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Synthesis of a new family of four deoxycholic acid derived chiral stationary phases and their evaluation in the HPLC resolution of racemic compounds

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Abstract

Four new chiral selectors obtained by suitable derivatization of the hydroxyl groups of the deoxycholic acid with two identical (homoderivatized) or different (heteroderivatized) arylisocyanates have been prepared and linked covalently to silica gel to obtain new chiral stationary phases (CSPs) for the HPLC separation of enantiomers. The CSPs containing two identical substituents are able to enantiodiscriminate different classes of racemic compounds, or the same racemates to a different extent, a property which depends on the different electronic character of the arylcarbamate moieties. The heteroderivatized CSPs retain the character of the two homoderivatized phases: however, the relative position of the two different arylcarbamate moieties on the deoxycholic backbone strongly affects the enantiodiscrimination capability of these two CSPs.

1. Introduction

One of the most simple, accurate and reliable methods for measuring the enantiomeric purity of organic compounds is HPLC on a chiral stationary phase (CSP), which allows the direct separation of enantiomers.[1] For this reason the last 15 years have witnessed the development and use of various CSPs based on cyclodextrines,[2] cellulose derivatives,[3] helical polymers,[4] proteins[5] and low molecular weight optically active compounds.[6] These last CSPs are very attractive not only for their effectiveness, but also because each molecule linked to the silica gel is considered to act independently (they are also named independent[7] CSPs), so that the factors governing the enantiodiscrimination process can be determined and, on this basis, the development of new, more efficient CSPs can be designed. Indeed, this has allowed

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the efficiency of some independent CSPs to be improved and new selectors, showing wider applicability, to be found.[6a]

It is now generally accepted that one interaction which plays an important role in the enantiodiscrimination process exhibited by the independent CSPs is the π–π one,[7] taking place between electronically complementary aromatic moieties present on the selector and the select and, therefore, a CSP showing wide applicability could be obtained by synthesizing a 'biselector system', i.e. a chiral molecule possessing two aromatic rings having different electronic properties, not interacting between themselves, so that they could be able to establish, independently, π–π enantioselective interactions with a wide range of racemic solutes. Of course, the biselector system must possess other functional groups as well, giving rise to further stereodependent interactions with the enantiomers of the analytes in order to have a full separation.[7]

Bile acids look very interesting candidates to act as scaffolds on which to attach the above mentioned aromatic rings. They possess a unique structure, endowed with hydroxyl groups having different reactivities, providing the opportunity for the attachment of molecular units: therefore new chiral molecules with properties determined by the nature and the arrangement of these units can be obtained. Indeed, bile acids have been used for preparing molecular tweezers,[8] supramolecular receptors,[9] chiral auxiliaries for asymmetric reactions,[10] scaffolds for combinatorial chemistry[11] and chiral stationary phases for HPLC.[12]

We addressed our attention to the use of deoxycholic acid **1**, one of the most common and readily available bile acids, for designing a series of chiral selectors for chromatography, in which structural and/or electronic perturbations could be introduced easily. Compound **1** possesses a carboxylic function, which can be exploited for linking to silica gel, and two hydroxyl groups, placed 6–6.5 Å apart[8] on a rigid backbone with different reactivities, owing to their different steric environments: by derivatizing the two hydroxyl moieties with two different aromatic groups, these would be separated sufficiently to act independently and the compound should behave as a biselector system (Fig. 1).

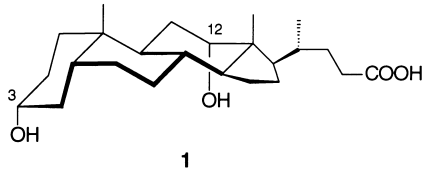

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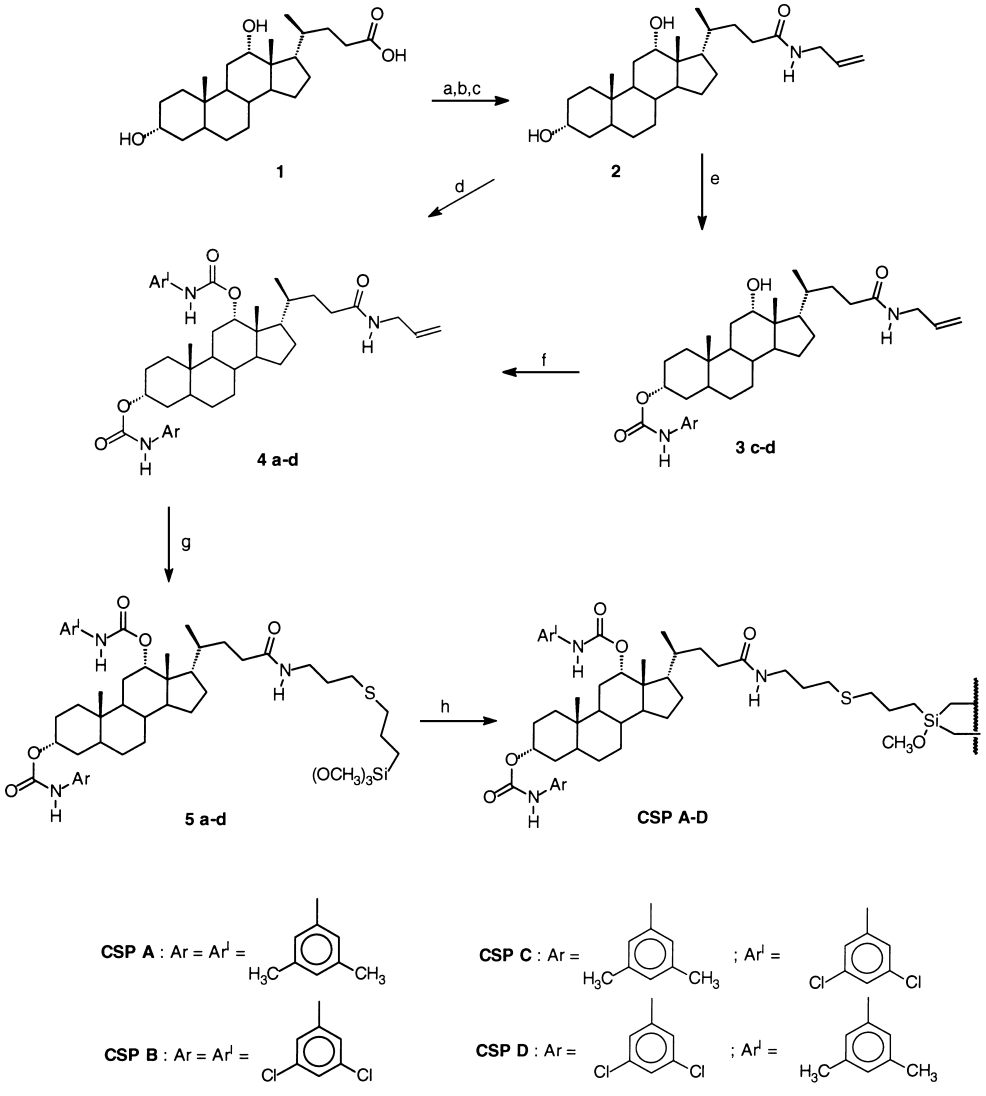
Figure 1.

