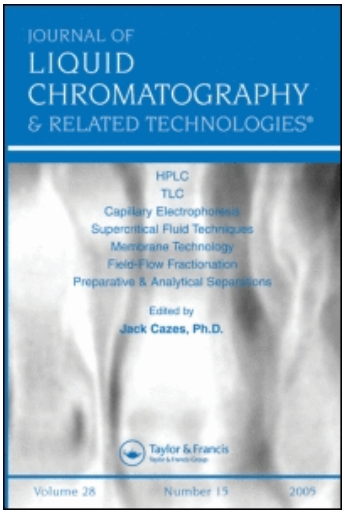

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Donor-Acceptor Chiral Centrifugal Partition Chromatography: Complete Resolution of Two Pairs of Amino-Acid Derivatives with a Chiral II Donor Selector

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DONOR-ACCEPTOR CHIRAL CENTRIFUGAL PARTITION CHROMATOGRAPHY: COMPLETE RESOLUTION OF TWO PAIRS OF AMINO-ACID DERIVATIVES WITH A CHIRAL II DONOR SELECTOR

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ABSTRACT

Two racemic compounds, namely N-(3,5-dinitrobenzoyl)-*tert*-butylvallnamide, and N-(3,5-dinitrobenzoyl)-*tert*-butylleucinamide, have been successfully completely resolved by centrifugal partition chromatography, using N-dodecanoyl-L-Proline-3,5-dimethylanilide as a chiral selector, and a biphasic system made with heptane, ethyl acetate, methanol and water. This opens the way to a better understanding of this class of separation by centrifugal partition chromatography.

INTRODUCTION

While resolution of optical isomers by liquid column chromatography using chiral stationary phases becomes more and more popular and diversified, with more than 100 commercial chiral solid phases[1], very few works have been published concerning this kind of separation by countercurrent chromatography or by centrifugal partition chromatography. Both techniques refer to support-free liquid-liquid chromatography with two immiscible liquids prepared by mixing two or more solvents or solutions; an instrument keeps one liquid stationary while the other liquid is pumped through it, and the chromatographic process occurs between the two liquid phases

Partial resolution of ± Norephedrine, using (R,R)-di-5-nonyltartrate as a chiral selector, and the biphasic system 1.2-dichloroethane / 0.5 M $NaPF_6$ in water, pH 4, has been obtained in around 4 days at 2 or 8 °C, using a Rotary Locular countercurrent chromatographic system (RLCCC) with 592 locules[2]. For this separation, the tartrate appears as an enantioselective ionophore toward the Norephedrine, ammonium salt[3].

Complete resolution of D,L-Isoleucine was obtained using N-dodecyl-L-Proline as a chiral selector, with the biphasic system 1-Butanol / Water, pH 5.5, and Cu^{II} ions as the metal center for Ligand Exchange Chromatography. Separation was performed with a droplet countercurrent chromatograph (DCCC) in approximately 2 days[4]. A racemic carboxylic acid was resolved using R-2-aminobutanol as a chiral selector and the biphasic system Chloroform / Methanol / Water, pH 7, in about 40 hours, with a DCCC[5]. For these two separations, interactions are mainly Ionic.

A few partial resolution of amino-acids or drug enantiomers using aqueous two phase systems and a protein as the chiral selector have also been published [6,7].

Combination of an enzyme reactor with a centrifugal partition chromatograph (CPC) has been used to resolve racemic amino-acid esters into the L free amino acid and the D amino-acid ester; incubation time was 5 hours, and CPC separation time was 3 hours[8].

Using a high performance centrifugal partition chromatograph (HPCPC)[9], we undertook some experiments in our laboratory, and before the successful separation described in "Results and Discussion", we had some unsuccessful experiences that we would like to summarize, as we think these experiences could help other groups working in this field.

1/ β-Cyclodextrin (β-CD) as a chiral selector :

β-CD stationary phases for column chromatography has been developed by D. W. Armstrong *et al.* [10]. β-CD has been reported to be very soluble in mixtures of water and dimethyl sulfoxide[11], and is readily soluble in the lower phase of any two-phase system WDT, made with Water / Dimethyl sulfoxide / Tetrahydrofuran[12] (up to 0.1 M, *i.e.* ≈57 g/l). We were using the system WDT 5 (water / DMSO / THF, 24.5 / 16.2 / 59.3, v/v/v, with (β-CD) = 0.05 M) to resolve the Trogger Base (2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5] diazocine[13], using the upper phase as the mobile phase. With this system the partition coefficient of the Trogger Base ($D \frac{\text{stationary}}{\text{mobile}}$) is 0.17 without the β-CD, and 0.42 with the β-CD, which seems to prove an interaction between the β-CD and the Trogger Base. But no detectable resolution occurred.

2/ (2R,3R)-di-n-butyl tartrate (DBT) as a chiral selector :

Chiral separations on reversed phase columns with DBT added to the mobile phase have been fully studied by C. Pettersson *et al.*[14]. Optimum selectivity is found at high loading of DBT on the support. Based on these results, we tested the ternary system Heptane / DBT / phosphate buffer in water,(PB), the ternary diagram of which is not available. Starting from the system DBT / PB, for which the organic phase is heavier than the aqueous phase, we added *aliquots of heptane*; the resulting liquid system is always biphasic, which is not surprising, but around the ratio HEP / DBT / PB 1/4/4 (v/v/v), the two phases have the same densities ($\Delta\rho$ = 0), and there is no settling; adding more heptane results in a DBT rich upper phase and a water rich lower phase, which can be used in CPC, despite the rather high viscosities of the two phases.

