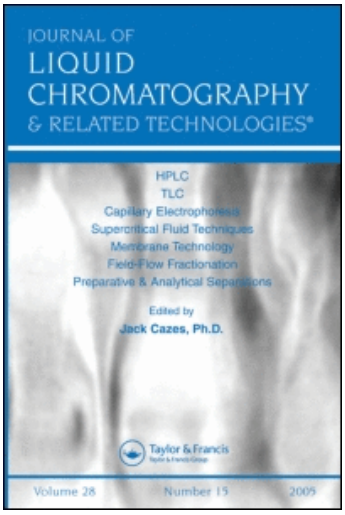

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Enantiomer Separation and Molecular Recognition with New Chiral Stationary Phases on 4-Chloro-3,5-dinitrobenzoic Acid Amides of α,β-Aminoalcohols and α-Arylethylamines

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ABSTRACT

Amides of 4-chloro-3,5-dinitrobenzoic acid (**1**) (CDNB) with enantiomerically pure α,β-aminoalcohols (**2–5**) and α-substituted ethylamines,[6–9] have been prepared as chiral selectors **10–17** and bound to aminopropyl silica gel affording chiral stationary phases **CSPs 1–8**. Comparative tests of their separation efficacy for 32 racemic analytes, representative of racemates with selected functionalities, has revealed important contribution of the π-acidic branching unit, the amide of 3,5-dinitro-4-alkylaminobenzoic acid, but limited contribution of both, hydrophilic hydroxy groups in the **CSPs 1–4** and π-basic aromatic units in the **CSPs 6–8**. Contribution to

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enantioselection efficacy of the γ-aminopropyl groups on the silica surface, in the vicinity to the chiral selector, is documented comparing the efficacy of **CSP 8** and **CSP 8′**.

Key Words: Chiral stationary phase; Brush-type; HPLC; 4-Chloro-3,5-dinitrobenzoic acid; Silica gel surface.

INTRODUCTION

Since the strong π-electron interactions are important for enantiorecognition on brush type of CSPs, most of them contain either strong π-donor or strong π-acceptor groups.[1] The most often π-donor group used is the naphthyl group,[2,3] and 3,5-dinitrobenzoyl group (DNB) is the most usual π-acceptor group,[4,5] whereas the most universal brush-type CSPs, Whelk-O1,[6] is composed of highly preorganized chiral cleft, which consists of an aromatic π-basic tetrahydrophenantrene ring and a π-acidic DNB group.[7] Mixed π-character of Whelk-O1 and its rigid structure are meritorious for success of this CSP.

In the last few years, we have developed a number of original brush-type CSPs that possess π-donor or π-acceptor properties, with 1,4,5,6-tetrachloro-1,3-dicyanobenzene (CTL) as the branching unit, which also can act as a weak or strong π-donor.[8–10] Assuming the branching structural element of chiral selector could be more involved in the chiral recognition process if it acts as a strong π-acceptor,[11] we have selected commercially available 4-chloro-3,5-dinitrobenzoic acid (CDNB) as the origin of a novel branching unit. Here we report on design and enantiomer separation with this novel type of CSPs that comprise amides of CDNB with either enantiomerically pure α,β-aminoalcohols, having hydrophilic hydroxyl group, or α-substituted ethylamines, having lipophilic, π-basic aromatic group.

EXPERIMENTAL

Chemicals

4-Chloro-3,5-dinitrobenzoic acid, (1*S*, 2*R*)-(−)-*cis*-1-amino-2-indanol, (1*R*, 2*S*)-(−)-norefedrine, L-alaninol, L-phenylglycinol, N,N,N-triethylamine, and (*R*)-(+)-1-(1-naphthyl)ethyl amine were obtained from Fluka (Buchs, Switzerland); (*R*)-(+)-1-phenylethyl amine, (*R*)-(+)-cyclohexylethyl

amine, N,N-dicyclohexylcarbodiimide (DCC), 2-ethoxy-1-ethoxycarbonyle-1,2-dihydrochinoline (EEDQ), and aminopropyltriethoxysilane (APTES) from Sigma-Aldrich (Aldrich Chimica, Milano, Italy); sodium hydroxide from Kemika (Zagreb, Croatia). HPLC silica gels Nucleosil 100-5 NH_2 and Nucleosil 100-5 were purchased from Macherey-Nagel (Düren, Germany). All the solvents used were purchased from J. T. Baker (Davenport, Holland) and distilled before use.

Some of the racemates used for the evaluation of columns were purchased from Sigma-Aldrich: *trans*-stilbenoxide (**TR 1**), benzoine (**TR 2**), Tröger base (**TR 3**), warfarine (**TR 4**), 1-(9-anthryl)-2,2,2-trifluoro-1-ethanol (**TR 5**) and α-arylethanols **TR 30–32**. The list of racemates prepared in our laboratory runs as follows: ethyl-7-chloro-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine-3-carboxylate (**TR 6**), ethyl-7-chloro-1,3-dimethyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine-3-carboxylate (**TR 7**), 7-chloro-3-(hydroxymethyl)-5-phenyl-3-benzyl-1H-benzo[f]1,4-diazepin-2-one (**TR 8**), N^1-(1-phenylethyl)-2,2-dimethylpropanamide (**TR 11**), N^1-(1-phenylethyl)benzamide (**TR 12**), N^1-(1-phenylethyl)-1-naphthamide (**TR 13**), N^1-phenyl-2-(4-isobutylphenyl)-propanamide (**TR 14**), N^1-(1-phenylethyl)-3,5-dinitrobenzamide (**TR 15**), and all 8 isopropyl esters of N-3,5-dinitrobenzoyl derivatives of amino acids (**TR 16** to **TR 23**). Racemates **TR 9** and **TR 10** were obtained from Dr. Z. Majer (Lorand Eötvös University, Budapest, Hungary), **TR 24** from Dr. I. Patonay (Lajos Kosuth University, Debrecen, Hungary), and dihydropyrimidine derivatives **TR 25** to **TR 29** from Dr. O. Kappe (Karl-Franzens University, Graz, Austria).

