CHAPTER IV

RESULTS AND DISCUSSION

4.1 Gas chromatographic separation of phenoxy acid methyl esters

Enantioseparation of phenoxy acid methyl esters on ASiMe and GSiMe columns was performed isothermally in the range of 70-220 °C with 10 °C increments. Each analyte was injected at least in duplicate with a split ratio of 100. From the chromatographic results, the retention factors (k′) and enantioselectivities (α) of analytes at each operating temperature could be obtained. Since the physical properties of analytes, such as boiling point and vapor pressure, are substantially varied, information from retention factors and enantioselectivities at specific temperature could not be directly compared. Therefore, thermodynamic parameters obtained over a temperature range will be investigated to provide better understanding about the interactions between the analytes and gas chromatographic stationary phases [22].

4.2 Thermodynamic investigation

Thermodynamic parameters responsible for the analyte-stationary phase interactions and enantioseparation were achieved through van't Hoff plot of ln k′ versus 1/T. All analytes provided linear relationship between ln k′ versus 1/T with the correlation coefficient (R^2) greater than 0.9990. The enthalpy (ΔH) and entropy (ΔS) changes for each enantiomer could be calculated from the slope and y-intercept, respectively. When the enantiomeric pairs were separated, the enthalpy and entropy differences (ΔΔH and ΔΔS) could be determined from the relationship between ln α and 1/T. Theoretically, ln α versus 1/T plots should be linear; however, the curvatures were observed in the temperature range examined for many analytes. This indicated that the change in temperature might be an important factor to a change in the interaction mechanism between analytes and the stationary phase. Alternatively, the ΔΔH and ΔΔS values could be calculated from the difference in ΔH and ΔS values

of two enantiomers derived from van't Hoff plot. Therefore, the $\Delta\Delta H$ and $\Delta\Delta S$ values in this research were acquired through the difference in ΔH and ΔS values.

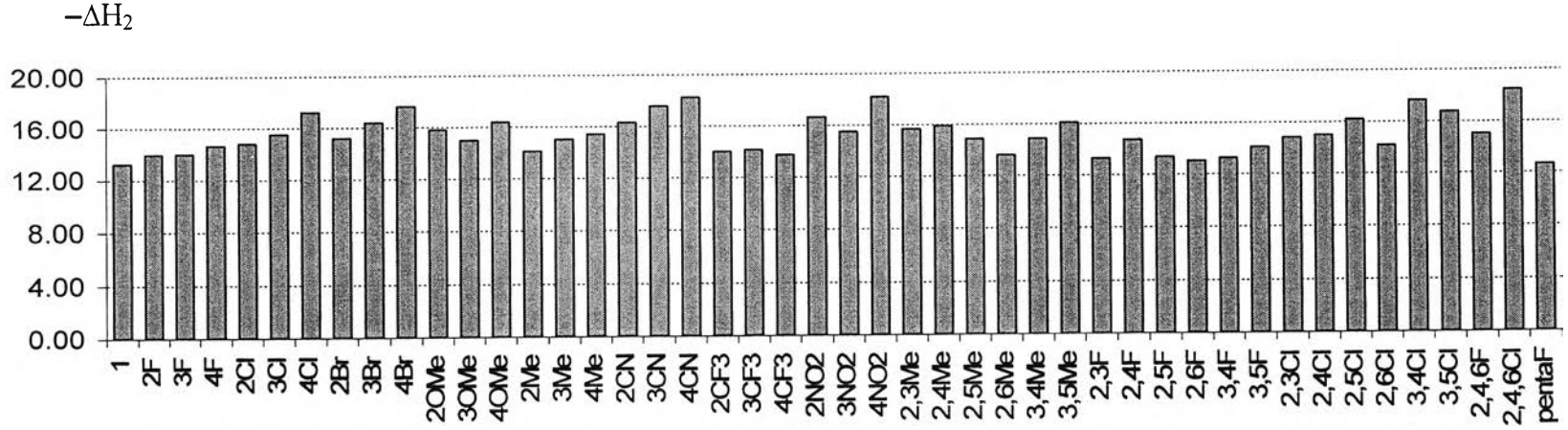
4.2.1 Enthalpy change ($-\Delta H$) and entropy change ($-\Delta S$)

The enthalpy change ($-\Delta H$) indicates the strength of interaction between an analyte and a stationary phase: the larger the value (more negative value), the stronger the interaction. While the entropy change ($-\Delta S$) signifies the loss of degree of freedom associated with the interaction between an analyte and a stationary phase.

The enthalpy ($-\Delta H_2$) and entropy ($-\Delta S_2$) changes of the more retained enantiomers of all phenoxy acid methyl esters on ASiMe and GSiMe columns were shown in Figures 4.1-4.2. On ASiMe column, all analytes exhibited similar $-\Delta H_2$ values of 15.25 ± 1.51 kcal/mol. This indicated that they all interacted with the stationary phase in a similar way and the major contribution towards the interaction on this phase would probably come from phenyl and ester groups of analytes. On GSiMe column, all analytes exhibited similar $-\Delta H_2$ values of 14.33 ± 1.09 kcal/mol. Previous study by Rodthongkum reported the $-\Delta H_2$ values of 14.94 ± 0.95 kcal/mol on BSiMe column [23]. It was noticed that the average strength of interaction between analytes and stationary phases slightly decreased from ASiMe > BSiMe > GSiMe, as the CD ring increased. Similar trend was observed for the entropy values.

Although the $-\Delta H_2$ values (as well as $-\Delta S_2$ values) of analytes on each column were similar, small trend could be observed for mono-substituted phenoxy acid methyl esters. The interaction strength of positional isomers on ASiMe and BSiMe were generally in the order of *para-* > *meta-* > *ortho*-isomers. This suggested that *para*-isomers may have appropriate conformation to form a more stable complex with ASiMe and BSiMe phases. However, the $-\Delta H_2$ values on GSiMe were in the order of *meta-* > *para-* ~ *ortho*-isomers.

(a)



(b)

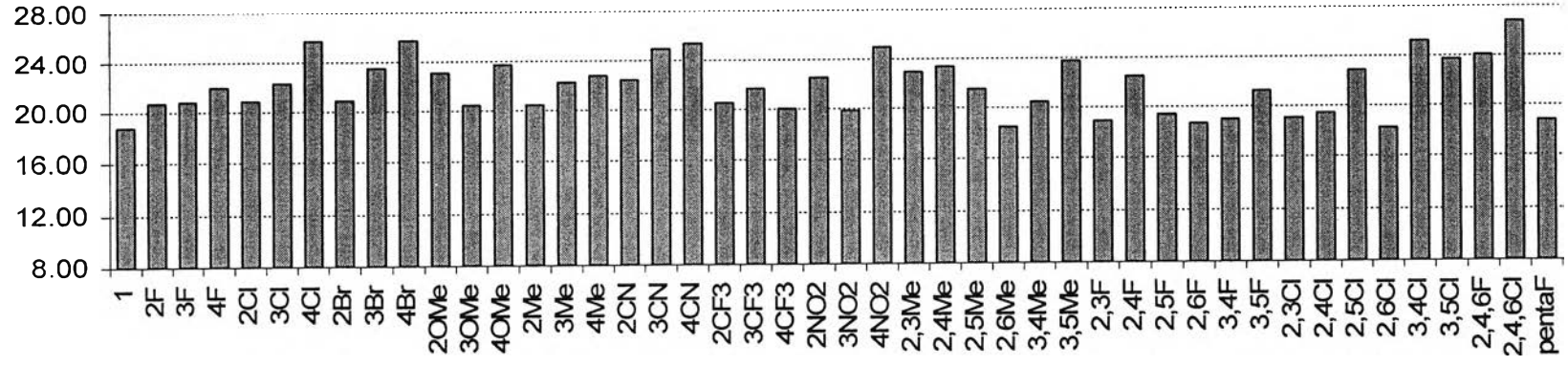


Figure 4.1 Thermodynamic values of the more retained enantiomers of phenoxy acid methyl esters on ASiMe column: (a) enthalpy change ($-\Delta H_2$, kcal/mol), $\bar{x}$ = 15.25, SD = 1.51; (b) entropy change ($-\Delta S_2$, cal/mol·K), $\bar{x}$ = 21.82, SD = 2.29.

