Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 1265–1275

TETRAHEDRON: *ASYMMETRY*

Synthesis of four cholic acid-based CSPs containing 2-naphthyl carbamate and 3,5-dinitrophenylcarbamate moieties and their evaluation in the HPLC resolution of racemic compounds

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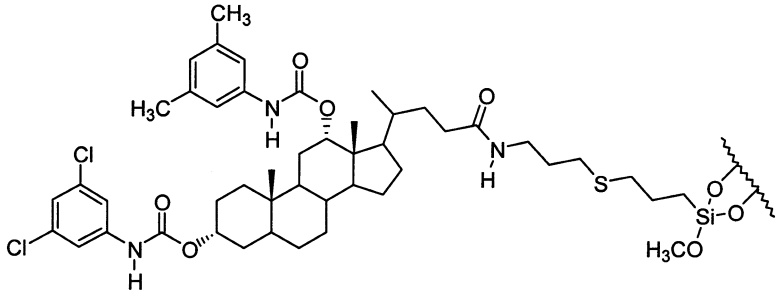
Received 8 May 2002; accepted 12 June 2002

Abstract—Four new chiral selectors, obtained by derivatising the hydroxy groups of cholic acid with 2-naphthylisocyanate and 3,5-dinitrophenylisocyanate have been prepared and linked to silica gel to obtain new chiral stationary phases (CSPs) for the HPLC separation of enantiomers. The CSP containing only 2-naphthylcarbamate groups is able to separate the enantiomers of π-acidic substrates, whereas the CSPs containing one 3,5-dinitrophenylcarbamate group and two 2-naphthyl carbamate moieties resolve π-acidic racemic compounds as well as π-basic substrates, with the observed enantiodiscriminating capabilities depending on the arrangement of the different carbamoyl units on the cholestanic backbone.

1. Introduction

Bile acids play an important role in the field of molecular recognition. They have been successfully used as molecular tweezers,[1] organic gelators,[2] optically active hosts in the formation of inclusion complexes[3] and chiral auxiliaries for asymmetric synthesis.[4] The recent use of bile acids as chiral selectors for the HPLC resolution of racemic compounds has opened new perspectives about the potential of these natural products. They have proven to be effective either as salts, which form micelles on the silica gel surface,[5] or as simple mono arylcarbamate derivatives.[6] In a different approach aimed at achieving biselector chiral stationary phases (CSPs), we previously described the synthesis of CSPs obtained by derivatising the hydroxyl groups of deoxycholic acid with 3,5-dichlorophenylisocyanate (whose aromatic ring has π-basic character) and 3,5-dimethylphenylisocyanate (having a π-acidic aromatic group) and using its carboxylic group for linking the chiral selectors to silica gel.[7] In this way, four CSPs have been obtained which have proven to be effective in the resolution of various racemic compounds. The enantiodiscrimination capabilities of these CSPs depended not only on the nature of the derivatising aromatic groups but also on their arrangement on the cholestanic skeleton.[7] In particular, **CSP 1** (Fig. 1), which possesses a 3,5-dimethylphenylcarbamate group



CSP 1

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

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PII: S0957-4166(02)00317-8

at the 12-position of the cholestanic skeleton and a 3,5-dichlorophenylcarbamate moiety at the 3-position, is able to resolve both π-acidic and π-basic racemates, so behaving as a biselector CSP.[7]

In an attempt to improve the enantiodiscrimination capabilities of these systems we addressed our attention toward the synthesis of bile acid derivatives possessing aromatic groups having stronger π-acid and π-basic character than 3,5-dichlorophenyl isocyanate and 3,5-dimethylphenyl isocyanate, respectively, in order to obtain chiral selectors capable, in principle, to establish stronger π–π interactions with the racemic compounds and, consequently, to afford better chromatographic separations. In addition, the enantiodiscriminating capability could also take advantage from the presence of a further aromatic carbamoyl group on the cholestanic backbone. Taking into account these considerations, we decided to use cholic acid as a chiral scaffold to which aromatic carbamoyl units could be linked, since this bile acid possesses three hydroxy groups which can be reacted selectively.[8] 2-Naphthyl isocyanate having stronger π-basic character than 3,5-dimethylphenyl isocyanate and 3,5-dinitrophenyl isocyanate, with greater π-acid character than 3,5-dichlorophenyl isocyanate, were chosen as derivatising groups of cholic acid. In this article we describe the synthesis of four cholic acid-derived CSPs, the first, containing only 2-naphthylcarbamoyl groups, and the others possessing two 2-naphthylcarbamoyl groups and one 3,5-dinitrophenyl carbamoyl moiety at different positions of the cholestanic skeleton (Fig. 2), and their use in the chromatographic resolution of racemic compounds.

2. Results and discussion

2.1. Synthesis of CSPs 2–5

The synthesis of selectors **8**, **10** and **13** is summarised in Scheme 1. The common starting point was the preparation of the *N*-methylallylamide derivative **7**, obtained by means of the mixed anhydride method:[9] in this way, a terminal double bond which can be used for linking the chiral selectors to silica gel, was introduced. *N*-Methylallylamine was chosen instead of allylamine, in order to avoid the formation, during the derivatisation of the hydroxy group at the 12-position of the cholestanic backbone, of a side product from the reaction of the amide nitrogen with the aryl isocyanate.[7] The reaction of **7** with a four-fold excess of 2-naphthylisocyanate in refluxing toluene afforded **8** in good yield. 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 mixed selector **10** can be obtained simply by adjusting the reaction temperature: at room temperature only the 3-OH group should react, whereas higher temperatures are required in order to derivatise the other hydroxy groups. Therefore, **7** was treated with a slight excess of 3,5-dinitrophenylisocyanate in THF at room temperature. However, under these mild reaction conditions, a small amount of the product derivatised at both the 3- and 12-positions was obtained, probably due to the highly electrophilic character of the 3,5-dinitrophenyl isocyanate, and hence, after chromatographic purification, pure **9** was obtained in only 65% yield. The reaction of **9** with an excess (2.5 equiv.) of 2-naphthylisocyanate in refluxing toluene afforded **10** in 70% yield after chromatographic purification.

In order to obtain **13**, the amide derivative **7** was reacted with a slight excess of 2-naphthylisocyanate in the presence of a catalytic amount of DMAP in THF as a solvent at room temperature. The mono-arylcarbamate derivative **11** was obtained as sole product, under these reaction conditions, so confirming that, using an arylisocyanate less reactive than 3,5-dinitrophenylisocyanate, the regioselectivity is guaranteed, simply by changing the reaction temperature. Although the reactivity of the two hydroxy groups at the 7- and 12-positions of the cholestanic backbone is similar and, hence, the use of protecting groups is required in order for selective derivatisation to be accomplished,[1a] much to our surprise the reaction of **11** with an excess of 3,5-dinitrophenylisocyanate in refluxing toluene afforded **12** as the sole product, which was obtained in 85% yield

