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Chiral Separation of Underivatized Amino Acids by Reactive Extraction with Palladium−BINAP Complexes

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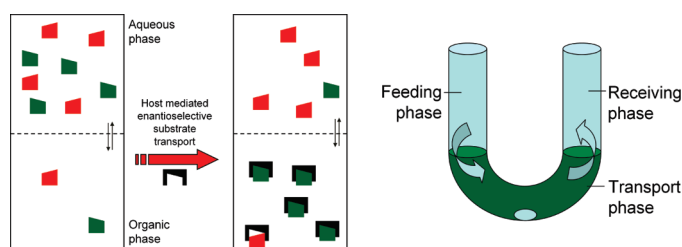
In answer to the need for a more economic technology for the separation of racemates, a novel system for reactive enantioselective liquid−liquid extraction (ELLE) is introduced. Palladium (*S*)-BINAP complexes are employed as hosts in the separation of underivatized amino acids. The system shows the highest selectivity for the ELLE of tryptophan with metal complexes as hosts reported to date and shows a good selectivity toward a range of natural and unnatural amino acids. Furthermore, the host can be prepared in situ from commerically available compounds. Bulk-membrane transport in the form of U-tube experiments demonstrates the enantioselective and catalytic nature of the transport. The dependency of the system on parameters such as pH, organic solvent, and host−substrate ratio has been established. ^{31}P NMR spectroscopy has been used to confirm the preferred enantiomer in the extraction experiments. The intrinsic selectivity was deduced by determination of the association constants of the palladium complex with the tryptophan enantiomers.

Introduction

The production and availability of enantiomerically pure compounds is of immediate importance to the pharmaceutical industry.[1] Methods available to provide enantiopure compounds include resolution of racemates, isolation from natural sources, fermentation, asymmetric catalysis, and biocatalysis, with resolution still being used most often.[2] Among the available resolution methods are resolution via crystallization of diastereomeric salts, also called classical

resolution,[3] kinetic resolution[4] with enzymes or chiral catalysts, or dynamic kinetic resolution.[5] Sometimes the unwanted isomer can be racemized so that it can be recycled.[6] Despite the theoretical maximum of 50% yield classic resolution is still a widely applied method in the fine chemical and pharmaceutical industries.[7] However, this production method is laborious, sometimes suffers reproducibility problems, and is relatively expensive.[8] Thus new effective separation methods are highly desirable. Among the methods being explored are resolution with in situ racemization,[9]

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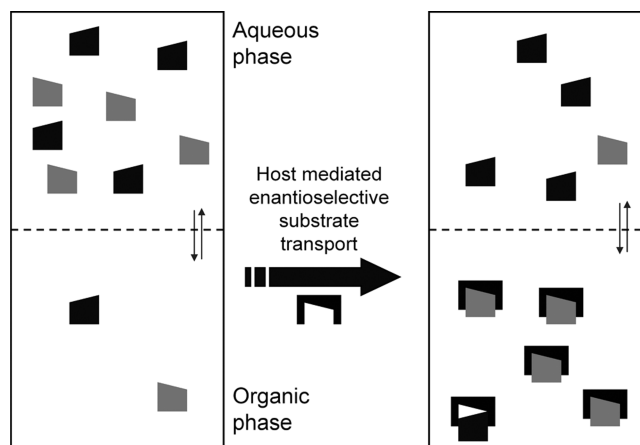
FIGURE 1. Schematic representation of ELLE: (gray symbols) (*S*)-substrate; (black symbols) (*R*)-substrate; and (black rectangles) host.

membrane-assisted separations,[10] diastereomer separation by distillation,[11] supercritical extraction,[12–14] fractional enantioselective extraction,[15,16] and chiral simulated moving bed (SMB).[17]

In enantioselective liquid−liquid extraction (ELLE), an enantiopure host is used as an extractant to react enantiospecifically and reversibly with a racemic substrate (Figure 1). If the host is confined to one phase in a biphasic system, an enantiomeric separation of the substrate can occur between the two phases in a single step. If the separation is imperfect, a fractional extraction scheme is needed.[18,19] A minimal selectivity of 1.5 is generally viewed as being necessary to avoid the requirement for an excessive number of fractional extraction steps.[20] An important feature of this system is its potential versatility. With a versatile host, for instance, the separation of racemates of an entire class of compounds is potentially achievable.

