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Enantiomeric Separation of Fused Polycycles by HPLC with Cyclodextrin and Macrocyclic Glycopeptide Chiral Stationary Phases

Xinxin Han, Qinhua Huang, Jie Ding, Richard C. Larock, and Daniel W. Armstrong
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Abstract: The enantiomeric separation of a series of 13 new chiral polycycles has been examined on both cyclodextrin-based and macrocyclic glycopeptide chiral stationary phases (CSPs) using HPLC in the normal phase, reversed phase, and polar organic modes. The most effective chiral selectors for the enantiomeric separation of these analytes are the 2,3-dimethyl-β-cyclodextrin (Cyclobond I-2000 DM) and hydroxypropyl-β-cyclodextrin (Cyclobond I-2000 RSP). The other Cyclobond-type and Chirobiotic (macrocyclic glycopeptide) CSPs only show enantioselectivity for a few of the racemic polycycles. The effects of mobile phase composition and analyte structure on chiral recognition and separation are considered.

Keywords: Fused polycycles, enantiomeric separation, chiral stationary phase, cyclodextrin, macrocyclic glycopeptide

INTRODUCTION

Fused polycycles exist widely in the natural world. Two pentacyclic proaporphine alkaloids, (−)-misramine (1) and (−)-labrandine (2), have been found in the Egyptian and Turkish flowering plant, *Roemeria hybrida*, respectively. A complex fused polycycle, dipuupehetriol (3), has been isolated from a Verongid sponge. From the Caribbean sponge *Smenospongia aurea*, aureol

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and its derivatives have been obtained (4). Esmeraldin A and B, derivatives of diphenazine, have been found in *Streptomyces antibioticus*, strain Tü 2706 (5).

Many fused polycycles are known to possess beneficial therapeutic activities. Dipuupehetriol has shown selectivity against the human lung cancer cell line A549 and the CV-1 cell line (3). Two analogs of aureol inhibit the growth of some gram-positive and gram-negative bacteria (4). Strong antitumor activities of hexacyclic derivatives of camptothecin have been reported (6). Some other tetracyclic compounds are inhibitors of kynurenine-3-hydroxylase (7) and poly(ADP-ribose)polymerase (8, 9).

Huang, Larock, and co-workers have recently prepared a set of new chiral fused polycycles (Fig. 1) (10), which includes 8 chromene derivatives, 2 quinoline derivatives, 2 isochromene derivatives, and 1 polycyclic diester. These compounds are obtained through palladium-catalyzed alkyl to aryl palladium migration, followed by intramolecular arylation. Since different enantiomers of a chiral compound can have different biological properties (11), separation of these new chiral polycycles and evaluation of their properties are desirable.

Cyclodextrin-based (12–23) and macrocyclic glycopeptide (24–35) chiral stationary phases (Fig. 2) are well known for their high enantioselectivities for separation of a variety of different chiral molecules. In this work, the enantiomeric selectivity of 8 cyclodextrin and 4 macrocyclic glycopeptide chiral stationary phases for 13 recently synthesized racemic fused polycycles have been investigated in the reversed phase, polar organic, and normal phase modes.

EXPERIMENTAL

Materials

Cyclobond I, II, III, DM, AC, RSP, DMP, SN; as well as the Chirobiotic V, R, T, and TAG CSPs (Fig. 2) were obtained from Advanced Separation

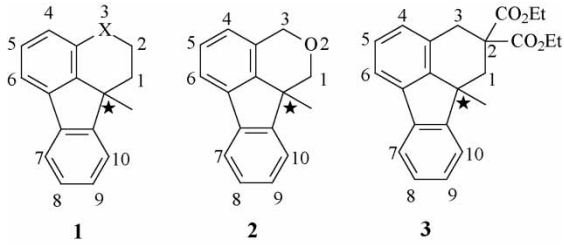


Figure 1. General structure and ring numbering conventions for the chiral polycycles. Structure **1** is a chromene (X = O) or quinoline ($X = NSO_2CF_3$) derivative. Structure **2** is an isochromene derivative. Structure **3** is a polycyclic diester. The carbon marked with an asterisk is the stereogenic center.