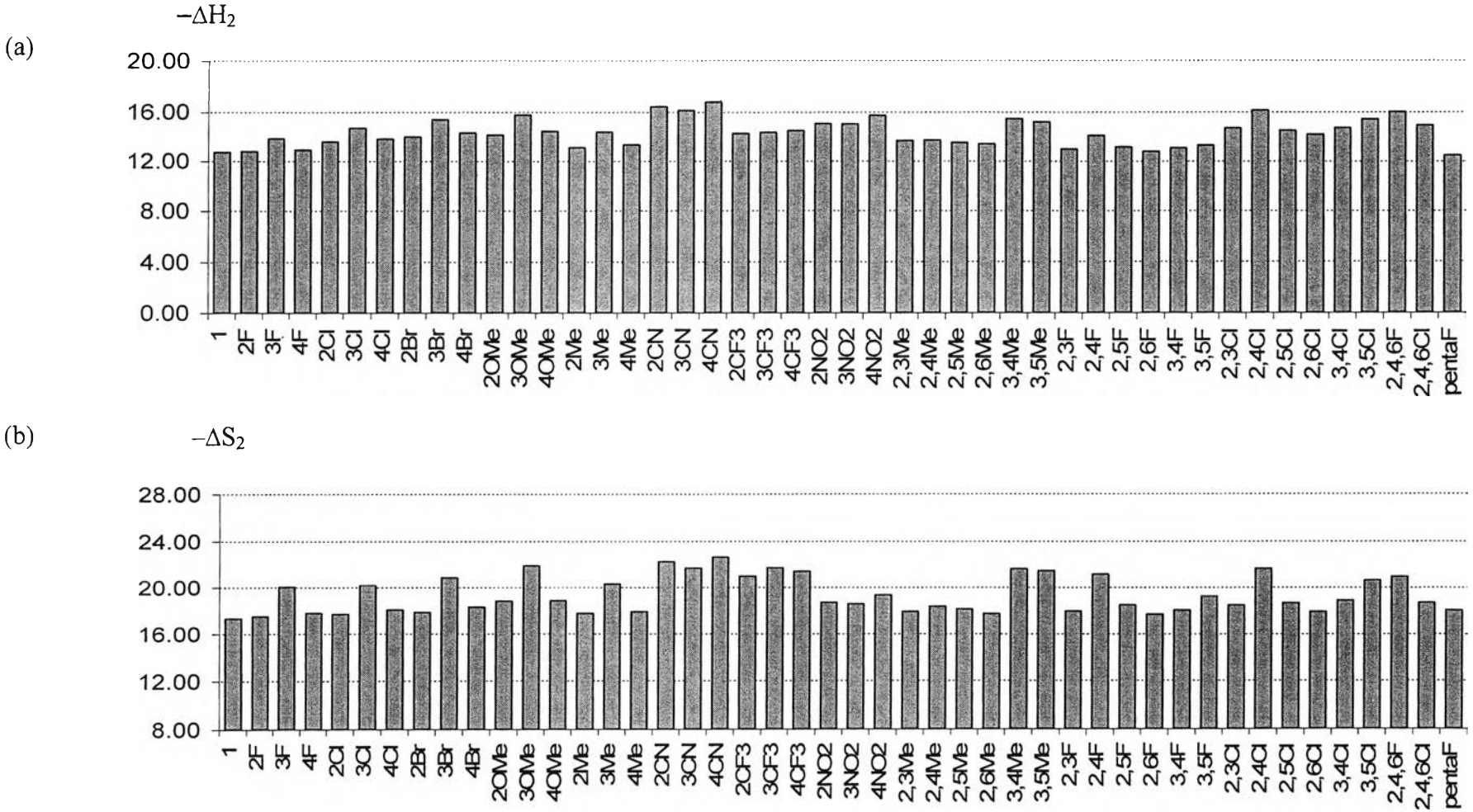


Figure 4.2 Thermodynamic values of the more retained enantiomers of phenoxy acid methyl esters on GSiMe column: (a) enthalpy change ($-\Delta H_2$, kcal/mol), $\bar{x}$ = 14.33, SD = 1.09; (b) entropy change ($-\Delta S_2$, cal/mol·K), $\bar{x}$ = 19.35, SD = 1.60.

4.2.2 Enthalpy difference (–ΔΔH) and entropy difference (–ΔΔS)

In this research, methyl 2-phenoxypropanoate (**1**) was regarded as a reference analyte. The influence of type, number and position of substituent on enantioseparation was systematically explored and discussed through the thermodynamic values. Additionally, thermodynamic values responsible for enantioseparation obtained from ASiMe and GSiMe columns will be compared to those previously attained from BSiMe column [23].

The –ΔΔH and –ΔΔS values studied in this research calculated from the difference in ΔH and ΔS of enantiomeric pairs through van't Hoff plot of ln k' versus 1/T. The –ΔΔH and –ΔΔS values of phenoxy acid methyl ester enantiomers on ASiMe and GSiMe columns were displayed in Figures 4.3-4.4. While the reference analyte (**1**) could not be enantioseparated on both ASiMe and GSiMe, substitution on the aromatic ring of analyte has significant effect on enantioseparation. ASiMe could separate larger number of phenoxy acid methyl esters with better degree of enantioseparation than GSiMe. From Figures 4.3-4.4, –ΔΔS values of analytes on both columns displayed similar trend as their corresponding –ΔΔH values; therefore, the enantioseparation on both columns will be discussed through –ΔΔH values only. Since the –ΔΔH values of all analytes were significantly different depending on the analyte structures (i.e. type, position, and number of substituents), detailed discussion of thermodynamic value will be classified into three groups according to the similarity of analyte structure.

