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Study of Chromatographic Enantioseparation of the Esters of *N*-Dinitrobenzoyl (*N*-DNB) and *N*-Benzoyl (*N*-B) α-Amino Acids on Novel Chiral Stationary Phases Containing Structurally Matching *N*-DNB and *N*-B-α-AA Amides in the Chiral Selector

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Enantioseparation ability and enantiopreference of chiral stationary phases **CSP 1**–**CSP 3**, containing a terminal *N*-3,5-dinitrobenzoyl (*N*-DNB) unit, and **CSP 4**, containing a terminal *N*-benzoyl (*N*-B) unit, are studied. Separation factors (α) for the two sets of test racemates (TR) that structurally match the chiral selector of these CSPs have been determined. The first set consists of seven *N*-DNB α-amino acid isopropylesters (**TR 1A**–**TR 7A**), and the second one of their *N*-B analogues (**TR 1**–**TR 7**). The best enantioseparation ($\alpha_{average}$ 1.27) is obtained when π-acceptor DNB unit is present in both TR and CSP. One π-acceptor unit, either in the analyte or in CSP, suffices for efficient enantioseparation ($\alpha_{average}$ 1.19). Interaction between π-neutral units in the CSP and test racemate does not afford effective enantioseparation ($\alpha_{average}$ 1.03). Using (*S*)-enantiomers of all TRs as standards, CD detection has revealed regular preference of the CSPs for the enantiomers containing amino acid amide of the same absolute configuration. The possible origin of such enantiopreference is discussed.

Key words
π–π interactions
chiral stationary phases
Pirkle-type
electron-deficient groups

INTRODUCTION

Pirkle-type CSPs have been known to be effective for the resolution of racemates containing either π-acid or π-basic groups.[1] Our efforts in this area have also resulted in several effective Pirkle-type CSPs.[2–4] Particularly effective were the tweezer-type CSPs, with two strongly π-acid 3,5-dinitrobenzoyl (DNB) groups per one molecule of chiral selector.[4] For these pseudo C_2-symmetric selectors, with two identical chiral units, an increase of the loading capacity was observed, however no increase of their chiral recognition ability. Chiral selectors in the Pirkle- or brush-type CSPs incorporate a π-acid or/and π-basic aromatic unit, for which it is argued that it provides π-π interaction with complementary aromatic groups in the analyte.[5,6] Besides, these CSPs retain more strongly DNB-α-amino acid esters of the same absolute configuration than those of the opposite configuration. General experience has shown that π-acidic CSPs can be used with a broad set of racemates, in several cases even with racemic compounds with π-acidic groups.[7] In contrast, π-basic stationary phases are only effective in the separation of racemates with strong π-acidic groups.

Here we report a comparative study of the contribution of the π-electron donor-acceptor, donor-donor, and acceptor-acceptor interaction to enantioselection in a set of new CSPs. To this aim the study of enantioseparation

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ability and enantiopreference of seven *N*-3,5-dinitrobenzoyl (*N*-DNB) and *N*-benzoyl (*N*-B) α-amino acid (α-AA) esters was performed. Four novel CSPs were prepared, three of them containing a terminal *N*-DNB unit and one an *N*-B unit. All CSPs comprise a linker unit consisting of 4-(alkyl)amino-3,5-dinitrobenzoyl amide moiety. All CSPs are thus structurally related, in that they comprise amides of *N*-DNB-α-AAs or *N*-B-α-AA. They also structurally match the test racemates, esters of the *N*-DNB and *N*-B derivatives of α-AAs.