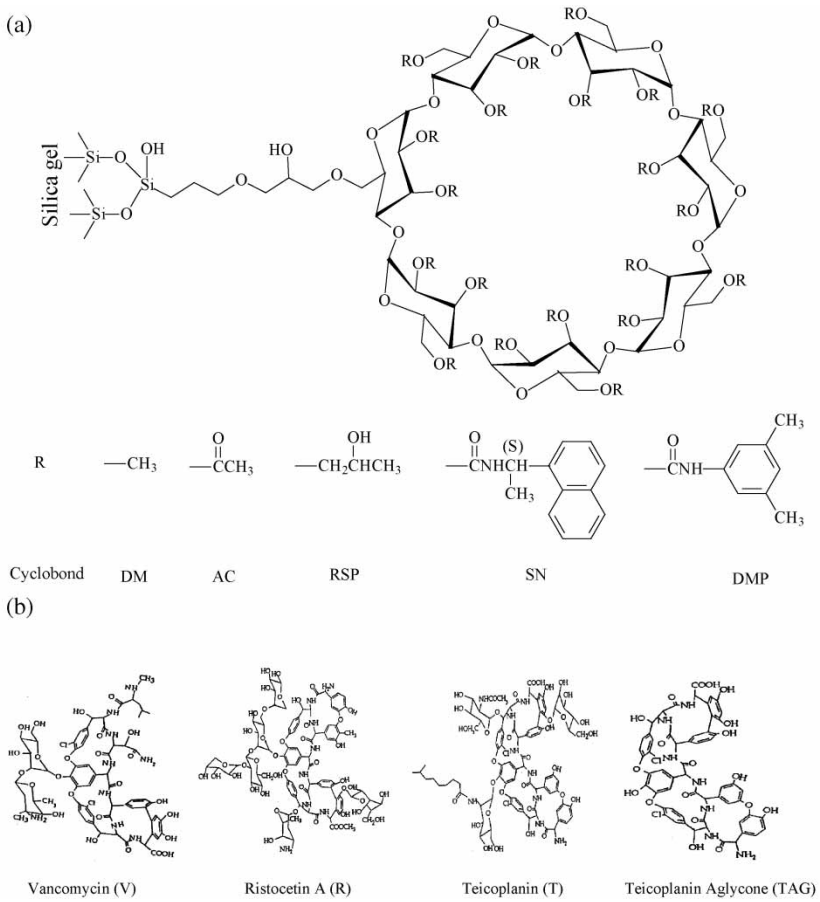


Figure 2. General structure of the (a) Cyclobond and (b) Chirobiotic CSPs (there can be 1–3 linkages for each cyclodextrin or macrocyclic glycopeptide molecule). R = H for Cyclobond I (β-cyclodextrin), II (γ-cyclodextrin), III (α-cyclodextrin). All derivatized cyclodextrin CSPs are made from β-cyclodextrin.

Technologies (Whippany, NJ, USA). All the stationary phases consist of chiral selectors bonded to 5 μm spherical porous silica gel (14, 15, 24). The chiral selectors are the native α-, β-, and γ-cyclodextrins, various derivatives of β-cyclodextrin, vancomycin, ristocetin A, teicoplanin, and teicoplanin aglycone (Fig. 2). The dimensions of the columns are 250 × 4.6 mm. HPLC grade methanol, acetonitrile, ethanol, and heptane were obtained from Fisher (Fairlawn, NJ, USA). The triethylamine and acetic acid used were ACS certified grade from Fisher. Water was deionized and filtered through active charcoal and a 5 μm filter. All chiral polycycles were prepared as previously reported via palladium-catalyzed alkyl to aryl migrations and cyclization (10).

Equipment

Chromatographic separations were carried out using an HP 1050 HPLC system with a UV VWD detector, an auto sampler, and computer-controlled Chem-station data processing software (Agilent Technologies, Palo Alto, CA, USA). The mobile phases were degassed by ultra-sonication under vacuum. UV detection was carried out at 254 nm for all of the compounds. All separations were carried out at room temperature ($\sim$23°C) and the flow rate of the mobile phase was 1.0 mL min^{-1}.

Column Evaluation

The performance of all stationary phases was evaluated in the reversed phase mode using acetonitrile/water and methanol/water mobile phases. Cyclobond I, II, III, AC, RSP, SN, and DMP and all Chirobiotic CSPs were evaluated in the polar organic mode using acetonitrile as mobile phase. Cyclobond SN and DMP and all Chirobiotic CSPs were evaluated in the normal phase mode using an ethanol/heptane mobile phase. Over the course of 1000 injections, no degradation of these columns was observed. When using a new mobile phase, 10 column volumes of solution were pumped through the column prior to injection of the analytes.