We decided to derivatize the hydroxyl groups of **1** as arylcarbamates, given that this kind of derivatization allows aromatic rings having different electronic properties to be introduced and, at the same time, to have NH carbamate moieties able to afford hydrogen bonding interactions with the racemic selectants. 3,5-Dimethylphenylisocyanate was chosen as the reagent possessing an electron rich aromatic ring and 3,5-dichlorophenylisocyanate was the derivatizing agent characterized by an electron-poor aromatic moiety. We report herein the synthesis of a new family of four CSPs derived from deoxycholic acid and the preliminary results obtained in the separation of racemic compounds.

2. Results and discussion

2.1. Synthesis of the CSPs

Our synthetic route to the homoderivatized and heteroderivatized CSPs is summarized in Scheme 1.



Scheme 1. Reagents and conditions: (a) tributylamine, dioxane; (b) ethyl chloroformate, 10°C; (c) allylamine, 10°C to rt; (d) ArNCO (2.5 equiv.) toluene, reflux; (e) ArNCO (1.2 equiv.) toluene–THF, DMAP, rt; (f) Ar^1NCO (1.2 equiv.), toluene, reflux; (g) 3-mercaptopropyltrimethoxysilane (5 equiv.), ArBN $CHCl_3$, reflux; (h) silica gel, toluene, reflux

Deoxycholic acid was converted to the corresponding allylamide by the mixed anhydride method,[13] in 88% yield. This derivatization allows a terminal double bond to be introduced, which is well known to be a suitable moiety for linking chiral selectors to silica gel.[14] The amide bond thus introduced also acts as a protecting group for the carboxylic function in the following reactions: the amide function is hardly hydrolyzable and therefore very resistant to possible acidic or basic chromatographic conditions. Furthermore, given that it is well known that the best results of enantioseparation are achieved when the chiral system is remote from the silica gel,[7a] the conversion of the carboxylic function to the *N*-allylamide moiety, followed by reaction of the terminal double bond with mercaptopropyltrimethoxysilane, allows the chiral auxiliary to be connected to the silica gel by means of a long tether (11 units from silica gel to cholestanic skeleton), which is a sufficient distance of the selector from the supporting material.

The heteroderivatized selectors were obtained exploiting the different reactivity of the 3- and 12-

hydroxyl groups: in fact, by reacting **2** with an isocyanate at room temperature in the presence of a catalytic amount of DMAP, only the hydroxyl group at the position 3 is converted to the corresponding carbamate, whereas the reaction of the 12-hydroxyl moiety takes place only on warming the reaction mixture to reflux in toluene. This allows the two different heteroderivatized selectors to be obtained simply by changing the order of introduction of the arylisocyanates. As shown in Scheme 1, the homoderivatized selectors were obtained simply by reacting **2** with the arylisocyanate at reflux in toluene. The derivatization reaction of the 12-OH group for preparing both the homo- and the heteroderivatized selectors afforded the corresponding carbamates only in moderate yield (~60%), even if an excess of arylisocyanate was employed. This occurs because a by-product, originating from the attack of the isocyanate on the amide nitrogen of the 3-arylcarbamoyl derivative, is formed to a significant extent during the reaction: however, this did not constitute a serious drawback, because the by-product was easily removed by flash chromatography.†

In order to prepare the CSPs, selectors **4a**–**d** were reacted with a fivefold excess of mercaptopropyltrimethoxysilane in the presence of AIBN for 20 h. Under these experimental conditions **4a**–**d** were fully converted to the corresponding trimethoxysilyl derivatives, which were separated from the excess of mercaptopropyltrimethoxysilane simply by washing with pentane. The grafting to silica gel was carried out in toluene at reflux for 24 h and the CSPs A–D, after being thoroughly washed and dried, were packed into stainless steel columns of 25 or 15 cm (internal diameter 4.6 mm).

2.2. Use of CSPs A–D in the resolution of some racemic compounds

Compounds **6**–**13** (Fig. 2), being representative examples of different classes of analytes, were used to test the enantiodiscriminating properties of CSPs A–D.