EXPERIMENTAL

Chemicals

Manufacturers of the chemicals used are quoted in the parentheses after each chemical: 4-chloro-3,5-dinitrobenzoic acid (Fluka, Buchs, Switzerland), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ; Fluka), L-α-phenylglycine (Sigma-Aldrich, Aldrich Chimica, Milano, Italy), L-leucine (Aldrich), L-alanine (Aldrich), ethylenediamine (Aldrich) and HPLC silica gel Nucleosil 100-5 NH_2 (Macherey-Nagel, Düren, Germany; w(C) = 2.46 %, w(H) = 0.89 % and w(N) = 0.96 %). *N*-3,5-dinitrobenzoyl derivatives of L-α-phenylglycine, L-leucine and L-alanine were prepared according the literature procedure.[8] Racemic analytes **TR 1A**–**TR 7A** were prepared from the set of D,L-amino acids (Sigma), and enantiomerically pure **TR 1**–**TR 7** were prepared from L-amino acids (Aldrich). All the solvents used were purchased from J. T. Baker (Davenport, Holland) and distilled before use.

Apparatus and Chromatography

IR: Perkin Elmer 297 spectrometer for KBr pellets. Elemental analyses were carried out by the Central Analytical Service (CAS) at the Ruđer Bošković Institute.

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 at 254 nm with a Knauer WellChrom K-2500 detector. A Jasco CD-2095 detector was used to determine the elution order of the enantiomers. Integration of the chromatograms was done with the Knauer Eurochrom 2000 software package. The following parameters were measured for the newly prepared columns:

k_1': capacity factor of the first eluted enantiomer, $(t_1–t_0)/t_0$;

k_2': capacity factor of the second eluted enantiomer, $(t_2–t_0)/t_0$;

α: selectivity factor, $\alpha = k_2'/k_1'$;

R_S: resolution factor, $R_S = 2(t_2–t_1)/(w_1+w_2)$; w is the baseline bandwidth obtained by drawing tangents to the inflection points of the chromatographic peak.

Packing of HPLC columns purchased from MsScientific (Berlin, Germany), 250 mm × 4.6 mm I.D., was performed by the slurry technique using a Knauer pneumatic HPLC-pump. n-Hexane, 2-propanol, dichloromethane and acetic acid used for HPLC chromatography were of analytical grade (J. T. Baker) and were redistilled before use. Analyte samples were prepared by dissolving *ca* 1 mg of the racemic compound in 1 ml of 2-propanol. For analytical purposes, 5 μl of freshly prepared solution were used.

Preparation of Chiral Stationary Phases ***CSP 1–CSP 4***

A mixture of 4-chloro-3,5-dinitrobenzoic acid (2.46 g, 9.96 mmol) and EEDQ (2.47 g, 9.96 mmol) in dry THF (60 ml) was stirred at ambient temperature for 2 h. To the obtained solution, silica gel Nucleosil 100-5 NH_2 (10.288 g; N 1.36 % and C 3.49 %) was added and stirred for an additional 18 h at ambient temperature. The modified silica gel was collected on G-4 filter, washed with tetrahydrofurane (2 × 20 ml) and acetone (2 × 20 ml), and dried at 70 °C for 4 h. 11.15 g of stationary phase **SP 1** was obtained; IR (KBr) ν/cm^{-1} 3450, 2910, 2840, 1630, 1540, 1340, 1250-1000, 785; *Anal*. found C 5.62, H 1.19, N 2.90 %. Based on the percent of C, 1.0 g of stationary phase **SP 1** contains *ca* 0.36 mmol of the bound organic molecule.

SP 1 (11.12 g) was suspended in dichloromethane (60 ml) and ethylenediamine (12 ml) was added. Reaction suspension was stirred for 1 h at ambient temperature and the product was collected on G-4 filter. The obtained modified silica was washed with dichloromethane (2 × 20 ml) and methanol (2 × 20 ml) and dried at 70 °C for 4 h to afford 11.20 g of stationary phase **SP 2**. A mixture of stationary phase **SP 2** (2.80 g), *N*-(3,5-dinitrobenzoyl)-L-α-amino acid (**2**–**4**, 1.34 mmol) and EEDQ (0.332 g, 1.34 mmol) in dry tetrahydrofurane (10 ml) was stirred for 24 h at ambient temperature. The obtained chiral stationary phase was collected on G-4 filter, washed with tetrahydrofurane (2 × 10 ml) and methanol (2 × 10 ml), and dried at 70 °C for 4 h.