Apparatus and Chromatography

IR: Perkin Elmer 297 spectrometer for KBr pellets. ^1H and ^{13}C NMR: Varian Gemini XL 300 spectrometer; δ in ppm relative to TMS as internal reference. Melting point: Electrothermal 9100 digital apparatus. Elemental analyses were carried out by the Central Analytical Service (CAS) at Ruder Boškovic 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. Integration of the chromatograms was made with the BDS software package (Barspec Ltd., Rehovot, Israel). The enantioselective analysis of product **18** was done by commercial column

Chiris-AD1, 150 mm × 4.6 mm ID (Iris Technologies, Lawrence, USA). For new prepared columns the following parameters were measured:

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 inflexion points of the chromatographic peak.

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. n-Hexane, 2-propanol, dichloromethane, and other 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 or dichloromethane. For analytical purposes 5 μL of freshly prepared solution were used.

Preparation of 3,5-Dinitrobenzamides 10–17

General Procedure

To the solution of CDNB (1.00 g; 4.0 mmol) and EEDQ (1.00 g; 4.0 mmol) in dichloromethane (30 mL), a solution of enantiomerically pure amino alcohol 2–5 (4.0Emmol) in dichloromethane (20 mL) was added. The obtained mixture was stirred for 16 h at ambient temperature and afforded the products **10–13** which were isolated by column chromatography or by filtration. Using the same procedure, but DCC (0.84 g, 4.0 mmol) as the condensing agent, α-arylethylamines 6–9 (4.0 mmol) were acylated, and products **14–17** isolated.

*(4-Chloro-3,5-Dinitrophenyl)-N-(2-Hydroxyisopropyl)Carboxamide (**10**)*

After chromatography on silica gel, eluent toluene/acetone 9 : 1, 1.02 g (83%) of product **10** as a yellow oil was obtained. IR (KBr): 3275, 3065, 2980, 1635, 1528, 1337, and 1305 cm^{-1}. ^1H NMR (acetone-d_6): 1.22 (3H, *d*, J = 7.0 Hz), 3.60 (2H, dd, J = 10.3 and 5.3 Hz), 4.05 (1H, *t*, J = 5.3 Hz), 4.20 (1H, m), 8.10 (1H, *d*, J = 5.4 Hz) and 8.72 (2H, s). ^{13}C NMR (acetone-d_6): 17.11, 49.48, 65.72, 122.11, 127.77, 136.96, 150.27, and 162.56. Anal. calcd.

for $C_{10}H_{10}O_6N_3Cl$ (303.65): C 39.55, H 3.31 and N 13.84. Found: C 39.58, H 3.26 and N 13.81%.

*N-((1S)-2-Hydroxy-1-Phenylethyl)(4-Chloro-3,5-Dinitrophenyl) Carboxamide (**11**)*

After chromatography on silica gel, eluent dichloromethane/methanol 100 : 3, 0.68 g (46%) of product **11** as yellow powder, mp 74–75°C was obtained. IR (KBr): 3270, 3060, 1637, 1533, and 1307 cm^{-1}. ^1H NMR ($CDCl_3$): 3.96 (1H, *d*, J = 5.2 Hz), 4.08 (2H, dd, J = 10.0 and 5.2 Hz), 5.21 (1H, m), 7.21–7.37 (5H, m), 8.48 (2H, s), and 8.94 (1H, *d*, J = 4.1 Hz). ^{13}C NMR ($CDCl_3$): 56.12, 64.77, 125.89, 126.10, 126.21, 127.59, 128.32, 134.00, 137.58, 148.76, and 161.88. Anal. calcd. for $C_{15}H_{12}O_6N_3Cl$ (365.72): C 49.25, H 3.30, and N 11.49. Found: C 48.99, H 3.24, and N 11.52%.

*(4-Chloro-3,5-Dinitrophenyl)-N-(2-Hydroxy-1-Methyl-2-Phenylethyl) Carboxamide (**12**)*

Solid product was obtained by filtration on G-4 filter. The product was dried at 50°C for 4 h and afforded 1.39 g (90%) of white powder, mp 212–213°C. IR (KBr): 3440, 3405, 3060, 2965, 2875, 1640, 1525, 1335, 990, 910, 730, 710, and 695 cm^{-1}. ^1H NMR (DMSO-d_6): 1.05 (3H, *d*, J = 6.5 Hz), 4.15 (1H, dd, J = 12.0 and 7.2 Hz), 4.75 (1H, *d*, J = 7.2 Hz), 5.72 (1H, m), 7.17–7.40 (5H, m), 8.77 (2H, s), and 8.88 (1H, *d*, J = 8.3 Hz). ^{13}C NMR (DMSO-d_6): 13.70, 52.06, 73.91, 121.29, 126.16, 126.93, 127.42, 127.98, 135.34, 143.28, 148.75, and 161.02. Anal. calcd. for $C_{16}H_{14}O_6N_3Cl$ (379.75): C 50.60, H 3.71, and N 11.06. Found: C 50.68, H 3.65, and N 11.02%.

*N-((2S,1R)-2-Hydroxyindanyl)-(4-Chloro-3,5-Dinitrophenyl) Carboxamide (**13**)*

Solid product was obtained by filtration on G-4 filter. The product was dried at 50°C for 4 h and afforded 1.18 g (77%) of yellowish powder, mp 218–219°C. IR (KBr): 3240, 3050, 2880, 1630, 1520, 1340, 1280, 1050, 960, 900, and 730 cm^{-1}. ^1H NMR (DMSO-d_6): 2.87 (1H, *d*, J = 16.1 Hz), 3.10 (1H, dd, J = 16.1 and 7.0 Hz), 4.23 (1H, bs), 4.54 (1H, s), 5.40–5.51 (1H, m), 7.11–2.26 (4H, m), 8.92 (2H, s), and 9.12 (1H, *d*, J = 8.6 Hz). ^{13}C NMR (DMSO-d_6): 39.88, 58.36, 72.26, 121.08, 124.75, 124.95, 126.40, 127.72, 127.84, 135.44, 140.93, 141.33, 148.67, and 162.54. Anal. calcd. for

$C_{16}H_{12}O_6N_3Cl$ (377.73): C 50.87, H 3.20, and N 11.12. Found: C 50.94, H 3.19, and N 11.14%.