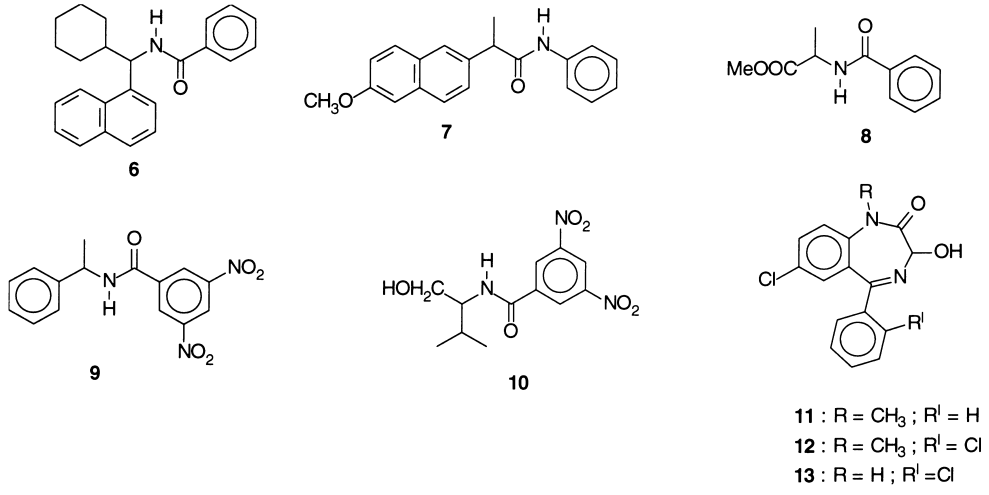


Figure 2.

As the data reported in Table 1 show, CSP B separates the π-donor racemic compounds **6**–**8** better than CSP A does, whereas it does not enantiodiscriminate π-acceptor analytes **9** and **10**, these being separated by CSP A. As far as the retention times are concerned, it is of note that π-donor racemic compounds **6**–**8** are strongly retained on CSP B, whereas lower k′ values are obtained on CSP A for the same analytes.

† The formation of the by-product could be avoided using the *N*-methyl-*N*′allylamide of the deoxycholic acid: work is in progress on this topic.

Table 1
Chromatographic resolution[a] of compounds **6–13** on CSPs **A–D**

		CSP A		CSP B		CSPC		CSP D		
entry	compound	k'[b]	α[c]	k'[b]	α[c]	k'[b]	α[c]	k'[b]	α[c]	eluent[d]
1	**6**	2.09	1.12	7.09[e]	1.45[e]	3.48	1.12	5.34	1.29	A
2	**7**	3.29	1.09	9.90	1.42	5.20	1.11	5.42	1.23	A
3	**8**	3.39	1.08	6.48	1.24	5.18	1.10	4.87	1.18	A
4	**9**	11.39	1.15	6.55	1.00	9.11[f]	1.07[f]	7.38	1.14	B
5	**10**	3.00	1.09	2.43	1.00	2.74	1.00	2.95	1.09	C
6	**11**	1.06	1.31	1.99	1.40	1.59	1.21	1.65	1.47	D
7	**12**	1.08	1.43	1.98	1.69	1.65	1.37	1.67	1.77	D
8	**13**	3.84	1.16	4.93	1.27	5.28	1.11	4.89	1.30	D

(a) chromatographic conditions : UV detection (λ=254 nm), t=25 °C, flow rate 1 ml/min (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 ; D = hexane-dichloromethane-propan-2-ol 70-30-3. (e) eluent B. (f) eluent hexane-dichloromethane-propan-2-ol 86-10-4.

On the contrary, π-acceptor racemates **9** and **10** are better retained on CSP A than on CSP B. Given that the only difference between these two phases is the electronic nature of the aryl moieties, linked to the deoxycholic skeleton by means of identical carbamate functions, the different retention times observed for the same compound on the two CSPs are certainly attributable to different π–π interactions, taking place between the aromatic ring of the selectant and those of the selectors. These results demonstrate the complementary characteristics of the deoxycholic based selectors, bearing the 3,5-dichlorophenyl and 3,5-dimethylphenyl moieties in the separation of racemic compounds possessing an aromatic π-donor or π-acceptor group.

The heteroderivatized CSP C, bearing a 3,5-dichlorophenylcarbamate moiety at position 12 and a 3,5-dimethylphenylcarbamate group at position 3, shows a low efficiency both in the separation of **6–8** with respect to CSP B and in the enantiodiscrimination of compound **9** with respect to CSP A; furthermore compound **10** is not resolved at all. The retention times for π-donor racemic compounds **6–8** are higher than those observed for the same analytes on CSP A and lower than those obtained on CSP B; likewise, the π-acceptor racemates **9** and **10** are better retained on CSP C than on CSP B, but less well retained than on CSP A. These data suggest that the two different aryl moieties of CSP C are still able to exhibit π–π interactions with the aromatic complementary groups present on the substrates, although, as the k′ values indicate, to a less extent than the homoderivatized phases. However, whilst the role played by the 3,5-dichlorophenylcarbamate moiety at position 12 in the enantiodiscrimination process of CSP C is preserved (even if the separations are poorer than CSP B, which possesses two 3,5-dichlorophenylcarbamate groups), the effect of the 3,5-dimethylphenylcarbamate moiety at position 3 is almost completely lost.

Interestingly, CSP D, the mixed phase bearing a 3,5-dichlorophenylcarbamate moiety at position 3 and a 3,5-dimethylphenylcarbamate group at position 12, shows, as expected, the same characteristics of CSP C, as far as the retention times are concerned, the k′ values being similar to those obtained with CSP C, but behaves quite differently with respect to CSP C as far as the enantiodiscrimination process is concerned. This phase is able to separate both π-donor and π-acceptor analytes: its enantiodiscriminating capability towards π-acceptor compounds **9** and **10** remains almost the same as CSP A, notwithstanding that it possesses only one 3,5-dimethylphenylcarbamate moiety, whereas it shows a slight decrease towards π-donor analytes **6–8** with respect to CSP B. This means that the chiral auxiliary of CSP D acts as a biselector system, being able to enantiodiscriminate racemic compounds possessing both π-donor and π-acceptor aromatic rings.

As far as the separation of the benzodiazepine enantiomers **11–13** is concerned, the data reported

in Table 1 show that this new family of selectors exhibits quite good enantioselectivity towards these pharmaceuticals, the α values ranging from 1.11 to 1.77: furthermore all the CSPs show the same trend in the enantioseparation of these racemates, the best separation being achieved always in the case of compound **12**.