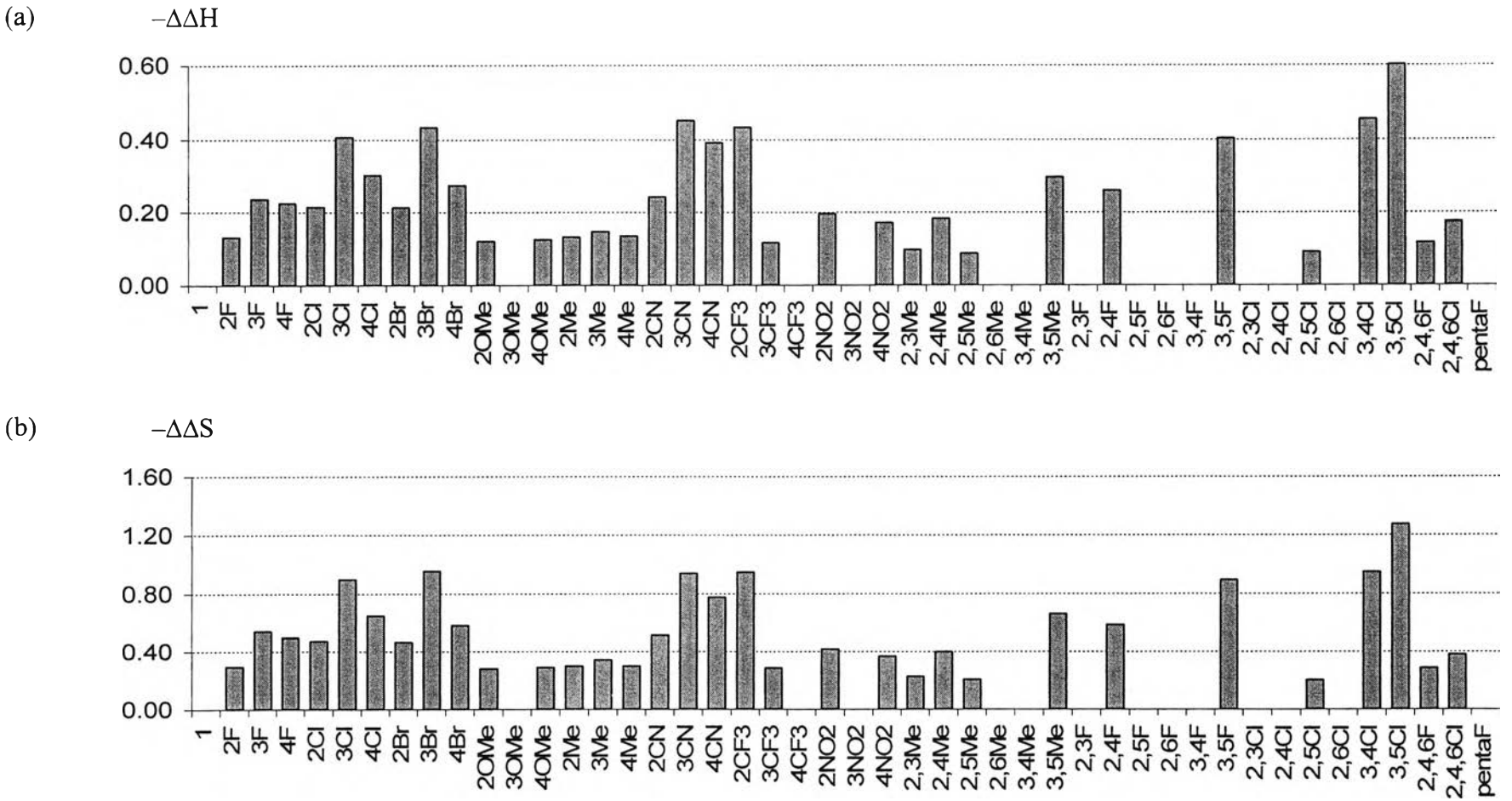


Figure 4.3 (a) Enthalpy difference (−ΔΔH, kcal/mol) and (b) entropy difference (−ΔΔS, cal/mol·K) of the enantiomers of phenoxy acid methyl esters on ASiMe column.

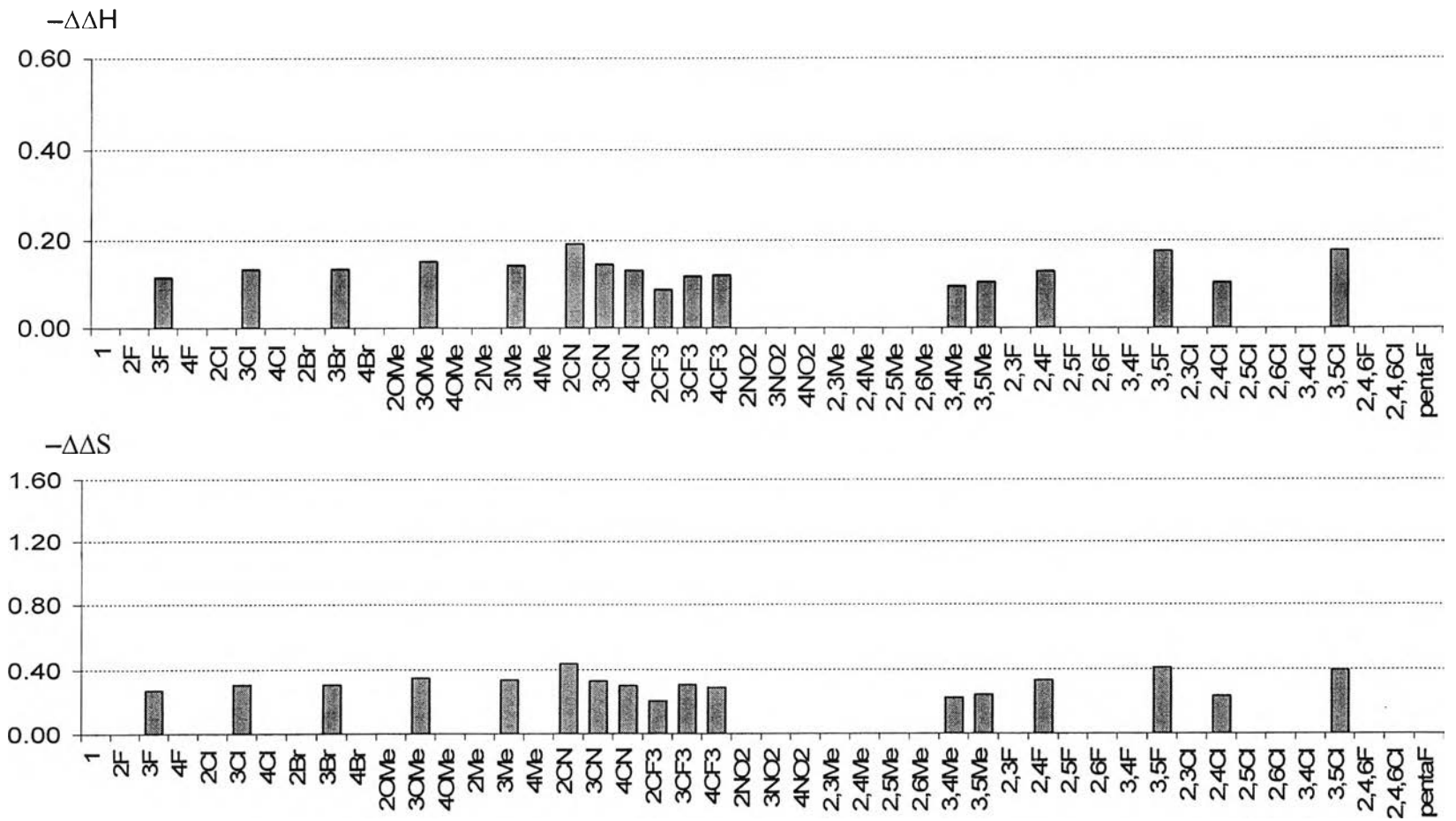
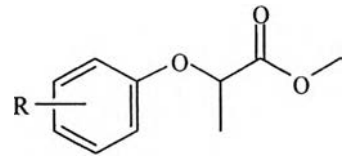


Figure 4.4 (a) Enthalpy difference (−ΔΔH, kcal/mol) and (b) entropy difference (−ΔΔS, cal/mol·K) of the enantiomers of phenoxy acid methyl esters on GSiMe column.