*N^1-[(1R)-1-Cyclohexylethyl]-4-Chloro-3,5-Dinitrobenzamide (**14**)*

0.70 g (97%) of product **14** as a slightly yellow powder, mp 216–217°C was obtained. IR (KBr): 3280, 3080, 2920, 2860, 1650, 1545, 1450, 1400, 1350, 1310, 1230, 1190, 1140, 1060, 990, 970, 940, 890, 840, 820, 750 i 700 cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.04–1.76 (11H, m), 1.05 (3H, *d*, J = 10.7 Hz), 4.06–4.07 (1H, m), 6.26 (1H, bs), and 8.38 (2H, s). ^{13}C NMR (DMSO-d_6): 18.57, 26.73, 26.92, 29.76, 30.05, 43.72, 51.92, 123.71, 126.79, 136.20, 150.24, and 162.05. Anal. calcd. for $C_{15}H_{18}O_5N_3Cl$ (355.77): C 50.63, H 5.09, and N 11.81. Found: C 51.45, H 5.32, and N 11.68%.

*N^1-[(1R)-1-Phenylethyl]-4-Chloro-3,5-Dinitrobenzamide (**15**)*

We obtained 1.40 g (98%) of product as yellow solid, mp 202–203°C. IR (KBr): 3340, 3280, 3090, 3080, 3040, 2980, 2940, 2880, 1690, 1610, 1550, 1500, 1450, 1350, 1290, 1130, 1060, 1010, 920, 830, 760, 740, 720, and 700 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.63 (3H, *d*, J = 7.0 Hz), 5.28 (1H, d*t*, J = 7.2 and 7.0 Hz), 7.30–7.54 (5H, m), 8.96 (2H, s), and 9.47 (1H, *d*, J = 7.8 Hz). ^{13}C-NMR ($CDCl_3$): 21.96, 49.38, 121.32, 126.19, 126.94, 127.38, 128.35, 135.06, 143.88, 148.79, and 160.86. Anal. calcd. for $C_{15}H_{12}O_5N_3Cl$ (349.72): C 51.51, H 3.45, and N 12.01. Found: C 52.01, H 3.62, and N 11.89%.

*N^1-[(1R)-1-(1-Naphthyl)Ethyl]-4-Chloro-3,5-Dinitrobenzamide (**16**)*

We obtained 1.55 g (95%) of 16 as a yellow powder, mp127–130°C. IR (KBr): 3600, 3380, 3230, 3150, 1790, 1700, 1600, 1495, 1210, 1070, 950, 930, 890, and 870 cm^{-1}. ^{1}H NMR (DMSO-d_6): 1.65 (3H, *d*, J = 6.6 Hz), 5.92–5.99 (1H, m), 7.33–8.76 (7H, m), 8.88 (2H, s), and 9.53 (1H, *d*, J = 7.3 Hz). ^{13}C NMR (DMSO-d_6): 21.26, 45.58, 122.81, 123.03, 125.55, 125.72, 126.41, 126.90, 127.42, 127.62, 128.78, 130.44, 133.45, 134.86, 139.38, 148.78, and 160.89. Anal. calcd. for $C_{19}H_{14}O_5N_3Cl$ (399.77): C 57.08, H 3.52, and N 10.51. Found: C 57.21, H 3.68, and N 10.06%.

*(R)-(+)-1-(9-Anthryl)Ethyl-4-Chloro-3,5-Dinitrobenzamide (**17**)*

Starting from *(R)*-1-(9-anthryl)Ethyl amine[12] we obtained 1.64 g (64%) of 17 as a yellow powder, mp 211–213°C, $[\alpha]^{20}_D = -375$ ($c = 2$ mg/mL

DMF). Enantiomeric purity was determined by Chiris-AD1 column, mobile phase n-hexane-dichloromethane-methanol (40 : 60 : 0.5), flow rate 1.0 mL/min, $t_{R1} = 6.8$ min, $t_{R2} = 8.2$ min. The estimated enantiomeric excess of **17** was 97.1%. IR (KBr): 3280, 3060, 1680, 1540, 1345, 1325, 1070, 900, and 744 cm^{-1}. ^1H NMR (DMSO-d_6): 2.05 (3H, *d*, J = 7.0 Hz), 6.59–6.69 (1H, m), 7.57–7.71 (4H, m), 8.20 (2H, *d*, J = 8.0 Hz), 8,65 (1H, s), 8.83 (2H, *d*, J = 8.0 Hz), 8.91 (2H, s), and 10.02 (1H, bs). ^{13}C NMR (DMSO-d_6): 20.84, 46.60, 121.61, 124.61, 124.99, 125.86, 127.49, 128.57, 129.53, 131.46, 134.76, 135.24, 148.83, and 161.32. Anal. calcd. for $C_{23}H_{16}N_3O_5Cl$ (449.86): C 61.41, H 3.59, and N 9.34. Found: C 61.23, H 3.59, and N 9.33%.

Preparation of Chiral Stationary Phases CSP 1–8

General Procedure

The suspension of chiral selector (0.60 g, 1.50 mmol) and silica gel Nucleosil 100-5 NH_2 (3.00 g, anal. found N 1.36, C 3.49%) in N,N-dimethylformamide (DMF, 15 mL) was stirred overnight at room temperature. The obtained chiral stationary phase was separated by vacuum filtration on G-4 filter, washed with DMF (2 × 20 mL) and methanol (3 × 20 mL), and dried at 70°C for 6 h.

CSP 1. 3.15 g of **CSP 1** was obtained. Anal. found: C 6.92, H 2.24, N 1.99%. As calculated on C%: 1.0 g of CSP contains 0.285 mmol of bound selector.