CSP 1: From **SP 2** and alanine derivative **2,** 2.94 g of product was obtained; *Anal*. found: C 8.98, H 2.18, N 1.22 %. Based on the percent of C, 1.0 g of chiral stationary phase **CSP 1** contains *ca* 0.26 mmol of bound selector.

CSP 2: From **SP 2** and leucine derivative **3,** 2.94 g of product was obtained; *Anal*. found: C 10.30, H 2.04, N 1.51 %. As calculated based on C %, 1.0 g of chiral stationary phase **CSP 2** contains *ca* 0.26 mmol of bound selector.

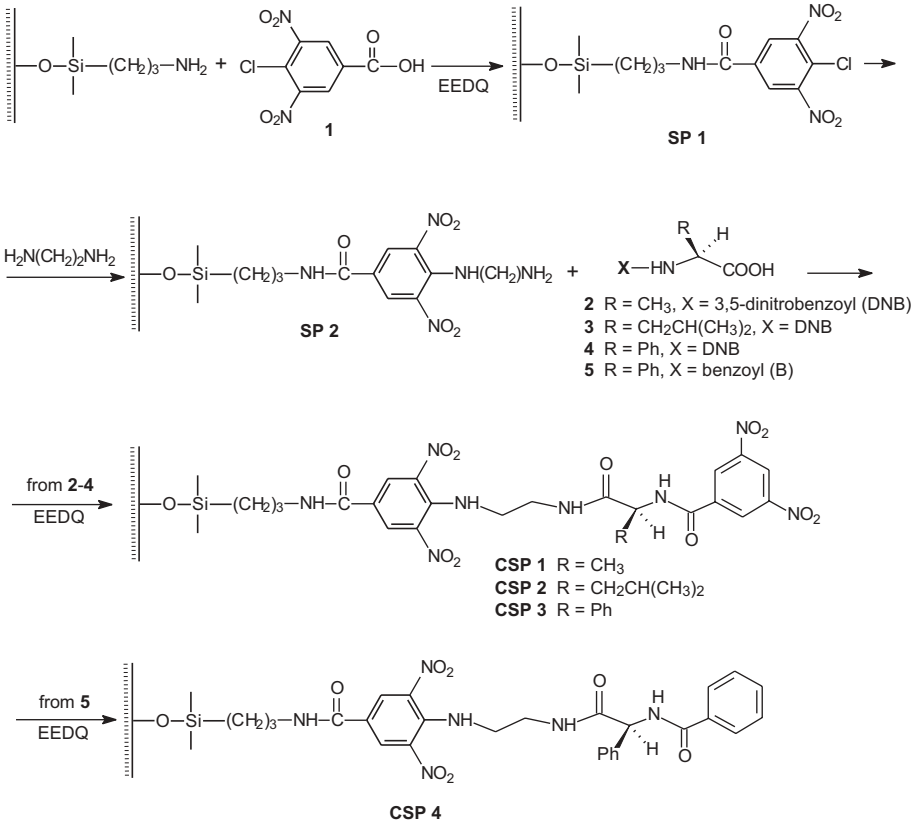
CSP 3: From **SP 2** and phenylglycine derivative **4,** 2.98 g of product was obtained; *Anal*. found: C 11.14, H 1.44, N 2.99 %. As calculated based on C %, 1.0 g of chiral stationary phase **CSP 3** contains *ca* 0.28 mmol of bound selector.

CSP 4: From **SP 2** and *N*-benzoylphenylglycine (**5**), 2.88 g of product was obtained; *Anal*. found: C 11.05, H 1.71, N 1.54. As calculated based on C %, 1.0 g of chiral stationary phase **CSP 4** contains *ca* 0.25 mmol of bound selector.