We selected the system HEP / DBT / PB 0.1 M, 25/25/50, v/v/v, which corresponds roughly to a solution 2 M of DBT in heptane as the organic upper phase, and an aqueous lower phase; adding hexafluorophosphate 0.1 M as a

counterion in the lower phase results in a triphasic system, part of the heptane being excluded from the DBT rich upper phase. After removing this heptane, the final biphasic system is then more than 2 M in DBT in the upper phase, which is then used as the stationary phase in CPC, and is quite stable under normal operating conditions. We tried to resolve ± Norephedrine with this system, but, due to the rather high viscosities of the two phases, we just observed a broad peak coming out, even at higher rotational speed[15] (1700 rpm). The flow rate was limited to 0.5 ml/min, because of the resultant hydrodynamic pressure[16].

3/ First attempt of charge transfer chiral chromatography :

The first charge transfer chiral selector tested in our laboratory was the N,N'-bis-[N-(3,5-dinitrobenzoyl)-(S)-phenylalanyl]-3,6-dioxa-1,8-octanediamine, (**1**).(Figure 1)(see Experimental). Racemic compounds similar to (**1**) display very high selectivity factors (α > 100) when chromatographed on a (S)-N-(2-naphtyl)alanine silica stationary phase[17].

Solubility of (**1**) is very low in most solvents, except in DMSO, and this is why we tested (**1**) with the biphasic system called WDT 3, *i.e* water / DMSO / THF, 16.7 / 25.1 / 58.2, v/v/v. In this system, up to 10 g/L of [I] can be dissolved in the upper phase (THF rich).

The system WDT 3, (**1**) = 10 g/L in upper phase, has been used in the descending mode to try to resolve the ± Lorazepam, which is known to be easily resolved on a N-(3,5-dinitrobenzoyl)-L-phenylalanine silica stationary phase[18]; the rotational speed was 1400 rpm and the flow rate was 3 ml/min. Even with the high partition coefficient observed for (**1**), on-line UV detection is not possible in these experiments because of the presence of amount of (**1**) in the mobile phase; TLC monitoring of the collected fraction was more convenient, but we did not observe a chiral resolution for the racemate, which had a partition coefficient of ≈3.6 in this system.

From experiment 1 and 2, we concluded that transposition of HPLC experiments where the observed selectivity factors are in the range 1.1 to 1.5, to CPC result in a chiral defeat, maybe because the chiral selector is not linked to a rigid matrix, which is known to play a role upon the enantioselectivity[19,20]. Experiment 3 will be further investigated, as its failure can be due to a poor choice of the racemate to be resolved.

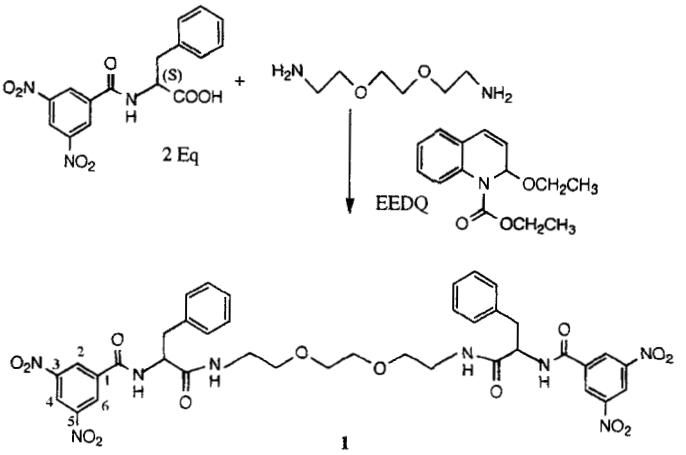

Figure 1 Synthesis of *N,N'-bis-[N-(3,5-dinitrobenzoyl)-(S)-phenylalanyl]-3,6-dioxa-1,8-octanediamine* (**1**).

This paper describe experiment 4, which was a success, and where the chiral selector is similar to a selector recently described by W.H. Pirkle *et al.*[21], which display high recognition for specific racemic compounds.

EXPERIMENTAL SECTION

Chemicals

N,N'-bis-[N-(3,5-dinitrobenzoyl)-(S)-phenylalanyl]-3,6-dioxa-1,8-octanediamine (**1**). To a solution of 3.6 g (25 mmol) of 3,6-dioxa-1,8-octanediamine and 17.9 g (50 mmol) of N-(3,5-dinitrobenzoyl)-(S)-phenylalanine[22] in 400 ml of tetrahydrofuran (THF), a solution of 12.4 g (50 mmol) of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in 50 ml of THF was added at room temperature. The mixture was refluxed during 3.5 hours, then the solution was evaporated. The residue was diluted with dichloromethane and washed with 1% ortophosphoric acid, 0.2M potassium hydroxide and distilled water. After drying over sodium sulfate, the solution was evaporated and the residual solid collected. Recrystallization from ethanol-water gave 10.4 g (51% yield) of a solid, m.p. 214°C. ^{1}H-NMR (200 MHz): δ

