# Enantioseparation and Chiral Recognition of $\alpha$ -Cyclohexylmandelic Acid and Methyl $\alpha$ -Cyclohexylmandelate on Hydroxypropyl- $\beta$ -Cyclodextrin as Chiral Selector: HPLC and Molecular Modeling

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Enantioseparations of (R/S)- $\alpha$ -cyclohexylmandelic acid [(R/S)-CHMA] and methyl (R/S)- $\alpha$ -cyclohexylmandelate [(R/S)-MCHMA] were performed on an achiral column (ODS) with 2-hydroxypropyl-B-cvclodextrin (HP-B-CD) as a chiral mobile phase additive. The influences of chromatographic conditions on the retention behavior of (R/S)-CHMA and (R/S)-MCHMA were studied in detail. Meanwhile, the thermodynamics parameters of enantioseparations for (R/S)-CHMA and (R/S)-MCHMA were determined to discuss driven power in the enantioseparation process. The inclusion complexation of HP- $\beta$ -CD with each enantiomer for (R/S)-CHMA and (R/S)-MCHMA was simulated by molecular docking to understand the chiral recognition mechanism of (R/S)-CHMA and (R/S)-MCHMA on HP-B-CD. The results showed that the chiral recognition ability of enantiometers of (R/S)-CHMA and (R/S)-MCHMA on HP- $\beta$ -CD is better than  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD and DM- $\beta$ -CD. Under the selected chromatographic conditions, baseline separations of enantiomers of (R/S)-CHMA and (R/S)-MCHMA were achieved. It is proved that the stoichiometry for (R/S)-CHMA-HP- $\beta$ -CD and (R/S)-MCHMA-HP-B-CD complexes is 1:1. However, the results of thermodynamics parameters analysis and molecular modeling show that the enantioseparations of CHMA and MCHMA on HP- $\beta$ -CD are enthalpy-driven processes and the primary driving forces responsible for chiral recognition are hydrophobic forces, dipole-dipole interaction, charge-transfer and hydrophobic interaction.

#### Introduction

 $\alpha$ -Cyclohexylmandelic acid (CHMA) and methyl  $\alpha$ -cyclohexylmandelate (MCHMA) are important pharmaceutical precursors that can be used as raw materials for producing multiple drugs, such as cyclandelate and oxybutynin with biological activity and good curative effect (1, 2). It is well known that the enantiomers of chiral drugs usually show different bioactivity in the human body. For example, (*S*)-oxybutynin has better pharmacological actions and lower side effects than its racemic mixture (2). Therefore, the enantioseparation of CHMA and MCHMA is necessary for producing chiral drugs, assessing the pharmacokinetic attributes of each enantiomer and controlling the enantiomeric purity of pharmaceutical preparations.

At present, several methods for the enantioseparations of (R/S)-CHMA and (R/S)-MCHMA have been reported. Tang *et al.* (3, 4) reported that enantiomers of CHMA were separated in a two-phase extraction system. Guo *et al.* (5) utilized

enantioselective liquid-liquid extraction to study the effect of the concentration of 2-hydroxypropyl-β-cyclodextrin (HPβ-CD), the concentration of CHMA, the mass fractions of C<sub>2</sub>H<sub>5</sub>OH and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, temperature and pH value on the chiral recognizition of CHMA enantiomers. Huang et al. (6, 7) reported the enantioseparation of racemic CHMA containing copper(II) N-dodecyl-(L)-hydroxyproline (CuN<sub>2</sub>) as a chiral carrier using hollow fiber-supported liquid membrane. Feitsma et al. (8) investigated the enantioseparation and elution order of CHMA on two β-CD bonded stationary phases using highperformance liquid chromatography (HPLC) and found that the elution order of the optical isomers is different on the two columns. We recently reported the enantioseparation of methyl mandelate (MMA) and MCHMA on permethylated  $\beta$ -cyclodextrin (PM- $\beta$ -CD) chiral stationary phase (9). The chromatographic separation of enantiomers of chiral compounds is a very efficient method for evaluting the pharmacokinetic attributes of each enantiomer and determining the enantiomeric purity in the process of phamaceutical preparation.

Cyclodextrins and their derivatives have been widely used as chiral selectors in the field of enantioseparation of chiral compounds (10–16). To understand the chiral recognition mechanism underlying the chiral separation using cyclodextrins and their derivatives as chiral selectors, great advances have been made using experimental (17, 18) and theoretical methods (19–24) during the past decade. However, to the best of our knowledge, the chiral recognition mechanism of CHMA and MCHMA on HP- $\beta$ -CD has not yet been reported.

The goal of this work was to establish a method for the enantioseparation of CHMA or MCHMA using cyclodextrins (CDs) as chiral mobile phase additive and to understand chiral recognition mechanism at the molecular level via the molecular modeling. To achieve our goal, the effect of chromatographic conditions (such as type and concentration of CD additive, pH, content of methanol and column temperature) on the enantionseparation of CHMA and MCHMA was first researched. Second, the thermodynamic parameters in the process of enantioseparations of CHMA and MCHMA using HP-B-CD as chiral additive were determined to discuss the driven power in the process of enantioseparations. Third, the interaction of HP-B-CD with enantiomers of CHMA and MCHMA was simulated by the molecular docking method and natural bond orbital (NBO) analysis was performed to discover why and how the chiral recognition takes place.

# **Materials and Methods**

# Chemicals

α-Cyclodextrin (α-CD) and γ-cyclodextrin (γ-CD) were purchased from Wuhan Xianghe Corporation (Wuhan, China). β-Cyclodextrin (β-CD) was purchased from Tailong Science & Technology Corporation (Wuhan, China). 2,6-Dimethyl-βcyclodextrin (DM-β-CD) was purchased from Kunshan Ruisikechemical Raw Material Corporation (Jiangsu, China). HP-β-CD was purchased from Shandong Xinda Fine Chemical Corporation (Shandong, China). (*R*/*S*)-CHMA, (*R*/*S*)-MCHMA, (*R*)-CHMA and (*S*)-MCHMA were provided by College of Pharmaceutical Sciences, Zhejiang University of Technology (Zhejiang, China).

## Apparatus

An Agilent 1100 series HPLC with variable wavelength detection (VWD) and a Chemstation workstation was purchased from Agilent Corporation (Santa Clara, CA). A Tigerkin ODS3 column was purchased from Dalian Sipore Corporation (Dalian, China). The solvent filter was purchased from Changcheng Glass Instrument Factory (Anhui, China).

#### **HPLC** measurements

The solutes used in this experiment were dissolved by methanol and these solutions were filtered through a 0.22- $\mu$ m filter before use.

## Chromatographic conditions

A mixture of methanol and water containing CDs as chiral additives was used as mobile phase; the flow rate was set at 0.8 mL/min; the sampling volume was 20  $\mu$ L; the ultraviolet (UV) detection wavelength was 221 nm. Sodium nitrate was used to determine the t<sub>0</sub> value.

# Molecular modeling

The starting structures of (R/S)-CHMA and (R/S)-MCHMA enantiomers were constructed with the help of Chem 3D Ultra (Version 8.0, CambridgeSoft; Cambridge, MA). These structures were first optimized by the semiempirical PM3 method implemented in the Gaussian 03 and then further optimized by the density functional theory (DFT) implemented in the Gaussian 03 at B3LYP/6-31 + g(d) level until all eigenvalues of the Hessian matrix were positive (25).

Using Chem 3D Ultra, the initial geometry of HP- $\beta$ -CD was constructed from the crystallographic parameters of  $\beta$ -CD taken from the Cambridge Structureal Database (CSD). The H-atoms in the 2-OH of each glucose unit for  $\beta$ -CD were replaced with 2-hydroxypropyl groups. Similarly, the structure was first optimized by the PM3 method and then further optimized by DFT at the B3LYP/6-31g level until all eigenvalues of the Hessian matrix were positive. The DFT-optimized structures of (*R/S*)-CHMA, (*R/S*)-MCHMA and HP- $\beta$ -CD were used for the molecular docking calculations.

The interactions of HP- $\beta$ -CD with (R/S)-CHMA and (R/S)-MCHMA enantiomers were simulated by molecular

docking using AutoDock 4.0 (26). The Lamarckian genetic algorithm (LGA) was selected for each enantiomers for the CHMA and MCHMA conformational search, and all parameters were the same for each docking. The partial atomic charges of HP-B-CD and each enantiomer were calculated using the Gasterier-Marsili (27) and Kollman methods (28), respectively. After merging non-polar hydrogen, the rotatable bonds were assigned. The grid maps were then calculated using AutoGrid. A grid map of dimensions  $60 \times 60 \times 60$  Å with a grid-point spacing of 0.375 Å was created for each enantiomer, which ensured an appropriate size of the each enantiomer-accessible space (29). The number of genetic algorithm runs for each inclusion complex and the number of evaluations for each run were set to 200 and 2.5 million, respectively. Other docking parameters such as crossover, mutation and elitism were set as default (0.08, 0.02 and 1, respectively). Based on RMS cluster tolerance between structures, the (R/S)-CHMA-HP- $\beta$ -CD and (R/S)-MCHMA-HP- $\beta$ -CD complexs were sorted into clusters. Finally, we obtained the dominating configuration of (R/S)-CHMA–HP- $\beta$ -CD and (R/S)-MCHMA–HP- $\beta$ -CD complexes with minimum binding free energy ( $\Delta G$ ).

# NBO analysis

NBO analysis for the dominating configuration of inclusion complexes with minimum binding free energy ( $\Delta$ G) obtained by molecular docking was performed at the B3LYP/6-31g level to obtain information about the intramolecular and intermolecular interactions such as hydrogen bonding, intermolecular charge transfer and dipole-dipole interaction of HP- $\beta$ -CD with (R/S)-CHMA and (R/S)-MCHMA. In the NBO analysis, the electronic delocalization interaction can be quantitatively described using the stabilization energy [ $E^{(2)}$ ], which is estimated by second order perturbation theory. The  $E^{(2)}$  can be calculated according to Eq. (1) (30, 31):

$$E^{(2)} = -n_{\sigma} \frac{\langle \sigma | F | \sigma \rangle}{\varepsilon_{\sigma*} - \varepsilon_{\sigma}} = -n_{\sigma} \frac{F_{i,j}^2}{\Delta E} = -n_{\sigma} \frac{F_{i,j}^2}{E_i - E_i} (1)$$

where  $\langle \sigma | F | \sigma \rangle$  denotes the Fock matrix elements between *i* and *j* orbitals;  $F_{i,j}$  is the off-diagonal NBO Fock matrix element;  $\varepsilon_{\sigma*}$  and  $\varepsilon_{\sigma}$  are the energies of  $\sigma$  and  $\sigma^*$  NBO orbitals,  $n_{\sigma}$  denotes the population of the donor  $\sigma$  orbital;  $E_i$  and  $E_j$  denote diagonal elements (orbital energies).

# **Results and Discussion**

## Influence of the species of cyclodextrin

The effect of the species of cyclodextrins on the enantioseparations of (R/S)-CHMA and (R/S)-MCHMA was evaluated. The experimental results showed that the enantiomers of (R/S)-CHMA and (R/S)-MCHMA were efficiently resolved using HP- $\beta$ -CD as chiral mobile phase additive, but not using other CDs, suggesting that the chiral recognition abilities of CDs for (R/S)-CHMA and (R/S)-MCHMA are closely related to the structure of CDs.

