

Figure 1. Proposed binding-site model for DHP/DHPM calcium antagonists (aryl-group up) and agonists (aryl-group down).⁹ The receptor sensitive groups are on the 'left-hand side' of the molecule.⁹

Since individual enantiomers of chiral DHPMs **1** have opposing pharmacological effects on the calcium channel, access to enantiomerically pure DHPMs is a requirement for the development of cardiovascular drugs of this structural type. In general, DHPMs **1** are readily available in racemic form through classical Biginelli dihydropyrimidine synthesis,¹⁰ or related methods.^{10,11} More recently, enantiomerically pure DHPMs were obtained by resolution of the corresponding racemic 5-carboxylic acids *via* diastereoisomeric ammonium salts,¹² or by separation of diastereoisomeric derivatives bearing chiral auxiliaries at N-3.⁷ Both of these methods, however, involve multi-step reaction sequences giving rise to only moderate overall yields of enantiomerically pure DHPMs.^{7,10,12}

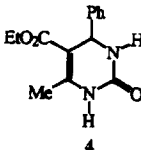
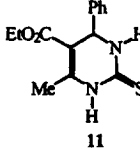
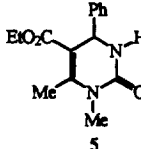
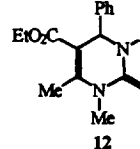
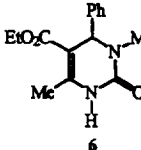
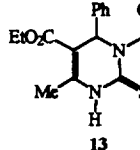
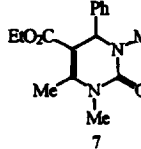
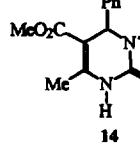
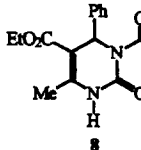
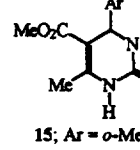
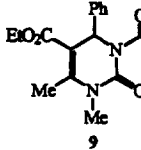
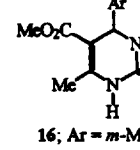
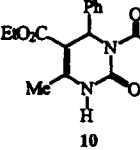
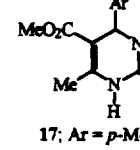
Due to recent advances in chromatographic enantioseparation techniques, enantioselective HPLC and related methods (*i.e.* simulated moving bed chromatography), have gained importance in the preparation of single-enantiomer drugs and intermediates.¹³ In the DHPM series only very few published examples of successful chromatographic enantioseparations exist.¹⁴ Here we report the first detailed and systematic study on the direct enantioseparation of DHPM derivatives of type **1** using a variety of commercially available chiral stationary phases (CSPs) in normal- and reversed-phase analytical HPLC. Apart from identifying a generally applicable CSP for these enantioseparations the correlation between structural features in DHPMs with the achieved separation on individual CSPs was of particular interest to us.

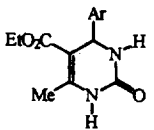
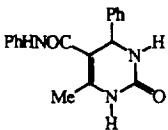
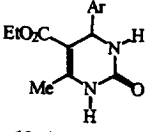
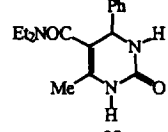
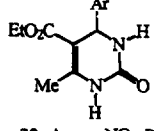
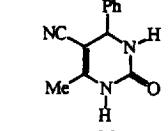
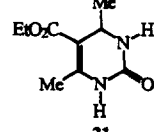
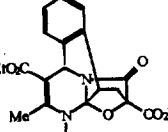
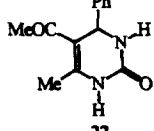
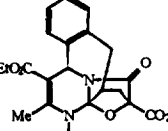
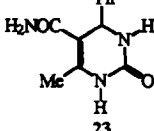
Results and discussion

For the present study we have selected a set of 29 representative DHPM derivatives (**4–32**, see Tables 1 and 2) that possess all of the important structural features^{4–9} of the biologically active analogs (*i.e.* **2**). DHPMs **4–32** were prepared according to literature procedures by standard three-component Biginelli condensation¹⁰ of the corresponding acetoacetates, aldehydes and urea derivatives (see Experimental). All substituents around the dihydropyrimidine ring were varied with the exception of the C6-methyl group which is mandatory for optimum calcium channel modulatory activity.^{4–9} The recently reported conformationally restricted polycyclic DHPM analogs **27–28**,¹ and the dihydropyrimidine-5-carboxylic acids **29–32** were also included.