(DMSO-d_6) 2.9-3.6 (m, 16H, $2CH_2Ar$, $2CH_2N$ and $4CH_2O$), 4.80 (m, 2H, 2CH), 7.1-7.4 (m, 10H, $2C_6H_5$), 8.33 (t, 2H, $2NHCH_2$), 8.94 (m, 2H, $2C^4H$), 9.02 (m, 4H, $2C^{2,6}H$), 9.45 (d, 2H, 2NHCH). IR (KBr): 3289 cm^{-1} (NH st), 1639 cm^{-1} (CO st, Amide I), 1537 cm^{-1} (N-C=O st si, Amide II and NO_2 st as), 1343 cm^{-1} (NO_2 st si), 1078 cm^{-1} (C-O st as). $[\alpha]_D^{22}$=-5.6° (c=1.0, THF). Analysis calculated for $C_{38}H_{38}N_8O_{14}$, C 54.94, H 4.61, N 13.49; found, C 55.04, H 4.55, N 13.15 %.

N-dodecanoyl-L-proline (**2**). L-Proline (11.6 g, 100 mmol) was dissolved in 150 ml of 1M sodium hydroxide solution and cooled in an ice-bath. The dodecanoyl chloride (24.1 g, 110 mmol) and 200 ml of 1M sodium hydroxide solution were added simultaneously over a period of 20 min. The solution was stirred at room temperature for 45 min and acidified with concentrated hydrochloric acid (pH 2-3). The solution was extracted with diethyl ether and the organic phase washed with a 12 % sodium chloride solution. After drying over sodium sulfate it was evaporated and 30 g (100% yield) of an oil collected (Figure 2).

N-dodecanoyl-L-proline-3,5-dimethylanilide (**3**). To a solution of 6.5 g (54 mmol) of freshly distilled 3,5-dimethylaniline and 15 g (50 mmol) of N-dodecanoyl-L-proline in 200 ml of THF, a solution of 12.6 g (50 mmol) of EEDQ in 100 ml of THF was added at room temperature. The mixture was allowed to react 24 Hrs at room temperature, then the solution was evaporated. The residue was diluted with dichloromethane and washed with 1% orthophosphoric acid, 0.2M sodium hydroxide and distilled water. After drying over sodium sulfate, the solution was evaporated and the residual collected. Recrystallization from ethanol-water gave 17.6 g (88% yield) of a white solid, m.p. 63°C. ^{1}H-NMR (200 MHz): δ ($CDCl_3$) 0.88 (t, 3H, CH_3), 1.25 (m, 18H, $9CH_2$), 1.67 (m, 2H, C^3H_2-Pro), 2.07 (m, 1H, C^2Ha-Pro), 2.27 (s, 6H, $ArCH_3$), 2.30 (m, 2H, CH_2CO), 2.60 (m, 1H, C^2Hb-Pro), 3.47 (m, 2H, CH_2N), 4.80 (d, 1H, CH), 6.70 (s, 1H, C^4'H), 7.17 (s, 2H, $C^{2',6'}H$), 9.62 (s, 1H, NH). IR (KBr): 3325 cm^{-1} (NH st), 1626 cm^{-1} (CO st, Amide I). $[\alpha]_D^{22}$=-66.9° (c=1.2, ethanol 96°). Analysis calculated for $C_{25}H_{40}N_2O_2.H_2O$, C 71.73, H 10.11, N 6.69; found, C 71.78, H 9.64, N 6.53 %. (Figure 2).

(±) N-(3,5-dinitrobenzoyl)amino acids. The appropriate racemic amino acid (23 mmol) was dissolved in 50 ml of 1M sodium hydroxide solution and

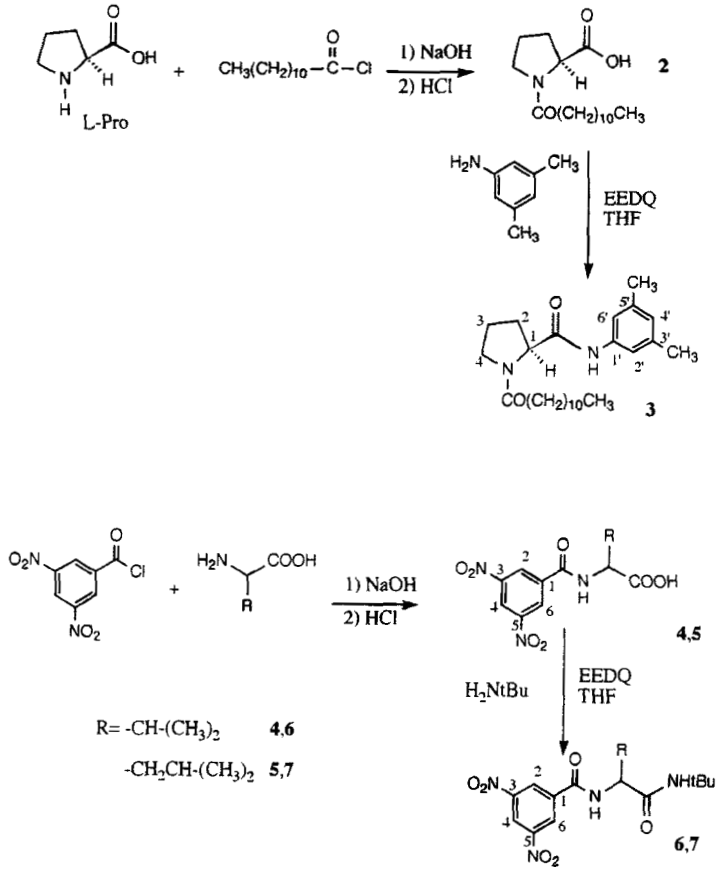

Figure 2 Synthesis of the chiral selector and of the racemics used in centrifugal partition chromatography.