However, (*S*)-CHMA was eluted earlier than (*R*)-CHMA, while the (*R*)-MCHMA is eluted earlier than (*S*)-MCHMA, suggesting that the intermolecular interactions of (*S*)-CHMA and (*R*)-MCHMA with HP- $\beta$ -CD were stronger than that of (*R*)-CHMA and (*S*)-MCHMA, respectively. In other words, the

## Table I

Effect of the Concentration of HP- $\beta$ -CD on the Retention Factors (k'), Separation Factors ( $\alpha$ ) and Resolutions ( $B_c$ ) for Enantiomers of CHMA and MCHMA\*

$\mathcal{C}_{\mathrm{HP-B-CD}}$ (mol/L)	CHMA				MCHMA				
	k's	k' <sub>R</sub>	α	R <sub>S</sub>	k' <sub>R</sub>	k's	α	$R_S$	
0.005 0.010 0.015 0.020	65.95 42.25 33.75 24.4	72.19 47.38 38.20 27.69	1.09 1.12 1.13 1.13	1.54 2.01 2.24 1.85	161.34 108.90 85.48 63.16	168.68 116.93 92.22 68.91	1.05 1.07 1.08 1.09	0.83 1.23 1.64 0.91	

\*Note: Column, Tigerkin ODS3; mobile phase, CH<sub>3</sub>OH-H<sub>2</sub>O (containing  $5 \sim 20 \text{ mmol/L}$ HP-β-CDs, 20 mmol/L KH<sub>2</sub>PO<sub>4</sub> and *n* mmol/L H<sub>3</sub>PO<sub>4</sub>, pH 2.5), 40:60 (v/v); flow rate, 0.8 mL/ min; column temperature, room temperature;  $k'_{\text{R}}$  and  $k'_{\text{S}}$  mean the retention factors of (*R*)-isomer and (*S*)-isomer, respectively.

inclusion complexes formed by HP- $\beta$ -CD with (*S*)-CHMA or (*R*)-MCHMA were more stable than with (*R*)-CHMA or the (*S*)-MCHMA. It is implied that there is an obvious different chiral recognition mechanism of HP- $\beta$ -CD with enantiomers of CHMA and MCHMA.

# Influence of concentration of HP-β-CD

The experimental results concerning the effect of the concentration of HP- $\beta$ -CD on the retention factors (k'), separation factors ( $\alpha$ ) and resolution factors (R<sub>s</sub>) for the (R/s)-CHMA and (R/s)-MCHMA under the selected conditions are summarized in Table I. Table I shows that when the concentration of HP- $\beta$ -CD is 15 mmol/L, the enantioseparation degrees of CHMA and MCHMA are better. This may be because the formation of the inclusion complexes of CHMA and MCHMA with HP- $\beta$ -CD was incomplete, leading to either partial or no resolution of enantiomers while the concentration of HP- $\beta$ -CD was lower. The increase of the concentration of HP- $\beta$ -CD can favor the formation of the inclusion complexes. Then, with the further increase of the concentration of HP- $\beta$ -CD, the self-associated interactions between molecules of HP- $\beta$ -CD will increase, leading to a reduction of enantioseparation degrees.

In most cases, the inclusion complex formed by the interaction of solute molecule with CDs is 1:1 stoichiometry. In some cases, two or more CD molecule can also associate with a single solute molecule. The relationship between the capacity factor (k') of the solute molecule in the chromatographic system and the concentration of CDs in the case of 1:1 stoichiometry can be expressed as Eq. (2) (32, 33):

$$\frac{1}{k'} = \frac{1}{\phi K C_A} + \frac{K_l C_{CDs}}{\phi K C_A} (2)$$

where *K* and *K*<sub>t</sub> are the corresponding equilibrium constant for the interaction of the solute molecule with the stationary phase absorption site and the corresponding equilibrium constant for the interaction of solute molecule with CDs, respectively;  $\phi$ denotes the phase ratio; *C*<sub>A</sub> and *C*<sub>CDs</sub> mean the stationary phase absorption site surface density or concentration and the concentration of CDs, respectively. The dependence of the reciprocal retention factors (1/k') of (*R*/*S*)-CHMA and (*R*/*S*)-MCHMA upon concentration of HP- $\beta$ -CD (*C*<sub>HP- $\beta$ -CD</sub>) is depicted in Figure 1. Figure 1 shows that there is a good linear correlation between 1/*k*<sup>'</sup> and *C*<sub>HP- $\beta$ -CD over the entire concentration range studied, which is shown in Eqs. (3) to (6). It is indicated that the complexes of (*R*/*S*)-CHMA and (*R*/*S*)-MCHMA with HP- $\beta$ -CD</sub>



**Figure 1.** Reciprocal retention factors of the enantiomers of CHMA and MCHMA upon concentration of HP- $\beta$ -CD: 1, (S)-CHMA; 2, (R)-CHMA; 3, (R)-MCHMA; 4, (S)-MCHMA.

Table II

Effect of Methanol Content of on the Retention Factors (k'), Separation Factors ( $\alpha$ ) and Resolutions (R\_S) for Enantiomers of CHMA and MCHMA\*

CMeOH (% v/v)	CHMA				MCHMA	Ą		
	k's	k' <sub>R</sub>	α	R <sub>S</sub>	k' <sub>R</sub>	k's	α	$R_S$
40	33.73	38.17	1.13	2.19	85.49	92.16	1.08	1.69
45	28.08	31.55	1.12	1.60	77.90	82.74	1.06	1.31
55	15.26	16.59	1.09	1.10	35.18	36.24	1.03	_
60	10.11	10.72	1.06	0.76	21.93	21.93	1.00	0

\*Note: Column, Tigerkin ODS3; mobile phase, CH<sub>3</sub>OH-H<sub>2</sub>O (containing 5  $\sim$  20 mmol/L HP- $\beta$ -CDs, 20 mmol/L KH<sub>2</sub>PO<sub>4</sub> and *n* mmol/L H<sub>3</sub>PO<sub>4</sub>, pH 2.5), 40:60 (v/v); flow rate, 0.8 mL/ min; column temperature, room temperature;  $k'_{\rm B}$  and  $k'_{\rm S}$  mean the retention factors of (*R*)-isomer and (*S*)-isomer, respectively.

with 1:1 stoichiometry are formed by inclusion interaction. Additionally, the apparent stability constants of (*R*)-CHMA and (*S*)-CHMA upon complexation with HP- $\beta$ -CD are (2.3 ± 0.6) × 102 M<sup>-1</sup> and (2.6 ± 0.8) × 102 M<sup>-1</sup>, respectively, as the apparent stability constants of (*R*)-MCHMA–HP- $\beta$ -CD and (*S*)-MCHMA–HP- $\beta$ -CD are (2.2 ± 0.5) × 102 and (1.9 ± 0.4) × 102 M<sup>-1</sup>, respectively, at the selected chromatographic conditions. It is generally suggested that the chiral discrimination ability of chiral compounds on HP- $\beta$ -CD is primarily based on the difference in the stability constant of each enantiomer upon complexation with CDs:

$$\frac{1}{k'_{\text{S,CHMA}}} = (0.00651 \pm 0.0019) + (1.66 \\ \pm 0.14)C_{\text{HP}-\beta-\text{CD}} \quad (r = 0.9889) \ (3)$$
$$\frac{1}{k'_{\text{R,CHMA}}} = (0.00635 \pm 0.0018) + (1.44 \\ \pm 0.13)C_{\text{HP}-\beta-\text{CD}} \quad (r = 0.9876) \ (4)$$
$$\frac{1}{k'_{\text{R,MCHMA}}} = (0.00287 \pm 0.00064) + (0.628 \\ \pm 0.046)C_{\text{HP}-\beta-\text{CD}} \quad (r = 0.9917) \ (5)$$
$$\frac{1}{k'_{\text{S,MCHMA}}} = (0.00295 \pm 0.00056) + (0.561 \\ \pm 0.041)C_{\text{HP}-\beta-\text{CD}} \quad (r = 0.9921) \ (6)$$

#### Influence of content of methanol in mobile phase

As shown in Table II, the k',  $\alpha$  and  $R_S$  for (R/S)-CHMA and (R/S)-MCHMA resolved on Tigerkin ODS3 using HP- $\beta$ -CD as



Figure 2. Chromatograms of enantioseparations of CHMA (A); MCHMA (B). Separation was performed on an ODS3 column with CH<sub>3</sub>OH:H<sub>2</sub>O (containing 15 mmol/L HP-β-CD and 20 mmol/L KH<sub>2</sub>PO<sub>4</sub>-H<sub>3</sub>PO<sub>4</sub>, pH 2.5), 40:60 (v/v) used as mobile phase; flow rate 0.8 mL/min; room temperature.

chiral mobile phase additive all decreased with the increase of methanol content in the mobile phase, indicating that methanol content in the mobile phase obviously affects the stability constant of (R/S)-CHMA and (R/S)-MCHMA upon complexation with HP- $\beta$ -CD. This may be because hydrophobic interactions between the solute and the stationary phase are decreased with the increase of methanol content. Meanwhile, at higher methanol contents, methanol competes with solute for preferred locations in the hydrophobic cavity and hydroxypropyl moieties of HP- $\beta$ -CD (34). As a result, the chiral recognition abilities of (R/S)-CHMA and (R/S)-MCHMA on HP- $\beta$ -CD decrease with the increase of methanol content.

#### Table III

Effect of pH Value of the Mobile Phase on the Retention Factors (k'), Separation Factors ( $\alpha$ ) and Resolutions ( $R_S$ ) for Enantiomers of CHMA and MCHMA\*

pН	CHMA				MCHMA						
pH C kt 2.5 3 2.8 3 3.1 2 3.5 2	k's	k' <sub>B</sub>	α	$R_S$	k' <sub>R</sub>	k's	α	$R_S$			
2.5 2.8 3.1 3.5	33.74 32.37 25.09 23.47	38.19 36.55 28.04 26.01	1.13 1.13 1.12 1.11	2.03 1.75 1.49 1.45	83.08 84.48 86.25 91.54	87.92 91.24 93.29 99.34	1.06 1.08 1.08 1.09	1.49 1.56 1.64 1.71			

\*Note: Column, Tigerkin ODS3; mobile phase,  $CH_3OH-H_2O$  (containing 5 ~ 20 mmol/L HP- $\beta$ -CDs, 20 mmol/L  $KH_2PO_4$  and *n* mmol/L  $H_3PO_4$ , pH 2.5), 40:60 (v/v); flow rate, 0.8 mL/ min; column temperature, room temperature;  $k'_R$  and  $k'_S$  mean the retention factors of (*R*)-isomer and (*S*)-isomer, respectively.

#### Influence of pH value

As acidic and dipolar solute, the HPLC retention of (R/S)-CHMA and (R/S)-MCHMA should be strongly related to the pH of the mobile phase. To better understand the effect of pH value on the retention behaviors and enantioselectivity of (R/S)-CHMA and (R/S)-MCHMA enantiomers, the k' and  $\alpha$  for (R/S)-CHMA and (R/S)-MCHMA were determined in the various pH mobile phases containing HP- $\beta$ -CD, and the results are shown in Table III.