Typically U-tubes[21] or membranes are employed in separation schemes where the chiral host is applied in a catalytic fashion. The use of a number of membranes in series has even allowed for complete separation.[22] Maier and Lindner have reported the use of a centrifugal partition

TABLE 1. Tryptophan Extraction with Palladium and Platinum (*S*)-BINAP Complexes[a]

entry	complex	aq	[host] (mM)	$D(\text{org/aq})^b$	$\alpha_{op}{}^c$
1	[PdCl$_2$((*S*)-BINAP)]	H$_2$O^d	1	0.1	2.2
2	[PdCl$_2$((*S*)-BINAP)]	H$_2$O	5	0.2	2.1
3	[PtCl$_2$((*S*)-BINAP)]	H$_2$O	1	0.1	1.9
4	[PtCl$_2$((*S*)-BINAP)]	H$_2$O	5	0.1	1.9
5	[PdCl$_2$((*S*)-BINAP)]	pH 7.0	1	0.5	2.4
6	[PdCl$_2$((*S*)-BINAP)]	pH 7.0	5	3.4	2.4
7	[PdCl$_2$((*S*)-BINAP)]	Trp-Na^e	1	0.5	2.4
8	[PdCl$_2$((*S*)-BINAP)]	Trp-Na	5	1.6	2.4
9	[PtCl$_2$((*S*)-BINAP)]	Trp-Na	1	0.7	2.2
10	[PtCl$_2$((*S*)-BINAP)]	Trp-Na	5	5.4	2.2

^{a}Conditions: organic solvent = dichloromethane; $T = 6$ °C; [substrate] = 2.0 mM, $V_{org} = V_{aq} = 0.40$ mL. ^{b}The distribution $D(\text{org/aq})$ of the substrate (AA) over the two phases is defined as the ratio of the concentration of the substrate over the two phases ($D = [\text{AA}]_{org}/[\text{AA}]_{aq}$). ^{c}The operational selectivity (α_{op}) is defined as the ratio of distribution of enantiomers ($\alpha_{op} = D_{\text{D-AA}}/D_{\text{L-AA}}$). ^{d}Double distilled water (dd). ^{e}Trp with 1 equiv of NaHCO$_3$ in dd.

chromatograph containing an MTBE solution of bis-1,4-(dihydroquinidinyl)phthalazine as the stationary chiral host solution and were able to fully separate the herbicide 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), which was fed as a solution in aqueous buffer as the mobile phase.[23] As these methods are not scalable we have developed the use of centrifugal separators as a highly efficient method for continuous extraction.[24–26] Applying a number of these in series allows for the full separation of a racemate.[27]

Over and above the technological aspects, the development of improved chiral host compounds is still required. In particular the enantioselective extraction of underivatized amino acids is one of the great challenges within the field of ELLE. Only a few studies have been reported in this field to date. Cram et al. have carried out pioneering work showing that high selectivities could be achieved for a range of amino acid perchlorate salts by using crown ethers with a functionalized BINOL backbone.[28,29] Rebek et al. developed a receptor that although not enantioselective, showed high selectivity for underivatized aromatic amino acids.[30] Subsequently, De Mendoza and co-workers combined a chiral guanidinium group with a crown-ether to extract tryptophan enantioselectively.[31] Metal complexes have been employed as hosts including lanthanide β-diketonate[32] and copper−proline complexes.[20,33] Copper complexes with chiral diamino

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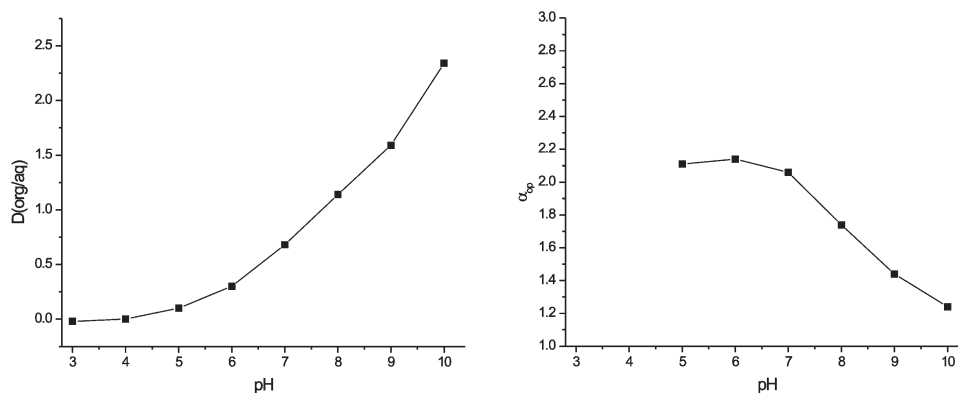
FIGURE 2. The dependency of the distribution and the operational selectivity (α_{op}) on pH. Conditions: organic solvent = dichloromethane; $T = 6\ °C$; metal precursor = [PdCl$_2$(CH$_3$CN)$_2$]; ligand = (*S*)-BINAP; [metal] = 1.0 mM; [ligand] = 1.0 mM; [substrate] = 2.0 mM.

ethane derivatives[34] or hydroxyproline derivatives[35] have been employed to transport amino acids through a liquid membrane enantioselectively. However, the challenge of developing a chiral metal complex that is facile to synthesize, which binds with high operational selectivity (α_{op}) to a variety of underivatized amino acids and can be applied in fractional ELLE, remains.