cooled in an ice-bath. The 3,5-dinitrobenzoyl chloride (5.3 g, 23 mmol) and 50 ml of 1M sodium hydroxide solution were added simultaneously over a period of 20 min. The solution was stirred at room temperature for 90 min and acidified with concentrated hydrochloric acid (pH 2-3). The resulting solid was collected by filtration and washed with water. Recrystallization from ethanol-water gave the corresponding N-(3,5-dinitrobenzoyl)amide.

(±) N-(3,5-dinitrobenzoyl)valine (**4**). Yield 41%. m.p. 187°C. ^{1}H-NMR (200 MHz): δ ($CDCl_3/CD_3OD$) 1.06 (d, 6H, $2CH_3$), 2.36 (m, 1H, CH), 4.74 (d, 1H, CHN), 9.09 (d, 2H, $C^{2,6}H$), 9.17 (d, 1H, C^4H). ^{13}C-NMR (50.3 MHz): δ ($CDCl_3/CD_3OD$) 17.8 and 18.9 ($2CH_3$), 30.9 (CH), 58.2 (CHN), 121.0 (C^4H), 127.6 ($C^{2,6}H$), 137.3 (C^1), 148.4 ($C^{3,5}$), 163.3 (CONH), 173.7 (COOH). IR (KBr): 3323 cm^{-1} (NH st), 1714 cm^{-1} (CO st, COOH), 1633 cm^{-1} (CO st, Amide I), 1538 cm^{-1} (N-C=O st si, Amide II and NO_2 st as), 1343 cm^{-1} (NO_2 st si) (Figure 2)

(±) N-(3,5-dinitrobenzoyl)leucine (**5**). Yield 67%. m.p. 181°C. ^{1}H-NMR (200 MHz): δ ($CDCl_3/CD_3OD$) 0.98-1.03 (m, 6H, $2CH_3$), 1.80 (m, 3H, $CHCH_2$), 4.77 (m, 1H, CHN), 8.54 (d, 1H, NH), 9.14 (m, 3H, ArH). ^{13}C-NMR (50.3 MHz): δ ($CDCl_3/CD_3OD$) 20.7 and 22.4 ($2CH_3$), 24.6 (CH), 39.7 (CH_2), 51.4 (CHN), 120.5 (C^4H), 127.4 ($C^{2,6}H$), 137.0 (C^1), 148.1 ($C^{3,5}$), 163.5 (CONH), 174.2 (COOH). IR (KBr): 3375 cm^{-1} (NH st), 1722 cm^{-1} (CO st, COOH), 1644 cm^{-1} (CO st, Amide I), 1538 cm^{-1} (N-C=O st si, Amide II and NO_2 st as), 1346 cm^{-1} (NO_2 st si) (Figure 2).

(±) N-(3,5-dinitrobenzoyl)-tert-butylamido derivatives of amino acids. To a solution of 9.6 mmol of N-(3,5-dinitrobenzoyl)amino acids and 10 mmol (2.5 g) of EEDQ in 50 ml of THF, 10 mmol (0.7 g) of *tert*-butylamine were added at room temperature. The mixture was refluxed during 18 hours and the resulting solution was evaporated. The residue was diluted with dichloromethane and washed with 5% sodium hydrogen carbonate, 1M hydrochloric acid and distilled water. After drying over sodium sulfate, the solution was evaporated and the residual solid collected. Recrystallization from ethanol-water gave the corresponding amide.

(±) N-(3,5-dinitrobenzoyl)-tert-butylvalinamide (**6**). Yield 89%. m.p. 235°C. ^{1}H-NMR (200 MHz): δ ($CDCl_3$) 1.01 (t, 6H, $2CH_3$), 1.38 (s, 9H, tBu), 2.25 (m, 1H, CH), 4.35 (t, 1H, CHN), 5.82 (s, 1H, NH), 7.78 (d, 1H, NHCOAr), 9.04 (d, 2H, $C^{2,6}H$), 9.17 (m, 1H, C^4H). ^{13}C-NMR (50.3 MHz): δ ($CDCl_3/CD_3OD$) 18.7 and 19.0 ($2CH_3$), 28.3 (tBu), 31.0 (CH), 51.6 (CqtBu), 60.6 (CHN), 121.0 (C^4H), 127.7 ($C^{2,6}H$), 137.3 (C^1), 148.4 ($C^{3,5}$), 163.0 (NHCOAr), 170.6 (CONH). IR (KBr): 3324 cm^{-1} (NH st), 1639 cm^{-1} (CO st, Amide I), 1544 cm^{-1} (N-C=O st si, Amide II and NO_2 st as), 1341 cm^{-1} (NO_2 st si) (Figure 2).