Table III shows that the retention times and resolutions of (R/S)-CHMA enantiomers decrease gradually with the increase of pH value in the mobile phase, as the retention times and resolutions of (R/S)-MCHMA increase gradually with the increase of pH value. The possible reasons for the acidic compound (R/S)-CHMA may be that one dissociation equilibrium exists in aqueous solution, leading to changing the apparent distribution coefficient of (R/S)-CHMA between mobile phase and stationary phase with the change of pH value in mobile phase solution. Additionally, the protonation degree of the neutral (R/S)-MCHMA enantiomers containing ester and hydroxyl moieties may increase with the decrease of pH value. In our experiment, the buffer solution was made of KH<sub>2</sub>PO<sub>4</sub> (20 mmol/L) and less H<sub>3</sub>PO<sub>4</sub>, so the ionic strength of the buffer solution increased from approximately 0.020 to 0.023 as the pH value decreased from 3.5 to 2.5. Therefore, increasing the ionic strength should increase the ion-dipole interaction of the neutral dipolar compound with ion in the mobile phase, with the result that the apparent oil-water partition coefficient of the neutral dipolar compound decreases. A similar phenomenon is also observed at  $pH = 2 \sim 5$  for neutral

## Table IV

Relationship Between Ink and T and Thermodynamic Parameters for the Enantioseparation of CHMA and MCHMA

Compounds		$\ln k' = A/$	T + B (r)*		$\Delta H$ (cal/mol)	$\Delta {\cal S} + {\sf Rln} \phi$ (cal/mol)	$\Delta$ ( $\Delta$ H) $^{\dagger}$ (cal/mol)	$\Delta (\Delta S)^{\ddagger}$ (cal/mol)	$\Delta (\Delta G)^{ m \$}$ (cal/mol)
		A B r							
(R/S)-CHMA	k's	614.6 698.1	1.512	0.9999	-1,221 -1,386	3.004	-165.0	-0.311	-73.25
(R/S)-MCHMA	k' <sub>R</sub> k' <sub>S</sub>	484.1 505.1	2.833 2.836	0.9991 0.9990	-961.9 -1,003.6	5.628 5.636	-43.0	0.008	-43.55

\*The equation shows the relationship between lnk and T, where A equals  $\Delta H/R$ , B equals  $\Delta S$  + Rln $\phi$ , r is correlation coefficient. R is gas constant (1.987 cal/mol). The temperature range studied is from 25 to 33°C.

 $^{\dagger}\Delta(\Delta H)$  is determined by the calculation of the difference of the enthalpic changes between the second-eluted enantiomer and the first-eluted enantiomer.

 $^{+}\Delta(\Delta S)$  is determined by the calculation of the difference of the entropic changes between the second-eluted enantiomer and the first-eluted enantiomer.

 ${}^{\$}\Delta(\Delta G)$  is calculated by  $\Delta G = \Delta H - T\Delta S$  at 298 K.

## Table V

Energies of the Complexes Obtained from Molecular Docking with AutoDock\*

Complexes	$\Delta G$	$\Delta E_1$	$\Delta E_2$	$\Delta E_3$
( <i>R</i> )-CHMA-HP-β-CD	-7.62	-7.87	-5.63	-2.24
( <i>S</i> )-CHMA-HP-β-CD	-8.04	-8.27	-5.90	-2.35
( <i>R</i> )-MCHMA-HP-β-CD	-6.24	-6.98	-6.84	-0.14
( <i>S</i> )-MCHMA-HP-β-CD	-5.89	-6.56	-6.51	-0.12

\*Note: Units are in kcal/mol.  $\Delta G$  is binding free energy change in the inclusion process, which is calculated in water solvent using a scoring function (38).  $\Delta E_1$  is intermolecular interaction energy, which means the energy of the interaction between HP- $\beta$ -CD and each enantionmer of CHMA and MCHMA and is a sum of Var der Waals energy, hydrogen bonding energy, desolvation free energy and electrostatic energy.  $\Delta E_2$  means the sum of Var der Waals energy, hydrogen bonding energy and desolvation energy.  $\Delta E_3$  is electrostatic energy.

andrographolide and dehydroandrographolide containing ester and hydroxyl moieties (35). Hence, retention times of the neutral (R/S)-MCHMA enantiomers are expected to decline as the pH value decreases.

# Influence of column temperature

The experimental results showed that, with the increase of column temperature, the eluting power for (R/S)-CHMA and (R/S)-MCHMA increases and the enantioseparation efficiency concomitantly decreases, which can be easily explained by the faster migration of the solute molecules through the chromatographic column and their lower affinity to the stationary phase.

Combining retention time and resolution, chromatographic conditions for better enantioseparation of CHMA and MCHMA were optimized. The optimized chromatographic conditions and chromatogram are shown in Figure 2.

## Thermodynamic parameters for enantioseparation

The relationship between the retention parameters (k' or  $\alpha$ ) and the column temperature (T) in the process of chromatographic enantioseparation can be described by Gibbs– Holmholtz and Van't Hoff equations (9) as follows:

$$\ln \mathbf{k}' = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \phi (7)$$
$$\ln \alpha = \frac{-\Delta (\Delta G)}{RT} = \frac{-\Delta (\Delta H)}{RT} + \frac{\Delta (\Delta S)}{R} (8)$$

where k' and  $\alpha$  should be defined as the retention factors and the separation factors, respectively; R and T are gas constant and absolute temperature (K), respectively;  $\phi$  is phase ratio;  $\Delta H$ and  $\Delta S$  denote the standard enthalpy and entropy of transfer of enantiomers from the mobile phase to the stationary phase, respectively;  $\Delta(\Delta H)$  and  $\Delta(\Delta S)$  denote the differences  $\Delta H_2 - \Delta H_1$ and  $\Delta S_2 - \Delta S_1$ , respectively, where subscripts 2 and 1 represent the more and the less retained enantiomers;  $\Delta(\Delta G)$  denotes a difference in Gibbs free energy of transfer from the mobile phase to the stationary phase between enantiomers. Therefore, apparent thermodynamic parameters in chromatographic separation process were also calculated from the plots of  $\ln k'$  or  $\ln \alpha$  versus 1/T. In most cases, the  $\Delta(\Delta H)$  value in enantioseparation process tends to be negative, favoring chiral recognition as temperature decreases, and the corresponding  $\Delta(\Delta S)$ value is generally also negative, counteracting chiral



**Figure 3.** Structures obtained by molecular docking for (R/S)-CHMA–HP- $\beta$ -CD and (R/S)-MCHMA–HP- $\beta$ -CD complexes.

recognition. This suggests that the enantioseparation is an enthalpy-driven process. Sometimes, the  $\Delta(\Delta H)$  and  $\Delta(\Delta S)$  for some chiral compounds are both positive, suggesting that the enantioseparation is entropy-driven. If the  $\Delta(\Delta H)$  and  $\Delta(\Delta S)$  are negative and positive, respectively, the enthalpic and entropic factors are both favorable to chiral recognition (36, 37).

The experimental results showed that the linear relationship between  $\ln k'$  or  $\ln \alpha$  and 1/T for (R/S)-CHMA and (R/S)S)-MCHMA are excellent (Table IV), indicating that the differences of enthalpic changes ( $\Delta H$ ) for (R/S)-CHMA and (R/S)-MCHMA enantiomers were invariable over the entire temperature range studied. It was suggested that the chiral recognition mechanism for CHMA and MCHMA on HP-B-CD did not change over the entire temperature range studied. Table IV also shows that the enthalpic changes ( $\Delta H$ ) for CHMA and MCHMA in the HPLC system using HP-β-CD as chiral additives are negative, which indicates that the enantioseparation of CHMA or MCHMA in the studied chromatographic system is exothermic and the transfer of solutes from the mobile phase to the CSP is enanthalpically favored. Although the differences of the enthalpic changes  $[\Delta(\Delta H)]$  between the second-eluted enantiomer and the first-eluted enantiomer for CHMA and MCHMA are both negative, the differences of the entropic

# Table VI

Partial Electron Donor Orbitals, Electron Acceptor Orbitals and the Corresponding E<sup>(2)</sup> Energies, Distances and Angles for (*R/S*)-CHMA-HP-β-CD Complexes Calculated by NBO Analysis at the B3LYP/ 6-31G Level\*

