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Effect of the presence of a free hydroxyl group on the enantiodiscrimination properties of cholic acid based CSPs bearing 2-naphthylcarbamate and 3,5-dinitrophenylcarbamate moieties in the HPLC resolution of racemic compounds

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Abstract—Two new chiral selectors, obtained by derivatizing two of the three hydroxyl groups of cholic acid with 2-naphthylisocyanate and 3,5-dinitrophenylisocyanate, have been prepared and linked to silica gel to obtain chiral stationary phases (CSPs) for the HPLC separation of enantiomers. The enantiodiscriminating capability of the two CSPs has been compared to that of the analogous CSP obtained from an exhaustively derivatized cholic acid based selector, in order to establish the effect of the presence of a free hydroxyl group on the enantiodiscrimination properties of this kind of selector. The chromatographic results demonstrate that the enantioselectivity of these selectors strongly depends on the position of the hydroxyl group on the cholestanic backbone.

1. Introduction

The preparation of broad spectrum independent chiral stationary phases (CSPs) is an attractive goal for scientists interested in enantioselective HPLC because in these phases the characteristics of the independent CSPs[1] (known interaction mode with the substrates, predictability of resolution) are coupled to wide applicability. For this reason efforts were made to find structural features that would guarantee efficiency and versatility to a chromatographic selector.[2]

Over the last few years, we have demonstrated that the use of bile acid derivatives, which possess aromatic units having different electronic characters, allows us to obtain CSPs showing both efficiency and versatility.[3] In fact, by linking to silica gel a deoxycholic acid derivative, which had a 3,5-dimethylphenylcarbamoyl moiety (π-basic) and a 3,5-dichlorophenylcarbamoyl group (π-acid), a CSP able to separate by HPLC both π-acid and π-basic racemic compounds with good enantioselectivity factors, was obtained.[4]

Even better results were obtained by linking to silica gel, a cholic acid derivative possessing 2-naphthylcarbamoyl groups as π-basic moiety and a 3,5-dinitrophenylcarbamoyl unit as π-acid moiety.[5] Both the efficiency and versatility of these CSPs were dependent not only on the nature of the moieties introduced but also on their relative position on the cholestanic skeleton.[3] Indeed the CSP, which afforded the best results was CSP **1** (Fig. 1) obtained by linking to silica gel the cholic acid derivative possessing 2-naphthylcarbamoyl moieties at the 3- and 7-position and 3,5-dinitrophenyl group at the 12-position of the cholestanic backbone.[5]

This CSP was able to resolve by HPLC π-acid and π-basic racemic compounds and also some underivatized racemates, such as binaphthols and alkylarylcarbinols.[5] These encouraging results prompted us to improve further the enantiodiscriminating properties of this kind of CSP by changing some structural characteristics of the cholic acid derived selector and, hence, we became interested in checking the effect of the presence of a free hydroxyl group. Taking into account that the versatility of CSP **1** is attributable to the presence of electronically different aromatic groups and that the best relative disposition of these groups on the cholic acid skeleton is that one where 3,5-dinitrophenylcarbamoyl moiety is

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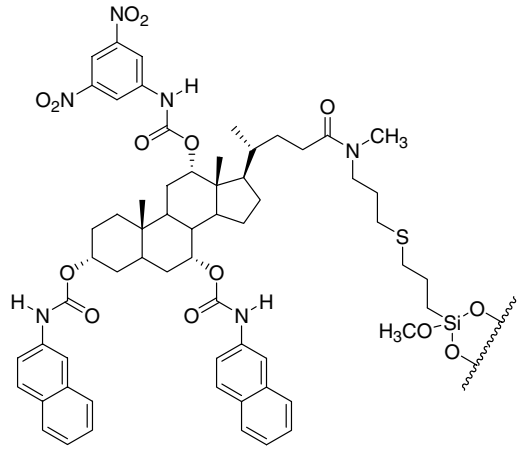


Figure 1. Structure of CSP **1**.

located at the 12-position, two parent CSPs having a free hydroxyl group can be obtained (Fig. 2).

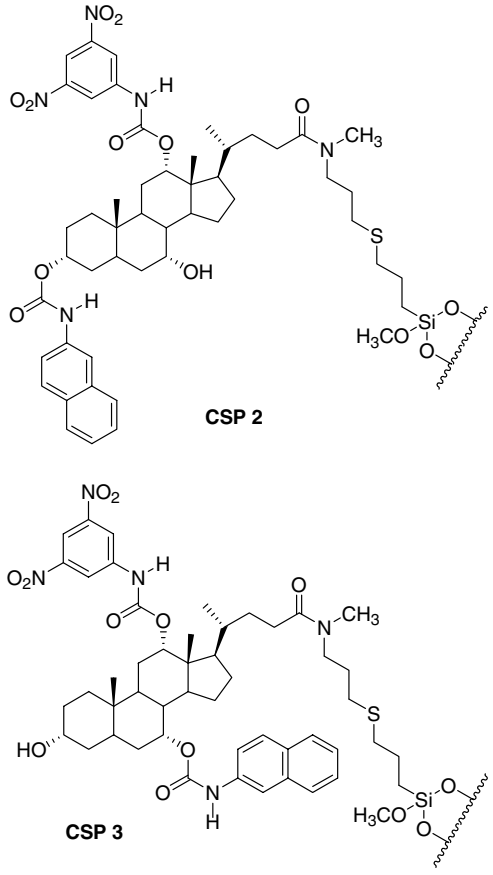


Figure 2. Structure of CSPs **2** and **3**.

Due to the different arrangement of the substituent groups on the cholestanic backbone, these two CSPs can behave differently as far as both efficiency and versatility are concerned.

Therefore, the synthesis of these CSPs and their use in the HPLC resolution of racemic compounds will not only allow us to shed light on the effect of a free hydroxyl group on the enantiodiscrimination properties of the cholic acid derived CSP **1**, but also to establish what is the best arrangement of the functional groups to achieve good enantioselectivity.