(±) N-(3,5-dinitrobenzoyl)-tert-butylleucinamide (**7**). Yield 84%. m.p. 238°C. ^{1}H-NMR (200 MHz): δ ($CDCl_3$) 0.92 (t, 6H, $2CH_3$), 1.37 (s, 9H, tBu), 1.63 (m, 3H,

$CHCH_2$), 4.60 (m, 1H, CHN), 5.97 (s, 1H, NH), 8.00 (d, 1H, NHCOAr), 9.08 (d, 2H, $C^{2,6}H$), 9.17 (m, 1H, C^4H). ^{13}C-NMR (50.3 MHz): δ ($CDCl_3/CD_3OD$) 21.6 and 22.4 ($2CH_3$), 24.6 (CH), 28.0 (tBu),40.7 (CH_2), 51.3 (CqtBu),53.0 (CHN), 120.8 (C^4H), 127.6 ($C^{2,6}H$), 137.1 (C^1), 148.3 ($C^{3,5}$), 162.9 (NHCOAr), 171.5 (CONH). IR (KBr): 3294 cm^{-1} (NH st), 1644 cm^{-1} (CO st, Amide I), 1556 cm^{-1} (N-C=O st si, Amide II and NO_2 st as), 1342 cm^{-1} (NO_2 st si) (Figure 2).

Centrifugal Partition Chromatography :

Apparatus. A series 1000 HPCPC (Sanki Eng. Limited, Nagaokakyo, Kyoto, Japan) was used[9]. It is a bench top CPC (30 x 45 x 45 cm, ≈ 60 kg); the column is a stacked circular partition disk rotor which contains 2136 channels with a total internal volume of around 240 ml. The column is connected to the injector and the detector through two high pressure rotary seals containing a drilled sapphire rod passing through two toroidal seals similar to those used with HPLC pump pistons. The partition disks are engraved with 1.5 x 0.28 x 0.21 cm channels connected in series by 1.5 x 0.1 x 0.1 cm ducts. A 4-port valve included in the series 1000 allows the HPCPC to be operated in either the descending or ascending mode. The HPCPC was connected to an HPLC system gold (Beckman, San Ramon, CA, USA), including a solvent delivery pump model 126 and a manual sample injector.

The solvent system was a mixture of Heptane / Ethyl acetate / Methanol / Water (see Results and Discussion); the flow rate was 5 ml/min, with a rotational speed of 1200 rpm. The CPC run was performed in the descending mode, and the backpressure was around 3 MPa.

HPLC

A HPLC Waters workstation (Waters Chromatography Division, Millipore Corp., Milford, MA) was used to control the optical purity of the CPC collected fractions. The column was a 10 x 0.46 cm, packed with N-(3,5-dinitrobenzoyl)-L-phenylalanine bonded to 5 μm silica (Chirachrom A1, Interchim, Montluçon, France), and the mobile phase a mixture of Heptane / 2-Propanol / Methanol, 63 / 27 / 10, v/v/v. The flow rate was 1 ml/min, and UV detection was performed at 254 nm.

TLC Monitoring

CPC experiments were monitored by TLC on Kieselgel 60 F 254 spezial (Riedel de Haën, Seelze, Germany), with Heptane Ethyl Acetate (1:1).

Recovering of Enantiomers

The CPC fractions were concentrated individually under reduced pressure to obtain an aqueous suspension which was then extracted with Heptane / Ethyl acetate (1:1). The organic phase was dried over sodium sulfate and the solution evaporated. The enantiomer was separated from the chiral selector by conventional column chromatography on silica gel (60-200 μm), using an Heptane / Ethyl acetate (1:1) mixture as eluant.

RESULTS AND DISCUSSION

Selection of the Solvent System.

As it is usual in centrifugal partition chromatography when a new mixture has to be studied, the first thing to do is to find a class of solvents where the mixture is freely soluble, then to achieve a biphasic system by adding non miscible solvents in which the selected solvents and the mixture will partition[23]. In our case both the Chiral selector (CS) and the racemic compounds (R) need to be freely soluble, and more, CS has to be mainly in one phase, which will be the stationary phase, while R have better to be well partitioned, and in favor of the other phase, if possible, and which will be the mobile phase.

We found CS and R to be readily soluble in Ethyl acetate (EtOAc) and in Methanol (MeOH), and we tested their partitioning in the quaternary system Heptane / EtOAc / MeOH / Water, which has been extensively described [23,24].

The system Heptane / EtOAc / MeOH / Water, 3 / 1/ 3 / 1 (v/v/v/v) allows CS to be more in the upper phase (which will be the stationary phase), with a partition coefficient, $D = \frac{C_{stationary}}{C_{mobile}}$, of around 3.1, and R more in the lower phase, with a partition coefficient (when CS is not present) of around 0.3 for the Valine derivative, and around 0.5 for the Leucine derivative (D was estimated by HPLC).

In presence of CS, the apparent partition coefficient of R, D', varies greatly, and it is not the same for the (+) and the (-) isomers, as shown on Table I.

It appears that one isomer has little or no affinity for the chiral selector, while the other isomers show a rather strong affinity for it.