Betcon donor         Electron acceptor         d (Å)         Angle (*)         Electron           Whith OHMA         PP(2)0225         BD*(1)0235+H236         2.149         101.0         1.64         PP(2)0225         BD*(1)0235+H236         2.080         105.3         2.46           PP(2)0225         BD*(1)0245+H236         2.080         105.3         2.46         14.9         2.60           Find GMA to HP.9-00         PP(2)0225         BD*(1)C47+H157         2.36         152.5         0.92         PP(2)0225         BD*(1)C47+H29         2.342         163.4         1.11           PP(2)0225         BD*(1)C47+H157         2.36         0.63         PP(1)0234         BD*(1)C47+H29         2.342         163.4         1.11           PP(2)0225         BD*(1)C47+H173         2.612         123.7         0.55         PP(1)0234         BD*(1)C7+H13         1.927         151.7         6.22           PP(1)0235         BD*(1)C49+H159         1.920         167.9         3.45         PP(2)0225         BD*(1)C17+H13         1.927         151.7         6.22           PP(1)0235         BD*(1)C24+H24         BD*(1)C24+H24         BD*(1)C24+H24         BD*(1)C24+H24         BD*(1)C24+H24         0.95         BD*(1)C24+H24         0.95         BD*(1)C24+H24	(R)-CHMA-HP-B-CD					(S)-CHMA-HP-β-CD					
Write         UPU         View         View <th< th=""><th>Electron donor</th><th>Electron acceptor</th><th>d (Å)</th><th>Angle (°)</th><th>E<sup>(2)†</sup></th><th>Electron donor</th><th>Electron acceptor</th><th>d (Å)</th><th>Angle (°)</th><th>E<sup>(2)</sup></th></th<>	Electron donor	Electron acceptor	d (Å)	Angle (°)	E <sup>(2)†</sup>	Electron donor	Electron acceptor	d (Å)	Angle (°)	E <sup>(2)</sup>	
IP (2) (225       B0*(1) (225+1226       2.149       101.0       1.64       IP (2) (225       B0*(1) (1) (25+1226       2.080       105.3       2.46         From CMAA to IP*9-C       IP       1.333       117.09       4.16       IP (2) (225       B0*(1) (1) (25+1226       1.956       114.9       2.64       114.9       2.64       114.9       2.64       1.11         IP (1) (225       B0*(1) (27+1175       2.332       152.5       0.92       IP (2) (225       B0*(1) (1) (24+1159       2.342       1.63.4       1.11         IP (2) (224       B0*(1) (102+1173       2.612       12.7       0.55       IP (1) (23+113       1.27       1.7.6       2.24         IP (2) (225       B0*(1) (12+1173       2.612       12.7       0.55       IP (2) (225       B0*(1) (12+1173       2.612       1.20       0.37       2.24       1.80       1.11       0.80         IP (2) (225       B0*(1) (12+11473       2.612       12.3       0.94       IP (2) (225       B0*(1) (12+1147       2.42       1.40       0.80         IP (2) (225       B0*(1) (12+1147       0.94       IP (2) (225       B0*(1) (12+1144       0.54       IP (2) (225       B0*(1) (12+1144       0.54       IP (2) (225       B0*(1) (12+1141       0.56	Within CHMA										
IP       DP*(1)0225+H226       1.933       117.09       4.16       IP       P(2)0235       B0*(1)0225+H226       1.956       114.9       2.60         Fm CHMA ho HP-CD       IP       2.31       IP       IP       100235       B0*(1)1024+H129       2.342       18.4       1.11         IP       IP       DP       IP       DP       IP       1063       IP       DP       IP       1063       IP       DP       IP       1063       IP       DP       IP       10234       BD*(1)C7+H73       2.274       126.2       160       DP       DP       IP       126.2       167.9       0.55       IP       IP       DP       IP       1.97       1.17       6.22       IP       14.1       1.66       DP       1.44       1.62       1.41       1.62       IP       0.35       DP       IP       1.97       1.41       1.62       1.41       1.62       1.41       1.62       1.41       1.62       1.41       1.62       1.41       1.62       1.62       BD*(1)C24+H23       BD*(1)C24+H24       1.41       1.66       1.62       1.41       1.66       1.62       1.41       1.66       1.62       1.41       1.66       1.62       1.41	LP(2)0225	BD*(1)0235-H236	2.149	101.0	1.64	LP(2)0225	BD*(1)0235-H236	2.080	105.3	2.46	
From CHMA to HP-B-D         2236         152.5         0.92         LP(2)0225         BD*(1)C47-H157         2.91         LP(1)0234         BD*(1)C60-H166         2.618         166.1         0.65           LP(1)0234         BD*(1)C60-H166         2.224         176.3         0.63         LP(1)0234         BD*(1)C72-H175         167.9         0.87           LP(1)0234         BD*(1)C47-H157         2.212         123.7         0.55         LP(2)0235         BD*(1)C17-H173         2.612         2.23         0.55         LP(2)0235         BD*(1)C17-H174         2.224         176.3         0.224           LP(2)0235         BD*(1)C149-H159         0.94         LP(2)0235         BD*(1)C17-H140         2.195         144.1         1.06           BD(1)C22-11238         BD*(1)C17-H140         0.97         LP(2)0235         BD*(1)C17-H140         0.80           BD(1)C22-11238         BD*(1)C168-H171         0.56         BD(1)C22-124         BD*(1)C17-H140         2.225           BD(1)C123-H248         BD*(1)C168-H171         0.56         BD(1)C22-H248         BD*(1)C17-H173         2.224           BD(1)C22-H226         BD*(1)C168-H171         0.55         BD(1)C22-H248         BD*(1)C17-H173         2.230           BD(1)C22-H226         BD*(1)C17-H172	LP(2)0235	BD*(1)0225-H226	1.933	117.09	4.16	LP(2)0235	BD*(1)0225-H226	1.956	114.9	2.60	
LP(1)0225       BD*(1)C47+H157       2.26       15.2       0.92       LP(2)0225       BD*(1)C61-H129       2.342       16.3.4       1.1         LP(2)0225       BD*(1)C61-H157       2.91       LP(1)0234       BD*(1)C61-H166       2.618       16.6.1       0.85         LP(2)0234       BD*(1)C60-H166       2.612       12.3.7       0.55       LP(1)0235       BD*(1)C5+H131       1.927       15.1.7       6.22         LP(1)0235       BD*(1)C5+H149       1.920       167.9       3.45       LP(2)0235       BD*(1)C1+H140       2.195       14.4.1       1.06         BD(1)C21+1228       BD*(1)C5+H149       1.920       167.9       3.45       LP(2)0235       BD*(1)C1+H140       2.195       14.4.1       1.06         BD(1)C22+1228       BD*(1)C5+H141       0.94       LP(1)0235       BD*(1)C1+H140       2.195       14.4.1       1.06         BD(1)C22+1248       BD*(1)C5+H141       0.56       BD(1)C22-124       BD*(1)C2+H145       2.53         BD(1)C22+H246       BD*(1)C2+H248       BD*(1)C2+H241       BD*(1)C2+H24       BD*(1)C2+H24       2.30         BD(1)C22+H245       BD*(1)C2+H241       BD*(1)C2+H24       BD*(1)C2+H24       BD*(1)C2+H24       2.32         BD(1)C22+H245       BD*(1)C2+H	From CHMA to HP-B-	CD									
IP (2) (225       BD*1 (1064) H166       2.24       176.3       0.63       IP (10234       BD*1 (1072+1175       2.24       126.2       186         IP (10234       BD*1 (1060) H166       2.612       123.7       0.55       IP (10235       BD*1 (1072+1175       2.74       126.2       186         IP (10234       BD*1 (1074) H173       2.612       123.7       0.55       IP (10235       BD*1 (1074) H173       1.927       15.7       6.22         IP (10235       BD*1 (1074) H159       0.94       IP (10235       BD*1 (1074) H174       2.195       144.1       1.06         ID (10272) H228       BD*1 (1058) H164       0.97       IP (10235       BD*1 (1074) H174       0.95       1.94         ID (1022+14238       BD*1 (1058) H174       0.56       BD (10220-H248       BD*1 (1058) H164       0.95         ID (1022+1424       BD*1 (1068) H171       0.56       BD (10224) H248       BD*1 (1024) H175       2.53         ID (1022+1424       BD*1 (1074) H175       0.55       BD (1023) H242       BD*1 (1024) H174       2.53         ID (1022+1244       BD*1 (1024) H174       0.56       BD (1023) H242       BD*1 (1024) H174       1.31         ID (1023) H245       BD*1 (1024) H174       0.56       BD (1023) H242	LP(1)0225	BD*(1)C47-H157	2.236	152.5	0.92	LP(2)0225	BD*(1)C3-H129	2.342	163.4	1.11	
LP(1)0234         BD*(1)C60-H166         2.24         17.6         0.63         LP(1)0234         BD*(1)C7-H175         2.274         12.6         1.60           LP(2)0234         BD*(1)C7-H173         2.612         123.7         0.55         LP(1)0235         BD*(1)C5-H131         1.927         15.7         6.27           LP(1)0235         BD*(1)C49-H159         1.920         167.9         3.45         LP(2)0235         BD*(1)C1-H140         2.195         14.41         1.06           DD(1)C21-H220         BD*(1)C58-H164         0.97         LP(2)0235         BD*(1)C1-H140         2.195         14.41         1.06           DD(1)C22-H248         BD*(1)C58-H164         0.56         BD(2)C22-C223         BD*(1)C10-H195         2.23           DD(1)C22-H248         BD*(1)C3-H140         0.56         BD(1)C22-H248         BD*(1)C1-H195         2.23           DD(1)C22-H244         BD*(1)C17-H140         0.55         BD(1)C23-H244         BD*(1)C2-H143         2.23           DD(1)C22-H248         BD*(1)C2-H143         0.54         BD(1)C23-H244         BD*(1)C2-H143         1.55           DD(1)C23-H244         BD*(1)C23-H244         BD*(1)C2-H143         1.55         BD(1)C23-H244         BD*(1)C2-H143         1.56           DD(1)C23-H24	LP(2)0225	BD*(1)C47-H157			2.91	LP(1)0234	BD*(1)C60-H166	2.618	166.1	0.85	
LP[2]0234       BD*(1)(167)-1173       0.67       LP[2)0234       BD*(1)(167)-1175       0.62         LP[1]0235       BD*(1)(24)-1159       1.920       167.9       3.45       LP[2)0235       BD*(1)(15+1131       1.927       151.7       6.22         LP[2)0235       BD*(1)(164)-1159       1.920       167.9       3.45       LP[2)0235       BD*(1)(15+114)       2.195       144.1       1.06         BD[2)(221-6220       BD*(1)(158+1164       0.97       LP[2)0235       BD*(1)(15)-11440       2.195       144.1       1.06         BD[2)(222-4224       BD*(1)(158+1164       0.97       LP[2)0235       BD*(1)(158+1164       0.95         BD[2)(222-4224       BD*(1)(158+1164       1.17       BD(1)(220-4224       BD*(1)(158+1164       0.95         BD[2)(222-424       BD*(1)(158+1164       1.17       BD(1)(225+1226       BD*(1)(158+1164       2.23         BD(1)(223+1244       BD*(1)(164+1157       0.55       BD(1)(1220-1424       BD*(1)(128+1143       1.55         BD(1)(223+1244       BD*(1)(124+1145       1.31       1.65       BD(1)(223+1244       BD*(1)(125+1143       1.55         BD(1)(223+1244       BD*(1)(124+1157       0.55       BD(1)(123+1444       BD*(1)(125+1143       1.55         BD(1)(223+124	LP(1)0234	BD*(1)C60-H166	2.224	176.3	0.63	LP(1)0234	BD*(1)C72-H175	2.274	126.2	1.60	