Group 1: Phenoxy acid methyl esters with mono-substitution on the aromatic ring



R = F, Cl, Br, OMe, Me, CN, CF_3, NO_2

Racemic phenoxy acid methyl esters in **group 1** are methyl 2-phenoxypropanoate derivatives with mono-substitution on the aromatic ring as shown above. Enthalpy differences (−ΔΔH values), representing the enantioseparation of phenoxy acid methyl esters in **group 1** on ASiMe and GSiMe columns are compared in Figure 4.5.

On ASiMe column, considering the effect of substituent position of analytes, it was found that *meta*-substituted phenoxy acid methyl esters tended to give high −ΔΔH values. Unfortunately, methoxy- and nitro-substituted analytes at *meta*-position (**3OMe** and **3NO_2**) gave no enantioseparation on this column. Although, most *ortho*-substituted phenoxy acid methyl esters showed low −ΔΔH values, all analytes with *ortho*-substitution could be enantioseparated. Interestingly, **2CF_3** has highest −ΔΔH value among all three trifluoromethyl-substituted phenoxy acid methyl esters. The relationships between ln α versus 1/T of three trifluoromethyl-substituted esters are shown in Figure 4.6. It is clear that **2CF_3** has higher enantioselectivity (α) at all temperatures. Additionally, **2CF_3** also showed the highest slope, indicating that the enantioseparation of **2CF_3** could be simply improved with a slight decrease in temperature. The separations of **2CF_3**, **3CF_3**, and **4CF_3** at 110 °C and 100 °C were shown in Figure 4.7.

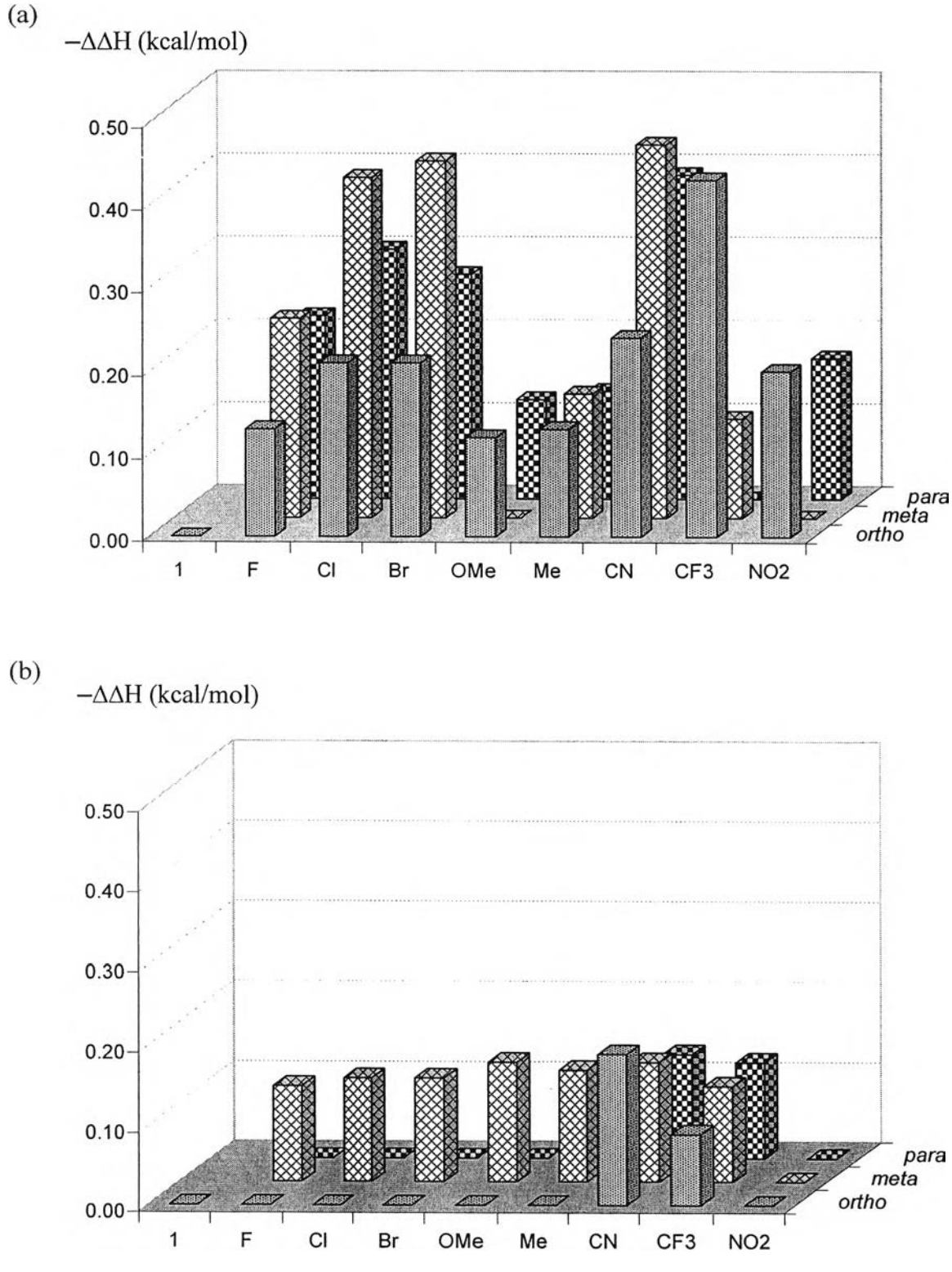


Figure 4.5 Enthalpy differences of the enantiomers of mono-substituted phenoxy acid methyl esters on (a) ASiMe and (b) GSiMe columns.

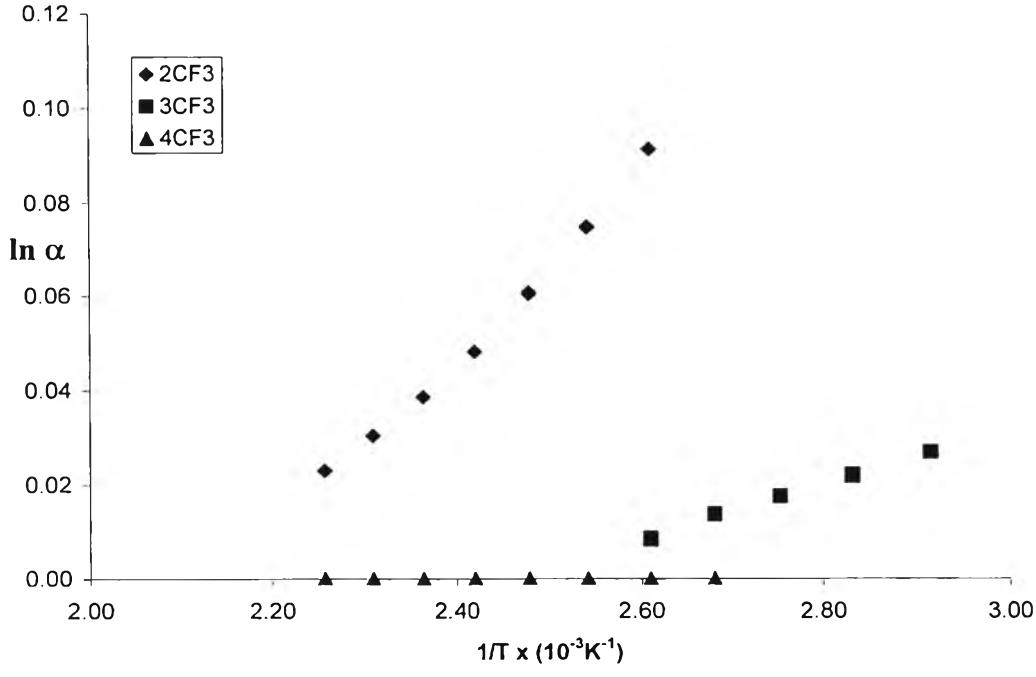


Figure 4.6 Plots of ln α versus 1/T of **$2CF_3$**, **$3CF_3$**, and **$4CF_3$** on ASiMe column.