Calculations

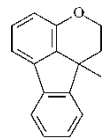
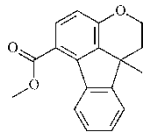
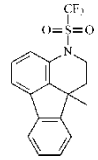
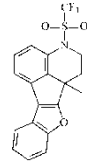
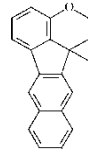
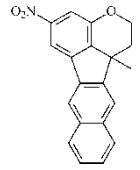
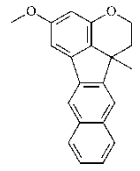
The dead time (t_0) was estimated using the peak resulting from the change in refractive index from the injection solvent on each chiral stationary phase. The retention factor (k) was calculated using the equation $k = (t_r - t_0)/t_0$. The enantioselectivity (α) was calculated using $\alpha = k_2/k_1$. The resolution factor (R_S) was calculated using the equation $R_S = 2 \times (t_{r2} - t_{r1})/(w_1 + w_2)$, where t_{r2} and t_{r1} are the retention times of the second and first enantiomers, respectively, and w_1 and w_2 are the corresponding base peak widths. The efficiency (number of theoretical plates, N) was calculated using $N = 16(t_r/w)^2$.

RESULTS AND DISCUSSION

Performance of the Chiral Stationary Phases

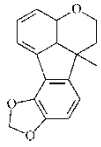
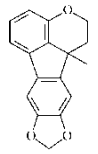
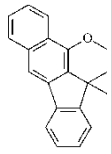
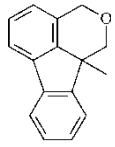
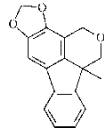
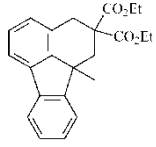
The chromatographic parameters for successful and unsuccessful separations are given in Tables 1–4. For the Cyclobond CSPs, enantiomeric separations were only observed in the reversed phase mode. No enantiomeric separations were achieved on these CSPs in the normal phase mode or the polar organic mode. Chirobiotic CSPs showed enantioselectivities for

Table 1. Retention factor of the first peak (k_1), enantioselectivity (α), and enantiomeric resolution (R_S) of all chiral polycycles on the Cyclobond RSP and DM CSPs in the reversed phase mode

#	Structure	CSP	k_1	α	R_S	Mobile phase (v/v)
1		RSP	7.34	1.09	1.0	$CH_3OH/H_2O = 35/65$
			5.43	1.10	1.4	$CH_3CN/H_2O = 20/80$
		DM	10.52	1.04	0.4	$CH_3OH/H_2O = 30/70$
			9.45	1.05	0.7	$CH_3CN/H_2O = 15/85$
2		RSP	5.59	1.17	1.8	$CH_3OH/H_2O = 40/60$
			6.02	1.11	1.5	$CH_3CN/H_2O = 20/80$
		DM	6.95	1.14	1.4	$CH_3OH/H_2O = 30/70$
			5.95	1.09	1.0	$CH_3CN/H_2O = 15/85$
3		RSP	6.34	1.12	1.5	$CH_3OH/H_2O = 40/60$
			7.03	1.14	1.8	$CH_3CN/H_2O = 20/80$
		DM	9.30	1.10	1.2	$CH_3OH/H_2O = 30/70$
			9.65	1.07	0.9	$CH_3CN/H_2O = 15/85$
4		RSP	4.76	1.17	1.7	$CH_3OH/H_2O = 50/50$
			4.97	1.15	2.1	$CH_3CN/H_2O = 25/75$
		DM	4.95	1.18	1.2	$CH_3OH/H_2O = 35/65$
			13.69	1.13	1.4	$CH_3CN/H_2O = 20/80$
5		RSP	6.55	1.10	1.3	$CH_3OH/H_2O = 50/50$
			7.46	1.10	1.5	$CH_3CN/H_2O = 25/75$
		DM	7.70	1.08	0.5	$CH_3OH/H_2O = 35/65$
			8.24	1.06	0.8	$CH_3CN/H_2O = 25/75$
6		RSP	10.32	1	0	$CH_3OH/H_2O = 50/50$
			9.34	1	0	$CH_3CN/H_2O = 25/75$
		DM	7.12	1.32	3.4	$CH_3OH/H_2O = 50/50$
			11.65	1.37	4.2	$CH_3CN/H_2O = 25/75$
7		RSP	7.57	1.11	1.3	$CH_3OH/H_2O = 50/50$
			7.21	1.12	1.5	$CH_3CN/H_2O = 25/75$
		DM	8.53	1.10	0.6	$CH_3OH/H_2O = 35/65$
			8.29	1.10	1.3	$CH_3CN/H_2O = 25/75$