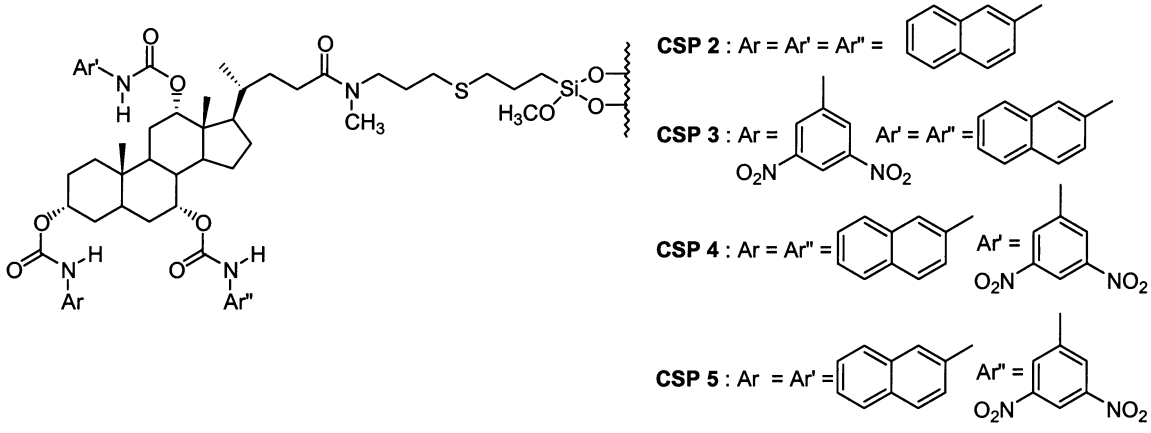
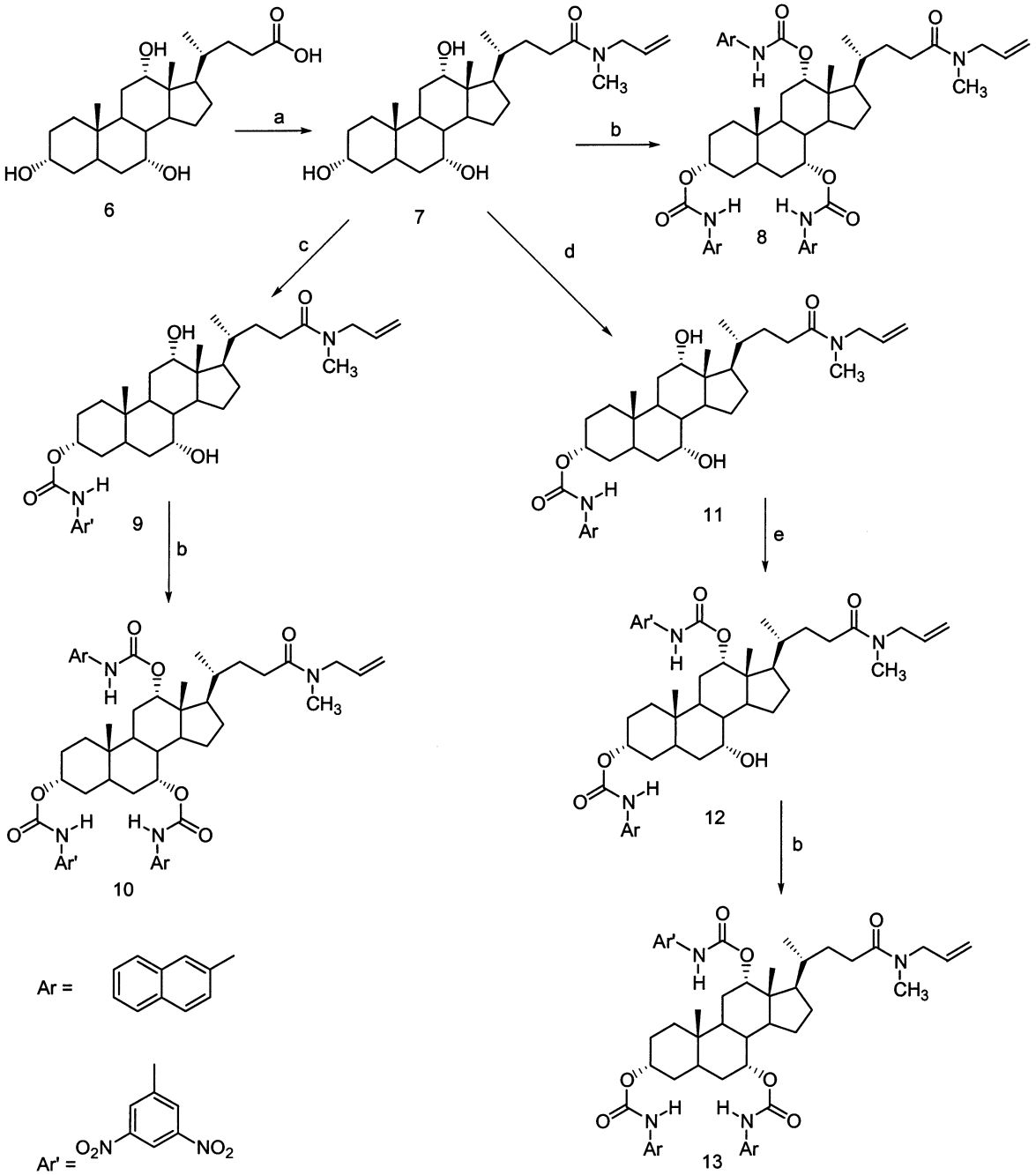


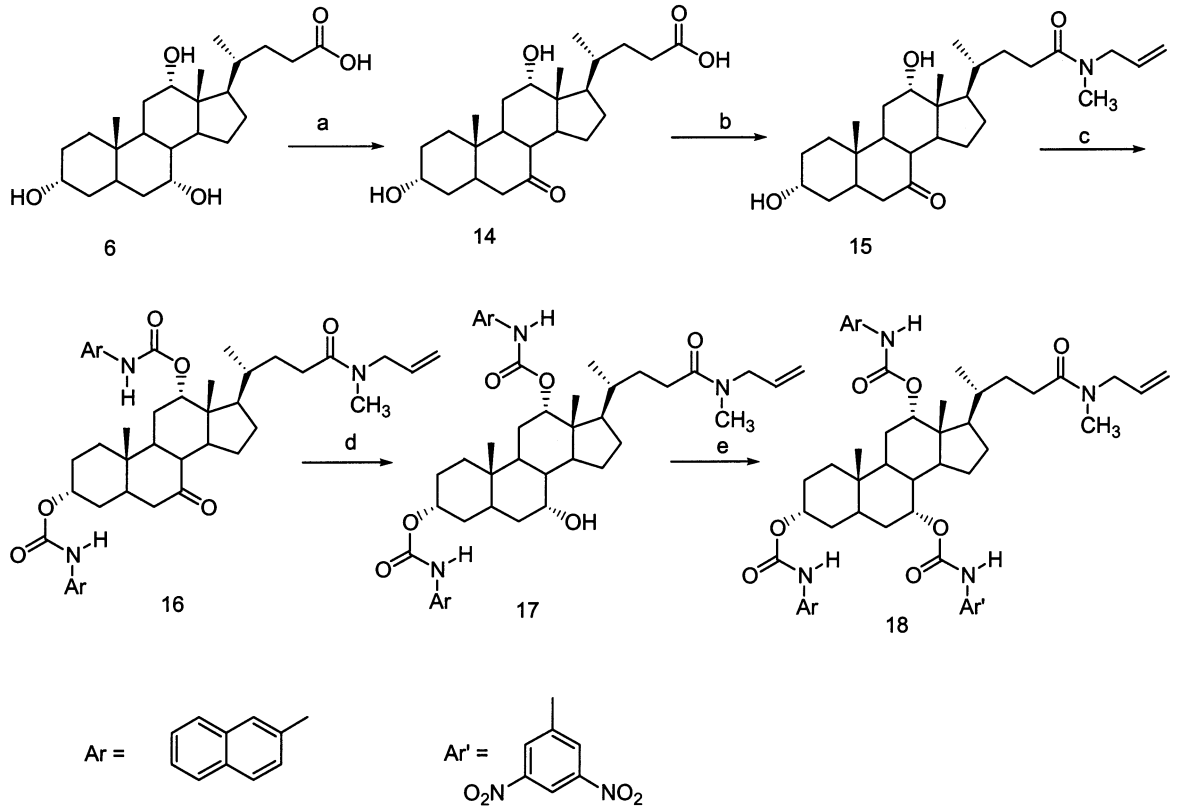
Figure 2. Structures of **CSPs 2–5**.



Scheme 1. *Reagents and conditions*: (a) i. Bu_3N, dioxane, 10°C, ii. EtOCOCl, iii. *N*-methylallylamine; (b) 2-naphthylisocyanate, DMAP, toluene, reflux; (c) 3,5-dinitrophenylisocyanate, THF, rt; (d) 2-naphthylisocyanate, DMAP, rt; (e) 3,5-dinitrophenylisocyanate, toluene, reflux.

after chromatographic purification. This probably happens because the crowding generated by the presence of a 3,5-dinitrophenylcarbamate at the 12-position prevents the approach of another 3,5-dinitrophenylcarbamate group to the 7-position. Additionally, using a four-fold excess of 3,5-dinitrophenyl isocyanate and prolonging the reaction time, no trace of the product having a 3,5-dinitrophenylcarbamate group at the 7-position can be detected. By reacting **12** with an excess of 2-naphthylisocyanate in refluxing toluene **13** was obtained in 60% yield, after chromatographic purification. The moderate yield is due to incomplete conversion of **12**, again probably because of the steric hindrance exerted by the 3,5-dinitrophenylcarbamate moiety at the 12-position.

