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Continuous Separation of Racemic 3,5-Dinitrobenzoyl-Amino Acids in a Centrifugal Contact Separator with the Aid of Cinchona-Based Chiral Host Compounds

Andrew J. Hallett, Gerard J. Kwant, and Johannes G. de Vries*[a]

Abstract: The resolution of racemates is mostly performed by crystallisation of diastereomeric salts. Direct physical separation could be much more efficient, but so far most concepts, with the exception of SMB, have proven to be non-scaleable. Here we report the first scalable process for the resolution of N-protected amino acid derivatives through selective extraction, with the aid of a catalytic amount of a chiral host compound based on Cinchona alkaloids. The method hinges on the use of centrifugal contact separators (CCSs) for fast mixing and separation. Although the highest *ee* obtained was only 80%, the concept can be extended through the use of a series of CCSs in countercurrent mode to effect full separation.

Keywords: amino acids · host–guest systems · liquid-phase separation · membranes · process intensification · transport

Introduction

Chiral molecules are of great importance in biological processes. A wide range of biological functions arise through molecular recognition, which often requires a strict matching of chirality. The two enantiomers will show different interactions with biological receptors or enzymes and hence give rise to different biological effects. The body, being extremely enantioselective, will interact with the components of a racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. One enantiomer may thus produce desired therapeutic activities, while the other may be inactive or, in the worst cases, produce unwanted effects. The efficient and economic production of enantiomerically pure compounds, particularly from racemic mixtures, is thus one of the major challenges facing the modern chemical industry. At present, the majority of commercially available drugs are both synthetic and chiral. However, a number of chiral drugs are still marketed as racemic mixtures. There are several methods for the preparation of enantiopure compounds, including resolution of racemates, fermentation, chiral pool synthesis and asymmetric synthesis.[1] Currently, most industrial processes are still based on the use of enantiopure materials obtained by classical resolution through crystallisation of diastereomeric salts. Nevertheless, resolutions with enzymes[2] and asymmetric catalysis[3] seem to be rapidly catching up.

Relatively few examples of processes for the separation of racemic or non-enantiomerically pure compounds by methods other than resolution of diastereomeric salts or enzymatic resolution can be found in the literature.[4] Certain synthetic receptors are capable of binding their substrates with sufficient power to draw them across phase boundaries: for example, from an aqueous medium into an organic solvent. If the receptor is enantioselective, one enantiomer of a racemic substrate will be preferentially transported, and some measure of resolution will be achieved. Chiral separation is thus achieved by the active transport of one enantiomer in a racemic solution from a feeding phase, through a transport phase containing a chiral host compound, into a receiving phase (Figure 1). The transport phase differs in polarity from the feeding and receiving phases (water and organic sol-

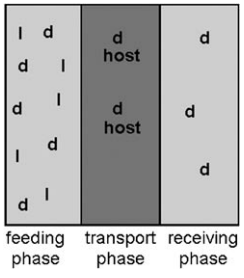

Figure 1. Chiral separation through enantioselective transport across phase boundaries.

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vent or vice versa). The chiral host compound selectively extracts one of the enantiomers of the racemic mixture from the feed phase and delivers it into the receiving phase. Usually the transport is driven by the concentration gradient of the substrate between the feeding and the receiving phase, although other incentives such as pH or temperature shifts can be applied if full transport is desired.

There are several possible forms of interaction between the host and the desired enantiomer (guest). These include ion-pair formation, hydrogen-bonding, π–π interactions and van der Waals interactions. These interactions result in one enantiomer binding preferentially to the host compound. Since the binding is reversible, the guest can also be released into the receiving phase, although this can be aided by altering, for example, the pH or temperature of the receiving phase.

A great variety of chiral host compounds for the separation of racemates have been reported in the literature. Chiral macrocyclic hosts, for example, have been used to separate amino acids, amino acid esters[5] and amines,[6] calixarenes to separate the enantiomers of amines,[7] guanidinium compounds for the separation of carboxylic acids and amino acids,[8] carbamoylated cinchona derivatives for the separation of carboxylic acids and N-protected amino acids,[9] tartaric borate derivatives for the separation of β-amino alcohols,[10] metal complexes for the separation of amino acid derivatives,[11] steroidal receptors for the separation of carboxylic acids,[12] and binaphthol hosts for the separation of amines and amino acid esters.[13]

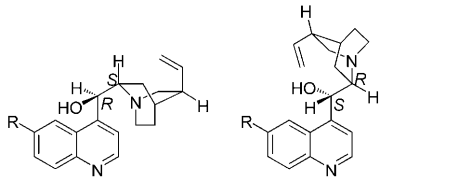
Historically, U-tubes or hollow fibre membrane systems have been used to demonstrate the ability of a chiral host to extract an enantiomer selectively from the feed phase and to deliver it to a receiving phase.[14,15] U-tubes cannot be scaled up, whereas the membrane systems have several associated problems when the process is scaled up for industrial use. The rate of mass transfer in such a device is too low for an industrial-scale process. Hollow fibre membranes are limited by diffusion rate, slowing down transport, and can only be used with water/nonpolar solvent combinations such as water/hexane.[15] Use of membranes that had been soaked with a solution of a chiral cinchona host allowed somewhat higher rates, but here crystallisation on the membrane surface formed an additional problem, which could be solved by the addition of an immobilised Cinchona alkaloid.[16] Maier has reported the use of a centrifugal partition chromatograph containing an MTBE solution of bis-1,4-(dihydroquinidinyl)phthalazine as the stationary chiral host solution and was able fully to separate the herbicide 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), which was fed dissolved in aqueous buffer as the mobile phase.[17] The only drawback was that full separation could be obtained only when a host–guest ratio of 1.0 was used.