RESULTS AND DISCUSSION

Preparation of **CSP 1**–**CSP 4** is outlined in Scheme 1, and follows previously reported protocols.[4,8] Amino group of aminopropyl silica, Nucleosil 100-5 NH_2, was

Scheme 1.



acylated by **1** to obtain the stationary phase **SP 1**. The next synthetic steps were completed on the solid phase. Ethylenediamine was first introduced as the second spacer, previously identified as optimal for such a tweezer-type as CSPs,[4] then acylation with *N*-DNB-α-AAs and *N*-B-PheGly afforded **CSP 1**–**CSP 3** and **CSP 4**, respectively.

CSP 1–**CSP 3** are characterised by the terminal DNB group, known as the very strong π-acidic group,[5] and moderately π-acid 4-alkylamino-DNB group as the branching unit. For the reference terminal unit, the π-neutral *N*-B group in the **CSP 4** was selected. The α-amino acid amides are the source of chirality in all these CSPs, which comprise two additional amide groups. Ar-N-H and DNB-CON-H hydrogen atoms are presumably hydrogen bonded to the neighbouring amide carbonyl and to one of the *ortho*-nitro groups in *para*-ethylamino-3,5-DNB unit, affording a shape-persistent core for these CSP.

No systemic study of the enantioseparation of π-donor and π-acceptor analytes (test racemates, TR) by the CSPs possessing high overall structural similarity and matching or mismatching π-electron density has been reported. Several examples of such separations were previously mentioned in the literature, but no general conclusions have been drown.[4,7,9] This study was aimed at determining the enantioselection ability of **CSP 1**–**CSP 4** for electron-deficient (π-acceptor) *vs.* electron rich (π-donor) analytes, the relative contribution of the branching *vs*. terminal π-unit, and the bias of enantioselection, *i.e.*, determination of the preferably bound enantiomer.

All columns filled with **CSP 1**–**CSP 4** were tested in enantioseparation of the two series of test racemates; isopropyl esters of α-amino acids containing either π-acidic *N*-DNB group (**TR 1A**–**TR 7A**), or π-neutral *N*-B group (**TR 1**–**TR 7**), Figure 1. Tables I and II present the chromatographic parameters obtained for separation of both series of test racemates with **CSP 1**–**CSP 4** using two eluting systems. They were selected in order to determine the contribution of the hydrogen bonding to the enantioselection. The first one, n-hexane/2-propanol (90:10), comprises weak hydrogen bonding alcohol, the second one contains methanol, known for its strong solvating and H-donor properties.[10]

As expected, separations obtained for racemic *N*-DNB derivatives **TR 1A**–**TR 7A** were more effective than separations for analogous *N*-B derivatives **TR 1**–**TR 7**. Best separations were registered with **CSP 2**, prepared from *(S)*-leucine, followed by **CSP 3** and **CSP 1**, derived from *(R)*-phenylglycine and *(S)*-alanine, respectively. The first three chiral selectors in **CSP 1**–**CSP 3** comprise two DNB units, and **CSP 4** only the branching *N*-DNB. Inversion of the elution order can therefore be expected for the last one, if the linker π-acceptor *N*-DNB unit significantly contributes to the overall π-π interaction with the analyte. Combined use of CD-detector and enantiomerically pure analytes as reference allowed determination of the elution order for all test racemates on all CSPs;

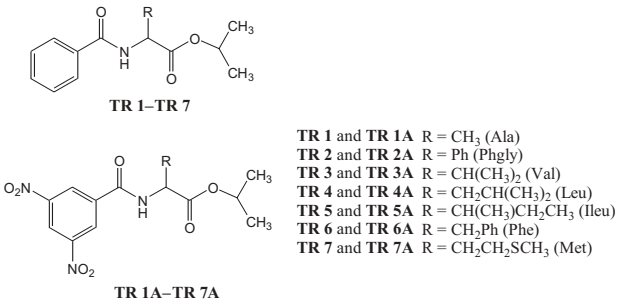


Figure 1. Racemic amino acid derivatives used for **CSP 1**–**CSP 4** evaluation.

