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Enantiomer separation of dimethyl dicarboxy α-biphenyl (DDB) and its analogues on a covalently bonded cellulose tris-(3,5-dimethylphenyl-carbamate) CSP

Dimethyl dicarboxy α-biphenyl (DDB) and its analogues represent atropisomers which have been resolved on the covalently bonded cellulose tris-(3,5-dimethylphenylcarbamate) (CDMPC) CSP. Different kinds of alcohols, tetrahydrofuran (THF), and chloroform were employed as mobile phase modifiers (MPMs), and their influence on the retention and separation of the enantiomers was investigated. Ternary mobile phases (hexane/2-propanol/THF, hexane/2-propanol/chloroform) were employed to investigate the separation of the five enantiomers. The advantages of the broader choice of solvents offered by the covalently bonded CDMPC CSP were discussed. The effect of structural variation of the enantiomers on their retention and separation was investigated.

Key Words: Enantiomeric separation; Mobile phase modifiers; Bonded type of CSP; Dimethyl dicarboxy biphenyl; Liquid chromatography

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1 Introduction

The individual isomers of many commercialized drugs often exhibit different pharmacological activity. The isolation of enantiomerically pure drugs is therefore becoming more and more important. HPLC had been proved to be one of the most useful methods for separating enantiomers on an analytical or a preparative scale [1, 2]. Enantiomers could first be changed to a mixture of diastereomeric compounds by the so-called *chiral auxiliary*, and then the mixture was separated by conventional HPLC. After that, the *chiral auxiliary* was released and the individual isomers obtained. This indirect method was time-consuming and inefficient because of the complex chemical reactions. Chiral stationary phase HPLC (CSP-HPLC) was a direct method for separating enantiomers, and isomers could be obtained after elution from the HPLC column. So CSP-HPLC has been widely used in the past two decades. Several kinds of CSPs were developed and used for analytical and preparative scale work [2]. Among them, the polysaccharide (cellulose or amylose) based CSPs proved to be some of the most widely used ones [3, 4].

The commonly used polysaccharide-based CSPs were prepared by coating polysaccharide derivatives on γ-aminopropylsilica [5]. The conventional mobile phase consisted of a mixture of hexane and alcohols. In such a mobile phase, most of the CSPs could remain stable for a long time. However, some solvents such as THF and chloroform can not be used in the mobile phase because the polysaccharide derivatives may dissolve in them or swell. These limitations of mobile phase composition represent a disadvantage in further method development. Covalently bonded polysaccharide derivative CSPs had been developed to overcome this problem [6–10]. Compared to coated-type CSPs, these bonded-type CSPs could be used with a much wider range of mobile phases.

DDB and its analogues (see **Figure 1**) have received considerable attention in recent years because of their clinical activity in the treatment of hepatitis [11]. They are axially chiral compounds and each isomer may exhibit different pharmacological activity. Several reports have been dedicated to the indirect resolution of DDB or its analogues [12–14]. However, recovery and efficiency were low. Direct resolution on coated-type polysaccharide derivatives was reported recently [15], but the low solubility of the sample in the mobile phase represented a limitation, especially on a preparative scale.

In this study, the direct resolution of dimethyl dicarboxy α-biphenyl (DDB) and its analogues on covalently bonded cellulose tris-(3,5-dimethylphenylcarbamate) (CDMPC) CSP was achieved. The influence of the mobile phase modifiers (MPMs) and the structure of the enantiomers on the chromatographic results was investigated. The possible resolution mechanism was considered.

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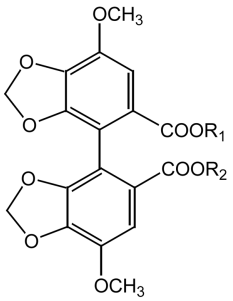

DDB1 $R_1, R_2=CH_3$-

DDB2 $R_1, R_2=CH_3CH_2$-

DDB3 $R_1, R_2=(CH_3)_2CH$-

DDB4 $R_1=CH_3$-, $R_2=CH_3CH_2$-

DDB5 $R_1=CH_3$-, $R_2=(CH_3)_2CH$-

Figure 1. Molecular structure of DDB and its analogous.

