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The enantiomeric recognition of dihydropyrimidonic compounds by chiral selectors derived from 4- or 2-chloro-3,5-dinitrobenzoic acid

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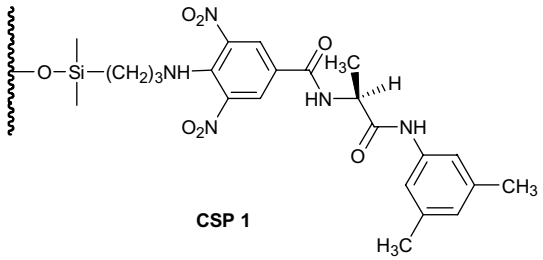
Abstract—Six new chiral packing materials for high performance liquid chromatography have been prepared from chiral selectors consisting of 4- or 2-chloro-3,5-dinitrobenzoic acid, L-alanine and different π-donor aromatic units. Comparative tests of these newly prepared CSPs on separation efficiency for 13 racemic dihydropyrimidonic (DHPM) analytes have revealed the strong contribution of the π-acceptor branching unit, as well as the important influence of the structure of the terminal π-donor unit. The role of the terminal aromatic group is primarily to increase the rigidity of the selector structure. Comparisons of the data revealed that selectors bound on the silica gel could be preorganized during the process of chiral recognition, resulting in the similar enantioseparation properties for DHPM analytes on both types of CSPs. However, some other compounds, such as amino alcohol β-agonists, exhibit very different enantioseparations.

1. Introduction

Today, the development of new chiral stationary phases (CSPs) is very important due to their use in the accurate analysis of chiral drugs and their metabolites in pharmaceutical and biological samples during drug research.[1–3] Furthermore there is a great demand for CSPs useful for preparative enantioseparation, especially on an industrial scale and for massive analytical usage.[4,5] Over the last few years we have designed and prepared a number of original brush-type CSPs that possess π-donor (π-basic)[6] and particularly π-acceptor (π-acidic) properties,[7] since it has been shown in general that π-acceptor CSPs can be used with a broad set of racemates and in several cases even with racemic compounds with π-acidic groups.[8]

Recently, we reported on a novel type of CSPs that comprise amides of 4-chloro-3,5-dinitrobenzoic acid with enantiomerically pure α,β-aminoalcohols or α-substituted aryl ethylamines.[9] These CSPs showed efficient enantioseparation of various racemates, particularly 4-aryl-3,4-dihydro-2(1*H*)-pyrimidones (DHPM),[10] compounds with known important pharmacological properties.[11,12] We also discussed the mechanism of enantioselection of DHPM on these CSPs supported by molecular modelling. To progress in the explanation of molecular recognition we prepared **CSPs 3–7**, which contain aromatic groups with different π-donor characteristics. Additionally, we prepared **CSP 2**, structurally similar to **CSP 1** that was proven to be the most efficient from the previous set. **CSP 2** was prepared from 2-chloro-3,5-dinitrobenzoic acid in order to bind the chiral selector to the silica surface from a different position and hence expand the area for the analytes approach to the dinitrobenzoyl unit. Therefore, for such a CSP, the more universal power of chiral recognition is expected. All newly prepared CSPs consist of an L-alanine unit, since it has been established that such types of CSPs with a simple methyl group at the stereogenic centre exhibit the best enantioseparation results.[10]



CSP 1

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2. Results and discussion

The chiral stationary phase **CSP 2**, prepared according to the scheme shown in Figure 1, contains a chiral selector composed of dinitrobenzoyl (DNB) and dimethylphenylamide (DMA) units, which are connected via L-alanine moiety. In comparison with the structure of **CSP 1**, the chiral selector of **CSP 2** is attached to silica through position 2 of DNB unit and therefore, from the formal structural point of view, the DNB unit is more open for the access of analytes. However, the liquid chromatographic separation of racemic compounds is generally unforeseen since in liquid media, both molecules, the selector and the selectant, can preorganize their structures. Considering the proximity of the silica gel surface, the possibility for the selector molecule in **CSP 2** to take a different orientation will have an impact on the chiral recognition mechanism.

The mechanism of chiral recognition during the chromatographic process is very difficult to ascertain because of the impossibility of direct monitoring of the processes. According to the literature data, the conclusion about the mechanism is mostly based on general deliberations, sometimes computer methods,[14] but rarely on using the NMR spectroscopy which requires a previous synthesis of CSP's analogues,[15] and recently on the using of IR and Raman spectroscopy of solid phases.[16,17] The latter is not completely pursuant to reality since, during the chromatography, chiral recognition evolves around the surface of solid carriers, not in the liquid phase. Therefore as the method, which could gather the most data about real interactions, we chose NMR spectroscopy. In order to use NMR spectroscopy, which can affirm the structural diversity and mode of chiral recognition, we had to synthesize the soluble analogues of **CSP 1** and **CSP 2** (Fig. 2). The chiral selectors **15** and **16** were prepared using *n*-butyl amine instead of aminopropyl silica, and applying the same synthetic procedure as for corresponding CSPs.