some chromatograms are presented in Figure 2. In all cases, except for the test racemate **TR 1** on **CSP 4** where the separation is very poor, the more strongly retained enantiomer of amino acid ester had the same configuration as the amino acid used for CSPs preparation. Such relation was repeatedly reported,[4,7,11] and its interpretation was offered by Hyun *et al.*[9,12]

For the separation of *N*-B derivatives **TR 1**–**TR 7** on **CSP 1**–**CSP3** with the mobile phase hexane/2-propanol 9:1, chromatographic parameters are in some cases close to the values obtained for *N*-DNB derivatives **TR 1A**–**TR 7A**. However, separations obtained for *N*-B derivatives **TR 1**–**TR 7** on **CSP 1**–**CSP 3** were deteriorated by methanol in the solvent mixture (hexane/dichloromethane/methanol 100:30:1), Table II. Samples **TR 1A**–**TR 7A** were not eluted with this mobile phase from column **CSP 4** and therefore the results for **CSP 4** are not included in Table III. The methanol molecule is smaller and a much better H-donor than 2-propanol.[10] It can better solvate the amide bonds in the chiral selector, and presumably perturbs the approach of the analyte to the chiral hole. The results with **CSP 1**–**CSP 3** *vs*. **CSP 4** indicate that solvation does not effect separation of *N*-DNB analytes;

TABLE I. Separation factors (α) and capacity factors of the first eluted enantiomer (k'_1, in parentheses) obtained for racemic analytes **TR 1**–**TR 7** and **TR 1A**–**TR 7A** on columns filled with **CSP 1**–**CSP 4**; column dimensions 250 mm × 4.6 mm ID; mobile phase hexane/2-propanol 9:1; flow rate 1.0 ml/min[a]

analyte	CSP 1	CSP 2	CSP 3	CSP 4	analyte	CSP 1	CSP 2	CSP 3	CSP 4
TR 1	1.12	1.00	1.22	1.02	**TR 1A**	1.16	1.27	1.19	1.22
	(4.77)	(7.25)	(3.57)	(3.08)		(9.06)	(7.41)	(9.40)	(8.02)
TR 2	1.12	1.27	1.25	1.05	**TR 2A**	1.19	1.40	1.29	1.24
	(5.03)	(3.73)	(3.92)	(3.05)		(10.98)	(7.74)	(13.03)	(11.01)
TR 3	1.10	1.23	1.16	1.00	**TR 3A**	1.20	1.37	1.26	1.00
	(3.00)	(2.28)	(2.11)	(2.53)		(6.90)	(5.12)	(7.33)	(2.10)
TR 4	1.18	1.41	1.18	1.06	**TR 4A**	1.21	1.48	1.21	1.25
	(3.15)	(2.34)	(2.13)	(2.01)		(5.58)	(3.98)	(5.99)	(5.21)
TR 5	1.14	1.29	1.22	1.00	**TR 5A**	1.23	1.45	1.31	1.17
	(2.64)	(2.01)	(1.91)	(1.61)		(7.53)	(4.47)	(9.32)	(5.79)
TR 6	1.05	1.11	1.16	1.05	**TR 6A**	1.12	1.22	1.27	1.16
	(5.73)	(4.34)	(4.48)	(3.64)		(12.34)	(8.82)	(13.20)	(10.24)
TR 7	1.16	1.62	1.29	1.04	**TR 7A**	1.20	1.36	1.24	1.26
	(6.86)	(5.08)	(5.56)	(4.24)		(13.92)	(10.04)	(15.88)	(13.55)

[a] *(S)*-enantiomers are always more retained, except for **TR 1** on **CSP 4**.