It has been reported that metal complexes can show good performance in enantioselective extraction.[36,37] Palladium phosphine complexes in general have shown the ability to bind underivatized amino acids.[38,39] On the other hand, the chiral bisphosphine BINAP has proven to be a highly versatile ligand in asymmetric catalysis.[40] Since it is possible to bind other ligands by exchange of the counterion it seemed logical to access palladium and platinum complexes of (*S*)-BINAP for their ability to extract amino acids in general and tryptophan (Trp) in particular in an enantioselective manner.

In this paper, we disclose our recent finding that the use of the metal complex [PdCl$_2$((*S*)-BINAP)] as a host results in the highest operational selectivity for underivatized tryptophan in an ELLE system using metal complexes reported to date. This system also shows good selectivity toward a range of natural and unnatural amino acids. Furthermore, the host can be prepared in situ from commercially available compounds.

Results and Discussion

Extraction Experiments. In Table 1 the results of the extraction of tryptophan with palladium and platinum (*S*)-BINAP complexes are summarized. In entries 1−4 it is shown that the complexes are able to extract tryptophan enantioselectively with a selectivity of 2.4 in the case of the palladium complex under completely neutral conditions. The distribution (D(org/aq)) is quite low, but can be

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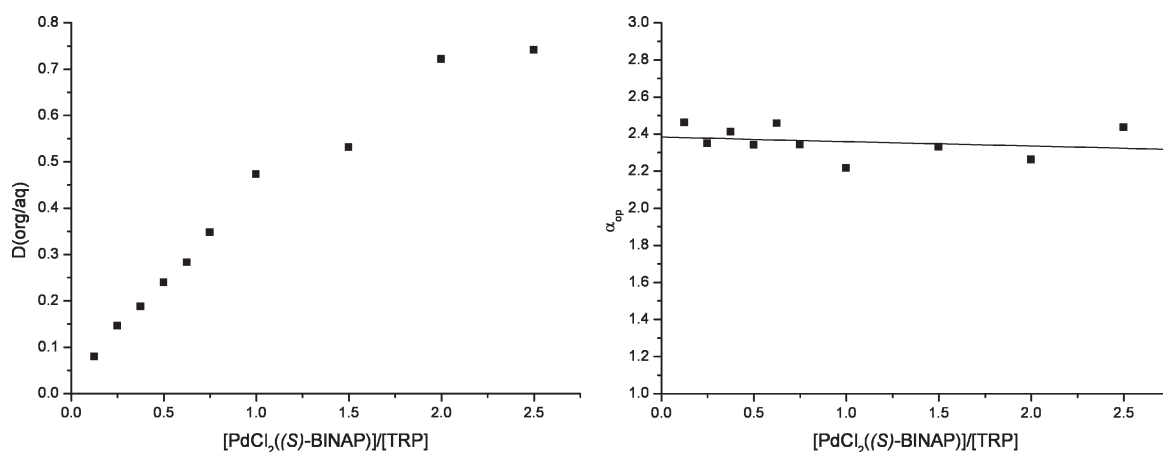(35) Pickering, P. J.; Chaudhuri, J. B. *Chirality* **1997**, *9*, 261–267.
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increased to 0.2 by increasing the host concentration (entry 2). In the case of a buffered aqueous solution at pH 7.0 (entry 5) the distribution is increased to 0.5 and the α_{op} increases to 2.4. A further increase of the distribution was obtained by increasing the host concentration (entry 6). When Trp-Na is used as a substrate (entry 7), the distribution and selectivity compare to that using a pH 7.0 buffered solution (entry 5). PtCl$_2$((*S*)-BINAP) shows a higher distribution (entries 9 and 10) albeit with a lower α_{op} (2.2).

The pH of the aqueous phase is in most cases a key issue in ELLE. Normally, the pH should affect the distribution of the substrate, but not the α_{op}.

In Figure 2, the relationship between the pH of the aqueous phase and the distribution and α_{op} is depicted. In the measured pH range, significant physical partition (i.e., the host-absent distribution of the substrate) was not observed (P(org/aq) = 0.0). Below a pH of 4, Trp does not show a significant distribution. As the pH increases from 5.0 to 7.0, the distribution rises from 0.1 to 0.7. The corresponding α_{op} remains constant at 2.1. When the pH is >8.0, the α_{op} drops, whereas at pH 10.0, $D = 2.3$ and $\alpha_{op} = 1.2$ are observed. The increased distribution in response to increasing pH suggests that the Trp anion complexes to the palladium complex.

The observation that the α_{op} stays constant in the pH range below 7.0 and the distribution below 1 shows that in this pH range, Trp binds enantioselectively to palladium, most probably in a 1:1 stoichiometry. At pH values above 8.0, it is possible that a second tryptophan binds to the complex ($D > 1$ and c(host) = 1.0 mM), providing an explanation for the decrease in α_{op}.