2 Experimental

2.1 Chemicals

Microcrystalline cellulose was obtained from Serva (Heidelberg, Germany). Silica gel (Kromasil, 5 μm, 200 Å, ca. 240 m^2/g) was purchased from Akzo Noble AB (Nacka, Sweden). Methacryloyl chloride and γ-(trimethoxysilyl) propyl methacrylate were obtained from Acros (New Jersey, USA). α,α′-Azobisisobutyronitrile (AIBN) was obtained from Sanpu Chemical Company (Shanghai, China). The CSP was prepared by radical co-polymerization of methacrylol cellulose derivatives (**Figure 2**) and γ-methacrylate propylated silica [16], and was packed in a stainless steel column (150 × 4.6 mm ID) by a slurry packing technique. Other reagents were all of analytical grade.

DDB and its analogues (DDB1, DDB2, DDB3, DDB4, and DDB5) were obtained from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China). Solutions of the DDBs (0.1 mg/mL) were prepared by dissolving them in ethanol.

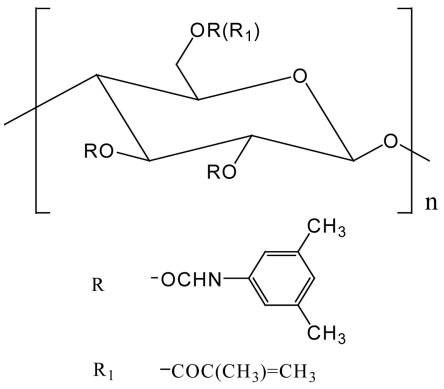

Figure 2. Structure of the cellulose derivative.

2.2 Apparatus and chromatographic conditions

HPLC separations were performed with a Elite P230 pump (Elite company, Dalian, China), a Spectra-200 UV detector (Spectra-Physics, San Jose, CA, USA), and a WDL-95 workstation (National Chromatographic R & A Center, Dalian, China).

The mobile phases were filtered and sonicated prior to use. Throughout this study, the separations were performed at room temperature (20 ± 0.5°C), and the flow rate was 0.5 mL/min. The injection volume was 5–10 μL for different enantiomers. UV detection was performed at 254 nm. The void time of the column was determined by 1,3,5-tri-*tert*-butylbenzene. The retention factor (k') was calculated from $(t_r - t_0)/t_0$, where t_r was the elution time of the enantiomer and t_0 was the void time of the column. The separation factor (α) was calculated from $(t_2 - t_0)/(t_1 - t_0)$. Resolution factor (Rs) was evaluated according to the expression $Rs = 1.18 \times (t_2 - t_1)/[(w_{1/2})_1 + (w_{1/2})_2]$, where $w_{(1/2)1}$ and $w_{(1/2)2}$ were the half band widths of the early and late eluted enantiomers, respectively.

3 Results and discussion

3.1 Enantiomer separation with conventional MPMs

Several kinds of primary alcohols and a secondary alcohol were employed as MPMs to carry out the enantiomer separation, and the chromatographic data are summarized in **Table 1**. As can be seen, the increased length/bulkiness of the alkyl portion on the primary alcohols resulted in decreased elution ability, and 2-propanol showed lower elution ability than 1-butanol. For all of the five enantiomers, the values of k_1' increased when the MPM was changed from ethanol to 1-butanol, and the largest values of k_1' were obtained when the MPM was 2-propanol. Enantioselectivity was also affected by the kind of MPM. For all the five enantiomers the values of α increased when the MPM was changed from ethanol to 1-propanol, and then decreased a little when the MPM was changed to 1-butanol (for DDB2, a slight increase was obtained). The best enantiomer separation was obtained when 2-propanol was employed as MPM for DDB1, DDB2, DDB4, and DDB5. For DDB3, 1-propanol was more suitable for obtaining higher enantioselectivity.

