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Journal of Chromatography A, 1031 (2004) 187–195
JOURNAL OF CHROMATOGRAPHY A
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Exhaustively substituted bile acids as chiral selectors for enantioselective chromatography Aim, use and perspectives

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Abstract

The use of chiral stationary phases (CSPs) obtained from cholic and deoxycholic acid derivatives in the HPLC resolution of racemic compounds is presented. The CSPs containing arylcarbamoyl derivatives of bile acids show enantiodiscriminating capabilities depending on the electronic character of the aryl substituents: the CSPs obtained starting from heteroderivatized selectors, i.e. bile acid derivatives containing both π-acidic and π-basic arylcarbamoyl moieties, show enantiodiscriminating capabilities strongly dependent on the arrangement of the electronically different arylcarbamates on the cholestanic backbone. The CSPs obtained starting from deoxycholic acid derivatives possessing both arylamido and arycarbamoyl substituents show enantiodiscriminating capabilities restricted to the resolution of benzodiazepine derivatives. Again, the enantioresolution properties depend not only on the electronic nature of the aromatic substituents but also on their arrangement on the cholestanic backbone. The comparison among the different families of bile acid based CSPs allows us to find likeness and differences in the enantiorecognition mechanism exhibited by the different chiral selectors.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Bile acids

1. Introduction

The use of bile acids in chiral recognition processes has received a great deal of attention in the last decade. These natural products, because of their unique structure endowed with several functional groups arranged in a rigid chiral backbone, have been used successfully in the construction of extended preorganised molecular architectures [1]. In addition, their enantiodiscriminating properties can suitably be tuned by reacting the original functional groups present in the parent compound with appropriate derivatizing agents. In this way, chiral auxiliaries for asymmetric couplings [2], as well as systems for the enantioselective extraction of aminoacids [3] or molecular tweezers [4,5] have been obtained.

More recently attention has been paid to the use of bile acids in enantioselective HPLC: both underivatised [6]and derivatised [7] bile acids have been linked to silica gel and used as chiral stationary phases (CSPs) for the HPLC resolution of racemic compounds. In a different approach, aimed at achieving biselector chiral stationary phases (CSPs), we addressed our attention toward the use of bile acids, as chiral scaffolds for obtaining this kind of CSPs. These natural products seems very suitable to this end because their hydroxy groups can be selectively derivatised allowing us to introduce on the cholestanic backbone aromatic moieties having different π-character. The presence of aromatic moieties having both π-donor and π-acceptor character makes possible, at least in principle, the π−π interaction of the chiral selector with a large range of racemic substrates [8].

To verify this hypothesis the hydroxy groups of deoxycholic and cholic acids were derivatised with different arylisocyanates and the carboxyl group present on the side chain was used for linking the bile acid derivatives to silica gel [9,10]. In addition, derivatives of deoxycholic acid containing both arylcarbamate and arylamide moieties having different π-character were synthesised and linked to silica gel [11].

Four different families of CSPs were obtained, having the structures reported in Fig. 1.

We present here the results obtained using the different bile acid based CSPs in the HPLC resolution of the racemic

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doi:10.1016/j.chroma.2003.10.135