Another important parameter in ELLE is the host concentration. Increasing the host concentration normally causes the distribution to increase. The α_{op} should be independent of the host concentration. In Figure 3 it is shown that the distribution increases up to 0.7 when the host concentration is increased to 2.5 mM. The α_{op} remains constant at 2.35. The linear fit depicted in the graph on the right shows an α_{op} between 2.32 and 2.38. These results show that excellent control of the distribution can be achieved by varying the host concentration while the α_{op} remains constant.

The organic solvent can have a profound influence on both distribution and the operational selectivity. In enantioselective host−guest complexations, the solvent can influence the

FIGURE 3. The dependency of the distribution and the operational selectivity (α_{op}) on the host concentration. Conditions: organic solvent = dichloromethane; $T = 6\ °C$; pH(aqueous phase) = 7.0; metal precursor = [PdCl$_2$(CH$_3$CN)$_2$]; ligand = (S)-BINAP; [substrate] = 2.0 mM.

TABLE 2. Solvent Effect on Enantioselective Extraction of Tryptophan with [PdCl$_2$((S)-BINAP)]^a

entry	solvent	D(org/aq)^a	α_{op}
1	dicholoromethane	0.5	2.4
2	1,2-dichloroethane	0.4	2.8
3	chloroform	0.6	1.9
4	chlorobenzene	0.3	1.2
5	toluene	1.0	1.2

^{a}Conditions: organic solvent = dichloromethane; $T = 6\ °C$; pH-(aqueous phase) = 7.0; metal precursor = [PdCl$_2$(CH$_3$CN)$_2$]; ligand = (S)-BINAP; [metal] = 1.0 mM; [ligand] = 1.0 mM; [substrate] = 2.0 mM.

TABLE 3. Substrate Scope^a

entry	substrate	D(org/aq)	α_{op}
1	Asp	0.7	1.1
2	His	1.2	2.0
3	Met	0.6	2.0
4	Pge^b	0.7	1.9
5	Phe	2.8	2.3
6	Trp	3.4	2.4
7	Val	0.7	2.1
8	Pgl^c	0.0	

^{a}Conditions: solvent = dichloromethane; $T = 6\ °C$; pH (aqueous phase) = 7.0; metal precursor: [PdCl$_2$(CH$_3$CN)$_2$]; ligand: (S)-BINAP; [PdCl$_2$(CH$_3$CN)$_2$] = 5.0 mM; [ligand] = 5.0 mM; [substrate] = 2.0 mM; ^{b}Pge = Phenylglycine; ^{c}Pgl = Phenylglycinol

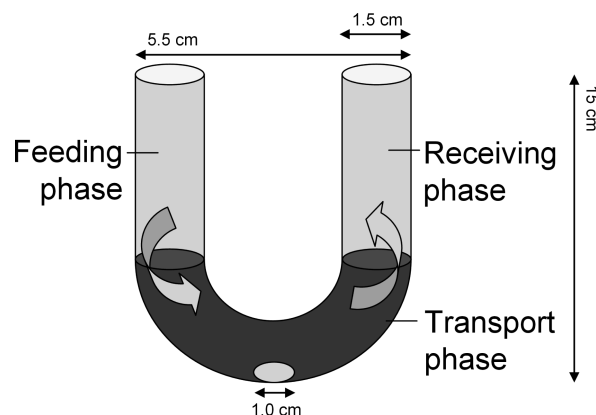noncovalent interactions and affect the conformation of the chiral partners.[41]

In Table 2, the distribution and operational selectivity of the ELLE of Trp by palladium BINAP complexes with five different organic solvents is shown. The chlorinated solvents show the same distribution trend, whereas the operational selectivities differ considerably. The highest selectivity is achieved with 1,2-dichloroethane (entry 2) inducing an α_{op} of 2.8 with a D of 0.4. In chlorobenzene (entry 4) no significant α_{op} was observed. The aromatic nature of the solvent is likely to influence the enantioselective complexation negatively, suggesting that $\pi-\pi$ interactions between tryptophan and the palladium complex are important in chiral recognition. Toluene (entry 5) stands out, being the only nonchlorinated solvent to show both high distribution and no significant operational selectivity. Visual inspection of the system showed a suspension in the organic layer, which confirms precipitation of the complex.

