# Enantiomer Separation of α-Dimethyl Dicarboxylate Biphenyl and Related Biphenyl Compounds by Normal Phase HPLC on Polysaccharide Based Chiral Stationary Phases

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**Cellulose tris(4-methylphenylcarbamate), amylose tris(3,5-dimethylphenylcarbamate) and amylose tris(phenylcarbamate) were prepared by the method reported by Okamoto and were coated onto an aminopropylated mesoporous spherical silica gel. These final products were used as chiral stationary phases of high performance liquid chromatography for the eighteen structurally related biphenyl compounds. The resolution was made using normal-phase methodology with a mobile phase consisting of *n*-hexane-alcohol (ethanol, 1-propanol, 2-propanol or 1-butanol). The effects of various aliphatic alcohols in the mobile phase were studied. The structural features of the solutes that influence their *k'* were discussed. A dominant effect of trifluoroacetic acid on chiral separation of acidic solutes was noted.**

**Keywords** enantiomer separation, HPLC, chiral stationary phase, α-dimethyl dicarboxylate biphenyl and related biphenyl compounds

## Introduction

α-Dimethyl dicarboxylate biphenyl (α-DDB, dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxy-biphenyl-2,2′-dicarboxylate) and related biphenyl compounds represent a class of anti-hepatitis drugs (Fig. 1). α-DDB is a synthetic mimic of natural product *Schizandrin C*[1] and was reported to have hepatitis-protective effect when hepatic damage was induced by carbon tetrachloride, thioacetamide, or *D*-galactosamine *in vivo* and *in vitro*.[2] α-DDB is used clinically for treatment of hepatitis in Orient and can reduce the levels of serum transaminases.[3] It has been reported that α-DDB and related biphenyl compounds also show potent inhibitory activity against HIV-1 replication in acutely infected H9 cell.[4,5] All biphenyl compounds studied are axially chiral compounds and may show different pharmacological activities as enantiomers.

High performance liquid chromatography (HPLC) has become the main tool for the separation of chiral compounds in pharmaceutical and agrichemical products. However, few chromatographic separation of α-dimethyl dicarboxylate biphenyl and structurally related biphenyl compounds has been reported in the literature. In a previous study we separated the enantiomers of some biphenyl compounds on cellulose tris (3, 5-dimethylphenylcarbamate) (CDMPC) CSP.[6] In this work, we studied the retention and separation of eighteen pairs of biphenyl enantiomers on three cellulose- or amylose-based CSP, *i.e.*, cellulose tris(4-methylphenylcarbamate) (CMPC), amylose tris(3,5-dimethylphenylcarbamate) (ADMPC) and amylose tris(phenylcarbamate) (ATPC). The influence of eluent composition under normal-phase conditions on the chiral resolution was investigated. It was noted that the structural variation of the solutes could influence their retention factors (*k'*). To our knowledge these CSP have never been developed for the enantioseparation of α-dimethyl dicarboxylate biphenyl and related biphenyl compounds.

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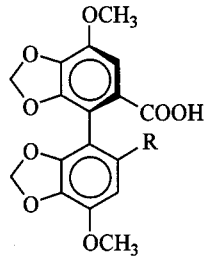
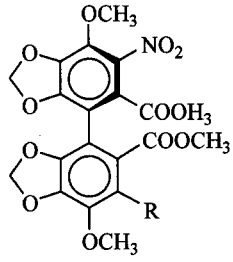
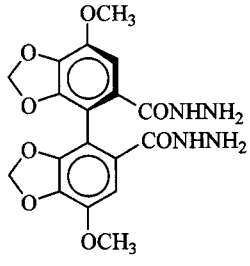


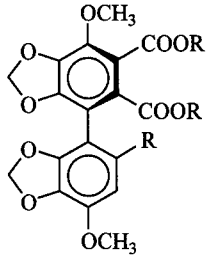
**1** R = CHO
**2** R = COOH
**3** R = $COOCH_3$
**4** R = $COOCH_2CH_3$
**5** R = $COOCH(CH_3)_2$