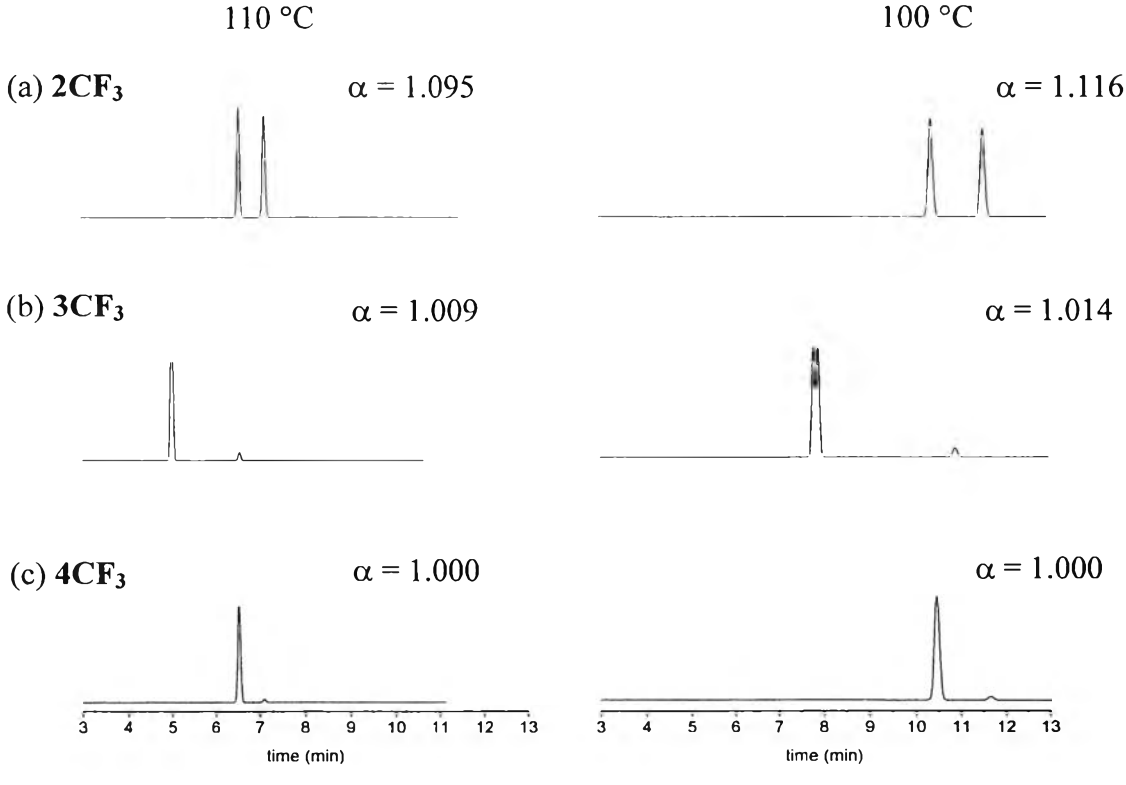


Figure 4.7 Chromatograms of (a) **$2CF_3$**, (b) **$3CF_3$**, (c) **$4CF_3$** on ASiMe column at (left) 110 °C and (right) 100 °C.

The type of substituent could affect enantiorecognition as well as the position of substituent. A small change in type of substituent from methyl (**3Me**) to methoxy (**3OMe**) eliminated enantioseparation (Figure 4.8). Among six *meta*-substituted phenoxy acid methyl esters that could be enantioseparated, $-\Delta\Delta H$ values increased in the order of **3CF$_3$** < **3Me** < **3F** < **3Cl** < **3Br** < **3CN**. In case of halogen-substituted analytes, it was observed that $-\Delta\Delta H$ values increased in the order of F < Cl ~ Br, according to the decreasing electronegativity of substituent (EN_F = 4.0, EN_{Cl} = 2.8, EN_{Br} = 2.7 [43]) and to the increasing size of substituent (r_F = 131 pm, r_{Cl} = 181 pm, r_{Br} = 196 pm [44]).

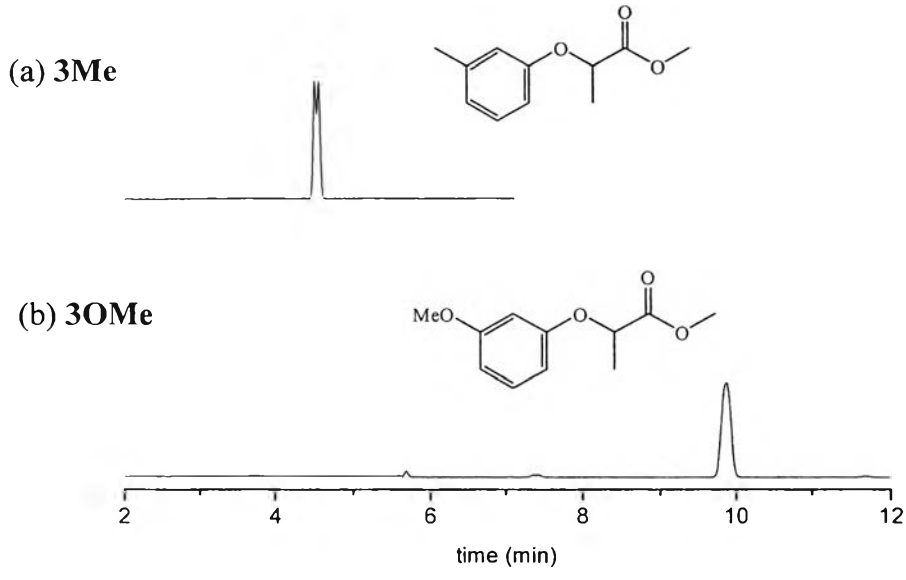


Figure 4.8 Chromatograms of (a) **3Me** and (b) **3OMe** on ASiMe column at 110 °C.

When the size of cyclodextrin ring became larger as in GSiMe, the enantioseparation of analytes was varied. For most of esters in **group 1**, $-\Delta\Delta H$ values obtained from GSiMe column were lower than those from ASiMe column (Figure 4.5). Some exceptions were observed. GSiMe could separate enantiomers of **3OMe** and **4CF$_3$** that could not be separated by ASiMe. Nonetheless, **3NO$_2$** could not be separated by either ASiMe or GSiMe.

It can be clearly seen that position of substituent has a strong impact on enantioseparation on GSiMe column, as most of *meta*-substituted esters could be separated, except for **3NO$_2$**. Even though the type of substituent did not much affect the $-\Delta\Delta H$ values of *meta*-substituted esters, it also played a role towards enantioseparation as all three isomers of only trifluoromethyl- and cyano-substituted phenoxy acid methyl esters could be enantioseparated on GSiMe column.

The enantioseparation abilities of ASiMe and GSiMe towards three halogen-substituted phenoxy acid methyl esters at *meta*-position were compared at 120 °C (Figure 4.9). It can be seen that ASiMe gave better enantioselectivities at lower analysis time than GSiMe for all three esters. From the relationships between ln α versus 1/T of three halogen-substituted esters (Figure 4.10), it was clear that all three analytes showed higher enantioselectivities at all temperatures examined and exhibited higher slope on ASiMe than on GSiMe. Overall, ASiMe would be a more suitable stationary phase for the enantioseparation of mono-substituted phenoxy acid methyl esters than GSiMe. The results obtained from ASiMe and GSiMe were different from those obtained from BSiMe [23] as BSiMe could separate all enantiomers of esters in **group 1** with high $-\Delta\Delta H$ values toward most of *para*-substituted esters.