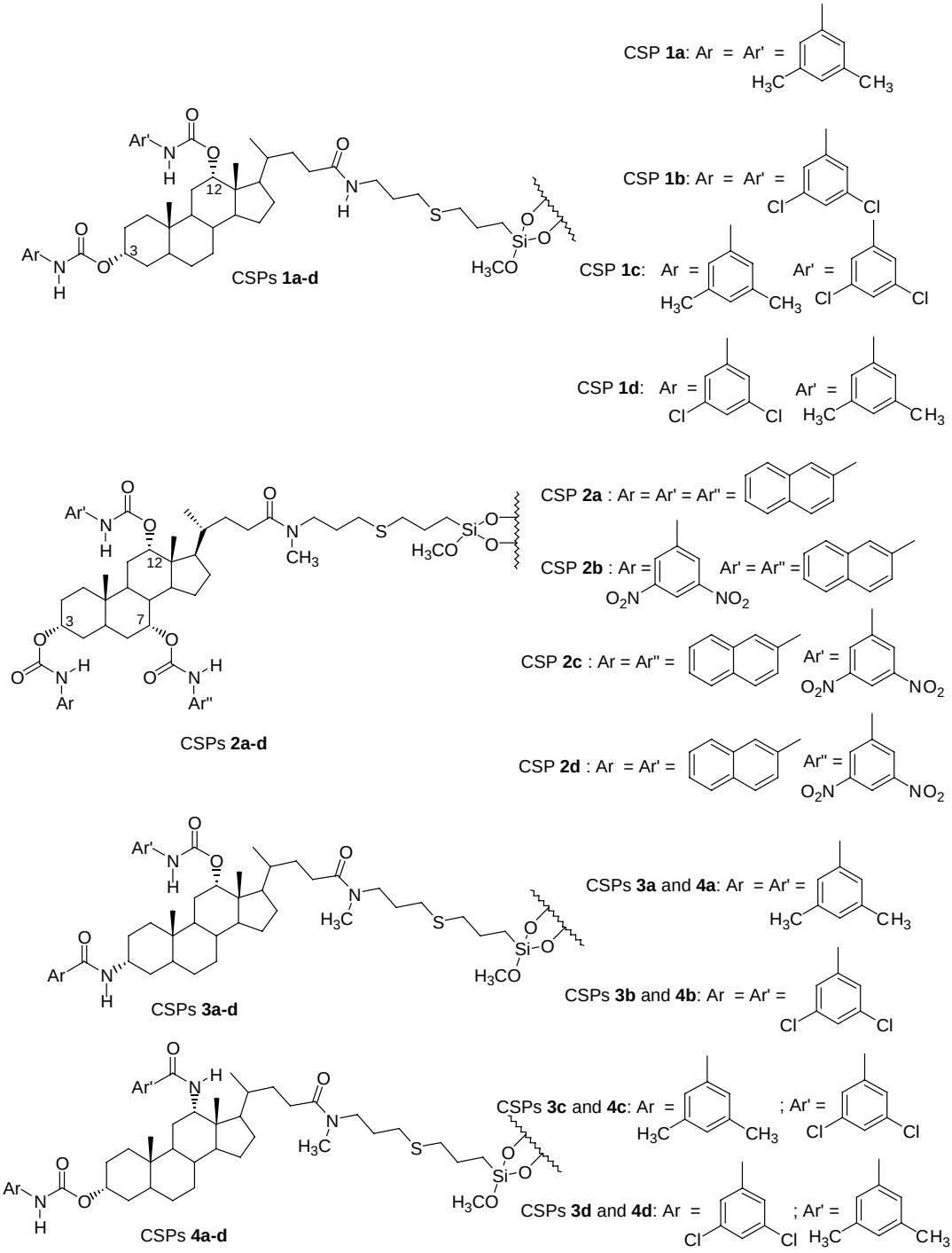


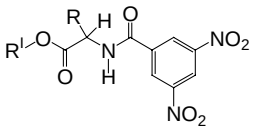
Fig. 1. CSP structures.

compounds reported in Fig. 2, focusing the attention on the capability of the mixed systems to work as biselector CSPs. The enantioresolution properties (α values and elution orders) toward selected racemic compounds of the four families of CSPs will be compared to gain some insight about likeness and differences of the enantiorecognition capability exhibited by the different bile acid based selectors.

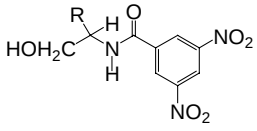
2. Experimental

2.1. Analysis

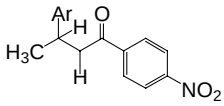
Liquid chromatography was carried out using a Jasco PU 980 pump equipped with a Jasco 975 UV detector and a Jasco 1595 CD detector.



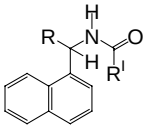
5a: R = benzyl, R^I = methyl
5b: R = isopropyl, R^I = methyl
5c: R = isopropyl, R^I = isopropyl
5d: R = methyl, R^I = methyl
5e: R = tert-butyl, R^I = methyl
5f: R = phenyl, R^I = methyl
5g: R = isopropyl, R^I = n-butyl
5h: R = isobutyl, R' = methyl
5i: R = sec-butyl, R' = methyl



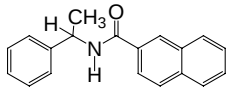
6a: R = phenyl
6b: R = isopropyl



7a: Ar = phenyl
7b: Ar = 1-naphthyl



8a: R = cyclohexyl, R^I = Phenyl
8b: R = methyl, R^I = phenyl
8c: R = methyl, R^I = 2-naphthyl
8d: R = cyclohexyl, R^I = t-butyl



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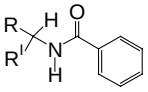
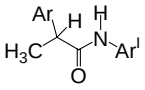
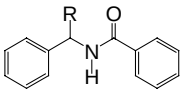


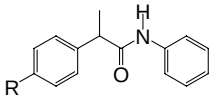
10a: R = methyl, R^I = COOMe
10b: R = i-propyl, R^I = COOMe
10c: R = methyl, R^I = CN