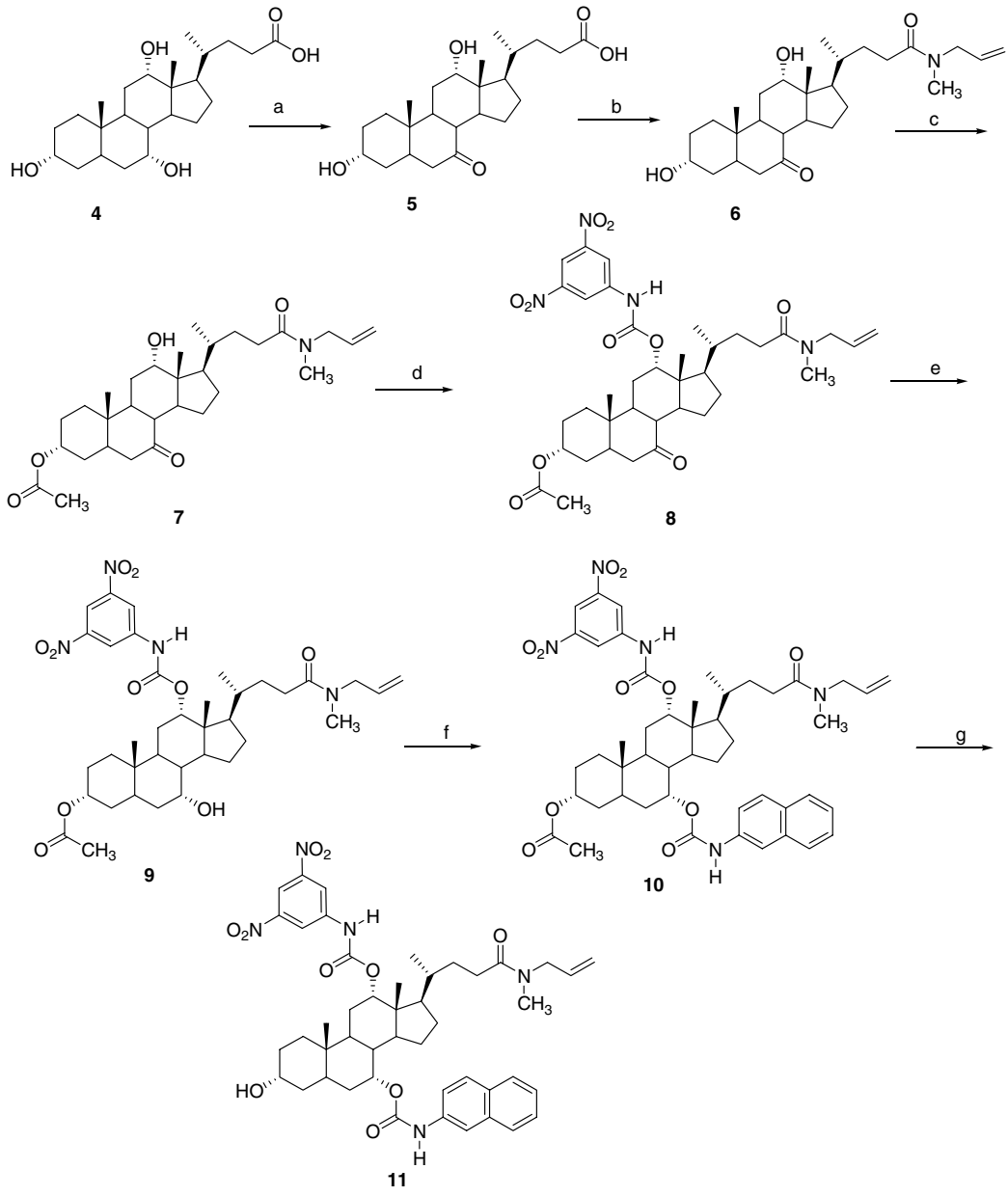
2. Results and discussion

2.1. Synthesis of the CSPs

The cholic acid based selector of CSP **2** was synthesized as previously described,[5] whereas the synthetic route to selector **11**, from which CSP **3** can be prepared, is summarized in Scheme 1. Due to the introduction of two different arylcarbamoyl moieties at the 7- and 12-position of the cholestanic backbone, selective protection of the 3 and 7 hydroxy groups of the cholic acid was mandatory. Since the hydroxy group at the 3-position of the cholestanic skeleton, which is equatorial is more reactive than the other two, which are axial, the selective protection of this group can be obtained easily because it will react preferentially with respect to the other hydroxyl groups with a great number of reactants. Selective protection of the 7-hydroxy group can be accomplished by means of its regioselective oxidation with NBS in alkaline solution,[6] because it is well known that the reduction, with $NaBH_4$, of the carbonyl function takes place with complete stereoselectivity,[7] the stereogenic centre being restored to the same absolute configuration as in cholic acid. Therefore, as shown in Scheme 1, **4** was reacted with NBS in a bicarbonate solution and ketoacid **5** was converted into the corresponding *N*-methylallylamide **6**, using the mixed anhydride method.[8]

The 3-hydroxyl group was protected as an acetate by reacting **6** with an excess of acetic anhydride at 80 °C.[9] The 3-acetyl derivative was obtained in 55% yield, after chromatographic purification, because conversion of the substrate in the acetylation reaction was not complete, even after prolonging the reaction time. The reaction of **7** with a 1.5-fold excess of 3,5-dinitrophenylisocyanate in refluxing toluene followed by reduction with $NaBH_4$ afforded **9** with complete stereoselectivity. In order to derivatize the hydroxyl group as a 2-naphthylcarbamate, **9** was reacted with 2 equiv of 2-naphthylisocyanate in refluxing toluene for 24 h. These reaction conditions allowed us to obtain compound **10** in 75% yield, after chromatographic purification. Selector **11** was eventually obtained by hydrolysis of the acetyl group by means of HCl in refluxing methanol.