So far, the only physical separation of racemates that is used on production scale is the chiral simulated moving bed (SMB) process. This method is rather costly because the chiral host is immobilised on a solid phase and the equipment is expensive because of the higher pressures. For an extraction-based separation process in a fine-chemical (multi-product) environment it is important to have equipment with high extraction efficiency and a small liquid hold-up that is compatible with a wide range of solvents. The small liquid hold-up facilitates fast start-up and reduces the required amount of the—often chemically complex—expensive host. Although existing mixer devices can be used for the extraction, phase separation may be very slow and is likely to become the capacity-limiting step.

Ideally we would like to develop a continuous process by using a device that combines fast mixing with fast separation in a small volume. Centrifugal contact separators (CCSs) such as those produced by CINC have the right characteristics.[18] The CINC Model V2 separator uses centrifugal force to separate two immiscible liquids of different densities. It operates both as a mixer and a separator. The immiscible liquids are rapidly mixed at speeds of up to 6000 rpm, giving excellent transport across phases. The centrifuge has an adjustable weir that can be adjusted to separate the aqueous and a variety of organic phases. We have previously reported on the use of this device for performing continuous two-phase catalytic reactions.[19] In this paper we report on the use of centrifugal contact separators for the separation of racemates with the aid of a chiral host compound.

Results and Discussion

Synthesis of Cinchona alkaloid host compounds: The Cinchona alkaloids (CAs) represent a class of compounds likely to provide enantioselective receptors because they have multiple functional groups and rigid skeletons with distinct conformational preferences. There are three positions in which the chemistry of the Cinchona alkaloid can be altered.

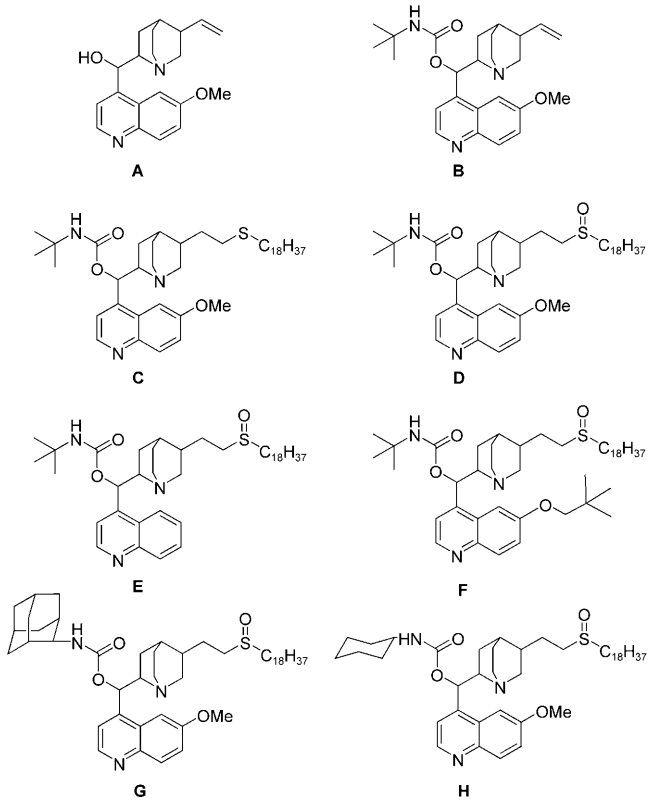


The hydroxy group can be allowed to react with, for example, an isocyanate to give a carbamoylated Cinchona alkaloid. The double bond can be employed to immobilise the CA to a silica support to create a chiral stationary phase or to add a long-chain lipophilic substituent. Finally, the methoxy group on the quinoline moiety can be altered to a range of alkoxy groups.

It has been shown by Lindner et al. that *O*-(1-adamantylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine can selectively extract one enantiomer of an N-protected amino acid derivative, such as 3,5-dinitrobenzoylleucine (DNB-Leu), from an aqueous buffered solution into an organic

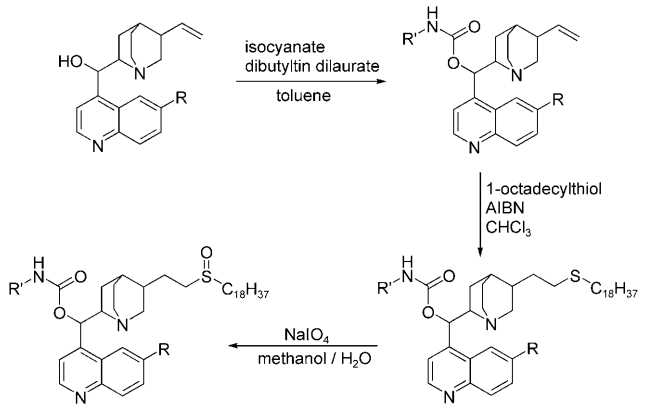
phase containing the chiral selector. These were stoichiometric experiments, in which two equivalents of racemate (with respect to the chiral host compound) were used. The variables explored were organic solvent, host/guest ratio, pH and buffer concentration of the aqueous phase and the structures of different N-protected leucine derivatives. Yields were 70–80% and *ee* values of 90% were observed in some cases.[20] Maier et al. have reported the use of these hosts for the separation of DNB-Leu in a membrane device. They achieved complete separation by using a number of membranes in series.[16] Here we report the results of experiments in which these host compounds are used in catalytic amounts for the extractive separation of racemates.

Since the long-chain lipophilic CAs had performed so well in the reported research we decided to use these host compounds to explore the use of CCSs for continuous chiral separations. We prepared a range of derivatised CAs as shown in Scheme 1. These CAs were examined for their



abilities selectively to extract DNB-Leu, 3,5-dinitrobenzoylalanine (DNB-Ala), 3,5-dinitrobenzoylvaline (DNB-Val), 3,5-dinitrobenzoylphenylalanine (DNB-Phe) and 3,5-dinitrobenzoylphenylglycine (DNB-PhG) from the aqueous phase into an organic phase. All of the synthesised CAs were prepared from either quinine or cinchonidine and so have the (8*S*,9*R*) configuration.