TABLE II. Separation factors (α) and capacity factors of the first eluted enantiomer (k'_1, in parentheses) obtained for racemic analytes **TR 1**–**TR 7** and **TR 1A**–**TR 7A** on columns filled with **CSP 1**–**CSP 3**; column dimensions 250 mm × 4.6 mm ID; mobile phase hexane/dichloromethane/methanol (100:30:1); flow rate 1.0 ml/min[a]

analyte	CSP 1	CSP 2	CSP 3	analyte	CSP 1	CSP 2	CSP 3
TR 1	1.09 (5.89)	1.00 (7.25)	1.00 (6.34)	**TR 1A**	1.18 (8.37)	1.39 (7.18)	1.24 (9.07)
TR 2	1.06 (2.76)	1.26 (1.72)	1.10 (2.80)	**TR 2A**	1.23 (6.95)	1.49 (5.66)	1.30 (8.38)
TR 3	1.00 (0.97)	1.00 (0.88)	1.00 (1.05)	**TR 3A**	1.21 (4.68)	1.43 (3.92)	1.30 (5.32)
TR 4	1.00 (0.99)	1.00 (0.96)	1.00 (1.00)	**TR 4A**	1.29 (5.28)	1.67 (4.37)	1.26 (6.50)
TR 5	1.00 (0.96)	1.09 (0.91)	1.00 (1.14)	**TR 5A**	1.28 (4.07)	1.54 (3.32)	1.29 (5.04)
TR 6	1.04 (3.12)	1.03 (1.57)	1.10 (2.88)	**TR 6A**	1.13 (7.89)	1.26 (6.40)	1.31 (8.50)
TR 7	1.13 (3.60)	1.24 (2.87)	1.23 (3.40)	**TR 7A**	1.22 (10.10)	1.45 (7.63)	1.31 (12.25)

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

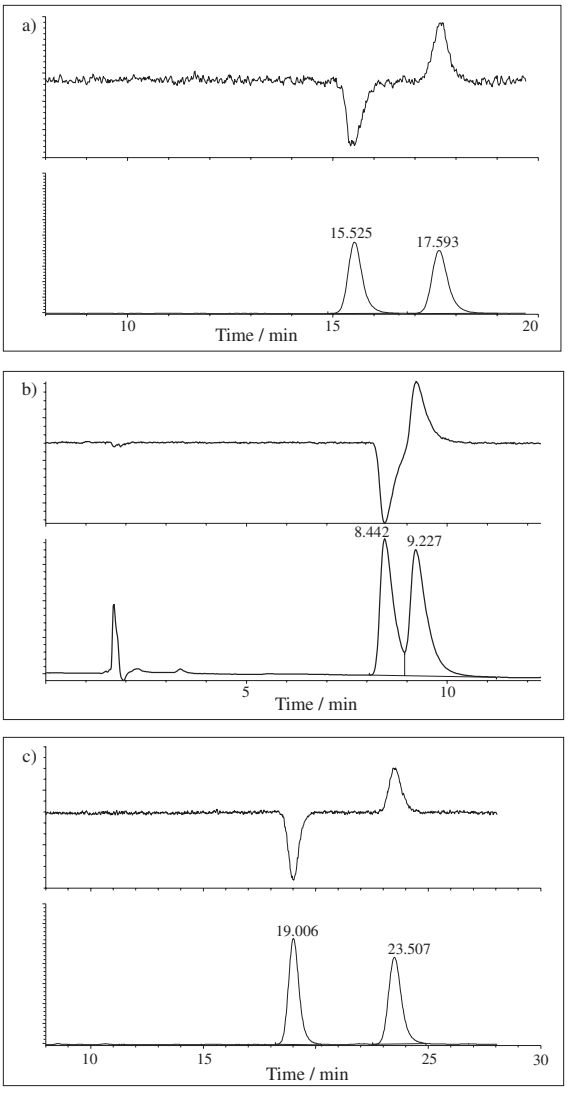


Figure 2. Several chromatograms obtained by the column filled with **CSP 2**; for **TR 2A** (a), **TR 6** (b) and for **TR 6A** (c). Upper frames were obtained by CD detection and lower frames by UV detection.