Selectors **11** and **12** were covalently linked to silica gel, as described in Scheme 2, by means of reaction with a 5-fold excess of 3-mercaptopropyltrimethoxysilane in the presence of AIBN in refluxing $CHCl_3$.[10] Under these reaction conditions, the selectors were fully converted into the corresponding silane derivatives, which were separated from the excess of 3-mercaptopropyltrimethoxysilane simply by washing with pentane. The grafting to silica gel was carried out in toluene at reflux for 24 h and the derivatized silica gels, after being thoroughly washed and dried, were employed for packing stainless steel columns of 15 cm (internal diameter 4.6 mm).



Scheme 1. Reagents and conditions: (a) NBS, 0.37 M $NaHCO_3$, rt to 85 °C; (b) (1) Bu_3N, dioxane, 10 °C, (2) EtOCOCl, (3) *N*-methylallylamine, 10 °C to rt; (c) acetic anhydride, toluene 80 °C, 5 h; (d) 3,5-dinitrophenylisocyanate, refluxing toluene; (e) $NaBH_4$, THF/MeOH, 0 °C to rt; (f) 2-naphthylisocyanate, refluxing toluene; (g) MeOH/HCl, reflux, 1 h.

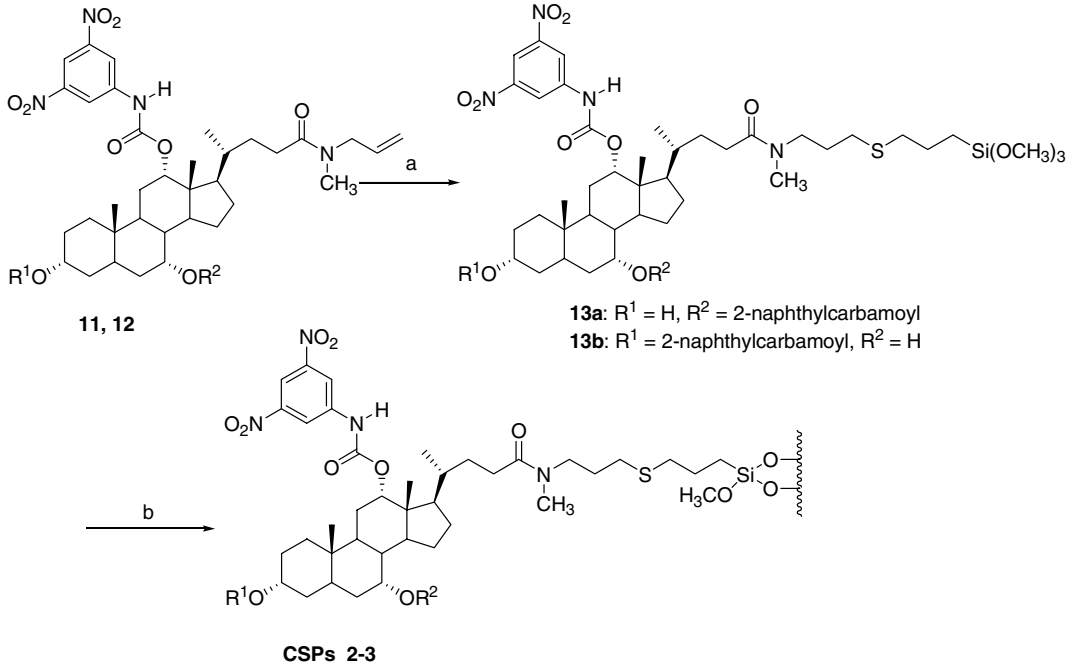
The amount of selector bonded to silica gel was determined by means of elemental analysis and results were comparable for both the CSPs.

2.2. Use of CSPs 2 and 3 in the chromatographic resolution of racemic compounds

The enantiodiscriminating capability of CSPs **2** and **3** was tested using the racemic compounds reported in Chart 1.

Some of these racemates were resolved by CSP **1**, so that a comparison of the enantioresolution properties of CSPs **2** and **3** with respect to those of CSP **1** could be easily made. Table 1 reports on the chromatographic results obtained using CSPs **2** and **3** in the HPLC separation of these racemic compounds compared with those obtained using CSP **1**.

CSP **2**, which possesses a free OH group at the 7-position of the cholestanic backbone, resolved, among the π-acid racemates (runs 1–5), only 4-nitrobenzamides **15a** and **15b** (runs 4 and 5). In contrast, CSP **3** was able to resolve the aminoacid derivative **14a** (run 1) not enantiodiscriminated by CSP **1** and compounds **14c** and **15a** with α values comparable to those obtained upon CSP **1**. These results suggest that the replacement of a 2-naphthylcarbamate moiety with a hydroxyl group results in worsening the enantiodiscriminating capabilities of this kind of selector towards π-acid racemic compounds, in



Scheme 2. Reagents and conditions: (a) 3-mercaptopropyltrimethoxysilane, AIBN, refluxing $CHCl_3$; (b) silica gel, refluxing toluene.

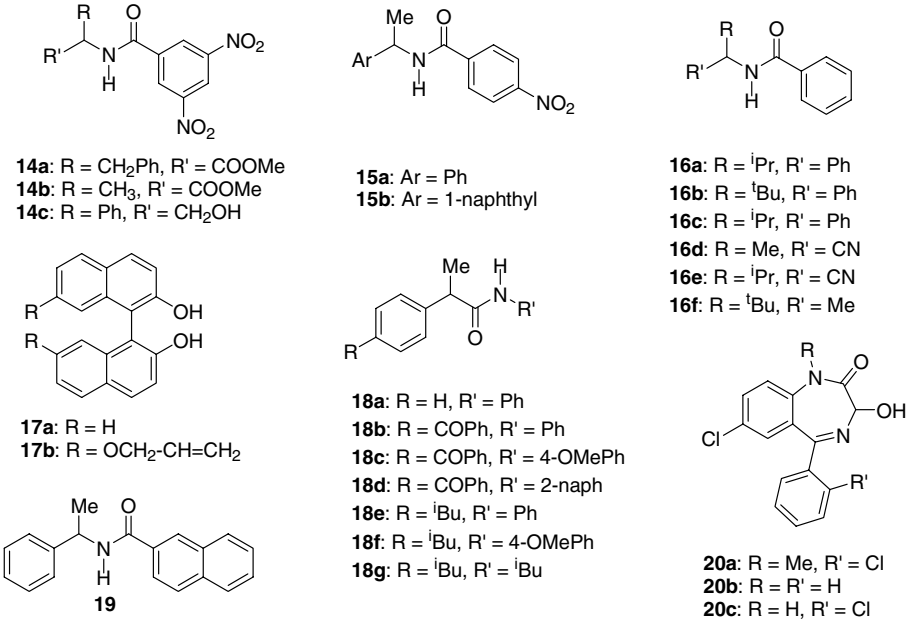


Chart 1.