CSP 2. 3.19 g of **CSP 2** was obtained. Anal. found: C 6.86, H 2.41, N 2.22%. As calculated on C%, 1.0 g of CSP contains 0.187 mmol of bound selector.

CSP 3. 3.19 g of **CSP 3** was obtained. Anal. found: C 7.04, H 2.26, N 2.13%. As calculated on C%, 1.0 g of CSP contains 0.184 mmol of bound selector.

CSP 4. We obtained 3.26 g of **CSP 4**. Anal. found: C 6.96, H 2.01, N 2.14%. As calculated on C%, 1.0 g of CSP contains 0.180 mmol of bound selector.

CSP 5. We obtained 3.34 g of **CSP 5**. Anal. found: C 7.62, H 2.34, N 1.97%. As calculated on C%: 1.0 g of CSP contains 0.180 mmol of bound selector.

CSP 6. Obtained was 3.21 g of **CSP 6**. Anal. found: C 7.41, H 2.81, N 2.25%. As calculated on C%, 1.0 g of CSP contains 0.217 mmol of bound selector.

CSP 7. 3.33 g of **CSP 7** was obtained. Anal. found: C 7.41, H 2.76, N 2.23%. As calculated on C%, 1.0 g of CSP contains 0.217 mmol of bound selector.

CSP 8. 3.29 g of **CSP 8** was obtained. Anal. found: C 9.96, H 1.70, N 1.77%. As calculated on C%, 1.0 g of CSP contains 0.221 mmol of bound selector.

Preparation of (R)-(+)-1-(9-Anthryl)ethyl-4-(γ-Triethoxysilyl-propyl) Amino-3,5-Dinitrobenzamide (18)

Compound **17** (0.45 g; 1.0 mmol) and 3-aminopropyltriethoxysilane (APTES, 5.0 mL) were heated on the bath at 70°C for 15 min. The crude product was separated on a silica column (30 g) with toluene-acetone (10 : 1) as eluent. Fractions with pure product were collected and evaporated to give 0.51 g (87%) of product 18. IR (KBr) 3260, 3040, 2935, 2905, 1618, 1510, 1265, 1068, and 720 cm^{-1}. ^1H NMR ($CDCl_3$) 0.51 (2H, *t*, J = 7.9 Hz), 1.06 (9H, *T*, J = 7.3 Hz), 1.60–1,70 (2H, m), 1.87 (3H, *d*, J = 7.1 Hz), 2.85–2.92 (2H, m), 3.65 (6H, *q*, J = 7.3 Hz), 6.48 (1H, m), 7.42–7.58 (5H, m), 8.04 (1H, *d*, J = 8.0 Hz), 8.35 (1H, m), 8.47 (1H, s), 8.71 (2H, bs), 8.75 (2H, s), and 9.62 (1H, *d*, J = 4.7 Hz). ^{13}C NMR ($CDCl_3$) 6.94, 18.72, 20.79, 23.01, 46.21, 48.84, 57.83, 119.42, 124.63, 124.84, 125.57, 127.17, 128.47, 129.39, 130.96, 131.40, 135.90, 137.14, 140.62, and 162.05. Anal. calcd. for $C_{32}H_{38}N_4O_5Si$ (586.75): C 65.51, H 6.52, and N 9.54. Found: C 65.68, H 6.48, and N 9.50%.

Preparation of Chiral Stationary Phase CSP 8′

Selector **18** (0.51 g, 0.87 mmol) and silica gel *Nucleosil* 100-5 (3.0 g) were suspended in dry toluene (10.0 mL) and heated under reflux for 24 h. The modified silica gel was collected on a G-4 filter, washed with toluene, then with 2-propanol and with *n*-hexane, and dried at 70°C for 5 h. 3.36 g of **CSP 8′** was obtained. Anal. found: C 7.99, H 1.39, N 3.52%. As calculated on C%, 1.0 g of CSP contains 0.238 mmol of bound selector.

RESULTS AND DISCUSSION

Starting from (CDNB)[1] and two groups of four primary amines, **2–5** and **6–9**, chiral selectors **10–17** were prepared, and bound to aminopropyl silica gel to give **CSPs 1–8**, Scheme 1.