LP(1)0234         BD*(1)(C3+1173         2.612         123.7         0.55         LP(1)0235         BD*(1)(C5+1131         1.927         15.7         6.22           LP(1)0235         BD*(1)(C4+1159         0.54         LP(2)0235         BD*(1)(C5+1131         2.24         2.24           BD/2)(221+0220         BD*(1)(1054+1164         0.97         LP(2)0235         BD*(1)(17)+1140         2.195         144.1         0.60           BD/2)(221+0220         BD*(1)(1058+1164         0.56         BD(2)(222-0224         BD*(1)(1058+1171         0.56         BD(1)(222+024         BD*(1)(1058+1171         0.56         BD1(10225+1226         BD*(1)(1058+1171         0.56         BD1(10225+1226         BD*(1)(1025+1143         2.53           BD(1)(222+1241         BD*(1)(1058+1171         0.56         BD1(10225+1226         BD*(1)(1025+1143         1.55           BD(1)(223+1244         BD*(1)(117+110         0.55         BD(1)(123-1424         BD*(1)(125+1143         1.55           BD(1)(223+1244         BD*(1)(125+1143         1.53         1.31         1.31         1.31           BD(1)(223+1245         BD*(1)(117+120         0.55         BD(1)(123+1244         BD*(1)(125+1143         1.31           BD(1)(223+1244         BD*(1)(123+1424         BD*(1)(123+1424         BD*(1)	LP(2)0234	BD*(1)C60-H166			1.08	LP(2)0234	BD*(1)C72-H175			0.87	
LP(1)0235       B0*(1)C49+H159       1.920       167.9       3.45       LP(2)0235       B0*(1)C17+H140       2.195       144.1       1.06         DP(2)0235       B0*(1)C3+H154       0.94       LP(2)0235       B0*(1)C17+H140       2.195       144.1       1.06         B01(2221+4238       B0*(1)C58+H164       0.97       LP(2)0235       B0*(1)C17+H140       0.80         B01(2221+4238       B0*(1)C58+H164       0.54       B01(1222+0C221       B0*(1)C58+H164       0.95         B01(10222+424       B0*(1)C58+H164       0.56       B01(10225+H226       B0*(1)C34-H150       2.53         B01(1022+424       B0*(1)C47+H157       0.56       B01(1023+H244       B0*(1)C28-H143       1.55         B01(10224+H24       B0*(1)C16+H138       5.98       B01(1023+H244       B0*(1)C28-H143       1.55         B01(1023+H245       B0*(1)C28+H144       B0*(1)C28-H143       1.51       1.31         B01(1023+H245       B0*(1)C28+H149       1.45       B01(1023+H244       B0*(1)C28+H143       1.80         B01(1023+H245       B0*(1)C28+H149       1.45       B01(1023+H244       B0*(1)C28+H143       1.80         B01(1023+H245       B0*(1)C28+H149       1.45       B01(1023+H244       B0*(1)C28+H236       1.33	LP(1)0234	BD*(1)C71-H173	2.612	123.7	0.55	LP(1)0235	BD*(1)C5-H131	1.927	151.7	6.22	
IP(2)0235       BD*1(1024+H159       0.94       IP(1)0235       BD*1(1017+H140)       2.195       144.1       1.06         BD/2)0219-0220       BD*1(1058+H164)       0.97       IP(2)0235       BD*1(107+H140)       1.94         BD/2)0219-0223       BD*1(1058+H164)       0.54       BD(2)022-0223       BD*1(1058+H164)       9.95         BD/1(0223+H240       BD*1(1058+H171)       0.56       BD(2)022-0223       BD*1(1023+H129)       2.32         BD/1(0224+H241)       BD*1(1054+H171)       0.56       BD(1)0223+H226       BD*1(1023+H143)       2.30         BD/1(023-H247       BD*1(1017+H20)       0.55       BD(1)023-H242       BD*1(1025+H143)       1.85         BD/1(023-H247       BD*1(1027+H145)       1.85       BD(1)023-H242       BD*1(1025+H143)       1.85         BD/1(023-H247       BD*1(1027+H44)       BD*1(1027+H44)       BD*1(1027+H45)       1.81         BD/1(023-H245       D*1(1027+H45)       1.80       1.81       1.80         BD/1(023-H247)       BD*1(1027+H44)       BD*1(1027+H45)       1.81         BD/1(023-H248)       2.711       138.8       0.53       IP(1)020       BD*1(1027+H45)       2.282       139.4       3.32         IP(1)07       BD*1(1023+H248       2.711       138.	LP(1)0235	BD*(1)C49-H159	1.920	167.9	3.45	LP(2)0235	BD*(1)C5-H131			2.24	
BD(2)C219-C220         BD*1(1)C58+H164         0.97         LP2(D235         BD*1(1)C17+H140         0.80           BD(1)C221+C224         BD*1(1)C58+H164         0.54         BD(2)C222-C221         BD*1(1)C58+H164         1.94           BD(1)C223+L240         BD*1(1)C58+H164         1.77         BD(1)C222-H228         BD*1(1)C17-H195         2.53           BD(1)C222+H248         BD*1(1)C47-H157         0.56         BD(1)C223-H242         BD*1(1)C36+H150         2.30           BD(1)C224+H24         BD*1(1)C47-H157         0.56         BD(1)C23-H242         BD*1(1)C23-H143         1.81           BD(1)C231-H244         BD*1(1)C27-H145         1.85         BD(1)C23-H244         BD*1(1)C27-H145         1.81           BD(1)C231-H244         BD*1(1)C27-H145         1.45         BD(2)C23-0234         BD*1(1)C1-H173         1.81           BD(1)C231-H245         BD*1(1)C47-H159         3.98         BD(1)C23-H246         2.282         139.4         3.21           BD(1)C232-H236         BD*1(1)C23-H245         2.063         1.34.7         2.96         LP(1)O20         BD*1(1)C27-H145         1.11           Frim HP_β-C to teCHMA         LP(1)O20         BD*1(1)C23-H246         2.478         19.9         0.56           LP(1)O31         BD*1(1)C23-H245	LP(2)0235	BD*(1)C49-H159			0.94	LP(1)0235	BD*(1)C17-H140	2.195	144.1	1.06	
B0(1)(221-H238         B0*(1)(281-H184         0.54         B0(2)(222-0221         B0*(1)(058-H171         1.94           B0(2)(223-0224         B0*(1)(058-H164         1.17         B0(1)(220-H248         B0*(1)(058-H174         0.55           B0(1)(222-H240         B0*(1)(058-H171         0.56         B0(2)(222-0228         B0*(1)(07-H195         2.53           B0(1)(222-H226         B0*(1)(07-H195         2.53         B0*(1)(023-H247         B0*(1)(07-H195         2.30           B0(1)(223-H247         B0*(1)(07-H195         0.55         B0(1)(023-H244         B0*(1)(025-H143         1.55           B0(1)(223-H244         B0*(1)(07-H195         2.30         1.55         B0(1)(023-H244         B0*(1)(025-H143         1.55           B0(1)(223-H244         B0*(1)(025-H143         0.94         B0(1)(023-H244         B0*(1)(027-H145         1.31           B0(1)(023-H245         B0*(1)(026-H166         1.45         B0(2)(023-0234         B0*(1)(017-H173         1.80           B0(1)(023-H246         B0*(1)(024-H165         1.45         B0(2)(023-H246         2.282         139.4         3.32           LP(1)07         B0*(1)(023-H246         2.063         134.7         2.96         LP(1002         B0*(1)(027-H265         2.282         139.4         3.32      <	BD(2)C219-C220	BD*(1)C58-H164			0.97	LP(2)0235	BD*(1)C17-H140			0.80	
BD(2)C223-C224         B0*(1)C58-H164         1.17         BD(1)C220-H248         BD*(1)C158-H164         0.55           BD(1)C223-H240         B0*(1)C69-H171         0.56         BD(1)C230-H241         BD*(1)C102-H125         2.22           BD(1)C224-H241         BD*(1)C17-H157         0.56         BD(1)C230-H241         BD*(1)C28-H130         2.30           BD(1)C230-H247         BD*(1)C17-H157         0.55         BD(1)C230-H242         BD*(1)C25-H143         1.65           BD(1)C231-H244         BD*(1)C17-H157         0.58         BD(1)C231-H244         BD*(1)C25-H143         1.31           BD(1)C231-H244         BD*(1)C25-H143         0.94         BD(1)C231-H244         BD*(1)C27-H145         1.77           BD(1)C231-H244         BD*(1)C27-H145         0.94         BD(1)C231-H244         BD*(1)C27-H145         1.77           BD(1)C231-H244         BD*(1)C27-H145         0.94         BD(1)C231-H244         BD*(1)C27-H145         1.77           BD(1)C231-H244         BD*(1)C23-H248         2.711         1.38         0.53         P(1)020         BD*(1)C25-H226         2.82         13.4         3.22           P(1)007         BD*(1)C23-H248         2.711         138.8         0.58         P(1)020         BD*(1)C23-H246         2.282         13.4	BD(1)C221-H238	BD*(1)C98-H184			0.54	BD(2)C220-C221	BD*(1)C69-H171			1.94	
BD(1)C223-H240         BD*(1)C69-H171         0.66         BD(2)C22-C223         BD*(1)C10-H195         2.53           BD(1)C224-H241         BD*(1)C39-H171         0.65         BD(1)C230-H241         BD*(1)C3-H129         2.23           BD(1)C232-H246         BD*(1)C17-H157         0.55         BD(1)C230-H244         BD*(1)C25-H143         1.65           BD(1)C231-H244         BD*(1)C25-H143         0.94         BD(1)C231-H244         BD*(1)C27-H145         1.31           BD(1)C231-H244         BD*(1)C24-H144         BD*(1)C27-H145         1.77         1.80           BD(1)C231-H244         BD*(1)C17-H173         1.80         1.80         1.80           BD(1)C232-H226         BD*(1)C49-H159         1.80         BD(1)C23-H224         BD*(1)C27-H145         1.80           BD(1)C232-H226         D*(1)C49-H159         1.81         BD(1)C23-H226         BD*(1)C27-H145         1.80           BD(1)C23-H226         D         1.31         BD(1)C23-H226         2.282         139.4         3.32           P(1)07         BD*(1)C23-H245         2.063         134.7         2.96         P(1)020         BD*(1)C23-H246         2.282         139.4         3.22           P(1)031         BD*(1)C23-H246         2.406         123.3         0.60	BD(2)C223-C224	BD*(1)C58-H164			1.17	BD(1)C220-H248	BD*(1)C58-H164			0.95	
BD(1)0225-H226         BD*(1)(1027-H127)         0.65         BD(1)0225-H226         BD*(1)(1024-H157)         0.56         BD(1)(230-H247         BD*(1)(17-H120)         1.65           BD(1)(223-H247         BD*(1)(11-H120)         0.55         BD(1)(223)-H244         BD*(1)(25-H143)         1.31           BD(1)(223)-H244         BD*(1)(125-H143)         0.94         BD(1)(223)-H244         BD*(1)(27-H145)         1.31           BD(1)(223)-H245         BD*(1)(1025-H143)         0.94         BD(1)(223)-H244         BD*(1)(27-H145)         1.31           BD(1)(223)-H246         BD*(1)(1025-H123)         1.33         BD(1)(223)-H244         BD*(1)(1027-H145)         1.80           BD(1)(223)-H246         BD*(1)(1049-H159)         3.98         BD(1)(1023)-H246         2.282         139.4         3.22           LP(1)007         BD*(1)(229-H248         2.711         138.8         0.53         LP(1)020         BD*(1)(023-H246         2.282         139.4         3.22           LP(1)071         BD*(1)(223-H245         2.063         134.7         2.96         LP(1)020         BD*(1)(023-H246         2.282         139.4         3.22           LP(1)074         BD*(1)(223-H245         2.063         134.7         2.96         LP(1)020         BD*(1)(023-H246         2.488	BD(1)C223-H240	BD*(1)C69-H171			0.56	BD(2)C222-C223	BD*(1)C107-H195			2.53	