The proper selection of chiral stationary phases (CSPs) composed of suitable chiral selectors (SOs) immobilized on support material is of great importance for developing effective HPLC enantioseparation methods. The selection of the eight CSPs used in this study was based on commercial availability and on the proven ability of these CSPs for the enantioseparation of molecules containing similar structural features as DHPMs. To evoke several different types of chiral recognition mechanisms a variety of selectors covering a broad spectrum of structural elements and secondary structures was chosen. These were the carbohydrate-based CSPs Chiralcel OD-H and ChiraDex, the glycopeptide-based CSPs Chirobiotic V and T, and the classical Pirkle-type CSPs (3*S*,4*R*)-Whelk-O1 and (*R,R*)-DPEDA for DHPMs **4–28**, and two chiral anion exchange-type CSPs for DHPM acids **29–32**. In order to facilitate the discussion the enantioseparation characteristics of CSPs 1–8 are discussed in four separated parts.

Table 1. Chromatographic data obtained by direct enantioseparation of DHPMs 4-28

DHPM	CSP ^a	MP ^b	k' ^{1c}	α^d	Rs ^e	DHPM	CSP ^a	MP ^b	k' ^{1c}	α^d	Rs ^e
 4	1	A	1.31	1.37	1.98	 11	1	A	0.71	1.08	0.29
	2	C	2.21	1.32	2.50		2	C	1.95	1.30	2.07
	3	D	0.30	4.75	10.93		3	E	2.06	1.26	3.02
	4	D	0.54	6.90	16.71		4	D	0.56	1.58	3.27
	5	A	2.05	-	-		5	A	n.e.	-	-
	6	F	2.53	1.14	1.23		6	F	2.14	1.10	0.85
 5	1	A	1.52	1.24	1.28	 12	1	A	0.93	1.10	0.50
	2	C	2.41	1.11	0.69		2	C	2.83	-	-
	3	E	1.88	1.13	1.56		3	E	2.36	-	-
	4	E	2.31	1.07	0.67		4	E	2.88	1.05	0.61
	5	A	3.29	-	-		5	A	n.e.	-	-
	6	A	1.48	-	-		6	F	1.87	1.05	0.57
 6	1	A	0.85	-	-	 13	1	A	0.58	1.88	3.54
	2	C	4.49	1.22	2.01		2	C	5.80	1.14	1.00
	3	E	1.74	1.31	3.32		3	E	2.24	1.36	3.87
	4	D	0.51	1.39	2.31		4	E	2.71	1.07	0.72
	5	A	1.92	-	-		5	A	n.e.	-	-
	6	F	1.69	1.29	2.55		6	A	0.75	-	-
 7	1	A	0.79	1.31	1.48	 14	1	A	1.29	1.36	1.93
	2	C	2.07	1.10	0.59		2	C	1.97	1.43	3.19
	3	D	0.24	-	-		3	D	0.28	4.45	10.60
	4	D	0.43	-	-		4	D	0.46	6.95	16.15
	5	A	4.13	1.09	0.95		5	A	2.18	1.07	0.47
	6	A	0.96	-	-		6	F	2.76	1.15	1.29
 8	1	A	1.03	1.51	2.88	 15; Ar = <i>o</i> -Me-Ph	1	A	1.43	-	-
	2	C	3.81	1.17	1.41		2	C	1.97	-	-
	3	E	1.60	-	-		3	D	0.30	4.16	10.14
	4	D	0.50	6.96	15.92		4	D	0.51	4.45	12.55
	5	A	2.28	1.25	2.07		5	A	1.99	-	-
	6	F	1.68	1.09	0.89		6	A	1.61	-	-
 9	1	A	1.59	1.08	0.41	 16; Ar = <i>m</i> -Me-Ph	1	A	1.12	1.45	2.15
	2	C	2.28	1.11	0.67		2	C	1.14	1.31	1.74
	3	E	1.85	1.13	1.53		3	D	0.31	3.21	7.95
	4	E	2.35	-	-		4	D	0.52	4.52	12.72
	5	A	4.40	1.16	1.63		5	A	2.06	1.15	0.78
	6	A	1.53	-	-		6	F	2.60	1.15	1.33
 10	1	A	0.79	1.29	1.65	 17; Ar = <i>p</i> -Me-Ph	1	A	1.15	1.65	2.61
	2	C	4.42	1.08	0.63		2	C	5.21	1.44	4.02
	3	D	0.15	-	-		3	D	0.28	3.81	8.85
	4	D	0.29	-	-		4	D	0.41	7.68	14.06
	5	A	2.18	-	-		5	A	2.27	1.15	0.98
	6	A	0.89	-	-		6	A	1.45	1.14	1.03