The preparation of the last chiral selector **18** required a different synthetic approach, which is summarised in Scheme 2. Since **18** possesses two 2-naphthylcarbamoyl groups at the 3- and 12-positions and a 3,5-dinitrophenylcarbamoyl group at the 7-position, and the presence of a 2-naphthylcarbamate moiety at the 12-position does not prevent the derivatisation of the hydroxy group at the 7-position, protection of this



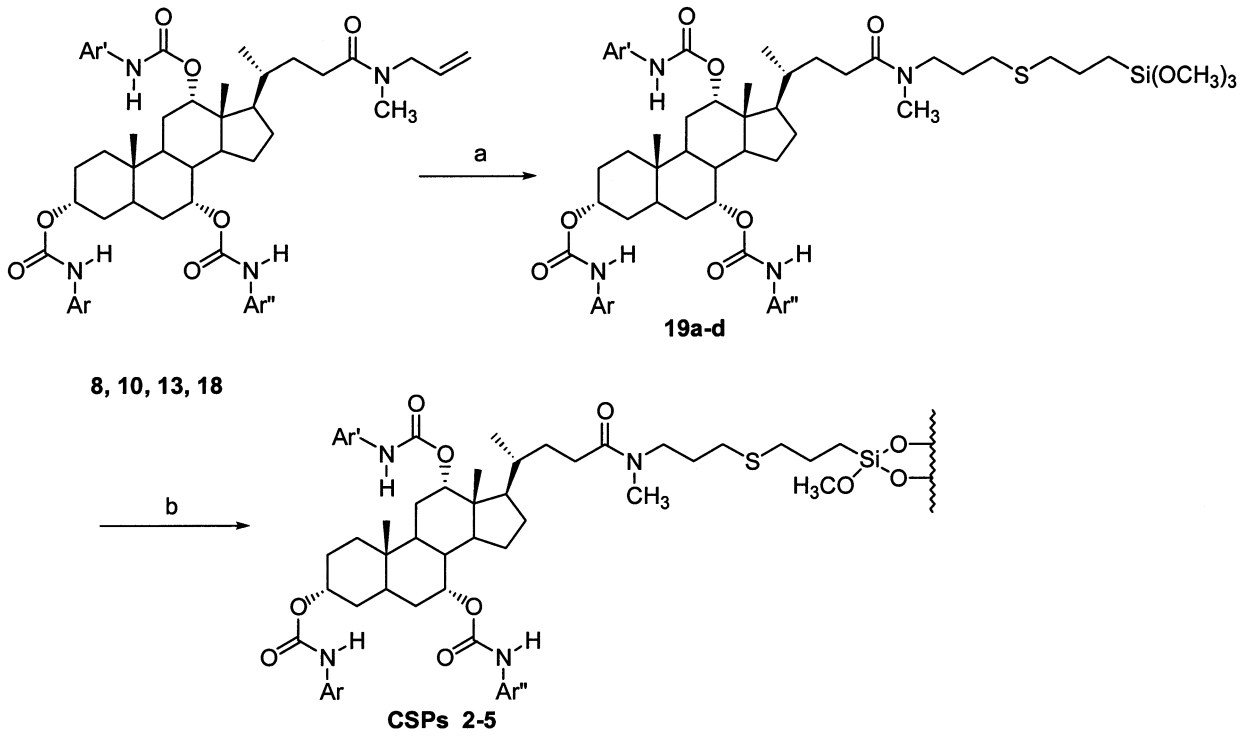
Scheme 2. *Reagents and conditions*: (a) NBS, 0.37 M $NaHCO_3$, rt to 85°C; (b) i. Bu_3N, dioxane, 10°C, ii. EtOCOCl, iii. *N*-methylallylamine, 10°C to rt; (c) 2-naphthylisocyanate, DMAP, toluene, reflux; (d) $NABH_4$, THF/MeOH 0°C to rt; (e) 3,5-dinitrophenylisocyanate, toluene, reflux.

group is mandatory. This protection can be accomplished by means of regioselective oxidation of the 7 hydroxy group of the cholic acid with NBS in alkaline solution, because it is well known that $NaBH_4$ reduction of the carbonyl function takes place with complete stereoselectivity and that the stereogenic centre is restored to the same absolute configuration as in **6**.[1a] Therefore, **6** was reacted with NBS in bicarbonate solution and the ketoacid **14** was converted into the corresponding *N*-methylallylamide **15**, using the mixed anhydride method. The reaction of **15** with a 2.5-fold excess of 2-naphthylisocyanate in refluxing toluene, followed by reduction with $NaBH_4$ afforded the disubstituted derivative **17** with complete stereoselectivity. The selector **18** was then obtained by treating **17** with an excess of 3,5-dinitrophenylisocyanate in refluxing toluene.

Selectors **8**, **10**, **13** and **18** were covalently linked to silica gel, as described in Scheme 3, by means of reaction with a five-fold excess of 3-mercaptopropyltrimethoxysilane in the presence of AIBN in refluxing $CHCl_3$.[7] 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 refluxing toluene over 24 h and, after being thoroughly washed and dried, the derivatised silica gels were employed for packing stainless steel columns of 15 cm (internal diameter 4.6 mm). The amount of selector bonded to the silica gel was determined in each case by means of elemental analysis and resulted in comparable loading values for all of the CSPs prepared.

2.2. Use of CSPs 2–5 in the chromatographic separation of racemic compounds

The racemic compounds reported in Chart 1 were used to test the enantiodiscriminating capability of CSPs **2**–**5**. The chromatographic results obtained in the separation of the π-acidic derivatives are reported in Table 1. **CSP 2**, which possesses only 2-naphthyl carbamate moieties is able to separate the enantiomers of all the selected π-acceptor racemates, with low to moderate enantioselectivity values. The best results are obtained in the case of the amino alcohol derivatives (runs 3 and 4), suggesting that the hydroxy group of the racemic substrate represents a further enantioselective interaction site with the chiral selector of the CSP. Furthermore, not only the 3,5-dinitrobenzoyl derivatives, but also the less π-acidic 4-nitrobenzamides **21** (runs 5 and 6) are resolved using **CSP 2**. Perusal of Table 1 gives an immediate idea of the different behaviour of the three mixed CSPs with respect to the resolution of π-acidic compounds. **CSP 3**, possessing a 3,5-dinitrophenylcarbamate group at the 3-position of the cholestanic backbone, is able to resolve only the phenylglycinol derivative **20c** (run 3), whereas **CSP 4**, which presents the 3,5-dinitrophenylcarbamate moiety at the 12-posi-



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

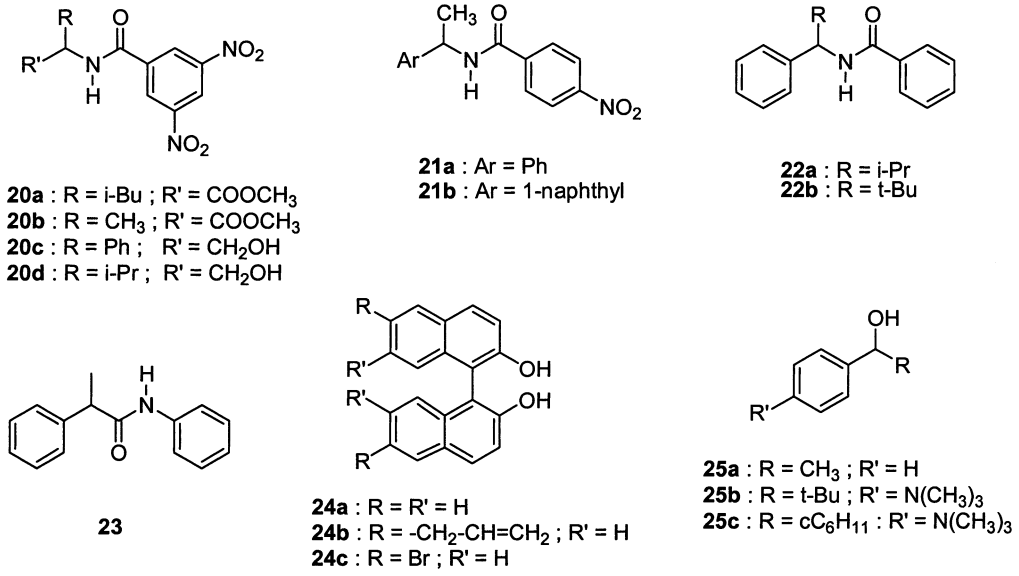


Chart 1.

tion, separates the enantiomers of all the derivatives apart from *N*-3,5-dinitrobenzoyl valinol **20d** (run 4). An intermediate behaviour is detected in the case of **CSP 5**, where the 3,5-dinitrophenylcarbamate group is at the 7-position of the cholestanic system: this phase resolves only four of the six π-acidic compounds, with enantioselectivity values comparable to those observed using **CSP 4**, the sole exception is represented by **20c** (run 3) which is resolved with a particularly high α value, even higher than that observed upon **CSP 2**. These chromatographic data indicate that the replacement of one of the three π-basic 2-naphthylcarbamate moieties with a π-acid 3,5-dinitrophenylcarbamate group affords CSPs which can discriminate between the enantiomers of π-acceptor racemic compounds, depending on the position where the substitution has taken place.