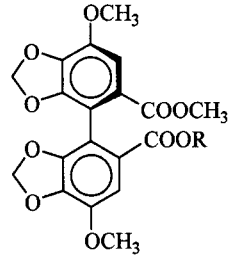
**6** R = H
**7** R = $NO_2$



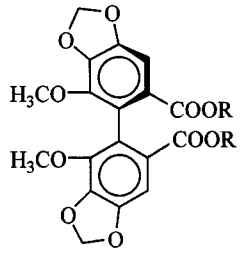
**8**



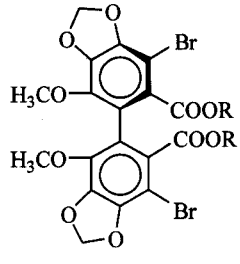
**9** R = $CH_3$
**10** R = $CH_2CH_3$
**11** R = $CH(CH_3)_2$



**12** R = $CH_2CH_3$
**13** R = $CH(CH_3)_2$



**14** R = $CH_3$
**15** R = $CH_2CH_3$
**16** R = $CH(CH_3)_2$



**17** R = $CH_2CH_3$
**18** R = $CH(CH_3)_2$

**Fig. 1** Structures of the racemic compounds.

## Experimental

*Chemicals*

Microcrystalline cellulose was purchased from The Fourth Reagent Factory of Shanghai (China) and amylose from Sigma (USA). Phenyl isocyanate and *p*-tolyl isocyanate were from Aldrich (Milwaukee, WI, USA) and 3,5-dimethylphenylisocyanate from ACROS (New Jersey, USA). 3-Aminopropyltriethoxysilane was a product of Liaoning Chemical Plant (China). The spherical silica gel (with a mean particle size of 5 $\mu$m, a mean pore diameter of 12 nm and a specific surface area of 110 $m^2 \cdot g^{-1}$) was made in our laboratory. The enantiomers of $\alpha$-DDB and related biphenyl compounds were kindly provided by prof. Junbiao Chang (Henan Institute of Chemistry, China).

*Apparatus and chromatographic conditions*

The HPLC system consisted of an M6000 pump (Waters, Milford, USA), a model SPD-1 UV variable wavelength detector (Shimadzu, Japan), and a model C-R2A chromatographic recorder (Shimadzu, Japan). The CSP prepared was packed into a 150 mm × 4.6 mm I.D. stainless-steel column by the conventional high pressure slurry-packing procedure. The mobile phases consisted of hexane and an alcohol modifier. The flow-rate was maintained at 1 $mL \cdot min^{-1}$ or 0.8 $mL \cdot min^{-1}$. All separations were performed at ambient temperature with UV detection set at 254 nm. The void volume of the columns was determined using tri-*tert*-butylbenzene.

*Preparation of* CSP

The three CSPs (CMPC, ATPC and ADMPC) were prepared by the method described by Okamoto.[7,8] The peaks due to the NH groups at around 3314 $cm^{-1}$ and the carbonyl groups at around 1715 $cm^{-1}$ were observed from the FT-IR spectra of the three CSPs, while the peaks of the hydroxyl groups at around 3350 $cm^{-1}$ almost completely disappeared. It was found that the cellulose and amylose were almost completely carbamoylated. The elemental analysis (Table 1) of the derivatives also showed that the hydroxy groups were almost quantitatively converted into the carbamate moieties. The structures of the derivatized subunits of the CSP can be found in the references.[9,10]

The dried CMPC, ATPC and ADMPC were dissolved in THF and coated on aminopropylated silica gel with a coating amount of 15% (*W*/*W*). Three kinds of CSPs were prepared by the same coating method.

**Table 1** Elemental analysis of cellulose and amylose derivatives

| Derivatives | Calculated(%) | | | Found(%) | | |
|---|---|---|---|---|---|---|
| | C | H | N | C | H | N |
| CMPC | 64.17 | 5.53 | 7.49 | 64.01 | 5.44 | 7.36 |
| ATPC | 62.42 | 4.85 | 8.09 | 62.05 | 4.73 | 8.17 |
| ADMPC | 64.56 | 6.18 | 6.98 | 63.99 | 5.80 | 6.61 |

## Results and discussion

The structures of the eighteen pairs of biphenyl compounds are shown in Fig. 1. Compounds **1**—**5** are carboxylic acids, **6**—**13** are $\alpha$-biphenyl dimethyl dicarboxylate (compound **6**) and its derivatives, and **14**—**19** are $\beta$-biphenyl dimethyl dicarboxylate (compound **14**) and its derivatives.