Comparing the enantiodiscrimination capability of CSP A and CSP B towards the benzodiazepines, we can observe that both the CSPs work rather well with this kind of racemic compound, although CSP B shows higher enantioselectivity. This means that both 3,5-dichlorophenylcarbamate and 3,5-dimethylphenylcarbamate linked to the deoxycholic scaffold constitute chiral selectors suitable for the enantioseparation of the benzodiazepines, even if to a different extent. Interestingly, CSP B, which affords better separations than CSP A, also shows higher retention times than CSP A, suggesting that the derivatization with the 3,5-dichlorophenylisocyanate is more suitable to afford stronger and more enantioselective interactions between the chiral selector and the benzodiazepine selectants.

The retention times of the benzodiazepines with the two heteroderivatized CSPs are intermediate between those obtained for CSP A and those observed for CSP B: therefore CSP C and CSP D behave, as far as the retention is concerned, toward the benzodiazepines just as toward the racemates **6**–**10**.

As far as the enantiodiscriminating capability is concerned, CSP C shows lower α values for all the benzodiazepines than CSP A and CSP B, whereas the enantioseparations obtained with CSP D are the most efficient. Given that CSP C and CSP D are different only in the relative position of the two aryl groups, the different behaviour exhibited in the separation of the benzodiazepine enantiomers is certainly attributable only to the different location of the two aryl moieties in the deoxycholic skeleton. So, when the 3,5-dimethylphenylcarbamate moiety is located at position 12 and 3,5-dichlorophenylcarbamate at position 3, as in CSP D, the action of the two aryl groups on the enantioseparation process is reinforced, whereas, when their position is exchanged, as in CSP C, they cannot work in a synergetic way, giving rise to worse separations.

In conclusion, starting from deoxycholic acid, a new family of efficient chiral selectors, containing two identical or different aromatic substituents, has been synthesized and used for the HPLC separation of racemic compounds. The results obtained in the enantioseparation of selected racemic compounds have allowed us to establish the complementary character of the selectors containing two 3,5-dimethylphenylcarbamate or two 3,5-dichlorophenylcarbamate moieties toward the enantioseparation of π-donor and π-acceptor analytes.

Deoxycholic acid has been shown to be suitable for the preparation of heteroderivatized selectors, which have been obtained by derivatizing the two hydroxyl groups with two different arylisocyanates. The two heteroderivatized selectors have been found to be non-equivalent in the enantioseparation of racemic compounds, their effectiveness depending on the relative position of the two different carbamate moieties on the deoxycholic backbone. Only when the two derivatizing groups take ‘matched’[15] positions, as in CSP D, does the chiral auxiliary behave as a biselector system, being able to separate both π-donor and π-acceptor analytes. On the contrary, if the two different arylcarbamates take ‘mismatched’[15] positions, as in CSP C, poorer separations are obtained.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-200 or a Bruker 200 MHz NMR spectrometer, using TMS as external standard. The following abbreviations are used: s=singlet,

d=doublet, dd=double doublet, t=triplet, m=multiplet, br=broad. TLC analyses were performed on silica gel 60 Macherey–Nagel sheets; flash chromatographic separations were carried out on adequate dimension columns using silica gel 60 (230–400 mesh). HPLC analyses were performed on a JASCO PU-980 intelligent HPLC pump equipped with a JASCO UV-975 detector. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Melting points were taken using a Kofler Reichert–Jung apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer 1710 spectrophotometer. Toluene, THF and dioxan were refluxed over sodium benzophenone and distilled before use. Allylamine and tributylamine were distilled over CaH_2. Unless otherwise specified the reagents were used without any purification.

Standard procedures were used for preparing racemic amides, the DNB derivative of the valinol and the anilide of naproxen.[16]

3.2. N-*Allyl-deoxycholan-24-amide* ***2***

To a solution of 6 g (0.0153 mol) of deoxycholic acid in 150 ml of dry dioxan, dry tri-*n*-butylamine (3.64 ml, 0.0153 mol) was added and the solution was kept at 10°C. Ethylchloroformate (1.47 ml, 0.0153 mol) in dry dioxan (5 ml) was added dropwise (10 min) and the reaction mixture stirred for 10 min at the same temperature. Dry allylamine (2.8 ml, 0.038 mol) in dry dioxan (5 ml) was added dropwise at 10°C and the mixture was stirred for 30 min at 10°C, then at room temperature for 2.5 h.

The reaction mixture was poured in water (400 ml) and the product was extracted with ethyl acetate (4×100 ml). The collected organic extracts were washed with 10% hydrochloric acid, water, a saturated solution of $NaHCO_3$ and water, in that order, then dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (SiO_2, dichloromethane:ethanol 88:12) affording 5.7 g (0.0134 mol, 88% yield) of the amide. Mp 89–90°C. $[\alpha]_D^{21}$ +39.7 (c 1.02, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 5.75 (m, 1H, CH=); 5.48 (t, 1H, NH); 5.08 (m, 2H, $=CH_2$); 3.89 (br t, 1H, H12); 3.81 (tt, 2H, C*H*$_2$-NH); 3.53 (m, 1H, H3); 2.10–0.75 (m, 28H, steroidal CH and CH_2 and OH); 0.92 (d, 3H, $CH_3$20); 0.82 (s, 3H, CH_3); 0.60 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.53 (C=O), 134.44 (*C*=CH_2), 116.07 (C=*C*H_2), 73.04 (C12), 71.60 (C3), 48.09, 46.98, 46.48, 42.09, 41.83, 36.38, 36.00, 35.26, 34.11, 33.58, 33.36, 31.69, 30.41, 28.56, 27.49, 27.15, 26.13, 23.66, 29.08, 17.38, 12.67. IR (KBr): 3380, 3310, 2925, 2861, 1645, 1549, 1017. Anal. calcd for $C_{27}H_{45}NO_3$: C, 75.13; H, 10.51; N, 3.24. Found: C, 75.17; H, 10.53; N, 3.25.

