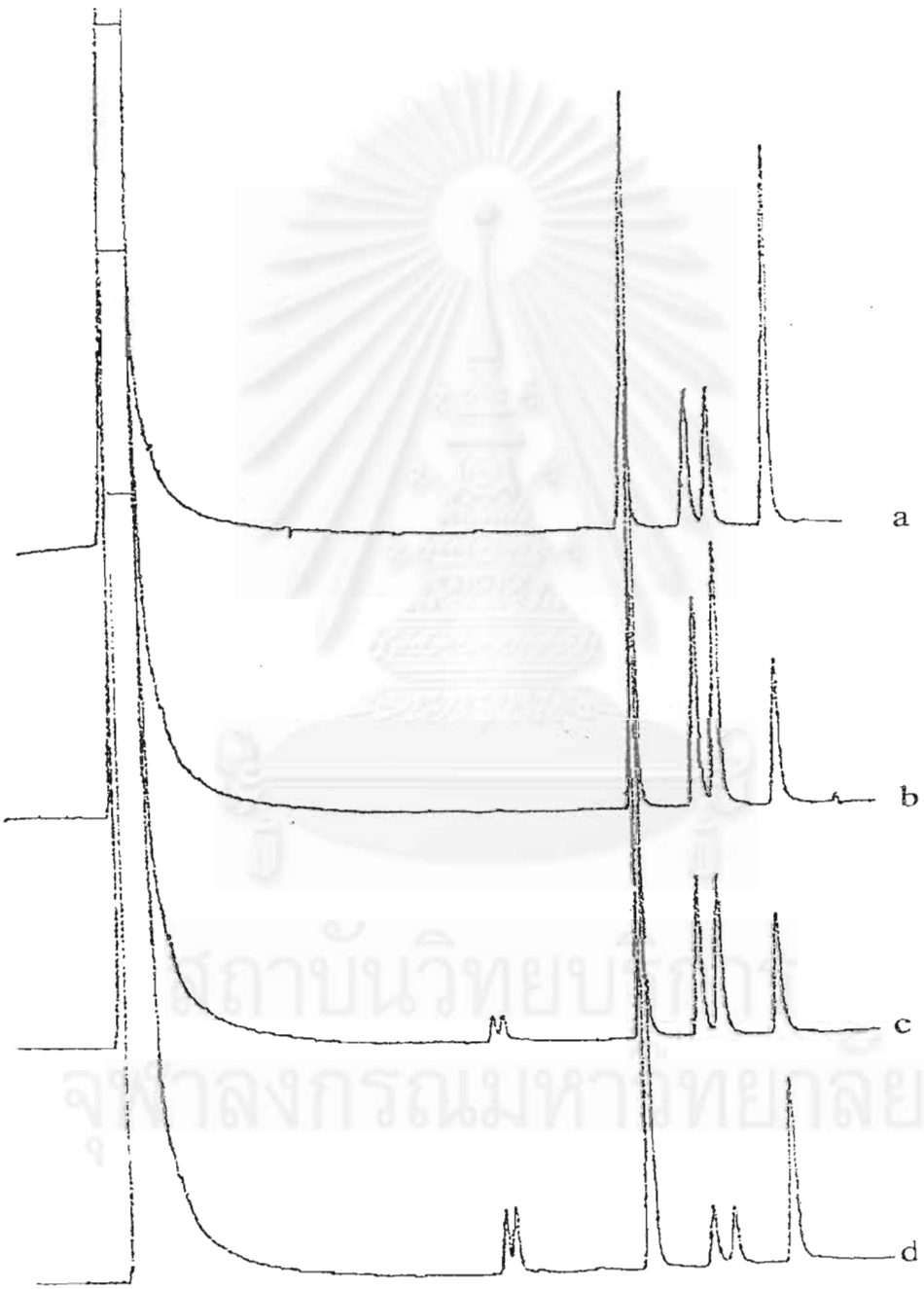


Figure 4.7 Separation of purified mixture from the reductive ring-opening of styrene oxide: (a) in the absence of cyclodextrin ; and in the presence of (b) underivatized cyclodextrin; (c) RAMEB ; and (d) TRIMEB. [0.6 mmol of starting epoxide and 0.4 mmol (0.7 eq) of selector]

TRIMEB still exhibited the same result. When a large amount of selector was used (1.0 eq), the white round solid was observed before the addition of reagent. The white solid formed in the reaction was probably an inclusion complex between styrene oxide and TRIMEB. This complex may protect the epoxide from being attacked by the reagent, thus large portion of epoxide was recovered at the end of reaction (40.0%). The relationship between the number of mole of selector and selectivity of the chiral product over the nonchiral product was summarized as in table 4.14.

Table 4.14 Effect of number of mole of selector on the selectivity and enantioselectivity of chiral product

amount of added cyclodextrin, mmol (equivalent)	% selectivity of 1-phenylethanol over 2-phenylethanol (% ee)		
	underivatized	RAMEB	TRIMEB
0.1 (0.2 eq)	43.7	46.9	37.7
0.4 (0.7 eq)	71.0 (11.0, *S*)	67.2	32.0
0.6 (1.0 eq)	71.1 (10.4, *S*)	74.2	33.9
1.0 (0.2eq)	66.7 (20.3, *S*)	68.1	43.0

The selectivity of 1-phenylethanol over 2-phenylethanol for the reaction without cyclodextrin was about 40 %

CHAPTER 5

CONCLUSION AND SUGGESTION FOR FUTURE WORK

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin was synthesized to use as a chiral gas chromatographic stationary phase and as a discriminator in reductive ring-opening of styrene oxide with sodium borohydride in water. The TRIMEB was mixed with polysiloxane before coating onto a capillary column. The mixture of styrene oxide, 1-phenylethanol and 2-phenylethanol could be analyzed for product distribution as well as enantiomeric excess of chiral components in the same chromatographic run. This is an advantage over the usual procedure which analyze the product distribution by non-chiral chromatographic technique and determine the enantiomeric excess by polarimetry or nuclear magnetic resonance spectroscopy.

The interactions between TRIMEB and all analytes in gas-liquid interface could be realized from the thermodynamic parameters obtained from gas chromatographic analysis. It is evident that the strength of interaction between TRIMEB and analytes is in the order: styrene oxide < 1-phenylethanol < 2-phenylethanol. For the chiral compounds, the S-enantiomers of both styrene oxide and 1-phenylethanol interact more strongly to TRIMEB than the R-enantiomers; therefore, the S-isomers elute after the R-isomers.

When TRIMEB was used as a chiral selector in reductive ring-opening of styrene oxide, no enantioselectivity on chiral product was observed. Product selectivity between 1-phenylethanol and 2-phenylethanol was also similar to the reaction without chiral selector, more 2-phenylethanol than 1-phenylethanol. When the amount of TRIMEB in the reaction was increased, similar results were still obtained. Moreover, a larger percentage of starting epoxide was left unreacted.

Two other types of cyclodextrin, underivatized and RAMEB (both possessing hydroxyl groups in their structures), were used in the same reaction. Both selectors offered different proportion of alcohol products, more 1-phenylethanol than 2-phenylethanol. However, enantioselectivity of 1-phenylethanol could only be obtained from underivatized cyclodextrin. This suggests that the hydroxyl groups presented in the structure of β-cyclodextrin have an influence on product distribution and enantioselectivity of chiral product. The uniformity of functional group of cyclodextrin was probably another factor influencing the enantioselectivity, as 1-phenylethanol extracted from reaction with RAMEB was racemic. Another interesting point discovered from this study was that the number of moles of cyclodextrin (and probably of styrene oxide) in the reaction plays more important role in selectivity and enantioselectivity of chiral product than the equivalent of selector.

One drawback from the use of cyclodextrin as discriminator in this study was the consumption of cyclodextrin in each reaction. Due to the limited amount of the synthesized selectors, the extraction efficiency of 0.04 M TRIMEB solution was used to calculate the product yield and selectivity for reactions containing underivatized cyclodextrin and RAMEB. The results could be improved if the extraction efficiencies of the mixture in underivatized cyclodextrin and RAMEB were determined at every concentration of selector added.

Finally, the information on the interaction between cyclodextrin and chiral analytes obtained from gas-liquid interface does not always correlate to the behavior in the solution. As it can be seen that TRIMEB could be used to separate enantiomers of styrene oxide and 1-phenylethanol by gas chromatography, but it showed no enantioselectivity in the solution. How cyclodextrin or its derivatives influences the product selectivity and enantioselectivity would be revealed if the association constant between enantiomers of styrene oxide and selector (especially TRIMEB) in aqueous solution could be determined.

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APPENDICES

Appendix A

Glossary

Capacity factor (k') is defined as the ratio of analyte masses in the stationary phase and mobiles phase. It is also equal to the ratio of the time of analyte molecules spend in the stationary phase (where they are stationary) to their time in the mobile phase (where they are transported down the column).

$$k' = \frac{t_r - t_m}{t_m}$$

where t_r is retention time of an analyte and t_m is time of an unretained compound.

Separation factor or *Selectivity (α)* is defined as the ratio of the capacity factor of the two adjacent peaks.

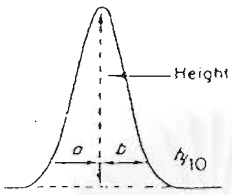
Phase ratio (β) is defined as the ratio of the volume of the mobile phase and stationary phase in the column. It depends only on the physical dimension of the column.

Number of theoretical plates or *Plate number (n)* is used as a measure of column efficiency. It is defined as the square of the ratio of the retention of analyte divided by the peak broadening.

$$n = 16\left(\frac{t_r}{w_b}\right)^2 = 5.54\left(\frac{t_r}{w_{h/2}}\right)^2$$

where w_b and $w_{h/2}$ is the width of the peak at its base and at half-height, respectively.

Asymmetric factor or *Tailing factor* (A_s) is usually for the indication of peak distortion (skew) which is normally measured as the ratio of the widths of the front and back of the peak at 10 % of the peak height.



$$A_s = \frac{b}{a}$$

Enantiomeric excess (ee) expresses the excess of one enantiomer over the other. In practice, it is often quoted as a percentage.

$$\%\,ee = \frac{E_1 - E_2}{E_1 + E_2} \cdot 100$$

where E_1 and E_2 are the amounts of the enantiomers (which directly obtained by peak integration of chromatograms) and E_1 is the major enantiomer.