It had been assumed that the retention of enantiomers on the polysaccharide derivative CSPs involved hydrogen bonding, dipole-dipole interaction, and π-π interaction [1, 17]. The carbamate residue on the CSP played an important role as the NH group and the C═O group could interact with the solute through these interactions. In the case of the five enantiomers, the C═O groups on the solute could undergo hydrogen bonding with the NH groups on the CSP, which is believed to be an important interaction for the retention and separation of the enantiomers. Dipole-dipole interactions could also occur between the C═O groups on the solute and their counterpart on the CSP. In the MPMs of alcohols, the OH groups could form hydrogen bonding with the C═O group on the CSP. Thus

Table 1. Chromatographic data obtained with different kinds of alcohols.

Mobile phase modifier	DDB1			DDB2			DDB3			DDB4			DDB5		
	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*
Ethanol	3.37	1.15	1.94	1.92	1.00	0.00	1.04	1.00	0.00	2.51	1.07	0.78	1.68	1.12	1.54
1-Propanol	4.38	1.17	1.82	2.13	1.04	0.30	1.07	1.10	0.56	3.09	1.09	0.94	1.97	1.14	1.56
1-Butanol	4.53	1.14	1.41	2.15	1.05	0.45	1.08	1.10	0.52	3.17	1.08	0.92	1.99	1.12	0.92
2-Propanol	6.30	1.25	2.05	2.91	1.06	0.42	1.36	1.08	0.82	4.34	1.14	1.45	2.55	1.19	2.06

Mobile phase composition: hexane/alcohol 75/25 (*v/v*).

Table 2. Chromatographic data obtained with different concentrations of 2-propanol.

Concentration of 2-propanol (*v/v*)	DDB1			DDB2			DDB3			DDB4			DDB5		
	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*
15	10.26	1.26	2.17	4.32	1.08	0.55	2.08	1.08	0.90	7.06	1.16	1.74	4.02	1.17	2.01
20	8.17	1.25	1.24	3.70	1.06	0.78	1.60	1.08	0.81	5.50	1.16	1.29	3.39	1.18	1.50
25	6.30	1.25	2.05	2.91	1.06	0.42	1.36	1.08	0.82	4.34	1.14	1.45	2.55	1.19	2.06
30	5.45	1.23	1.76	2.56	1.05	0.45	1.15	1.07	0.42	3.78	1.13	0.88	2.25	1.18	1.58

Mobile phase composition: hexane/2-propanol (*v/v*).

the use of alcohols in the mobile phase reduced the dipole-dipole interactions between the CSP and the enantiomers. When the MPM was changed from ethanol to 2-propanol, the length/bulkiness of the alkyl portion of the alcohols increased, and their ability to interact with the carbamate residues decreased. Thus the solutes can interact more strongly with the CSP, and the retention factors increased with this tendency. In addition, solute-CSP complexes could be formed through the inclusion of the enantiomers in the chiral grooves in the highly ordered structure of the CSPs, and chiral discrimination between the enantiomers was due to their different steric fits in the chiral grooves [17]. The interaction of MPMs with CSP may affect the steric nature of the chiral grooves, which correspondingly affected the separation factor of the enantiomers. Thus, the separation factors of the enantiomers varied with the structure of the MPMs. Among the four alcohols investigated, 2-propanol is probably the most effective MPM for the separation of the enantiomers.

Different concentrations of 2-propanol were employed to investigate the influence of concentration on the retention and separation of the enantiomers. As can be seen in **Table 2**, with the concentration of 2-propanol increasing, the retention factors of all five enantiomers decreased. This could be attributed to the increased ability of the 2-propanol to interact with the carbamate group. On the other hand, the increment of the concentration of 2-propanol had little effect on the separation factors (α), which meant that the change of concentration of the alcohol had little effect on the conformation of the chiral grooves. The enantioselectivity was mainly determined by the structure but not the concentration of MPMs. The chromatograms for the separation of the five enantiomers with 2-propanol (30%) as MPM are presented in **Figure 3** (mobile phase I).