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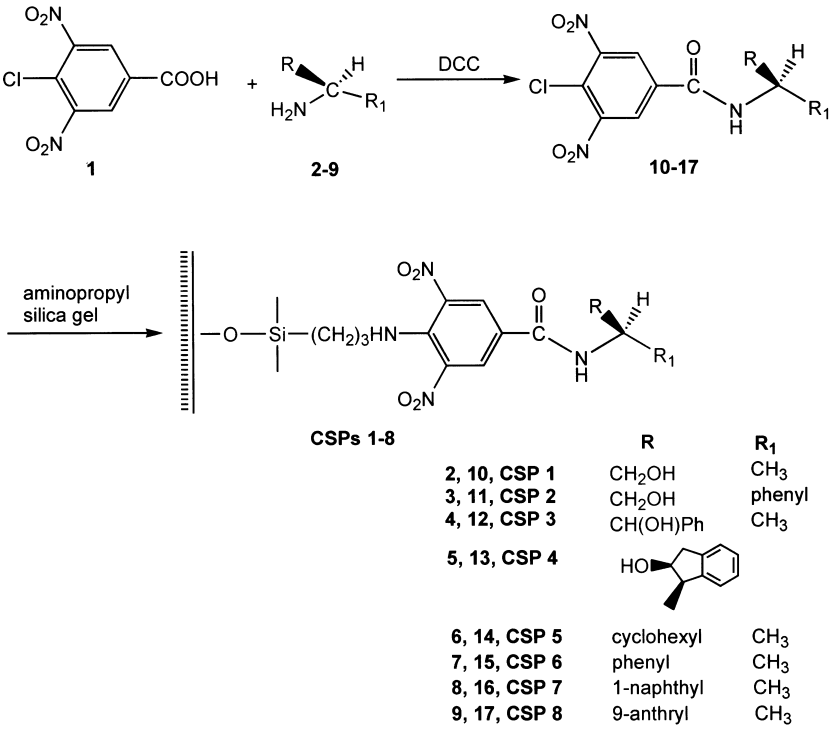
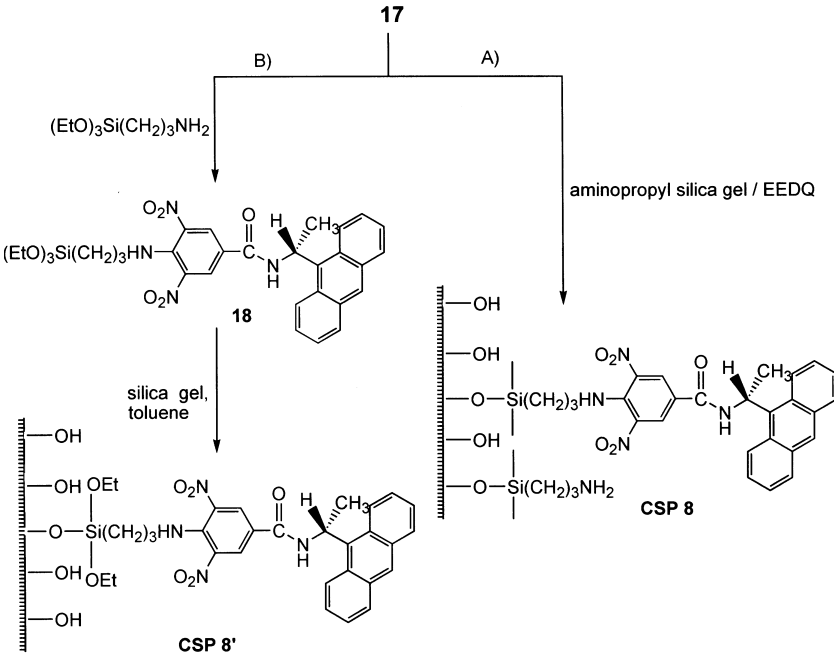


	R	R_1
2, 10, CSP 1	CH_2OH	CH_3
3, 11, CSP 2	CH_2OH	phenyl
4, 12, CSP 3	CH(OH)Ph	CH_3
5, 13, CSP 4	HO	
6, 14, CSP 5	cyclohexyl	CH_3
7, 15, CSP 6	phenyl	CH_3
8, 16, CSP 7	1-naphthyl	CH_3
9, 17, CSP 8	9-anthryl	CH_3

Scheme 1.

Since we recently observed decisive contribution of the free γ-aminopropyl groups on the silica surface to enantiorecognition of 2-aryloxypropionic acids,[13] we have prepared, by a modified approach, **CSP 8′** with the same chiral selector as **CSP 8** but avoided the free aminopropyl groups, Scheme 2.

Tetrasubstituted benzene rings in the **CSPs 1–8** retains π-acid properties, though the electron-donating amino group replaces electron-acceptor chlorine atoms. Cumulative Hammett σ^* value for 4-amino-3,5-dinitro-1-carboxamido benzene could be roughly estimated as $+1.26$,[14] compared to -1.15 and -2.35 for diamino- and triamino-dicyano-chlorobenzene branching units, present in our recently reported CSPs.[8–10] This estimation reveals strong π-acceptor properties of the former and π-donor properties of the later branching aromatic unit. The first group here reported CSPs containing enantiopure α,β-aminoalcohols **2–5** with (**3, 5**) or without (**2, 4**) an additional aromatic ring. The second group of CSPs (CSPs **6–8**) contains enantiopure



Scheme 2.

π-basic α-arylaminoethanes (**7–9**) and α-cyclohexylaminoethane **(6)** as a reference void of any π-basic aromatic unit, but capable for the Van der Waals interactions. The branching aromatic π-acid unit is connected to a chiral unit via an amide bond that can act as H-bond donor–acceptor and also confers certain rigidity to the whole assembly.

Screening of the columns filled with **CSPs 1–4**, Table 1, revealed the ternary mixture n-hexane/dichloromethane/methanol (100 : 30 : 1) as a better solvent system than a traditional binary mixture n-hexane/2-propanol; contribution of dichloromethane can be ascribed to the promotion of hydrogen-bonding interaction. Among **CSPs 1–4**, the best efficacy with most racemates exhibit **CSP 4**, derivative of bicyclic (+)-2-aminoindanol, and the least effective enantioseparation was obtained with **CSP 1**, derived from conformationally flexible L-alaninol, confirming that conformationally rigidity of the CSP is important for chiral recognition. The results of enantioseparation for the set of 29 test racemates (**TR 1**–**TR 29** are listed in Fig. 1) and the chromatographic resolution parameters are presented in the Tables 1 and 2. No one of the CSPs effected resolution with the first three racemates, **TR 1–3**,

Table 1. Results obtained for evaluation of HPLC columns filled with **CSPs 1–4**, columns dimension 250 mm × 4.6 mm ID, and **CSP 2′***.