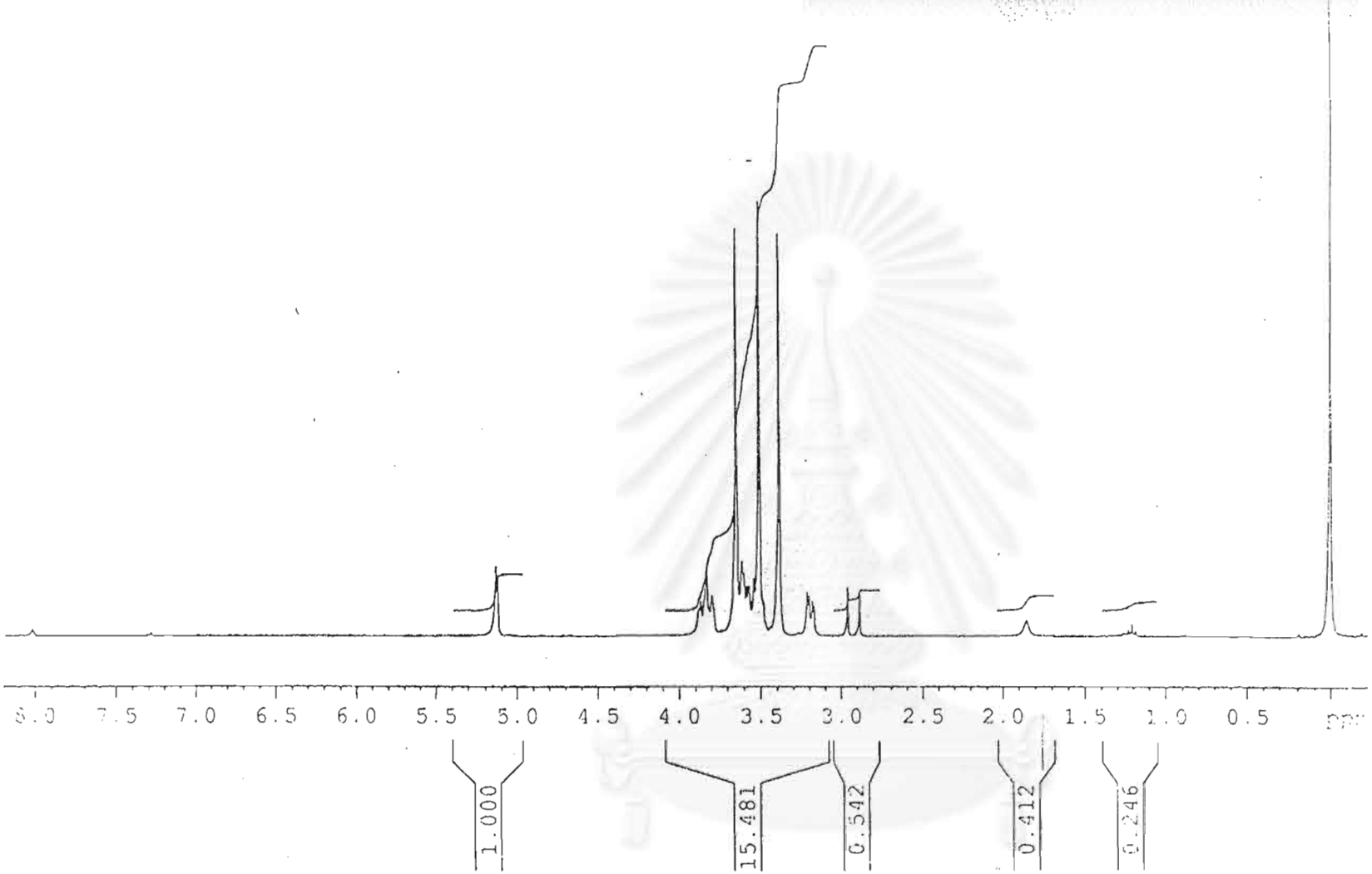


Figure B1 H 300 MHz NMR spectrum of the synthesized heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) in $CDCl_3$.

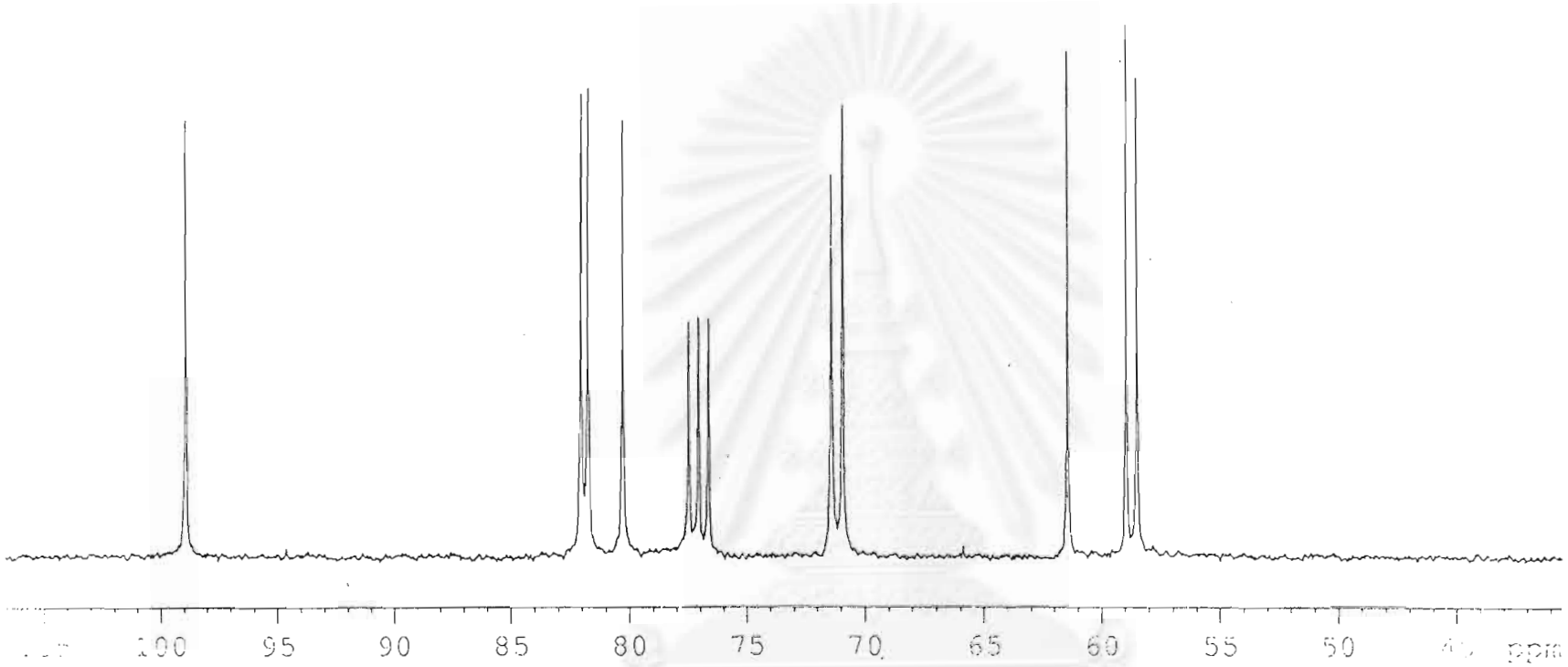


Figure B2 ^{13}C 75 MHz NMR spectrum of the synthesized heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TRIMEB) in $CDCl_3$.

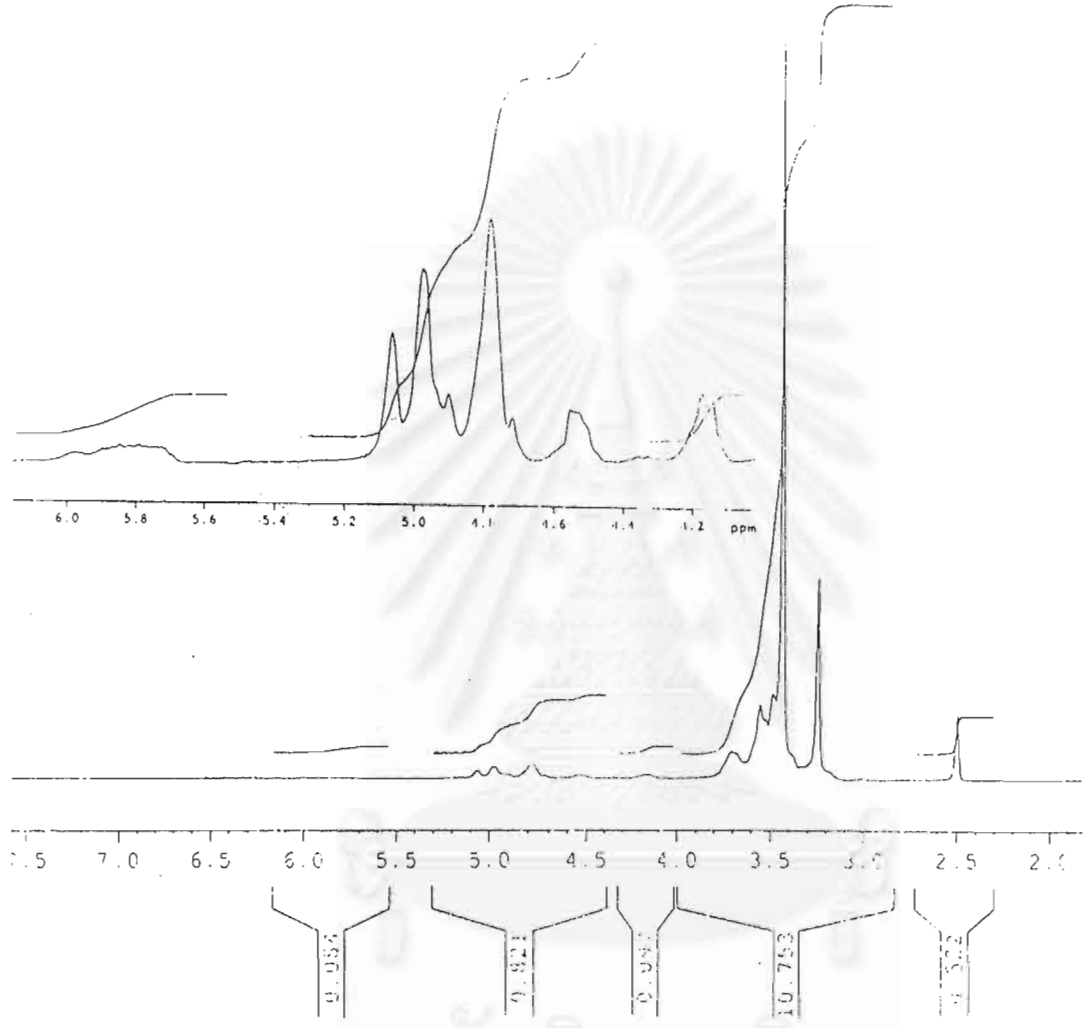
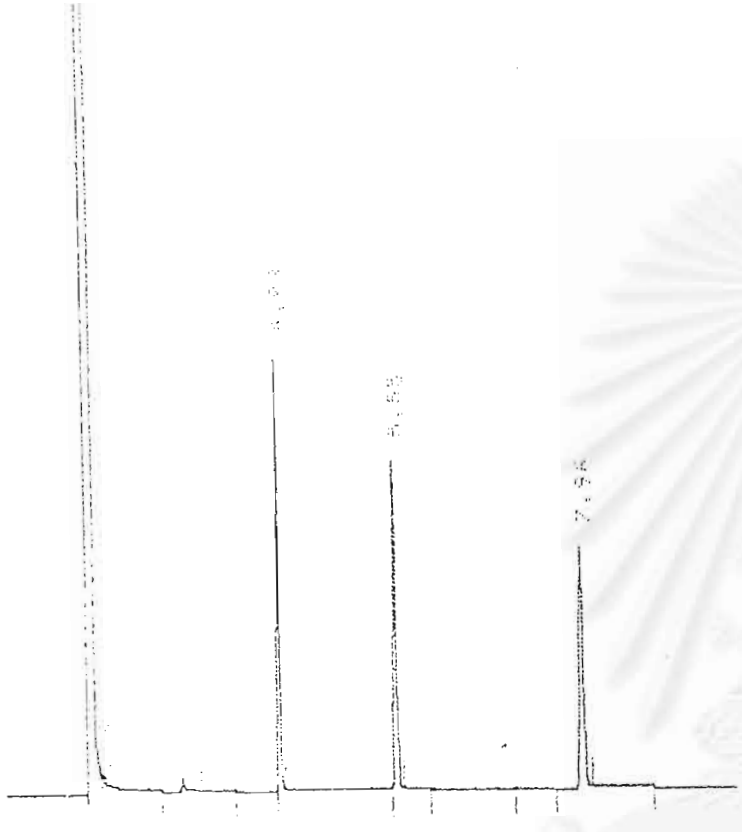


Figure B3 H 300 MHz NMR spectrum of the synthesized randomly methylated β-cyclodextrin (RAMEB) in d_6-DMSO.