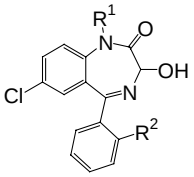
11a: Ar = 4-isopropylphenyl, Ar^I = 4-methoxyphenyl
11b: Ar = 2-(7methoxy)naphthyl, Ar^I = phenyl
11c: Ar = 4-isopropylphenyl, Ar^I = phenyl



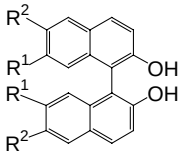
12a: R= i-Pr;
12b: R= t-Bu



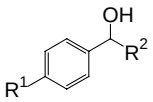
13a: R=H;
13b: R=COPh



16a: R^1=CH_3; R^2=H;
16b: R^1=CH_3; R^2=Cl;
16c: R^1=H; R^2=H;
16d: R^1=H; R^2=Cl;



14a: R^1=R^2=H;
14b: R^1=$CH_2CH{=}CH_2$; R^2=H;
14c: R^1=H, R^2=Br;



15a: R^1=H; R^2= CH_3;
15b: R^1=$N(CH_3)_2$; R^2=Bu^t;
15c: R^1=$N(CH_3)_2$; R^2=Cy;

Fig. 2. Compounds separated.

2.2. Materials

CSPs **1**–**4** were prepared as described elsewhere [9–11].

3. Results and discussion

3.1. Use of CSPs *1a–d*

Table 1 reports on the chromatographic resolution of electronpoor racemic compounds upon CSPs **1a**–**d**.

CSP **1a**, having π-basic character due to the presence of two electronrich aromatic substituents on the cholestanic backbone, resolves the π-acidic racemic analytes except compounds **5b** and **5d** (entries 2 and 4). The structural characteristics which contribute to the resolution of the aminoacid derivatives are the presence of an aromatic group, as in the case of phenylalanine and phenylglycine derivatives, and the nature of the ester group. In fact, CSP **1a** does not resolve the methylester of valine derivative **5b** (entry 2), whereas is able to separate the enantiomers of the corresponding isopropyl and butyl esters (entries 3 and 7). CSP **1b**, possessing two π-acidic aromatic groups, shows worse enantiodiscriminating properties toward this class of racemic compounds, being able to resolve only the paranitrobenzamides **7a**–**b** with the same enantioselectivity factors as CSP **1a** (entries 9 and 10), and showing little separations in the case of three aminoacid derivatives (entries 3, 5 and 7). The mixed CSP **1c**, which possess a π-basic aromatic group at the position 3 of the cholestanic backbone and a π-acidic moiety at the position 12, shows enantioresolution capability only toward the paranitrobenzamides **7a**–**b** (entries 9 and 10). On the contrary, CSP **1d**, having a π-basic group at the position 12 and a π-acidic moiety at the position 3 of the cholestanic backbone, results very efficient in the resolution of π-acidic racemic compounds. The enantiodiscriminating capability of CSP **1d** is better than CSP **1a**, although this last possesses two π-basic aromatic moieties. Not only CSP **1d** resolves all the π-acidic compounds listed in Table 1, but also, in several cases, the enantioselectivity factors are higher than those obtained using CSP **1a** (entries 2–5 and 7).

The chromatographic results obtained in the separation of the enantiomers of π-basic analytes are reported in Table 2.

Perusal of this table shows that CSPs **1a**–**d** are able to resolve this class of racemates. The omoderivatised CSPs show a behaviour depending on the electronic character of their aromatic substituents: CSP **1b** possessing two π-acceptor aromatic groups affords better separations than CSP **1a**, which have two π-donor aromatic groups. This suggests that the π−π interaction play an important role in the enantiorecognition process of π-donor racemic analytes. CSP **1b** affords the best separation when the racemic compounds possess a naphthalene moiety (entries 1–4 and 10) which can enlarge the chiral cavity, because it is longer than the phenyl group, and extend the possibility of π−π interaction because of its stronger π-donor character. CSP **1c** shows, as expected because of the presence of only one π-acidic aromatic moiety, a lower enantioselectivity than CSP **1b** in the resolution of the compounds listed in Table 2; in addition, its enantiodiscriminating capability results comparable or, in some cases (entries 1–3), inferior with respect to CSP **1a**. On the contrary, CSP **1d** separates the enantiomers of all the compounds listed in Table 2. The values of the enantioselectivity factors are intermediate between those obtained with CSP **1b**, and those obtained with CSP **1a**: anyway, baseline separation of the chromatographic peaks is obtained in all the cases (Fig. 3).