3.2 Enantiomer separation with THF or chloroform as MPM

The bonded type of polysaccharide derivative CSPs is expected to extend the choice of solvents beyond the coated type CSPs, making it possible to investigate the enantiomer separation with THF or chloroform as MPM. In contrast to the conventionally adopted MPM (protic solvent), THF is a kind of aprotic solvent and chloroform is neither a protic nor an aprotic solvent. The use of such solvents would change the type of interaction between the MPM and the CSP. Also, CDMPC could be dissolved or swollen in both THF and chloroform, and the addition of those solvents in the mobile phases would probably lead to the alternation of the steric nature of the chiral grooves, which would further affect the separation results of the enantiomers. Furthermore, a high percentage of THF or chloroform in the mobile phase may solve the problem of low sample solubility in the preparative separation. Although such was the original intention for the development of bonded type of CSPs, few reports had paid attention to this aspect [7, 8, 18].

The chromatographic data for different concentrations of THF as MPM are presented in **Table 3**. When THF was adopted as MPM, hydrogen bonding could take place between the oxygen atom on the molecule of THF and the

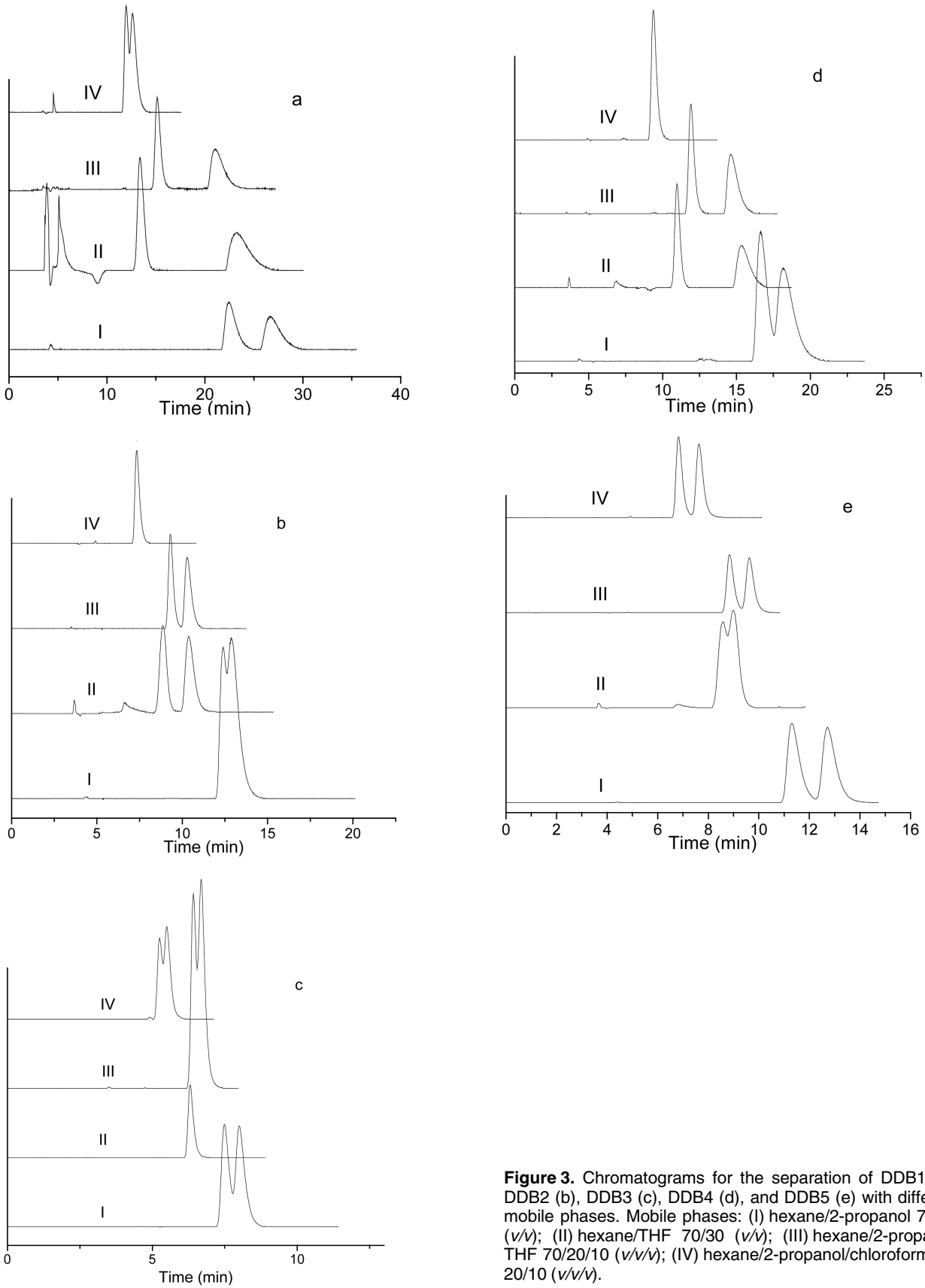

Figure 3. Chromatograms for the separation of DDB1 (a), DDB2 (b), DDB3 (c), DDB4 (d), and DDB5 (e) with different mobile phases. Mobile phases: (I) hexane/2-propanol 70/30 (*v/v*); (II) hexane/THF 70/30 (*v/v*); (III) hexane/2-propanol/THF 70/20/10 (*v/v/v*); (IV) hexane/2-propanol/chloroform 70/20/10 (*v/v/v*).

Table 3. Chromatographic data obtained with different concentrations of THF.

Concentration of THF (*v/v*)	DDB1			DDB2			DDB3			DDB4			DDB5		
	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*
15	20.27	3.07	13.57	9.06	1.65	3.26	2.76	1.00	0.00	13.70	2.22	8.47	7.62	1.08	1.16
20	9.80	2.82	10.30	4.59	1.53	2.97	2.06	1.00	0.00	6.85	2.02	6.37	4.27	1.10	1.29
25	4.48	2.29	4.73	2.49	1.39	2.90	1.17	1.00	0.00	3.51	1.79	3.97	2.34	1.09	0.77
30	2.84	2.00	4.11	1.55	1.28	1.77	0.88	1.00	0.00	2.15	1.59	3.98	1.47	1.08	0.54

Mobile phase composition: hexane/THF (*v/v*).