DHPM	CSP ^a	MP ^b	k' ^{1c}	α^d	Rs ^e	DHPM	CSP ^a	MP ^b	k' ^{1c}	α^d	Rs ^e
 18; Ar = <i>o</i> -NO ₂ -Ph	1	A	1.87	2.67	1.45	 24	1	A	2.75	-	-
	2	C	1.90	-	-		2	C	2.39	1.35	2.72
	3	D	0.34	4.65	11.88		3	D	0.31	1.43	1.86
	4	D	0.55	2.92	7.47		4	D	0.47	2.85	6.78
	5	A	5.02	-	-		5	A	8.40	-	-
	6	F	4.38	1.31	2.82		6	A	3.08	1.25	1.34
 19; Ar = <i>m</i> -NO ₂ -Ph	1	A	2.35	-	-	 25	1	A	1.65	1.29	1.18
	2	C	1.56	-	-		2	C	2.32	1.26	1.46
	3	D	0.35	3.11	8.15		3	E	1.27	1.27	2.50
	4	D	0.57	3.99	12.18		4	D	0.52	3.90	10.57
	5	A	3.90	-	-		5	A	6.28	1.09	0.46
	6	F	3.98	1.08	0.69		6	A	2.45	1.38	1.77
 20; Ar = <i>p</i> -NO ₂ -Ph	1	A	2.11	1.29	1.50	 26	1	A	1.70	1.20	1.28
	2	C	6.67	1.18	1.95		2	C	2.33	1.50	3.13
	3	D	0.29	3.00	7.29		3	D	0.27	2.72	6.39
	4	D	0.48	7.29	15.66		4	D	0.36	8.67	12.71
	5	A	3.96	-	-		5	A	3.93	-	-
	6	A	1.80	1.05	0.45		6	F	3.44	1.26	2.17
 21	1	A	0.75	1.26	1.25	 27	1	A	3.66	2.52	7.57
	2	C	1.20	-	-		2	B	6.23	1.34	3.48
	3	D	0.20	2.33	4.09		3	E	2.63	-	-
	4	D	0.34	3.85	9.34		4	E	3.90	1.06	0.66
	5	A	1.43	-	-		5	B	11.23	1.32	1.65
	6	F	1.80	-	-		6	F	5.70	1.08	0.82
 22	1	A	1.88	1.37	1.93	 28	1	A	3.72	1.48	2.71
	2	C	3.19	1.26	2.28		2	B	0.23	1.46	0.71
	3	D	0.31	3.23	8.43		3	E	2.58	1.10	1.18
	4	D	0.44	6.04	14.47		4	E	4.28	-	-
	5	A	4.33	1.13	0.71		5	B	10.07	1.64	3.58
	6	F	4.29	1.18	1.37		6	F	5.43	1.08	0.81
 23	1	A	2.66	-	-	^a CSPs: 1: Chiralcel OD-H, 2: ChiraDex, 3: Chirobiotic V, 4: Chirobiotic T, 5: 3S,4R-Whelk-O1, 6: R,R-DPEDA;					
	2	C	1.20	-	-	^b Mobile phase compositions:					
	3	D	0.29	1.70	3.21	A: 2-propanol/n-heptane=20/80; B: 40/60; F: 10/90; C:					
	4	D	0.34	4.53	10.52	MeOH/H ₂ O=10/90; D: acetonitrile/H ₂ O=40/60; E: 20/80.					
	5	A	n.e.	-	-	Flow: CSP 1, 2, 5, 6: 0.8 mL/min; CSP 3 and 4: 1 mL/min					
	6	A	4.39	-	-	^c k' ¹ = (t ₁ -t ₀)/t ₀ ^d α = k' ² /k' ¹ ^e Rs = 1.18 (t ₂ -t ₁)/(w _(0.5) ¹ + w _(0.5) ²)					

Carbohydrate-based chiral stationary phases (CSP 1 and CSP 2)

Naturally occurring chiral sources such as starch or cellulose, native or in derivatized form are of growing interest as chiral SOs. Okamoto¹⁵ has shown that the coating of cellulose derivatives onto silica can give very enantioselective CSPs. CSP 1 (Chiralcel OD-H) is structurally a 3,5-dimethylphenyl-carbamoylated cellulose. Besides the supramolecular effects¹⁶ stemming from the helical secondary structure (forming chiral cavities and/or bays),¹⁷ the aromatic substituents provide $\pi\pi$ -interactions and the carbamate functionalities allow for additional hydrogen donor-acceptor sites.