Table 2 reports on the chromatographic results obtained in the resolution of π-basic racemates upon CSPs **3**–**5**. As expected, **CSP 2** did not give any resolution of these compounds, because of the lack of a π-acid group which gives rise to π–π interactions with the substrates.[10] All three mixed CSPs are able to

Table 1. Chromatographic resolution of racemic π-acceptor compounds on CSPs **2**–**5**[a]

Run	Compound	CSP 2		CSP 3		CSP 4		CSP 5		Eluent[e]
		k'[b]	α[c] (o.e.)[d]	k'[b]	α[c]	k'[b]	α[c] (o.e.)[d]	k'[b]	α[c] (o.e.)[d]	
1	**20a**	4.32	1.11	4.56	1	4.47	1.05	3.46	1.05	A
2	**20b**	7.35	1.12	7.66	1	7.96	1.18	6.05	1	A
3	**20c**	8.85	1.22	8.08	1.20	10.18	1.20	13.40[f]	1.66	B
4	**20d**	7.50	1.15	5.63	1	6.77	1	10.75[f]	1	B
5	**21a**	3.55[g]	1.05 (+)	3.26	1	3.04	1.07 (+)	9.48[h]	1.06 (+)	A
6	**21b**	3.95	1.16 (+)	5.07	1	3.62	1.06 (+)	13.10	1.08 (+)	C

[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 70/30/5, B=hexane/dichloromethane/2-propanol 70/30/7, C=hexane/dichloromethane/2-propanol 70/30/3.
[f] Eluent A.
[g] Eluent C.
[h] Eluent C.

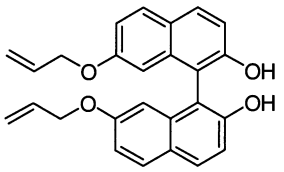
Table 2. Chromatographic resolution of π-donor racemic compounds on CSPs **3**–**5**[a]

Run	Compound	CSP 3		CSP 4		CSP 5		Eluent[e]
		k'[b]	α[c] (o.e.)[d]	k'[b]	α[c] (o.e.)[d]	k'[b]	α[c] (o.e.)[d]	
1	**22a**	3.76	1.09 (+)	3.06	1.09 (+)	3.08	1.12 (+)	D
2	**22b**	5.11	1.05 (+)	1.98[f]	1.06 (+)	3.97	1.12 (+)	E
3	**23**	3.79	1.08 (−)	3.69	1.15 (−)	3.39	1.06 (−)	D
4	**24a**	1.53	1	1.36	1.18 (+)	1.16	1	A
5	**24b**	1.22	1	1.06	1.40 (+)	–	–	A
6	**24c**	2.66	1	2.52	1.09 (+)	2.04	1	A
7	**25a**	3.82	1	3.70	1.04	3.87	1	E
8	**25b**	2.17	1	1.76	1.06	2.13	1	E
9	**25c**	3.69	1	3.07	1.11	3.52	1	E

[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 (240 nm for compounds **24**) of the first eluted enantiomer.
[e] A=hexane/dichloromethane/2-propanol 70/30/5, D=hexane/dichloromethane/2-propanol 80/20/1, E=hexane/dichloromethane/2-propanol 90/10/1.
[f] Eluent D.

resolve π-basic amide derivatives with the enantioselectivity depending on the position of the 3,5-dinitrophenylcarbamate group on the cholestanic backbone. CSPs **3** and **4** show the same behaviour toward the chromatographic separation of the enantiomers of amides **22**, whereas **CSP 5** affords slightly better resolution of these compounds, which is independent of the structure of the substrates (runs 1 and 2). On the contrary, the resolution of the anilide **23** is better using **CSP 4** than those seen with CSPs **3** and **5**. As far as underivatised compounds, i.e. binaphthol derivatives and alkylarylcarbinols, are concerned, only **CSP 4** has proven effective (runs 4–9) affording resolution dependent on the structure of the substrates. The chiral recognition toward binaphthol derivatives is influenced by the presence of substituents at the 6,6′- and 7,7′-positions of the binaphthyl system in an opposite mode: the 7,7′-diallyloxy-2,2′-dihydroxy-1,1′-binaphthyl **24b** (run 5, Fig. 3) is better resolved than BINOL **24a** (run 4), whereas the enantiomers of 6,6′-dibromo-2,2′-dihydroxy-1,1′-binaphthyl **24c** (run 6) are separated less well with respect to BINOL.

Even the resolution of the alkylarylcarbinols depends on the nature of the examined racemates. When the aromatic ring of the substrate possesses an electron-donating group, such as the dimethylamino moiety of **25c**, the racemate is resolved with a higher α value (run 9). This suggests that the π–π interaction between the electronically complementary aromatic moieties present on the chiral selector of **CSP 4** (3,5-dinitrophenyl) and the substrate (4-dimethylaminophenyl) play an important role in the enantiodiscrimination of these racemates. On the contrary, the presence of a *tert*-butyl group on the stereogenic centre of the alcohol is deleterious to the enantiodiscrimination and the separation of the enantiomers of a *tert*-butyl carbinol is poor (run 8).



24b

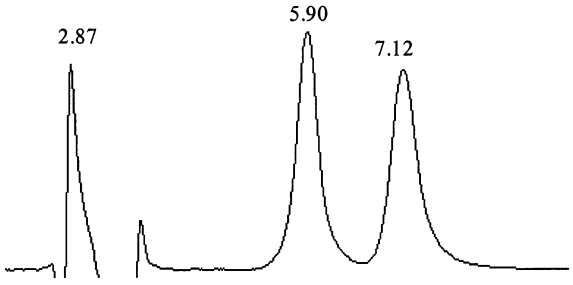


Figure 3. Chromatographic resolution of **24b** upon **CSP 4**: for chromatographic conditions see Table 2.

3. Conclusions

The chromatographic data concerning the resolution of both π-acceptor and π-donor racemic compounds upon CSPs **2**–**5** allow us to reach some conclusions about the behaviour of these CSPs. The separation of the enantiomers of π-acid compounds, and the resolution of π-basic racemates depend not only on the electronic nature of the aromatic carbamate moieties but also on the relative position that these functions have on the cholestanic backbone. **CSP 2**, possessing only 2-naphthyl carbamate moieties is able to separate the enantiomers of all the examined π-acid substrates, whereas no resolution of π-basic racemates is obtained. The mixed CSPs **3**–**5**, having a 3,5-dinitrophenylcarbamate moiety in three different position of the cholestanic skeleton, show enantiodiscrimination depending on the positioning of the π-acceptor moiety. In particular, **CSP 4**, which possesses a 3,5-dinitrophenylcarbamate group at the 12-position of the cholestanic backbone has proven to be the most versatile of these CSPs: as a matter of fact it was able to resolve almost all of the π-acid racemates and π-basic substrates examined, including underivatized compounds, thus demonstrating that the use of adequately derivatised bile acids represents a promising approach to the achievement of broad-spectrum CSPs. In this case, and as we have previously shown,[7,11] the enantioselective behaviour of the bile acid derivatives is strongly dependent on the arrangement of the aromatic substituents on the cholestanic backbone. Studies aimed at clarifying the reasons for the different behaviour of the CSPs **3**–**5** are now in progress and the results will be reported in due course.