NH group on the CSP, so there is competition between THF and DDBs to interact with the NH group on the CSP. The consecutive increment of the concentration of THF in mobile phases would lead to increased competition ability, and this resulted in a decrement of the retention factors. A notable result was that the decrement of retention factors originating from the increment of the concentration of THF was much larger than that with 2-propanol as MPM. As the use of THF reduced the hydrogen bonding between the CSP and the enantiomers, while the use of 2-propanol reduced the dipole-dipole interactions, it seemed that hydrogen bonding played a more important role than dipole-dipole interactions in the retention. In addition, the shape/size of the chiral grooves might play a role in controlling the retention [19].

When the MPM was changed from 2-propanol to THF, the separation results of the five enantiomers were greatly changed. As can be seen in Table 3, the separation factors of DDB1, DDB2, and DDB4 were greatly improved. For example, DDB2 and DDB4 were not satisfactorily resolved on using a mobile phase containing 2-propanol as MPM, but were baseline separated with THF as MPM (Figure 3.b and Figure 3.d). However, the separation factors of DDB3 and DDB5 were decreased. For DDB3, no resolution was observed at any concentration of THF in the mobile phase. Obviously, the interaction between THF and the NH group and/or the swelling of CDMPC might change the properties of the chiral grooves. This change was positive for the resolution of DDB1, DDB2, and DDB4, but negative for DDB3 and DDB5. In addition, the concentration of THF strongly affected the separation factors of some enantiomers. For DDB1, DDB2, and DDB4, the separation factors decreased greatly with increasing concentration of THF. However, for DDB3 and DDB5, little effect was observed. This indicated that, in contrast to the case of 2-propanol, the concentration of THF strongly affected the conformation of the chiral grooves. The chromatograms for the separation of the five enantiomers with THF as MPM (30%) are shown in Figure 3 (mobile phase II).

Chloroform was also used as MPM for the enantiomer separation. The five enantiomers could not be eluted (in 60 min) even with high concentrations of chloroform (40%), although at this concentration the polarity of the mobile phase was higher than of that containing the same concentration of 2-propanol and THF (for chloroform $P' = 4.1$, for 2-propanol and THF $P' = 3.9$ [20]). Thus polarity did not seem to be the dominating factor in controlling the retention. As chloroform is hardly likely to interact with the chiral active site on the CSP, the existence of competitive solvents in the mobile phase seemed to be important for the separation of the enantiomers. The enantiomers could be easily eluted when the concentration of chloroform in the mobile phase was up to 50%, but no separation thereof could be obtained.