BD(1)0225-H226       BD*(1)C47-H157       0.56       BD(1)C230-H241       BD*(1)C36-H150       2.30         BD(1)C230-H244       BD*(1)C117-H20       0.55       BD(1)C230-H242       BD*(1)C25-H143       1.65         BD(1)C231-H244       BD*(1)C16-H138       5.98       BD(1)C231-H244       BD*(1)C27-H145       1.31         BD(1)C231-H245       BD*(1)C60-H166       1.45       BD(2)C233-0234       BD*(1)C17-H173       1.80         BD(1)C231-H246       BD*(1)C24-H28       2.711       138.8       0.53       LP(1)O20       BD*(1)O23-H226       2.282       139.4       3.32         LP(1)07       BD*(1)C23-H248       2.711       138.8       0.53       LP(1)O20       BD*(1)O23-H226       2.282       139.4       3.32         LP(1)031       BD*(1)C23-H245       2.063       134.7       2.96       LP(1)O20       BD*(1)O23-H236       2.38       128.7       2.79         LP(1)031       BD*(1)C23-H245       2.066       123.3       0.60       LP(1)042       BD*(1)C23-H244       2.478       119.9       0.56         LP(1)064       BD*(1)C23-H246       2.108       119.8       5.09       LP(1)042       BD*(1)C23-H244       2.478       119.9       0.56         LP(1)064       BD*(1)C23-H246 </td <td>BD(1)C224-H241</td> <td>BD*(1)C69-H171</td> <td></td> <td></td> <td>0.65</td> <td>BD(1)0225-H226</td> <td>BD*(1)C3-H129</td> <td></td> <td></td> <td>2.22</td>	BD(1)C224-H241	BD*(1)C69-H171			0.65	BD(1)0225-H226	BD*(1)C3-H129			2.22	
BD(1)(230-H247       BD*(1)(117-H20       0.55       BD(1)(230-H242       BD*(1)(25-H143       1.65         BD(1)(221-H244       BD*(1)(16-H138       5.98       BD(1)(231-H244       BD*(1)(25-H143       1.31         BD(1)(223)-H245       BD*(1)(260-H166       1.45       BD(2)(233-0234       BD*(1)(27-H145       1.80         BD(1)(223)-H246       BD*(1)(202-H128       1.45       BD(2)(233-0234       BD*(1)(17-H173       1.80         BD(1)(223)-H236       BD*(1)(223-H248       2.711       138.8       0.53       LP(1)020       BD*(1)(225-H226       2.282       139.4       3.32         LP(1)07       BD*(1)(223-H248       2.711       138.8       0.53       LP(1)020       BD*(1)(223-H246       2.282       139.4       3.32         LP(1)031       BD*(1)(223-H245       2.063       134.7       2.96       LP(1)020       BD*(1)(236-H236       2.288       128.7       2.79         LP(1)053       BD*(1)(223-H246       2.063       134.7       2.96       LP(1)020       BD*(1)(236-H236       2.288       128.7       2.79         LP(1)053       BD*(1)(223-H246       2.066       123.3       0.60       LP(1)042       BD*(1)(231-H244       2.468       120.5       0.70         LP(1)054	BD(1)0225-H226	BD*(1)C47-H157			0.56	BD(1)C230-H241	BD*(1)C36-H150			2.30	
BD(1)C231-H244       BD*(1)C16-H138       5.98       BD(1)C231-H244       BD*(1)C25-H143       1.31         BD(1)C231-H245       BD*(1)C25-H143       0.94       BD(1)C231-H244       BD*(1)C27-H145       1.77         BD(2)C233-0234       BD*(1)C60-H166       1.45       BD(2)C233-0234       BD*(1)C71-H173       1.80         BD(1)C23+H236       BD*(1)C29-H248       2.711       138.8       0.53       LP(1)020       BD*(1)0235-H226       2.282       139.4       3.32         LP(1)07       BD*(1)C23+H245       2.063       134.7       2.96       LP(1)020       BD*(1)0235-H226       2.282       139.4       3.22         LP(1)031       BD*(1)C23+H245       2.063       134.7       2.96       LP(1)020       BD*(1)0235-H226       2.282       139.4       3.22         LP(1)031       BD*(1)C23+H245       2.063       134.7       2.96       LP(1)020       BD*(1)C23-H246       2.282       139.4       3.20         LP(1)053       BD*(1)C23+H245       2.108       119.8       5.09       LP(1)042       BD*(1)C23-H244       2.468       120.5       0.70         LP(1)064       BD*(1)C23+H246       2.108       137.0       0.57       LP(1)010       BD*(1)C22-H245       2.472       118.9       0.59	BD(1)C230-H247	BD*(1)C117-H20			0.55	BD(1)C230-H242	BD*(1)C25-H143			1.65	
BD(1)C231-H245       BD*(1)C25-H143       0.94       BD(1)C231-H244       BD*(1)C27-H145       1.77         BD(2)C233-0234       BD*(1)C60-H166       1.45       BD(2)C233-0234       BD*(1)C71-H173       1.80         BD(1)0235-H236       BD*(1)C49-H159       3.98       BD(1)0235-H236       BD*(1)C27-H145       1.81         P(1)07       BD*(1)C229-H248       2.711       138.8       0.53       LP(1)020       BD*(1)0235-H236       2.282       139.4       3.32         LP(1)031       BD*(1)C231-H245       2.063       134.7       2.96       LP(1)020       BD*(1)C235-H236       2.282       139.4       3.32         LP(1)053       BD*(1)0225-H226       2.666       123.3       0.60       LP(1)020       BD*(1)0235-H236       2.478       119.9       0.56         LP(1)054       BD*(1)0235-H236       2.108       119.8       5.09       LP(1)042       BD*(1)C23-H244       2.478       119.9       0.56         LP(1)054       BD*(1)0235-H236       2.108       119.8       5.09       LP(1)042       BD*(1)C23-H244       2.478       119.9       0.59         LP(1)074       BD*(1)C23-H240       2.394       137.0       0.57       LP(1)010       BD*(1)C22-H245       2.472       118.9       0.59	BD(1)C231-H244	BD*(1)C16-H138			5.98	BD(1)C231-H244	BD*(1)C25-H143			1.31	
BD(2)C233-0234 BD(1)C25-H236 From HP-β-CD to CHMA         BD*(1)C29-H248 From HP-β-CD to CHMA         1.45         BD(2)C233-0234 BD(1)C23-H236         BD*(1)C17-H140         1.11           LP(1)07         BD*(1)C229-H248         2.711         138.8         0.53         LP(1)020         BD*(1)0235-H236         2.282         139.4         3.32           LP(1)031         BD*(1)C231-H245         2.063         134.7         2.96         LP(1)020         BD*(1)0235-H236         2.238         128.7         2.79           LP(1)053         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)0235-H236         2.478         11.9         0.56           LP(1)064         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)C23-H241         2.478         11.9         0.56           LP(1)064         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)C231-H244         2.468         120.5         0.70           LP(1)064         BD*(1)0225-H226         2.108         119.8         5.09         LP(1)042         BD*(1)C231-H244         2.460         1.52           LP(1)074         BD*(1)C23-H240         2.394         137.0         0.57         LP(1)010 <t< td=""><td>BD(1)C231-H245</td><td>BD*(1)C25-H143</td><td></td><td></td><td>0.94</td><td>BD(1)C231-H244</td><td>BD*(1)C27-H145</td><td></td><td></td><td>1.77</td></t<>	BD(1)C231-H245	BD*(1)C25-H143			0.94	BD(1)C231-H244	BD*(1)C27-H145			1.77	
BD(1)0235-H236         BD*(1)C49-H159         3.98         BD(1)0235-H236         BD*(1)C17-H140         1.11           From HP-β-CD to CHMA         LP(1)07         BD*(1)C229-H248         2.711         138.8         0.53         LP(1)020         BD*(1)C225-H226         2.822         139.4         3.32           LP(1)031         BD*(1)C231-H245         2.063         134.7         2.96         LP(1)020         BD*(1)C235-H236         2.238         128.7         2.79           LP(2)031         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)C230-H241         2.478         119.9         0.56           LP(1)053         BD*(1)0235-H236         2.108         119.8         5.09         LP(1)042         BD*(1)C230-H241         2.478         119.9         0.56           LP(1)064         BD*(1)0235-H236         2.108         119.8         5.09         LP(1)042         BD*(1)C231-H244         2.468         120.5         0.70           LP(2)064         BD*(1)C23-H240         2.394         137.0         0.57         LP(1)010         BD*(1)C222-H249         2.472         118.9         0.59           LP(2)010         BD*(1)C23-H244         2.593         149.8         1.26         LP(2)0110         BD*(1	BD(2)C233-O234	BD*(1)C60-H166			1.45	BD(2)C233-O234	BD*(1)C71-H173			1.80	
From HP-β-CD to CHMA       LP(1)07       BD*(1)C229-H248       2.711       138.8       0.53       LP(1)020       BD*(1)0225-H226       2.282       139.4       3.32         LP(1)031       BD*(1)C231-H245       2.063       134.7       2.96       LP(1)020       BD*(1)0235-H236       2.238       128.7       2.79         LP(1)053       BD*(1)0225-H226       2.666       123.3       0.60       LP(1)042       BD*(1)C231-H244       2.478       119.9       0.56         LP(1)054       BD*(1)0225-H226       2.666       123.3       0.60       LP(1)042       BD*(1)C230-H241       2.478       119.9       0.56         LP(1)064       BD*(1)0225-H236       2.108       119.8       5.09       LP(1)042       BD*(1)C231-H244       2.468       120.5       0.70         LP(2)054       BD*(1)0225-H236       2.108       119.8       5.09       LP(1)062       BD*(1)C221-H249       2.470       149.0       1.52         LP(1)074       BD*(1)C223-H240       2.394       137.0       0.57       LP(1)010       BD*(1)C222-H250       2.472       118.9       0.59         LP(2)0110       BD*(1)C223-H244       2.593       149.8       1.26       LP(2)0110       BD*(1)C222-H250       2.472       1.07	BD(1)0235-H236	BD*(1)C49-H159			3.98	BD(1)0235-H236	BD*(1)C17-H140			1.11	
LP(1)07         BD*(1)C229-H248         2.711         138.8         0.53         LP(1)020         BD*(1)0225-H226         2.282         139.4         3.32           LP(1)031         BD*(1)C231-H245         2.063         134.7         2.96         LP(1)020         BD*(1)0235-H236         2.238         128.7         2.79           LP(2)031         BD*(1)C231-H245         0.58         LP(2)020         BD*(1)0235-H236         2.478         119.9         0.56           LP(1)053         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)0231-H244         2.478         119.9         0.56           LP(1)064         BD*(1)0235-H236         2.108         119.8         5.09         LP(1)042         BD*(1)C231-H244         2.468         120.5         0.70           LP(1)074         BD*(1)C23-H240         2.394         137.0         0.57         LP(1)010         BD*(1)C22-H250         2.472         118.9         0.59           LP(2)0110         BD*(1)C231-H244         2.593         149.8         1.26         LP(2)0110         BD*(1)C22-H250         2.472         18.9         0.57           BD(1)C16-H138         BD*(1)C231-H244         2.593         149.8         1.26         LP(2)0110         BD	From HP-β-CD to CHN	AN									