The apparent partition coefficient, D', results from a quadratic equilibrium scheme (Figure 3). On this Figure, R means either (+) or (-) isomer, and :

$$D_{CS} = \frac{(\overline{CS})}{(CS)}, \quad D_R = \frac{(\overline{R})}{(R)}, \quad D_{RCS} = \frac{(\overline{RCS})}{(RCS)} \qquad \text{(Partition Coefficients)}$$

$$\overline{K} = \frac{(\overline{RCS})}{(\overline{R})(\overline{CS})}, \quad K = \frac{(RCS)}{(R)(CS)} \qquad \text{(Complex Formation Constants)}$$

(overlined term means : in the stationary phase).

The apparent coefficient for R is :

$$D' = \frac{(\overline{R}) + (\overline{RCS})}{(R) + (RCS)} = D_R \times \frac{1 + \overline{K} D_{CS} (CS)}{1 + K(CS)} \qquad [1]$$

Assuming the (+) isomer to be the most retarded, the selectivity factor, $\alpha_{+,-}$ is then (since $D_{R+} = D_{R-}$)[25]:

$$\alpha_{+,-} = \frac{D'_+}{D'_-} = \frac{1 + \overline{K}_+ D_{CS} (CS)}{1 + \overline{K}_- D_{CS} (CS)} \times \frac{1 + K_- (CS)}{1 + K_+ (CS)} \qquad [2]$$

Simplification occurs when $K_- = \overline{K}_- = 0$ (no complex formation between the (-) enantiomer and CS, which is roughly our case for the Valine der., see Table I). We have then :

$$\alpha_{+,-} = \frac{1 + \overline{K}_+ D_{CS} (CS)}{1 + K_+ (CS)} \qquad [3]$$

When 1 can be neglected, compared to the other terms, we get :

$$\alpha_{+,-} = \frac{\overline{K}_+}{K_+} D_{CS} \qquad [4]$$

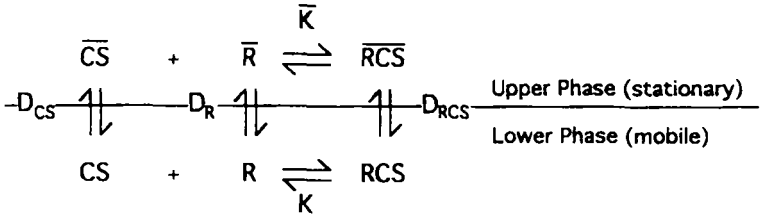

Figure 3 Quadratic scheme describing the equilibrium involved in donor-acceptor chiral centrifugal partition chromatography. D is a partition coefficient, and K is a complex formation constant.

Table I: Apparent partition coefficients, D', of (±) Val and (±) Leu Derivatives

	D' when (CS) = 0	D' when $(CS)_{upper\ phase} \approx 0.019$ M, $(CS)_{lower\ phase} \approx 0.006$M ($D_{CS} \approx 3.1$)
(-) Val Der.	≈ 0.3	0.45
(+) Val Der.		1.5
(+) Leu Der.	≈ 0.5	0.5
(-) Leu Der.		5.5

Equation [4] means that the chiral separation will be better if the chiral selector is mainly in the stationary phase (D_{CS} large), and if complex formation is favored in the stationary phase too, *i.e.* electron donor acceptor interaction more important in the stationary phase than in the mobile phase, which means that the stationary phase should have a lower dielectric constant than the mobile phase (which is our case).

CPC Runs

Collected fractions of the CPC runs were monitored by TLC, since on-line UV detection is not possible, due to the presence of CS in the mobile phase.

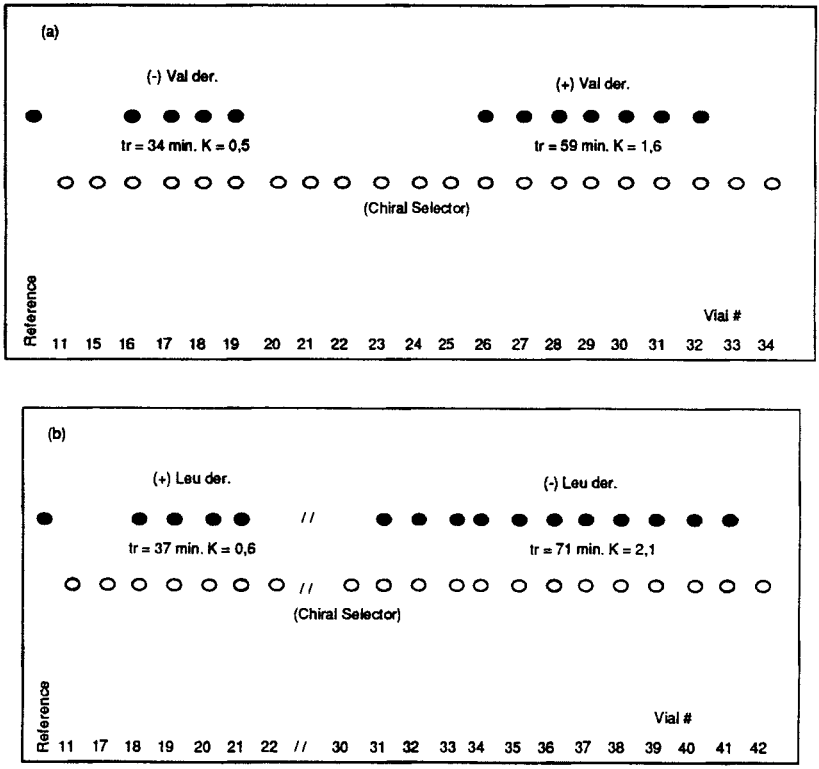

Figure 4 TLC control of the CPC runs.