In preparative separation, selectivity factor, separation time, and solubility of the sample should all be seriously considered with regard to the total cost. Although the five enantiomers could be resolved on a coated-type CDMPC CSP [15], the low solubility of the samples in hexane-alcohol mixtures was a limitation for preparative scale work. To the best of our knowledge, the solubility of DDBs was much higher in THF than in alcohols. With covalently bonded CDMPC CSPs, the high percentage of THF in the mobile phase not only improved the sample solubility, but also enhanced the separation factors and shortened the separation times (for DDB1, DDB2, and DDB4). This is advantageous for preparative purposes, and is one of the reasons that the bonded-type of polysaccharide derivative CSPs are superior to their coated-type of counterparts.

3.3 Enantiomer separation with ternary mobile phases

With conventional MPMs, the alcohols could undergo hydrogen bonding with NH on the CSP and thus compete with the DDBs for chiral or achiral sites; however, the resolutions with these mobile phases were unsatisfactory in some cases. With *unconventional* MPMs, THF/chloroform shows advantages for the separation of various enantiomers, but competitive solvents are needed in some cases. We therefore coupled the two kinds of MPMs to investigate separation using ternary mobile phases.

As can be seen in **Table 4**, the k_1' values of the five enantiomers with ternary mobile phases (mobile phase a and

Table 4. Chromatographic data obtained with ternary mobile phase.

Mobile phase	DDB1			DDB2			DDB3			DDB4			DDB5		
	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*	k_1'	α	*Rs*
a	3.34	1.51	3.52	1.68	1.17	1.64	0.84	1.10	0.62	2.43	1.32	2.98	1.54	1.15	1.53
b	2.44	1.08	0.76	1.10	1.00	0.00	0.51	1.14	0.66	1.70	1.00	0.00	0.96	1.24	1.72

Mobile phases: (a) hexane/2-propanol/THF 70/20/10 (*v/v/v*); (b) hexane/2-propanol/chloroform 70/20/10 (*v/v/v*).

b, which were equivalent to mobile phase III and IV in Figure 3, respectively) were lower than those with the conventional mobile phases (hexane/2-propanol). These reductions may have originated from the change of properties of CDMPC and/or the increased competition ability of the mixed MPMs.

A notable result is that THF and chloroform had different effects on the separation factors of the five enantiomers. In the case of mobile phase a, the separation factors of DDB1, DDB2, and DDB4 were greatly enhanced, but those of DDB3 and DDB5 were little changed (compared to the mobile phase with 2-propanol as MPM). In the cases of mobile phase b, the separation factors of DDB3 and DDB5 were enhanced, but those of DDB1, DDB2, and DDB4 were reduced.

The use of the ternary mobile phases led to more complicated retention and resolution mechanisms compared to those of binary ones. Even though, they still show some advantages, especially in the separation of DDB3 and DDB5, for which the best resolution was only achieved by using the ternary mobile phase composed of hexane/2-propanol/chloroform (70/20/10) (Table 4 and Figure 3).

3.4 Influence of enantiomer structure on retention and resolution

It could be observed in Figure 1 that the five enantiomers have very similar structures, and the only difference lay in the substituent on the ester at position 2 and 2′. The steric bulk of the substituent increased according to the following sequence, DDB1 < DDB4 < DDB2 < DDB5 < DDB3. It was expected that the increased steric bulk would make it more difficult for the C═O to interact with the chiral site on the CSP, and/or result in lower degree of inclusion of the enantiomers in the chiral grooves, which would lead to a decrease of the retention factors. This assumption was supported by experiment. As can be seen in Table 1 to Table 4, with all tested mobile phases, the retention factors of the five enantiomers decreased in above sequence.