It has been assumed that the separation of enantiomers on the cellulose- and amylose-based CSP was due to the formation of solute-CSP complexes between the enantiomers and the chiral cavities in the higher order structures of the CSP.[9-11] In the CSP with carbamate derivatives, such as the CSP used in our study, the binding of the solutes to the CSPs was achieved through interactions between the solutes and the polar carbamate groups on the CSP.[9,10] The carbamate groups on the CSP can interact with solutes through hydrogen bonding using the C = O and NH groups and through dipole-dipole interactions using the C = O moiety. In our case, the C = O group on all of the solutes and the NH group on the stationary phases could form hydrogen bonding. Dipole-dipole interactions could also occur between the C = O group on the solutes and the C = O group on the stationary phases. Wainer *et al.*[11] have reported that the solute-CSP complex, formed between a solute having aromatic functionalities and cellulose-based CSP, can be stabilized by insertion of the aromatic portion of the solute into the chiral cavity. In this case, this type of stabilization interaction might also occur due to the presence of the aromatic functionalities on the solutes. Chiral discrimination between the enantiomers was due to the differences in their steric fit in the chiral cavity.[9-11]

*Enantioseparation of biphenyl carboxylic acids*

The chromatographic data are summarized in Table 2. The resolution of racemic carboxylic acids **1**—**5** was influenced greatly by the eluting systems used. These compounds were not eluted from the column within reasonable time ( > 120 min) when a mixture of hexane-2-propanol (80:20, $V:V$) was employed. By the addition of a small amount (0.2%) of trifluoroacetic acid (TFA) to the mixture, efficient separation of the carboxylic acids was attained. The acid may weaken the adsorption of the solutes on the aminopropylated silica gel which was used as a support of ADMPC[12]. Without the addition of TFA, the acidic analytes interacted with residual amino groups and residual silanols of the silica gel substrate through hydrogen bonding. The presence of TFA in the mobile phase masks these groups. The acid may also depress the dissociation of the acidic solutes. Thus, the achiral interactions between the stationary phase and the acidic enantiomers are remarkedly reduced.

**Table 2** Chiral resolution of carboxylic acids **1**—**5** on ADMPC CSP

| Sample | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ |
|---|---|---|---|---|
| 1 | 15.67 | 19.59 | 1.25 | 1.28 |
| 2 | 8.38 | 11.40 | 1.36 | 1.55 |
| 3 | 9.39 | 12.11 | 1.29 | 1.25 |
| 4 | 8.03 | 9.64 | 1.20 | 1.02 |
| 5 | 6.78 | 7.53 | 1.11 | 0.85 |

Mobile phase: hexane/2-propanol/TFA (80:20:0.2, $V:V:V$); flow rate: 0.5 mL/min. $k_1'$ = capacity factor of the first-eluted enantiomer; $k_2'$ = capacity factor of the second-eluted enantiomer; separation factor ($\alpha$) = $k_2'/k_1'$; resolution factor ($R_s$) = $2(t_2 - t_1)/(W_1 + W_2)$, where $t_1$ and $W_1$ are the retention time and bandwidth of the first eluted enantiomer, and $t_2$ and $W_2$ are the retention time and bandwidth of the second eluted enantiomer, respectively.

*Enantioseparation of biphenyl compounds* **6**—**19**

The resolution results of compounds **6**—**19** on CMPC, ADMPC and ATPC CSPs are summarized in Tables 3, 4 and 5, respectively.

Enantiomers of **7** were completely resolved on ADMPC CSP. Enantiomers of **6**, **8**, **9**, **10**, **12**, **13**, **15** and **17** were partly separated. The CSP provided better separation of enantiomers of **6**, **8** and **9** compared to the other phases. A variation of solvent had a significant influence on resolution ($R_s$). The retention factors ($k'$) decreased on changing the mobile phase modifier from 2-propanol through ethanol to 1-propanol and then to 1-butanol. The maximum separation for most enantiomers was achieved with the mobile phase hexane/ethanol.