particular when the OH is located at the 7-position. In fact in this case, 2-naphthylcarbamoyl moiety, responsible for the interaction with π-acid racemates, is linked to the less stereochemically demanding[11] 3-position and this most likely prevents an efficient enantioselective interaction with these racemic compounds. In contrast when the π-basic arylcarbamoyl group is linked at the 7-position, as in CSP **3**, π-acid racemic compounds bearing a phenyl group (runs 1, 3 and 4) were resolved, probably because the enantiodiscriminating capability of this selector takes advantage of the spatial proximity of the two different arylcarbamoyl moieties, which could allow the π-acid racemic compounds having these structural features to experience two contemporary π–π interactions (phenyl-3,5-dinitrophenylcarbamoyl and 3,5-dinitrophenyl-2-naphthylcarbamoyl).

Better results were obtained in the chromatographic resolution of π-basic racemic compounds. CSP **2** is able to resolve the racemates enantiodiscriminated by CSP **1** (runs 6–11) except for **16b** (run 7). However, the enantioselectivity factors are in general lower than those obtained with CSP **1** (except for **15b**, **18b** and **20b**), suggesting that the replacement of the naphthylcarba-

Table 1. Chromatographic resolution[a] of racemic compounds on CSPs **1**–**3**

Run	Compound	CSP **2**			CSP **3**			CSP **1**		Eluent[e]
		k'[b]	α[c] (e.o.)[d]	Rs[j]	k'[b]	α[c] (e.o.)[d]	Rs[j]	k'[b]	α[c] (e.o.)[d]	
1	**14a**	3.75	1		2.22	1.14	0.88	4.87	1[f]	A
2	**14b**	4.85	1		3.80	1		7.96	1.18	A
3	**14c**	15.01	1		13.21	1.15	0.78	10.18	1.20[g]	A
4	**15a**	9.26	1.05	0.54	1.76	1.11	1.05	3.04	1.07[f]	A
5	**15b**	2.99	1.14	1.02	1.93	1		3.62	1.06[f]	A
6	**16a**	5.72	1.02		2.68	1.17	1.12	3.06	1.09[h]	B
7	**16b**	4.72	1[i]		2.85	1.16[i]	1.23	1.98	1.06	C
8	**17a**	3.57	1.15[h]	1.18	3.5	1.44	1.38	1.36	1.18[f]	D
9	**17b**	3.72	1.06[h]	0.55	3.13	1.46	1.51	1.06	1.40[f]	D
10	**18a**	2.28	1.13	0.82	1.58	1.19	0.83	3.69	1.15	C
11	**18b**	17.12	1.13	0.92	10.50	1.15	1.07	6.59	1.04	B
12	**20a**	3.91	1		2.51	1.16 (+)	1.15	5.74	1.08 (+)[g]	A
13	**20b**	32.71	1.26 (+)[h]	0.89	9.19	1.20 (+)	1.21	7.71	1.06 (+)[g]	A
14	**20c**	10.16	1.11 (+)	1.20	7.95	1.19 (+)	1.32	7.35	1.16 (+)[f]	A

[a] Chromatographic conditions: UV detection (λ 254 nm), flow 1 mL/min, $T = 25$ °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–2-propanol 70:30:3; B = hexane–dichloromethane–2-propanol 90:10:1; C = hexane–dichloromethane–2-propanol 80:20:1; D = hexane–dichloromethane–2-propanol 90:10:2; E = hexane–dichloromethane–2-propanol 70:30:5, F = hexane–dichloromethane–2-propanol 70:30:7.
[f] Eluent E.
[g] Eluent F.
[h] Eluent C.
[i] Eluent = hexane–dichloromethane–2-propanol 95:5:1.
[j] Resolution factor.

moyl moiety at the 7-position with a hydroxyl group weakens the enantioresolution capability of the selector towards this class of racemic compounds. This probably happens because of enlargement of the cavity of the selector of CSP **2** with respect to that of CSP **1** selector,[12] due to the absence of the hindering 2-naphthylcarbamoyl moiety at position 7. It is known that the racemic compounds interact with similar selectors, by approaching them from the cleft formed by the concave part of the cholestanic backbone and the appended groups,[13] therefore its enlargement can make it possible that the enantiomers of a racemic substrate fit to the same extent in the cleft and hence their enantiodiscrimination is prevented. A different situation was found in the case of CSP **3**, which was able to resolve all the π-basic racemic compounds enantiodiscriminated by CSP **1** with higher values of the enantioselectivity factors (runs 6–11), in particular in the case of the two binaphthols **17a** and **17b** (runs 8 and 9). These results can be explained by taking into account that the chiral cleft of CSP **3** selector has an intermediate size between those of CSP **1** and CSP **2** selectors, since the 3-position lacks 2-naphthylcarbamoyl moiety and the two arylcarbamoyl groups are closer when located at the 7- and 12-positions.[12] This makes it possible for easier access of the substrates into the cleft of CSP **3** than into the cavity of CSP **1** and, due to the spatial proximity of the two arylcarbamoyl groups, we can suppose that these racemates can establish π–π face to face interaction with 3,5-dinitrophenylcarbamoyl moiety together with a π–π face to edge interaction with 2-naphthylcarbamoyl group,[14] which gives rise to the better enantiodiscriminating capability of CSP **3**. These interactions are not possible in the case of CSP **1** probably because the narrower cavity prevents a deep access of the substrates.[15]