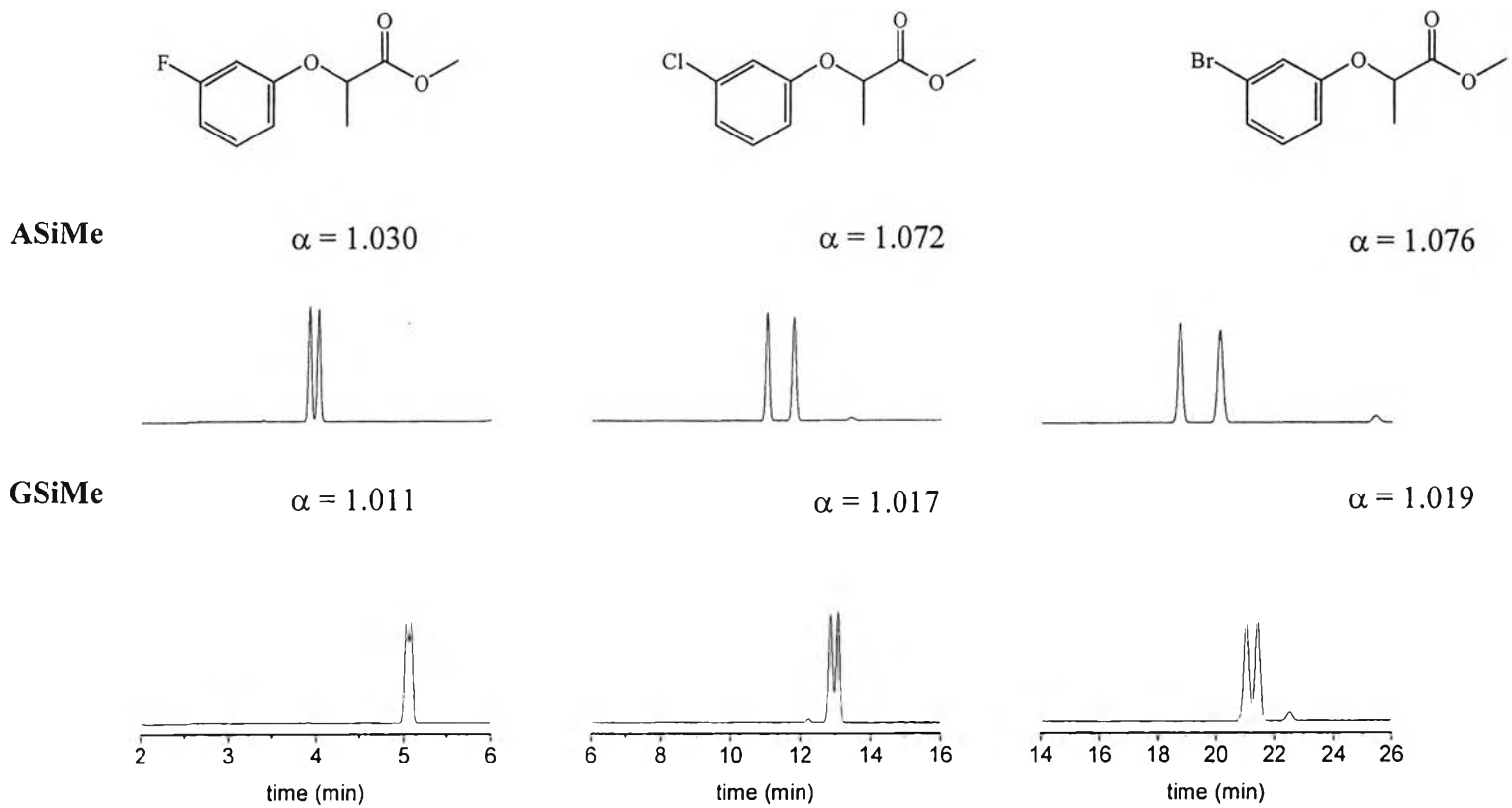


Figure 4.9 Chromatograms of **3F**, **3Cl**, and **3Br** on ASiMe and GSiMe columns at 120 °C.

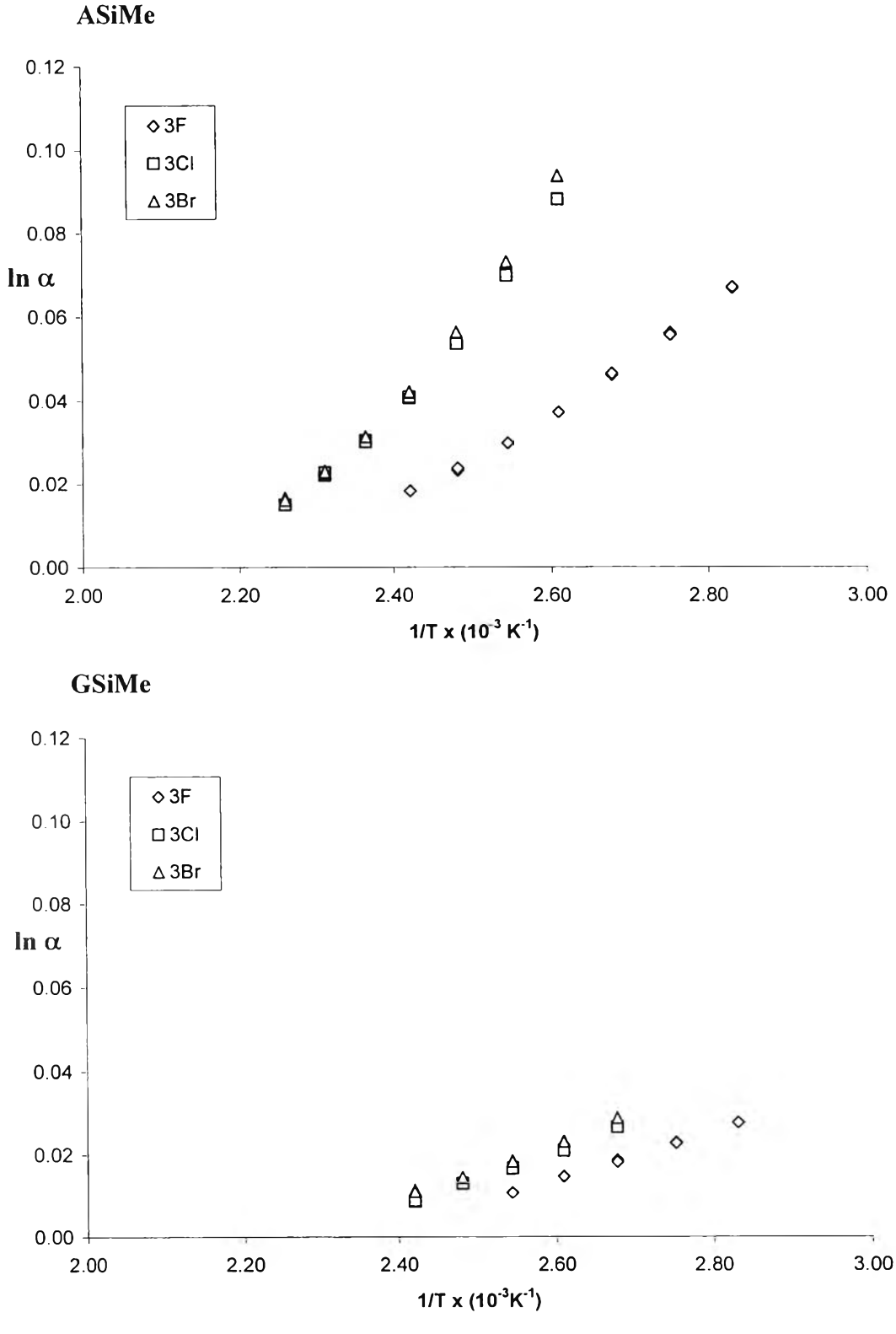
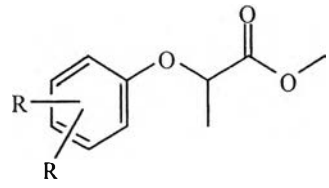


Figure 4.10 Plots of ln α versus 1/T of **3F**, **3Cl**, and **3Br** on ASiMe and GSiMe columns.