Two-phase extraction experiments: Two-phase extraction experiments were carried out in beakers. In principle, these



R = OMe; R' = adamantyl, *t*Bu, cyclohexyl
R = H, O-neopentyl; R' = *t*Bu

Scheme 1. Preparation of lipophilic carbamoylated CAs.

are only suited to give data on the equilibrium distributions of enantiomers between the phases and the binding to the host. Under the right mixing conditions (fast mixing), however, they give information on the binding rate (intrinsic) as well. The model is based on a "simple" mass balance for the two enantiomers. Binding of an enantiomer to the host in the organic phase is described by a Langmuir isotherm (illustrated for enantiomer 1):

$$C_{H,1} = C_{H,0}\,\frac{K_1 C_1}{1+K_1 C_1+K_2 C_2} \qquad (1)$$

where C_1 and C_2 are the concentrations of the two enantiomers in the aqueous phase and $C_{H,0}$ is the initial (or total) host concentration. The total concentration of an enantiomer in the organic phase is assumed to be composed of the amount bound to the host and the physically extracted matter, described by the distribution coefficient m_1:

$$C_{Org,1} = m_1 C_1 + C_{H,0}\,\frac{K_1 C_1}{1+K_1 C_1+K_2 C_2} \qquad (2)$$

The model results are calculated from the mass balance for every enantiomer over the equilibrium cell:

$$V_{a,1}(C_{1,o}-C_1) = V_{Org}(C_{Org,1,o}-C_{Org,1}) \qquad (3)$$

where the intrinsic selectivity is defined as the ratio of adsorption constants (K_1/K_2) and the operational selectivity as the ratio of concentrations for the organic phase ($C_{Org,1}/C_{Org,2}$) and for the aqueous phase (C_1/C_2). From the analysis we may obtain the values for the initial concentration of each enantiomer in the aqueous phase, the concentration of each enantiomer in the aqueous phase at equilibrium and thus the total yield, the yield of each enantiomer and the enantiomeric excess.

Two-phase extraction experiments can be used to determine the ideal conditions for selective chiral extraction.

There are a number of variables to consider, including the pH of the aqueous phase, the relative concentrations of host and guest, the organic solvent and the temperature.

Enantioselective extraction of *N*-(3,5-dinitrobenzoyl)-D,L-leucine: The CAs were initially used in the two-phase extraction of D,L-DNB-Leu (Table 1). In these experiments, D,L-DNB-Leu in a buffered aqueous phase (2 mL, 1 mmol solution) was mixed with a CA in an organic solvent (2 mL, 0.5 mmol solution). There are therefore two molecules of amino acid derivative (or one of each enantiomer) for each molecule of CA. The theoretical maximum yield is 50%. In all cases it was ascertained that the true equilibrium had been reached by following the extraction over time until constant values were obtained. The highest yields and selectivities were observed when the aqueous feed phase was at pH 6. The lowest yields, and generally selectivities, were observed at pH 9.

Table 1. Yields and selectivities for extraction of *N*-(3,5-dinitrobenzoyl)-D,L-leucine.

Exp.	Host	Organic solvent	pH	Yield [%]	*ee* [%]
1	CA **D**	heptane	6	38	85[a]
2	CA **D**	dichloromethane	6	27	54
3	CA **D**	*tert*-butyl methyl ether	6	71	14
4	CA **D**	dichloroethane	6	26	57
5	CA **D**	toluene	6	20	1
6	CA **D**	heptane	7	21	96[a]
7	CA **D**	dichloromethane	7	14	3
8	CA **D**	*tert*-butyl methyl ether	7	27	7
9	CA **D**	dichloroethane	7	18	5
10	CA **D**	toluene	7	7	40
11	CA **D**	heptane	9	3	29
12	CA **D**	dichloromethane	9	7	15
13	CA **D**	*tert*-butyl methyl ether	9	8	20
14	CA **D**	dichloroethane	9	7	12
15	CA **D**	toluene	9	4	6
16	CA **E**	dichloromethane	6	7	64
17	CA **F**	heptane	6	8	2
18	CA **F**	dichloromethane	6	25	67
19	CA **F**	*tert*-butyl methyl ether	6	83	6
20	CA **G**	heptane	6	24	20
21	CA **G**	dichloromethane	6	36	59
22	CA **G**	*tert*-butyl methyl ether	6	86	6
23	CA **H**	heptane	6	17	23
24	CA **H**	dichloromethane	6	16	42
25	CA **H**	*tert*-butyl methyl ether	6	83	6
26	CA **A**	dichloromethane	6	49	13
27	CA **B**	dichloroethane	6	32	55

[a] The high enantioselectivities obtained in these experiments are probably due to classical resolution by crystallisation of a diastereomeric salt. Vide infra.

The use of heptane as the organic solvent seemed to induce the highest selectivities. Chlorinated and aromatic solvents can disrupt the interactions—such as the hydrogen bonding and the π–π stacking—between the host and the guest. Use of *tert*-butyl methyl ether resulted in unexpectedly high yields of 70–80% for all host complexes. Since the theoretical maximum yield is 50%, a control experiment with *tert*-butyl methyl ether and no host complex was performed; this resulted in a similar high yield, showing that in this case direct extraction had taken place, bypassing the intermediacy of the chiral host. The highest yields and selectivities obtained in solvents other than heptane were generally obtained with hosts containing the adamantyl carbamoyl group and the neopentoxy substituent on the quinoline. Chlorinated solvents gave similar results in analogous experiments: CA **D**, for example, gave yields of 27 and 26% and selectivities of 54 and 57% in dichloromethane and 1,2-dichloroethane, respectively (Experiments 2 and 4 in Table 3).

Enantioselective extraction of other DNB-amino acids: Two-phase extraction experiments were also performed with other DNB-protected amino acids, including D,L-DNB-Phe, D,L-DNB-Ala, D,L-DNB-Val and D,L-DNB-PhG (Table 2). For a full list of two-phase extraction experiments with all amino acid derivatives see the Supporting Information.

Table 2. Yields and selectivities for extraction of DNB-Leu.