(*continued*)

Table 1. Continued

#	Structure	CSP	k_1	α	R_S	Mobile phase (v/v)
8		RSP	5.48	1.23	2.5	$CH_3OH/H_2O = 40/60$
			5.00	1.24	2.6	$CH_3CN/H_2O = 20/80$
		DM	8.81	1.18	1.8	$CH_3OH/H_2O = 35/65$
			5.82	1.13	1.5	$CH_3CN/H_2O = 20/80$
9		RSP	12.77	1.03	0.3	$CH_3OH/H_2O = 40/60$
			9.80	1.05	0.6	$CH_3CN/H_2O = 20/80$
		DM	3.82	1.18	1.9	$CH_3OH/H_2O = 50/50$
			4.96	1.12	1.5	$CH_3CN/H_2O = 25/75$
10		RSP	11.62	1.13	1.5	$CH_3OH/H_2O = 50/50$
			11.58	1.14	1.8	$CH_3CN/H_2O = 25/75$
		DM	9.04	1	0	$CH_3OH/H_2O = 35/65$
			9.52	1.03	0.3	$CH_3CN/H_2O = 25/75$
11		RSP	5.23	1.07	0.8	$CH_3OH/H_2O = 35/65$
			3.68	1.07	1.0	$CH_3CN/H_2O = 20/80$
		DM	5.18	1	0	$CH_3OH/H_2O = 35/65$
			7.34	1	0	$CH_3CN/H_2O = 15/85$
12		RSP	5.12	1.10	1.3	$CH_3OH/H_2O = 40/60$
			5.06	1.11	1.5	$CH_3CN/H_2O = 20/80$
		DM	7.78	1.17	2.0	$CH_3OH/H_2O = 35/65$
			5.04	1.12	1.6	$CH_3CN/H_2O = 20/80$
13		RSP	2.28	1	0	$CH_3OH/H_2O = 40/60$
			3.17	1	0	$CH_3CN/H_2O = 20/80$
		DM	5.48	1.46	4.0	$CH_3OH/H_2O = 35/65$
			6.16	1.26	2.5	$CH_3CN/H_2O = 20/80$

several of these compounds in the reversed phase mode, but no enantiomeric separations were observed in the polar organic mode. Only separations for compounds **3** and **4** were observed for Chirobiotic CSPs in the normal phase mode. For all of the CSPs, enantiomeric separations ($Rs > 0.3$) of all the 13 analytes and baseline separations for 11 of them were achieved. The performance of all of the CSPs is summarized in Fig. 3. Obviously, the Cyclobond I-2000 RSP and DM CSPs are the most effective for the enantiomeric separation of these chiral polycycles. Eleven enantiomeric and 8 baseline separations were obtained with the Cyclobond I-2000 RSP CSP

Table 2. Retention factor of the first peak (k_1), enantioselectivity (α), and enantiomeric resolution (R_S) of chiral polycycles separated on the Cyclobond AC, I, DMP, and II CSPs in the reversed phase mode

Compound #	CSP	k_1	α	R_S	Mobile phase (v/v)
2	AC	2.52	1.30	2.0	$CH_3OH/H_2O = 40/60$
8	AC	5.15	1.07	0.6	$CH_3OH/H_2O = 30/70$
10	AC	8.27	1.11	1.1	$CH_3OH/H_2O = 40/60$
2	I	1.95	1.35	1.1	$CH_3OH/H_2O = 30/70$
4	I	2.10	1.40	0.7	$CH_3OH/H_2O = 40/60$
1	DMP	3.74	1.04	0.6	$CH_3OH/H_2O = 60/40$
2	DMP	4.81	1.04	0.4	$CH_3OH/H_2O = 60/40$
4	DMP	8.66	1.04	0.4	$CH_3OH/H_2O = 60/40$
5	DMP	11.25	1.02	0.3	$CH_3OH/H_2O = 60/40$
6	DMP	9.81	1.10	1.5	$CH_3OH/H_2O = 70/30$
9	DMP	5.89	1.13	1.8	$CH_3OH/H_2O = 60/40$
3	II	2.16	1.08	0.8	$CH_3OH/H_2O = 30/70$
13	II	4.35	1.42	2.1	$CH_3OH/H_2O = 30/70$