A feature of a successful ELLE system is the substrate scope. Several natural and one unnatural amino acid have been extracted to test their distribution and operational selectivity. From the data in Table 3 it is evident that aspartic acid shows good distribution of 0.7 but, unfortunately, the α_{op} is not significant. The second carboxylic acid group of Asp does not seem to have a positive effect on D and α_{op} compared to Trp. The distributions summarized in entries 2−7 show a wide range of amino acid enantiomers that can be successfully separated by extraction. All of the amino acids other then Asp tested could be extracted with an α_{op} of at least 1.9. The amino acids with a benzyl, phenyl, or an

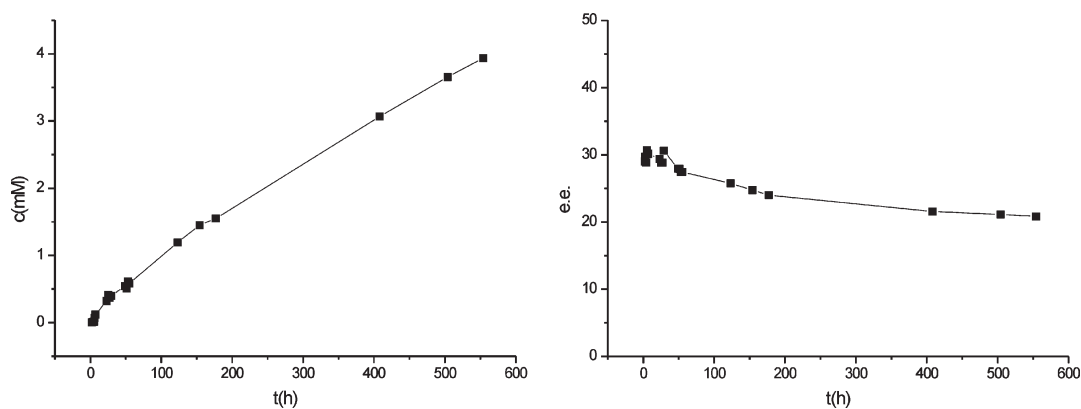(41) Schug, K. A.; Lindner, W. *Chem. Rev.* **2005**, *105*, 67–113.

**FIGURE 4.** Scheme of the U-tube used for bulk-membrane transport.

isopropyl side group could be extracted with high operational selectivities. These results demonstrate the versatility of the [PdCl$_2$((S)-BINAP)] complex as an enantioselective extractant. The nonsignificant distribution of phenylglycinol (entry 8) illustrates that the carboxylic acid group is required for extraction.

After separation of the two liquid phases the amino acid attached to the metal complex can be re-extracted into a second aqueous phase at a different pH, i.e., back extraction. Back-extraction of Trp is possible by using a 1 M HCl (aq) solution. Trp-Na was extracted with 1 equiv of [PdCl$_2$((S)-BINAP)]; subsequent back-extraction yielded the expected

FIGURE 5. Transport rate and ee values of the bulk-membrane transport. Conditions: organic solvent = dichloromethane; $T = 6\,°C$. Lag time of the transport through the transport phase (8 h) is omitted.
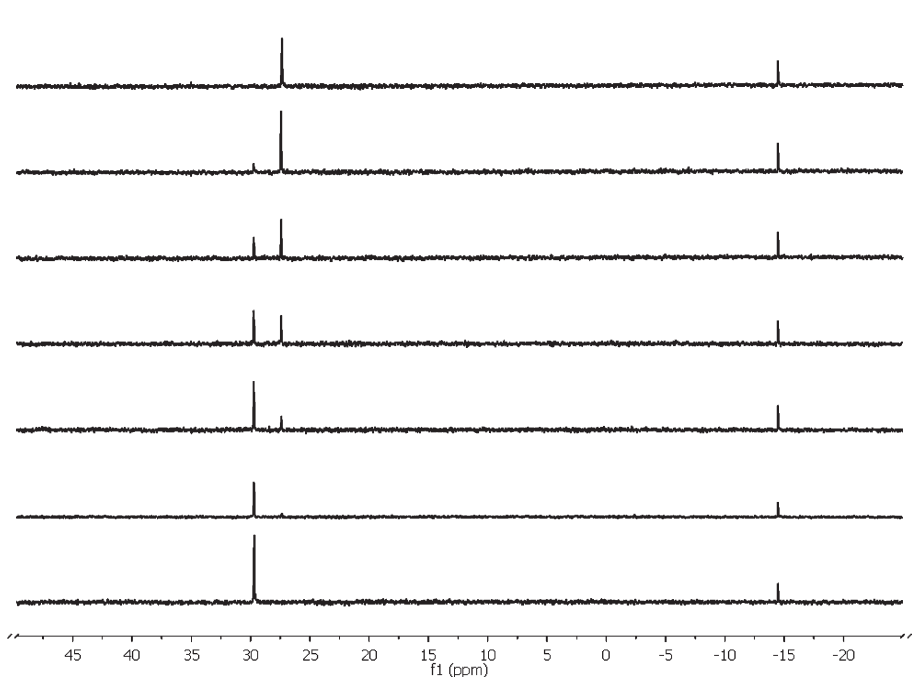
FIGURE 6. ^{31}P NMR spectra of the titration of the palladium complex upon addition of D-Trp (spectra 1−7: [D-Trp] = 0.0, 0.5, 1.0, 1.5, 2.0, 5.0, 10.0 mM). Extraction conditions: organic solvent = CDCl$_3$; $T = 6\,°C$.

values of c(aq) = 0.20 mM with the predicted ee of 36%. The remaining complex was successfully re-employed in a subsequent extraction, demonstrating the full reversibility of the system.