In order to make a complete assignation of the chemical shifts in the ^{13}C and ^{1}H NMR, various one- and two-dimensionals were performed. On the basis of information from all the quoted spectra, the signals in the ^{1}H and ^{13}C NMR for both selectors was successfully and completely established. NOESY spectra of compound **15** offered information about the interactions of protons through space, and about the geometry of the selector structure. This spectrum revealed the interaction between H(1) on the stereogenic C-atom and protons belonging to the methyl group attached to the chiral C-atom, with the protons of both aromatic groups: with H(6) at the *ortho*-position of the DNB unit and with H(10) at the *ortho*-position of the DMA group. Furthermore, there are mutual special interactions of protons of two distant aromatic units separated by the chiral dipeptide spacer; H(6) interacts with all protons of DMA group. These data show the structure of the chiral cavity whose walls are created by lateral aromatic units, one rich, and the other poor with π-electrons. The interactions of the protons of the DMA group with protons of the aminobutyl chain on the opposite side of molecule were also observed. The reasons for this lie in the curved geometry of the molecule and the mobility of the aliphatic chain. The aliphatic chain, which replaces the spacer towards the silica, showed none of the interactions with the protons of central part of molecule. This

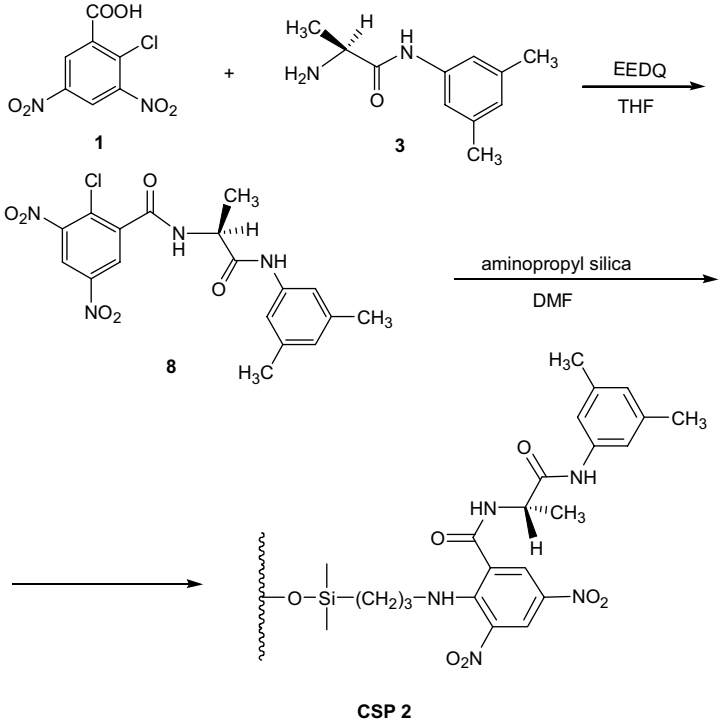


Figure 1. Synthetic route for **CSP 2**.

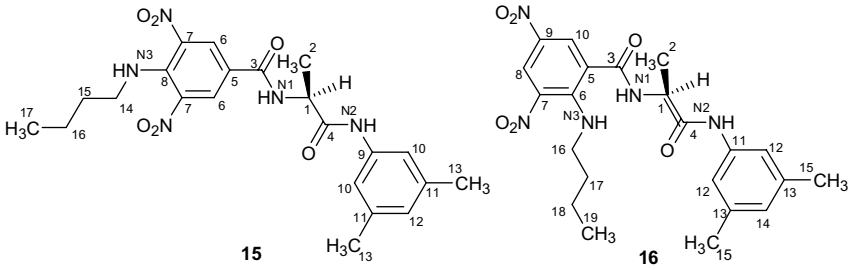


Figure 2. Structures of soluble chiral selectors **15** and **16**. Note that atom numbering does not follow IUPAC rules.

proves that the spacer and silica surface cannot provide significant contribution to enantiorecognition either for selector **15**, or for **CSP 1**.

The solubility of selector **16**, compared to **15**, is considerably weaker, which indicates its different structural properties. The NOESY spectrum shows similar interactions between the protons of the aromatic units as for **15**, revealing the analogous structures of the chiral cavity in both. The presence of noticeable interactions between the aminobutyl chain and all the protons of the chiral cavity indicates the strong influence of the spacer and silica surfaces during the chiral recognition process on **CSP 2**. Furthermore the structure of **16** offers the possibility of hydrogen bonding between the proton on N(3) of aminobutyl chain with the oxygen of the neighbouring carbonyl group that close stable six-membered ring, and probably weaken the solubility. This situation is influential on the appearance of the ^{1}H NMR spectrum where reduced mobility and interactions with protons of chiral cavity cause broadening of some signals of the butyl chain. From the data obtained from NMR it can be concluded that selector **16** is more rigid than **15** and their structural characteristics are sufficiently different to achieve different enantioseparation.

The column filled with **CSP 2** was tested for separations of DHPM racemic compounds **TRs 1**–**13**, as shown in Figure 3. To assure comparable results, the chromatographic analyses were performed by applying the same conditions as with **CSP 1**.[10] Tuning of different mobile phases revealed a ternary mixture of hexane/2-propanol/acetic acid (180:20:1) as the eluent of choice for our analyses. Acetic acid played the role of reducing the achiral interactions of polar DHPM analytes with residual γ-aminopropyl groups at silica surface.[18] The parameters and conditions obtained by these analyses are presented in Table 1.