Therefore, the introduction of a 3,5-dimethylphenylcarbamate at position 12 and a 3,5-dichlorophenylcarbamate at position 3 of the cholestanic backbone allows to obtain a biselector system, capable, once linked to silica gel, to

Table 1
Chromatographic resolution[a] of π-acidic racemic compounds upon CSPs **1a**–**d**

Entry	Compound	CSP **1a**		CSP **1b**		CSP **1c**		CSP **1d**		Eluent[d]
		K'[b]	α[c]	K'[b]	α[c]	K'[b]	α[c]	K'[b]	α[c]	
1	**5a**	12.10	1.16	3.03	1.00	9.25	1.00	3.29	1.14	A
2	**5b**	9.19	1.00	2.58[e]	1.00	7.42	1.00	7.15	1.09	A
3	**5c**	5.09	1.10	1.51[e]	1.07	4.56	1.00	3.98	1.20	A
4	**5d**	3.32[e]	1.00	6.11[e]	1.00	12.40	1.00	10.45	1.11	A
5	**5e**	6.67	1.06	1.76[e]	1.07	5.29	1.00	5.04	1.13	A
6	**5f**	12.31	1.16	3.23[e]	1.00	10.38	1.00	9.81	1.11	A
7	**5g**	5.57	1.09	4.61	1.06	4.84	1.00	4.72	1.20	A
8	**6a**	3.00	1.09	2.43	1.00	2.74	1.00	2.95	1.09	C
9	**7a**	3.46	1.11	3.80	1.10	3.01	1.08	3.08	1.11	B
10	**7b**	1.92	1.21	4.65	1.21	3.23	1.10	2.10	1.20	B

[a] Chromatographic conditions: UV detection (λ = 254 nm), $T = 25\,^{\circ}C$.
[b] Retention factor of the first eluted enantiomer.
[c] Enantioselectivity factor.
[d] A: hexane–dichloromethane–propan-2-ol (85:15:1); B: hexane–dichloromethane–propan-2-ol (70:30:1); C: hexane–dichloromethane–propan-2-ol (75:20:5).
[e] Eluent B.

Table 2
Chromatographic resolution[a] of π-basic racemic compounds upon CSPs **1a–d**

Entry	Compound	CSP **1a**		CSP **1b**		CSP **1c**		CSP **1d**		Eluent[d]
		K'[b]	α[c]	K'[b]	α[c]	K'[b]	α[c]	K'[b]	α[c]	
1	**8a**	2.09	1.12	7.09[e]	1.45	3.48	1.12	5.34	1.29	A
2	**8b**	3.09	1.15	5.14[e]	1.42	4.69	1.10	8.60	1.18	A
3	**8c**	4.27	1.13	5.32[e]	1.58	5.88	1.10	7.26	1.24	A
4	**8d**	1.14	1.00	13.52[e]	1.34	1.72	1.00	5.14	1.07	A
5	**9**	3.94	1.17	4.37[e]	1.13	5.06	1.16	5.84	1.15	A
6	**10a**	3.39	1.08	6.48	1.24	5.18	1.10	4.87	1.18	A
7	**10b**	1.15	1.07	1.46[e]	1.24	1.66	1.12	1.85	1.16	A
8	**10c**	3.30	1.00	8.05	1.11	8.32[f]	1.00	7.38	1.09	B
9	**11a**	2.59	1.09	7.19	1.23	3.18	1.08	4.05	1.14	A
10	**11b**	3.29	1.09	9.90	1.42	5.20	1.11	5.42	1.23	A
11	**11c**	1.07	1.09	2.92	1.21	1.52	1.07	1.56	1.13	A

[a] Chromatographic conditions: UV detection ($\lambda = 254$ nm), $T = 25\,^{\circ}$C.
[b] Retention factor of the first eluted enantiomer.
[c] Enantioselectivity factor.
[d] A: hexane–dichloromethane–propan-2-ol (85:15:1) and B: hexane–dichloromethane–propan-2-ol (70:30:1).
[e] Eluent B.
[f] Eluent A.

separate the enantiomers of both π-acceptor and π-donor racemic compounds.

3.2. Use of CSPs *2a–d*

CSPs **2a–d**, obtained by derivatization of cholic acid, possess three arylcarbamoyl substituents having stronger π-donor (naphthyl) and π-acceptor (3,5-dinitrophenyl) character. Since cholic and deoxycholic acid have the same absolute configuration at the stereogenic centers, the higher substitution degree together with the stronger π-donor and π-acceptor character of the substituents should afford better enantiodiscriminating capabilities with respect to CSPs **1a–d**. The chromatographic results obtained using CSPs **2a–d** in the HPLC resolution of both π-acceptor and π-donor racemic compounds are reported in Table 3.

Perusal of the table gives an immediate idea about the differences between this family of CSPs and CSPs **1a–d**: CSPs **2a–d** are able to resolve racemic compounds having different structure with respect to those enantiodiscriminated by CSPs **1a–d**: only the enantiomers of compounds **5a–e**, **6b** and **7a–b** (entries 1–4 and 8–10), which are resolved by CSPs **1a–d**, are separated also by CSPs **2a–d**.