Bulk-Membrane Transport. Reversibility of the amino acid binding to the [PdCl$_2$((S)-BINAP)] complex was also proven by bulk-membrane transport experiments. The U-tube depicted in Figure 4 was used to transport Trp from the aqueous feeding phase to the aqueous receiving phase through the organic (DCM) transport phase, using the [PdCl$_2$((S)-BINAP)] complex as an enantioselective carrier. Figure 5 shows the transport rate and the ee of the receiving phase. After 100 h, the amount of transported Trp exceeds the amount of carrier present in the transport phase. The transport rate is constant during the transport, which suggests a constant concentration of substrate in the transport phase. The pH of the feeding phase starts at 8.1 and declines during the transport process up to 7.4. Up to 4 equiv of Trp

with respect to the carrier are transported in this experiment, albeit that the ee decreases from 30% to 20%. These results demonstrate the enantioselective and reversible nature of the system. The low rate is due to the poor phase mixing in the U-tube and should not be seen as indicative of the rate of a preparative separation.

^{31}P NMR Spectroscopy. The ^{31}P NMR titration spectra in Figures 6 and 7 show the ^{31}P NMR shifts of BINAP in the two diastereomeric complexes formed from [PdCl$_2$((S)-BINAP)] and both enantiomers of Trp. (S)-BINAP is used as an internal standard ($c = 0.2$ mM), since an excess showed no change in extraction. The [PdCl$_2$((S)-BINAP)] complex appears as a singlet absorption at 29.7 ppm.

At higher concentrations of D-Trp (Figure 6), the absorption at 29.7 ppm decreases in intensity, whereas a singlet absorption grows at 27.4 ppm. At $c = 10.0$ mM, the initial absorption is below detection limits, leaving only the new singlet absorption. Together with ES-MS data
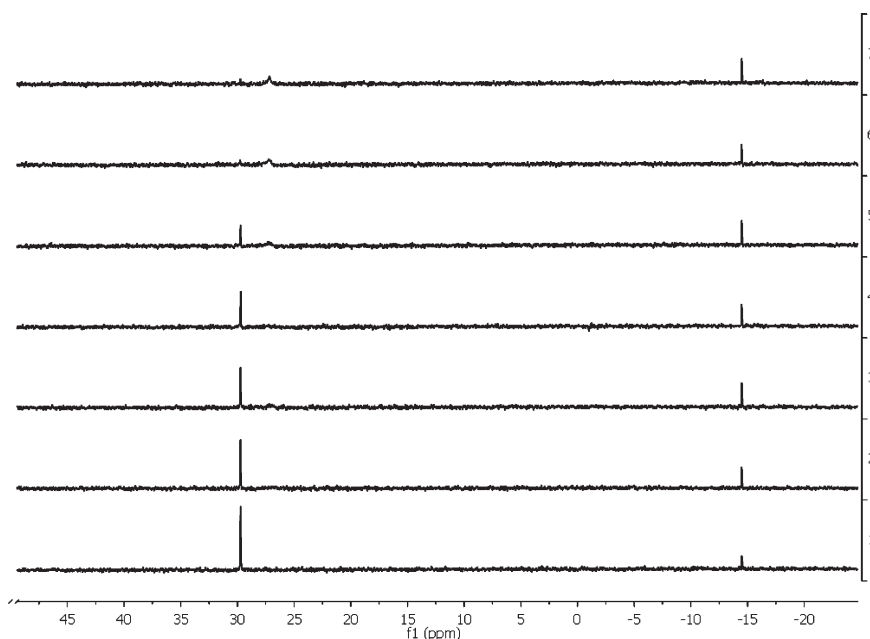
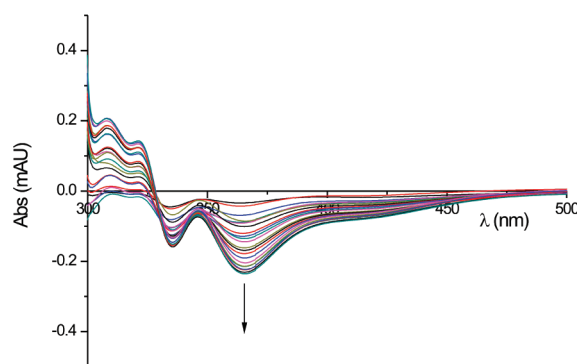**FIGURE 7.** ^{31}P NMR spectra of the titration of the palladium complex upon addition of L-Trp (spectra 1−7: [L-Trp]=0.0, 0.5, 1.0, 1.5, 2.0, 5.0, 10.0 mM). Extraction conditions: organic solvent = CDCl$_3$; T = 6 °C.
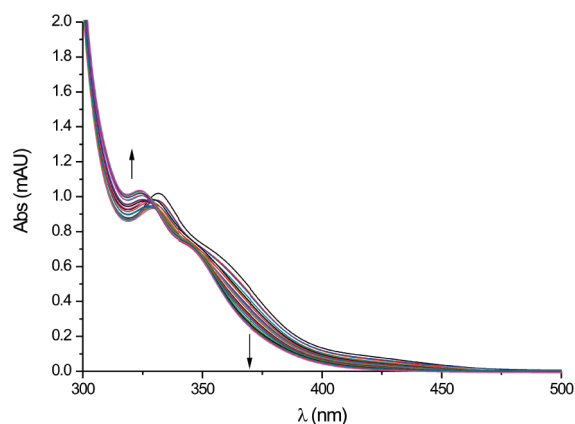
FIGURE 8. UV−vis spectra of the palladium complex upon addition of D-Trp ([D-Trp] = 0.0−10.0 mM). Extraction conditions: solvent = dichloromethane; T = 6 °C.