The trend observed with the π-basic racemates was also confirmed in the chromatographic separation of the benzodiazepines **20** (runs 12–14) that CSP **3** resolved not only better than CSP **2** but also than CSP **1**. In order to check if the differences in enantiodiscriminating capability of the three CSPs can be attributed to a change of the enantiorecognition mechanism, the elution order of compounds **20** was determined. Knowledge of the elution order allows to establish with which enantiomer of the substrate the CSP forms the more stable diastereoisomeric adsorbate. If the elution order of the same compounds is the same upon different CSPs, then the more stable diastereoisomeric adsorbate is formed with the same enantiomer of the racemate and this suggests that the enantiorecognition mechanism must be similar on different CSPs. CSPs **1**–**3** showed for compounds **20** the same elution order, so suggesting that the differences in enantiodiscriminating capability are not attributable to a change of the chiral recognition mechanism in passing from one to another CSP.

The good chromatographic results obtained with CSPs **2** and **3** towards the resolution of π-basic racemic compounds enantiodiscriminated by CSP **1**, prompted us to verify the enantioresolution properties of these CSPs towards other π-basic racemates, not resolved by CSP **1**. Table 2 reports on the chromatographic results obtained

Table 2. Chromatographic resolution[a] of π-basic racemic compounds on CSPs **2** and **3**

Run	Compound	CSP **2**			CSP **3**		
		k'^{b}	α^{c}	Rs[d]	k'^{b}	α^{c}	Rs[d]
1	**16c**	3.55	1		1.34	1.19	1.26
2	**16d**	20.61	1		16.53	1.13	0.88
3	**16e**	5.83	1		4.41	1.17	1.23
4	**16f**	2.24	1		1.34	1.14	0.95
5	**18c**	15.24	1.07	0.61	10.26	1.14	1.22
6	**18d**	12.06	1		6.72	1.12	1.16
7	**18e**	2.97	1.17[e]	0.86	0.80	1.38	1.15
8	**18f**	7.14	1.12[e]	1.15	1.75	1.20	0.91
9	**18g**	0.85	1		1.25	1.23	1.03
10	**19**	6.07	1.10	1.09	2.99	1.17	1.18

[a] Chromatographic conditions: UV detection (λ 254 nm), flow 1 mL/min, $T = 25$ °C, eluent hexane–dichloromethane–2-propanol 80:20:1.
[b] Retention factor of the first eluted enantiomer.
[c] Enantioselectivity factor.
[d] Resolution factor.
[e] Eluent = hexane–dichloromethane–2-propanol 90:10:1.

using CSPs **2** and **3** in the resolution of various arylamides **16c–f** and **19** and *N*-aryl or *N*-alkyl amides of arylpropionic acids. Perusal of Table 2 shows that CSP **3** was not only more efficient but also more versatile than CSP **2**, which separated the enantiomers of only four racemates (runs 5, 7, 8 and 10), with lower enantioselectivity factors than those observed on CSP **3**. Various benzamides were well resolved on CSP **3** (runs 1–4) suggesting that the presence of an aryl group linked to the stereogenic centre of these compounds is not mandatory.

3. Conclusions

Chromatographic data concerning the HPLC resolution of both π-acid and π-basic compounds on CSPs **2** and **3** compared to those related on the use of CSP **1** allows us to reach some conclusions about the effect of the presence of a free OH group on the enantiodiscrimination capability of cholic acid based mixed selectors. The effect of the free hydroxyl group depends on its position on the cholestanic backbone. The presence of a hydroxyl group at the 7-position, as in CSP **2**, does not significantly improve the enantiodiscrimination ability of this kind of selector either towards π-acid or π-basic racemic compounds. On the contrary, when the OH group is at the 3-position of cholic acid system, as in CSP **3**, a more efficient and versatile chiral selector is obtained, in particular towards π-basic racemic compounds. In fact CSP **3** is able not only to resolve the π-basic racemates enantiodiscriminated by CSP **1** with higher α values, but also to separate the enantiomers of π-basic compounds not resolved by CSP **1**. On the basis of these results we can suppose that the different enantiodiscriminating capability is due to different sizes of the chiral cleft formed by the concave part of the cholic acid with the appended arylcarbamoyl groups. When this cleft is too large, as in CSP **2**, both enantiomers of the racemic compounds interact to the same extent. In contrast, when the cleft is too narrow, as in CSP **1**, a deep access of the enantiomers into the cavity, and hence their enantioselection, is prevented. The medium sized chiral cleft of CSP **3** allows the enantiomers to enter and to experience enantioselective interactions with both the arylcarbamoyl moieties, which give rise to their chromatographic separation.[15]

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on an NMR Varian Gemini 200 or on a Varian VXR-300 in $CDCl_3$ or in DMSO-d_6, using TMS as internal standard. The following abbreviations were used: s = singlet; d = doublet; dd = double doublet; t = triplet; ddt = double double triplet; m = multiplet.

TLC analyses were performed on silica gel plates Macherey-Nagel 60 F_{254}. Chromatographic purifications were performed using silica gel Macherey-Nagel, 70–230 or 230–400 for flash chromatography. Optical rotations were measured at the sodium D-line on a digital polarimeter JASCO-DIP 360: concentration, solvent and temperature are those specified for every measure. Melting points were taken using a Kofler Reichert-Jung apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1710 spectrophotometer. HPLC analyses were performed on a Jasco PU-980 chromatograph, equipped with a JASCO UV-975 detector and a circular dichroism JASCO J-710 detector. Elemental analyses were carried out at Dipartimento di Scienze e Tecnologie Chimiche dell'Univesità degli Studi di Udine.