**Table 3** Influence of eluents on the resolution of compounds **6—19** on ADMPC CSP [a]

| Sample | Eluent | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hexane + 20% 2-propanol | | | | Hexane + 20% ethanol | | | | Hexane + 20% 1-propanol | | | | Hexane + 20% 1-butanol | | | |
| | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ |
| **6** | 16.21 | 17.84 | 1.10 | 0.85 | 14.96 | 16.31 | 1.09 | 0.85 | 11.39 | 12.83 | 1.13 | 1.04 | 8.07 | 8.55 | 1.06 | 0.62 |
| **7** | 23.42 | 28.30 | 1.21 | 1.35 | 24.81 | 27.62 | 1.11 | 1.15 | 17.12 | 20.14 | 1.18 | 1.25 | 12.18 | 14.00 | 1.15 | 0.85 |
| **8** | 8.88 | 9.67 | 1.09 | 0.82 | 8.89 | 9.87 | 1.11 | 1.03 | 7.21 | 7.72 | 1.07 | 0.75 | 5.37 | 5.69 | 1.06 | 0.58 |
| **9** | 8.33 | 9.08 | 1.09 | 0.85 | 7.69 | 8.55 | 1.11 | 1.05 | 6.16 | 6.52 | 1.06 | 0.71 | 4.80 | 5.09 | 1.06 | 0.68 |
| **10** | 6.07 | 6.61 | 1.09 | 0.80 | 5.48 | 6.02 | 1.10 | 0.92 | 4.59 | 4.83 | 1.05 | 0.62 | 3.21 | 3.37 | 1.05 | 0.60 |
| **11** | 4.39 | | 1.00 | 0.00 | 4.04 | | 1.00 | 0.00 | 2.79 | | 1.00 | 0.00 | 2.39 | | 1.00 | 0.00 |
| **12** | 7.26 | 7.91 | 1.09 | 0.88 | 6.93 | 7.74 | 1.12 | 1.10 | 5.31 | 5.65 | 1.06 | 0.65 | 4.45 | 4.76 | 1.07 | 0.69 |
| **13** | 6.63 | | 1.00 | 0.00 | 5.90 | 6.34 | 1.07 | 0.81 | 5.19 | | 1.00 | 0.00 | 3.75 | | 1.00 | 0.00 |
| **14** | 4.66 | | 1.00 | 0.00 | 4.91 | | 1.00 | 0.00 | 2.95 | | 1.00 | 0.00 | 2.94 | | 1.00 | 0.00 |
| **15** | 3.50 | 3.98 | 1.14 | 1.04 | 3.51 | 4.01 | 1.14 | 1.20 | 2.64 | 2.99 | 1.13 | 0.85 | 2.25 | 2.52 | 1.12 | 0.74 |
| **16** | 2.38 | | 1.00 | 0.00 | 2.79 | | 1.00 | 0.00 | 1.89 | | 1.00 | 0.00 | 1.48 | | 1.00 | 0.00 |
| **17** | 5.33 | 5.71 | 1.07 | 0.75 | 5.04 | 5.30 | 1.05 | 0.66 | 3.55 | | 1.00 | 0.00 | 3.35 | | 1.00 | 0.00 |
| **18** | 4.48 | | 1.00 | 0.00 | 3.78 | | 1.00 | 0.00 | 2.64 | | 1.00 | 0.00 | 2.60 | | 1.00 | 0.00 |
| **19** | 3.09 | | 1.00 | 0.00 | 2.64 | | 1.00 | 0.00 | 1.90 | | 1.00 | 0.00 | 1.88 | | 1.00 | 0.00 |

[a] The flow-rate was 0.8 mL/min.