CPC conditions : heptane / ethyl acetate / methanol / water 3/1/3/1
Descending mode, Flow rate = 5 ml/min
Rotational speed : 1200 rpm; Backpressure ≈ 3 MPa
$V_{stat}/V_{mob} \approx 1$; (CS) = 0.01M, in upper phase
Sample : 2 ml of a 0.001 M aminoacid der.
Collected fractions every 2 min (10 ml).

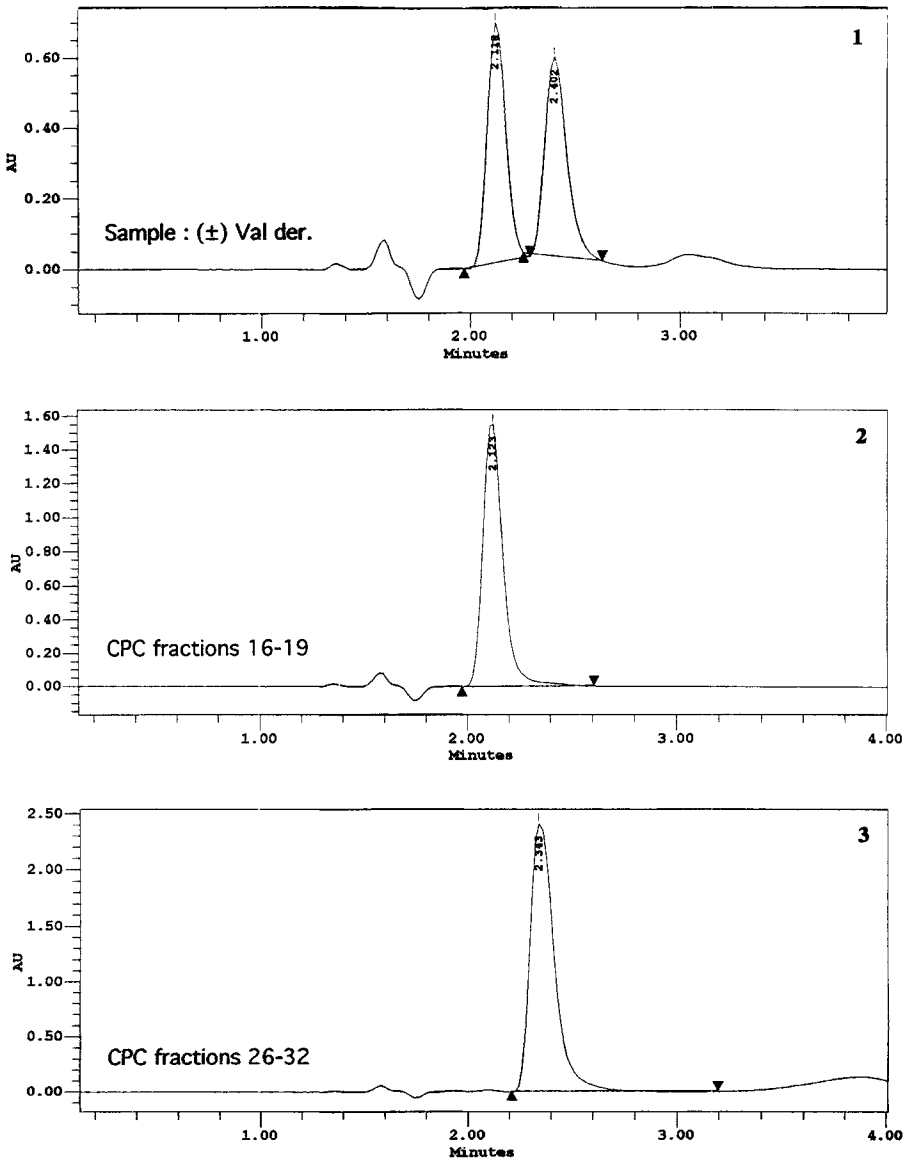

Figure 5 HPLC control of the CPC separation of the Val der. enantiomers : •1 original sample ; •2 CPC fractions 16-19, after removal of CS, and corresponding to (-) Val der.; •3 CPC fractions 26-32, after removal of CS, and corresponding to (+) Val der.