Exp.	Host	Organic solvent	pH	Yield [%]	*ee* [%]
28	CA **A**	dichloroethane	6	58	5
29	CA **B**	dichloroethane	6	46	19
30	CA **D**	dichloroethane	6	46	19
31	CA **F**	dichloroethane	6	37	30
32	CA **G**	dichloroethane	6	52	19
33	CA **H**	dichloroethane	6	22	19
34	CA **D**	dichloroethane	7	13	38
35	CA **F**	dichloroethane	7	10	55
36	CA **G**	dichloroethane	7	20	30
37	CA **H**	dichloroethane	7	6	2
38	CA **D**	dichloroethane	9	0	0
39	CA **D**	heptane	6	50	43
40	CA **D**	chloroform	6	33	15
41	CA **D**	toluene	6	14	6

Use of a pH 6 aqueous phase results in the highest yields, as in the case of the DNB-Leu substrate. With this racemate, however, pH 7 had given the highest selectivities, whereas at pH 9 no extraction was observed. As in the case of *N*-(3,5-dinitrobenzoyl)leucine, extractions in heptane gave the best result (50% yield, *ee* = 43%, Exp. 39, Table 2). Use of toluene again resulted in the lowest yield and selectivity (14% yield, *ee* = 6%, Exp. 41).

The yields and selectivities obtained with CA **D** in hexane (Figure 2) and in 1,2-dichloroethane (Figure 3) are shown for each of the amino acid derivatives used. In this case the yield is represented as the percentage of the maximum that can be extracted. With CA **D** in heptane the highest selectivities are observed in the separation of DNB-Leu and DNB-Val. Selectivities for DNB-Phe and DNB-PG are moderate. However, the yield in the extraction of DNB-Phe is excellent. Both the yield and selectivity for DNB-Ala are low. With CA **D** in 1,2-dichloroethane the yields are similar to those above but the selectivities are generally lower, with the exception of DNB-Ala, for which the *ee* has greatly in-

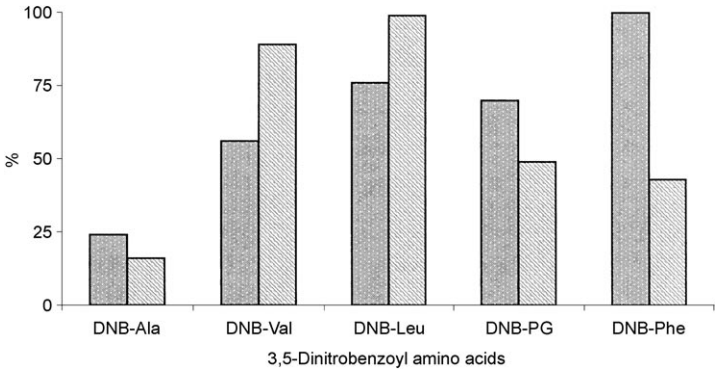


Figure 2. Extraction of DNB-protected amino acids by CA **D** in heptane; yield: ■, *ee*: ■.

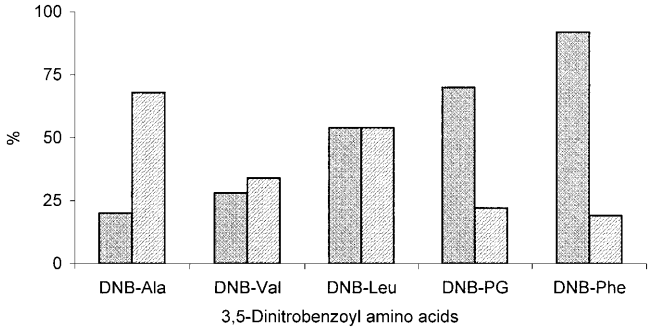


Figure 3. Extraction of DNB-protected amino acids by CA **D** in 1,2-dichloroethane; yield: ■, *ee*: ■.

creased. Again, the leucine and valine derivatives are extracted with higher selectivities than the phenylalanine and phenylglycine derivatives.

Enantioselective enrichment by centrifugal separation: A centrifugal separator (CCS) is in essence a centrifuge (Figure 4). The immiscible liquid phases are introduced by pump in the narrow annular mixing zone between the outside of the rotor and the inside of the outer housing. Here, very efficient and fast mixing between the two phases occurs, which is highly conducive towards high mass transfer. The dispersion is then sucked inside the centrifuge, where the two phases are gradually but very efficiently separated whilst moving upwards, after which they leave the device through separate exits. In addition to the fast mixing and separating, another major advantage of the CCS over hollow fibre membranes is the ability to use a variety of polar and nonpolar solvents. There are two different designs of transport experiments: with and without recycling. In our experiments we have employed the recycling setup (Figure 5), in which the aqueous phases are fed into the CCSs and, after separation from the organic phase, are returned to the original container. Two CCS units and three peristaltic pumps are employed in the setup for extraction experiments (Figure 5).

The only criteria for the use of solvents in the CCS are that the two chosen solvents must be immiscible and of different densities. An aqueous phase can therefore be mixed with a range of organic solvents such as alkanes, cycloalkanes, halogenated solvents (chloroform, dichloromethane, 1,2-dichloroethane etc.), higher alcohols and aromatic solvents. The aqueous phase can include buffer solutions to maintain constant pH values, or salts such as KBr to provide counter-transport of—in this case—anions.