**Table 4** Influence of eluents on the resolution of compounds **6—19** on CMPC CSP [a]

| Sample | Alcohol concentration(%) | Eluent | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Hexane/ethanol | | | | Hexane/1-propanol | | | | Hexane/1-propanol | | | |
| | | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ |
| **6** | A | 11.5 | | 1.00 | 0.00 | 16.18 | | 1.00 | 0.00 | 23.54 | 25.89 | 1.10 | 0.60 |
| **7** | A | 20.25 | 23.56 | 1.16 | 0.92 | 26.61 | 34.58 | 1.30 | 1.25 | 40.72 | 57.28 | 1.41 | 1.25 |
| **8** | A | 23.63 | | 1.00 | 0.00 | 23.54 | 28.45 | 1.21 | 0.80 | 46.56 | 58.45 | 1.26 | 0.80 |
| **9** | A | 18.63 | 20.25 | 1.09 | 0.65 | 25.63 | 31.21 | 1.22 | 0.80 | 43.51 | 54.49 | 1.25 | 0.82 |
| **10** | A | 6.50 | 7.79 | 1.20 | 0.90 | 7.33 | 9.90 | 1.35 | 1.25 | 13.02 | 18.36 | 1.41 | 1.30 |
| **11** | C | 11.19 | | 1.00 | 0.00 | 14.06 | | 1.00 | 0.00 | 24.15 | | 1.00 | 0.00 |
| **12** | A | 10.53 | 13.02 | 1.24 | 1.01 | 12.80 | 18.63 | 1.46 | 1.35 | 22.02 | 32.23 | 1.46 | 1.40 |
| **13** | A | 5.15 | 8.22 | 1.60 | 1.35 | 5.72 | 11.08 | 1.94 | 2.50 | 10.01 | 20.95 | 2.09 | 3.00 |
| **14** | A | 3.43 | 9.68 | 2.82 | 3.00 | 3.67 | 12.13 | 3.31 | 4.50 | 6.23 | 21.50 | 3.45 | 5.00 |
| **15** | A | 2.28 | 3.41 | 1.50 | 1.30 | 2.36 | 3.62 | 1.53 | 1.30 | 3.63 | 5.84 | 1.61 | 1.35 |
| **16** | D | 10.01 | 11.87 | 1.19 | 0.95 | 12.33 | 15.82 | 1.28 | 1.25 | 18.52 | 26.58 | 1.44 | 1.35 |
| **17** | B | 5.96 | | 1.00 | 0.00 | 6.93 | | 1.00 | 0.00 | 13.11 | | 1.00 | 0.00 |
| **18** | C | 11.52 | | 1.00 | 0.00 | 12.41 | | 1.00 | 0.00 | 23.51 | | 1.00 | 0.00 |
| **19** | C | 8.12 | | 1.00 | 0.00 | 8.26 | | 1.00 | 0.00 | 13.25 | | 1.00 | 0.00 |

[a] Eluents A, hexane/alcohol (70:30, *V*:*V*); B, hexane/alcohol (80:20, *V*:*V*); C, hexane/alcohol (95:5, *V*:*V*); D, hexane/alcohol (98:2, *V*:*V*). The flow-rate was 1.0 mL/ min.

On the other hand, high $\alpha$-values were obtained for **10** and **12—16** on CMPC CSP, and all twelve isomers were completely separated (Table 4). This CSP showed stronger retention of the solutes compared to the other CSPs. The retention factors ($k'$) decreased on changing the mobile phase modifier from 2-propanol to 1-propanol and to ethanol as expected from the higher polarity of the ethanol. The maximum separation for all of the enantiomers was achieved with the mobile phase hexane/2-propanol.

Table 5 shows the resolution results on ATPC CSP. The enantiomers of **6**, **8**, **9**, **10**, **12**, **13** and **19** were partly separated on this phase. The enantiomers of **7**, **17**, **18** and **19** were better resolved on this CSP than on the other phases.

**Table 5** Capacity factors ($k_1'$ and $k_2'$), enantioselectivity ($\alpha$) and resolution ($R_s$) obtained on ATPC CSP for compounds **6—19**[a]

| Sample | Eluent | $k_1'$ | $k_2'$ | $\alpha$ | $R_s$ |
|---|---|---|---|---|---|
| **6** | A | 18.43 | 20.81 | 1.13 | 0.82 |
| **7** | A | 28.23 | 40.64 | 1.44 | 2.55 |
| **8** | A | 10.26 | 11.70 | 1.14 | 0.90 |
| **9** | B | 18.42 | 21.17 | 1.15 | 0.98 |
| **10** | B | 11.09 | 12.57 | 1.13 | 0.85 |
| **11** | B | 6.27 | | 1.00 | 0.00 |
| **12** | B | 13.64 | 15.65 | 1.15 | 1.02 |
| **13** | B | 10.05 | 11.50 | 1.14 | 0.98 |
| **14** | B | 8.25 | | 1.00 | 0.00 |
| **15** | B | 5.83 | | 1.00 | 0.00 |
| **16** | B | 3.33 | | 1.00 | 0.00 |
| **17** | C | 14.20 | 16.82 | 1.18 | 1.15 |
| **18** | C | 9.48 | 11.11 | 1.17 | 1.05 |
| **19** | B | 3.95 | 4.54 | 1.15 | 0.85 |

[a] Eluents A, hexane/2-propanol (80:20, $V:V$); B, hexane/2-propanol (90:10, $V:V$); C, hexane/2-propanol (94:6, $V:V$). The flow-rate was 1.0 mL/min.