As observed in the case of CSPs **1a–d**, also the enantioseparations upon CSPs **2a–d** depend on the electronic nature of the arylcarbamate substituents on the cholestanic backbone. CSP **2a**, possessing three π-donor 2-naphthylcarbamate groups is able to resolve only π-acceptor racemic analytes, with enantioselectivities depending on the racemate structure. The best resolved compounds are the aminoalcohol derivatives **6a–b**. The enantioselectivity factors are in general higher than those observed in the resolution of the

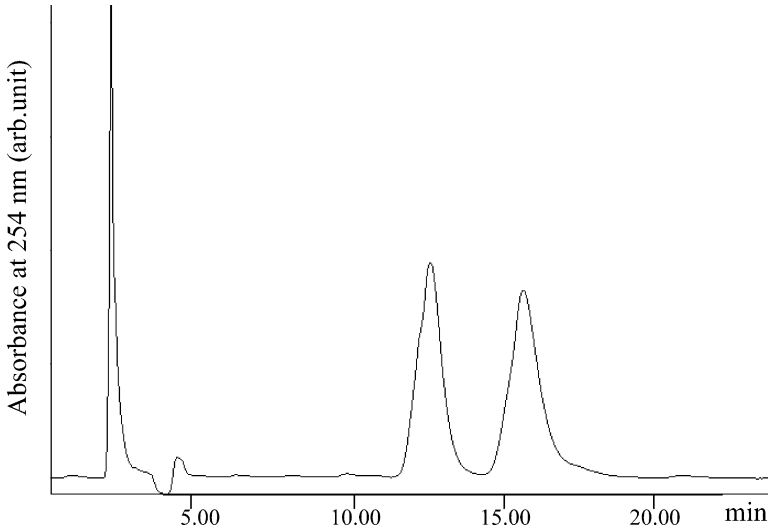


Fig. 3. Chromatographic resolution of compound **8a** upon CSP **1d**: for chromatographic conditions see Table 2.

Table 3
Chromatographic resolution[a] of racemic compounds upon CSPs **2a–d**

Entry	Compound	CSP **2a**		CSP **2b**		CSP **2c**		CSP **2d**		Eluent[d]
		k'[b]	α[c]	k'[b]	α[c]	K'[b]	α[c]	k'[b]	α[c]	
1	**5a**	3.93	1.08	3.85	1	4.87	1	3.48	1	A
2	**5b**	4.38	1.09	4.52	1	4.87	1	3.48	1	A
3	**5d**	7.35	1.12	7.66	1	7.96	1.18	6.05	1	A
4	**5e**	5.51	1.08	3.25	1	3.72	1	2.61	1	A
5	**5h**	4.32	1.11	4.56	1	4.47	1.03	3.46	1.05	A
6	**5I**	3.83	1.08	4.00	1	4.32	1	3.33	1	A
7	**6a**	8.85	1.22	8.08	1.20	10.18	1.20	13.40[e]	1.66	B
8	**6b**	7.50	1.15	5.63	1	6.77	1	10.75[e]	1	B
9	**7a**	3.55[f]	1.05	3.26	1	3.04	1.07	9.84[g]	1.06	A
10	**7b**	3.95	1.16	5.07	1	3.62[e]	1.06	13.10[g]	1.08	C
11	**12a**	–	–	3.76	1.09	3.06	1.09	3.08	1.12	D
12	**12b**	–	–	5.11	1.05	1.98[h]	1.06	3.97	1.12	E
13	**13a**	–	–	3.79	1.08	3.69	1.15	3.39	1.06	D
14	**13b**			3.82	1.25	6.59	1.04	10.93	1.08	D
15	**14a**			1.53	1	1.36	1.18	1.16	1	A
16	**14b**			1.22	1	1.06	1.40	–	–	A
17	**14c**			2.66	1	2.52	1.09	2.04	1	A
18	**15a**			3.82	1	3.70	1.04	3.87	1	E
19	**15b**			2.17	1	1.76	1.06	2.13	1	E
20	**15c**			3.69	1	3.07	1.11	3.52	1	E

[a] Chromatographic conditions: UV detection ($\lambda = 254$ nm), $T = 25\,^{\circ}$C.
[b] Retention factor of the first eluted enantiomer.
[c] Enantioselectivity factor.
[d] A: hexane–dichloromethane–propan-2-ol (70:30:5); B: hexane–dichloromethane–propan-2-ol (70:30:7); C: hexane–dichloromethane–propan-2-ol (70:30:3); D: hexane–dichloromethane–propan-2-ol (80:20:1) and E: hexane–dichloromethane–propan-2-ol (90:10:1).
[e] Eluent A.
[f] Eluent C.
[g] Eluent F (hexane–dichloromethane–propan-2-ol (90:10:5)).
[h] Eluent D.