3.3. *3-Arylcarbamoyl*-N-*allyl-deoxycholan-24-amide* ***3c,d****: general procedure*

To a solution of 3.7 mmol of **1** in dry THF (50 ml) 4.5 mmol of arylisocyanate and 25 mg (0.2 mmol) of DMAP were added and the mixture was stirred at room temperature for 15 h. The THF was evaporated under reduced pressure and the crude product was purified by column chromatography (SiO_2, dichloromethane:acetone 90:10).

3.3.1. *3-(3,5-Dimethylphenyl)carbamoyl*-N-*allyl-deoxycholan-24-amide* ***3c***

Yield 1.7 g, 78%. Mp 120–122°C. $[\alpha]_D^{25}$=61.7 (c=1.02, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.04 (s, 2H, aromatics); 6.65 (s, 2H, aromatic and NH carbamate); 5.75 (m, 1H, CH=); 5.62 (t, 1H, NH); 5.08 (m, 2H, $=CH_2$); 4.58 (m, 1H, H3); 3.89 (br t, 1H, H12); 3.81 (t, 2H, C*H*$_2$-NH); 2.10–0.75 (m, 27H, steroidal CH and CH_2 and OH); 0.92 (d, 3H, $CH_3$20); 0.82 (s, 3H, CH_3); 0.60 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.56 (amide C=O), 153.45 (carbamate C=O), 138.42 (aromatic), 138.03 (aromatic) 134.30 (*C*=CH_2), 124.74 (aromatic), 116.42 (aromatic), 116.09 (C=*C*H_2), 74.90 (C3), 73.10

(C12), 48.16, 47.10, 46.42, 41.64, 35.67, 35.07, 34.85, 34.03, 33.57, 33.21, 32.43, 31.54, 28.56, 27.44, 26.88, 26.73, 26.00. 23.56, 22.94, 21.27, 17.30, 12.61. IR (KBr): 3335, 3077, 2936, 2861, 1713, 1653, 1616, 1540, 1448, 1373, 1330, 1270, 1222, 1081, 1017. Anal. calcd for $C_{36}H_{54}N_2O_4$: C, 74.70; H, 9.40; N, 4.84. Found: C, 74.75; H, 9.42; N, 4.85.

3.3.2. 3-(3,5-Dichlorophenyl)carbamoyl-N-*allyl-deoxycholan-24-amide* ***3d***

Yield 1.6 g, 70%. Mp 118–120°C. $[\alpha]_D^{24}$=56.6 (c=1.12, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$ δ): 7.95 (br s, 1H, NH carbamate); 7.42 (d, 2H, aromatics); 6.92 (t, 1H, aromatic) 5.75 (m, 2H, CH= and NH amide); 5.08 (m, 2H, $=CH_2$); 4.61 (m, 1H, H3) 3.89 (t, 1H, H12); 3.81 (t, 2H, CH_2-NH); 2.10–0.75 (m, 27H, steroidal CH and CH_2 and OH); 0.92 (d, 3H, $CH_3$20); 0.82 (s, 3H, CH_3); 0.60 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$ δ): 173.68 (amide C=O), 153.14 (carbamate C=O), 140.77 (aromatic), 135.07 (aromatic) 134.15 (*C*=CH_2), 122.66 (aromatic), 116.66 (aromatic), 116.40 (C=*C*H_2), 75.44 (C3), 73.31 (C12), 48.25, 47.41, 46.56, 42.00, 41.82, 35.33, 34.67, 34.15, 33.71, 33.45, 32.45, 31.72, 28.66, 27.66, 26.92, 26.53, 26.10, 23.66, 23.01, 17.32, 12.73. IR (KBr): 3284, 3077, 2935, 2870, 1715, 1655, 1590, 1535, 1443, 1405, 1372, 1307, 12407, 1214, 1110, 1061, 1023, 985, 920. Anal. calcd for $C_{34}H_{48}Cl_2N_2O_4$: C, 65.90; H, 7.81; Cl, 11.44; N, 4.52. Found: C, 65.94; H, 7.82; Cl, 11.47; N, 4.54.

3.3.3. 3,12-Diarylcarbamoyl-N-*allyl-deoxycholan-24-amide* ***4a***,***b****: general procedure*

A mixture of 3 mmol of **1**, dry toluene (25 ml) and arylisocyanate (10.2 mmol) was stirred under reflux for 20 h. The toluene was removed under vacuum and the crude product was purified by column chromatography (SiO_2, dichloromethane:acetone 95:5).

3.3.4. 3,12-Bis-(3,12-dimethylphenyl)carbamoyl-N-*allyl-deoxycholan-24-amide* ***4a***

Yield 1.4 g, 64%. Mp 128–130°C. $[\alpha]_D^{24}$=90.3 (c=0.975, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.04 (s, 2H, aromatics); 6.89 (s, 2H, aromatics); 6.65 (br s, 1H, NH carbamate); 6.65 (s, 1H, aromatic); 6.60 (s, 1H, aromatic); 6.48 (br s, 1H, NH carbamate); 5.72 (m, 1H, CH=); 5.52 (br t, 1H, NH amide); 5.08 (m, 3H, $=CH_2$ and H12); 4.58 (m, 1H, H3); 3.78 (t, 2H, CH_2-NH); 2.20 (s, 6H CH_3); 2.18 (s, 6H, CH_3); 2.10–0.75 (m, 26H, steroidal CH and CH_2); 0.82 (s, 3H, CH_3); 0.79 (d, 3H, $CH_3$20); 0.68 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.27 (amide C=O), 153.10 (carbamate C=O), 138.52 (aromatic), 138.45 (aromatic), 138.04 (aromatic), 137.80 (aromatic), 134.26 (*C*=CH_2), 124.74 (aromatic), 116.39 (aromatic), 116.14 (aromatic) 116.01 (C=*C*H_2), 76.37 (C12), 75.00 (C3), 48.38, 47.64, 45.12, 41.75, 35.47, 34.31, 34.48, 34.26, 33.66, 33.45, 32.56, 31.44, 27.35, 26.80, 25.86, 25.79, 23.37, 22.83, 21.28, 17.59, 12.41. IR (KBr): 3342, 3075, 2936, 2861, 1715, 1656, 1634, 1608, 1554, 1544, 1447, 1378, 1327, 1268, 1223, 1191, 1159, 1081, 1012. Anal. calcd for $C_{45}H_{63}N_3O_5$: C, 74.45; H, 8.75; N, 5.79. Found: C, 74.49; H, 8.77; N, 5.80.

