DOI: 10.1002/ejoc.200701101

Resolution of Racemic *N*-Benzyl α-Amino Acids by Liquid-Liquid Extraction: A Practical Method Using a Lipophilic Chiral Cobalt(III) Salen Complex and Mechanistic Studies

Pawel Dzygiel,[a] Toby B. Reeve,[a] Umberto Piarulli,*[b] Martin Krupicka,[c] Igor Tvaroska,[c] and Cesare Gennari*[a]

Keywords: Amino acids / Cobalt / Recognition / Resolution / Salen ligands

The efficient resolution of racemic *N*-benzyl α-amino acids (*N*-Bn-AA) has been achieved by a liquid-liquid extraction process using the lipophilic chiral salen–cobalt(III) complex [Co^{III}(**3**)(OAc)]. As a result of the resolution by extraction, one enantiomer (*S*) of the *N*-benzyl α-amino acid predominated in the aqueous phase, while the other enantiomer (*R*) was driven into the organic phase by complexation to cobalt. The complexed amino acid (*R*) was then quantitatively released by a reductive ($Co^{III} \rightarrow Co^{II}$) counter-extraction with aqueous sodium dithionite or L-ascorbic acid in methanol. The reductive cleavage allowed to recover the [Co^{II}(**3**)] complex in good yield, which could be easily re-oxidized to [Co^{III}(**3**)(OAc)] with air/AcOH and reused with essentially no loss of reactivity and selectivity. Investigation on the nitrogen substitution indicates that the presence of a single benzyl group on the amino acid nitrogen is important to obtain high enantioselectivity in the extraction process. The kinetic vs. thermodynamic nature of the resolution process was also investigated with an enantiomeric exchange experiment, which shows that the liquid-liquid extraction with [Co^{III}(**3**)(OAc)] is an equilibrium process operating under thermodynamic control. In the absence of a suitable crystal structure of the [Co^{III}(**3**)(*N*-Bn-AA)] complexes, computational and spectroscopic studies were used to investigate how the *N*-benzyl α-amino acids are accommodated in the "binding pocket" of the chiral cobalt complex.

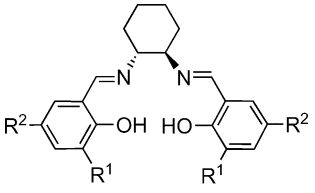
Introduction

Traditional methods for the separation of racemates include crystallization of diastereomeric salts,[1] chiral chromatography[2] and enzymatic resolution.[3] Each of these methods may have certain advantages (efficiency, practicality, economy, etc.) over the others, for a particular chiral compound. However, the selection and optimization of the method may take considerable time and effort for each separate case and the optimized procedure may not always be general for a certain class of chiral compounds. Therefore, there is a continuous need for alternative general strategies, able to resolve racemic mixtures in a cost, time and waste saving manner.[4] A promising methodology relies on the ability of a chiral selector to discriminate between the two enantiomers of a racemate, thus making the enantiomeric separation of racemic mixtures possible, for example, by transport across a chiral membrane,[5] or by liquid-liquid extraction with a chiral host, in the case of hydrophilic substrates.[6] This latter protocol involves the extraction of one enantiomer into an organic phase by selective coordination to a hydrophobic selector, to leave the uncomplexed enantiomer in an aqueous phase. The attraction of this method is that it circumvents the use of excessive handling of solids, which is associated with classical resolution by crystallization of diastereomeric salts; on a production scale this is often the slowest step in the process.

The development of chiral hosts for the enantioselective recognition and separation of α-amino acids and their derivatives is an extensively explored area. Numerous receptors have been proposed over the years, but unfortunately most of them suffer from several drawbacks, like limited efficiency, laborious synthesis, difficult recovery of the selector after release of the complexed enantiomer. A simultaneous binding of both the amino and the carboxylic group is usually required to achieve chiral discrimination.[7] Chiral transition metal complexes, due to their spatial and chemical properties (e.g., Lewis acidity, different charge of the metal ion, availability of different binding sites in a chiral environment), are ideal species for such selection. In fact,