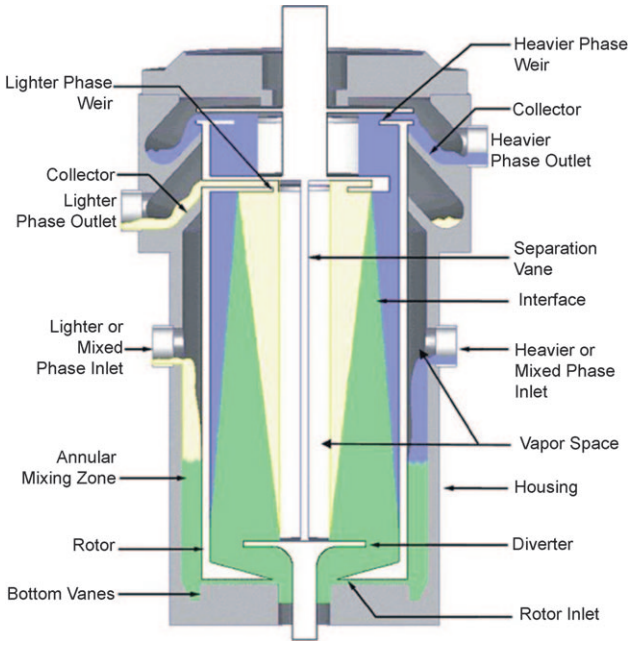


Figure 4. Schematic cross-section of a centrifugal contact-separator (Courtesy of CINC-Solutions, the Netherlands).

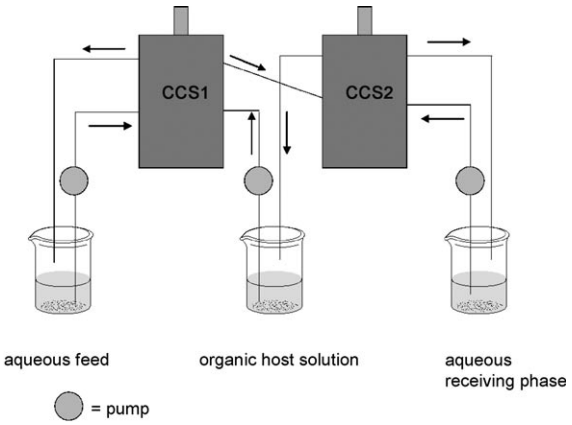


Figure 5. Setup of the CINC model V2 separators for extraction experiments.

A variety of CA hosts were tested in the separation of D,L-DNB-protected amino acids under different conditions. Samples both from the feed phase and from the receiving phase were taken over time. The first transport experiment performed in the CCS made use of the CA host *O*-(1-*tert*-butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (CA **D**) as the host compound and heptane as the solvent. This is the combination that gave the highest yield (38%)

and selectivity (85%) in the two-phase extraction experiments (Exp. 1). However, there were difficulties in dissolving DNB-Leu at pH 6 at higher concentrations, so the feed phase was prepared at pH 6.5. The receiving phase was prepared at pH 9 in order to "strip" the amino acid derivative from the host. After three hours of operation, there was no DNB-Leu observed in the receiving phase. The concentration of DNB-Leu in the feed phase had decreased by 10% (equal to the concentration of host compound) and a white solid was present in the feed CCS at the completion of the experiment. It appeared that DNB-Leu was forming a salt with the host compound (as the concentration in the feed phase was reduced) but was not being delivered to the receiving phase. KBr was added to the receiving phase to aid the removal of the substrate from the host molecule. Again, after three hours, no DNB-Leu was observed in the receiving phase and there was precipitation of the host/guest on complexation. In retrospect, this experiment suggests that the high *ee* obtained in the 2:1 screening experiments was due to classical resolution through preferential crystallisation of one of the diastereomeric salts. To overcome this problem, a more polar solvent—1,2-dichloroethane—was employed (Table 3).

It can be seen that the change of solvent results in complete transport of DNB-Leu from the feed phase to the receiving phase (Figure 6). It can also be seen that enantioselective transport takes place. The concentration of the *R* enantiomer (◆) in the feed phase is reducing at a faster rate than that of the *S* enantiomer (■). This is also observed in the receiving phase, where the concentration of the *R* enantiomer (▲) is increasing at a faster rate than that of the *S* enantiomer (●). Eventually both enantiomers are transported completely to the receiving phase. The transport is relatively fast with the CCS (ca. 90 min for 5 mmol of amino acid) in comparison with enantiomeric separations using hollow fibre membranes.[15] The course of *ee* change in feeding and receiving phases in this experiment clearly shows that initially (*S*)-DNB-Leu is transported with an *ee* of 58%. (Figure 6b) This number quickly erodes over time as increasingly also the *R* enantiomer is being transported, due by the fact that the rate of transport is proportional to the concentration in the feeding phase. Interestingly, it would also be possible to recover the *R* enantiomer from the feeding phase, where after 90 min an *ee* of 83% has been reached, although the yield of DNB-Leu has by now declined to 20%. The conditions used in this experiment will from now on be defined as the standard conditions, and in all other experiments performed their deviations from these conditions will be highlighted. The results of other experiments will also be compared with the results from these standard conditions.

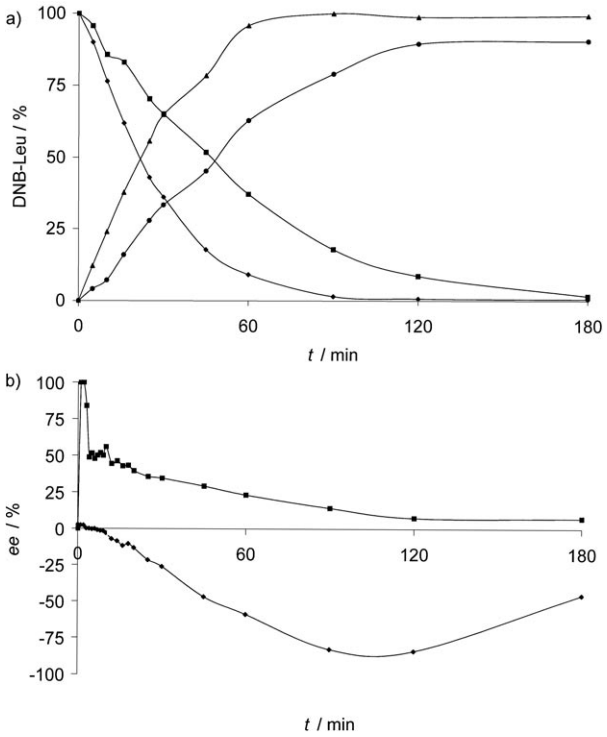


Figure 6. a) Enantiomeric separation of *N*-(3,5-dinitrobenzoyl)-D,L-leucine with use of CA **D** and two CCSs (■ = (*S*) (feed), ◆ = (*R*) (feed), ▲ = (*R*) (receiving), ● = (*S*) (receiving)). b) *ee* of feed (◆), *ee* of receiving (■).