The separation capabilities of CSP 1 were evaluated by normal-phase HPLC for racemic DHPMs 4–28 (Table 1). The mobile phase used was 2-propanol/n-heptane=20/80. We found that this carbamoylated cellulose derivative is a very effective CSP resolving a wide range of DHPM racemates. About 80% of all analytes presented in Table 1 were separated by this CSP. One of the interesting effects observed with this CSP was the pronounced influence of the substituents on the C4-aryl ring

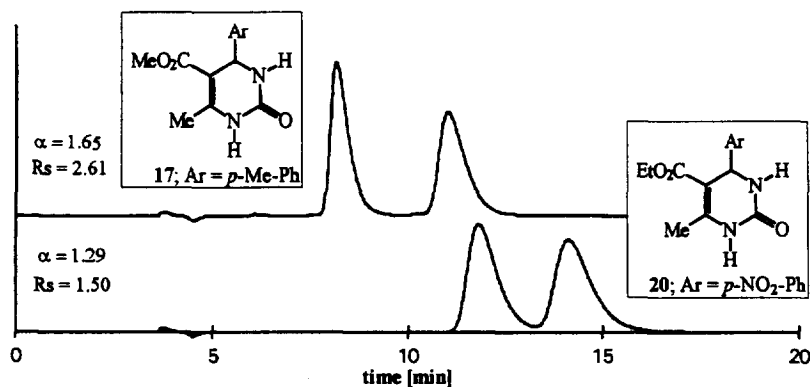


Figure 2. Enantioseparation of DHPMs 17 and 20 on Chiralcel OD-H (see Table 1).

(electron-withdrawing vs electron-donating) on the enantioseparation characteristics. The unsubstituted phenyl compound **14** acted as lead compound in this series and showed an α -value of 1.36. Comparing the *o*-, *m*-, and *p*-methyl substituted analogs (entries **15**–**17**) DHPM **15** was not separated on CSP 1 but **16** and **17** were well separated with α -values of 1.45 and 1.65, respectively (Table 1, Figure 2). This may indicate that this DHPM can not adopt a suitable conformation for interaction with the chiral selector. Recent computational studies on the conformational hypersurface of DHPMs **14**–**17** have shown that for the *o*-substituted analog **15** a preference for a conformer exists in which the aryl substituent (Me) is oriented synperiplanar to C4-H as indicated in Figure 1 (no such preference is found for the unsubstituted (**14**) or *m*-substituted (**16**) derivative).¹⁸ On the other hand the *o*-nitro substituted DHPM **18** was separated with the largest α -value (2.67) on this CSP, whereas the *m*-nitro derivative **19** was not separated and the *p*-nitro derivative **20** displayed a smaller α -value of 1.29 compared with the lead compound **14** (Figure 2). These data may indicate that in the case of nitro substitution on the aromatic ring, intercalation as well as $\pi\pi$ -interaction play an important role.

Chiral inclusion or host–guest type selectors^{17,19} encompass well-defined, relatively small molecules such as crown ethers or cyclodextrins. Cyclodextrins (CDs), cyclic oligomers of glucose bonded through α -1,4 linkages are both suited for application in HPLC^{20–22} as well as in capillary electrophoresis (CE).^{23–25} Recently, α -, β - and γ -cyclodextrins and derivatives thereof have found great interest as chiral selectors due to their unique structural features.^{20–22} CSP 2 (ChiraDex), an underivatized β -cyclodextrin SO, represents this host–guest complexation type CSP. This β -cyclodextrin (CD) selector consists of 7 glucose molecules forming a cone. The inner surface of the cone is lipophilic and at the ‘mouth’ of the cavities hydroxyl groups are located. Chiral analytes may penetrate into the lipophilic chiral cavity of the SO cone by interaction with the chiral residual hydroxyl groups at the mouth of the cone. Typical analytes to be resolved with this kind of selector possess aromatic functionalities appended to a stereogenic center on the selectand as well as polar or hydrogen bonding functionalities.¹⁹

In the case of DHPMs **4**–**28** α -values obtained with CSP 2 ranged between 1.08 and 1.50. As expected and shown in Table 1 the aromatic moiety at the C4 position of the dihydropyrimidine ring plays an important role in the chiral recognition mechanism. DHPM **14** was well separated with a relative retention of 1.43. However, a decreasing α -value with increasing bulkiness of the aromatic moiety was observed (entries **15**–**20**). It appears that spatially restricted molecules cannot penetrate into the chiral cavity and thus no enantioseparation occurs due to the lack of stereoselective interactions. 76% of DHPMs **4**–**28** could be resolved with this CSP.