Comparison of chromatographic results for DHPM compounds obtained on **CSP 1** and **CSP 2** showed their similar enantioseparation properties; some of the analyzed DHPMs were better separated with **CSP 1**, and some with **CSP 2**. This is a little surprising considering the results obtained by NMR spectroscopy. Although NMR analysis revealed different structures of the selectors in relation to the silica surface, the data obtained from chromatographic analyses showed that this factor has no marked influence on enantioseparation. It seems that during the chiral recognition process, the structure of the chiral selector is preorganized in such a way that

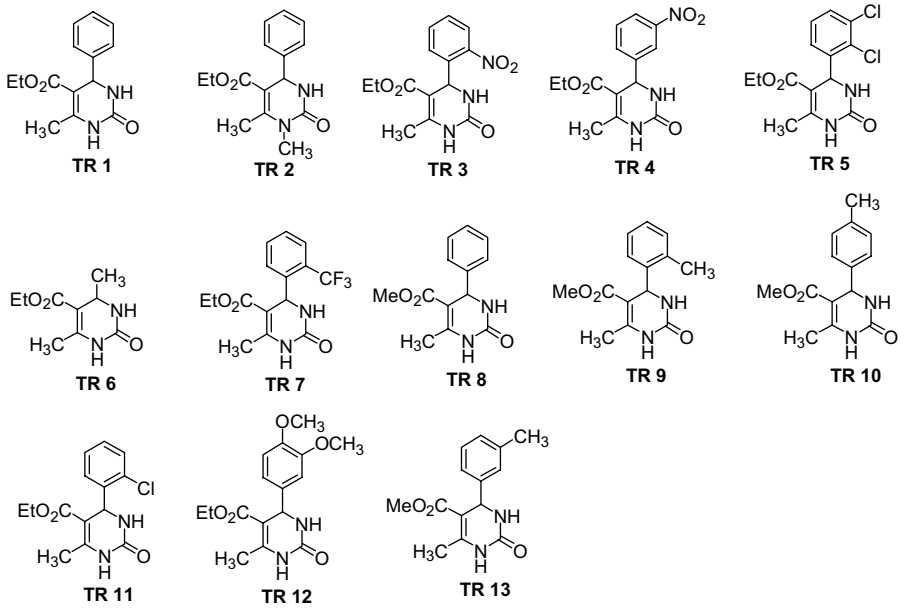


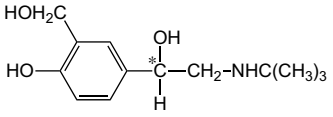
Figure 3. 3,4-Dihydro-2(1*H*)-pyrimidone racemates used for evaluation of **CSPs 1**–**7**.

Table 1. Results obtained for the enantioseparation of racemic analytes **TR 1**–**TR 13** on the columns filled with chiral stationary phases **CSP 1** and **CSP 2**; column dimension 250 mm × 4.6 mm ID; *n*-hexane–2-propanol–acetic acid (180:20:1); flow rate 2.0 mL/min[a]

Anal.	CSP 2			CSP 1		
	k'_1	α	R_S	k'_1	α	R_S
TR 1	4.31	1.15	1.91	4.26	1.18	2.19
TR 2	3.16	1.20	2.09	3.11	1.18	2.08
TR 3	7.82	1.22	2.64	8.12	1.14	1.51
TR 4	7.95	1.08	0.95	9.31	1.23	3.22
TR 5	4.12	1.22	2.03	4.32	1.10	0.96
TR 6	4.98	1.14	1.47	4.70	1.18	1.71
TR 7	3.06	1.20	1.94	2.81	1.10	0.93
TR 8	5.12	1.13	1.56	5.40	1.20	1.84
TR 9	4.52	1.20	2.36	4.58	1.14	1.21
TR 10	4.41	1.11	1.38	3.43	1.04	0.31
TR 11	4.35	1.18	2.39	4.01	1.10	0.92
TR 12	16.57	1.10	1.23	16.26	1.36	3.12
TR 13	4.53	1.11	1.51	4.48	1.18	1.62

[a] By CD detection (*S*)-enantiomers were proven to always be the more retained ones.

interaction points are moved away from the silica surface, resulting in a similar character and ability to separate DHPM derivatives for the both, **CSP 1** and **CSP 2**. The observed distinction in the structure of the selector is small and offers the possibility for the fine adjustments of separation that differs from compound to compound. However, unlike DHPM derivatives, which on both the investigated CSPs were separated equally, some preliminary results showed that various other compounds, such as some aminoalcohol β-agonists,[19,20] have been separated very differently. For example, by analysis of albuterol **17**, and clenbuterol **18**, the good baseline separations were obtained for both racemates, but only on **CSP 1** (Fig. 4a and b). On **CSP 2** the enantiomers of these substances were not resolved (Fig. 4c).[21]



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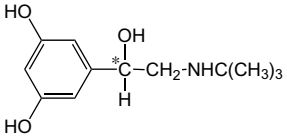

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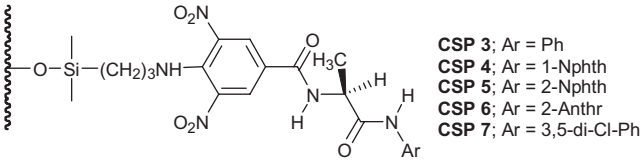
Continuing our research we prepared six new **CSPs 3**–**7**, which mutually differ along the terminal π-donor aromatic unit. The synthetic path for their preparation followed the procedure described for the preparation of a similar set of CSPs.[10] All necessary selectors were prepared in high yields, and their binding to aminopropyl silica gel progressed without any problem. The obtained CSPs usually contained 0.21–0.25 mmol of selector per 1.0 g of the material. Results obtained by chromatographic analysis of DHPM racemates **TRs 1**–**13** are shown in Table 2.



All the newly prepared CSPs separated the enantiomers of the DHPM derivatives. The best separations were achieved with **CSP 5**, prepared from 2-aminonaphthalene. This CSP showed much better separations than **CSP 1** and **CSP 2**. Unlike **CSP 5**, the weakest separations were obtained with **CSP 4**, which consists of a terminal group prepared from 1-aminonaphthalene. For example, the chromatograms obtained for analyte **TR 8** with the columns filled with **CSPs 1**, **4**, **5** and **7** are shown in Figure 5. It is clear that on three columns, the peaks of the enantiomers are separated very well, but on **CSP 4** (Fig. 5b) the separation is poor.