4. Experimental

^{1}H and ^{13}C NMR spectra were recorded on a 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 purification 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-600 detector. Elemental analyses were carried out at Laboratorio di Microanalisi, Dipartimento di Farmacia, Università di Pisa.

Toluene, THF and dioxane were heated under reflux 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 recrystallised from petroleum ether. Ethyl chloroformate was distilled under nitrogen atmosphere just before use. *N*-Bromosuccinimide was recrystallised from water. 3-Mercaptopropyltrimethoxysilane was distilled under reduced pressure before use. Unless otherwise specified the reagents were used without any purification.

4.1. *N*-Allyl-*N*′-methylcholan-24-amide, 7

Dry tributylamine (8.7 mL, 36.70 mmol) was added to a solution of cholic acid **6** (15.00 g, 36.70 mmol) in dry dioxane (270 mL); the mixture was cooled to 10°C and ethylchloroformate (3.5 mL, 36.70 mmol) in dry dioxane (12 mL) was added dropwise. After 10 min, *N*-methylallylamine (8.8 mL, 91.80 mmol) in dry dioxane (15 mL) was dropwise added. The resulting mixture was stirred at 10°C for additional 30 min, and at room temperature overnight. Then it was poured into water (500 mL) and the crude product was extracted with ethyl acetate (5×50 mL); the combined organic extracts were washed with 10% HCl (2×50 mL), water, 10% $NaHCO_3$ and water, in that order, then dried over anhydrous Na_2SO_4. After removing the solvent at reduced pressure, **7** was obtained (10.80 g, 23.4 mmol, 64%): mp = 76–80°C; $[\alpha]_D^{22} = +2.6$ (*c* 1.00; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.65 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.00 (d, 3H, CH_3 21), 1.10–2.70 (m, 27H, steroidal CH and CH_2, OH 3, 7, 12), 2.88–2.94 (s, 3H, N-CH_3), 3.40 (m, 1H, CH 3), 3.80–4.00 (m, 4H, $CH_2CH{=}CH_2$, CH 7 and CH 12), 5.15 (m, 2H, $CH_2{=}CH$), 5.75 (ddt, 1H, $CH{=}CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.5, 17.5, 22.4, 23.2, 26.3, 27.5, 28.1, 29.9, 30.4, 31.1, 31.4, 34.6, 34.7, 35.3, 35.6, 39.5, 41.4, 41.6, 46.4, 46.9, 52.2, 68.4 (C 7), 71.6 (C 3), 73.0 (C 12), 116.6 and 117.0 (=CH_2), 132.7 and 133.1 (-CH=), 173.4

and 174.0 (C=O). IR (KBr, cm^{-1}): 3421, 2934, 1654, 1628, 1458, 1406, 1261, 1078, 1047, 914, 796.

4.2. *N*-Allyl-*N*′-methyl-3,7,12-tris(2-naphthyl)carbamoyloxycholan-24-amide, 8

2-Naphthylisocyanate (1.10 g, 6.50 mmol) and DMAP (0.012 g, 0.1 mmol) was added to a solution of **7** (0.85 g, 1.85 mmol) in dry toluene (25 mL) and the resulting mixture was heated under reflux for 40 h. The solvent was removed under reduced pressure, and the crude product was purified by flash chromatography (SiO_2, CH_2Cl_2/acetone = 95:5), affording **8** (1.31 g, 1.35 mmol, 73%): mp = 134–137°C; $[\alpha]_D^{20} = +118.3$ (*c* 1.01; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.80 (s, 3H, CH_3), 0.90 (d, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.00–2.40 (m, 24H, steroidal CH and CH_2), 2.80 (s, 3H, N-CH_3), 3.65–4.00 (m, 2H, C$\underline{H}_2$CH=CH_2), 4.55 (m, 1H, CH 3), 4.95–5.25 (m, 4H, CH=C$\underline{H}_2$, CH 7 and CH 12), 5.65 (m, 1H, C$\underline{H}$=CH_2), 6.90 (s, 1H, NH), 7.10–7.50 (m, 11H, aromatics and NH), 7.60–8.10 (m, 12H, aromatics); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.2, 17.9, 22.4, 23.0, 25.8, 26.8, 27.0, 27.2, 29.1, 29.8, 30.4, 30.7, 30.8, 31.1, 31.5, 33.5, 34.4, 34.6, 34.9, 38.0, 40.7, 43.6, 45.5, 47.5, 47.7, 49.9, 52.1, 72.4 (C 7), 75.0 (C 12), 76.8 (C 3), 114.7, 115.2, 116.5, 117.0, 117.7, 119.1, 119.2, 124.5, 124.7, 126.3, 126.6, 127.2, 127.4, 127.5, 128.5, 128.7, 129.9, 130.0, 132.4, 132.9, 133.8, 133.9, 135.3, 135.4, 135.5, 152.9 (carbamate C=O), 153.3 (carbamate C=O), 153.4, (carbamate C=O), 173.2 and 173.6 (amide C=O); IR (KBr, cm^{-1}): 3402, 3297, 3052, 2939, 2068, 1718, 1633, 1605, 1534, 1506, 1432, 1396, 1358, 1215, 1045, 954, 853, 812, 746. Anal. calcd for $C_{61}H_{68}N_4O_7$: C, 75.59; H, 7.07; N, 5.78. Found: C, 75.69; H, 7.11; N, 5.67%.

4.3. *N*-Allyl-*N*′-methyl-3-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 9

3,5-Dinitrophenylisocyanate (0.79 g, 3.76 mmol) was added to a solution of **7** (1.58 g, 3.42 mmol) in dry THF (40 mL) and the resulting mixture was stirred for 65 h at room temperature: the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (SiO_2; CH_2Cl_2/acetone = 80:20) affording **9** (1.49 g, 2.22 mmol, 65%): mp = 143°C; $[\alpha]_D^{23} = +96.7$ (*c* 1.00; CH_2Cl_2,); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.70 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.05 (d, 3H, CH_3 21), 1.05–2.60 (m, 26 H, steroidal CH and CH_2, OH 7 and 12), 3.05 (s, 3H, N-CH_3), 3.80–4.20 (m, 4H, C$\underline{H}_2$CH=CH_2, CH 7 and CH 12), 4.60 (m, 1H, CH 3), 5.05–5.35 (m, 2H, CH=C$\underline{H}_2$), 5.65–5.95 (ddt, 1H, C$\underline{H}$=CH_2), 8.65 (t, 1H, aromatic), 9.05 (s, 2H, aromatics), 9.65 (s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.6, 17.5, 22.3, 23.2, 26.4, 26.6, 27.8, 28.2, 29.6, 30.0, 31.6, 34.2, 34.7, 34.8, 35.3, 35.4, 39.6, 41.1, 41.9, 46.5, 47.6, 50.4, 52.5, 68.2, 73.1, 76.3, 111.5, 117.0, 118.0, 132.3, 142.4, 148.7, 153.4 (carbamate C=O), 174.2 and 174.5 (amide C=O); IR (KBr, cm^{-1}): 3446, 3110, 2940, 1734, 1617, 1544, 1466, 1420, 1345, 1245, 1221, 1071, 914, 820, 730.