alone. The Cyclobond I-2000 DM CSP was able to separate 12 analytes, with 5 baseline separations. The other Cyclobond and Chirobiotic CSPs were not as effective as the former two CSPs. Only a few analytes were resolved on these other CSPs. For the separation of these neutral chiral fused-ring polycycles, the Cyclobond CSPs are superior to the Chirobiotic CSPs. However, for compounds **3** and **4**, high enantioselectivities and resolutions were observed on Chirobiotic T and Tag columns.

Table 3. Retention factor of the first peak (k_1), enantioselectivity (α), and enantioresolution (R_S) of chiral polycycles separated on the Chirobiotic V, R, T, and Tag CSPs in the reversed phase mode

Compound #	CSP	k_1	α	R_S	Mobile phase (v/v)
2	V	3.04	1.20	1.7	$CH_3OH/H_2O = 30/70$
4	V	6.62	1.03	0.3	$CH_3OH/H_2O = 30/70$
13	V	3.42	1.08	0.5	$CH_3OH/H_2O = 30/70$
2	R	6.89	1.22	1.3	$CH_3OH/H_2O = 20/80$
9	R	3.81	1.14	0.9	$CH_3OH/H_2O = 30/70$
11	R	2.59	1.09	0.4	$CH_3OH/H_2O = 30/70$
12	R	3.40	1.08	0.5	$CH_3OH/H_2O = 30/70$
3	T	6.58	1.66	4.9	$CH_3OH/H_2O = 40/60$
4	T	11.9	1.13	1.4	$CH_3OH/H_2O = 40/60$
3	Tag	6.39	1.86	3.6	$CH_3OH/H_2O = 50/50$
4	Tag	3.44	1.87	3.4	$CH_3OH/H_2O = 60/40$

Table 4. Retention factor of the first peak (k_1), enantioselectivity (α), and enantiomeric resolution (R_S) of chiral polycycles separated on the Chirobiotic V, R, T, and Tag CSPs in the normal phase mode

Compound #	CSP	k_1	α	R_S	Mobile phase (v/v)
3	V	8.00	1.04	0.8	HEP/EtOH = 99/1
4	V	8.32	1.06	0.9	HEP/EtOH = 99/1
3	R	7.40	1.04	0.6	HEP/EtOH = 99/1
4	R	7.99	1.04	0.4	HEP/EtOH = 99/1
3	T	4.94	1.44	3.2	HEP/EtOH = 98/2
4	T	5.14	1.30	2.3	HEP/EtOH = 98/2
3	Tag	2.08	3.29	3.1	HEP/EtOH = 80/20
4	Tag	1.61	3.50	2.7	HEP/EtOH = 80/20

Effect of Mobile Phase Composition

Based on studies reported in our previous publications, the pH of the reversed phase mobile phase has little effect on the enantiomeric separation of hydrophobic compounds that lack ionizable groups (21–23, 35). Two organic modifiers, acetonitrile, and methanol were examined for separation of all of the analytes on all CSPs. In most cases, the organic modifiers have only small effects on the enantioselectivity, but they do affect resolution to some extent (Table 1). For example, Cyclobond I-2000 RSP and DM CSPs showed similar enantioselectivities for compound **1** when using a methanol/water or acetonitrile/water mobile phase. However, the enantiomeric resolution was better when using an acetonitrile/water mobile phase

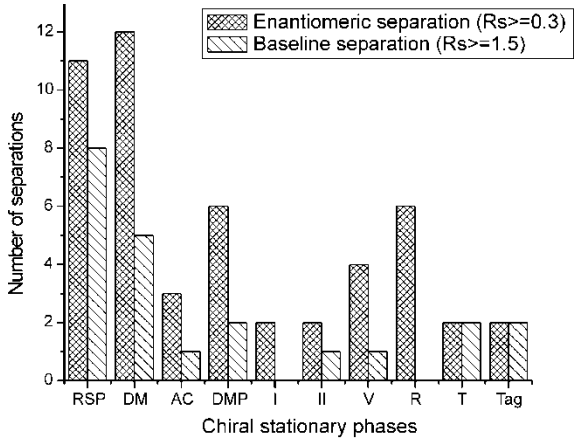


Figure 3. Summary of the number of baseline and partial separations obtained on different Cyclobond and Chirobiotic CSPs.