[a] Dipartimento di Chimica Organica e Industriale, Centro di Eccellenza C.I.S.I., Università degli Studi di Milano,
Via G. Venezian, 21, 20133 Milano, Italy
Fax: +39-02-5031-4072
E-mail: cesare.gennari@unimi.it
[b] Dipartimento di Scienze Chimiche e Ambientali, Università degli Studi dell'Insubria,
Via Valleggio, 11, 22100 Como, Italy
Fax: +39-031-238-6449
E-mail: umberto.piarulli@uninsubria.it
[c] Laboratory of Computational Chemistry, Institute of Chemistry, Slovak Academy of Sciences,
Dúbravská cesta 9, 84538 Bratislava, Slovakia
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

transition-metal complexes have been used in several such molecular recognition studies,[8] including the selective crystallization from a racemic mixture of a complex of one enantiomer bound to the chiral selector.[9] Molecular recognition with chiral transition-metal complexes has also been demonstrated in various practical methods for the separation of enantiomers including chiral HPLC,[10] transport across a liquid membrane,[11] micelle-enhanced ultrafiltration[12] and two-aqueous-phase co-micellar systems.[13] In the cases described above, the complexes were usually based on the coordination of ligands derived from amino acids or amines to Cu^{II} ions,[9–13] although enantioselective complexes of other metal ions, such as Co^{II} and Ni^{II}, have also been reported.[8,9a] In one notable case, a chiral cobalt(II) complex derived from ligand **1** (Figure 1), namely [Co^{II}(**1**)], was used in an enantioselective resolution of *N*-benzylalanine.[14] Treating [Co^{II}(**1**)] with 2 equiv. of racemic *N*-Bn-Ala in 5:1 MeOH-H_2O/air, followed by extraction with $CHCl_3$/H_2O, led to enantiomeric excesses of 94% (*R*) and 93% (*S*) for the complexed ([Co^{III}(**1**)(*N*-Bn-Ala)]) and the uncomplexed amino acid, respectively.[15] The major drawback of this approach resided in the necessity to use rather harsh conditions (reduction with $NaBH_4$) to release the complexed amino acid.



(*R*,*R*)-**1** $R^1 = R^2 = H$
(*R*,*R*)-**2** $R^1 = CH_3$, $R^2 = H$
(*R*,*R*)-**3** $R^1 = R^2 = t$Bu

Figure 1. Chiral salen ligands.

It is well known that the interaction of (salen)cobalt(II) complexes with easily ionizable compounds, such as carboxylic or sulfonic acids, in the presence of air causes oxidation of the Co^{II} to a Co^{III} species, with concurrent uptake of the counter-anion ($RCOO^-$ or RSO_3^-). Both the (salen)-cobalt(II) and cobalt(III) complexes are only sparingly soluble in water and readily solubilized in a number of organic solvents, and have found synthetic application in several asymmetric transformations, such as: i) the renowned hydrolytic kinetic resolution (HKR) of epoxides (Co^{3+}), ii) the enantioselective cyclopropanation with alkyl diazoacetate esters (Co^{2+}), iii) the asymmetric borohydride reduction of aromatic ketones (Co^{2+}), iv) the asymmetric Baeyer–Villiger oxidation (Co^{3+}).[16]

We have recently communicated a novel approach to the resolution of racemic *N*-benzyl α-amino acids by liquid-liquid extraction, using the lipophilic chiral (salen)cobalt(III) complex [Co^{III}(**3**)(OAc)] (see Scheme 2), in excellent yield and enantioselectivity.[17] As a result of the resolution by extraction, one enantiomer of the *N*-benzyl α-amino acid predominates in the aqueous phase, while the other enantiomer is driven into the organic phase by complexation to the cobalt center. The complexed amino acid can then be released by a reductive ($Co^{III} \rightarrow Co^{II}$) counter-extraction into an aqueous phase. The original chiral cobalt(III) complex can be regenerated and reused with essentially no loss of reactivity and selectivity. Enantiomerically pure *N*-benzyl α-amino acids have found various important synthetic applications,[18] and can easily be transformed into α-amino acids by hydrogenolysis.[19]

Herein we report a full account on this work, where the scope of the extraction was investigated considering both the lateral chain of the different amino acids and the substitution at the nitrogen atom. In addition, a rationale for the origins of the observed enantioselectivity is proposed based on spectroscopic and computational studies.

Results and Discussion

Extraction of N–Bn–alanine

At the beginning of our investigations, we planned to develop a liquid-liquid extraction process with a lipophilic chiral complex and a hydrophilic racemic substrate. As mentioned in the introduction, [Co^{II}(**1**)] had already been used in the resolution of racemic *N*-benzylalanine by Fujii and co-workers;[14,15] therefore, we began our extraction experiments by mixing an aqueous solution of racemic *N*-benzylalanine (2 equiv.) with a dichloromethane solution of the chiral selector [Co^{II}(**1**)] (1 equiv.). The resulting biphasic solution was mixed thoroughly and the two layers were separated. As expected, the interaction of [Co^{II}(**1**)] with *N*-benzylalanine induced the air oxidation of the metal ion to cobalt(III) with concurrent formation of the cobalt(III) complex [Co^{III}(**1**)(*N*-Bn-Ala)] (Scheme 1). This complex was isolated in nearly quantitative yield (99%) from the organic phase, and was characterized by HRMS (ESI) as well as IR, ^1H and ^{13}C NMR spectroscopy. Unfortunately, no conclusive evidence regarding the diastereomeric composition of the complex could be obtained by the ^1H- and ^{13}C-NMR spectra (see also the Supporting Information). In a control experiment, *N*-benzylalanine was partitioned between water and dichloromethane without the selector, and no *N*-benzylalanine was extracted into the organic phase. The absolute configuration (*S*) and the *ee* value of uncomplexed *N*-Bn-Ala (56% *ee*) were determined by HPLC analysis of the aqueous phase (Table 1, entry 1); reduction of the Co^{III} complex with $NaBH_4$ led to the recovery of (*R*)-*N*-Bn-Ala with a consistent *ee* value.