4.4. *N*-Allyl-*N*′-methyl-3-(3,5-dinitrophenyl)carbamoyloxy-7,12-bis(2-naphthyl)carbamoyloxy-cholan-24-amide, 10

2-Naphthylisocianate (0.93 g, 5.50 mmol) and DMAP (0.015 g, 0.12 mmol) were added to a solution of **9** (1.47 g, 2.19 mmol) in dry toluene (40 mL) and the resulting mixture was heated under reflux for 40 h; the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (SiO_2, CH_2Cl_2/acetone = 95:5), affording **10** (1.41 g, 1.40 mmol, 64%): mp = 155–158°C; $[\alpha]_D^{21} = +87.3$ (*c* 0.99; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.80 (s, 3H, CH_3), 0.90 (d, 3H, CH_3 21), 0.95 (s, 3H, CH_3), 1.00–2.50 (m, 24H, steroidal CH and CH_2), 2.75 and 2.80 (s, 3H, N-CH_3), 3.60–3.95 (m, 2H, C$\underline{H}_2$CH=CH_2), 4.55 (m, 1H, CH 3), 4.90–5.25 (m, 4H, CH=C$\underline{H}_2$, CH 7 and CH 12), 5.50–5.70 (m, 1H, C$\underline{H}$=CH_2), 7.20–7.50 (m, 8H, naphthylics and carbamate NH), 7.60–7.80 (m, 6H, naphthylics), 7.90 (s, 2H, naphthylics), 8.30–8.60 (m, 4H, 3,5-dinitrophenylics and carbamate NH); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.2, 17.7, 22.3, 22.4, 23.0, 25.6, 25.8, 26.8, 27.0, 27.2, 29.1, 29.2, 30.0, 30.5, 30.8, 31.3, 31.5, 33.6, 34.2, 34.4, 34.6, 35.0, 38.1, 40.7, 43.5, 45.5, 47.5, 48.0, 52.1, 72.7 (C 7), 76.0 (C 12), 77.6 (C 3), 111.9, 114.8, 115.2, 116.6, 117.1, 117.7, 119.1, 119.7, 124.7, 126.4, 126.5, 127.2, 127.3, 127.5, 128.6, 130.0, 132.3, 132.8, 133.8, 135.7, 140.7, 148.4, 152.2 (carbamate C=O), 152.5 (carbamate C=O), 153.3 (carbamate C=O), 173.4 and 173.8 (amide C=O); IR (KBr, cm^{-1}): 3392, 2937, 1734, 1636, 1544, 1430, 1343, 1222, 1069, 816, 728. Anal. calcd for $C_{57}H_{64}N_6O_{11}$: C, 67.84; H, 6.39; N, 8.33. Found: C, 68.02; H, 6.41; N, 8.19%.

4.5. *N*-Allyl-*N*′-methyl-3-(2-naphthyl)carbamoyloxycholan-24-amide, 11

2-Naphthylisocyanate (1.44 g, 8.50 mmol) and DMAP (0.052 g, 0.43 mmol) were added to a solution of **7** (3.57 g, 7.74 mmol) in dry THF (100 mL) and the resulting mixture was stirred at room temperature for 16 h, then filtered to remove 2-naphthylurea; the solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography (SiO_2; CH_2Cl_2/acetone = 80:20) to afford **11** (3.42 g, 5.42 mmol, 70%): mp = 123–129°C; $[\alpha]_D^{29} = +46.9$ (*c* 1.07; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.68 (s, 3H, CH_3). 0.88 (s, 3H, CH_3), 0.98 (d, 3H, CH_3 21), 1.00–2.48 (m, 26H, steroidal CH and CH_2, OH 7 and 12), 2.88 and 2.92 (s, 3H, N-CH_3), 3.82–4.02 (m, 4H, C$\underline{H}_2$CH=CH_2, CH 7 and CH 12), 4.58 (m, 1H, CH 3), 5.06–5.22 (m, 2H, CH=C$\underline{H}_2$), 5.64–5.82 (ddt, 1H, C$\underline{H}$=CH_2), 7.19 (s, 1H, carbamate NH), 7.29–7.44 (m, 3H, naphthylics), 7.68–7.75 (m, 3H, naphthylics), 7.98 (s, 1H, naphthylic); ^{13}C NMR (50 MHz, $CDCl_3$, δ):12.5, 17.6, 22.4, 23.2, 26.6, 27.5, 28.3, 30.4, 31.1, 31.4, 34.4, 34.7, 35.4, 35.6, 39.6, 41.2, 41.9, 46.5, 47.1, 52.2, 68.2 (C 7), 73.0 (C 12), 75.3 (C 3), 114.6, 116.6, 117.1 (=CH_2), 119.3, 124.4, 126.3, 127.4, 127.5, 128.6, 130.0, 132.7, 133.2 (CH=), 134.0, 135.3, 153.5 (carbamate C=O), 173.5 and 173.9 (amide C=O); IR (KBr, cm^{-1}): 3435, 3056, 2938, 2869, 1706, 1629, 1550, 1506, 1466, 1433, 1400, 1359, 1233, 1047, 913, 854, 814, 746.

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

3,5-Dinitrophenylisocyanate (0.76 g, 3.64 mmol) was added to a solution of **11** (1.48 g, 2.35 mmol) in dry toluene (30 mL) and the resulting mixture was heated under reflux for 16 h, then allowed to cool to room temperature. The solvent was removed under reduced pressure and the solid obtained was purified by flash chromatography (SiO_2, CH_2Cl_2/acetone = 88:12) to afford **12** (1.64 g, 1.95 mmol, 83%): mp = 145–149°C, $[\alpha]_D^{22} = +96.6$ (*c* 0.90; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.75 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 0.95 (d, 3H, CH_3 21), 1.00–2.45 (m, 25H, steroidal CH and CH_2, OH 7), 2.90 (s, 3H, N-CH_3), 3.80–4.05 (m, 3H, C$\underline{H}_2$CH=CH_2, CH 7), 4.55 (m, 1H, CH 3), 5.05–5.25 (m, 3H, CH=C$\underline{H}_2$, CH 12), 5.60–5.85 (ddt, 1H, C$\underline{H}$=CH_2), 6.91 (s, 1H, carbamate NH), 7.27–7.50 (m, 3H, naphthylics), 7.65–7.80 (m, 3H, naphthylics), 7.90 (s, 1H, naphthylic), 8.60 (d, 1H, 3,5-dinitrophenylic), 8.65 (t, 2H, 3,5-dinitrophenylics), 8.80 (s, 1H, carbamate NH); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.1, 17.8, 22.4, 22.9, 25.5, 27.1, 27.4, 27.5, 29.9, 30.6, 31.0, 31.3, 33.7, 34.6, 34.7, 34.8, 35.1, 35.7, 39.1, 41.1, 41.4, 45.2, 47.7, 47.9, 50.1, 52.2, 68.0 (C 7), 75.5 (C 12), 76.0 (C 3), 112.2, 114.6, 116.7 and 117.2 (=CH_2), 118.3, 119.1, 124.6, 126.5, 127.3, 127.4, 128.8, 130.0, 132.4, 132.8, 133.6, 135.6, 141.3, 141.4, 148.7, 152.9, 153.0 (carbamate C=O), 153.4 (carbamate C=O), 173.5 and 173.6 (amide C=O); IR (KBr, cm^{-1}): 3403, 2939, 1734, 1628, 1544, 1432, 1344, 1224, 1047, 910, 854, 807, 731.

4.7. *N*-Allyl-*N*′-methyl-3,7-bis(2-naphthyl)carbamoyloxy-12-(3,5-dinitrophenyl)carbamoyloxychol-an-24-amide, 13

2-Naphthylisocyanate (0.52 g, 3.08 mmol) and DMAP (13 mg, 0.10 mmol) were added to a solution of **12** (1.62 g, 1.93 mmol) in dry toluene (25 mL) and the resulting mixture was heated under reflux for 16 h, then allowed to cool to room temperature; the solvent was removed at reduced pressure, and the obtained solid was purified by flash chromatography (SiO_2, CH_2Cl_2/acetone = 95:5) affording **13** (1.11 g of 1.1 mmol, yield 57%): mp = 135–140°C, $[\alpha]_D^{22} = +63.1$ (*c* 0.91; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.77 (s, 3H, CH_3), 0.90 (d, 3H, CH_3 21), 0.95 (s, 3H, CH_3), 1.05–2.50 (m, 24H, steroidal CH and CH_2), 2.85 (s, 3H, N-CH_3), 3.70–4.05 (m, 2H, C$\underline{H}_2$CH=CH_2), 4.55 (m, 1H, CH 3), 5.05–5.25 (m, 4H, CH=C$\underline{H}_2$, CH 7 and 12), 5.65 (ddt, 1H, C$\underline{H}$=CH_2), 7.00–8.10 (m, 16H, naphthylics and carbamate NH), 8.20–8.70 (m, 3H, 3,5-dinitrophenyilics); ^{13}C NMR (50 MHz, DMSO-d_6, δ): 11.4, 17.1, 21.8, 22.2, 24.9, 26.3, 26.6, 28.2 29.1, 30.4, 31.1, 33.4, 34.1, 34.5, 37.3, 38.7, 39.1, 39.6, 40.0, 40.1, 40.3, 40.4, 42.5, 45.0, 46.6 and 46.9, 70.9 (C 7), 74.7 (C 12), 77.3 (C 3), 110.8, 113.8, 113.9, 114.3, 115.5, 117.1 and 117.4 (=CH_2), 119.3, 119.4, 119.5, 123.6, 125.6, 125.7, 126.4, 126.5, 126.6, 127.5, 127.7, 128.0, 128.1, 133.1, 133.2, 133.3, 136.3, 141.4, 148.1, 148.2, 152.8 (carbamate C=O), 152.9 (carbamate C=O), 171.7 (amide C=O); IR (KBr, cm^{-1}): 3290, 2937, 1733, 1635, 1606, 1544, 1506, 1432, 1344, 1223, 1046, 955, 854, 809, 747. Anal. calcd for $C_{57}H_{64}N_6O_{11}$: C, 67.84; H, 6.39; N, 8.33. Found: C, 67.98; H, 6.37; N, 8.21%.