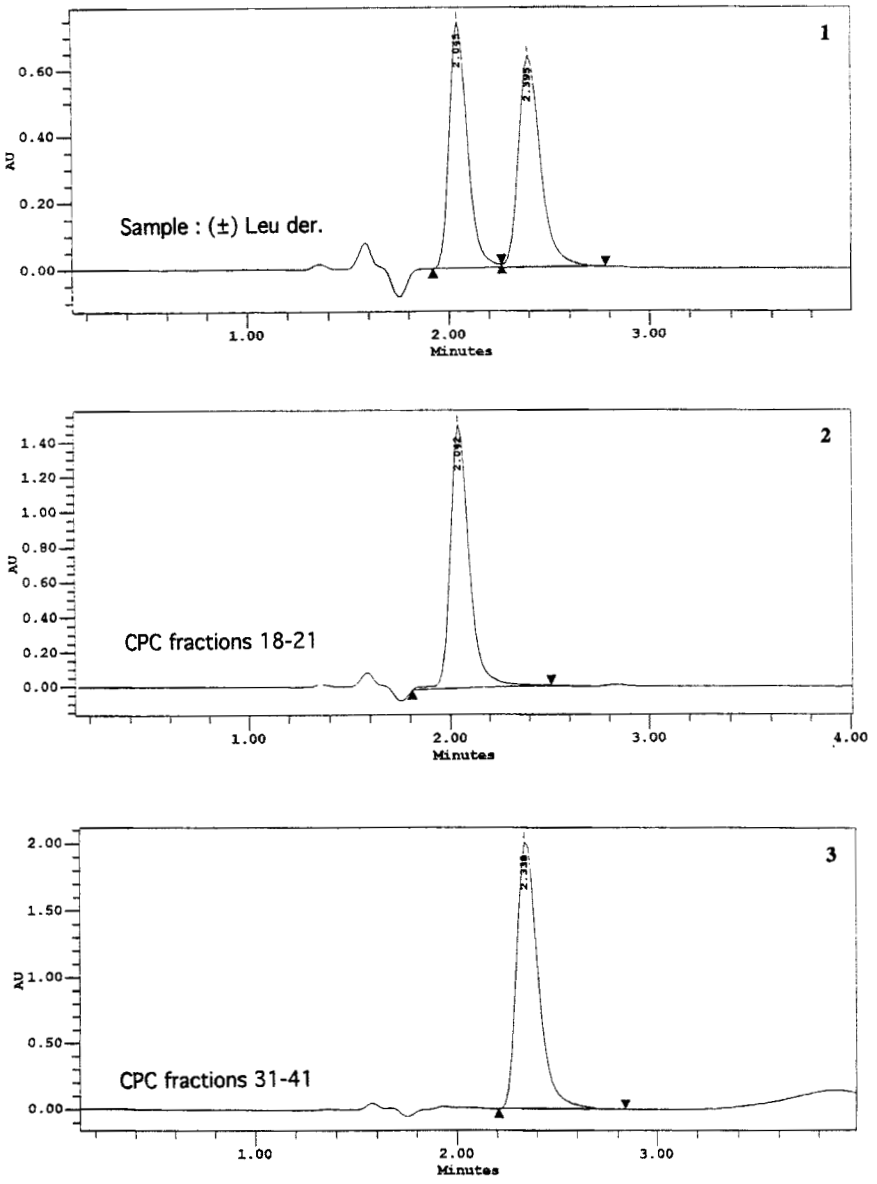

Figure 6 HPLC control of the CPC separation of the Leu der. enantiomers : •1 original sample ; •2 CPC fractions 18-21, after removal of CS, and corresponding to (+) Leu der.; •3 CPC fractions 31-41, after removal of CS, and corresponding to (-) Leu der.

Figure 4 shows two typical TLC control and CPC conditions, one is for the resolution of the (±) Val derivative, the other for the resolution of the (±) Leu derivative. There is more than 70 ml between the end of the first peak and the beginning of the second peak, for (±) Val der., and more than 120 ml for (±) Leu der.. The first peaks correspond to the (-) Valine der. (α=-6.9°, c = 1.0, chloroform), and to the (+) Leucine der. (α=+9.0°, c = 1.1, chloroform). The approximate selectivity factors (estimated from the middle of the black dots (Fig. 4) are 3.2 for (±) Val der., and 3.5 for (±) Leu der.. CS, which is in every fraction, is easily removed by simple column chromatography on silica (see experimental); the pure enantiomer comes first, and CS remains on the column, and is recovered by washing with EtOAc as eluant.

Remark : The elution orders of R(±) and CS, both in TLC and open column chromatography, are opposite to the one expected from the relative partition coefficients of the studied species in the biphasic system Heptane / EtOAc / MeOH /Water 3/1/3/1, where CS appears as "less polar" than the racemics. This highlights the unique selectivity of centrifugal partition chromatography compared to silica based chromatography, and which is achieved by a fine tuning of the solvent system in order to get a particular and often narrow specificity. In this particular case, we observed that CS was totally in methanol in the system heptane / methanol, and was precipitating if a small amount of water was added, which means that CS does not "like" neither heptane nor water. When ethyl acetate is added, then CS redissolves, mainly in the heptane/ethyl acetate upper phase. This mechanism could be called preferential solvation and desolvation.

Figure 5 & 6 show the HPLC chromatograms corresponding to the two CPC runs.

CONCLUSION

Total separation of two pairs of optical isomers in less than one and a half hour by Donor Acceptor Chiral Centrifugal Partition Chromatography (DAC-CPC) is a real encouragement in finding some new selectors specially tailored for CPC resolution of specific racemic mixtures. CPC generally display a higher α than HPLC does for a given pair of molecules differing only slightly, but it seems

that this is not the case for chiral differences. We are now undertaking systematic studies to find the rules which will direct us to new and powerful selectors. To our knowledge, this is the first time a complete resolution of non-ionic racemics has been acheived in countercurrent chromatography.

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25 if the (-) isomer is the most retarded, all the discussion remains the same by just inverting (+) for (-)

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