π-acceptor racemic compounds upon CSP **1a**: it is to note that CSP **2a** is able to resolve π-acceptor racemates, like **5b**, **6b** and **5d**, which are not enantiodiscriminated by CSP **1a**. These results suggest that the introduction of three naphthyl substituents on the cholestanic backbone, having a stronger π-donor character than the 3,5-dimethylphenyl moieties, affords a more enantioselective CSP towards this class of racemic compounds. The enantiodiscriminating capabilities of the mixed CSPs **2b–d** depend on the arrangement of the different arylcarbamoyl substituents on the cholestanic backbone, as already observed in the case of the mixed CSPs **1c–d**. CSP **2b** is able to resolve only compound **6a**, among the π-acceptor analytes, whereas both CSP **2c** and **2d** separate also the enantiomers of the 4-nitrobenzamides **7a–b** with very similar enantioselectivities (entries 9–10) and some aminoacid derivatives. It is to note that CSP **2c** affords the best resolution of **5d** (entry 6), whereas CSP **2d** separates the enantiomers of **6a** with the highest enantioselectivity factor (entry 7). As far as the resolution of π-donor racemic compounds is concerned, the three mixed CSPs are able to separate the enantiomers of the four derivatised racemic compounds **12a–b** and **13a–b** (entries 11–14). A general trend cannot be found, as CSP **2d** results more efficient in the resolution of **12a–b**, whereas the compounds well resolved upon CSPs **2b** and **2c** are **13b** (entry 14) and **13a** (entry 13), respectively. CSP **2c** is able to resolve also underivatised racemic compounds, like binaphthols **14**, and alkylarylcarbinols **15**: this CSP results very efficient especially in the resolution of binaphthols affording good chromatographic separations (Fig. 4).

The differences in the enantioresolution properties of CSPs **2a–d** cannot be attributed to differences in the molecular conformation of the four selectors. In fact, a combined circular dichroism–molecular mechanics study has demonstrated that the conformation of the four selectors is very similar and does not depend on the arrangement of the different arylcarbamoyl groups on the cholestanic backbone [12]. Therefore, the different enantiodiscriminating characteristics of the four selectors are likely due to the different stereochemical environment of the three arylcarbamoyl substituted positions. The results obtained in the chromatographic resolution of π-basic racemic compounds suggests that the position 12 possesses the most favourable stereochemical environment: in fact, CSP **2c**, possessing a 3,5-dinitrophenylcarbamoyl moiety at this position affords not only the best resolution of derivatised compounds but also is able to separate the enantiomers of underivatised π-basic racemates (entries 15–20). The highest α value obtained in the resolution of **6b** upon CSP **2d** can be explained, taking into account that this selector possesses a π-basic

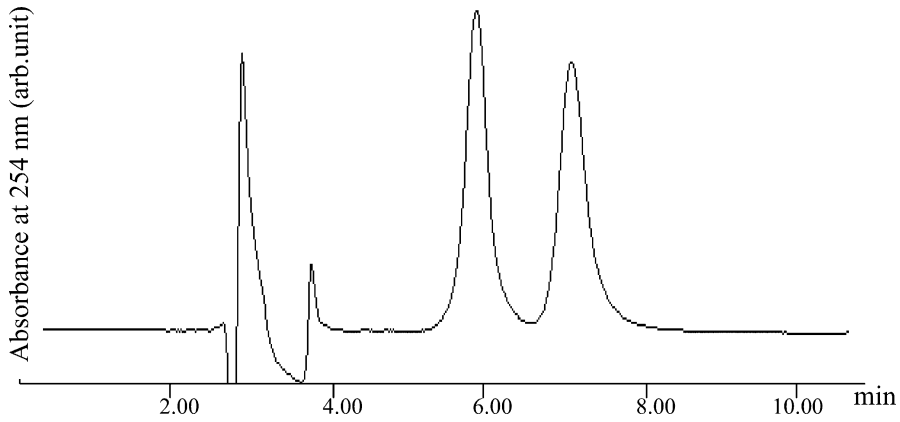


Fig. 4. Chromatographic resolution of compound **14b** upon CSP **2c**: for chromatographic conditions see Table 3.

2-naphthylcarbamoyl moiety at the position 12 and is devoid of the same group at the position 7. This arrangement results remarkably favourable to the enantiodiscrimination of this π-acidic racemate because not only the π-basic moiety is on most enantioselective position, but also the two sterically demanding 2-naphthylcarbamoyl groups are farther than in CSPs **2a** and **2b**, as demonstrated in the previous study [12]. Therefore, the substrate can go deeply into the chiral cavity [13] of the substituted cholestanic system and this allows a stronger enantioselective interaction with the arylcarbamate groups directed toward the inner of the cavity [12].