LP(1)031         BD*(1)C231-H245         2.063         134.7         2.96         LP(1)020         BD*(1)0235-H236         2.238         128.7         2.79           LP(2)031         BD*(1)C231-H245         0.58         LP(2)020         BD*(1)0235-H236         1.03           LP(1)053         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)C20-H241         2.478         119.9         0.56           LP(1)064         BD*(1)0235-H236         2.108         119.8         5.09         LP(1)042         BD*(1)C20-H244         2.468         120.5         0.70           LP(1)064         BD*(1)0235-H236         1.33         LP(1)062         BD*(1)C221-H249         2.470         149.0         1.52           LP(1)074         BD*(1)C223-H240         2.394         137.0         0.57         LP(1)010         BD*(1)C222-H250         2.472         118.9         0.59           LP(2)0110         BD*(1)C23-H244         2.593         149.8         1.26         LP(2)0110         BD*(1)C22-H250         2.472         118.9         0.57           BD(1)C16-H138         BD*(1)C231-H244         2.593         149.8         1.26         LP(2)0110         BD*(1)C22-H250         2.472         18.9         0.57 </td <td>LP(1)07</td> <td>BD*(1)C229-H248</td> <td>2.711</td> <td>138.8</td> <td>0.53</td> <td>LP(1)020</td> <td>BD*(1)0225-H226</td> <td>2.282</td> <td>139.4</td> <td>3.32</td>	LP(1)07	BD*(1)C229-H248	2.711	138.8	0.53	LP(1)020	BD*(1)0225-H226	2.282	139.4	3.32	
LP(2)031       BD*(1)C231-H245       0.58       LP(2)020       BD*(1)0235-H236       1.03         LP(1)053       BD*(1)0225-H226       2.666       123.3       0.60       LP(1)042       BD*(1)0230-H241       2.478       119.9       0.56         LP(1)064       BD*(1)0235-H236       2.108       119.8       5.09       LP(1)042       BD*(1)0231-H244       2.460       140.0       1.52         LP(2)064       BD*(1)0235-H236       1.33       LP(1)062       BD*(1)0222-H249       2.470       149.0       1.52         LP(2)010       BD*(1)C223-H240       2.394       137.0       0.57       LP(1)010       BD*(1)C222-H250       2.472       118.9       0.59         LP(2)010       BD*(1)C23-H244       2.593       149.8       1.26       LP(2)0110       BD*(1)C222-H250       2.472       18.9       0.59         BD(1)C16-H138       BD*(1)C231-H244       2.593       149.8       1.26       LP(2)0110       BD*(1)C222-H250       3.08         BD(1)C16-H138       BD*(1)C231-H244       2.64       LP(2)0110       BD*(1)C225-H26       3.08         BD(1)C17-H140       BD*(1)C231-H244       .606       1.07       BD(1)C27-H143       BD*(1)C231-H244       .61         BD(1)C27-H143       BD*(1)	LP(1)031	BD*(1)C231-H245	2.063	134.7	2.96	LP(1)020	BD*(1)0235-H236	2.238	128.7	2.79	
LP(1)053         BD*(1)0225-H226         2.666         123.3         0.60         LP(1)042         BD*(1)(230-H241         2.478         119.9         0.56           LP(1)064         BD*(1)0235-H236         2.108         119.8         5.09         LP(1)042         BD*(1)(221-H244         2.468         120.5         0.70           LP(2)064         BD*(1)(223-H240         2.394         137.0         0.57         LP(1)010         BD*(1)(222-H249         2.470         149.0         1.52           LP(2)010         BD*(1)(223-H240         2.394         137.0         0.57         LP(1)010         BD*(1)(222-H250         2.472         118.9         0.56           LP(2)010         BD*(1)(223-H240         2.593         149.8         1.26         LP(2)0110         BD*(1)(222-H250         2.472         118.9         0.56           BD(1)C16-H138         BD*(1)(2231-H244         2.593         149.8         1.26         LP(2)0110         BD*(1)(222-H250         0.57           BD(1)C16-H138         BD*(1)(2231-H244         2.64         LP(2)0110         BD*(1)(225-H226         3.08           BD(1)(102-H143         BD*(1)(2231-H244         2.64         LP(2)0110         BD*(1)(2231-H244         3.08           BD(1)(22-H143         BD*(1)(2231-H2	LP(2)031	BD*(1)C231-H245			0.58	LP(2)020	BD*(1)0235-H236			1.03	
LP(1)064         BD*(1)0235-H236         2.108         119.8         5.09         LP(1)042         BD*(1)C231-H244         2.468         120.5         0.70           LP(2)064         BD*(1)0235-H236         1.33         LP(1)062         BD*(1)C221-H249         2.470         149.0         1.52           LP(1)074         BD*(1)C223-H240         2.394         137.0         0.57         LP(1)010         BD*(1)C222-H250         2.472         118.9         0.59           LP(2)0110         BD*(1)C223-H240         2.593         149.8         1.26         LP(2)0110         BD*(1)C222-H250         2.472         118.9         0.59           BD(1)C16-H138         BD*(1)C231-H244         2.593         149.8         1.26         LP(2)0110         BD*(2)C222-C223         0.57           BD(1)C16-H138         BD*(1)C231-H244         2.64         LP(2)0110         BD*(1)C225-H226         3.08           BD(1)C17-H140         BD*(1)C235-H236         1.07         BD(1)C25-H143         BD*(1)C231-H224         0.70           BD(1)C25-H143         BD*(1)C231-H244         3.23         3.08         3.08         3.08           BD(1)C25-H143         BD*(1)C231-H244         3.23         3.23         3.08         3.08         3.01         3.01	LP(1)053	BD*(1)0225-H226	2.666	123.3	0.60	LP(1)042	BD*(1)C230-H241	2.478	119.9	0.56	
LP(2)064         BD*(1)0235-H236         1.33         LP(1)062         BD*(1)C221-H249         2.470         149.0         1.52           LP(1)074         BD*(1)C223-H240         2.394         137.0         0.57         LP(1)010         BD*(1)C222-H250         2.472         118.9         0.59           LP(2)0110         BD*(1)C223-H240         2.593         149.8         1.26         LP(2)0110         BD*(1)C222-H250         2.472         118.9         0.59           BD(1)C16-H138         BD*(1)C231-H244         2.593         149.8         1.26         LP(2)0110         BD*(1)C22-H226         0.57           BD(1)C16-H138         BD*(1)C231-H244         2.64         LP(2)0110         BD*(1)C22-H226         3.08           BD(1)C17-H140         BD*(1)O235-H236         1.07         BD(1)C25-H143         BD*(1)C231-H224         0.70           BD(1)C25-H143         BD*(1)C231-H224         0.61         3.02         3.02         3.03           BD(1)C27-H145         BD*(1)C231-H224         3.23         3.23         3.23         3.23         3.23         3.23           BD(1)C27-H145         BD*(1)C230-H224         3.23         3.23         3.23         3.23         3.23           BD(1)C27-H145         BD*(1)C230-H224	LP(1)064	BD*(1)0235-H236	2.108	119.8	5.09	LP(1)042	BD*(1)C231-H244	2.468	120.5	0.70	
LP(1)074         BD*(1)C223-H240         2.394         137.0         0.57         LP(1)0110         BD*(1)C222-H250         2.472         118.9         0.59           LP(2)010         BD*(1)C223-H240         2.593         149.8         1.26         LP(2)0110         BD*(1)C222-H250         0.66           BD(1)C16-H138         BD*(1)C231-H244         2.64         LP(2)0110         BD*(2)C222-C223         0.57           BD(1)C16-H138         BD*(1)C231-H244         2.64         LP(2)0110         BD*(1)C25-H236         1.07           BD(1)C17-H140         BD*(1)C231-H234         1.07         BD(1)C27-H143         BD*(1)C231-H234         0.61           BD(1)C27-H145         BD*(1)C231-H244         0.61         3.08         3.08         3.08           BD(1)C27-H145         BD*(1)C231-H244         0.61         3.02         3.03         3.03	LP(2)064	BD*(1)0235-H236			1.33	LP(1)062	BD*(1)C221-H249	2.470	149.0	1.52	
LP(2)0110 BD*(1)C223-H240 2.593 149.8 1.26 LP(2)0110 BD*(1)C222-H250 0.66 BD(1)C16-H138 BD*(1)C231-H244 2.64 LP(2)0110 BD*(2)C222-C223 0.57 BD(1)C3-H129 BD*(1)O25-H226 3.08 BD(1)C17-H140 BD*(1)O25-H226 1.07 BD(1)C25-H143 BD*(1)O231-H244 0.61 BD(1)C25-H143 BD*(1)C231-H244 0.61 BD(1)C25-H143 BD*(1)C231-H244 3.23 BD(1)C25-H143 BD*(1)C231-H244 3.23 BD(1)C25-H143 BD*(1)C231-H244 3.23 BD(1)C25-H143 BD*(1)C231-H244 3.23 BD(1)C25-H143 BD*(1)C231-H244 3.23 BD(1)C25-H145 BD*(1)C230-H241 3.80	LP(1)074	BD*(1)C223-H240	2.394	137.0	0.57	LP(1)0110	BD*(1)C222-H250	2.472	118.9	0.59	
BD(1)C16-H138 BD*(1)C231-H244 2.64 LP(2)O110 BD*(2)C222-C223 0.57 BD(1)C25-H129 BD*(1)O225-H226 3.08 BD(1)C17-H140 BD*(1)O235-H236 1.07 BD(1)C25-H143 BD*(1)C231-C232 0.70 BD(1)C25-H143 BD*(1)C231-H244 0.61 BD(1)C25-H143 BD*(1)C231-H244 3.23 BD(1)C25-H145 BD*(1)C231-H244 3.23 BD(1)C26-H150 BD*(1)C231-H244 3.80	LP(2)0110	BD*(1)C223-H240	2.593	149.8	1.26	LP(2)0110	BD*(1)C222-H250			0.66	
BD(1)C3-H129       BD*(1)0225-H226       3.08         BD(1)C17-H140       BD*(1)0235-H236       1.07         BD(1)C25-H143       BD*(1)C231-C232       0.70         BD(1)C25-H143       BD*(1)C231-H244       0.61         BD(1)C25-H145       BD*(1)C231-H244       3.23         BD(1)C26-H150       BD*(1)C230-H241       3.80	BD(1)C16-H138	BD*(1)C231-H244			2.64	LP(2)0110	BD*(2)C222-C223			0.57	
BD(1)C17-H140       BD*(1)C235-H236       1.07         BD(1)C25-H143       BD*(1)C231-C232       0.70         BD(1)C25-H143       BD*(1)C231-H244       0.61         BD(1)C25-H145       BD*(1)C231-H244       3.23         BD(1)C26-H150       BD*(1)C230-H241       3.80						BD(1)C3-H129	BD*(1)0225-H226			3.08	
BD(1)C25-H143       BD*(1)C231-C232       0.70         BD(1)C25-H143       BD*(1)C231-H244       0.61         BD(1)C27-H145       BD*(1)C231-H244       3.23         BD(1)C36-H150       BD*(1)C230-H241       3.80						BD(1)C17-H140	BD*(1)0235-H236			1.07	
BD(1)C25-H143BD*(1)C231-H2440.61BD(1)C27-H145BD*(1)C231-H2443.23BD(1)C36-H150BD*(1)C230-H2413.80						BD(1)C25-H143	BD*(1)C231-C232			0.70	
BD(1)C27-H145 BD*(1)C231-H244 3.23 BD(1)C36-H150 BD*(1)C230-H241 3.80						BD(1)C25-H143	BD*(1)C231-H244			0.61	
BD(1)C36-H150 BD*(1)C230-H241 3.80						BD(1)C27-H145	BD*(1)C231-H244			3.23	
						BD(1)C36-H150	BD*(1)C230-H241			3.80	