3.3.5. 3,12-Bis-(3,5-dichlorophenyl)carbamoyl-N-*allyl-deoxycholan-24-amide* ***4b***

Yield 1.44 g, 62%. Mp 148–151°C. $[\alpha]_D^{25}$=85.0 (c=1, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.42 (d, 2H, aromatics); 7.29 (d, 3H, aromatics and NH carbamate); 6.98 (t, 1H, aromatic); 6.96 (t, 1H, aromatic); 6.83 (br s, 1H, NH carbamate); 5.68 (m, 2H, CH= and NH amide); 5.05 (m, 3H, $=CH_2$ and H12); 4.54 (m, 1H, H3); 3.78 (t, 2H, CH_2-NH); 2.30–0.75 (m, 26H, steroidal CH and CH_2); 0.82 (s, 3H, CH_3); 0.78 (d, 3H, $CH_3$20); 0.64 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.47 (amide C=O), 152.80 (carbamate C=O), 152.71 (carbamate C=O), 140.49 (aromatic), 140.05 (aromatic), 135.26 (aromatic), 135.16 (aromatic), 133.97 (*C*=CH_2), 122.98 (aromatic), 122.88 (aromatic), 117.01 (aromatic), 116.64 (aromatic) 116.45 (C=*C*H_2), 77.37 (C12), 76.07 (C3), 49.51, 47.62, 45.19, 41.91, 41.79, 35.46, 34.66, 34.41, 34.32, 33.65, 33.56, 32.52, 31.51, 27.38, 26.64, 25.88, 25,66, 23.37, 22.81,

17.61, 12.42. IR (KBr): 3438, 3075, 2936, 2861, 1715, 1649, 1592, 1533, 1447, 1410, 1250, 1212. Anal. calcd for $C_{41}H_{51}Cl_4N_3O_5$: C, 60.97; H, 6.36; Cl, 17.56; N, 5.20. Found: C, 61.00; H, 6.37; Cl, 17.59; N, 5.22.

3.4. 3-Arylcarbamoyl-12-arylcarbamoyl′-N-allyl-deoxycholan-24-amide: general procedure

To a solution of **3** (2.2 mmol) in dry toluene (25 ml), the arylisocyanate (2.7 mmol) was added and the solution was stirred at reflux for 24 h. The toluene was evaporated at reduced pressure and the crude product was purified by flash chromatography (SiO_2, dichloromethane:acetone 95:5).

*3.4.1. 3-(3,5-Dimethylphenyl)carbamoyl-12-(3,5-dichlorophenyl)carbamoyl-N-allyl-deoxycholan-24-amide **4c***

Yield 1.1 g, 65%. Mp 130–132°C. $[\alpha]_D^{25}$=86.1 (c=1.03, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.42 (br s, 3H, aromatic and NH carbamate); 6.89 (br s, 3H, aromatics); 6.60 (s, 1H, aromatic); 6.55 (br s, 1H, carbamate); 5.72 (m, 1H, CH); 5.52 (br t, 1H, NH amide); 5.08 (m, 3H, =CH_2 and H12); 4.58 (m, 1H, H3); 3.78 (t, 2H, C*H*$_2$-NH); 2.20 (s, 6H CH_3); 2.10–0.75 (m, 26H, steroidal CH and CH_2); 0.79 (d, 3H, $CH_3$20); 0.77 (s, 3H, CH_3); 0.68 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.31 (amide C=O), 153.33 (carbamate C=O), 152.34 (carbamate C=O), 140.63 (aromatic), 136.63 (aromatic), 137.64 (aromatic), 135.03 (aromatic), 134.13 (*C*=CH_2), 124.93 (aromatic), 122.63 (aromatic), 117.03 (aromatic), 116.31 (aromatic), 116.4 (10C=*C*H_2), 76.98 (C12), 75.34 (C3), 49.22, 47.77, 45.09, 41.67, 35.34, 34.95, 34.25, 33.96, 33.76, 33.67, 32.71, 31.48, 27.44, 27.06, 26.90, 25.97, 25.39, 23.32, 22.83, 21.38, 17.60, 12.47. IR (KBr): 3321, 3075, 2957, 2861, 1715, 1651, 1616, 1589, 1541, 1450, 1407, 1375, 1260, 1242, 1218, 1194, 1159, 1114, 1087. Anal. calcd for $C_{43}H_{57}Cl_2N_3O_5$: C, 67.35; H, 7.49; Cl, 9.25; N, 5.48. Found: C, 67.39; H, 7.51; Cl, 9.27; N, 5.51.

*3.4.2. 3-(3,5-Dichlorophenyl)carbamoyl-12-(3,5-dimethylphenyl)carbamoyl-N-allyl-deoxycholan-24-amide **4d***

Yield 1.0 g, 60%. Mp 137–139°C. $[\alpha]_D^{25}$=77.9 (c=0.78, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): 7.22 (d, 2H, aromatics); 7.02 (s, 2H, aromatics); 6.92 (d, 1H, aromatic); 6.71 (br s, 1H, carbamate); 6.69 (br s, 1H, NH carbamate); 6.62 (s, 1H, NH carbamate); 5.72 (m, 1H, CH=); 5.49 (br s, 1H, NH amide); 5.05 (m, 3H, =CH_2 and H12); 4.55 (m, 1H, H3); 3.69 (t, 2H, C*H*$_2$-NH); 2.22 (s, 6H CH_3); 2.10–0.75 (m, 26H, steroidal CH and CH_2); 0.84 (s, 3H, CH_3); 0.71 (d, 3H, CH_3); 0.68 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.31 (amide C=O), 153.33 (carbamate C=O), 152.34 (carbamate C=O), 140.63 (aromatic), 136.63 (aromatic), 137.64 (aromatic), 135.03 (aromatic), 134.13 (*C*=CH_2), 124.93 (aromatic), 122.63 (aromatic), 117.03 (aromatic), 116.31 (aromatic), 116.4 (10C=*C*H_2), 76.98 (C12), 75.34 (C3), 49.60, 47.69, 45.22, 41.99, 41.80, 35.63, 34.96, 34.66, 34.44, 34.04, 33.54, 32.46, 31.56, 27.40, 26.85, 26.74, 26.01, 25.86, 23.47, 22.90, 21.32, 17.66, 12.46. IR (KBr): 3438, 3075, 2936, 2862, 1711, 1647, 1590, 1536, 1449, 1409, 1296, 1243, 1216, 1190. Anal. calcd for $C_{43}H_{57}Cl_2N_3O_5$: C, 67.35; H, 7.49; Cl, 9.25; N, 5.48. Found: C, 67.40; H, 7.52; Cl, 9.27; N, 5.50.