Group 2: Phenoxy acid methyl esters with di-substitution on the aromatic ring



R = F, Cl, Me

Racemic phenoxy acid methyl esters in **group 2** are methyl 2-phenoxypropanoate derivatives with difluoro-, dichloro-, and dimethyl-substitutions on the aromatic ring as shown above. The $-\Delta\Delta H$ values representing the enantioseparation of di-substituted phenoxy acid methyl esters on ASiMe and GSiMe columns were illustrated in Figure 4.11. It still can be seen that ASiMe exhibited better chiral recognition towards di-substituted esters than GSiMe.

For di-substituted phenoxy acid methyl esters, the position of substituent on the aromatic ring still played a key role on enantioseparation as phenoxy acid methyl esters with substituents at 3,5-position (*meta*-position) showed high $-\Delta\Delta H$ values (Figure 4.11). These results correspond with those obtained from **group 1** that *meta*-substitution enhanced enantioseparation. Among all phenoxy acid methyl esters studied in this research, **3,5Cl** gave highest $-\Delta\Delta H$ value on ASiMe. The separations of four dichloro-substituted phenoxy acid methyl esters, with *meta*-substitution, on ASiMe and GSiMe were compared in Figure 4.12. However, none of all 2,6-substituted analytes could be separated on ASiMe, BSiMe or GSiMe, except for small-sized substituent **2,6F** that could be separated on BSiMe column. This suggested that steric position hindered the interaction between analytes and cyclodextrin derivative and, thus, no enantioseparation was observed.

Considering the effect of type of substitution, a trend could be observed on both columns, but more clearly on ASiMe column. The $-\Delta\Delta H$ values of 3,5-substituted analytes have a tendency to increase in the order of Me < F < Cl on ASiMe and Me < F ~ Cl on GSiMe. In contrary, fluoro-substituted analytes seemed to promote enantioseparation on BSiMe column [23].

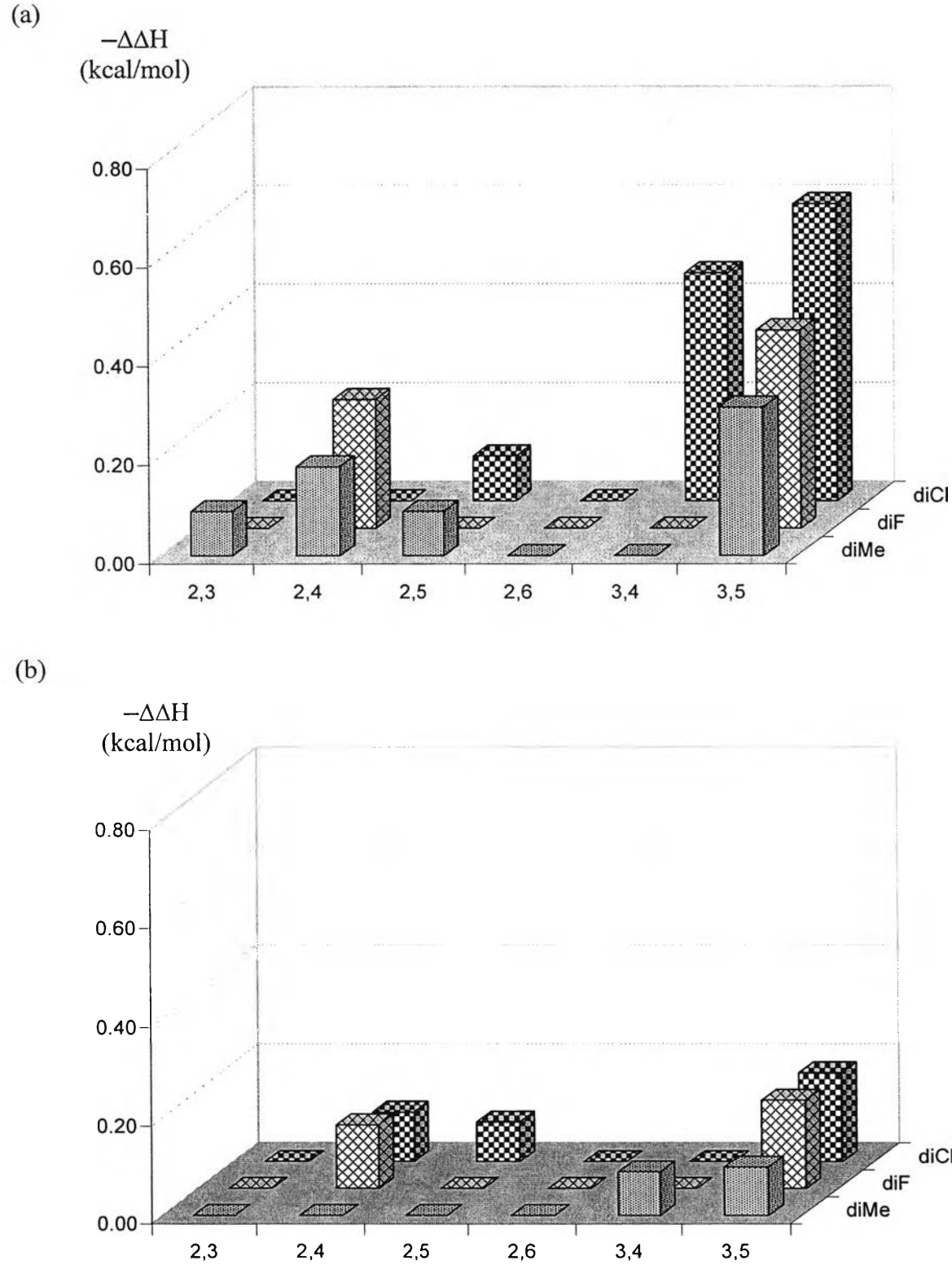


Figure 4.11 Enthalpy differences of the enantiomers of di-substituted phenoxy acid methyl esters on (a) ASiMe and (b) GSiMe columns.