\*Note: BD denotes  $\sigma$  bonding orbital; BD\* denotes  $\sigma^*$  antibonding orbital; LP denotes valence lone pair. For BD and BD\*, (1) denotes  $\sigma$  orbital, (2) denotes  $\pi$  orbital. For LP, (1) and (2) denote first and second lone pair electrons, respectively.  $E^{(2)}$  denotes the stabilization energy.

<sup>†</sup>Unit of E<sup>(2)</sup> is in kcal/mol.

changes  $[\Delta(\Delta S)]$  between the second-eluted enantiomer and the first-eluted enantiomer for CHMA and MCHMA are negative and positive, respectively. It is indicated that the (*S*)-CHMA– HP- $\beta$ -CD system was more ordered than (*R*)-CHMA–HP- $\beta$ -CD. Moreover, over the temperature range studied, the differences in the enthalpic and entropic changes for CHMA and MCHMA enantiomers in chromatographic separation process were fitted with  $|\Delta(\Delta H)| > |T\Delta(\Delta S)|$ , which indicated the enantioseparation of CHMA and MCHMA was an enthalpy-driven processes.

# Molecular modeling and NBO analysis

To understand chiral recognition of enantiomers of CHMA and MCHMA on HP- $\beta$ -CD, the inclusion interactions between HP- $\beta$ -CD and each enantionmer of CHMA and MCHMA were further studied using molecular modeling techniques, and NBO analysis were carried out to complement the experimental results. The molecular modeling results obtained by molecular docking using AutoDock are presented for the dominating configuration of inclusion complexes with minimum binding free energy ( $\Delta G$ ) in Table V. Table V shows that the intermolecular

interaction energy ( $\Delta E_1$ ) of the (R)-CHMA–HP- $\beta$ -CD complex is greater than that of the (S)-CHMA–HP- $\beta$ -CD complex as the  $\Delta E_1$  value of the (S)-MCHMA–HP- $\beta$ -CD complex is greater than that of the (R)-MCHMA–HP- $\beta$ -CD complex, indicating that the (S)-CHMA–HP- $\beta$ -CD and (R)-MCHMA–HP- $\beta$ -CD complexes are more stable than (R)-CHMA–HP- $\beta$ -CD and (S)-MCHMA–HP- $\beta$ -CD complex; this is consistent with the results observed in chromatographic experiments. Meanwhile, the  $\Delta(\Delta G)$  value is in order of (R/S)-CHMA–HP- $\beta$ -CD > (R/S)-MCHMA–HP- $\beta$ -CD, impling that the enantioselection of (R/S)-CHMA on HP- $\beta$ -CD is better than that of (R/S)-MCHMA, which is consistent with the results observed in chromatographic experiments, namely, that  $\alpha_{(R/S)$ -CHMA is greater than  $\alpha_{(R/S)$ -MCHMA-

The dominating configurations for the (R/S)-CHMA–HP- $\beta$ -CD and (R/S)-MCHMA–HP- $\beta$ -CD complexes obtained by molecular docking using AutoDock are presented in Figure 3. As shown in Figure 3, although (R/S)-CHMA and (R/S)-MCHMA are both inserted into the HP- $\beta$ -CD cavity from the wider rim of HP- $\beta$ -CD, the binding geometries of these complexes are fully different. For the (R/S)-CHMA–HP- $\beta$ -CD

## Table VII

Partial Electron Donor Orbitals, Electron Acceptor Orbitals and the Corresponding E<sup>(2)</sup> Energies, Distances and Angles for (R/S)-MCHMA-HP-β-CD Complexes Calculated by NBO Analysis at the B3LYP/ 6-316 Level\*

(R)-MCHMA-HP-β-C	D				(S)-MCHMA-HP-β-CD					
Electron donor	Electron acceptor	d (Å)	Angle (°)	E <sup>(2)†</sup>	Electron donor	Electron acceptor	d (Å)	Angle (°)	$E^{(2)^{+}}$	
Within MCHMA										
LP(1)0234	BD*(1)0225-H226	2.035	117.8	1.64	LP(2)0219	BD*(1)C225-H248	2.531	117.3	0.56	
LP(2)0234	BD*(1)0225-H226	2.035	117.8	5.12	LP(2)0219	BD*(1)C228-H237	2.401	99.8	0.50	
					LP(2)0234	BD*(1)0219-H220	2.082	116.9	3.57	
					LP(1)0235	BD*(1)C221-H252	2.456	102.8	0.53	
From MCHMA to HP-	β-CD									
LP(1)0225	BD*(1)C5-H131	2.380	132.6	1.23	LP(1)0219	BD*(1)C49-H159	2.388	132.2	2.00	
LP(2)0225	BD*(1)C3-H129	1.998	146.2	5.88	LP(2)0219	BD*(1)C38-H152	2.731	153.9	0.55	
LP(1)0234	BD*(1)C14-H136	2.218	136.4	3.21	LP(1)0234	BD*(1)C36-H150	1.913	149.2	6.29	
LP(1)0234	BD*(1)C16-H138	2.289	131.3	0.74	LP(2)0234	BD*(1)C36-H150			2.06	
BD(2)C220-C221	BD*(1)08-H78			1.33	LP(2)0235	BD*(1)C25-H143	2.399	140.6	0.83	
BD(2)C220-C221	BD*(1)C107-H195			1.24	BD(1)C223-H244	BD*(1)C61-H167			0.69	
BD(1)C220-H252	BD*(1)C3-H129			0.54	BD(1)C223-H245	BD*(1)C49-H159			0.59	
BD(2)C222-C223	BD*(1)C69-H171			1.48	BD(1)C223-H245	BD*(1)C61-H167			3.15	
BD(1)C223-H249	BD*(1)C58-H164			1.95	BD(1)C225-H248	BD*(1)C38-H152			1.56	
BD(1)0225-H226	BD*(1)C3-H129			1.53	BD(1)C226-H251	BD*(1)C27-H145			3.01	
BD(1)C229-H241	BD*(1)C49-H159			0.80	BD(2)C228-C229	BD*(1)C58-H164			2.33	
BD(1)C229-H241	BD*(1)C61-H167			0.52	BD(1)C228-H237	BD*(1)C47-H157			0.68	
BD(1)C229-H242	BD*(1)C61-H167			0.56	BD(1)C229-H238	BD*(1)C58-H164			2.35	
BD(1)C231-H239	BD*(1)C38-H152			1.59	BD(2)C230-C231	BD*(1)C69-H171			1.85	
BD(1)C231-H240	BD*(1)C38-H152			1.46	BD(1)C230-H239	BD*(1)C69-H171			2.33	
BD(1)C232-H245	BD*(1)C16-H138			0.76	BD(1)C231-H240	BD*(1)C3-H129			1.47	
BD(2)C233-O234	BD*(1)C16-H138			1.17	BD(2)C233-O234	BD*(1)C36-H150			1.22	
BD(1)C236-H254	BD*(1)C25-H143			3.68						
From HP-β-CD to MC	HMA									
LP(1)020	BD*(1)0225-H226	1.830	151.9	16.66	LP(1)09	BD*(1)C231-H240	2.448	129.9	1.05	
LP(2)020	BD*(1)0225-H226			1.05	LP(1)022	BD*(1)C222-H243	2.531	126.7	0.74	
LP(1)022	BD*(1)C230-H238	2.724	144.8	0.66	LP(1)022	BD*(1)C226-H250	2.127	138.5	1.94	
LP(2)022	BD*(1)C232-H245	2.718	145.9	0.64	LP(2)022	BD*(1)C226-H250			1.67	
LP(1)062	BD*(1)C223-H249	2.747	137.4	0.50	LP(1)031	BD*(1)C236-H253	2.299	128.9	2.08	
BD(1)C3-H129	BD*(1)0225-H226			0.52	LP(1)044	BD*(1)C225-H249	2.405	120.6	0.70	
BD(1)C25-H143	BD*(1)C236-H254			2.61	LP(1)053	BD*(1)0219-H220	2.219	111.9	2.92	
BD(1)C58-H164	BD*(1)C223-H249			1.28	BD(1)C3-H129	BD*(1)C231-H240			2.63	
BD(1)C109-H198	BD*(1)C221-H251			0.54	BD(1)022-H81	BD*(1)C226-H250			0.84	
					BD(1)C27-H145	BD*(1)C226-H251			1.08	
					BD(1)C36-H150	BD*(2)0233-0234			0.50	
					BD(1)C38-H152	BD*(1)C225-H248			0.68	
					BD(1)C47-H157	BD*(1)C228-H237			0.70	

\*Note: BD denotes  $\sigma$  bonding orbital; BD\* denotes  $\sigma^*$  antibonding orbital; LP denotes valence lone pair. For BD and BD\*, (1) denotes  $\sigma$  orbital, (2) denotes  $\pi$  orbital. For LP, (1) and (2) denote first and second lone pair electrons, respectively.  $E^{(2)}$  denotes the stabilization energy.

<sup>†</sup>Unit of E<sup>(2)</sup> is in kcal/mol.

complexes, the phenyl and cyclohexyl groups in the CHMA molecule are located on the wider rim of HP- $\beta$ -CD as the carboxylic acid moiety is located on the narrow rim of HP- $\beta$ -CD. For the (R/S)-MCHMA–HP- $\beta$ -CD complexes, the phenyl and ester groups in CHMA molecule are close to the wider rim of HP- $\beta$ -CD as cyclohexyl group is located on the the narrow rim of HP- $\beta$ -CD. It is indicated that the chiral recognition mechanism is closely related to the configurations of inclusion complexes.

To have a better estimation of the chiral recognition of HP- $\beta$ -CD with (R/S)-CHMA and (R/S)-MCHMA, NBO analysis was carried out to predict hydrogen bonding between host and guest. In the NBO analysis, the stabilization energy [ $E^{(2)}$ ] can be used to characterize the hydrogen bonding interaction between the LP(Y) lone pair of the proton acceptor and BD\*(X–H) anti-bond of proton donor, which reflects the delocalization trend of electrons from electron donor to electron acceptor orbitals (39, 40). It is generally suggested that the E<sup>(2)</sup> value is larger than 2.0 kcal/mol for the strong hydrogen bonding interaction (41). The partial electron donor

orbitals, electron acceptor orbitals and the corresponding  $E^{(2)}$ , S)-MCHMA-HP-β-CD complexes calculated by NBO analysis at the B3LYP/6-31G level are summarized in Tables 6 and 7. Tables 6 and 7 show that there are intermolecular hydrogen bonding interactions between (R/S)-CHMA and HP- $\beta$ -CD in both inclusion complexes, suggesting that there are four strong hydrogen bonding interactions and six weak hydrogen bonding interactions in the (R)-CHMA-HP- $\beta$ -CD complex, as there are three strong hydrogen bonding interactions and eight weak hydrogen bonding interactions in the (S)-CHMA-HP-B-CD complex. The H ... O distances and angles at the H atom in these hydrogen bondings (O-H ... O or C-H ... O) herein range from 1.920 to 2.711 Å and from 118.9° to 176.3°, respectively, which fall within the reported data [less than 3.2 Å and greater than  $90^{\circ}$  (42)]. Similarly, there are three strong hydrogen bonding interactions and five weak hydrogen bonding interactions in the (R)-MCHMA-HP- $\beta$ -CD complex as there are five strong hydrogen bonding interaction and five weak hydrogen bonding interactions in the (S)-MCHMA – HP- $\beta$ -CD complex. It is also found that the  $E^{(2)}$  values of intramolecular C–H ... O

interactions for CHMA and MCHMA in complexes are greater than 2 kcal/mol, suggesting that there are intramolecular and intermolecular hydrogen bonding interactions in (R/S)-CHMA – HP- $\beta$ -CD and (R/S)-MCHMA–HP- $\beta$ -CD complexes. Additionally, there are many electron delocalization interactions of  $\sigma$  bonding orbital with antibonding orbital between host and guest molecules, indicating that the primary driving forces in the chiral recognitions of (R/S)-CHMA and (R/S)-MCHMA on HP- $\beta$ -CD are hydrogen bonding interaction, dipole-dipole interaction, charge-transfer and hydrophobic interaction, which lead to the formation of different geometric structures of (R/S)-CHMA–HP- $\beta$ -CD and (R/S)-MCHMA– HP- $\beta$ -CD complexes.

# Conclusion

Enantioseparations of (R/S)-CHMA and (R/S)-MCHMA can achieve baseline separation under selected chromatographic conditions, based on the formation of inclusion complexes of (R/S)-CHMA and (R/S)-MCHMA with HP- $\beta$ -CD. The stoichiometry for (R/S)-CHMA-HP- $\beta$ -CD and (R/S)-MCHMA-HP- $\beta$ -CD complexes is 1:1. The chiral recognition of (R/S)-CHMA and (R/S)-MCHMA on HP- $\beta$ -CD is dependent on the different configurations of inclusion complexes. However, the primary driving forces in the chiral recognitions of (R/S)-CHMA and (R/S)-MCHMA on HP- $\beta$ -CD are hydrogen bonding interaction, dipole-dipole interaction, charge-transfer and hydrophobic interaction.

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