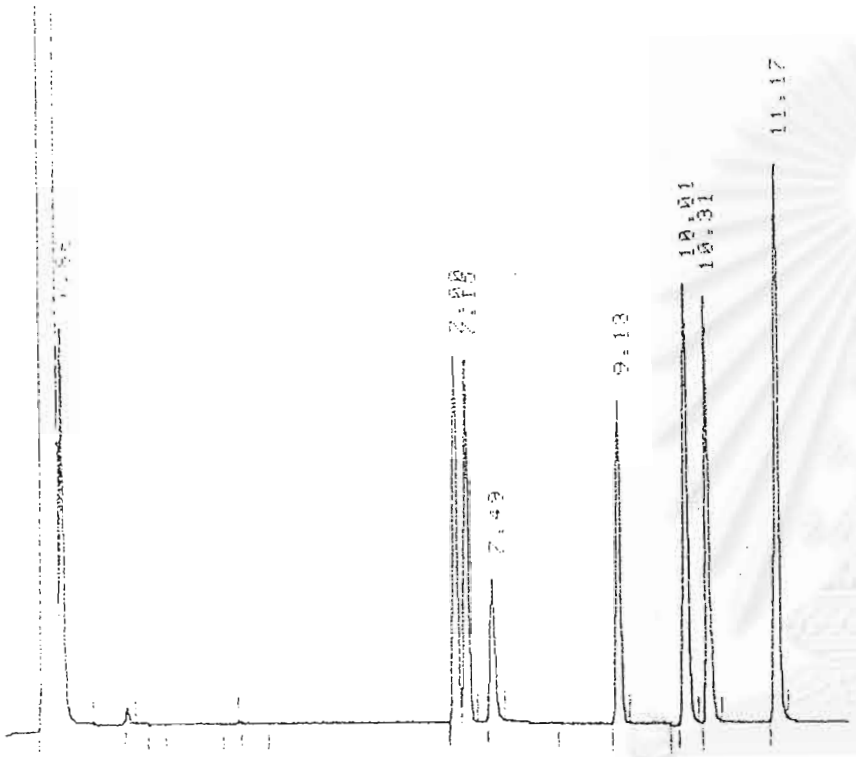
RT	AREA	TYPE	AR/HT
1.55	5943500	DSBB	0.019
2.34	326	D BV	0.023
3.51	4843	BB	0.026
4.67	4860	PB	0.033
6.48	4990	PB	0.046

Figure B4 The separation of n-tetradecane, n-pentadecane and n-hexadecane on a OV-1701 (reference column) ; 31.77 m long, 0.25 mm i.d., 0.25 μm film thickness, at 160 °C isothermal.



RT	AREA	TYPE	AR/HT
1.55	1.25E+07	SBB	0.032
4.03	5635	BB	0.031
5.55	5680	BB	0.042
7.96	5921	BB	0.059

Figure B5 The separation of n-tetradecane, n-pentadecane and n-hexadecane on a 17% w/w TRIMEB in OV-1701 (cyclodextrin column) ; 31.72 m long, 0.25 mm i.d., 0.25 μm film thickness, at 160 °C isothermal.



RT	AREA	TYPE	AR/HT
1.62	1.03E+07	SBB	0.033
1.73	768370	TBP	0.037
1.86	3784	TPB	0.034
7.00	10149	BV	0.067
7.15	10216	VB	0.068
7.49	4327	BB	0.072
9.13	8813	BB	0.066
10.01	12678	PB	0.070
10.31	12725	BB	0.072
11.17	15552	PB	0.067

Figure B6 The separation of standard styrene oxide, 1-phenylethanol, 2-phenylethanol and naphthalene on a 17% w/w TRIMEB in OV-1701 (cyclodextrin column) ; 31.72 m long, 0.25 mm i.d., 0.25 μm film thickness, separation condition: initial temperature 110 °C, then at 2 °C/min to 120 °C, then at 5 °C/min to 155 °C.

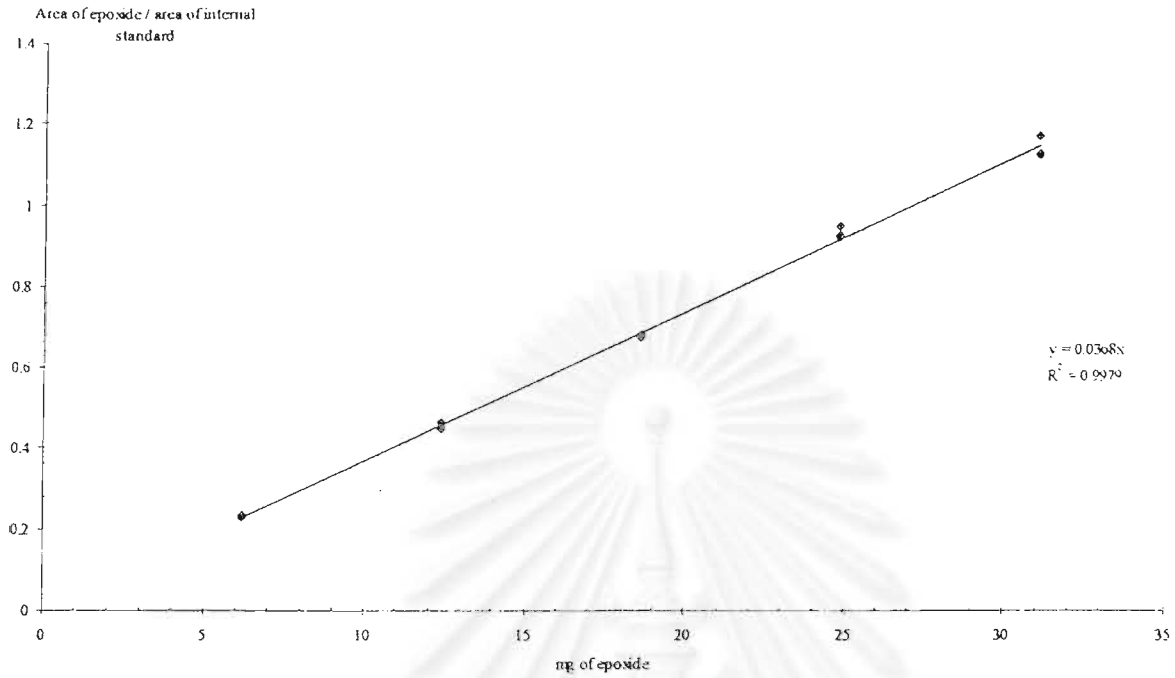


Figure B7 The calibration curve for determination of (*R*)-(+)-styrene oxide in reaction mixture.

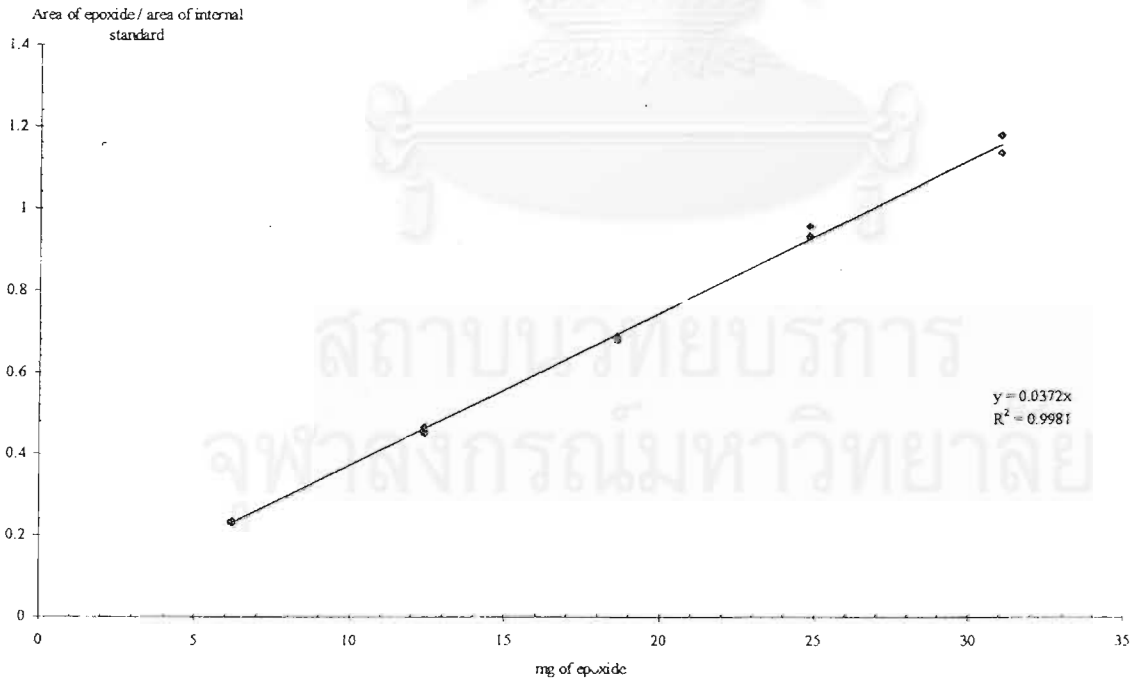


Figure B8 The calibration curve for determination of (*S*)-(-)-styrene oxide in reaction mixture.

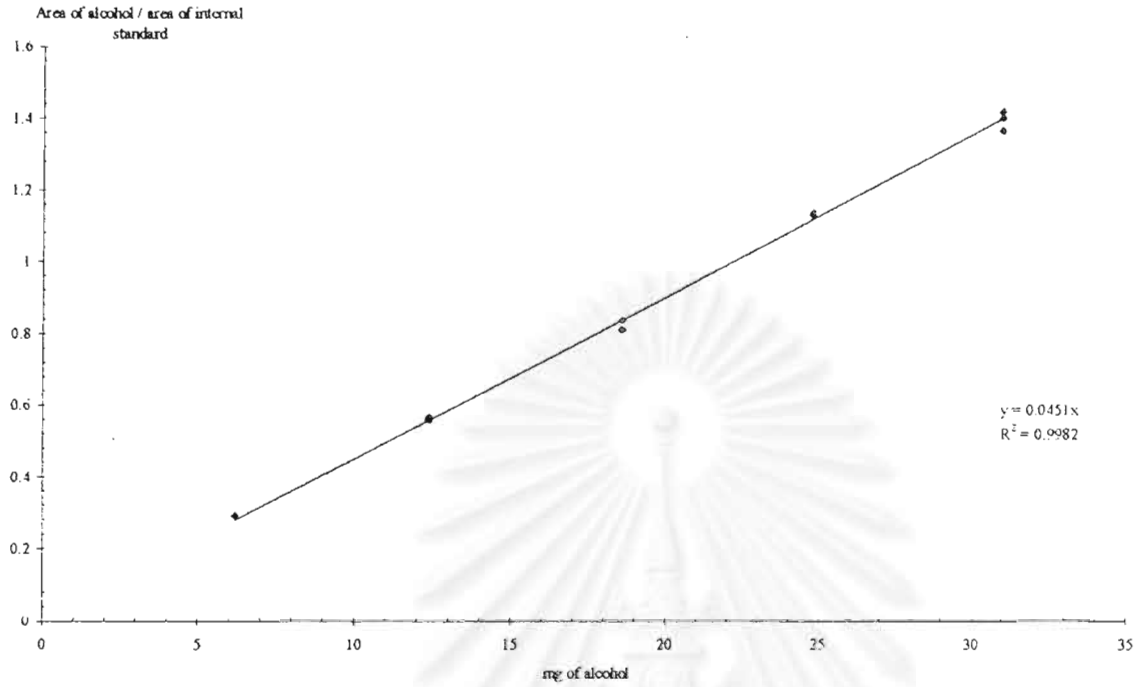


Figure B9 The calibration curve for determination of (*R*)-(+)-1-phenylethanol in reaction mixture.