*3.3. Use of CSPs **3a–d** and **4a–d** and comparison among all the CSPs*

The replacement of an arylcarbamoyl group of CSPs **1a–d** with an arylamide moiety gives rise to CSPs **3a–d** or CSPs **4a–d** depending on the position where the arylamide group is placed on the cholestanic backbone. This structural change affords less versatile CSPs than the previous ones: these CSPs are able to resolve only benzodiazepines **16**. Since the enantiomers of this class of racemic compounds are separated also by CSPs **1a–d** and **2a–d**, we can use the chromatographic results obtained in the HPLC resolution of benzodiazepines **16** for comparing the enantiorecognition properties of the four families of CSPs. These results are reported in Table 4.

CSPs **1a–d** are able to resolve benzodiazepines **16** with enantioselectivity factors depending on the racemic substrate as well as the electronic nature of the arylcarbamoyl substituents of the CSPs and their arrangement on the cholestanic backbone (entries 1–4). As far as the racemate structure is concerned, the best enantioselectivity factors are observed always in the resolution of **16b**, whereas **16d** possesses structural features which afford poorer separations. The presence of π-acceptor arylcarbamoyl substituents on the cholestanic backbone seems more favourable to the HPLC resolution of benzodiazepines: CSP **1b** (entry 2) affords better separation than CSP **1a** (entry 1). The two mixed CSPs behave in a rather different manner: CSP **1c** affords the lowest enantioselectivity factors in the resolution of all the benzodiazepines (entry 3), whereas CSP **1d**, in which the position of the two different arylcarbamoyl substituents is exchanged with respect to CSP **1c**, gives the best results in terms of enantioseparations (entry 4). These results indicates that the two electronically different substituents are in a matched relationship in CSPs **1d**, affording better enantioseparations of the racemic compounds, whereas they are mismatched when arranged as in CSP **1c**. When the arylcarbamoyl group at position 3 of the cholestanic backbone is replaced by an arylamide moiety CSPs **3a–d** are obtained, which are able to resolve benzodiazepine **16** in some cases even better than CSPs **1a–d**. The behaviour towards this kind of racemates is similar to that observed with CSPs **1a–d**: again, the best resolved compound is **16b** upon all the CSPs. As far as the dependence of the enantiodiscriminating capabilities of the CSPs on the arrangement of the different substituents on the cholestanic backbone is concerned, CSP **3b**, which possesses two π-acceptor aromatic moieties affords the best resolutions (entry 6). The replacement of the arylcarbamate moiety with an arylamide group at position 12 of the cholestanic skeleton affords less enantioselective CSPs (entries 9–12): only **16b** is resolved by all the CSPs of this family, whereas the enantiomers of **16d** are never separated. Therefore, although CSPs **4a–d** show lower enantiodiscriminating capabilities than those exhibited by CSPs **3a–d**, the trend in the resolution of **16** is the same and also within these CSPs the best phase results that bearing two 3,5-dichlorophenyl moieties on the cholestanic backbone (entry 10). The elution orders of racemates **16** is the same upon CSPs **1a–d**, CSPs **3a–d** and CSPs **4a–d** (Table 4): this, together with the similar trend in the resolution of **16a–d**, suggests that a very similar enantiorecognition mechanism acts when these compounds are eluted upon the three families of CSPs. Therefore, the differences found in the enantiodiscriminating properties of the phases are probably due only to more or less favourable arrangement of the different substituents to the chiral recognition of benzodiazepines.

A different situation is found in the enantioseparation of **16a–d** upon CSPs **2a–d** (entries 13–16). There is not a ben-