Table 3. Conditions for enantioselective transport in two CCSs.

composition of feeding phase and flow rate	0.1 M phosphate buffer (pH 6.5), 5 mM substrate, 500 mL H_2O, 625 mL min^{-1}
composition of receiving phase and flow rate	0.1 M phosphate buffer (pH 9), 0.1 M KBr, 1000 mL H_2O, 625 mL min^{-1}
transport phase: host compound, concentration, solvent and flow rate	CA **D**, 0.5 mM, 500 mL 1,2-dichloroethane, 625 mL min^{-1}
rotational speed	feed CCS 3500 rpm stripping CCS 3500 rpm

The CA host *O*-(1-*tert*-butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (CA **D**) was used for this experiment because it had given the highest yield and selectivity in two-phase extraction experiments. However, this had been the case when the organic solvent used was heptane. The highest yield and selectivity observed in 1,2-dichloroethane had been achieved with the CA host *O*-(1-adamantylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (CA **G**). This host led to a yield of 36 and 59% enantioselectivity in 1,2-dichloroethane (Exp. 21), in comparison with a yield of 27% and a selectivity of 54% for CA **D** in the same solvent (Exp. 2). The CCS extraction experiment was repeated under identical conditions with the CA **G** host (Figure 7).

Consistently with the two-phase extraction experiments, the transport is faster in this experiment than in the standard one. Also, the selectivity has slightly increased. The highest selectivity in the two-phase experiments had been

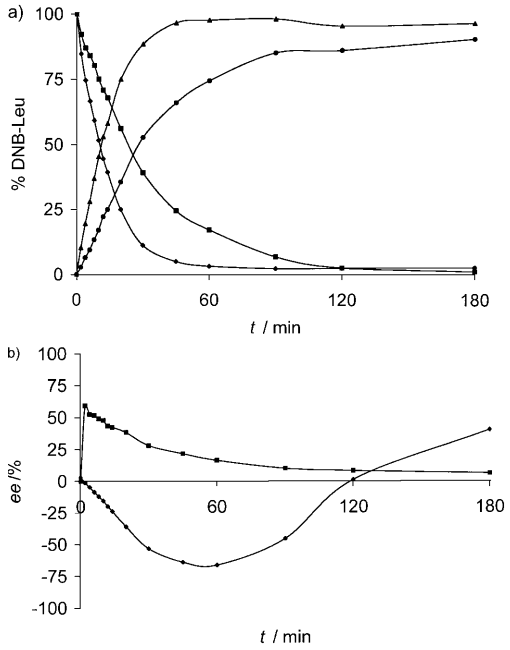


Figure 7. Enantiomeric separation of DNB-D,L-leucine with CA **G** with the setup of Figure 5 (for legend see Figure 6).

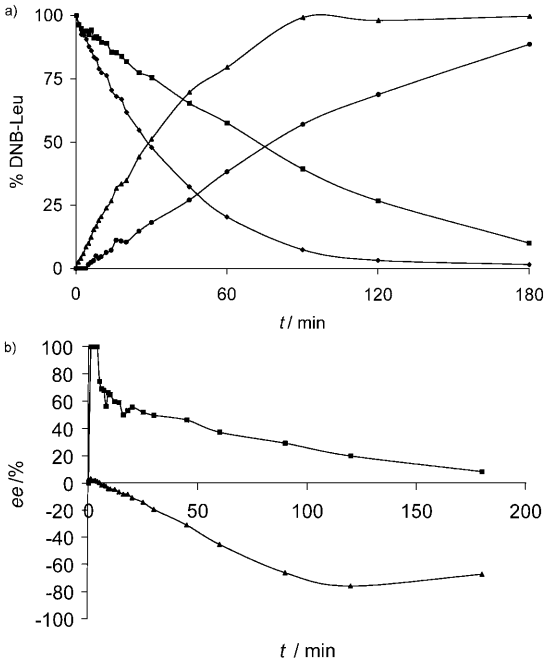


Figure 8. Enantiomeric separation of 3,5-dinitrobenzoyl-D,L-leucine with CA **F** and CCS extractors (for legend see Figure 6).

obtained with the CA host *O*-(1-*tert*-butylcarbamoyl)-6-neopentoxy-11-octadecylsulfinyl-10,11-dihydrocinchonidine (CA **F**). This host had induced a selectivity of 67%, but the yield had been relatively low at 25% (Exp. 18). Again, the CCS extraction experiment was performed under the standard conditions with CA **F** host (Figure 8). Host CA **F** gave a greater selectivity but a lower rate of transport, in accordance with the two-phase extraction experiments.

Many extraction experiments using the centrifugal separators were performed with different hosts and substrates and different flow rates, rotational speeds and concentrations. For a complete listing and graphical representation of each experiment see the Supporting Information. Use of CA **B** resulted in faster transport but slightly lower selectivity.

Without KBr in the receiving phase, the transport was much slower. A number of explanations for this effect are possible. It is assumed that at the pH used during transport the host is present mostly in its protonated form. This means that when the DNB-LEU is stripped into the receiving phase an exchange of counter-ions occurs. If no bromide is present, more polar anions such as hydroxide or phosphate would lead to a poorly soluble host compound. In addition, the thermodynamics for anion exchange may become unfavourable. For fast transport the host should not all be in the host–guest form, because this would lead to the extraction becoming the rate-determining step. A subtle balance between counter-ions is thus necessary, and this so far seems to be optimal with bromide.