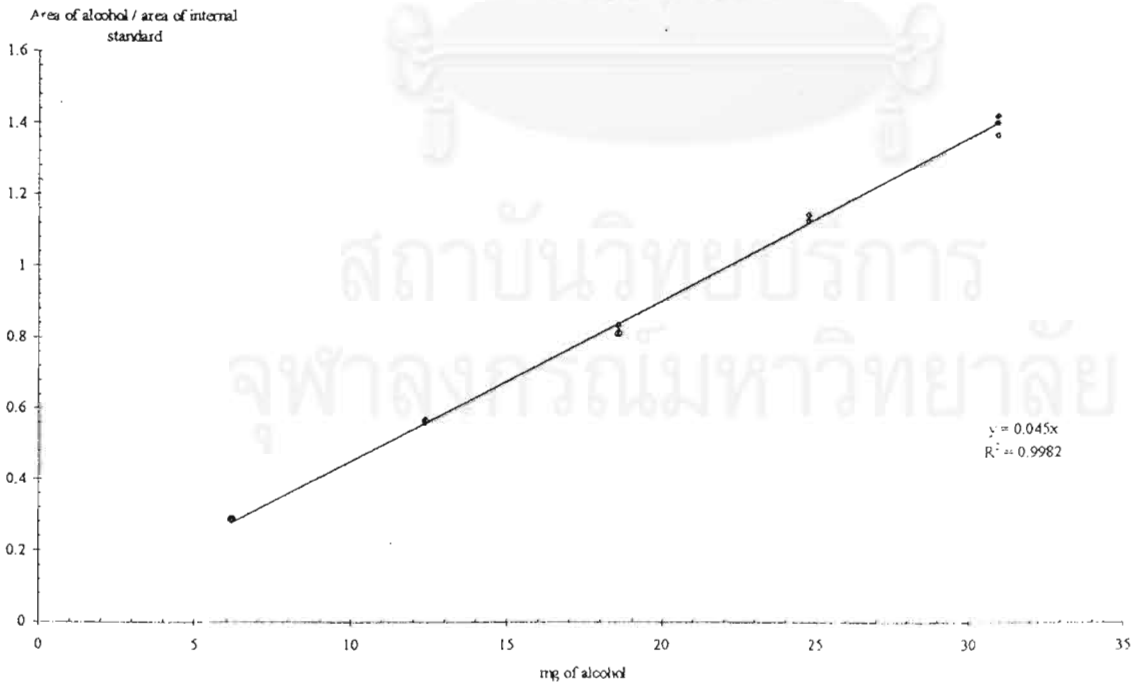


Figure B10 The calibration curve for determination of (*S*)-(-)-1-phenylethanol in reaction mixture.

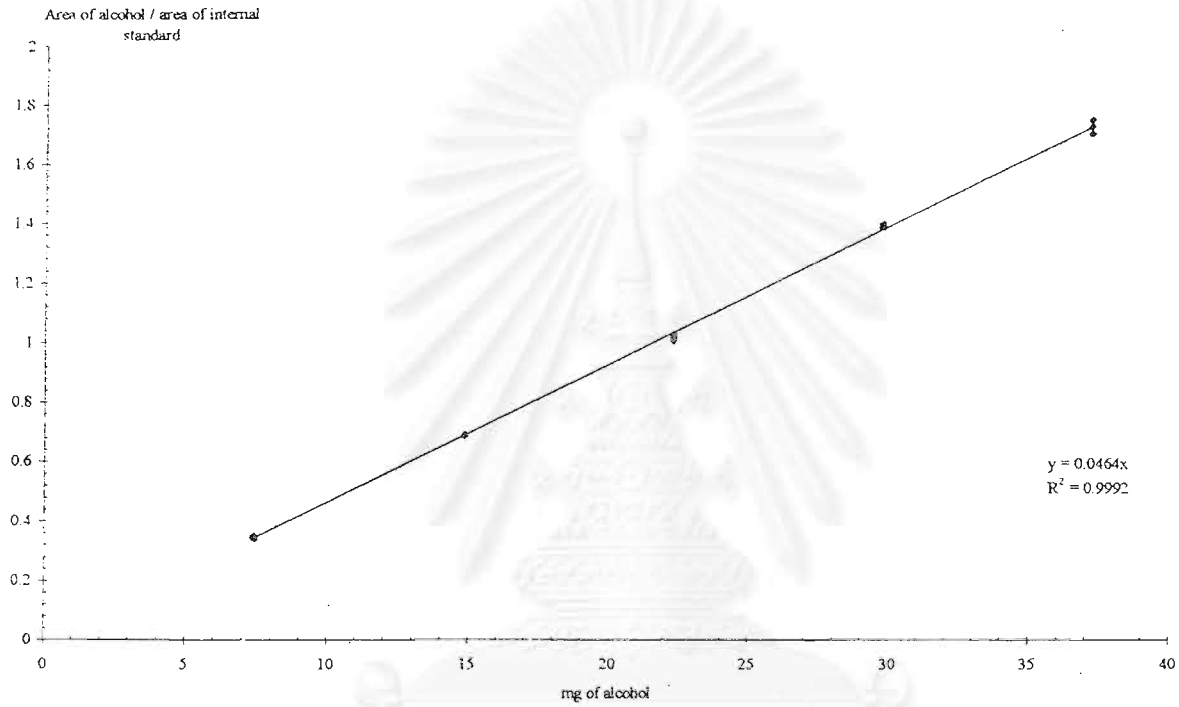
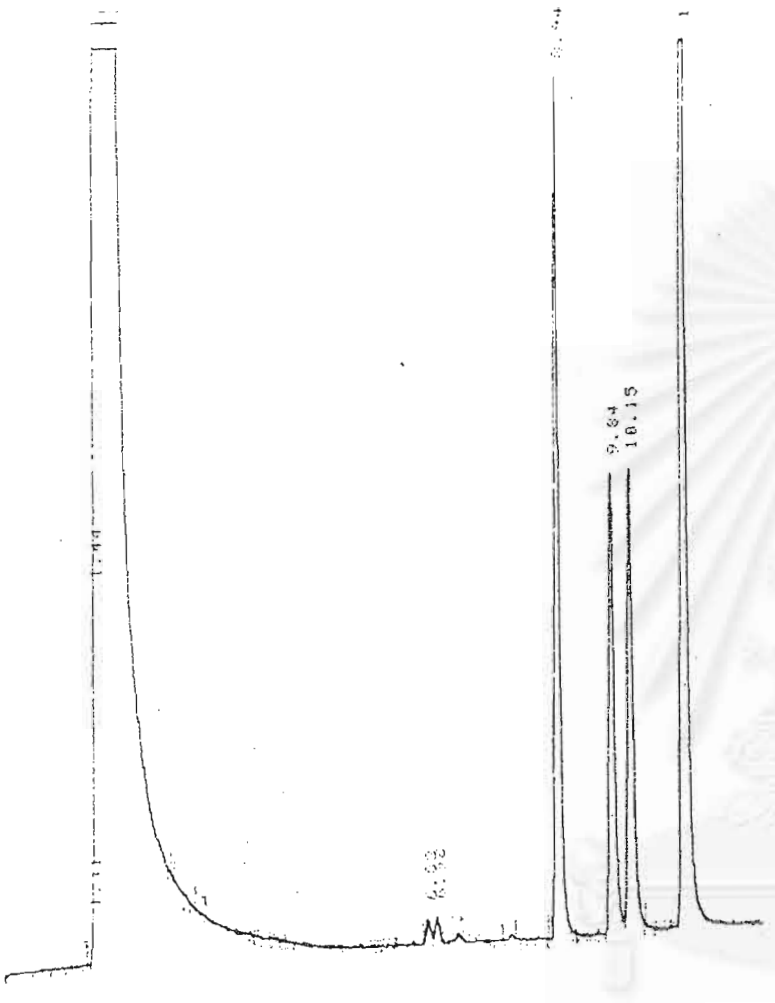
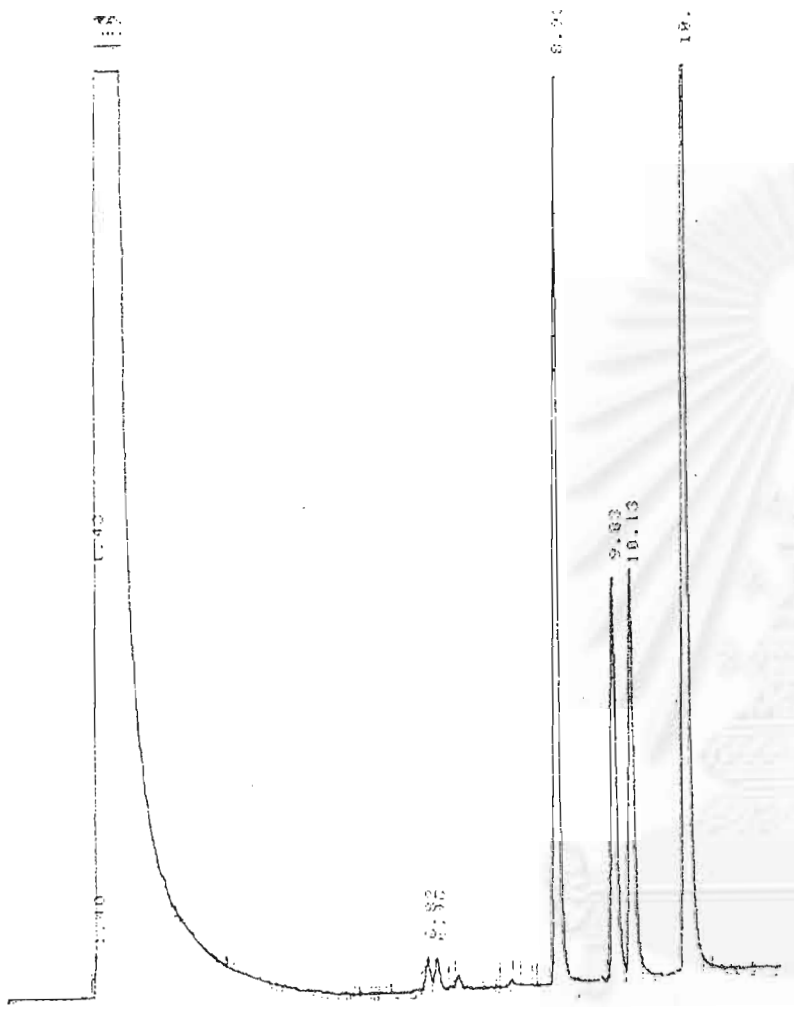