Analyte	Mobile phase	CSP 1			CSP 2			CSP 3			CPS 4		
		k'_2	α	R_S	k'_2	α	R_S	k'_2	α	R_S	k'_2	α	R_S
TR 2	A	0.19	1.0	0	2.12	1.04	nm	0.25	1.0	0	2.41	1.06	0.32
TR 3	A	1.16	1.0	0	1.41	1.0	0	1.33	1.0	0	1.47	1.12	0.79
TR 5	A	3.81	1.03	0.64	5.42	1.15	1.65	3.99	1.04	0.62	4.02	1.05	0.10
TR 6	A	9.79	1.07	nm	36.02	1.0	0	11.92	1.0	0	44.41	1.17	0.86
TR 7	A	1.30	1.06	0.45	1.51	1.0	0	1.15	1.0	0	1.23	1.0	0
TR 12	A	2.98	1.0	0	4.25	1.09	0.89	4.14	1.0	0	3.33	1.07	0.59
TR 13	A	3.37	1.0	0	5.70	1.10	0.93	3.97	1.02	nm	3.82	1.04	0.15
	B	2.29	1.0	0	4.79	1.15	1.18	3.79	1.0	0	3.63	1.06	0.47
TR 14	A	1.45	1.0	0	2.02	1.12	0.86	1.50	1.0	0	1.55	1.0	0
TR 15	A	7.85	1.0	0	15.31	1.10	0.95	11.60	1.03	0.37	14.22	1.0	0
	B	2.00	1.0	0	3.81	1.0	0	3.20	1.0	0	4.22	1.06	0.66
TR 16	A	4.03	1.05	0.73	8.12	1.15	1.34	5.73	1.07	0.93	7.81	1.20	2.09
TR 17	A	1.56	1.02	nm	3.48	1.11	0.83	2.95	1.0	0	4.15	1.17	2.40
TR 18	A	2.31	1.12	0.54	5.12	1.07	0.41	3.76	1.07	0.91	5.26	1.25	1.45
TR 19	A	1.53	1.24	0.90	3.34	1.05	0.29	2.79	1.12	0.79	3.71	1.22	2.16
TR 20	A	2.50	1.05	0.36	6.54	1.18	1.60	4.34	1.04	0.51	6.36	1.14	1.54
TR 21	A	2.34	1.07	0.47	6.04	1.11	0.86	4.20	1.06	0.71	6.34	1.23	2.30

(*continued*)

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Table 1. Continued.

Analyte	Mobile phase	CSP 1			CSP 2			CSP 3			CPS 4		
		k'_2	α	R_S	k'_2	α	R_S	k'_2	α	R_S	k'_2	α	R_S
TR 22	A	4.07	1.05	0.68	8.64	1.07	0.48	6.26	1.08	0.96	9.12	1.21	2.38
TR 23	A	3.17	1.0	0	5.20	1.08	0.44	3.95	1.07	0.88	5.87	1.16	1.80
TR 30	C	6.69	1.0	0	7.85	1.0	0	11.03	1.0	0	5.29	1.0	0
TR 31	A	1.30	1.0	0	2.72	1.0	0	2.24	1.05	0.41	3.48	1.0	0
	C	1.69	1.0	0	4.42	1.0	0	6.06	1.0	0	2.99	1.0	0
TR 32	C	8.85	1.0	0	15.7	1.0	0	17.21	1.0	0	1.17	1.0	0

*A = *n*-hexane/dichloromethane/2-propanol 100 : 30 : 1, flow 1.0 mL/min; B = *n*-hexane/2-propanol 99 : 1, flow 1.0 mL/min.
Note: nm = nonmeasurable.

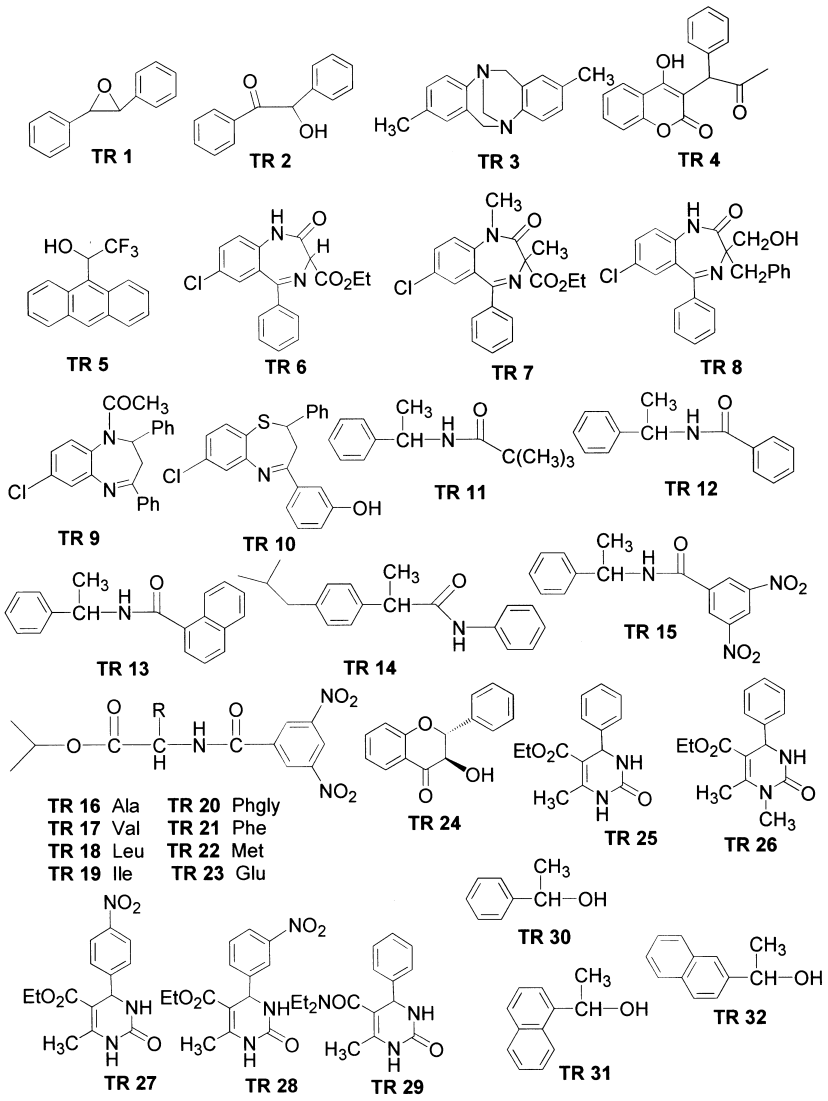

Figure 1. Structures of test racemates used for evaluation of newly prepared CSPs.

Table 2. Results obtained for evaluation of HPLC columns filled with **CSPs 5-8**; columns dimension 250 mm × 4.6 mm ID; mobile phase *n*-hexane/2-propanol 8 : 2; flow rate 1.0 mL/min.