The experiment described here (a real biphasic liquid–liquid extraction) differs substantially from the conditions used by Fujii et al. (homogeneous 5:1 MeOH/H_2O solution, see ref.[14,15]). The results obtained by Fujii et al. (*ee* = 93–94%) could be nicely reproduced by performing this separation under the original homogeneous conditions.

In order to optimize the enantioselectivity of our extraction protocol, we decided to investigate the role of substituted aromatic rings in the salen ligand. Extraction using the 2,2′-dimethyl-substituted analogue [Co^{II}(**2**)] under the same conditions again led to the formation of a cobalt(III)

Table 1. Extraction of racemic *N*-Bn-Ala using chiral (salen)cobalt(II) and cobalt(III) complexes at 10 °C.

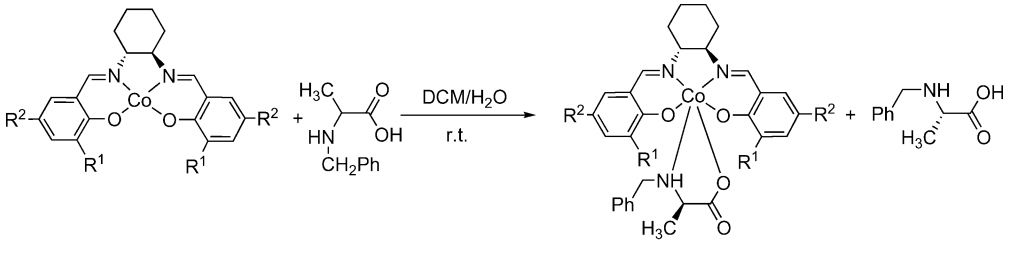
Entry	Host complex	Product	Equiv. extracted	% *ee* (absol. configuration)[a]
1	(*R*,*R*)-[Co^{II}(**1**)]	[Co^{III}(**1**)(*N*-Bn-Ala)]	0.99[b]	56 (*S*)
2	(*R*,*R*)-[Co^{II}(**2**)]	[Co^{III}(**2**)(*N*-Bn-Ala)]	0.96[b]	39 (*S*)
3	(*R*,*R*)-[Co^{II}(**3**)]	–	0[b]	–
4	(*R*,*R*)-[Co^{III}(**1**)(OAc)]	[Co^{III}(**1**)(*N*-Bn-Ala)]	0.92	55 (*S*)
5	(*R*,*R*)-[Co^{III}(**2**)(OAc)]	[Co^{III}(**2**)(*N*-Bn-Ala)]	0.99[c]	54 (*S*)
6	(*R*,*R*)-[Co^{III}(**3**)(OAc)]	[Co^{III}(**3**)(*N*-Bn-Ala)]	**0.99**	**93** (*S*)
7	(*R*,*R*)-[Co^{III}(**3**)(OAc)][d]	[Co^{III}(**3**)(*N*-Bn-Ala)]	**0.98**	**93** (*S*)
8	(*R*,*R*)-[Co^{III}(**3**)(OTf)]	[Co^{III}(**3**)(*N*-Bn-Ala)]	0.92	85 (*S*)
9	(*R*,*R*)-[Co^{III}(**3**)(PF_6)]	[Co^{III}(**3**)(*N*-Bn-Ala)]	0.92	88 (*S*)

[a] Determined on uncomplexed *N*-Bn-Ala by chiral HPLC analysis of the aqueous phase (see Exp. Sect. and Supporting Information). [b] Extractions were run at room temperature. [c] Extraction time of 48 h was necessary, compared to 24 h in all other cases. [d] Second cycle: [Co^{III}(**3**)(OAc)] was obtained from [Co^{III}(**3**)(*N*-Bn-Ala)] after reductive cleavage and re-oxidation (see text and Supporting Information).