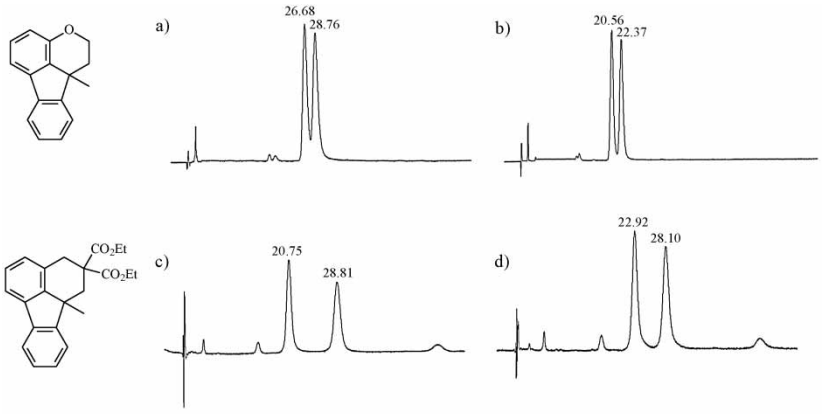


Figure 4. Chromatograms showing the difference in the separation when using two different organic modifiers in the reversed phase mode. Chromatograms (a) and (b) were obtained using the Cyclobond I-2000 RSP CSP. Chromatograms (c) and (d) were obtained using the Cyclobond I-2000 DM CSP. The mobile phase composition (volume ratio) in each case was as follows: (a) and (c) $CH_3OH/H_2O = 35/65$, (b) and (d) $CH_3CN/H_2O = 20/80$. Enantioselectivity: (a) $\alpha = 1.09$, (b) $\alpha = 1.10$, (c) $\alpha = 1.46$, (d) $\alpha = 1.26$. Number of theoretical plates of the first peak: (a) $N_1 = 3200$, (b) $N_1 = 4300$.

due to an increase in the efficiency (Fig. 4a and 4b). The theoretical plate number of the first peak, N_1, is 3200, when methanol was used as the organic modifier, while N_1 is 4300, when acetonitrile was used. Similar trends were observed for the separation of compounds **3**–**5**, **7**, and **9**–**12** on the Cyclobond I-2000 RSP column and compounds **4**–**7** on the Cyclobond I-2000 DM column. The resolution usually increases when using acetonitrile as the organic modifier due to an increase in the efficiency of the column. However, it should be noted that in a few special cases, better resolution was observed when methanol was used as the organic modifier, because of higher enantioselectivity. One typical example is the separation of compound **13** on the Cyclobond I-2000 DM CSP. Higher enantioselectivity, which resulted in better resolution, was observed when using a methanol/water mobile phase as opposed to an acetonitrile/water mobile phase (Fig. 4c and 4d).

Effects of the Structure of the Analyte

Although all analytes have similar molecular skeletons, as well as stereogenic centers, a small difference in the structure of these analytes away from the stereogenic center produces large effects on these enantiomeric separations. These effects are illustrated using the following examples.

Both the Cyclobond I-2000 RSP and DM columns displayed higher enantioselectivities for compound **2**, which has a methyl ester substituent at the 6 position, than compound **1** without such a group, when a methanol/water mobile phase was used. Therefore, the resolution for compound **2** is higher than compound **1** on these two columns. Compound **2** also can be easily separated on the Cyclobond I-2000 AC, I, Chirobiotic V, and R columns, while no enantioselectivity was observed for compound **1** on these CSPs. Another example is the separation of compounds **11** and **12**. The methylenedioxy group at the 4 and 5 positions of the polycycle enhanced the enantiomeric resolution. Baseline separation of compound **12** was achieved on the Cyclobond DM CSP, while no selectivity for compound **11** was found on this column due to the lack of substituents. In general, Cyclobond CSPs showed higher enantioselectivities for the racemic polycycles with substituents than the analogous compounds without substituents. A substituent on any chiral compound can provide steric interactions that adjust the geometry of the inclusion complexation, thereby providing a more or less favorable enantioselective binding site. Obviously, in these specific cases, the substituent on the polycycle resulted in an inclusion complex that enhanced the enantiomeric recognition between the racemic analytes and the derivatized cyclodextrin, thereby improving the separations.