*3.5. 3,12-Diarylcarbamoyl-N-(3-trimethoxysilylpropylthio)propyl-deoxycholan-24-amide **5a–d**: general procedure*

To a solution of **5**, 1.4 mmol in dry $CHCl_3$ (10 ml) freshly distilled 3-mercaptopropyltrimethoxysilane (1.3 ml, 7 mmol) and AIBN (0.05 g, 0.3 mmol) were added and the mixture was stirred under reflux for 20 h. The solvent was eliminated by evaporation under reduced pressure and the residual oil was

dispersed in pentane (40 ml), giving a solid which was filtered and washed with pentane (5×30 ml). The pure product **5a**–**d** was obtained in quantitative yield.

*3.5.1. 3,12-Bis-(3,5-dimethylphenyl)carbamoyl-*N*-(3-trimethoxysilylpropylthio)propyl-deoxycholan-24-amide **5a***

Yield 1.29 g. Mp 89–91°C. $[\alpha]_D^{26}$=71.6 (c=1, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): 7.04 (s, 2H, aromatic); 6.89 (s, 2H, aromatic); 6.65 (br s, 1H, NH carbamate); 6.65 (s, 1H, aromatic); 6.60 (s, 1H, aromatic); 6.48 (sl s, 1H, NH carbamate); 5.52 (br s, 1H, NH amide); 5.08 (br s, 1H, H12); 4.58 (m, 1H, H3); 3.57 (s, 9H, OCH3); 3.33 (dd, 2H, CH_2-CO); 2.55 (t, 4H, CH_2-S); 2.20 (s, 6H CH_3); 2.18 (s, 6H, CH_3); 2.10–0.75 (m, 32H, steroidal CH and CH_2, chain CH_2); 0.82 (s, 3H, CH_3); 0.79 (d, 3H, $CH_3$20); 0.68 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.43 (amide C=O), 153.11 (carbamate C=O), 138.76 (aromatic), 138.64 (aromatic), 138.00 (aromatic), 137.82 (aromatic), 124.96 (aromatic), 116.31 (aromatic), 76.74 (C12), 75.12 (C3), 50.51, 50.46, 49.56, 47.66, 45.27, 41.61, 38.66, 35.64, 34.36, 34.74, 34.43, 34.07, 33.60, 32.59, 31.60, 29.41, 29.21, 27.44, 26.88, 26.03, 25.67, 23.49, 22.98, 21.36, 21.32, 17.66, 12.47. IR (KBr): 3331, 3075, 2936, 2861, 1725, 1704, 1650, 1613, 1549, 1447, 1378, 1271, 1223, 1191, 1159, 1079, 1015. Anal. calcd for $C_{51}H_{79}N_3O_8SSi$: C, 66.41; H, 8.63; N, 4.56; S, 3.48; Si, 3.04. Found: C, 66.45; H, 8.65; N, 4.58; S, 3.45; Si, 3.05.

*3.5.2. 3,12-Bis-(3,5-dichlorophenyl)carbamoyl-*N*-(3-trimethoxysilylpropylthio)propyl-deoxycholan-24-amide **5b***

Yield 1.4 g. Mp 110–112°C. $[\alpha]_D^{28}$=78.3 (c=0.95, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): 7.42 (d, 2H, aromatics); 7.29 (d, 3H, aromatics and NH carbamate); 6.98 (t, 1H, aromatic); 6.96 (t, 1H, aromatic); 6.78 (br s, 1H, NH carbamate); 5.60 (t, 1H, NH amide); 5.02 (br, 2H, H12); 4.54 (m, 1H, H3); 3.48 (s, 9H, OCH_3); 3.26 (dd, 2H, CH_2-NH); 2.48 (t, 4H, CH_2-S); 2.30–0.75 (m, 32H, steroidal CH and CH_2, chain CH_2); 0.82 (s, 3H, CH_3); 0.78 (d, 3H, $CH_3$20); 0.64 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.64 (amide C=O), 152.76 (carbamate C=O), 152.63 (carbamate C=O), 140.33 (aromatic), 140.02 (aromatic), 135.28 (aromatic), 135.20 (aromatic), 123.13 (aromatic), 116.96 (aromatic), 116.68 (aromatic) 76.39 (C12), 76.00 (C3), 50.54, 49.65, 47.69, 45.28, 41.79, 36.76, 35.58, 34.99, 34.48, 34.02, 33.69, 33.47, 31.62, 29.47, 29.13, 27.51, 26.69, 25.91, 23.44, 22.95, 18.30, 17.65, 12.42. IR (KBr): 3342, 3085, 2936, 2861, 1734, 1709, 1653, 1634, 1589, 1538, 1449, 1437, 1410, 1378, 1306, 1247, 1215, 1191, 1162, 1114, 1071. Anal. calcd for $C_{47}H_{67}Cl_4N_3O_8SSi$: C, 56.23; H, 6.73; Cl, 14.12; N, 4.19; S, 3.19; Si, 2.80. Found: C, 56.26; H, 6.74; Cl, 14.15; N, 4.20; S, 3.18; Si, 2.81.