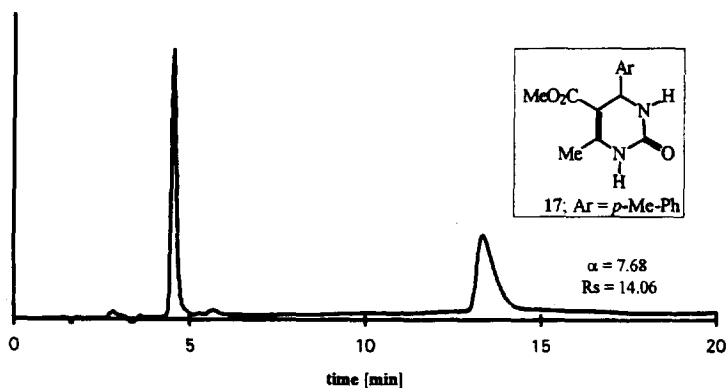


Figure 3. Enantioseparation of DHPM 17 on Chirobiotic T (CSP 4).

Glycopeptide antibiotic-based chiral stationary phases (CSP 3 and CSP 4)

Apart from polysaccharides, other molecules of the chiral pool such as amino acids, peptides and proteins²⁶ can be used as SOs in enantioselective HPLC.¹⁷ Recently, the glycopeptide antibiotics vancomycin and teicoplanin have been introduced as chiral selectors both as CSPs immobilized onto silica^{27–29} and as additives in CE.^{30,31} These large molecules with molecular weights of 1449 for vancomycin (18 stereogenic centers) and 1885 for teicoplanin (23 stereogenic centers) possess various interaction sites such as $\pi\pi$ -donor/acceptor, dipole–dipole, hydrogen-donor/acceptor, electron-donor/acceptor, cation–anion interactions, steric attraction and/or repulsion. In the present study both the vancomycin-based (CSP 3, Chirobiotic V) and the teicoplanin-based (CSP 4, Chirobiotic T) SOs were evaluated with respect to their applicability toward the separation of chiral DHPM derivatives (Figure 3).

The structurally related glycopeptide antibiotics vancomycin and teicoplanin showed similar but not identical enantioselectivities. In some cases they even produced complementary separations (*e.g.* DHPMs **8,9** and **27,28**). The best enantioseparations were achieved in the reversed-phase HPLC mode. Typical mobile-phase compositions contained water with 40–60% of acetonitrile as organic modifier. Comparing CSP 3 and 4 with respect to the retention behavior we observed that under identical mobile phase compositions the retention times on CSP 4 were somewhat larger. In most cases the teicoplanin-based CSP (CSP 4) displayed also larger α -values. No predictions about the chiral recognition mechanisms could be made using these structurally very complex chiral selectors. The chromatographic data displayed in Table 1 clearly demonstrate that CSPs 3 and 4 are by far the most selective CSPs for this type of heterocycles. The capacity value (k') was very low, almost eluting the first enantiomer with the void volume. The selectivities ranging from 1.10 to 4.75 for CSP 3 and from 1.05 to 8.67 for CSP 4 respectively, also indicate that there are extreme energy differences between the diastereoisomeric associates on the chiral surface of the CSP and the enantiomers of this class of analytes. Only two examples, *viz* DHPMs **7** and **10** could not be separated by the glycopeptide CSPs. All other derivatives (92%) were separated by at least one of the two CSPs. Note that DHPM-5-carboxamide **23** could only be separated by these CSPs. On the other hand, the conformationally restricted DHPMs **27** and **28**, readily separable on other CSPs, surprisingly caused problems on both CSP 3 and CSP 4.

Pirkle-type chiral stationary phases (CSP 5 and CSP 6)

CSP 5 and CSP 6 represent the classic Pirkle-type CSPs. The chiral selector used in CSP 5 was recently reported by Pirkle and coworkers.^{32,33} The structure of CSP 5 (3S,4R-Whelk-O1) displayed in Figure 4 can be seen as semirigid frame with a π -acidic 3,5-dinitrobenzamide and a π -basic aromatic group attached to it. The two aromatic rings are forming a chiral cleft. Additionally, the

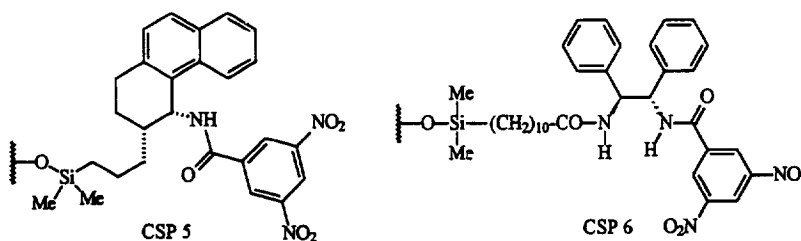


Figure 4. Chiral selector units for CSP 5 (3*S*,4*R*-Whelk-O1) and CSP 6 (*R,R*-DPEDA).