Another interesting example is the separation of chromene derivatives **5**–**7**. These three compounds have similar structures, except for differing substituents in the 5 position of the polycycle. Compound **5** has a proton, while compounds **6** and **7** have nitro and methoxy groups, respectively. The methoxy group has a small effect on the enantiomeric separation on the Cyclobond I-2000 RSP and DM CSPs. Both CSPs showed similar enantioselectivities for compounds **5** and **7** (Fig. 5). Conversely, the nitro group affects enantiomeric separation greatly. Although Cyclobond I-2000 RSP CSP was not able to separate the enantiomers of compound **6**, the enantiomeric separation was improved for this compound on Cyclobond I-2000 DM CSP compared with compounds **5** and **7** (Fig. 5).

A comparison of the separation of the structural isomers **8** and **9** is also interesting. A change in the position of the methylenedioxy substituent resulted in different enantioselectivities for these two compounds on the Cyclobond I-2000 RSP column. Using the same mobile phase on the Cyclobond I-2000 RSP CSP, compound **8** (with the methylenedioxy substituent at the 7 and 8 positions) showed lower retention, but higher enantioselectivity, than compound **9** (with the same group at the 8 and 9 positions). Clearly, the location of the same substituents on the polycycles also affected the enantiomeric separations of these compounds.

Although there is no significant difference for the separations of two somewhat similar quinoline derivatives **3** and **4** on the Cyclobond DM and RSP CSPs, the Chirobiotic T and TAG CSPs showed different enantioselectivity for these two analytes. In the reversed phase mode, the Chirobiotic T column showed much higher enantioselectivity for compound **3** than

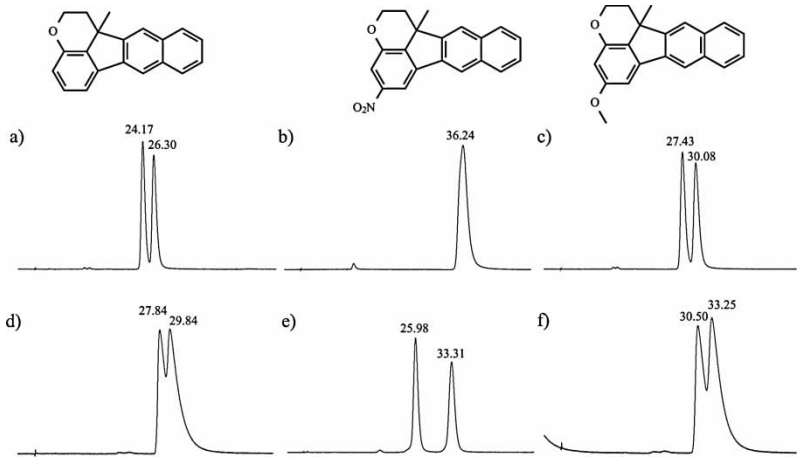


Figure 5. The effects of different analyte substituents on the enantiomeric separation. Chromatograms (a), (b), and (c) were obtained using the Cyclobond I-2000 RSP CSP. Chromatograms (d), (e), and (f) were obtained using the Cyclobond I-2000 DM CSP. The mobile phase composition (volume ratio) in each case was as follows: (a), (b), (c), and (e) $CH_3OH/H_2O = 50/50$, (d) and (f) $CH_3OH/H_2O = 35/65$. Enantioselectivity α: (a) $\alpha = 1.10$, (c) $\alpha = 1.11$, (d) $\alpha = 1.08$, (e) $\alpha = 1.32$, (f) $\alpha = 1.10$.