Figure B11 The calibration curve for determination of 2-phenylethanol in reaction mixture from the reaction



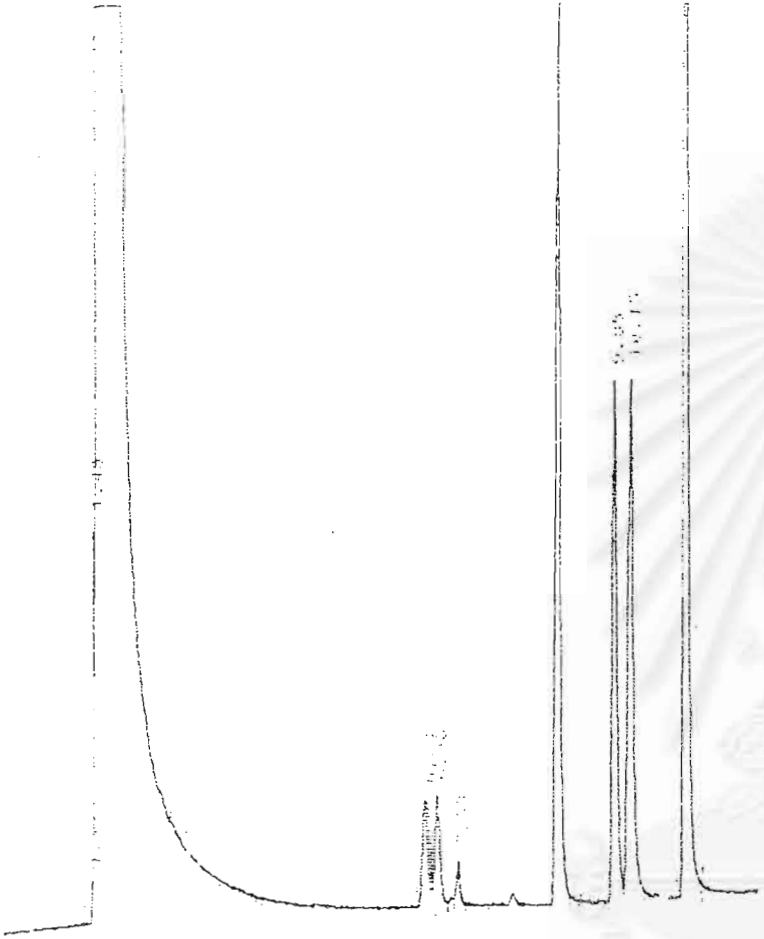
RT	AREA	TYPE	AR/HT
1.41	68	PB	0.007
1.44	2089	BH	0.012
1.49	1.33E+07	^ SHH	0.028
1.56	3.62E+07	^ SHB	0.077
1.64	312619	TBP	0.037
1.77	39558	TPB	0.033
6.83	837	BV	0.073
6.98	944	VV	0.076
8.94	39306	PV	0.088
9.84	23635	PV	0.099
10.15	23475	VV	0.098
11.00	67038	PB	0.099

Figure B12 Separation of purified mixture from the reductive ring –opening of styrene oxide in the absence of cyclodextrin. (0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$), separation condition same as figure B6.



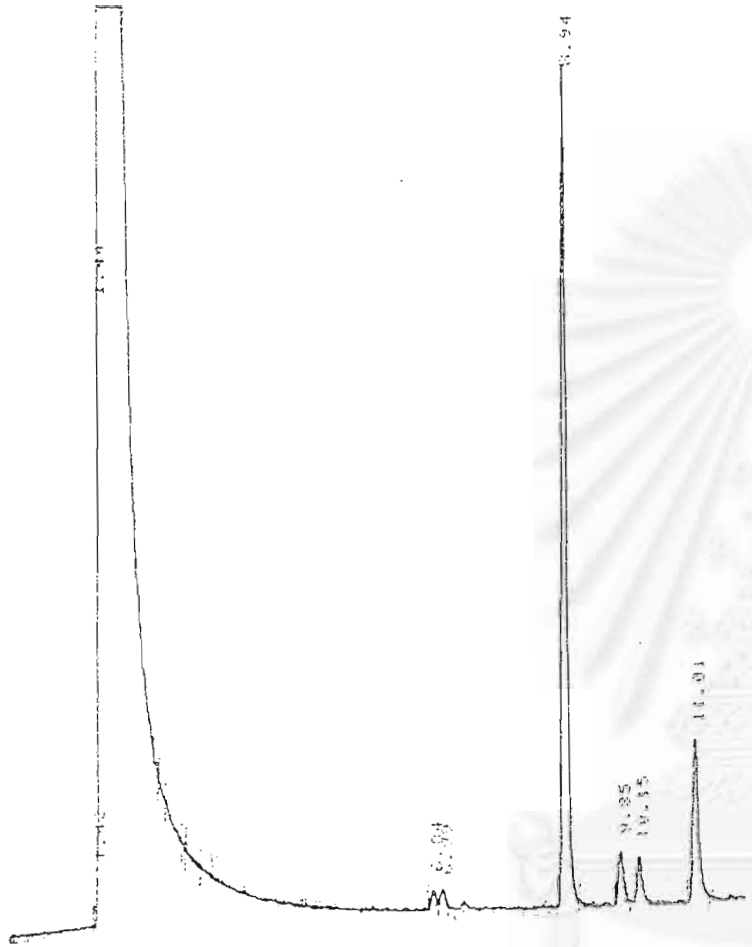
RT	AREA	TYPE	AR/HT
1.40	361	PV	0.015
1.43	2865	VH	0.013
1.48	1.30E+07	^ SHH	0.028
1.54	3.49E+07	^ SHB	0.074
1.64	923260	TBP	0.036
1.76	38754	TPB	0.032
6.82	1323	BV	0.080
6.96	1555	VP	0.091
8.93	41258	BV	0.088
9.83	20295	PV	0.098
10.13	20494	VV	0.097
10.99	55830	PV	0.101

Figure B13 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of TRIMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.06 mmol (0.1 eq) of selector], separation condition same as figure B6.



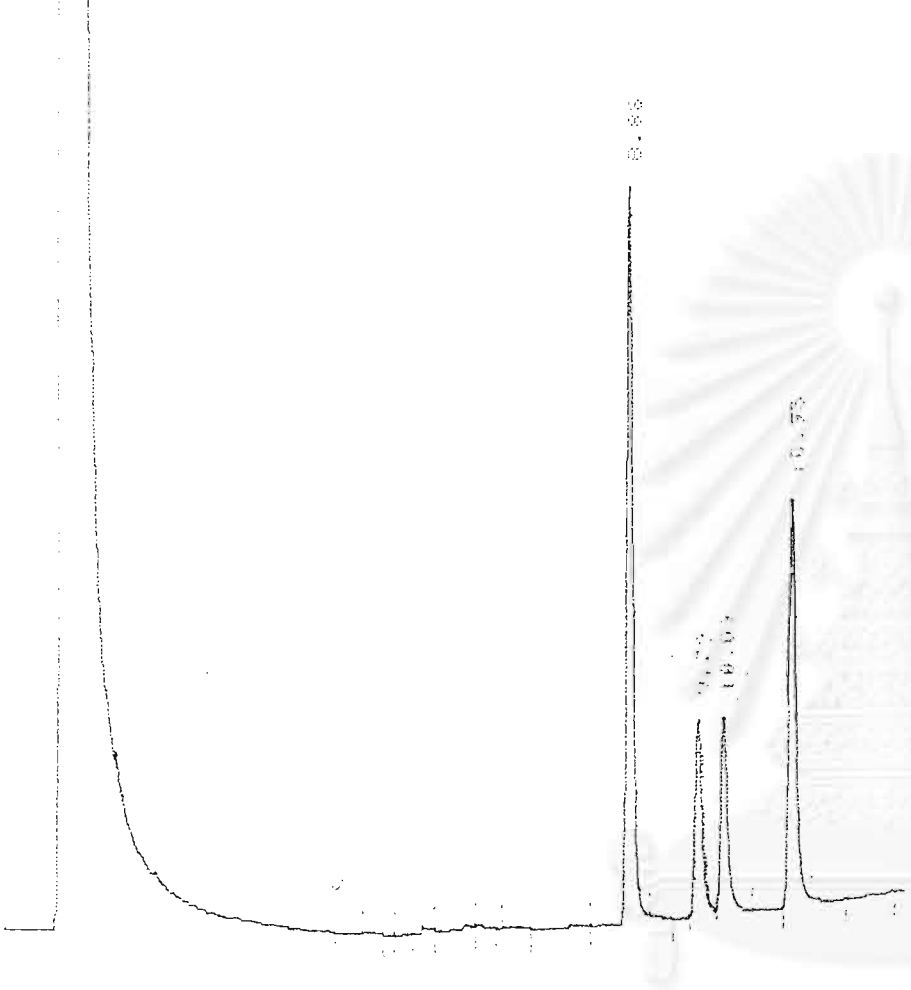
RT	AREA	TYPE	AR/HT
1.42	391	BV	0.016
1.45	2901	VH	0.014
1.49	1.15E+07	^ SHH	0.024
1.56	3.63E+07	^ SHB	0.077
1.65	814110	TBP	0.038
1.77	45868	TPB	0.033
6.83	4895	PV	0.088
6.98	5409	VV	0.093
8.95	41329	BV	0.088
9.85	26580	PV	0.098
10.15	26589	VV	0.099
11.00	70791	PV	0.099

Figure B14 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of TRIMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.12 mmol (0.2 eq) of selector], separation condition same as figure B6.



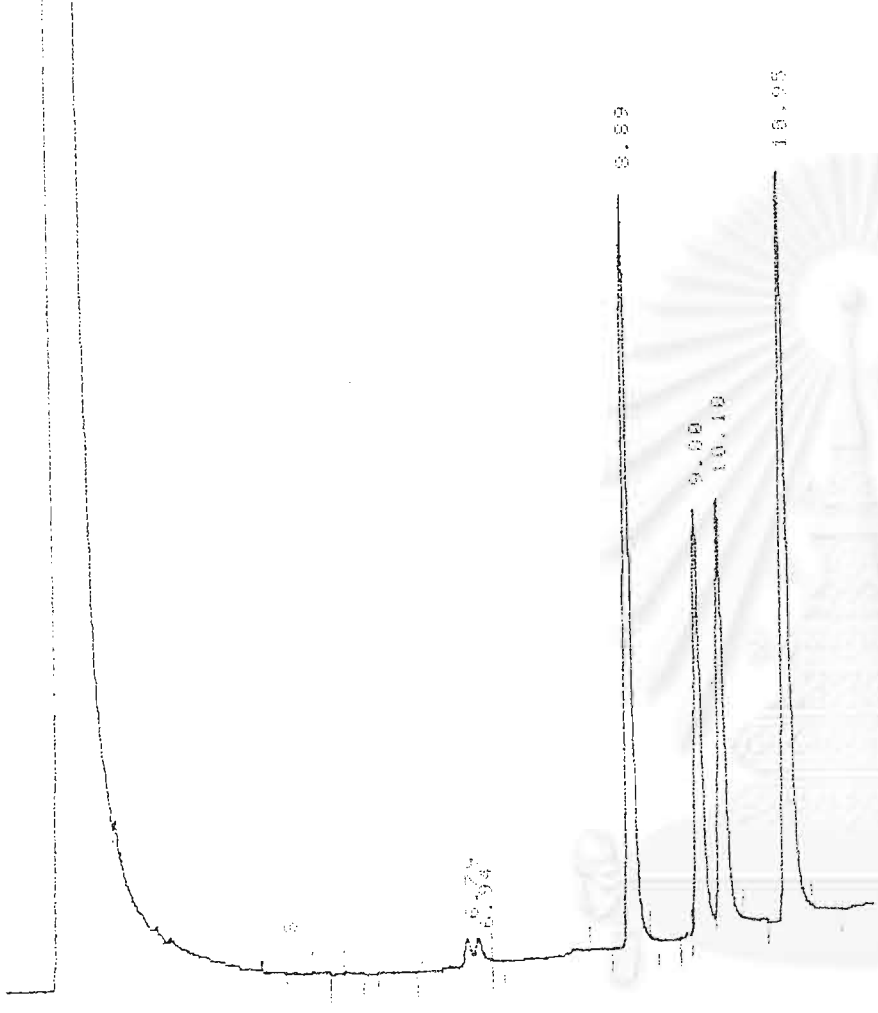
RT	AREA	TYPE	AR/HT
1.42	248	PV	0.010
1.44	4711	VH	0.015
1.49	1.36E+07	^ SHH	0.029
1.56	3.54E+07	^ SHB	0.075
1.65	828510	TBP	0.038
1.77	36459	TPB	0.032
6.84	731	BV	0.077
6.99	852	VP	0.087
8.94	39914	BP	0.092
9.85	2151	BP	0.089
10.15	2496	PP	0.103
11.01	8434	PV	0.100

Figure B15 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of TRIMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.18 mmol (0.3 eq) of selector], separation condition same as figure B6.