Toluene, THF and dioxane were refluxed over sodium-benzophenone and distilled before use. *N*-Methylallylamine, pyridine and tributylamine were distilled over CaH_2. Chloroform was distilled before use. 2-Naphthoylchloride was distilled under reduced pressure and 3,5-dinitrobenzoylchloride was recrystallized from petroleum ether. Ethyl chloroformate was distilled

under nitrogen atmosphere just before use. *N*-Bromosuccinimide was recrystallized from water. 3-Mercaptopropyltrimethoxysilane was distilled under reduced pressure before use. Unless otherwise specified, the reagents were used without any purification. Compounds **6** and **12** were synthesized as previously reported and matched the reported characteristics.[5]

4.2. *N*-Allyl-*N*′-methyl-3-acetyl-7-oxo-12-hydroxycholan-24-amide, 7

Acetic anhydride (2.05 mL, 21.7 mmol) in dry toluene (3.15 mL) was added to a solution of amide **6** (3 g, 6.5 mmol) in dry toluene (3.15 mL) and the resulting mixture was stirred for 5 h at 80 °C, then cooled to room temperature and diluted in CH_2Cl_2. The solvent was removed under reduced pressure and the crude product purified by flash chromatography (SiO_2, CH_2Cl_2–acetone = 75:25) affording **7** (1.7 g, 3.4 mmol, 52%): mp = 74–78 °C; $[\alpha]_D^{20} = +17.3$ (*c* 0.99, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.67 (s, 3H, CH_3); 0.96 (d, 3H, CH_3 21); 1.17 (s, 3H, CH_3); 0.90–2.50 (m, 28H, CH and CH_2 steroidal, OH 12); 2.16 (s, 3H, $OCOCH_3$); 2.89 and 2.93 (s, 3H, N–CH_3); 3.87–3.98 (m, 3H, C*H*$_2$–CH=CH_2 and CH 12); 4.66 (m, 1H, CH 3); 5.06–5.28 (m, 2H, C*H*$_2$=CH); 5.75 (ddt, 1H, C*H*=CH_2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.8, 17.6, 21.2, 22.7, 24.1, 25.8, 27.5, 29.1, 33.0, 33.6, 34.6, 34.9, 35.9, 40.7, 45.1, 45.7, 46.5, 49.4, 72.0 (C 12), 72.8 (C 3), 170.5 (C=O acetyl), 211.0 (C=O carbonylic). IR (KBr, cm^{-1}): 3813, 3440, 2948, 1732, 1709, 1629, 1468, 1392, 1364, 1244, 1080, 1049, 924, 802, 597, 400.

4.3. *N*-Allyl-*N*′-methyl-3-acetyl-7-oxo-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 8

3,5-Dinitrophenylisocyanate (1.21 g, 5.8 mmol) was added to a solution of **7** (1.5 g, 3.0 mmol) in dry toluene (45 mL) and the resulting mixture heated at reflux and stirred overnight, then allowed to cool to room temperature. The solvent was removed under reduced pressure and the crude product purified by flash chromatography (SiO_2, CH_2Cl_2–acetone = 92:8) affording **8** (1.7 g, 2.4 mmol, 80%): mp = 138–145 °C; $[\alpha]_D^{21} = +63.1$ (*c* 1, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.67 (s, 3H, CH_3); 0.96 (d, 3H, CH_3 21); 1.17 (s, 3H, CH_3); 0.90–2.50 (m, 27H, CH and CH_2 steroidal); 2.16 (s, 3H, $OCOCH_3$); 2.89 and 2.96 (s, 3H, N–CH_3); 3.85–4.01 (m, 2H, C*H*$_2$–CH=CH_2); 4.63 (m, 1H, CH 3); 5.04–5.28 (m, 3H, C*H*$_2$=CH and CH 12); 5.71 (ddt, 1H, C*H*=CH_2); 8.65 (t, 1H, aromatic); 8.76 (d, 2H, aromatics); 9.06 (d, 1H, NH carbamate); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.4, 17.8, 21.2, 22.7, 23.9, 25.9, 26.4, 27.6, 30.2, 30.9, 31.3, 33.1, 33.5, 33.8, 34.6, 35.1, 36.8, 38.4, 42.0, 45.1, 45.2, 45.7, 46.6, 46.9, 48.9, 50.3, 52.3, 72.9 (C 3), 112.1, 116.9 (=CH_2), 117.2, 117.9, 132.4 (−CH=), 141.5, 146.5, 148.7, 152.9 (C=O carbamate), 170.4 (C=O acetyl), 173.5, 174.0 (C=O amide), 211.0 (C=O carbonylic). IR (KBr, cm^{-1}): 3854, 3838, 3751, 3244, 3117, 2956, 2364, 2345, 1734, 1710, 1654, 1617, 1546, 1466, 1424, 1345, 1245, 1222, 1066, 1048, 1022, 919, 897, 806, 765, 732, 669, 656, 400.

4.4. *N*-Allyl-*N*′-methyl-3-acetyl-7-hydroxy-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 9