their separation is similar with both mobile phases. It can be concluded that the π-deficient *N*-DNB group of the analyte enables strong interactions with the π-deficient *N*-DNB group of **CSP**s **1–3** and disrupts H-bonding with methanol. These results also suggest that the primary role of π-π interactions is to enhance attraction of the analytes. This attractive interaction enables the analyte molecule to enter the chiral hole where the analyte enantiomers are differently oriented, depending on the H-bonding and van der Waals interactions, and therefore distinguished by different stability of their complexes.[13]

As mentioned in the Introduction, the *N*-DNB-α-AA unit is present in many chiral stationary phases. Although the contribution of this π-acid unit to chiral recognition is repeatedly evidenced,[14] its relative contribution, as compared, *e.g.*, to the *N*-B-α-AA unit, is not evaluated. *N*-DNB-Ala, *N*-DNB-Leu, and *N*-DNB-Phgly are present in the CSPs formerly prepared by Pirkle,[15] Hyun,[9,12] and by us.[4]

Table III gives the α-values in relation to the π-electron character of the aromatic groups on the chiral selector and analyte. These results reveal that separation of the π-acceptor-acceptor pair is most effective, followed by separation of the π-acceptor and π-neutral aromatic moieties, either in CSP or in analyte, while the least effective are separations when both, CSP and analyte, contain π-neutral aromatic moiety. An interesting point are the similarly efficient separations of *N*-DNB derivatives **TR 1A–TR 7A** on **CSP 4** ($\alpha_{average}$ 1.19) and of *N*-B derivatives **TR 1–TR 7** on **CSP 1–CSP 3** ($\alpha_{average}$ 1.20). If the chiral recognition process is exclusively governed by π-π interactions, *e.g.*, according to the model of the molecular recognition proposed by Hunter *et al.*,[16,17] the interaction between two π-deficient *N*-DNB groups will have a more significant effect on enantioseparation than the interaction between one *N*-DNB and one *N*-B group. The results obtained for **TR 1A–TR 7A** on **CSP 1–CSP 3** and **CSP 4** reveal only a slightly higher enantioselection capacity of the former three CSPs ($\alpha_{average}$ 1.27) than the last one ($\alpha_{average}$ 1.19).

It is remarkable that **CSP 1–CSP 4**, all based on *N*-DNB-α-AA amide chiral selector, preferably bind **TR 1A–TR 7A** and **TR 1–TR 7** of the same absolute configuration, Tables I and II. We assume that the origin of the preferred binding of the enantiomers structurally highly similar to CSP with the same absolute configuration, lies in the »head to tail« orientation of the analyte in the complex with the chiral selector of **CSP 1–CSP 3** and of **CSP 4**.

TABLE III. π-Electron character of the aromatic groups in interactions, and their influence on the enantioseparation

π-character of the interacting group	CSP	TR	α_{min}–α_{max}	$\alpha_{average}$
CSP acceptor TR acceptor	**CSP 1**	**TR 1A–TR 7A**	1.12–1.23	1.19
CSP acceptor TR neutral	**CSP 1**	**TR 1–TR 7**	1.05–1.18	1.12
CSP acceptor TR acceptor	**CSP 2**	**TR 1A–TR 7A**	1.22–1.48	1.36
CSP acceptor TR neutral	**CSP 2**	**TR 1–TR 7**	1.00–1.62	1.28
CSP acceptor TR acceptor	**CSP 3**	**TR 1A–TR 7A**	1.19–1.31	1.25
CSP acceptor TR neutral	**CSP 3**	**TR 1–TR 7**	1.16–1.29	1.21
CSP neutral TR acceptor	**CSP 4**	**TR 1A–TR 7A**	1.00–1.26	1.19
CSP neutral TR neutral	**CSP 4**	**TR 1–TR 7**	1.00–1.06	1.03