RT	AREA	TYPE	AR/HT
1.48	1.16E+07	SBH	0.025
1.54	2.81E+07	^ SHB	0.060
1.64	680200	TBP	0.033
1.76	21127	TPB	0.030
8.88	27364	PB	0.089
9.79	8398	PV	0.101
10.09	8875	VB	0.107
10.95	17164	PB	0.101

Figure B16 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of underivatized cyclodextrin. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.12 mmol (0.2 eq) of selector], separation condition same as figure B6.

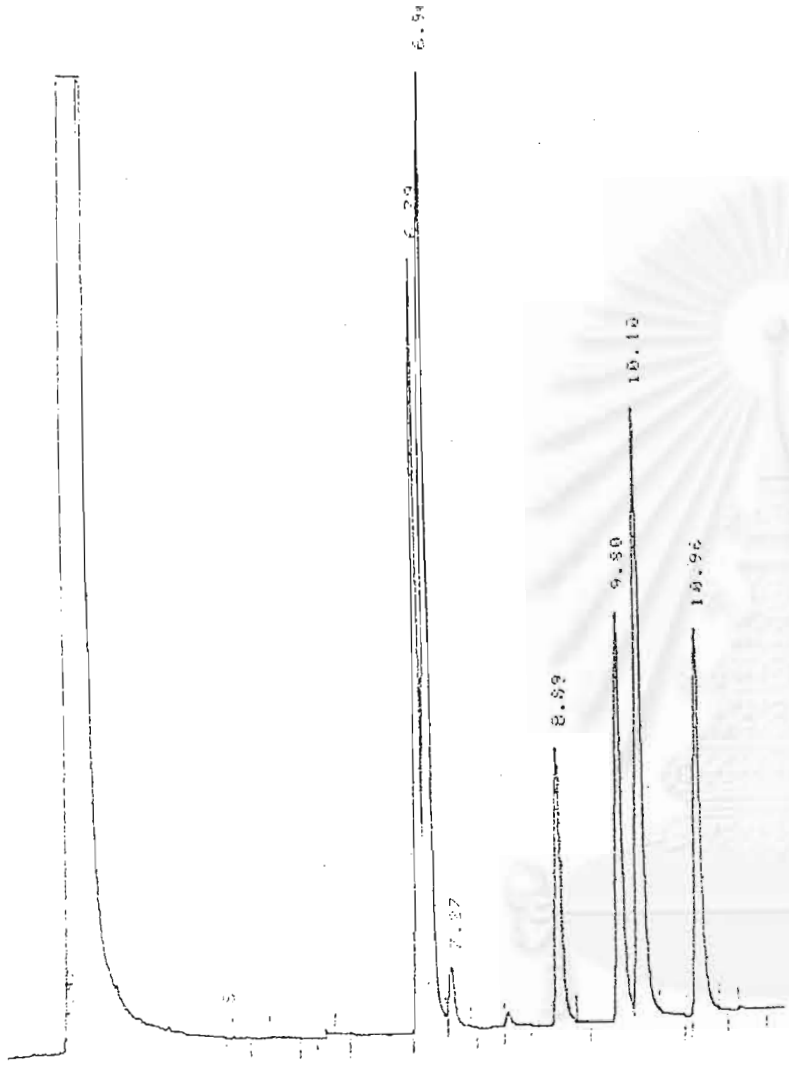


RT	AREA	TYPE	AR/HT
1.48	1.16E+07	SBH	0.025
1.55	2.76E+07	^ SHB	0.058
1.65	665580	TBP	0.033
1.77	18743	TPB	0.030
6.79	847	PV	0.080
6.94	911	VB	0.087
8.89	27957	PB	0.090
9.80	18154	BV	0.102
10.10	18107	VB	0.102
10.95	31926	PB	0.103

Figure B17 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of RAMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.12 mmol (0.2 eq) of selector], separation condition same as figure B6.

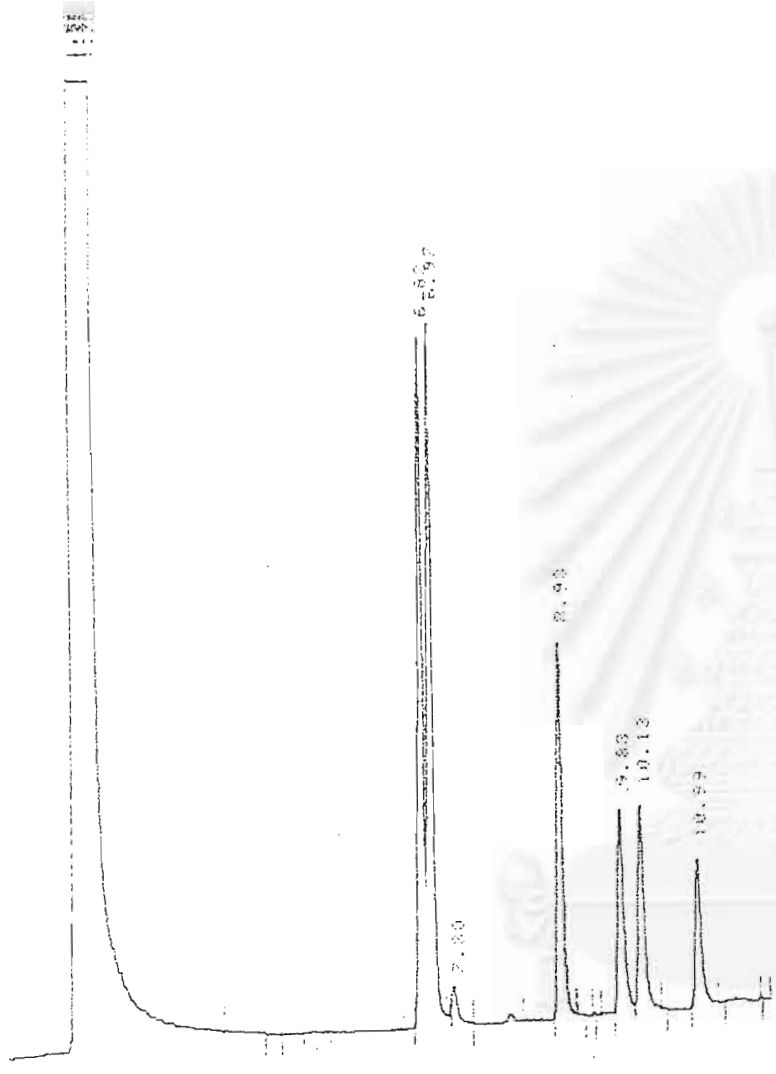
RT	AREA	TYPE	AR/HT
1.50	1.04E+07	SHH	0.024
1.56	2.55E+07	^ SHB	0.054
1.66	232710	TBP	0.034
1.79	29313	TPB	0.037
6.84	73646	PV	0.086
6.98	83422	VB	0.097
7.32	3314	BB	0.081
8.17	721	BB	0.087
8.95	13670	PB	0.092
9.85	11691	PV	0.105
10.15	11703	VB	0.108
11.01	31300	PB	0.108

Figure B18 Separation of purified mixture from the reductive ring–opening of styrene oxide in the absence of cyclodextrin. (5 mmol of starting epoxide and 10 mmol of $NaBH_4$), separation condition same as figure B6.



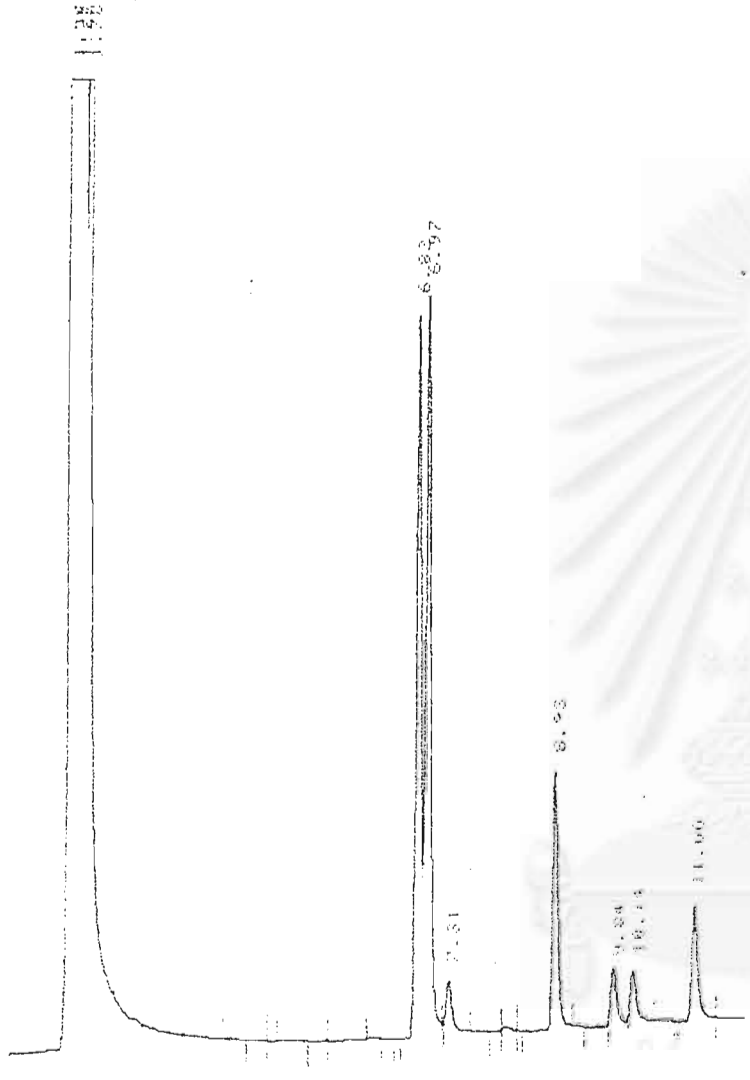
RT	AREA	TYPE	AR/HT
1.42	1792	BH	0.185
1.49	1.01E+07	SHH	0.023
1.55	2.36E+07	^ SHB	0.050
1.65	235310	TBP	0.032
1.77	19595	TPB	0.034
6.79	31635	PV	0.084
6.94	47757	VB	0.095
7.27	2120	BB	0.082
8.89	12572	PB	0.093
9.80	20325	PV	0.102
10.10	31362	VB	0.106
10.96	20220	PB	0.107

Figure B19 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of underivatized cyclodextrin. [5 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 1 mmol (0.2eq) of selector], separation condition same as figure B6.