Table 4
chromatographic resolution[a] of benzodiazepines **16**

Entry	CSP	**16a**		**16b**		**16c**		**16d**		Eluent[e]
		k'[b]	α[c] (o.e.)[d]	k'[b]	α[c] (o.e.)[d]	k'[b]	α[c] (o.e.)[d]	k'[b]	α[c] (o.e.)[d]	
1	**1a**	1.06	1.31 (−)	1.08	1.43 (−)	3.84	1.16 (−)	4.09	1.11 (−)	A
2	**1b**	1.99	1.40 (−)	1.98	1.69 (−)	4.93	1.27 (−)	5.25	1.17 (−)	A
3	**1c**	1.59	1.21 (−)	1.65	1.37 (−)	5.28	1.11 (−)	5.56	1.03 (−)	A
4	**1d**	1.65	1.47 (−)	1.67	1.77 (−)	4.89	1.30 (−)	5.03	1.21 (−)	A
5	**3a**	2.02	1.00	2.01	1.25 (−)	8.51	1.16 (−)	8.08	1.00	A
6	**3b**	3.18	2.00 (−)	2.22	2.67 (−)	9.25	1.71 (−)	8.08	1.51 (−)	A
7	**3c**	1.95	1.13 (−)	2.35	1.39 (−)	10.58	1.12 (−)	10.39	1.00	A
8	**3d**	1.93	1.48 (−)	2.03	2.04 (−)	7.69	1.57 (−)	7.97	1.41 (−)	A
9	**4a**	2.09	1.00	2.15	1.00	11.73	1.00	13.00	1.00	A
10	**4b**	2.89	1.39 (−)	2.83	2.28 (−)	10.13	1.33 (−)	11.28	1.00	A
11	**4c**	2.51	1.24 (−)	2.37	1.28 (−)	9.41	1.00	10.35	1.00	A
12	**4d**	2.34	1.00	2.32	1.17 (−)	10.41	1.00	10.31	1.00	A
13	**2a**	5.62	1.11 (+)	4.64	1.10 (+)	4.99	1.13 (+)	6.31	1.20 (+)	B
14	**2b**	5.37	1.17 (+)	6.35	1.10 (+)	10.65	1	10.28	1.12 (+)	B
15	**2c**	5.57	1	5.74	1.08 (+)	7.71	1.06 (+)	7.35	1.16 (+)	B
16	**2d**	4.44	1.40 (+)	5.09	1.30 (+)	9.11	1.21 (+)	8.34	1.23 (+)	B

[a] Chromatographic conditions: UV detection ($\lambda = 254$ nm), flow 1 ml/min, $T = 25\,^{\circ}$C.
[b] Retention factor of the first eluted enantiomer.
[c] Enantioselectivity factor.
[d] Sign of the circular dichroism at 254 nm of the first eluted enantiomer.
[e] A: hexane–dichloromethane–propan-2-ol (70:30:3) and B: hexane–dichloromethane–propan-2-ol (70:30:5).

zodiazepine which results best resolved upon all the four phases: in fact, the resolution of **16d** is better upon CSPs **2a** and **2c**, whereas **16a** is better resolved upon CSPs **2b** and **2c**. In general, the enantioselectivity factors are lower than those observed upon CSPs **1a**–**d** and **3a**–**d**: only CSP **2d** affords enantioselectivities comparable to those obtained with CSPs **1a**–**d** (entry16). However, the most remarkable difference is the inversion of the elution orders observed upon CSPs **2a**–**d** with respect to that found upon the other phases. This difference points out that CSPs **2a**–**d** undergo a different enantiorecognition mechanism in the chromatographic resolution of **16** with respect the deoxycholic derived CSPs, attributable to the different dervatisation of the cholestanic system, as well as to the presence of a third arylcarbamoyl substituent on the position 7 of the cholestanic backbone.

4. Conclusions

The different derivatisation of cholic and deoxycholic acids afford chiral selectors that once linked to silica gel give rise to CSPs having enantiodiscriminating properties which depend on the type of derivatisation. The introduction upon the deoxycholic backbone of 3,5-dichlorophenylcarbamate or 3,5-dimethylphenylcarbamate moieties affords CSPs able to resolve, respectively, π-basic or π-acidic derivatised racemic compounds. The introduction of the two different moieties affords mixed CSPs, whose enantiodiscriminating capabilities depend on the arrangement of these moieties on the cholestanic backbone: the matched relationship between the two groups gives rise to a CSP able to separate the enantiomers of both π-acidic and π-basic analytes. The replacement of an arylcarbamate moiety with an arylamide group gives CSPs showing lower versatility, being able to resolve only a class of racemates. The introduction on the cholic acid scaffold of 2-naphthylcarbamate and 3,5-dinitrophenylcarbamate groups afford CSPs which resolve both π-donor and π-acceptor racemic compounds with enantioselectivities which, again, depend on the arrangement of these aromatic moieties on the cholestanic backbone. The matched disposition of these groups afforded a CSP able to resolve even some underivatised racemic compounds. The comparison among the different CSPs allowed us to find similarity and differences in their enantiorecognition mechanism. In particular, the differences in the elution orders of benzodiazepines pointed out a difference in the chiral recognition mechanism exhibited by the different families of bile acid derived CSPs. Our results suggest that arylcarbamoyl derivatives of bile acids can be successfully used as chiral selectors in enantioselective chromatography. Since their enantiodiscriminating properties depend on the type of derivatisation, by changing isocyanate new and more efficient selectors can be found, suitable for the HPLC resolution of different racemic compounds.

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