Comparisons of the 3D models of **CSP 4** and **CSP 5**, (Fig. 6)[22] showed the importance of the steric disturbance made by the hydrogen atom attached to the 8-position of the naphthalene ring. This hydrogen atom obstructs the entry of the analytes into the chiral cavity, resulting in very weak selections on **CSP 4**. **CSP 3** separations are slightly weaker than with **CSP 5**, but the peaks are broader, which causes worse resolution. The reason for this probably lies in the fact that the structure of the selector with the terminal phenylamide group is less rigid than with the 2-naphthylamide group. Although the complex between the selector and analyte

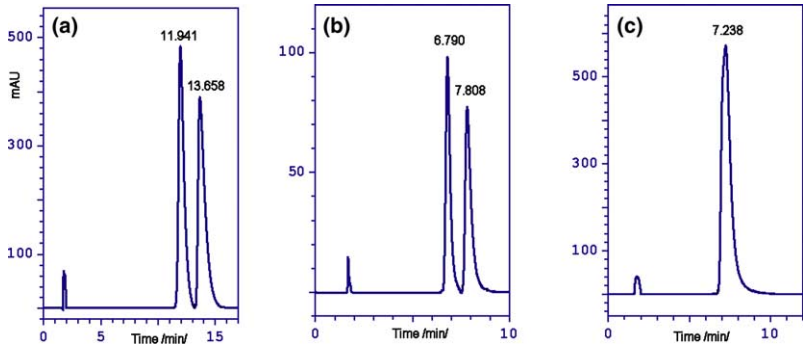


Figure 4. Chromatograms obtained for **17** (a), **18** (b) on **CSP 1**, and also for **17** (c) on **CSP 2**. Mobile phase: *n*-hexane–2-propanol–acetic acid (180:20:1); flow rate 2.0 mL/min.

Table 2. Results obtained for the enantioseparation of racemic analytes **TR 1–13** on the columns filled with chiral stationary phases **CSP 3–7**; columns dimension 250 mm × 4.6 mm ID; *n*-hexane–2-propanol–acetic acid (180:20:1); flow rate 2.0 mL/min[a]

Anal.	CSP 3			CSP 4			CSP 5			CSP 6			CSP 7		
	k'_1	α	R_S	k'_1	α	R_S	k'_1	α	R_S	k'_1	α	R_S	k'_1	α	R_S
TR 1	4.08	1.20	1.44	4.05	1.08	0.42	4.64	1.31	2.80	4.43	1.21	2.41	5.09	1.29	3.36
TR 2	3.36	1.23	1.53	3.62	1.09	0.57	4.05	1.30	3.21	3.81	1.17	1.42	4.85	1.33	3.76
TR 3	7.79	1.18	1.30	8.44	1.08	nm	10.62	1.19	1.75	9.44	1.12	1.71	10.50	1.24	2.34
TR 4	8.69	1.27	1.81	9.38	1.12	0.84	10.71	1.40	3.23	8.91	1.29	3.19	10.10	1.43	3.93
TR 5	4.38	1.09	0.71	4.53	1.0	0	5.35	1.15	1.55	5.27	1.13	1.33	5.74	1.14	1.97
TR 6	4.48	1.14	0.82	4.55	1.07	nm	5.45	1.31	2.63	5.01	1.24	2.28	3.99	1.10	1.20
TR 7	2.67	1.10	0.47	2.87	1.0	0	3.20	1.15	1.53	3.26	1.12	1.06	3.60	1.13	1.42
TR 8	4.59	1.26	1.38	5.03	1.03	nm	5.68	1.30	2.60	5.37	1.21	2.51	6.31	1.32	2.69
TR 9	4.47	1.13	1.22	4.75	1.0	0	5.16	1.21	2.26	5.00	1.16	1.34	5.89	1.22	2.02
TR 10	4.34	1.23	1.91	3.00	1.0	0	5.40	1.32	2.91	4.69	1.21	1.88	6.00	1.28	2.62
TR 11	3.92	1.09	0.63	4.10	1.0	0	4.84	1.16	1.76	4.66	1.12	1.24	5.51	1.14	1.72
TR 12	17.39	1.0	0	15.33	1.08	1.07	18.08	1.52	4.02	17.34	1.35	3.78	21.15	1.60	4.07
TR 13	4.37	1.18	1.35	4.30	1.0	0	5.01	1.29	2.78	4.69	1.20	1.69	5.15	1.25	2.73

nm = non-measurable.

[a] The more retained are always (*S*)-enantiomers.

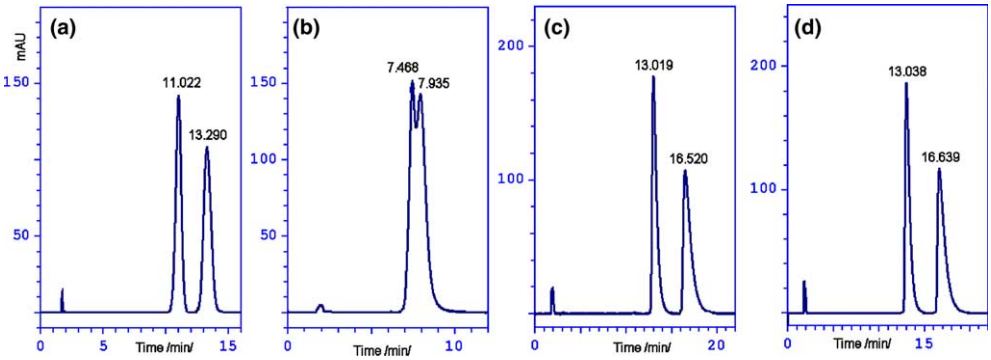


Figure 5. Chromatograms obtained for **TR 8** on **CSP 1** (a), **CSP 4** (b), **CSP 5** (c) and **CSP 7** (d). Mobile phase: *n*-hexane–2-propanol–acetic acid (180:20:1); flow rate 2.0 mL/min.