The results obtained demonstrated that CMPC, ADMPC and ATPC CSPs showed similar resolving ability for the separation of $\alpha$-biphenyl dimethyl dicarboxylate and its derivatives (compounds **6—13**). Partial separation or complete separation of all these enantiomers was obtained. However, the three CSPs exhibited different resolving abilities for the separation of $\beta$-biphenyl dimethyl dicarboxylate and its derivatives (compounds **14—19**). The separation of enantiomers of **14—19** were rather poor

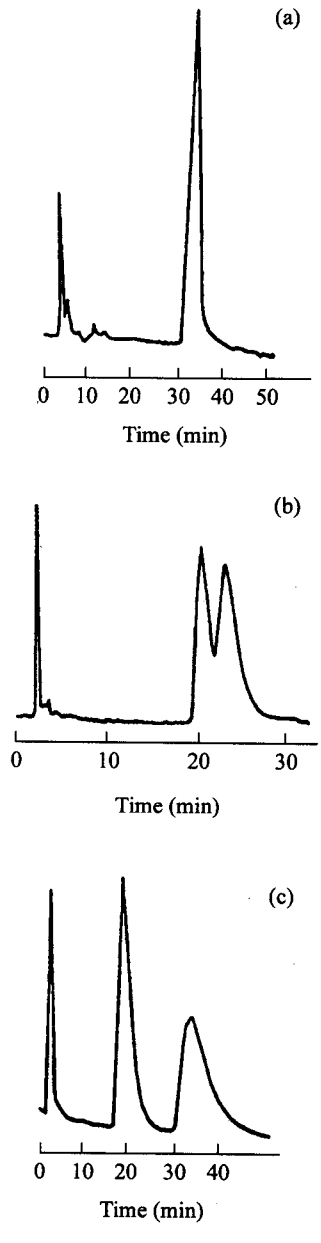



**Fig. 2** Chromatograms of the separation of compound **13** using hexane/2-propanol as the mobile phase. HPLC conditions: (a) ADMPC column with hexane/2-propanol (80:20, $V:V$) as the mobile phase; (b) ATPC column with hexane/2-propanol (90:10, $V:V$) as the mobile phase; (c) CMPC column with hexane/2-propanol (70:30, $V:V$) as the mobile phase. Chromatographic conditions same as Table **3**, **4**, **5** and Experimental.

on ADMPC CSP. Only enantiomers of **15** and **17** could be partly resolved. CMPC CSP exhibited the overall best performance for the separation of enantiomers of **14—16**. All six isomers were completely separated on this CSP. However, the enantiomers of brominated derivatives **17—**

**19** could not be resolved on the phase. On the contrary, the enantiomers of **17**—**19** were better resolved on ATPC CSP while the enantiomers of **14**—**16** were not resolved on this phase.

It is noticeable from Tables 3, 4 and 5 that the capacity factors ($k'$) of enantiomers depended on the number of methylene units in alkyl substituent (R) within the examined homologous series (compare compounds **9**, **10**, with **11**; **12** with **13**; **14**, **15** with **16**; **17**, **18** with **19**). The $k'$ values decreased with an increase of the length of alkyl chain from methyl to isopropyl under the same mobile phase conditions. This suggests that steric and hydrophobic interactions caused by the alkyl groups of the solutes and the CSP have a prominent effect in stabilizing the solute-stationary phase complex.

From Tables 3, 4 and 5, it can also be found that the $\beta$-biphenyl dimethyl dicarboxylate and its derivatives showed shorter retention time than their corresponding isomers, $\alpha$-biphenyl dimethyl dicarboxylate and its derivatives (compare compound **9** with **14**, **10** with **15**, and **11** with **16**). This might indicate that the steric conformations of $\alpha$-biphenyl compounds fit the chiral cavities in the CSP more appropriately than that of $\beta$-biphenyl compounds.

The difference in chiral recognition of the three CSP might be due to the different configuration of the glucose residue and higher order structure.[10] The cellulose derivative has been reported to exist in a conformation of a left-handed threefold (3/2) helix. The amylose derivative can be regarded as a left-handed fourfold (4/1) helix.[13,14] In addition, the derivatization groups on the CMPC, ADMPC and ATPC CSPs had significant structural differences. Compared to ATPC CSP, the CMPC CSP had one methyl group on the phenyl ring while the ADMPC CSP had two, increasing the bulkiness of the aromatic functionality. These differences could lead to the difference in higher order structure of the three CSPs, resulting in difference in chiral recognition ability.