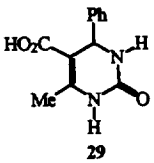
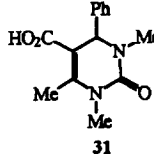
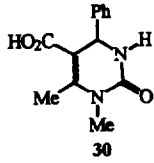
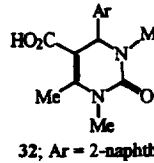
amide N–H may serve as hydrogen bonding interaction site. With these structural characteristics, CSP 5 was rationally designed³⁴ for the separation of enantiomers containing an aromatic moiety and hydrogen bond acceptor near the stereogenic center. The SO of CSP 6 developed by Uray³⁵ (*R,R*-N-DNP-DPEDA) is shown in Figure 4 and displays similar interaction characteristics with a more flexible backbone compared to CSP 5. Efficient enantioseparations of some DHPMs similar to **4–28** on CSP 6 have already been reported.^{14c}

Both CSPs were run in normal-phase mode using mixtures of *n*-heptane/2-propanol as mobile phase compositions. Although CSP 5 and 6 are somewhat similar in design they showed remarkable differences in terms of chiral recognition. Statistically, 42% of DHPMs **4–28** were separated on CSP 5 and 68% on CSP 6. By comparing DHPMs **4–7**, representing dihydropyrimidines with different methylation positions at N1 and N3, the following enantioseparation effects were noted (Table 1): CSP 6 seems to rely on a hydrogen bonding donor at position N1 for chiral recognition. Chiral recognition of CSP 5 on the other hand is not related to the hydrogen bond interaction sites located at N1 and/or N3. Remarkably, it was impossible to separate the N1/N3 unsubstituted DHPM **4** on CSP 6, possibly due to concurrency of the two hydrogen bonding sites. In order to specify the role of the aromatic substituent in DHPMs on the chiral recognition, the chromatographic behavior of a number of aryl substituted DHPMs was studied (entries **14–20**). In accordance with the π -acidic nature of the selectors of CSP 5 and 6 a variation of the π -basicity of the analyte should result in a change in enantioselectivity. Under chosen conditions lead compound **14** showed α -values of 1.07 and 1.15 for CSP 5 and 6, respectively. Compared to the somewhat more π -basic DHPMs **15–17** no significant differences in stereoselectivity were observed (the exception being the *o*-methyl analog **15** where no enantioseparation was achieved. As another interesting effect we notice that CSP 5 was unable to separate the nitro substituted DHPMs **18–20**. Under identical conditions CSP 6 could separate also these π -acidic DHPM derivatives, however with smaller α -values than for the unsubstituted derivative. The high selectivity value of the *o*-nitro derivative **18** ($\alpha=1.31$) may be attributed to steric effects. As expected, both Pirkle type CSPs were unable to separate 4-methyl-DHPM **21** lacking the important aromatic interaction site. In addition to CSP 5 and CSP 6 we have also evaluated the Pirkle naphthyl-type CSP³⁶ for the enantioseparation of DHPMs **4–28**. However, under chosen mobile phase conditions (*n*-heptane/2-propanol=80/20) only the *m*-nitro and *p*-nitro substituted π -acidic DHPMs (entries **19**, **20**) were separated, both displaying an α -value of 1.12 (data not included in Table 1).

Anion exchange-type chiral stationary phases (CSP 7 and 8)

Apart from the dihydropyrimidine-5-carboxylic ester derivatives **4–28** described in Table 1 we have also attempted the enantioseparation of the corresponding carboxylic acids **29–32**³⁷ (Table 2) employing recently developed chiral ion exchanger type CSPs.³⁸ Quinine-based CSP 7 (2,6-diisopropylphenyl carbamate, Figure 5) and CSP 8 (*tert*-butyl carbamate, Figure 5)³⁸ are weak chiral ion exchangers that work preferentially in the reversed-phase mode where ionic type interactions between the selector (SO) and the selectand (SA) are the primary binding and/or retention mechanism. The strength of these ionic interactions can easily be balanced by the buffer strength. Besides ion exchanging mechanisms adsorption to the lipophilized silica surface can be named as minor retention