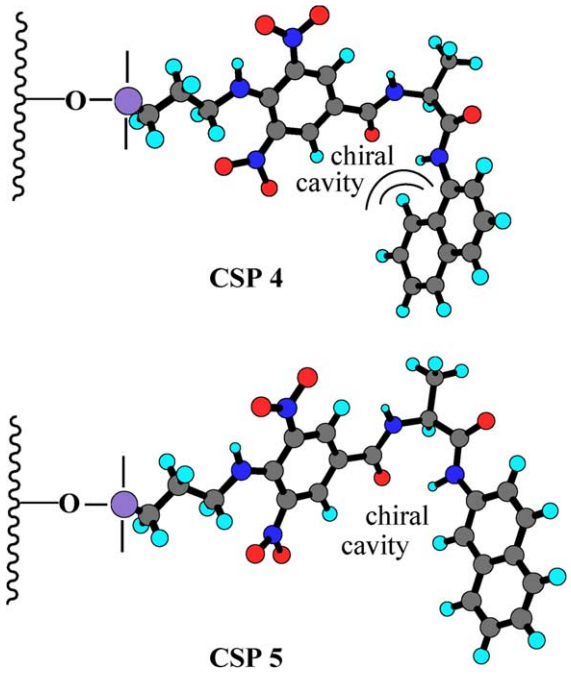


Figure 6. Structures of **CSP 4** and **CSP 5** obtained by molecular modelling.[22]

has been realized, there are many possibilities for the different orientations of the approaching analytes, which manifest in the broadening of the peaks. **CSP 6**, which possesses a 2-anthrylamide as a terminal group, has shown good separation properties, but is nevertheless weaker than **CSP 5**. A possible explanation is that the elongated anthryl system partly obstructs access to chiral cavity and does not additionally contribute to the rigidity of the selector structure. Only **CSP 7**, prepared from 3,5-dichloroaniline, shows results on the same scale with **CSP 5**, and in some cases even little better. Obtained chromatographic data indicate that the 3,5-dichlorophenylamide unit reduces the number of different orientations of the approaching analyte and offers the possibility for additional interactions with electronegative chlorine atoms.

As shown, all selectors possess a terminal aromatic group with equal ability to form π–π interactions with analytes. Furthermore, the terminal aromatic groups differ in size and position of connection to the chiral dipeptide moiety, which defines the walls of chiral cavity. For all such groups it is a prerequisite that they do not disturb the entrance of analytes towards the chiral cavity and DNB unit where complexation evolves

primarily by hydrogen bonding. According to the difference in the separation of DHPM enantiomers on investigated CSPs, it can be concluded that the π–π aromatic interactions are of a little importance. However, increasing the size of the aromatic moiety facilitates the rigidity of the selector, resulting in a uniform approach of analyte to the chiral selector and better enantioseparation.

3. Conclusion

Soluble analogues of **CSP 1** and **CSP 2** were investigated by NMR spectroscopy and both the CSPs were evaluated under the same chromatographic conditions. Comparison of the data revealed that selectors bound on the silica gel could be preorganized during the process of chiral recognition, resulting in the similar enantioseparation properties. However, while such a situation is valid it is only true for the case of DHPM analytes. Another class of compounds, such as some amino alcohol β-agonists whose enantiomers are separated by these CSPs, **CSP 1** and **CSP 2** recognize very differently. Additionally, six new CSPs were prepared in order to study the influence of the terminal aromatic group on the separation of DHPM enantiomers. The new CSPs investigated herein generally display very good separation abilities. The best separations of the DHPMs enantiomers were achieved with **CSP 5** and **CSP 7**, prepared from 2-aminonaphthalene and 3,5-dichloroaniline, respectively. Finally, we deduced that the main role of the terminal aromatic groups is to define the wall of the chiral cavity and to strengthen the rigidity of the chiral selector, which then results in a uniform approach of analyte to the chiral selector and good enantioseparation.

4. Experimental

4.1. Chemicals

4-Chloro-3,5-dinitrobenzoic acid, *N*-Boc-L-α-alanine, *N*,*N*,*N*-triethylamine, 3,5-dimethylaniline **2**, 1-aminonaphthalene **4**, 2-aminonaphthalene **5**, 2-aminoanthracene **6** and 3,5-dichloroaniline **7** were obtained from Fluka (Buchs, Switzerland); *N*,*N*-dicyclohexylcarbodiimide (DCC) and 2-ethoxy-1-ethoxycarbonyle-1,2-dihydrochinoline (EEDQ) from Sigma–Aldrich (Aldrich Chimica, Milano, Italy); sodium hydroxide and aniline from Kemika (Zagreb, Croatia) and 2-chloro-3,5-dinitrobenzoic acid **1** from Lancaster Synthesis GmbH (Frankfurt am Main, Germany). HPLC silica gel *Nucleosil 100-5 NH_2* was purchased from Macherey-Nagel (Düren, Germany) and Separon SGX NH_2, 5 μm, from Tessek (Prague, Czech Rep.). All the solvents used were purchased from J. T. Baker (Davenport, Holland) and distilled before use. Dihydropyrimidine derivatives **TR 1**–**TR 13** were obtained from Dr. O. Kappe (Karl-Franzens University, Graz, Austria) or synthesized in our laboratory.[13] Albuterol **17** and clenbuterol **18** were purchased from Sigma–Aldrich.