FIGURE 9. Differential UV−vis spectra of the palladium complex upon addition of D-Trp ([D-Trp] = 0.0−10.0 mM). Extraction conditions: solvent = dichloromethane; T = 6 °C.

taken from the organic phase (MS (ES) m/z 931.5 (M$^+$)), which confirms the complexation of D-Trp to the palladium BINAP complex, a [PdCl((S)-BINAP)(D-Trp)] complex is proposed.

Figure 7 shows the spectra obtained upon addition of L-Trp to the palladium complex. The observed spectra are different compared to the spectra with D-Trp (Figure 6). As in Figure 6, the initial absorption at 29.7 ppm decreases in intensity upon increasing the L-Trp concentration. The newly formed complex, however, shows a broad, ill-defined absorption, in the range of 26.1−28.1 ppm. The difference between the absorption at 27.4 ppm of the [PdCl((S)-BINAP)(D-Trp)] complex and the broad absorption at 26.1−28.1 ppm of [PdCl((S)-BINAP)(L-Trp)] is evidence for a difference in complexation between the two Trp enantiomers. The singlet absorption in the case of D-Trp suggests a symmetrical complex with respect to the phosphorus atoms on the ^{31}P

NMR time scale. The broad, ill-defined absorption in the case of L-Trp is indicative of fast exchange processes suggesting a less stable complex. This agrees with the observation that D-Trp is the preferred enantiomer in the extraction experiments.

UV−Vis Spectrosopy and Association Constants. The relationship between the α$_{op}$ and the association constants between both enantiomers of the substrate and the host was investigated by titration, using UV−vis spectroscopy. The association constants of the palladium complex with the enantiomers of tryptophan in a biphasic system were determined accordingly.

The UV−vis spectra shown in Figures 8 and 9 show the effect of increased complexation of D-Trp to the palladium complex. An isosbestic point is maintained at 328 nm, which slightly shifts, probably due to a small absorption of Trp at this wavelength.[42] Above 328 nm, titration with D-Trp results in a decrease in the absorption of the palladium complex.

(42) Nishijo, J.; Yonetani, I.; Iwamoto, E.; Tokura, S.; Tagahara, K.; Sugiura, M. *J. Pharm. Sci.* **1990**, *79*, 14–18.
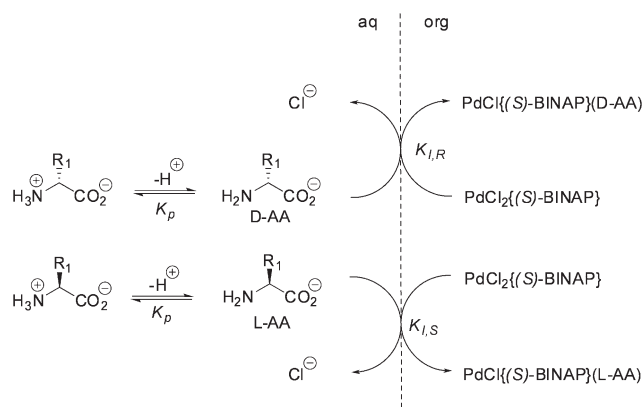
FIGURE 10. Proposed extraction model.

Nonlinear regression of the titration data at 368 nm yielded the following association constants:

$$K_{a(D\text{-}Trp)} = 856 \pm 46$$

$$K_{a(L\text{-}Trp)} = 539 \pm 33$$

The association constant of D-Trp is higher than that of L-Trp. This is in agreement with the preferred extraction of D-Trp in a typical extraction experiment. If the intrinsic selectivity is defined as the ratio of the K_a values, then $\alpha_{int} = 1.6$. This value is somewhat lower than the α_{op} observed in the extraction experiments (vide supra). Nevertheless, the intrinsic selectivity corresponds reasonably well to the operational selectivity.