	CSP 5			CSP 6			CSP 7			CSP 8		
Analyte	k_2'	α	R_S	k_2'	α	R_S	k_2'	α	R_S	k_2'	α	R_S
TR 4	2.43	1.0	0	1.95	1.08	0.34	2.11	1.08	0.35	r	—	—
TR 5	0.44	1.0	0	0.43	1.0	0	0.27	1.0	0	0.94	1.05	0.21
TR 6	1.15	1.05	0.51	1.75	1.0	0	1.69	1.0	0	6.73	1.0	0
TR 7	0.97	1.0	0	0.81	1.0	0	0.78	1.07	0.82	1.41	1.10	0.42
TR 8	0.79	1.0	0	0.98	1.0	0	0.90	1.0	0	3.82	1.02	nm
TR 9	2.42	1.07	nm	4.45	1.09	0.86	2.19	1.11	0.68	9.33	1.03	nm
TR 10	0.29	1.0	0	0.40	1.0	0	0.27	1.0	0	1.00	1.10	nm
TR 11	3.00	1.0	0	0.89	1.11	0.68	0.53	1.17	0.92	1.44	1.10	1.02
TR 12	2.04	1.0	0	1.79	1.11	0.95	1.83	1.11	1.07	4.58	1.10	0.65
TR 13	4.12	1.0	0	3.59	1.11	1.48	3.12	1.07	0.66	2.63	1.12	0.24
TR 14	0.87	1.0	0	0.74	1.09	0.52	0.61	1.09	0.57	1.76	1.11	1.10

TR 15	1.79	1.0	0	2.46	1.13	1.17	4.63	1.46	4.20	1.67	1.19	0.22
TR 16	1.05	1.0	0	1.17	1.11	0.92	1.50	1.36	2.08	5.55	1.05	0.52
TR 17	0.87	1.0	0	0.93	1.19	1.17	1.33	1.62	3.80	4.53	1.15	1.47
TR 18	0.74	1.0	0	0.79	1.27	1.41	1.11	1.79	4.00	3.31	1.17	2.00
TR 19	1.15	1.0	0	0.89	1.20	1.32	1.29	1.61	3.31	4.31	1.12	0.81
TR 20	1.17	1.0	0	1.54	1.0	0	2.99	1.26	2.33	9.97	1.09	0.96
TR 21	1.03	1.0	0	1.27	1.18	1.47	2.18	1.36	2.83	6.64	1.06	0.72
TR 22	1.37	1.0	0	1.50	1.20	1.44	2.24	1.59	4.35	8.52	1.12	1.35
TR 23	0.92	1.0	0	1.11	1.28	1.32	1.68	1.41	2.47	5.83	1.03	nm
TR 24	0.79	1.0	0	0.98	1.0	0	0.38	1.0	0	1.37	1.25	1.35

Notes: r = retained on column. nm = nonmeasurable.

whereas **TR 25–29** were completely retained on the column with the mobile phase used in this screening.

During chromatographic study of the HPLC columns filled with **CSPs 1–4**, we became aware of the paper of Malyshev and Vinogradov,[15] where the study of the column containing chiral selector 3, prepared by the "on column binding" of 3 to γ-aminopropyl silica, was reported. The column was prepared by repeated elution of the commercial column filled with γ-aminopropyl silica with solution of 9, followed by the washing with less polar solvent. This method does not allow even approximate determination of the loading of chiral selector and it was, therefore, interesting to compare the reported results with efficacy of our **CSPs 1–4**, in particular with **CSP 2**, Table 1. To the list of test racemates routinely tested in our former work,[8–10] we added primary alcohols **TR 30–32**, Fig. 1, tested on the reported column.[15]

For enantioseparation of analyte **TR 13** by our **CSP 2**, we obtained two peaks; $k'_2 = 4.79$ and $\alpha = 1.15$. Although our column was larger than the one reported[15] (250 mm × 4.6 mm versus 150 mm × 3.3 mm), we obtained two times lower k'_2. Since the retention times in our column are shorter then in the reported column, the later seem to have more unreacted amino groups of γ-aminopropyl silica gel, and, consequently, lower loading. Neither ours, nor the reported column, has separated racemate **TR 15**; capacity factor k'_2 obtained for our column is 3.81 and for the reported one is 2.70, i.e. k'_2 on our column is 1.4 times larger, which contradicts the previous conclusions. The authors did not report any data for elemental analysis, whereas for our stationary phases, elemental analysis shows ca 0.2 mmol of chiral selector per gram of material, as usually found for brush-type CSPs. For α-arylethanols (**TR 30–32**) our column exhibited 3.53, 2.48, and 1.55 times larger k'_2 then reported by Malyshev et al.,[15] but no separation was obtained.

Some interesting aspects concerning relative efficacy of enantioselection with **CSPs 5–8** are summed up in Table 2. With **CSP 5**, only partial resolution was recorded for two racemates; the highest efficacy was reached for **CSP 7**, reflecting an optimal ratio between π-donor capacity and steric hindrance of the aryl group on the stereogenic center. Data in Table 2, in particular comparison of the α-values for **TR 11** to **TR 23** vs. **TR 5** to **TR 10**, reveal the amide group in the racemic analyte as a key functionality for chiral recognition, not the π-donor capacity of the aromatic units. This result is evident for **CSP 8**, where substantial contribution of the π–π interaction was expected. Condensed heterocycles **TR 6** to **TR 10** were particularly badly resolved, presumably because of the large steric requirements risen by the non-planar, annulated 7-membered heterocyclic ring.