Increasing the speed of rotation in each CCS from 3500 to 4800 rpm resulted in lower selectivity. This is due to the characteristics of the equipment: at higher speed the volume of the mixed phase reduces, leading to shorter contact time and less efficient extraction.[19] Conversely, lowering the speed of mixing to 2800 rpm resulted in slower transport but higher selectivity. Having the feed CCS set at 2800 rpm and the stripping CCS set at 4800 rpm resulted in a higher selectivity but slower transport. The results are almost identical to those obtained when both CCS extractors were set at 2800 rpm. This suggests that in the current setup the rotational speed of the feed CCS is more important for controlling yield and selectivity than the rotational speed of the stripping CCS.

In the experiments described thus far, the concentration of host has been equal to 10% of the concentration of substrate. Increasing the concentration of host results, as expected, in faster transport but lower selectivity. On lowering the flow rate of the transport phase by a third and, at the same time, increasing the concentration of host by a third, the same number of molecules passed through the CCS extractors per minute but at a higher concentration, which resulted in faster transport but lower selectivity.

Changing the substrate to DNB-Phe resulted in generally fast transport but with lower selectivity than observed with DNB-Leu (For CAs **B**, **D** and **F**). Use of CA **D** led to slower transport of DNB-Val than of the other two substrates.

Outlook

In order to achieve full separation of both enantiomers a number of CCSs in series has to be used, with the aqueous and organic streams running in countercurrent directions. One enantiomer will be obtained in pure form from the

aqueous stream (exit of CCS no. 1); the other enantiomer will be continuously extracted from the organic phase in CCS no. 5. We are currently studying this system in more detail, allowing us to calculate the necessary number of CCSs and the other process parameters.[21]

We would expect it to be possible to separate an entire class of compounds, such as DNB-amino acids, with a single host compound. Although there will be differences in selectivity for each substrate, these can be compensated for by using more CCSs in series. Early calculations show it is possible to produce 10–20 kg of pure enantiomer per week in the setup shown in Figure 9. With the largest commercially

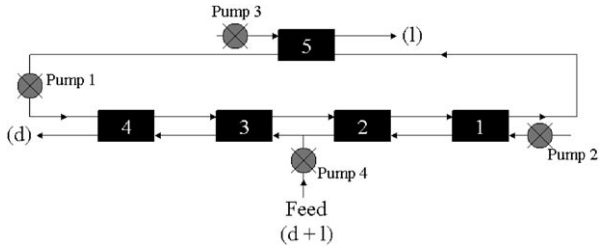


Figure 9. Full separation of racemates with a series of CCSs in a countercurrent setup.

available CCS a production of 5–10 tons per week should be feasible. If the host compound is sufficiently stable—and so far we have not detected any decomposition products—it should be possible to achieve turnover numbers on host of 400–700 per week, which leads to perfectly acceptable economics. We believe that the method described in this paper has potential similar to that of chiral SMB for the ton-scale separation of racemates, but at much reduced cost.

Other host compounds will need to be developed for the separation of other classes of racemates. A patent for this continuous flow system has been filed.[22]

Conclusion

In conclusion, we have shown that it is possible to separate a racemic mixture of an *N*-acylated amino acid by extraction across a liquid membrane containing a chiral host compound based on a Cinchona alkaloid. We have now shown for the first time that it is possible to perform this separation in continuous mode by use of a catalytic amount of chiral host compound in a centrifugal contact separator. Further work directed towards full separation of racemates by the same principles is in progress.

Experimental Section

General: All starting materials were purchased from commercial chemical suppliers and were used as received. Organic solvents were purchased from Fluka, were of dried reagent grade and were used without further purification.

HPLC analyses of the 3,5-dinitrobenzoyl-protected amino acids were carried out with a Chirobiotic T column (250×4.6 mm ID) from Astec, Inc. CAT 12024. A mixture of acetonitrile (75%), methanol (25%), acetic acid (0.25%) and triethylamine (0.25%) (polar ionic mode: pim mode) was used as eluent. The analysed samples contain feeding (or receiving) phase (0.1 mL) and eluent (0.9 mL). The flow rate was 1.2 mL min^{-1}. The injection volume was 20 μL, and detection was performed with a spectrophotometer at UV 270 nm. All analyses were performed at ambient temperature.

Retention times for the first-eluting enantiomer L-DNB-Leu and the second-eluting enantiomer D-DNB-Leu were 3.92 and 7.00 min, respectively. Retention times for the first-eluting enantiomer L-DNB-Phe and the second-eluting enantiomer D-DNB-Phe were 3.74 and 6.13 min, respectively. Retention times for the first-eluting enantiomer L-DNB-Val and the second-eluting enantiomer D-DNB-Val were 3.68 and 5.41 min, respectively. Retention times for the first-eluting enantiomer L-DNB-Ala and the second-eluting enantiomer D-DNB-Ala were 4.26 and 5.41 min, respectively. Retention times for the first-eluting enantiomer L-DNB-Phg and the second-eluting enantiomer D-DNB-Phg were 3.39 and 10.66 min, respectively.

Conditions for two-phase extraction experiments: Two-phase extraction experiments were carried out with a mixture of the CA host compound in an organic solvent (2 mL, 0.5 mmol solution) and the substrate in a buffered aqueous solution (2 mL, 1 mmol solution). The phases were stirred in a sample vial (20 mL) overnight and the mixture was then allowed to stand for 1 h in order to allow phase separation. Concentrations of each enantiomer in the aqueous phase were determined by chiral HPLC analysis. Extraction yields and selectivity were calculated by comparison with the analysis of the original starting aqueous phase.

Conditions for CCS extraction experiments: For the CCS extraction experiments, two CINC model V2 separators were employed with use of the recycle setup. The feed and transport phases were thus pumped into the first CCS with peristaltic pumps (Watson Marlow 101U/R) set at 70 rpm, giving a flow rate of 625 mL min^{-1} for each phase. After mixing and separation of the phases, the aqueous phase was returned to the original feed solution, whereas the organic phase was transferred to a second CINC model V2 separator. The receiving phase was pumped into the second CINC model V2 separator with a peristaltic pump (Watson Marlow 101U/R) also set at 70 rpm, giving a flow rate of 625 mL min^{-1}. After mixing and separation of the phases, the aqueous phase was returned to the original receiving solution and the organic phase was returned to the original transport solution. There are therefore three closed loops in the system: two aqueous and one organic.