Compound **8** (1.6 g, 2.2 mmol) was dissolved in a mixture of dry THF (4 mL) and methanol (20 mL) cooled to 0 °C, and $NaBH_4$ (0.14 g, 3.7 mmol) added slowly. When gas evolution ceased, the mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed at reduced pressure, and the remaining solid dissolved in ethyl acetate. The solution was washed with 10% $KHCO_3$ (2 × 30 mL) and brine (2 × 30 mL), then dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the crude product was purified by flash chromatography (SiO_2, CH_2Cl_2–acetone = 80:20) affording **9** (1.3 g, 1.8 mmol, 81%): mp = 135–142 °C; $[\alpha]_D^{22} = +56.4$ (*c* 0.99, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3); 0.89 (s, 3H, CH_3); 0.96–2.40 (m, 28H, CH and CH_2 steroidal and OH 7); 2.16 (s, 3H, $OCOCH_3$); 2.88 and 2.92 (s, 3H, N–CH_3); 3.80–4.01 (m, 3H, C*H*$_2$–CH=CH_2 and CH 7); 4.51 (m, 1H, CH 3); 5.05–5.20 (m, 3H, C*H*$_2$=CH and CH 12); 5.67 (ddt, 1H, C*H*=CH_2); 8.66 (t, 1H, aromatic); 8.76 (d, 2H, aromatics); 9.08 (d, 1H, NH carbamate); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.1, 17.8, 21.3, 22.4, 22.9, 25.6, 26.6, 27.4, 27.6, 30.8, 34.6, 35.1, 38.4, 39.0, 41.0, 43.4, 45.2, 47.7, 50.1, 52.2, 68.0 (C 7), 74.3 (C 12), 78.5 (C 3), 112.2, 116.7 (=CH_2), 118.0, 132.7 (−CH=), 141.4, 148.8, 152.8 (C=O carbamate), 170.6 (C=O acetyl). IR (KBr, cm^{-1}): 3854, 3821, 3751, 3736, 3712, 3689, 3676, 3650, 3447, 3115, 2941, 2872, 2346, 1734, 1624, 1548, 1466, 1424, 1344, 1246, 1224, 1193, 1075, 1025, 1009, 897, 806, 769, 732, 656, 611, 400.

4.5. *N*-Allyl-*N*′-methyl-3-acetyl-7-(2-naphthyl)carbamoyloxy-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 10

2-Naphthylisocyanate (0.65 g, 3.6 mmol) and DMAP (0.14 g, 1.1 mmol) were added to a solution of **9** (1.2 g, 1.7 mmol) in dry toluene (40 mL) and the resulting mixture heated at reflux and stirred for 24 h, then filtered to remove 2-naphthylurea; the solvent was evaporated under reduced pressure and the obtained solid purified by flash chromatography (SiO_2, CH_2Cl_2–ethyl acetate = 85:15) to afford **10** (1.1 g, 1.2 mmol, 74%): mp = 144–148 °C; $[\alpha]_D^{23} = +87.1$ (*c* 0.97, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.76 (s, 3H, CH_3); 0.86 (d, 3H, CH_3 21), 0.93 (s, 3H, CH_3); 0.96–2.40 (m, 27H, CH and CH_2 steroidal); 2.03 (s, 3H, $OCOCH_3$); 2.83 and 2.87 (s, 3H, N–CH_3); 3.73–3.95 (m, 2H, C*H*$_2$–CH=CH_2); 4.52 (m, 1H, CH 3); 4.90–5.14 (m, 4H, C*H*$_2$=CH and CH 12 and CH 7); 5.63 (ddt, 1H, C*H*=CH_2); 7.25–7.80 (m, 9H, H naphthylics and NH carbamates), 8.01–8.70 (m, 3H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.1, 14.1, 17.6, 20.9, 21.1, 22.4, 22.8, 25.7, 26.8, 27.3, 29.1, 30.1, 30.6, 31.3, 33.5, 34.3, 34.9, 35.0, 37.8, 40.6, 43.8, 45.4, 47.4, 50.1, 52.2, 72.0 (C 7), 73.9 (C 12), 78.4 (C 3), 112.0, 114.7, 116.9 (=CH_2), 117.6, 117.9, 119.2, 124.6, 126.3, 127.2, 127.4, 128.7, 130.0, 132.1, 133.8 (−CH=), 135.4, 141.3, 141.4, 148.6, 152.9 (C=O carbamate), 153.1 (C=O carbamate), 167.7, 170.3 (C=O acetyl), 173.7, 174.0 (C=O amide).

IR (KBr, cm^{-1}): 3853, 3838, 3750, 3735, 3690, 3676, 3649, 3385, 3108, 2941, 2871, 2361, 2343, 1733, 1634, 1605, 1547, 1506, 1472, 1432, 1344, 1245, 1224, 1127, 1071, 956, 920, 893, 854, 807, 731, 668, 655, 473, 400.

4.6. *N*-Allyl-*N*′-methyl-3-hydroxy-7-(2-naphthyl)carbamoyloxy-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 11

Compound **10** (1.06 g, 1.2 mmol) was dissolved in a mixture of MeOH (30 mL) and HCl (0.80 mL) and the resulting mixture heated at reflux and stirred for 1 h. After cooling to room temperature, the mixture was poured into water and extracted with CH_2Cl_2. The combined organic extracts were washed with 10% HCl (2×20 mL), 10% $NaHCO_3$ (2×20 mL) and water then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product purified by chromatography (SiO_2, CH_2Cl_2–ethyl acetate = 70:30) affording **11** (0.85 g, 1.0 mmol, 84%): mp = 159–163 °C; $[\alpha]_D^{18} = +93.4$ (*c* 0.98, CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$, δ): 0.70 (s, 3H, CH_3); 0.83 (d, 3H, CH_3 21), 0.87 (s, 3H, CH_3); 0.96–2.40 (m, 28H, CH and CH_2 steroidal and OH 3); 2.81 and 2.84 (s, 3H, N–CH_3); 3.48 (m, 1H, CH 3); 3.75–3.95 (m, 2H, C*H*$_2$–CH=CH_2); 4.94–5.10 (m, 4H, C*H*$_2$=CH, CH 7 and CH 12); 5.63 (ddt, 1H, C*H*=CH_2); 7.25–7.93 (m, 9H, H naphthylics and NH carbamates), 8.40–8.70 (m, 3H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.1, 14.1, 17.6, 20.9, 21.1, 22.4, 22.8, 25.7, 26.8, 27.3, 29.1, 30.1, 30.6, 31.3, 33.5, 34.9, 35.0, 37.8, 40.6, 43.8, 45.4, 47.4, 50.1, 52.2, 71.8 (C 7), 72.2 (C 12), 77.4 (C 3), 112.0, 114.7, 116.2 (=CH_2), 117.6, 117.9, 119.2, 124.6, 126.5, 127.2, 128.7, 130.0, 132.1, 133.8 (—CH=), 135.4, 141.3, 141.4, 148.6, 152.8 (C=O carbamate), 153.3 (C=O carbamate), 167.7, 173.7, 174.0 (C=O amide). IR (KBr, cm^{-1}): 3854, 3838, 3751, 3690, 3676, 3643, 3629, 3384, 2930, 2362, 2344, 1734, 1607, 1547, 1507, 1432, 1344, 1245, 1224, 1119, 1073, 1000, 956, 898, 855, 808, 732, 669, 655, 610, 474, 400.