4.2. Apparatus and chromatography

One- and two-dimensional homo- and heteronuclear ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 600 spectrometer, operating at 600.133 MHz for the ^1H nucleus and 150.917 MHz for the ^{13}C nucleus. Samples were measured from DMSO-d_6 solutions at 22 °C (295 K) in 5 mm NMR tubes. Chemical shifts, in ppm, are referred to TMS as internal standard. FID resolution in ^1H NMR and ^{13}C NMR spectra was 0.29 and 0.54 Hz per point, respectively. The following measurement techniques were used: standard ^1H, ^{13}C gated proton decoupling, APT, COSY, phase sensitive mode NOESY, HMQC and HMBC. Proton decoupling was performed by Waltz-16 modulation. COSY and NOESY were measured using 2048 points in F2 dimension and 512 increments in F1 dimension. The latter was subsequently zero-filled to 1024 points. Increments were obtained by 4 scans (COSY) and 16 scans (NOESY), 8012.82 Hz spectral width and a relaxation delay of 1.5 s. Mixing time was 0.8 s. The FID resolution was 3.91 and 15.65 Hz/point in F2 and F1 dimensions, respectively. The HMQC spectra ($^1J_{C,H}$ was set to 145 Hz) were recorded with 2048 points in F2 dimension and 256 increments in F1 dimension, subsequently zero-filled to 1024 points. For each increment, 64 scans were collected, using a relaxation delay of 1.0 s. The spectral widths were 6067.96 Hz (F2) and 25000 Hz (F1), with the corresponding resolutions of 2.96 and 97.65 Hz/point in F2 and F1 dimensions, respectively. The HMBC spectra were measured with 2048 points and 8012.82 Hz spectral width in F2 dimension and a relaxation delay of 1.0 s. The additional delay of 0.065 s was used for detecting the long-range C–H couplings. The spectral width in the F1 dimension was 33,560 Hz, while 256 increments were recorded, each by 64 scans. The FID resolution was 3.912 and 131.08 Hz per point in F2 and F1 dimensions, respectively. The 2D NMR spectra were measured in pulsed field gradient mode (*z*-gradient).

IR: Bruker ABB Bomem spectrometer for KBr pellets. Melting point: Electrothermal 9100 MP digital apparatus. The Central Analytical Service (CAS) at Ruđer Bošković Institute carried out elemental analyses.

Chromatography was performed with a Knauer WellChrom Maxi-Star K-1000 pump (Knauer GmbH, Berlin, Germany) using a Knauer HPLC 6-port-valves injector with a 20 μL loop. Detection was performed with a Knauer WellChrom K-2500 detector ($\lambda = 254$ nm). To determine the elution order of the enantiomers Jasco CD-2095 detector was used ($\lambda = 254$ nm). Integration of the chromatograms was made with the Knauer Eurochrom 2000 software package. For new prepared columns the following parameters were measured: k'_1: capacity factor of the first eluted enantiomer; k'_2: capacity factor of the second eluted enantiomer; α: selectivity factor; R_S: resolution factor.

The packing of HPLC columns purchased from Max Stevenson (Berlin, Germany), dimension 250 mm ×

4.6 mm ID, was performed by a slurry technique using a Knauer pneumatic HPLC-pump. Solvents used for HPLC chromatography were analytical grade from J. T. Baker, and redistilled before use. The samples of analytes are prepared by dissolving ca. 1 mg of the racemic compound in 1 mL of 2-propanol. For analytical purposes 5 μL of freshly prepared solutions were used.

4.3. Chemistry

4.3.1. Preparation of chiral selectors 8–13. The chiral selectors **8**–**13** were prepared under the general procedure described previously.[10] The structures of the prepared compounds were confirmed by ^{1}H and ^{13}C NMR spectra.

4.3.1.1. (2*S*)-2-Chloro-*N*-[1-(3,5-dimethyl-phenylcarbamoyl)-ethyl]-3,5-dinitrobenzamide 8. Yield: 53%. ^{1}H NMR (DMSO-d_6) δ 1.42 (3H, d, J = 7.1 Hz), 2.22 (6H, s), 4.64 (1H, dq, J = 7.1 and 6.9 Hz), 6.69 (1H, s), 7.23 (2H, s), 8.54 (1H, d, J = 2.6 Hz), 9.02 (1H, d, J = 2.6 Hz), 9.21 (1H, d, J = 6.9 Hz, N–H), 10.02 (1H, s, N–H). ^{13}C NMR (DMSO-d_6) δ: 18.03, 21.11, 49.84, 117.24, 120.80, 125.03, 126.23, 128.69, 137.76, 138.74, 139.82, 145.91, 148.60, 162.81, 170.23.

4.3.1.2. (2*S*)-4-Chloro-3,5-dinitro-*N*-(1-phenylcarbamoyl-ethyl)-benzamide 9. Yield: 68%. ^{1}H NMR (DMSO-d_6) δ 1.40 (3H, d, J = 7.1 Hz), 4.58 (1H, dq, J = 7.1 and 7.0 Hz), 6.98 (1H, dt, J = 6.5 and 1.0 Hz), 7.24 (2H, t, J = 7.5 Hz), 7.55 (2H, d, J = 8.5 Hz), 8.81 (2H, s), 9.24 (1H, d, J = 7.0 Hz, N–*H*), 10.07 (1H, s, N–*H*). ^{13}C NMR (DMSO-d_6) δ 17.66, 50.35, 119.34, 121.41, 123.37, 127.46, 128.64, 134.44, 136.86, 148.67, 161.66, 170.65.