The separation is in opposite way between the ADMPC CSP and the CMPC CSP and this could be correlated to the recognition mechanism of the enantioseparation involving hydrogen bonding, dipole-dipole interaction, $\pi$-$\pi$ interaction and inclusion into the chiral groove.[9] In CMPC CSP the carbamate residue appears to be important to induce efficient chiral discrimination. The alcohols, as mobile phase modifiers, compete for the chiral sites with the solute. The enantiomeric resolution increased as the size of the alcohol increased with 2-propanol giving the maximum resolution. This might be due to the decrease in the capacity of larger alcohols to compete for hydrogen bonding sites because of steric hindrance.[15] In ADMPC CSP the best results are obtained with smaller alcohol (ethanol added to the mobile phase). A loss of resolution are observed when using 1-propanol or 2-propanol. This suggests that hydrogen bonding interactions are probably not the predominant type while the separation might involve more $\pi$-$\pi$ interactions between the aromatic moiety of the solute and the stationary phase.

## Conclusions

A series of biphenyl compounds with anti-hepatitis activity were separated into enantiomers by HPLC on three CSPs, namely, CMPC, ADMPC and ATPC CSPs. The three phases perform in a complementary fashion. The CMPC phase provided more baseline separations of these biphenyl compounds than the other phases. The presence of a small amount of TFA as mobile phase additive benefits the enantioseparation of acidic chiral compounds via reducing the hydrogen bonding interaction of solute active sites with residual amino groups and residual silanols in the CSP packing. The mobile phases had some influence on the $k'$ and $\alpha$ values. The retention of a solute depends on its steric conformation and the chain length of its alkyl portion. The good separation of optical isomers of these biphenyl compounds makes this chromatographic method suitable for quantifying optical purity and for studies in pharmacological distribution.

## References

1 Xie, J.-X.; Zhou, J.; Zhang, C.-Z.; Yang, J.-H.; Jin, H.-Q.; Chen, J.-X. *Acta Pharmcol. Sin.* **1982**, *17*, 23 (in Chinese).

2 Liu, G. T. *J. Biomed. Lab. Sci.* **1989**, *2*, 230.

3 Liu, G. T. In *Advances in Chinese Medical Materials Research*, Eds.: Chang, H. M.; Yeung, H. W.; Tso, W. W.; Koo, A. **1987**, pp. 257—267.

4 Xie, L.; Xie, J. X.; Kashiwada, Y.; Cosentino, L. M.; Liu, S. H.; Pai, R. B.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1995**, *38*, 3003.

5 Chen, D.-F.; Zhang, S.-X.; Xie, L.; Xie, J.-X.; Chen, K.; Kashiwada, Y. *Bioorg. Med. Chem.* **1997**, *5*, 1715.

6 Liu, Y.-Q.; Lao, W.-J.; Zhang, Y.-H.; Jiang, S.-X.;

Chen, L.-R. *Chromatographia* **2000**, *52*, 190.
7 Okamoto, Y.; Kawashima, M.; Hatada, K. *J. Chromatogr.* **1986**, *363*, 173.
8 Chankvetadze, B.; Yashima, E.; Okamoto, Y. *J. Chromatogr. A* **1995**, *694*, 101.
9 Okamoto, Y.; Kaida, Y. *J. Chromatogr. A* **1994**, *666*, 403.
10 Okamoto, Y.; Yashima, E. *Angew. Chem., Int. Ed. Engl.* **1998**, *38*, 1020.
11 Wainer, I. W.; Stiffin, R. M.; Shibata, T. *J. Chromatogr.* **1987**, *411*, 139.
12 Okamoto, Y.; Aburatani, R.; Kaida, Y.; Hatada, K. *Chem. Lett.* **1988**, 1125.
13 Vogt, U.; Zugenmaier, P. *Ber. Bunsengs. Phys. Chem.* **1985**, *89*, 1217.
14 Kennedy, J. H. *J. Chromatogr.* **1996**, *725*, 219.
15 Kirkland, K. M. *J. Chromatogr. A* **1995**, *718*, 9.

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