Table 2. Chromatographic data obtained by direct enantioseparation of DHPMs 29–32

DHPM	CSP ^a	MP ^b	k' ^{1c}	α^c	R _s ^c	e.o. ^d	DHPM	CSP ^a	MP ^b	k' ^{1c}	α^c	R _s ^c	e.o. ^d
 29	7	G	2.15	1.15	1.34	-	 31	7	G	4.39	-	-	-
	8	G	1.73	1.03	0.25	-		8	G	3.41	1.13	1.26	R<S
 30	7	G	3.27	1.17	1.76	-	 32; Ar = 2-naphthyl	7	G	8.36	1.28	2.83	R<S
	8	G	2.69	-	-	-		8	G	7.31	1.59	4.80	R<S

^a CSP 7, CSP 8: see Figure 5. General HPLC conditions: Flow: 1 mL/min

^b Mobile phase composition: G: MeOH/ ammonium acetate (0.1 M) = 80/20, pH = 6.00 (glacial acetic acid)

^c see Table 1;

^d e.o. = elution order

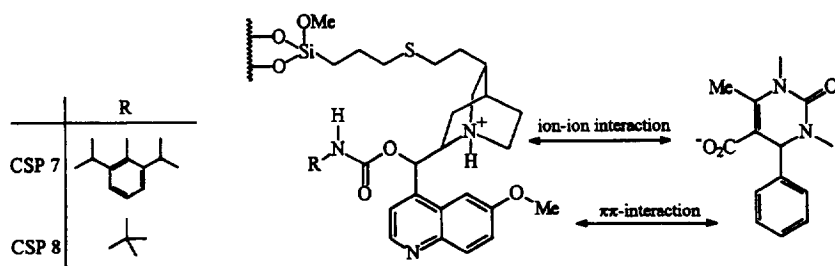


Figure 5. Quinine-based CSP 7 and CSP 8 and proposed chiral recognition principle.

mechanism. Separation of the DHPM acid enantiomers is caused by simultaneous multiple interactions (e.g. ionic and $\pi\pi$) between the two enantiomers and the chiral selector (Figure 5).

The DHPM carboxylic acids displayed in Table 2 (entries 29–32) were separated with α -values ranging from 1.03 to 1.59. CSP 7 and 8 seem to be complementary in terms of chiral recognition since the N1/N3 unsubstituted DHPM 29 separated well on CSP 7, the N1/N3 dimethylated DHPM 31 was not separated on CSP 7 under chosen conditions (Table 2). CSP 8 essentially displayed the opposite effect, although DHPM 29 was recognized with a very small α -value of 1.03. DHPM 32, the 2-naphthyl analog of DHPM 31, was separated on both quinine type CSPs, indicating that the nature of the aromatic moiety attached to the stereogenic center also plays a role in the chiral recognition process ($\pi\pi$ -interaction). Since we had the enantiomerically pure DHPMs (*S*)-31 and (*S*)-32 in our hands (previously prepared by classical diastereoisomeric resolution)¹² we were able to determine the elution order of enantiomers for these particular cases (Figure 6, Table 2).

Summary and conclusion

In the present study 29 variously substituted chiral 4-aryldihydropyrimidine derivatives (DHPMs 4–32) were separated into individual enantiomers by direct enantioselective HPLC employing different types of CSPs. In all but one case (DHPM 12) baseline separation of enantiomers could be achieved ($\alpha \geq 1.13$, $R_s \geq 1.26$) with at least one of the eight CSPs. More than half (55%) of the DHPMs investigated were separated with $\alpha \geq 2.50$, the highest value achieved being $\alpha = 8.67$ for DHPM 26. In

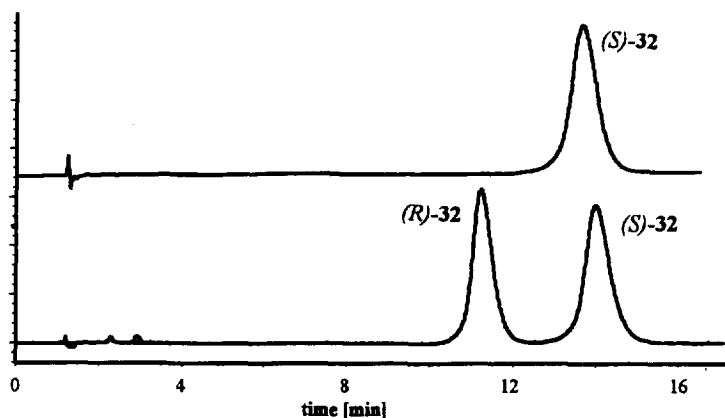


Figure 6. Enantioseparation of DHPM 32 on CSP 8 and HPLC trace of (S)-32.