The analytes **TR 4** and **TR 25–29**, containing strong H-donor/acceptor groups, were strongly and non-selectively retained. Such behavior of **CSPs 5–8**

towards these two groups of analytes has indicated strong contribution of the free γ-aminopropyl groups to the non-selective interactions. Because of their incomplete conversion, such behavior is observed for many Pirkle-type columns.[16] It is known from the literature, that only 10–20% of silanol groups on silica surface are bound to chiral selectors;[17,18] for **CSPs 5–8** an average value of ca 15%, *i.e.* 0.6 μmol/m^2, was found, see Experimental. Such partial loading leaves free on the silica surface a large percentage of silanol and aminopropyl groups. Structure of all chiral selectors study, herewith, is characterized by the bulky groups placed ahead of the sterogenic center and π-acid unit, acting as a fence[19] and obstructing approach of the analyte toward amide bonds at the stereogenic center, allowing them to interact with the γ-amino groups on the spacer. In order to reduce this interaction, we have varied polarity of the elution systems. Addition of trifluoroethanol, known as exclusive proton donor,[19,20] to get mobile phase n-hexane/dichloromethane/trifluoroethanol (100 : 40 : 2), has diminished enantioseparation. Contrary, mobile phase comprising THF as exclusive, weak proton acceptors (n-hexane/THF 7 : 3), gave much better separations, similar to those obtained when standard hexane/2-propanol mobile phase was used. Further tuning revealed ternary mixture hexane/2-propanol/acetic acid (180 : 20 : 1) as the eluent of choice for **CSP 8**. With this mobile phase, several good separations were obtained, in particular for aromatic H-bond donor–acceptor analytes, Table 3

Table 3. Comparison of results obtained for several racemates by columns filled with **CSP 8** and **CSP 8′**; columns dimension 250 mm × 4.6 mm ID; mobile phases were *n*-hexane/2-propanol 8 : 2 and hexane/2-propanol/acetic acid 180 : 20 : 1 for analytes **TR 4** and **TR 25–29** analysed on **CSP 8**; flow rate 1.0 mL/min.

	CSP 8′			CSP 8		
Analyte	k'_1	α	R_S	k'_1	α	R_S
TR 4	2.73	1.10	0.74	12.82	1.24	4.09
TR 15	6.32	1.32	2.78	1.67	1.19	0.22
TR 18	1.44	1.18	1.08	3.31	1.17	2.00
TR 24	0.73	1.19	0.87	1.37	1.25	1.35
TR 25	1.39	1.07	0.42	4.82	1.10	1.21
TR 26	1.75	1.05	0.41	4.33	1.08	1.07
TR 27	3.44	1	0	10.85	1.15	2.07
TR 28	2.68	1.09	0.75	11.33	1.13	1.82
TR 29	8.12	1	0	26.50	1.04	0.52

and Fig. 2, which were completely retained on the column when standard mobile phases were used.

We have recently observed synergistic interaction of carboxylic analyte, α-aryloxypropionic acids, with the chiral selector and achiral free γ-aminopropyl units on silica.[13] In order to reveal this effect in representative **CSP 8**, we have prepared **CSP 8′** according to the Scheme 2, voided of the excess of aminopropyl groups on the silica surface. Generally, the analytes were more easily flushed from **CSP 8′** and enantioseparations were worse than with **CSP 8**, Table 3. This reveals that multiple cooperative interactions with chiral selectors and achiral units required to get, as recently defined "amplification of the chiral environment",[21] is not achieved with the analytes studied with these two columns.

CONCLUSION

This study revealed that CDNB represents useful branching π-acid units in the preparation of novel CSPs for enantioseparation by HPLC. Preparation and testing of new CSPs revealed definitive contribution of the amide group as

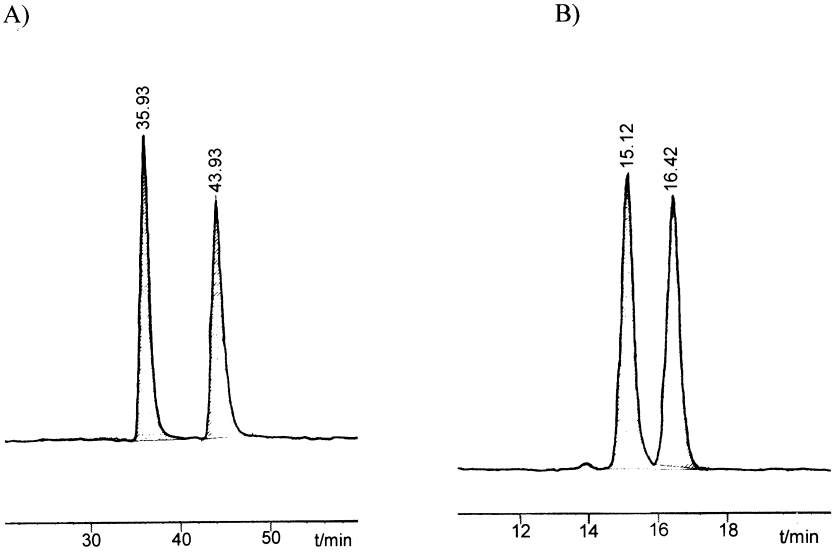

Figure 2. Chromatograms obtained for **TR 4** (A) and **TR 25** (B) on column filled with **CSP 8**, 250 mm × 4.6 mm ID, mobile phase: n-hexane/2-propanol/acetic acid (180 : 20 : 1), flow 1.0 mL/min.

H-bonding donor–acceptors. Significant contribution of the terminal π-donor aromatic ring is observed only for **CSP 8** with extended aromatic systems, which interacts with analytes **TR 27** and **TR 28** containing nitrophenyl groups in proper position to match π-donor units. Therefore, **CSP 8** proved effective in separation of polar racemates, but not as universal as commercial Whelk-O1. There is overall shape similarity between Whelk-O1 and **CSP 8** though conformational freedom of **CSP 8** is larger. Beside, the selectors in **CSPs 6–8** are bound to silica extending π-basic units ahead of the π-acidic ones, such as to rise perturbation on the approach of analyte to the chiral hole. Reversal of π-donor and π-acceptor units within chiral selectors present in **CSPs 6–8**, is expected to form chiral holes open to the analyte; we are presently searching for such effective couples of CDNB with chiral units to ensure open chiral topology and improved enantioselection.

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