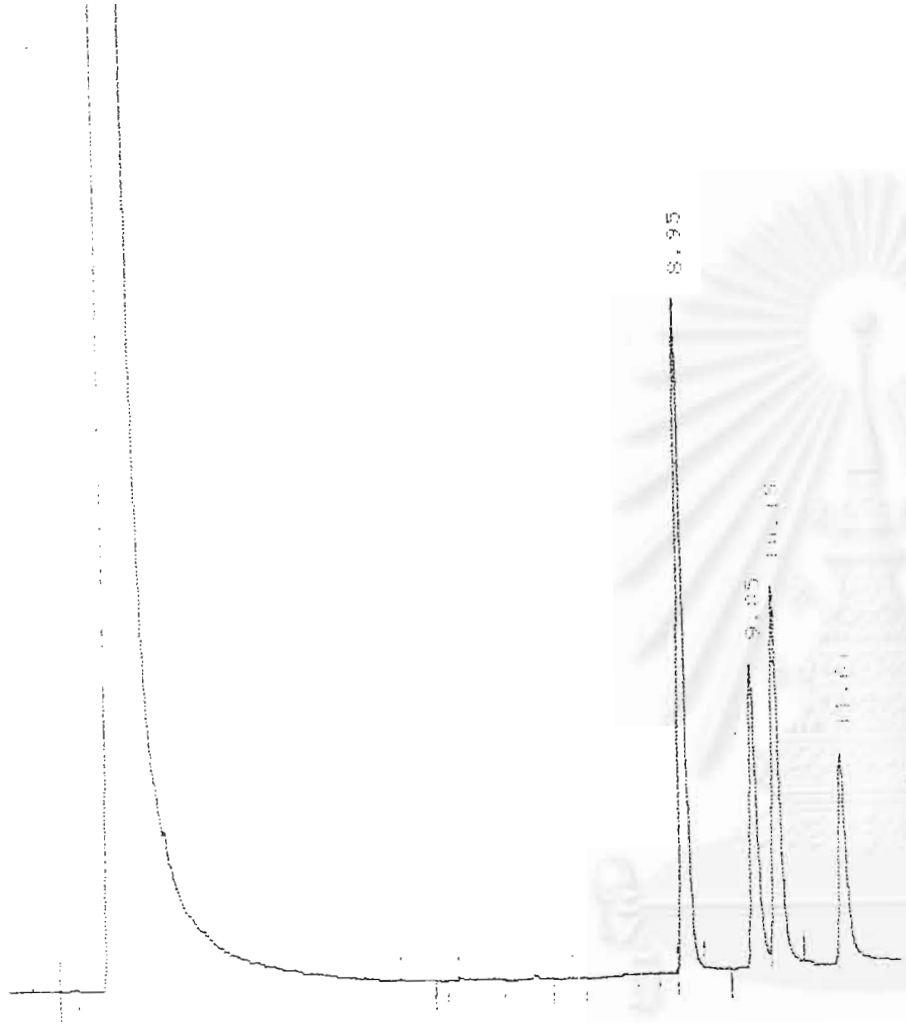
RT	AREA	TYPE	AR/HT
1.50	1.09E+07	SBH	0.024
1.56	2.46E+07	^ SHB	0.052
1.66	327390	TBP	0.033
1.78	24012	TPB	0.034
6.82	28145	PV	0.084
6.97	31457	VB	0.092
7.30	1307	BB	0.078
8.93	16427	BB	0.089
9.83	10233	PV	0.101
10.13	10458	VB	0.102
10.99	7544	BB	0.103

Figure B20 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of RAMEB. [5 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 1 mmol (0.2eq) of selector], separation condition same as figure B6.



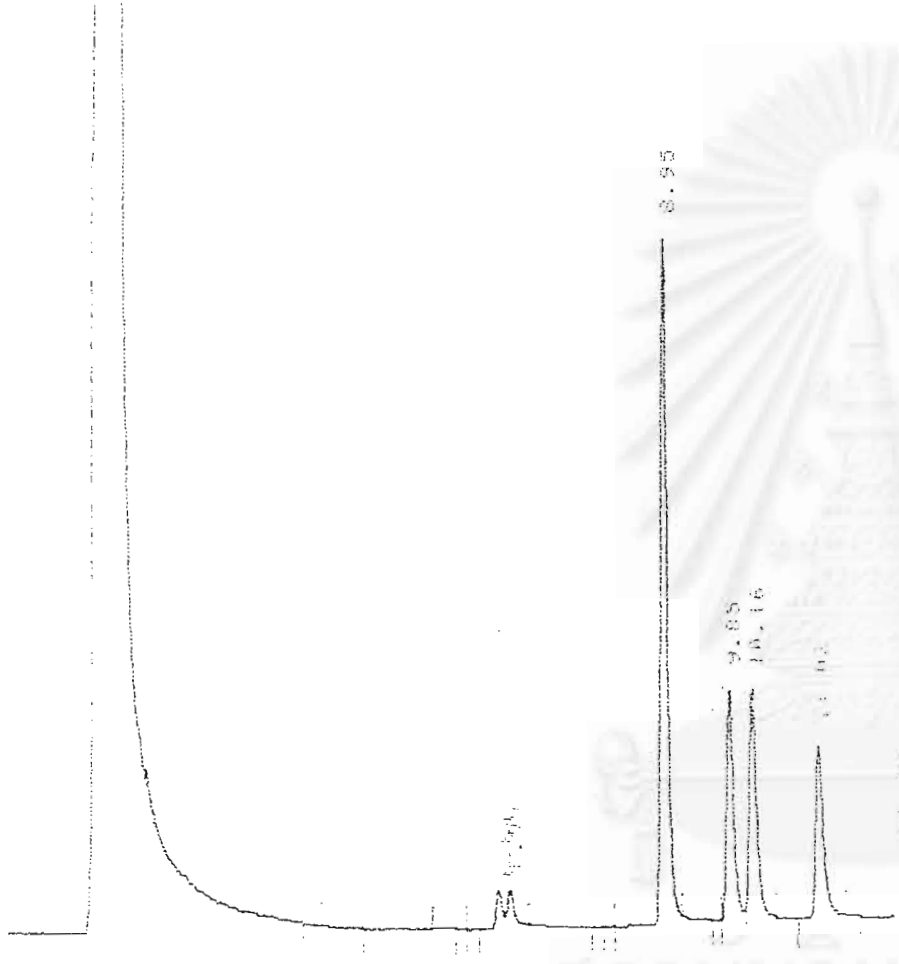
RT	AREA	TYPE	AR/HT
1.50	9600600	DSBH	0.023
1.56	2.32E+07	^ SHB	0.049
1.66	227470	TBP	0.033
1.78	22268	TPB	0.036
6.82	29754	PV	0.084
6.97	33800	VB	0.094
7.31	1892	PB	0.083
8.93	11408	PB	0.090
9.84	2861	BV	0.101
10.14	2915	VB	0.113
11.00	5767	BB	0.103

Figure B21 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of TRIMEB. [5 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 1 mmol (0.2eq) of selector], separation condition same as figure B6.



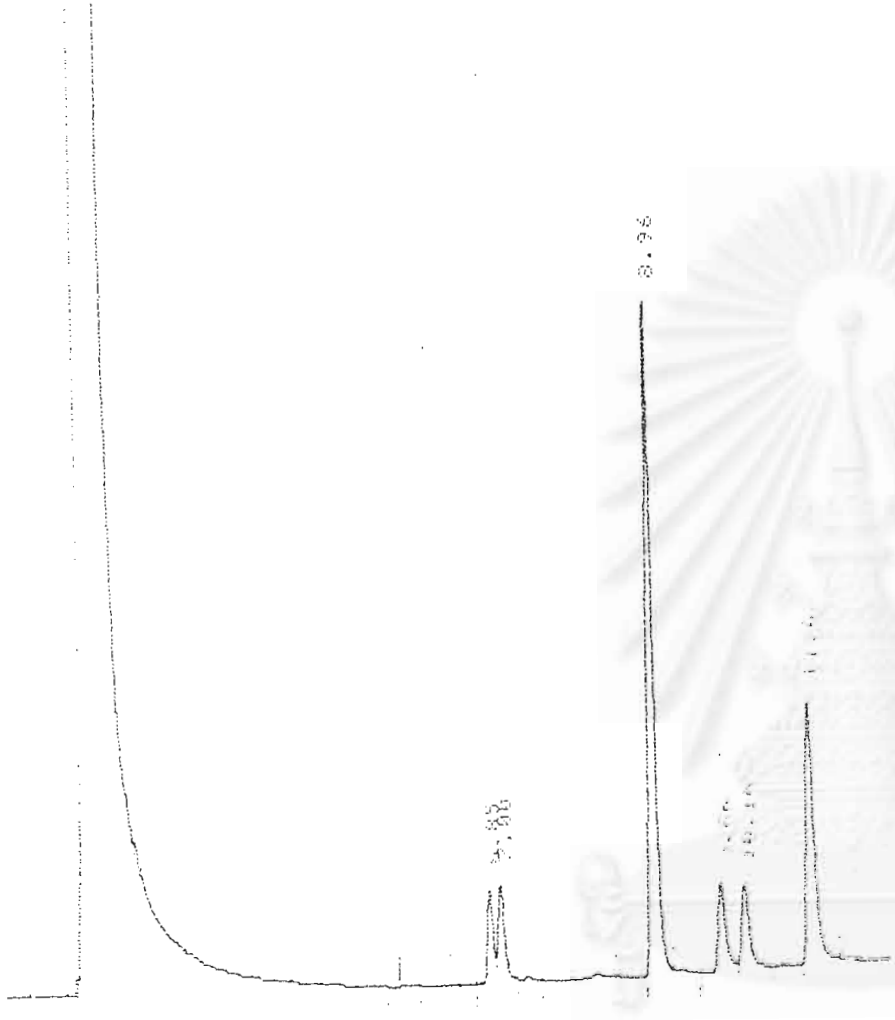
RT	AREA	TYPE	AR/HT
1.48	1.18E+07	^ SBH	0.026
1.54	2.35E+07	^ SHB	0.058
1.65	577890	TBP	0.033
1.77	10245	TPB	0.028
8.95	26147	PB	0.093
9.85	13556	PV	0.107
10.15	16899	VB	0.107
11.01	9846	BB	0.112

Figure B22 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of underivatized cyclodextrin. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.4 mmol (0.7eq) of selector], separation condition same as figure B6.