*3.5.3. 3-(3,5-Dimethylphenyl)carbamoyl-12-(3,5-dichlorophenyl)carbamoyl-*N*-(3-trimethoxysilyl-propylthio)propyl-deoxycholan-24-amide **5c***

Yield 1.34 g. Mp 107–110°C. $[\alpha]_D^{25}$=60.0 (c=0.98, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.42 (br s, 3H, aromatic and NH carbamate); 6.89 (br s, 3H, aromatic); 6.60 (s, 1H, aromatic); 6.55 (br s, 1H, carbamate); 5.42 (br s, 1H, NH amide); 5.08 (br s, 1H, H12); 4.58 (m, 1H, H3); 3.57 (s, 9H, OCH_3); 3.33 (dd, 2H, CH_2-CO); 2.55 (t, 4H, CH_2-S); 2.10–0.75 (m, 32H, steroidal CH and CH_2, chain CH_2); 0.79 (d, 3H, $CH_3$20); 0.77 (s, 3H, CH_3); 0.68 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.65 (amide C=O), 153.34 (carbamate C=O), 152.67 (carbamate C=O), 138.56 (aromatic), 137.61 (aromatic), 135.04 (aromatic), 124.89 (aromatic), 122.51 (aromatic), 116.96 (aromatic), 116.17 (aromatic), 76.99 (C12), 75.26 (C3), 50.47, 49.25, 47.57, 45.11, 41.81, 38.68, 35.38, 34.96, 34.29, 35.80, 33.67, 32.71, 31.53, 29.34, 29.09, 27.44, 27.02, 26.68, 25.93, 25.50, 23.34, 22.84, 21.38, 17.58, 12.44. IR (KBr): 3326, 3085, 2957, 2861, 1731, 1704, 1650, 1586, 1533, 1442, 1410, 1323, 1260, 1218, 1191, 1154, 1095, 1015.

Anal. calcd for $C_{49}H_{73}Cl_2N_3O_8SSi$: C, 61.10; H, 7.64; Cl, 7.36; N, 4.36; S, 3.33; Si, 2.92. Found: C, 61.16; H, 7.63; Cl, 7.34; N, 4.37; S, 3.32; Si, 2.90.

*3.5.4. 3-(3,5-Dichlorophenyl)carbamoyl-12-(3,5-dimethylphenyl)carbamoyl-*N*-(3-trimethoxysilyl-propylthio)propyl-deoxycholan-24-amide* ***5d***

Yield 1.34 g. Mp 105–107°C. $[\alpha]_D^{25}$=77.3 (c=0.85, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): 7.21 (d, 2H, aromatic); 7.02 (s, 2H, aromatic); 6.92 (t, 1H, aromatic); 6.80 (br s, 1H, carbamate); 6.72 (br s, 1H, carbamate); 6.62 (s, 1H, aromatic); 5.64 (br s, 1H, NH amide); 5.00 (br s, 1H, H12); 4.55 (m, 1H, H3); 3.50 (s, 9H, OCH3); 3.24 (dd, 2H, CH_2-NH); 2.46 (t, 4H, CH_2-S); 2.10–0.75 (m, 32H, steroidal CH and CH_2, chain CH_2); 0.84 (s, 3H, CH_3); 0.71 (d, 3H, CH_3); 0.68 (s, 3H, CH_3). ^{13}C NMR (200 MHz, $CDCl_3$, δ): 173.46 (amide C=O), 153.24 (carbamate C=O), 152.51 (carbamate C=O), 140.56 (aromatic), 138.60 (aromatic), 137.24 (aromatic), 135.20 (aromatic), 124.96 (aromatic), 123.00 (aromatic), 116.57 (aromatic), 116.36 (aromatic), 76.73 (C12), 75.66 (C3), 49.54, 47.59, 45.23, 41.73, 38.64, 35.58, 34.94, 34.63, 34.39, 34.01, 33.54, 32.40, 31.34, 29.36, 29.16, 27.42, 26.81, 26.72, 26.01, 25.80, 23.45, 22.92, 22.84, 21.34, 17.69, 12.43. IR (KBr): 3331, 3085, 2936, 2861, 1720, 1651, 1586, 1538, 1453, 1410, 1373, 1245, 1218, 1191, 1079. Anal. calcd for $C_{49}H_{73}Cl_2N_3O_8SSi$: C, 61.10; H, 7.64; Cl, 7.36; N, 4.36; S, 3.33; Si, 2.92. Found: C, 61.14; H, 7.62; Cl, 7.38; N, 4.37; S, 3.34; Si, 2.93.

3.6. Preparation of the CSPs A–D: general procedure

A solution of the silane **5** (12 mmol) in 15 ml of dry toluene was added dropwise to 3.5 g of spherical silica gel (200 Å, 5 μm), previously dried at 180°C at 0.1 mm Hg for 15 h, slurried in 15 ml of dry toluene and the mixture was gently stirred at reflux for 24 h. The mixture, cooled at room temperature, was filtered and washed with toluene (3×30 ml), dichloromethane (3×30 ml), methanol (3×30 ml), THF (3×30 ml) and pentane (3×30 ml), then dried at 50°C at 0.1 mm Hg.

The amount of chiral selector linked to silica gel was determined by elemental analysis.

CSP A: C% 9.36; H% 1.44; N% 0.63 corresponding to 0.155 mmol/g and 0.442 μmol/m^2.

CSP B: C% 10.11; H% 1.34; N% 0.68; Cl% 2.45 corresponding to 0.173 mmol/g and 0.494 μmol/m^2.

CSP C: C% 8.62; H% 1.30; N% 0.61 corresponding to 0.148 mmol/g and 0.423 μmol/m^2.

CSP D: C% 9.51; H% 1.38; N% 0.57 Cl% 1.16 corresponding to 0.154 mmol/g and 0.440 μmol/m^2.

The derivatized silica gels were slurried in dichloromethane and packed into 25 cm (15 cm for CSP D) stainless steel columns at 400 bar using acetone.

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