The separation factors of the enantiomers were also affected by the molecular structure. It had been reported that the separation of enantiomers was due to the differences in their steric fits in the chiral grooves of the CSP [17]. So with the same mobile phase, those which fitted properly in the chiral grooves could be separated well. DDB3 and DDB5 usually exhibited different separation behavior from DDB1, DDB2, and DDB4. This may be due to the significantly greater steric bulk of the isopropyl group. In addition, the use of the MPM would affect the conformation of the chiral grooves, and different kinds of MPM should be employed to achieve the best separation of the different DDBs.

4 Conclusion

DDB and its analogues could be well resolved on covalently bonded CDMPC CSP. The structure of the alcohol affected the retention factors and separation factors of the enantiomers, but the concentration of the alcohol only affected the retention factors. Among the conventional MPMs, 2-propanol (30%) was the most efficient solvent for enantiomer separation. The use of THF/chloroform as MPM changed the chromatographic behavior of the enantiomers in comparison to 2-propanol as MPM. Separation of some enantiomers was greatly enhanced, and their solubility in the mobile phase was improved, which showed some advantages on the preparative scale. The best resolution of DDB3 and DDB5 could only be achieved with a ternary mobile phase. In the separation of the enantiomers, hydrogen bonding between the NH group on the CSP and the C═O groups on the solutes might play an important role. In addition, the steric bulk of the substituting group of the ester on the solute molecules also affected the retention behavior of the enantiomers.

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References

[1] Y. Okamoto, E. Yashima, *Angew. Chem. Int. Ed.* **1998**, *37*, 1021.
[2] N.M. Maier, P. Franco, W. Lindner, *J. Chromatogr. A* **2001**, *906*, 3.
[3] L. Miller, C. Weyker, *J. Chromatogr. A* **1990**, *511*, 97.
[4] Y. Okamoto, Y. Kaida, *J. Chromatogr. A* **1994**, *666*, 403.
[5] Y. Okamoto, M. Kawashima, K. Hatada, *J. Am. Chem. Soc.* **1984**, *106*, 5357.

[6] Y. Okamoto, R. Aburatani, S. Miura, K. Hatada, *J. Liq. Chromatogr.* **1987**, *10*, 1613.

[7] E. Yashima, H. Fukaya, Y. Okamoto, *J. Chromatogr. A* **1994**, *677*, 11.

[8] C. Minguillón, P. Franco, L. Oliveros, P. Lopez, *J. Chromatogr. A* **1996**, *728*, 407.

[9] X.M. Chen, H.F. Zou, Q. Zhang, J.Y. Ni, Z.Z. Zhang, *J. Chromatogr. Sci.* **2002**, *40*, 315.

[10] X.M. Chen, Y.Q. Liu, F. Qin, L. Kong, H.F. Zou, *J. Chromatogr. A* **2003**, *1010*, 185.

[11] L. Xie, J.X. Xie, Y. Kashiwada, L.M. Cosention, S.H. Liu, R.B. Pai, Y.C. Chen, K.H. Lee, *J. Med. Chem.* **1995**, *38*, 3003.

[12] T.D. Nelson, A.I. Meyers, *J. Org. Chem.* **1994**, *59*, 2577.

[13] B.H. Lipshutz, Z.P. Liu, F. Kayser, *Tetrahedron Lett.* **1994**, *35*, 5567.

[14] F.W. Yeng, J.Y. Chiu, C.J. Wang, *U.S. Pat.* 5 504 221, **1996**.

[15] Y.Q. Liu, W.J. Lao, Y.H. Zhao, S.X. Jiang, L.R. Chen, *Chromatographia* **2000**, *52*, 190.

[16] X.M. Chen, F. Qin, Y.Q. Liu, X.D. Huang, H.F. Zou, *J. Chromatogr. A* **2004**, *1034*, 109.

[17] I.W. Wainer, R.M. Stiffin, T. Shibata, *J. Chromatogr.* **1987**, *411*, 139.

[18] P. Franco, C. Minguillon, L. Oliveros, *J. Chromatogr. A* **1998**, *793*, 239.

[19] R.M. Wenslow, Jr., T. Wang, *Anal. Chem.* **2001**, *73*, 4190.

[20] L.R. Snyder, J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, 2nd ed. John Wiley and Sons, New York 1979, p. 246.