The tubing used for the experiments depends on the solvents. For the aqueous phases, Marprene (Watson Marlow, 8 mm 5/16″, serial number 902.0080.016) was used in the peristaltic pumps to drive the solutions to the CCSs. For the organic phase (chlorinated solvents), Viton (Watson Marlow, 8 mm 5/16″, serial number 970.0080.016) was used in the peristaltic pump. All of the outlets of the CCSs were fitted with chemically resistant T-tubes, allowing air to enter the CCS in order to prevent a pressure build-up.

The tubing from the outlets of the CCSs also depends on the solvents. For the aqueous phase, Sta-Pure Silicone (Watson Marlow, 16 mm/22 mm, serial number 910.0159.032) was used to return the phases to the original solutions, although any tubing can be employed for this. For the organic phase (chlorinated solvents), Íso-Versinic, Verneret (Merck Eurolab, 15 mm/21 mm, serial number VERN770460-01) was used to transport the phase between the two CCSs and to return the phase to the original solution.

The experiment was started by setting the CCSs to the required rpm and pumping the organic phase (the heavier phase) into the first CCS. After 5–10 seconds, the feed phase was also pumped into the first CCS. Once the aqueous solution could be seen returning to the feed phase, the receiving phase was pumped into the second CCS. When only one phase is within the CCSs, the solution will exit by the heavy-phase outlet.

The solutions were pumped continuously through the system for 3 h, during which samples were taken both from the feed and from the re-

ceiving phases. Samples were taken at various times between 0 and 180 minutes and were analysed by chiral HPLC.

For a typical experiment (Exp. 3), the feed phase consisted of 3,5-dinitrobenzoyl-D,L-leucine (0.82 g, 5 mmol) in distilled water (500 mL). The solution was set at pH 6.5 with a phosphate buffer (0.1 M). The transport phase was *O*-(1-*tert*-butylcarbamoyl)-11-octadecylsulfinyl-10,11-dihydroquinine (CA **D**) (0.182 g, 0.5 mmol) in 1,2-dichloroethane (500 mL). The receiving phase consisted of KBr (11.9 g, 0.1 M) in distilled water (1000 mL). The solution was set at pH 9 with a phosphate buffer (0.1 M). All solutions were pumped into the CCSs at 625 mL min^{-1}, and the CCSs were set at 3500 rpm.

Synthesis of chiral hosts: The chiral hosts based on Cinchona alkaloids were synthesised as described by Lindner and Lämmerhofer.[23] Quinine, cinchonidine or 6-neopentoxycinchonidine were first carbomoylated with 1-adamantyl, 1-cyclohexyl or 1-*tert*-butyl isocyanate. The resulting carbamate was then treated with the long-chain thiol to give the thioether, which could in turn be oxidised to the desired product.

6-Hydroxycinchonidine: 6-Hydroxycinchonidine was prepared by the literature procedure;[24] BBr_3 (80 mL, 1 M solution in CH_2Cl_2, 80.0 mmol) was thus slowly added at −78 °C to a solution of quinine (6.51 g, 20.1 mmol) in CH_2Cl_2 (500 mL). The mixture was allowed to reach ambient temperature and was then heated to 40 °C for 1 h. After the mixture had cooled to 5 °C, NaOH (150 mL, 10 % soln.) was added. The aqueous phase was separated, washed with CH_2Cl_2 (150 mL) and acidified with HCl (50 mL, 37 % soln.). The acidic solution was in turn brought to pH 9.5 with NH_4OH (58 %), and the product was extracted with butan-1-ol (2 × 200 mL). After drying over Na_2SO_4 and filtering, the solvent was removed under reduced pressure. Yield 5.04 g, 16.2 mmol (81 %).

6-Neopentoxy-cinchonidine: 6-Neopentoxy-cinchonidine was prepared by the literature procedure;[25] finely powdered caesium carbonate (7.56 g, 23.2 mmol, dried at 120 °C in vacuo for 16 h) and neopentyl bromide (3 mL, 23.8 mmol) were thus added to a solution of 6-hydroxycinchonidine (5.04 g, 16.2 mmol, dried at 120 °C in vacuo for 1 h) in *N*-methylpyrrolidone (50 mL). The mixture was heated to 130 °C with stirring for 24 h, after which it was allowed to reach ambient temperature. Water (300 mL) was added, and the product was extracted with toluene (5 × 100 mL). The combined organic phases were washed with water (3 × 200 mL) and brine (3 × 200 mL) and were then dried over $MgSO_4$. The mixture was filtered, and the solvent was removed under reduced pressure. Yield 2.93 g, 7.5 mmol (45 %).

(3,5-Dinitrobenzoyl)-protected amino acids: The *N*-(3,5-dinitrobenzoyl)-protected amino acids were prepared in quantitative yields by the literature procedure.[26] Thus, to obtain *N*-(3,5-dinitrobenzoyl)-D,L-leucine, 3,5-dinitrobenzoyl chloride (9.35 g, 40.5 mmol) was added to a suspension of D,L-leucine (5.32 g, 40.5 mmol) in dry THF (100 mL). The mixture was cooled in an ice bath, and propylene oxide (2.9 mL, 41.4 mmol) was added dropwise. After stirring for 2 h at ambient temperature, the solution was filtered through celite, and the solvent was removed under reduced pressure to give the desired product. *N*-(3,5-Dinitrobenzoyl)-D,L-valine, *N*-(3,5-dinitrobenzoyl)-D,L-alanine, *N*-(3,5-dinitrobenzoyl)-D,L-phenylalanine and *N*-(3,5-dinitrobenzoyl)-D,L-phenylglycine were prepared in a similar manner.

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