Chiral recognition on the Pirkle-type CSPs is taken to occur through the π-π parallel stacking interactions of the two aromatic moieties.[11,12,18] It is known that only the electron-deficient, π-acceptor or π-acid, aromatics prefer to stack in a nearly parallel fashion.[17,19–21] On the contrary, electron-rich, π-donor or π-basic, aromatic units do not stack well because of mutual repelling of the aromatic π-clouds in any stacked orientation except for the edge-on geometry. Either of the π-π interactions between two *N*-DNB units in the analyte and chiral selector in **CSP 1**–**CSP 4**, offset-stacking or edge-to-face orientation, allows formation of up to three hydrogen bonds in the test racemate-chiral selector [TR-CS] complex. Two of them would suffice to get an effective chiral recognition. Structural details of the complex [TR-CS], *i.e.*, the origin of the preferred binding of the enantiomer possessing the same absolute configuration as CS, can be deduced from the combined experimental and computational approach. Elucidation of the structures of the representative couple of diastereomeric complexes between enantiomeric *N*-DNB-α-AA esters such as TR and *N*-DNB-α-AA amides in chiral selectors is envisaged.

CONCLUSIONS

In conclusion, the presence of the terminal DNB group in the **CSP 1**–**CSP 3** and in the analyte, though not the sole factor important for chiral discrimination, significantly contributes to the enantioseparation. Terminal DNB group in the **CSP 1**–**CSP 3** enables separation of both π-acid and π-basic analytes, the former with higher efficacy than the latter. Terminal *N*-B group in **CSP 4** enables effective enantioseparation of **TR 1A**–**TR 7A**, which contain the π-acceptor *N*-DNB group. The same elution order of enantiomers on **CSP 1**–**CSP 3** and **CSP 4** indicates a limited contribution of the branching *N*-DNB unit to the orientation of the analyte. The attractive π-π interactions presumably allow the entry of both enantiomers into the chiral hole, which then orient differently, governed by H-bonding and van der Waals interactions.

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SAŽETAK

Studija kromatografske enantioseparacije estera *N*-dinitrobenzoil (*N*-DNB) i *N*-benzoil (*N*-B) α-aminokiselina na novim kiralnim nepokretnim fazama čiji kiralni selektori sadrže strukturno slične *N*-DNB i *N*-B α-AA amide

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Proučavane su sklonosti i mogućnosti odjeljivanja enantiomera na kiralnim nepokretnim fazama **CSP 1**–**CSP 3** koje sadrže terminalnu *N*-3,5-dinitrobenzoilnu (*N*-DNB) jedinicu te na **CSP 4** koja sadrži terminalnu *N*-benzoilnu jedinicu. Određeni su faktori odjeljivanja (α) za dva skupa test racemata (TR) koji su strukturno slični kiralnim selektorima ispitivanih kiralnih nepokretnih faza. Prvi se skup sastoji od sedam izopropilnih estera *N*-DNB α-aminokiselina (**TR 1A**–**TR 7A**), a drugi od njihovih *N*-B analoga (**TR 1**–**TR 7**). Prosječno najbolja odjeljivanja enantiomera ($\alpha_{average}$ 1.27) su dobivena kada je π-akceptorska DNB jedinica prisutna i u TR i u CSP. Jedna π-akceptorska jedinica, ili u analitu ili u CSP, dovoljna je za učinkovitu enantioseparaciju ($\alpha_{average}$ 1.19), međutim, interakcija između π-neutralnih jedinica i u CSP i u TR ne omogućuje dobru enantioseparaciju ($\alpha_{average}$ 1.03). Uporabom *(S)*-enantiomera test racemata kao standarda i CD detekcijom utvrđeno je da kiralne nepokretne faze ovoga tipa imaju veću sklonost prema enantiomerima apsolutne konfiguracije iste kao kiralni selektor. Razmatran je mogući razlog takve sklonosti.