4.7. Preparation of silane derivatives 13: representative procedure

3-Mercaptopropyltrimethoxysilane (0.8 mL, 4.2 mmol) was added to a solution of the selector (0.8 mmol) in $CHCl_3$ (10 mL) and the resulting mixture heated at reflux until TLC analysis showed complete conversion of the substrate. After cooling, the solvent was removed at reduced pressure, and the remaining oil was dispersed in pentane (30 mL); the precipitate was filtered and washed again with pentane (5×30 mL) to afford the pure product.

4.7.1. *N*-Methyl-*N*′-[(trimethoxysylylpropylthio)propyl]-3-hydroxy-7-(2-naphthyl)carbamoyloxy-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 13a.

The mixture was heated at reflux for 48 h and 0.93 g of product **13a** was obtained: mp = 97–102 °C; $[\alpha]_D^{16} = +82.6$ (*c* 1.01, CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$, δ): 0.70 (d, 2H, CH_2), 0.79 (s, 3H, CH_3); 0.91 (d, 3H, CH_3 21), 0.94 (s, 3H, CH_3); 0.96–2.40 (m, 36H, CH and CH_2 steroidal, CH_2 chain and OH 3); 2.81 and 2.84 (s, 3H, N–CH_3); 3.45 (m, 1H, CH 3); 3.54 (s, 9H, $Si(OCH_3)_3$); 4.99–5.14 (m, 2H, CH 7 and CH 12); 7.25–7.80 (m, 9H, H naphthylics and NH carbamates), 8.09–8.75 (m, 3H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 8.7, 9.2, 12.6, 18.2, 18.8, 19.0, 23.1, 23.5, 26.0, 27.5, 27.8, 28.7, 29.2, 31.3, 32.3, 33.5, 34.8, 35.2, 38.2, 39.0, 39.4, 39.8, 40.2, 40.5, 40.7, 40.9, 41.1, 41.5, 43.6, 46.0, 47.0, 47.9, 49.5, 50.6, 50.8, 58.4, 58.7, 66.1, 72.0 (C 7), 74.2 (C 12), 78.4 (C 3), 81.8, 112.2, 114.7, 118.1, 120.3, 125.1, 127.1, 127.9, 128.2, 129.1, 129.9, 130.1, 134.4, 137.8, 142.4, 149.3, 154.2 (C=O carbamate), 173.1 (C=O amide). IR (KBr, cm^{-1}): 3750, 3381, 2938, 2363, 2344, 1729, 1634, 1606, 1547, 1506, 1466, 1432, 1344, 1244, 1222, 1079, 1001, 955, 897, 808, 768, 731, 655, 474, 400.

4.7.2. *N*-Methyl-*N*′-[(trimethoxysylylpropylthio)propyl]-3-(2-naphthyl)carbamoyloxy-7-hydroxy-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 13b.

The mixture was heated at reflux for 30 h and 1.01 g of product **13b** was obtained: mp = 89–95 °C, $[\alpha]_D^{22} = +56.9$ (*c* 1.02, CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$, δ): 0.70 (d, 2H, CH_2), 0.75 (s, 3H, CH_3); 0.90 (s, 3H, CH_3); 0.95 (d, 3H, CH_3 21); 0.92–2.45 (m, 35H, CH and CH_2 steroidal, and chain CH_2); 2.95 (s, 3H, N–CH_3); 3.55 (s, 9H, $Si(OCH_3)_3$); 4.60 (m, 1H, CH 3) 5.05–5.25 (m, 2H, CH 7, CH 12); 6.91 (s, 1H, NH carbamate); 7.27–7.50 (m, 3H, H naphthylics); 7.65–7.80 (m, 3H, H naphthylics); 7.90 (s, 1H, H naphthylic); 8.60 (t, 1H, H *p*-3,5-dinitrophenylic); 8.65 (d, 2H, H *o*-3,5-dinitrophenylics); 8.80 (s, 1H, NH carbamate); IR (KBr, cm^{-1}): 3444, 2944, 2855, 2366, 2344, 1733, 1716, 1694, 1633, 1600, 1544, 1505, 1466, 1433, 1344, 1505, 1466, 1433, 1344, 1244, 1222, 1188, 1077, 994, 900, 822, 805, 766, 733, 477.

4.8. General procedure for the preparation of CSPs

A solution of 3-mercaptopropyltrimethoxysylanic derivative in dry toluene (20 mL) was added dropwise to a suspension of spherical silica gel (2.5 g) [previously dried under reduced pressure ($p = 0.01$ mmHg) at 180 °C for 15 h] in dry toluene (15 mL). The resulting mixture was heated at reflux, with gentle stirring, for 24 h. After cooling to room temperature, the silica was filtered and washed sequentially with toluene (3×30 mL), methanol (3×30 mL), acetone (3×30 mL), CH_2Cl_2 (3×30 mL) and pentane (2×30 mL), in that order, then dried under reduced pressure ($p = 0.01$ mmHg) at 45 °C for 8 h. The amount of selector linked to silica gel was then determined by elemental analysis.

CSP **2**: C, 9.26; H, 1.50; N, 0.98% corresponding to 0.233 mmol/g.

CSP **3**: C, 8.95; H, 1.56; N, 0.87% corresponding to 0.207 mmol/g.

Two 15 cm stainless steel columns were slurry packed with each of these materials, using conventional high pressure packing techniques.

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15. This is only an interpretation of the chromatographic data, based on the knowledge of the conformations of these kinds of selectors[12] and on a known mode of interaction,[13] and not a hypothesis of an enantiorecognition mechanism, which deserves further studies to be elucidated: work is currently in progress on this topic.