4.3.1.3. (2*S*)-4-Chloro-*N*-[1-(naphthalen-1-ylcarbamoyl)-ethyl]3,5-dinitrobenzamide 10. Yield: 64%. ^{1}H NMR (DMSO-d_6) δ 1.59 (3H, d, J = 7.0 Hz), 4.82–4.86 (1H, m), 7.48–7.61 (4H, m), 7.80 (1H, d, J = 8.0 Hz), 7.95 (1H, d, J = 8.0 Hz), 8.06 (1H, d, J = 7.6 Hz), 8.93 (2H, s), 9.37 (1H, d, J = 7.0 Hz, N–H), 10.14 (1H, s, N–H). ^{13}C NMR (DMSO-d_6) δ 17.83, 50.32, 121.47, 122.51, 122.91, 125.55, 125.82, 125.98, 126.11, 127.59, 128.12, 128.37, 133.29, 133.72, 134.57, 148.70, 161.95, 171.63.

4.3.1.4. (2*S*)-4-Chloro-*N*-[1-(naphthalen-2-ylcarbamoyl)-ethyl]-3,5-dinitrobenzamide 11. Yield: 69%. ^{1}H NMR (DMSO-d_6) δ 1.44 (3H, d, J = 7.1 Hz), 4.63 (1H, dq, J = 7.1 and 7.0 Hz), 7.38 (1H, t, J = 8.4 Hz), 7.41 (1H, t, J = 7.8 Hz), 7.57 (1H, dd, J = 7.0 and 2.0 Hz), 7.73–7.86 (3H, m), 8.23 (1H, s), 8.84 (2H, s), 9.29 (1H, d, J = 7.0 Hz, N–*H*), 10.31 (1H, s, N–*H*). ^{13}C NMR (DMSO-d_6) δ 17.59, 50.46, 115.55, 120.06, 121.39, 124.60, 126.36, 127.21, 127.39, 127.43, 128.28, 129.78, 133.29, 134.43, 136.40, 148.66, 161.72, 170.91.

4.3.1.5. (2*S*)-4-Chloro-*N*-[1-(anthracen-2-ylcarbamoyl)-ethyl]-3,5-dinitrobenzamide 12. Yield: 91%. ^{1}H NMR (DMSO-d_6) δ 1.53 (3H, d, J = 7.1 Hz), 4.73 (1H, dt, J = 7.1 and 6.7 Hz), 7.42–7.50 (2H, m), 7.62 (1H, d, J = 7.9 Hz), 8.01–8.08 (3H, m), 8.42–8.53 (3H, m), 8.91 (2H, s), 9.37 (1H, d, J = 6.7 Hz, N–H), 10.43 (1H, s, N–H). ^{13}C NMR (DMSO-d_6) δ 17.61, 50.49, 114.33, 120.99, 121.40, 124.91, 124.94, 125.56, 125.80, 127.43, 127.62, 127.99, 128.51, 128.77, 130.42, 131.48, 131.65, 134.41, 135.81, 148.65, 161.72, 171.07.

4.3.1.6. (2*S*)-4-Chloro-*N*-[1-(3,5-dichloro-phenylcarbamoyl)-ethyl]-3,5-dinitrobenzamide 13. Yield: 50%. ^{1}H NMR (DMSO-d_6) δ 1.39 (3H, d, J = 7.1 Hz), 4.50 (1H, dq, J = 7.1 and 7.0 Hz), 7.23 (1H, t, J = 1.8 Hz), 7.62 (2H, d, J = 1.8 Hz), 8.81 (2H, s), 9.29 (1H, d, J = 7.0 Hz, N–*H*), 10.41 (1H, s, N–*H*). ^{13}C NMR (DMSO-d_6) δ 17.22, 50.59, 117.46, 121.50, 122.62, 127.42, 134.06, 134.26, 141.21, 148.68, 161.81, 171.38.

4.3.2. Preparation of chiral stationary phases CSPs 2–7. A suspension of each from the set of chiral selectors **8**–**13** (1.00 mmol), silica gel (3.00 g; *Nucleosil 100-5 NH_2* or Separon SGX NH_2, 5 μm) and diisopropylamine (1.0 mL) in tetrahydrofurane (15 mL) was stirred overnight at ambient temperature. The modified silica gel was collected on a G-4 filter, washed with tetrahydrofurane and methanol and dried at 70 °C for 4 h. Both used starting silica gels exhibited very similar separation characteristics. Since we wanted to compare the new CSPs with the **CSP 1** originally prepared from Nucleosil silica gel,[10] we herein report the analytical data only for CSPs prepared from Nucleosil 100-5 NH_2 (C 2.46, N 0.96%).

CSP 2. Prepared from **8**, yield 3.235 g. Anal. found C 7.29, H 1.82 and N 2.03%. As calculated on % C 1.0 g of CSP contain 0.22 mmol of bound selector.
CSP 3. Prepared from **9**, yield 3.195 g. Anal. found C 6.31, H 1.62 and N 1.80%. As calculated on % C 1.0 g of CSP contain 0.21 mmol of bound selector.
CSP 4. Prepared from **10**, yield 3.213 g. Anal. found C 7.32, H 1.85 and N 2.05%. As calculated on % C 1.0 g of CSP contain 0.21 mmol of bound selector.
CSP 5. Prepared from **11**, yield 3.241 g. Anal. found C 7.74, H 1.90 and N 2.17%. As calculated on % C 1.0 g of CSP contain 0.22 mmol of bound selector.
CSP 6. Prepared from **12**, yield 3.092 g. Anal. found C 9.66, H 1.89 and N 2.36%. As calculated on % C 1.0 g of CSP contain 0.25 mmol of bound selector.
CSP 7. Prepared from **13**, yield 3.183 g. Anal. found C 7.93, H 1.94 and N 2.22%. As calculated on % C 1.0 g of CSP contain 0.22 mmol of bound selector.