4.8. *N*-Allyl-*N*′-methyl-3,12-dihydroxy-7-oxocholan-24-amide, 15

Cholic acid **6** (3.00 g, 7.34 mmol) was added to a solution of $NaHCO_3$ (120 mL, 0.372 M) warmed at about 60°C, until the solid was completely dissolved. After cooling to room temperature, NBS (3.27 g, 18.37 mmol) was added and the resulting mixture was stirred for 17 h at room temperature and for 2 h at 80–85°C, then allowed to cool to room temperature. The mixture was acidified with aqueous HCl (6 M, 100 mL): the yellow precipitate was isolated by filtration and washed with water, vigorously scratching. The product was dissolved in acetone and dried over anhydrous Na_2SO_4; the solvent was removed under reduced pressure affording the crude product **14** (3.27 g), which was then dissolved in dry dioxane (60 mL) and cooled to 10°C; dry tributylamine (1.9 mL, 8.00 mmol) was added; then ethyl chloroformate (0.8 mL, 8.00 mmol) in dry dioxane (5 mL) was dropwise added, and, after 10 min, *N*-methylallylamine (1.9 mL, 20.11 mmol) in dry dioxane (5.0 mL) was added dropwise. The resulting mixture was stirred at 10°C for 30 min and at room temperature overnight, then poured into water (100 mL), and the crude product was extracted with ethyl acetate (5×10 mL); the combined organic extracts were washed with a 10% aqueous HCl solution (2×10 mL), water, 10% $NaHCO_3$ solution and water, in that order, then dried over anhydrous Na_2SO_4. After removing the solvent at reduced pressure, pure **15** (2.80 g, 0.9 mmol, 83%) was obtained: mp = 71–75°C; $[\alpha]_D^{23} = -7.0$ (*c* 0.88; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.65 (s, 3H, CH_3), 0.95 (d, 3H, CH_3 21), 1.15 (s, 3H, CH_3), 0.90–2.50 (m, 25H, steroidal CH and CH_2, OH 3 and 12), 2.75–2.90 (m, 1H, CH 8), 2.95 (s, 3H, N-CH_3), 3.50–3.68 (m, 1H, CH 3), 3.90–4.05 (m, 3H, C$\underline{H}_2$CH=CH_2 and CH 12), 5.08–5.29 (m, 2H, C$\underline{H}_2$=CH), 5.65–5.90 (ddt, 1H, C$\underline{H}$=CH_2); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.7, 17.6, 22.8, 24.3, 27.6, 29.2, 29.7, 31.3, 34.1, 34.6, 35.2, 35.9, 37.3, 40.9, 45.3, 45.5, 46.0, 46.4, 46.5, 49.5, 70.9 (C 12), 72.0 (C 3), 116.9 and 117.0 (=CH_2), 132.8 and 133.0 (-CH=), 173.7 and 173.9 (amide C=O), 211.5 (carbonil C=O); IR (KBr, cm^{-1}): 3422, 2936, 1706, 1628, 1458, 1400, 1290, 1067, 923.

4.9. *N*-Allyl-*N*′-methyl-3,12-bis(2-naphthyl)carbamoyloxy-7-oxocholan-24-amide, 16

2-Naphthylisocyanate (2.01 g, 11.87 mmol) and DMAP (0.032 g, 0.25 mmol) were added to a solution of the amide **15** (2.18 g, 4.75 mmol) in dry toluene (65 mL) and the resulting mixture was heated under reflux for 23 h, then allowed to cool to room temperature; the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (SiO_2, CH_2Cl_2/acetone = 92:8) affording **37** (1.82 g, 2.28 mmol, 48%): mp = 137°C, $[\alpha]_D^{26} = +120.3$ (*c* 0.86; CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.78 (s, 3H, CH_3), 0.90 (d, 3H, CH_3 21), 1.20 (s, 3H, CH_3), 1.00–

2.40 (m, 23H, steroidal CH and CH_2), 2.90–2.94 (m, 4H, N-CH_3 and CH 8), 3.80–4.00 (m, 2H, $CH_2CH{=}CH_2$), 4.65 (m, 1H, CH 3), 5.00–5.25 (m, 3H, $CH{=}CH_2$, CH 12), 5.6–5.8 (ddt, 1H, $CH{=}CH_2$), 6.70 (s, 1H, carbamate NH), 7.06 (s, 1H, carbamate NH), 7.24–7.48 (m, 6H, naphthylics), 7.68–7.80 (m, 6H, naphthylics), 7.87 (s, 1H, naphthylic), 8.01 (s, 1H, naphthylic); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.6, 17.9, 22.7, 24.1, 26.3, 26.6, 27.5, 29.6, 30.0, 31.1, 33.5, 33.6, 34.7, 34.8, 37.0, 42.1, 45.0, 45.2, 45.3, 45.6, 45.7, 45.8, 46.9, 48.9, 49.2, 73.9 (C 12), 75.8 (C 3), 114.8, 114.9, 116.8, 116.9 119.2, 124.6, 124.7, 126..4, 126.6, 127.3, 127.4, 127.5, 128.7, 128.9, 130.2, 133.9, 135.3, 135.5, 152.9 (carbamate C=O), 153.0 (carbamate C=O), 173.3 (amide C=O), 211.1 (carbonil C=O); IR (KBr, cm^{-1}): 3284, 2942, 1718, 1630, 1607, 1585, 1543, 1507, 1473, 1432, 1358, 1232, 1052, 954, 854, 813, 746.

4.10. *N*-Allyl-*N*′-methyl-3,12-bis(2-naphthyl)carbamoyloxycholan-24-amide, 17

Compound **16** (1.79 g, 2.24 mmol) was dissolved in a mixture of dry THF (4 mL) and methanol (20 mL) cooled to 0°C, and $NaBH_4$ (0.13 g, 3.45 mmol) was 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 was dissolved in ethyl acetate; the solution was washed with 10% $NaHCO_3$ (2×30 mL) and brine (2×30 mL), then dried over anhydrous Na_2SO_4. After removing the solvent, **17** was obtained (1.54 g, 1.93 mmol, 86%): mp = 133–135°C, $[\alpha]_D^{24} = +125.1$ (*c* 1.47; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.80 (s, 3H, CH_3), 0.95 (s, 6H, CH_3 and CH_3 21), 1.00–2.50 (m, 25H, steroidal CH and CH_2, OH 7), 2.88 (s, 3H, N-CH_3), 3.75–4.00 (m, 3H, $CH_2CH{=}CH_2$, CH 7), 4.50–4.65 (m, 1H, CH 3), 5.00–5.25 (m, 3H, $CH{=}CH_2$, CH 12), 5.60–5.80 (ddt, 1H, $CH{=}CH_2$), 6.65 (s, 1H, carbamate NH), 7.05 (s, 1H, carbamate NH), 7.25–7.50 (m, 6H, naphthylics), 7.65–7.80 (m, 6H, naphthylics), 7.90 (s, 1H, naphthylic), 8.05 (s, 1H, naphthylic); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.3, 17.9, 22.5, 23.1, 25.8, 27.0, 27.3, 27.8, 31.1, 34.7, 34.8, 35.0, 35.7, 124.5, 124.6, 126.4, 126.5, 127.3, 127.4, 128.7, 128.8, 130.1, 130.2, 132.7, 133.9, 134.0, 135.6, 153.2 (carbamate C=O), 173.4 (amide C=O); IR (KBr, cm^{-1}): 3399, 3298, 3054, 2938, 2868, 1716, 1633, 1537, 1505, 1471, 1433, 1397, 1358, 1232, 1126, 1047, 954, 909, 853, 813, 745.