compound **4** and the enantiomeric resolution of compound **3** is about 3.5 times that of compound **4**. However, on Chirobiotic TAG column, the enantioselectivity of both compounds **3** and **4** increased (Fig. 6). Although higher enantioselectivity was observed for compound **3** on the Chirobiotic TAG than the Chirobiotic T column, the resolution was worse on the Chirobiotic TAG column due to the low efficiency (N1 is 1400 on Chirobiotic TAG CSP and 2600 on Chirobiotic T CSP). The enantiomeric resolution for compound **4** was significantly greater on the Chirobiotic TAG column than on the Chirobiotic T column, because of the increase in the enantiomeric selectivity. In the normal phase mode, high enantiomeric resolutions of compounds **3** and **4** were observed on both Chirobiotic T and TAG CSPs. The Chirobiotic TAG column showed much higher enantioselectivities (more than twice) for these two compounds compared to the Chirobiotic T column. However, no great increase in separation was observed due to the poor efficiency of the Chirobiotic TAG column. The results in the normal phase (Table 4) indicated that the steric effect of the bulky sugar groups on the teicoplanin decreased the chiral recognition of these two compounds. On the contrary, these repulsive steric interactions of the Chirobiotic T column decreased the retention and increased the efficiency greatly compared with the Chirobiotic TAG column. In addition, compounds **3** and **4** are the only compounds, which can be separated in normal phase mode on all Chirobiotic CSPs.

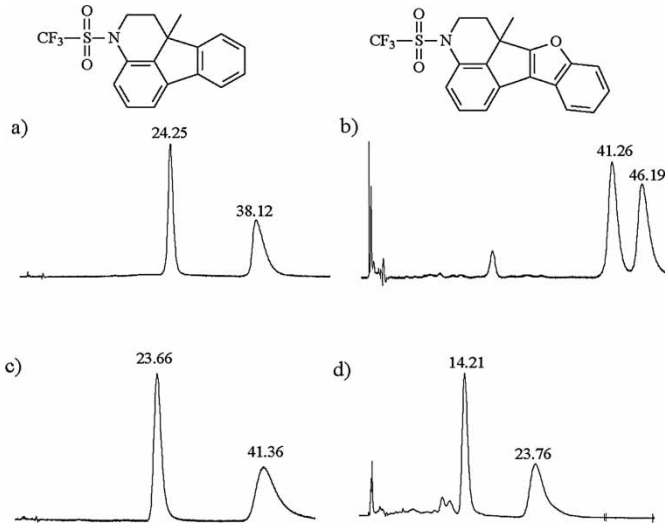


Figure 6. Comparison of the separations of compounds **3** and **4** on Chirobiotic T and TAG CSPs in reversed phase mode. Chromatograms (a) and (b) were obtained using the Chirobiotic T CSP. Chromatograms (c) and (d) were obtained using the Chirobiotic TAG CSP. The mobile phase composition (volume ratio) in each case was as follows: (a) and (b) $CH_3OH/H_2O = 40/60$, (c) $CH_3OH/H_2O = 50/50$, (d) $CH_3OH/H_2O = 60/40$. Enantioselectivity α: (a) $\alpha = 1.66$, (b) $\alpha = 1.13$, (c) $\alpha = 1.86$, (d) $\alpha = 1.87$. Number of theoretical plates of the first peak N_1: (a) $N_1 = 2600$, (c) $N_1 = 1400$.

CONCLUSIONS

All of the 13 chiral fused polycycles examined were separated on Cyclobond and Chirobiotic CSPs and 11 of them were baseline separations. Cyclobond I-2000 DM and RSP CSPs are the most broadly applicable CSPs for the separation of these chiral compounds. Although Chirobiotic CSPs are not as effective as Cyclobond CSPs for these analytes, high enantioselectivities and resolutions for two analytes were observed on the Chirobiotic T and TAG columns in the reversed phase and normal phase modes. The reversed phase mode is the best mobile phase for these separations. Enantiomeric separations of only two analytes were observed in the normal phase mode on Chirobiotic CSPs and no enantioselectivity was found in the polar organic mode on any CSP. Similar enantioselectivities were found for analytes when either acetonitrile or methanol were used in the reversed phase mode. Generally, the acetonitrile/water mobile phases showed higher efficiencies than methanol/water mobile phases. For some special cases, the enantioselectivity in the methanol/water mobile phase was higher than with the acetonitrile/water mobile phase. The structure of the individual analytes

greatly affected the enantiomeric separation. Chiral analytes with substituents generally were better separated than their unsubstituted parent compounds.

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