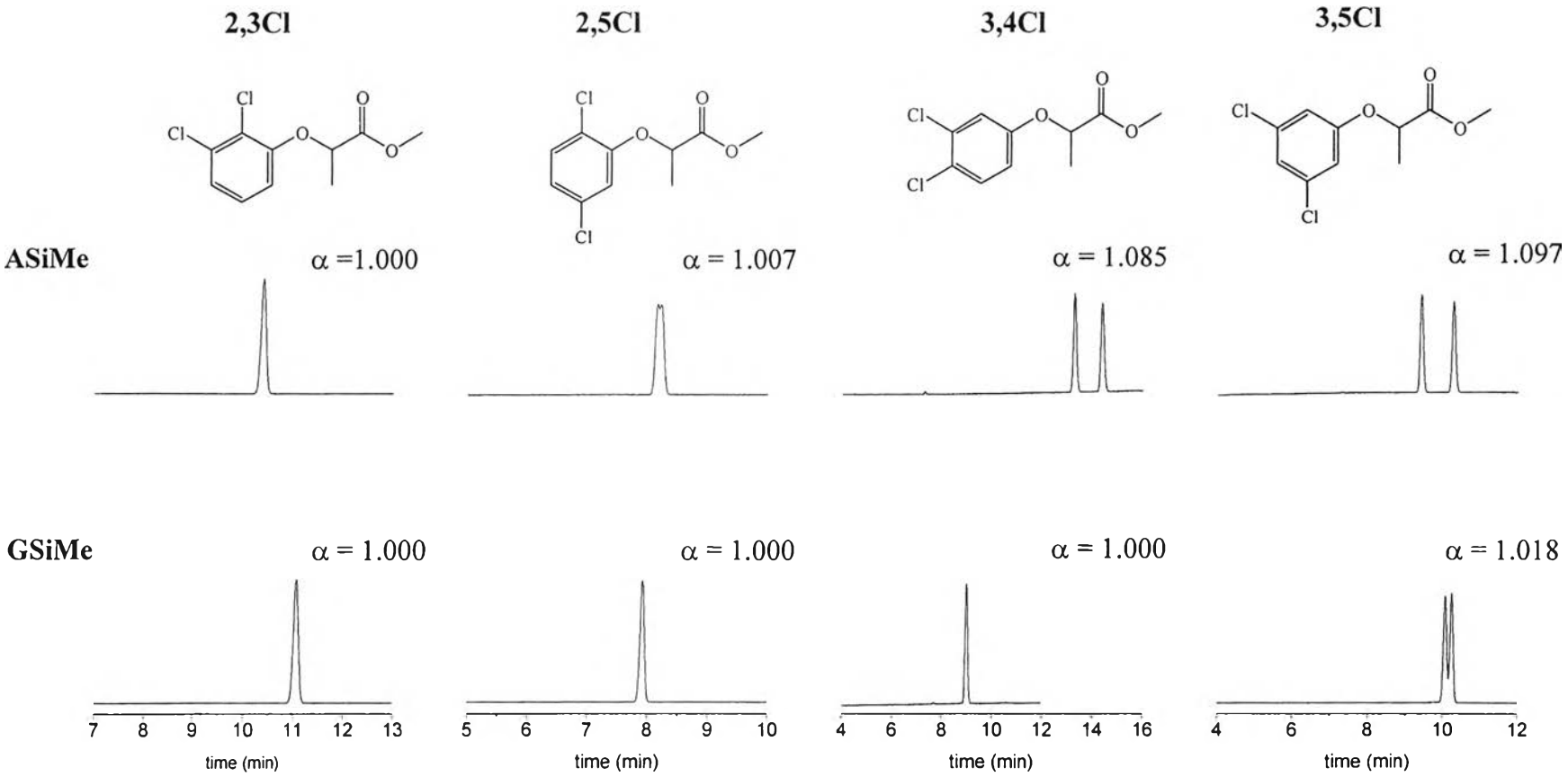
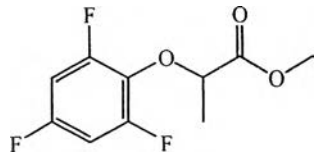
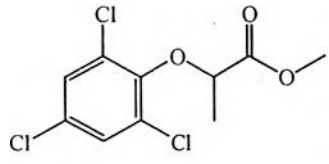


Figure 4.12 Chromatograms of **2,3Cl**, **2,5Cl**, **3,4Cl**, and **3,5Cl** on ASiMe and GSiMe columns at 140 °C.

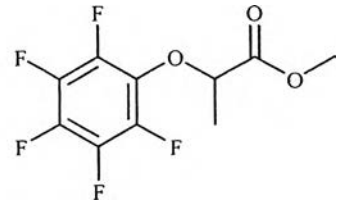
Group 3: Other phenoxy acid methyl esters with substitution on the aromatic ring



2,4,6F



2,4,6Cl



pentaF

Enantiomeric separation of reference **1** and esters in **group 3** on three chiral columns were compared (Figure 4.13). While ASiMe could not separate enantiomers of unsubstituted (**1**) or penta-substituted (**pentaF**) esters, it could separate enantiomers of **2,4,6F** and **2,4,6Cl**. Unfortunately, GSiMe could not resolve enantiomers of all four analytes. This indicated the importance of the size of cyclodextrin ring. For BSiMe, it could separate enantiomers of **1**, **2,4,6F**, and **pentaF**, but not **2,4,6Cl** [23]. It is interesting to see that an addition of one more substituent at *para*-position as in **2,4,6F** and **2,4,6Cl** (compared to **2,6F** and **2,6Cl** in **group 2**) could introduce the enantioseparation on small-sized cyclodextrin derivative, ASiMe. Unfortunately, other tri-, tetra- and penta-substituted analytes and their isomers are not available; thus, a common trend on the effect of number of aromatic-substituent on enantioseparation could not be proposed.

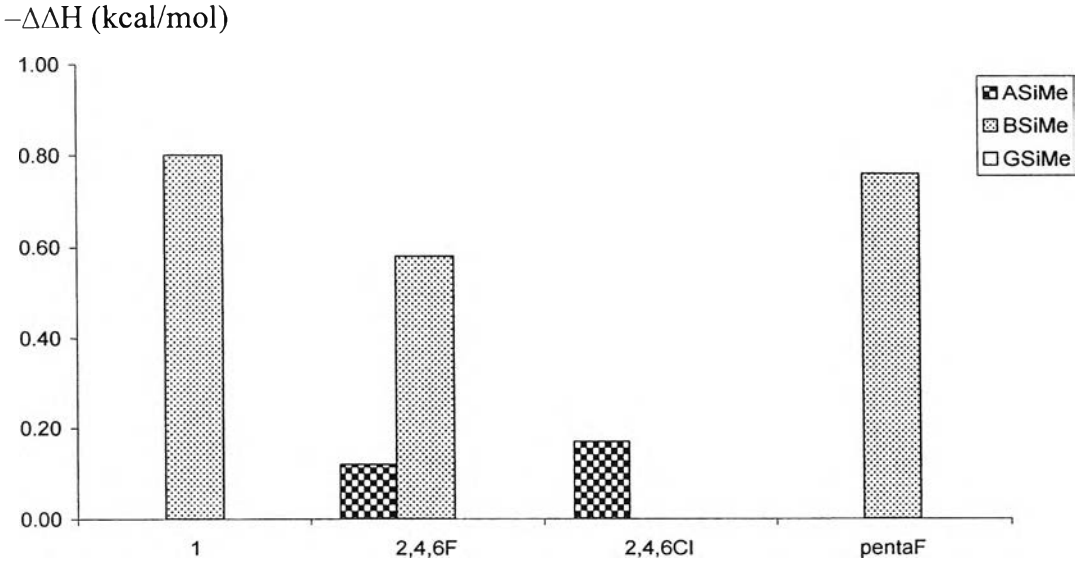


Figure 4.13 Enthalpy differences of **1**, **2,4,6F**, **2,4,6Cl**, and **pentaF** on ASiMe, BSiMe, and GSiMe columns.