4.3.3. (2*S*)-4-Butylamino-*N*-[1-(3,5-dimethyl-phenylcarbamoyl)-ethyl]-3,5-dinitrobenzamide 15. Suspension of (2*S*)-4-chloro-*N*-[1-(3,5-dimethyl-phenylcarbamoyl)-ethyl]-3,5-dinitrobenzamide[10] **14** (0.5 mmol; 0.210 g) and *n*-butylamine (1.5 mmol, 0.15 mL, previously dried overnight under the solid KOH) in anhydrous tetrahydrofurane (10 mL) was stirred overnight at room temperature. After evaporation and chromatography on silica gel, using a mixture of solvents CH_2Cl_2–CH_3OH (10:0.5) as eluent, 193 mg (85%) of **15** was isolated as orange powder; mp 194.0–195.0 °C; $[\alpha]_D^{20} = +113.0$ (*c* 1 mg/mL, DMF). IR (KBr) ν 3560.5, 3496.9, 3332.7, 2967.5, 2935.7, 1666.7, 1631.2, 1555.2, 1535.1, 1514.8,

1466.7, 1431.3, 1322.4, 1284.0, 1226.3, 920.1, 844.2 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.95 (3H, t, $J = 7.5$ Hz, CH_3), 1.41 (2H, m, $J = 7.5$ and 6.4 Hz, CH_2), 1.65 (3H, d, $J = 7.0$ Hz, CH_3–C*), 1.63–1.71 (2H, m, CH_2), 2.20 (6H, s, CH_3-arom.), 3.04 (2H, m, CH_2), 5.03 (1H, q, $J = 7.0$, C*–*H*), 6.67 (1H, s, 4-xylyl-*H*), 7.12 (2H, s, 2,6-xylyl-*H*), 8.24 (1H, s, N–*H*), 8.63 (1H, t, $J = 5.4$ Hz, N–*H*), 9.02 (1H, s, N–*H*), 9.06 (2H, s, DNB–arom.-*H*). ^{13}C NMR (DMSO-d_6) δ 13.43 (*C*H_3), 18.21 (*C*H_3–C*), 19.72 (*C*H_2), 21.10 (2*C*H_3), 31.83 (*C*H_2), 46.19 (*C*H_2), 50.65 (*C**H), 117.51, 118.69, 125.96, 130.96, 136.63, 137.43, 138.75, 141.25, 163.5, 171.41. Anal. Calcd for $C_{22}H_{27}O_6N_5$ (457.486): C, 57.76; N, 15.30; H, 5.95. Found: C, 57.71; N, 15.39; H, 6.01.

4.3.4. (2*S*)-2-Butylamino-*N*-[1-(3,5-dimethyl-phenylcarbamoyl)-ethyl]-3,5-dinitrobenzamide 16. A suspension of benzamide **8** (0.5 mmol; 0.210 g) and *n*-butylamine (1.5 mmol, 0.15 mL, previously dried overnight under the solid KOH) in anhydrous tetrahydrofurane (10 mL) was stirred overnight at room temperature. After evaporation and chromatography on silica gel, using a mixture of solvents CH_2Cl_2–CH_3OH (10:0.5) as eluent, 173 mg (76%) of **16** was isolated as yellow powder; mp 238.5–239.5 °C; $[\alpha]_D^{20} = -57$ (*c* 1 mg/mL, DMF). IR (KBr) *v* 3443.9, 3338.2, 3269.2, 3105.0, 3057.4, 2967.5, 2935.7, 2872.1, 1667.8, 1648.8, 1608.1, 1537.5, 1523.0, 1456.9, 1440.6, 1356.4, 1326.5, 1314.8, 1296.7, 1280.3, 1225.1, 1176.2, 1135.4, 1098.4, 930.9, 920.9, 844.8, 745.3, 723.6 cm^{-1}. ^1H NMR (DMSO-d_6) δ 0.88 (3H, t, $J = 7.3$ Hz, CH_3), 1.28–1.37 (2H, m, CH_2), 1.55–1.63 (2H, m, CH_2), 1.62 (3H, d, $J = 6.6$ Hz, CH_3–C*), 2.29 (6H, s, CH_3-arom.), 3.14–3.20 (2H, m, CH_2), 4.75 (1H, q, C*–*H*), 6.79 (1H, s, 4-xylyl-*H*), 6.99 (1H, s, N–*H*), 7.14 (2H, s, 2,6-xylyl-*H*), 7.89 (1H, s, N–*H*), 8.40 (1H, s, DNB–arom.-*H*), 9.00 (1H, s, N–*H*), 9.05 (1H, s, DNB–arom.-*H*). ^{13}C NMR (DMSO-d_6) δ 13.54 (*C*H_3), 17.93 (*C*H_3), 19.85 (*C*H_2), 21.29 (2*C*H_3-arom.), 31.94 (*C*H_2), 46.55 (*C*H_2), 50.51 (*C**–H), 117.68, 122.65, 125.73, 126.73, 129.90, 133.41, 136.88, 1138.95, 147.27, 154.73, 166.64, 169.04. Anal. Calcd for $C_{22}H_{27}O_6N_5$ (457.486): C, 57.76; N, 15.30; H, 5.95. Found: C, 57.84; N, 15.52; H, 6.05.

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