Proposed Extraction Model. As a preliminary model, based on the results presented here, we propose the interface model for extraction.[34,43] If the pH of the aqueous phase is at 7.0, the amino acid is in equilibrium between the zwitterionic form and the anionic form. No significant physical partition has been observed under these conditions ($P(org/aq) = 0.0$). Hence, complexation of the amino acid with the metal complex is expected to occur at the liquid−liquid interface as a counterion exchange with a chloride, according to the model shown in Figure 10.

According to the model shown in Figure 10, the distribution for D-AA can be defined as:

$$D_{D\text{-}AA} = \frac{[D\text{-}AA]_{org,total}}{[D\text{-}AA]_{aq,total}} = \frac{[PdCl((S)\text{-}BINAP)(D\text{-}AA)]_{org}}{[D\text{-}AA]_{aq}}$$

The equilibria at the interface can be defined as:

$$K_{i,D} = \frac{[PdCl((S)\text{-}BINAP)(D\text{-}AA)]_{org}[Cl^-]_{aq}}{[D\text{-}AA]_{aq}[PdCl_2((S)\text{-}BINAP)]_{org}}$$

In this way the distribution ratio for one enantiomer becomes:

$$D_{D\text{-}AA} = K_{i,D}\frac{[PdCl_2((S)\text{-}BINAP)]_{org}}{[Cl^-]_{aq}}$$

Which gives an operational selectivity that can be expressed as:

$$\alpha_{op} = \frac{D_{D\text{-}AA}}{D_{L\text{-}AA}} = \frac{K_{i,D}}{K_{i,L}}$$

Observations on all of the studied parameters are in line with this model. The pH has an influence on the distribution, but not on the α_{op}, as does the host concentration. Furthermore, the α_{op} corresponds to the intrinsic selectivity and thus to the association constants of the host and the substrate.

Conclusions

In conclusion, we have developed a new method of efficient enantioselective extraction of underivatized amino acids using a readily available host that showed unprecedented selectivities for tryptophan. Bulk membrane transport experiments proved that the system is fully reversible. The intrinsic selectivity determined corresponds well with the operational selectivity. ^{31}P NMR spectroscopy suggests that a more stable complex is formed in the case of the preferred enantiomer. An extraction model featuring a counterion exchange extraction mechanism at the liquid−liquid interface is proposed. Further investigations are underway to expand the scope, increase the selectivity, and elucidate the further details of the extraction mechanism.

Experimental Section

General procedure for extraction experiments: *cis*-[PdCl$_2$-(CH$_3$CN)$_2$]$_2$ and (*S*)-BINAP were dissolved in dcm in equimolar amounts and the mixture was stirred overnight. The solution was diluted to the desired concentration and used in the extraction experiments. The palladium and platinum complexes were synthesized and purified following literature procedures and spectroscopic and analytical data correspond with those reported.[44] The isolated complexes were used as a host, giving similar results compared to the aforementioned in situ generated host in the case of the palladium complex. The racemic amino acid was dissolved in double distilled water (adding 1 equiv of sodium bicarbonate in the case of Trp-Na) or in the appropiate sodium phosphate buffer ($c = 0.100$ M) at a concentration of $c = 2.0$ mM. The two stock solutions were put together in a vial in equivolumous amounts (0.40 mL) and stirred overnight at 6 °C. All extractions were performed at least in duplo. A sample of the aqueous phase was analyzed by using a RP-HPLC equipped with a Crownpak(+) column.

General procedure for titration experiments: Extractions were carried out as indicated above with the aqueous phase at pH 7.0. The amino acid was enantiomerically pure and its concentration was varied between 0.20 and 10.0 mM. The equivolumous amounts of the liquid phases of the extraction were increased to $V = 0.6$ mL. In the ^{31}P NMR titrations, CDCl$_3$ was used as solvent. CDCl$_3$ gave extraction results which compared well with CHCl$_3$.

Bulk-membrane transport experiments: Experiments were done in duplicate. Feeding phase: double distilled water, $V = 5.0$ mL; c(Trp-Na) = 20.0 mM. Transport phase: dichloromethane, $V = 10.0$ mL, c([PdCl$_2$((*S*)-BINAP)]) = 0.5 mM. Receiving phase: 0.100 M HCl (aq). The phases were placed into the U-tube depicted in Figure 4. The tube was placed in a cooled chamber at $T = 6$ °C. The magnetic stirrer was set to 900 rpm.

(43) Pickering, P. J.; Chaudhuri, J. B. *Chem. Eng. Sci.* **1997**, *52*, 377–386.

(44) Celentano, G.; Benincori, T.; Radaelli, S.; Sada, M.; Sannicolò, F. *J. Organomet. Chem.* **2002**, *643*, 424–430.

When a sample of 0.2 mL was taken out of the receiving phase, it was replaced by 0.2 mL of 0.100 M HCl (aq).

Supporting Information Available: Titration graphs and nonlinear regression fits. This material is available free of charge via the Internet at http://pubs.acs.org.