4.11. *N*-Allyl-*N*′-methyl-3,12-bis(2-naphthyl)carbamoyloxy-7-(3,5-dinitrophenyl)carbamoyloxy-cholan-24-amide, 18

3,5-Dinitrophenylisocyanate (1.03 g, 4.94 mmol) was added to a solution of **17** (1.52 g, 1.90 mmol) in dry toluene (25 mL), and the resulting mixture was heated under reflux for 22 h. After cooling to room temperature, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (SiO_2, $CHCl_3$/acetone = 90:10) to give **18** (1.57 g, 1.56 mmol, 82%): mp = 156°C, $[\alpha]_D^{33} = +95.2$ (*c* 0.93; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.75 (s, 3H, CH_3), 0.88 (d, 3H, CH_3 21), 0.95 (s, 3H, CH_3), 1.00–2.50 (m, 24H, steroidal CH and CH_2), 2.85 (s, 3H, N-CH_3), 3.70–4.00 (m, 2H, $CH_2CH{=}CH_2$), 4.50–4.75 (m, 1H, CH 3), 4.95–5.30 (m, 4H, $CH{=}CH_2$, CH 7 and 12), 5.52–5.68 (ddt, 1H, $CH{=}CH_2$), 7.10–8.70 (m, 20H, naphthylics and 3,5-dinitrophenylics, carbamate NH); ^{13}C NMR (50 MHz, $CDCl_3$, δ): 12.4, 17.8, 22.4, 23.0, 26.1, 27.1, 27.2, 27.5, 29.3, 29.4, 29.7, 30.3, 30.8, 31.0, 31.5, 33.7, 34.6, 34.7, 34.8 35.0, 37.9, 40.7, 44.0, 45.6, 47.2, 47.4, 50.1, 52.3, 73.8 (C 7), 75.8 (C 12), 76.4 (C 3), 111.8, 115.0, 116.8, 117.2 ($=CH_2$), 118.1, 119.1, 119.4, 124.7, 126.6, 127.2, 127.4, 127.6, 128.7, 128.8, 129.9, 130.0, 132.3, 132.7, 133.7, 133.9, 135.3, 135.9, 140.8, 148.2, 152.5 (carbamate C=O), 153.6 (carbamate C=O), 173.7 and 174.1 (amide C=O); IR (KBr, cm^{-1}): 3392, 3056, 2938, 1733, 1633, 1605, 1547, 1506, 1432, 1344, 1220, 1126, 1045, 955, 894, 853, 810, 730. Anal. calcd for $C_{57}H_{64}N_6O_{11}$: C, 67.84; H, 6.39; N, 8.33. Found: C, 67.76; H, 6.41; N, 8.25%.

4.12. Preparation of silane derivatives 19: representative procedure

3-Mercaptopropyltrimethoxysilane (0.8 mL, 4.25 mmol) was added to a solution of the selector (0.85 mmol) in $CHCl_3$ (10 mL) and the resulting mixture was heated under 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.

4.12.1. *N*-Methyl-*N*′-[(trimethoxysylylpropylthio)propyl]-3,7,12-tris(2-naphthyl)carbamoyloxycholan-24-amide, 19a. The mixture was heated under reflux for 20 h and 0.99 g of product **19a** was obtained: mp = 90–95°C; $[\alpha]_D^{26} = +94.6$ (*c* 0.91; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.70 (d, 2H, CH_2), 0.80 (s, 3H, CH_3), 0.90 (d, 3H, CH_3 21), 0.95 (s, 3H, CH_3), 1.05–2.60 (m, 34H, steroidal CH and CH_2, chain CH_2), 2.80 and 2.87 (s, 3H, N-CH_3), 3.30 (m, 2H, N-CH_2), 3.55 (s, 9H, $Si(OCH_3)_3$), 4.55 (m, 1H, CH 3), 5.00–5.25 (m, 2H, CH 7 and CH 12), 7.00–8.10 (m, 24H, naphthylics and NH); IR (KBr, cm^{-1}): 3299, 2939, 1726, 1635, 1605, 1542, 1506, 1432, 1359, 1228, 1072, 955, 854, 813, 747.

4.12.2. *N*-Methyl-*N*′-[(trimethoxysylylpropylthio)propyl]-3-(3,5-dinitrophenyl)carbamoyloxy-7,12-bis(2-naphthyl)-carbamoyloxylcholan-24-amide, 19b. The mixture was heated under reflux for 48 h and 0.96 g of product **19b** was obtained: mp = 110°C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.75 (d, 2H, CH_2), 0.85 (s, 3H, CH_3), 0.90 (d, 3H, CH_3 21), 0.95 (s, 3H, CH_3), 1.00–2.60 (m, 34H, steroidal CH and CH_2, chain CH_2), 2.80 and 2.85 (s, 3H, N-CH_3), 3.10–3.40 (m, 2H, N-CH_2), 3.50 (s, 9H, $Si(OCH_3)_3$), 4.55 (m, 1H, CH 3), 4.95–5.25 (m, 2H, CH 7 and CH 12), 7.10–8.10 (m, 16H, naphthylics and NH), 8.20–8.60 (m, 4H, 3,5-dinitrophenylics and NH).

4.12.3. *N*-Methyl-*N*′-[(trimethoxysylylpropylthio)-propyl]-3,7-bis(2-naphthyl)carbamoyloxy-12-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 19c. The mixture was heated under reflux for 20 h and 1.02 g of product **19c** was obtained: mp = 94°C; $[\alpha]_D^{25} = +42.3$ (*c* 0.87; CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.70 (d, 2H, CH_2), 0.75 (s, 3H, CH_3), 0.85 (d, 3H, CH_3 21), 0.95 (s, 3H, CH_3), 1.00–2.60 (m, 34H, steroidal CH and CH_2, aliphatic chain CH_2), 2.75 and 2.95 (s, 3H, N-CH_3), 3.10–3.40 (m, 2H, N-CH_2), 3.50 (s, 9H, Si$(OCH_3)_3$), 4.55 (m, 1H, CH 3), 4.90–5.25 (m, 2H, CH 7 and CH 12), 7.10–8.10 (m, 16H, naphthylics and NH 3 and 7), 8.50–8.80 (m, 4H, 3,5-dinitrophenylics and NH); IR (KBr, cm^{-1}): 3392, 2941, 1728, 1636, 1606, 1545, 1507, 1432, 1344, 1233, 1073, 955, 809, 748.

4.12.4. *N*-Methyl-*N*′-[(trimethoxysylylpropylthio)-propyl]-3,12-bis(2-naphthyl)carbamoyloxy-7-(3,5-dinitrophenyl)carbamoyloxycholan-24-amide, 19d. The mixture was heated under reflux for 48 h and 0.96 g of product **19d** was obtained: mp = 128–130°C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.80 (s, 3H, CH_3), 0.94 (d, 3H, CH_3 21), 0.98 (s, 3H, CH_3), 1.00–2.60 (m, 34H, steroidal CH and CH_2, chain CH_2), 2.60–2.95 (s, 3H, N-CH_3), 3.50 (s, 9H, Si$(OCH_3)_3$), 4.50–4.70 (m, 1H, CH 3), 5.00–5.25 (m, 2H, CH 7 and CH 12), 7.10–8.70 (m, 20H, aromatics and NH); IR (KBr, cm^{-1}): 3299, 2941, 1730, 1606, 1546, 1508, 1433, 1345, 1221, 1073, 956, 896, 854, 812, 730.

4.13. General procedure for the preparation of CSPs

A solution of the 3-mercaptopropyltrimethoxysilanic 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 under reflux, with gentle stirring, for 24 h. After cooling to room temperature, the silica was filtered and washed sequentially with toluene (3×30 mL), CH_2Cl_2 (3×30 mL), methanol (3×30 mL), THF (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, 18.07; H, 2.26; N, 1.37%; corresponding to 0.245 mmol/g.

CSP 3: C, 14.50; H, 1.93; N, 1.80% corresponding to 0.214 mmol/g.

CSP 4: C, 13.97; H, 1.91; N, 1.72% corresponding to 0.205 mmol/g.

CSP 5: C, 12.55; H, 1.84; N, 1.51% corresponding to 0.180 mmol/g.

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

Acknowledgements

This work was supported by MURST (Progetto Nazionale Stereoselezione in Sintesi Organica: Metodologie e Applicazioni).

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