general, N1 and/or N3 alkylated derivatives (**5–7**, **9**, **12**) were less well-resolved than their unsubstituted analogs (**4**, **8**, **11**). Similarly, the 2-thioxo analogs **11** and **12** were significantly less well-resolved than the corresponding 2-oxo-DHPMs **4** and **5**. Note that even the 4-methyl-DHPM **21** could be separated by some CSPs. A critical analysis of the data presented in Tables 1 and 2 shows that it is difficult to predict and rationalize the enantioseparation capabilities of individual CSPs. Even small structural changes on the DHPM skeleton may result in a dramatic change in enantioselectivity. Nevertheless, by covering a broad spectrum of CSPs as used in this study the chances of achieving good enantioseparation for a particular DHPM derivative are very high. It should be also pointed out that the pharmacologically relevant DHPMs (Figure 1, *e.g.* X=NO₂, N1-H), in particular analogs with an N3-acyl functionality (*cf.* **1**, **2**) were readily separated by most CSPs.

We therefore have demonstrated that enantioselective HPLC can be an effective tool for obtaining pharmacologically relevant DHPM derivatives in enantiomerically pure form. Although this work was mainly exploratory and analytical in intent (separations were not optimized) the high separation coefficients and resolution values achieved with most CSPs leave little doubt that this method could also be used in preparative chiral chromatography for the large-scale production of DHPM derivatives. Furthermore, the HPLC application described herein may also be employed as an analytical tool in biomedical research.

Experimental

Racemic DHPM derivatives **4–32** were synthesized following literature procedures: **4**,³⁹ **5**,⁴⁰ **6**,⁴¹ **7**,⁴² **8–10**,⁴¹ **11**,⁴⁰ **12,13**,⁴¹ **14–17**,¹⁸ **18**,⁴³ **19–21**,³⁹ **22**,⁴⁴ **23–25**,⁴⁵ **26**,⁴¹ **27,28**,¹ **29,30**,⁴⁶ **31,32**.¹² Acetonitrile (ACN), methanol (MeOH), n-heptane and 2-propanol for the preparation of the mobile phases were HPLC grade and purchased from Aldrich (Steinheim, Germany). Ammonium acetate was obtained from Merck (Darmstadt, Germany). HPLC grade water (purified by a Milli-Q-Plus filtration unit from Millipore) was used to prepare the mobile phases. Mobile phases were filtered through a Nalgene nylon membrane filter (0.2 mm) (Nalge Company, New York, USA) and degassed before use. The pH of the mobile phases refers to the apparent pH (pH_a) and was measured with an Orion-pH-meter, model 520A. Details about mobile phase compositions are given in Tables 1 and 2. All other chemicals used were of p.a. quality.

All high performance liquid chromatographical measurements carried out in this work employed a Hewlett Packard HP 1050 compact system with variable wavelength detector (VWL) and a HP^{2D}HPLC Chemstation version A.02.05. The chiral stationary phases used for the direct enantioseparations of the racemic DHPM **4–28** were: CSP 1: Chiralcel OD-H (J.T. Baker, Netherlands) (250×4.6 mm i.d., 5 mm), CSP 2: ChiraDex (Merck, Darmstadt) (250×4.6 mm i.d., 5 mm), CSP 3: Chirobiotic V (Astec,

Whippany, USA) (250×4.6 mm i.d., 5 mm), CSP 4: Chirobiotic T (Astec, Whippany, USA) (250×4.6 mm i.d., 5 mm), CSP 5: 3*S*,4*R*-Whelk-O1 (Merck, Darmstadt) (250×4.6 mm i.d., 5 mm), CSP 6: (*R,R*)-DPEDA (*N'*-Undec-10-enoyl-(*R,R*)-*N*-3,5-dinitrobenzoyl-1,2-diphenyl-1,2-diaminoethane) provided by G. Uray³⁵ (250×4.0 mm i.d., 5 mm). The direct separation of the racemic DHPM carboxylic acids 29–32 was accomplished by using quinine carbamate based chiral ion exchangers CSP 7 and 8 (150×4.0 mm i.d., 5 mm) provided by W. Lindner.³⁸ The temperature during the analyses was adjusted to 25°C. The flow rate was set to 0.8 mL/min, unless stated otherwise. UV detection was performed at 254 nm.

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