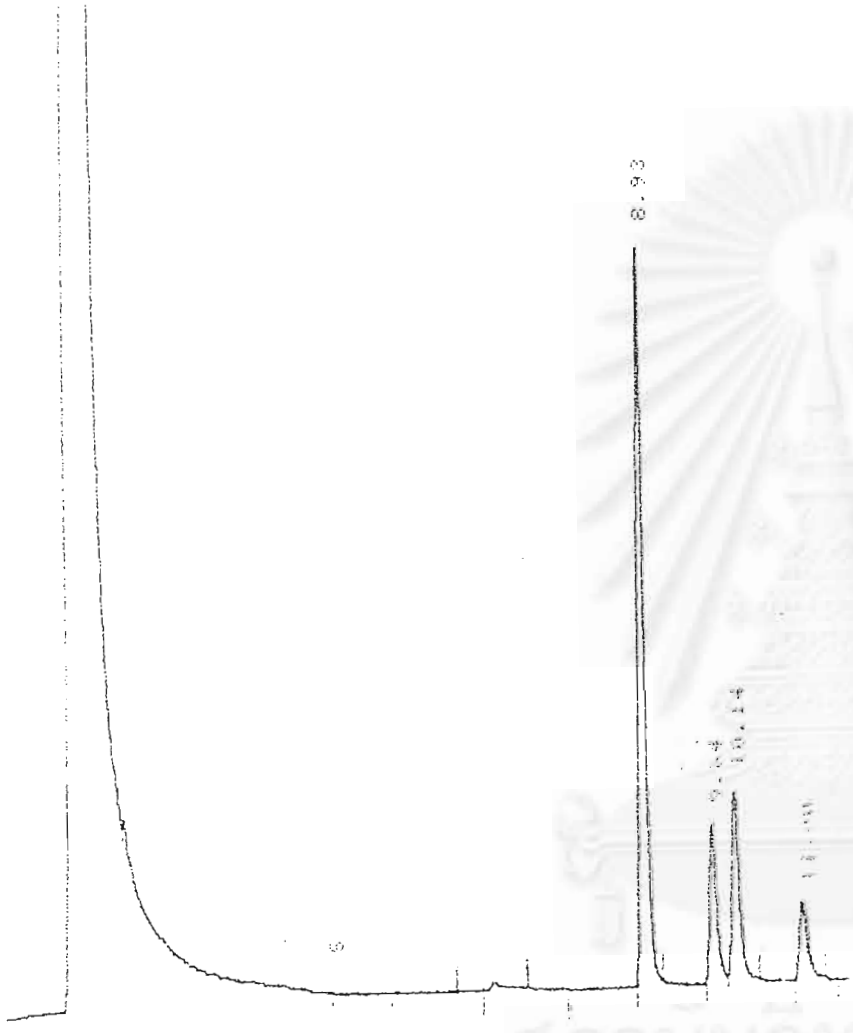
RT	AREA	TYPE	AR/HT
1.49	1.18E+07	SBH	0.026
1.55	2.76E+07	^ SHB	0.058
1.65	571270	TBP	0.033
1.77	10556	TPB	0.028
6.84	1394	PV	0.088
6.99	1531	VB	0.097
8.95	25957	PB	0.092
9.85	10191	BV	0.106
10.16	10429	VB	0.109
11.02	8063	PB	0.113

Figure B23 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of RAMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.4 mmol (0.7eq) of selector], separation condition same as figure B6.



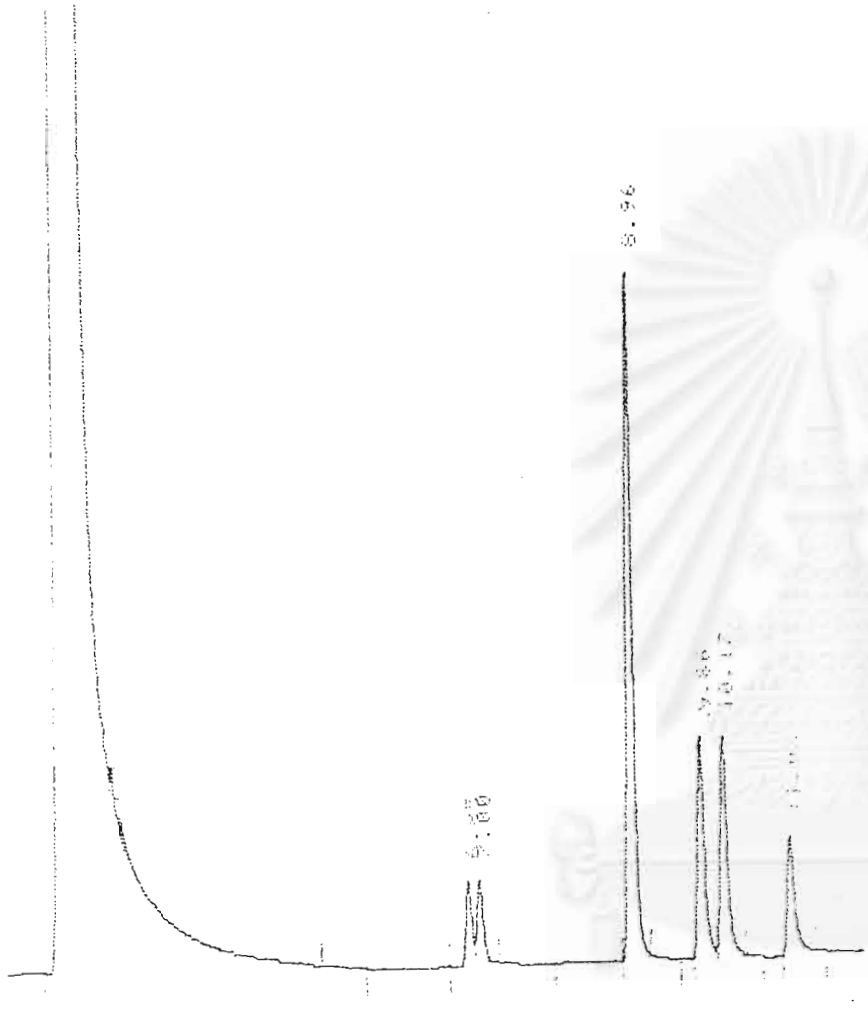
RT	AREA	TYPE	AR/HT
1.50	1.18E+07	SPH	0.026
1.56	2.72E+07	^ SHB	0.058
1.67	561630	TBP	0.034
1.79	11040	TPB	0.028
6.85	3473	PV	0.087
7.00	3993	VV	0.097
8.96	25891	PB	0.092
9.86	4050	PV	0.111
10.16	4025	VB	0.112
11.03	12412	PB	0.112

Figure B24 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of TRIMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.4 mmol (0.7eq) of selector], separation condition same as figure B6.



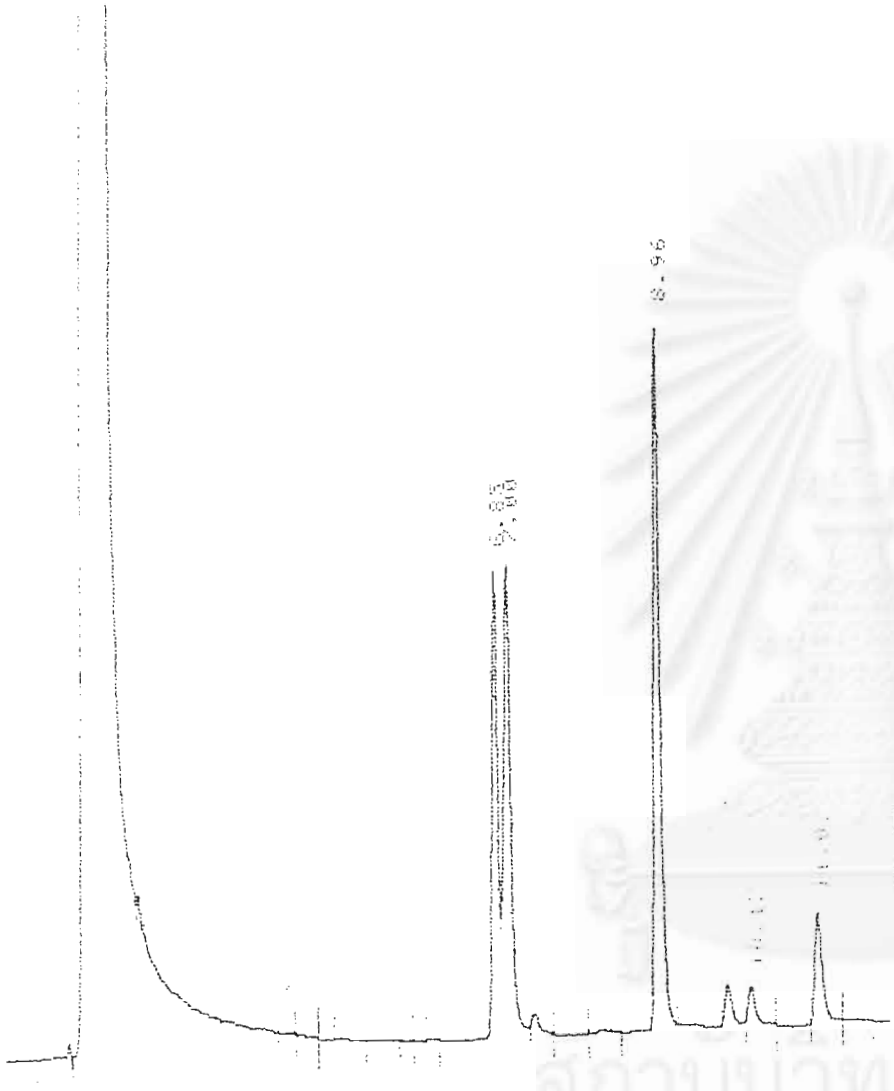
RT	AREA	TYPE	AR/HT
1.48	1.19E+07	SBH	0.026
1.54	2.89E+07	^ SHB	0.061
1.64	586730	TBP	0.034
1.76	12119	TPB	0.029
8.93	28788	BB	0.094
9.84	7140	BV	0.109
10.14	8995	VB	0.114
11.00	3609	PB	0.112

Figure B25 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of underivatized cyclodextrin. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.6 mmol (1eq) of selector], separation condition same as figure B6.



RT	AREA	TYPE	AR/HT
1.49	1.15E+07	SPH	0.025
1.55	2.99E+07	^ SHB	0.063
1.65	563870	TBP	0.035
1.77	11839	TPB	0.029
6.85	3095	PV	0.087
7.00	3439	VB	0.096
8.96	27053	PB	0.094
9.86	9915	PV	0.106
10.17	10139	VB	0.109
11.03	5574	PB	0.114

Figure B26 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of RAMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.6 mmol (1eq) of selector], separation condition same as figure B6.



RT	AREA	TYPE	AR/HT
1.49	1.19E+07	^ SPH	0.026
1.55	2.67E+07	^ SHB	0.057
1.66	569530	TBP	0.033
1.78	10755	TPB	0.028
6.85	17064	PV	0.088
7.00	19464	VV	0.099
8.96	26440	PB	0.091
10.17	1618	BB	0.105
11.03	4902	BB	0.108

Figure B27 Separation of purified mixture from the reductive ring–opening of styrene oxide in the presence of TRIMEB. [0.6 mmol of starting epoxide, 10 mmol of $NaBH_4$ and 0.6 mmol (1eq) of selector], separation condition same as figure B6.

VITA

Mr. Nopporn Song-im was born on September 3, 1975 at Bangkok. He received a Bachelor of Science Degree in Industrial Chemistry from Faculty of Science, King Mongkut's Institutes of Technology Ladkrabang in 1997. After graduation, he has been employed as a lecturer in the Department of Chemistry in the same institute. After one year working, he has temporarily left for studying in the master degree program at Chulalongkorn University and he has been a graduate student, studying for a M.Sc. in Analytical Chemistry.