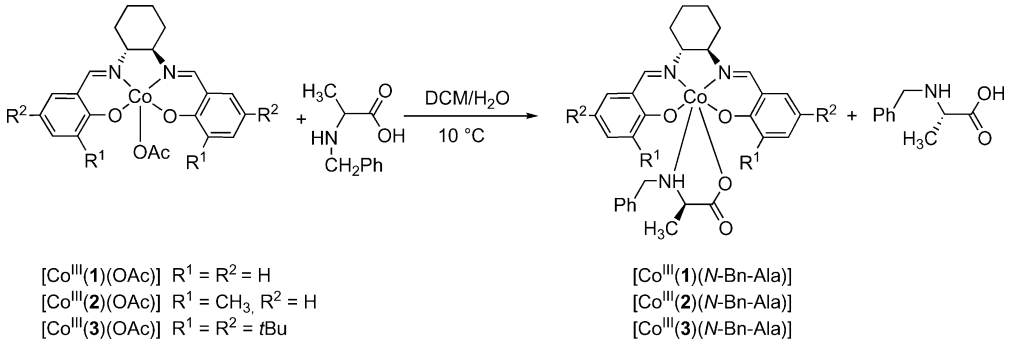
species, [Co^{III}(**2**)(*N*-Bn-Ala)], through air oxidation of the metal (Scheme 1).

In this case, *N*-benzylalanine was also extracted in high yield (96%), but a decreased enantioselectivity (39% *ee* in favor of the *S* enantiomer) was observed in the unbound *N*-benzylalanine (Table 1, entry 2). The 2,2′,4,4′-tetra-*tert*-butyl-substituted chiral ligand **3** can be easily prepared by a high-yielding one-pot method,[16,20] or is commercially available on a large scale along with its cobalt(II) complex [Co^{II}(**3**)]. When complex [Co^{II}(**3**)] was employed in the extraction of *N*-benzylalanine by using our procedure, no oxidation to cobalt(III) occurred, and consequently no complexation/extraction of *N*-benzylalanine was observed in the organic phase (Table 1, entry 3). This is only apparently a disappointing result and is in reality the key for a successful extraction/counter-extraction cycle. In fact, the attempted release of the coordinated amino acid from [Co^{III}(**1**)(*N*-Bn-Ala)] or [Co^{III}(**2**)(*N*-Bn-Ala)] by using different reductive (Na_2SO_3, $Na_2S_2O_4$, $Na_2S_2O_3$, Na_2S) or hydrolytic (HCl, CF_3COOH, CF_3SO_3H) methods constantly failed; in general, no reductive cleavage occurred, or it may have occurred but the (salen)cobalt(II) complexes were not compatible with the released amino acid, i.e. were immediately reoxidized by air to form again the starting cobalt(III) complexes. The only successful reductive cleavage was the previously mentioned reduction with $NaBH_4$,[14] where the amino acid is quantitatively released but the (salen)cobalt(II) complex is mostly destroyed. On the contrary, the [Co^{II}(**3**)] complex is compatible with the released amino acid (is not oxidized by air in the presence of the amino acid), and this allows *in principle* the realization of an extraction/reductive-cleavage cycle. Moreover, the increased stability of [Co^{II}(**3**)] towards oxidation[21] means that the ternary complex [Co^{III}(**3**)(*N*-Bn-AA)] (AA = α-amino acid) should have an increased susceptibility towards reduction back to cobalt(II), thus making the extraction/reductive cleavage cycle potentially easier (see section below: Reductive Cleavage).

At this point, (salen)cobalt(III) acetate complexes [Co^{III}(**1**–**3**)(OAc)] (Scheme 2) were tested in the extraction of *N*-Bn-Ala. These complexes were readily prepared from complexes [Co^{II}(**1**–**3**)] by oxidation with air in the presence of acetic acid.[22] During this investigation, it became apparent that there was a significant correlation between temperature and enantioselectivity, with extractions performed at 10 °C giving the optimal results.[23] For example, the enantioselectivity of extraction of *N*-benzylalanine using complex [Co^{III}(**3**)(OAc)] improved substantially (from 75.0% to 93.0% *ee*) when the temperature was lowered from 25 °C to 10 °C, while extractions run at room temperature (not thermostatted) gave erratic results (*ee* = 83 ± 10%). The results of extraction using one equivalent of complexes [Co^{III}(**1**–**3**)(OAc)] with two equivalents of racemic *N*-benzylalanine, under thermostatted conditions at 10 °C, are summarized in Table 1 (entries 4–6). In all cases, extraction of *N*-benzylalanine was very efficient and the co-



Scheme 1. Resolution of a racemic mixture of *N*-benzylalanine by liquid–liquid extraction using salen–cobalt(II) complexes.



Scheme 2. Resolution of a racemic mixture of *N*-benzylalanine by liquid-liquid extraction using (salen)cobalt(III) complexes.

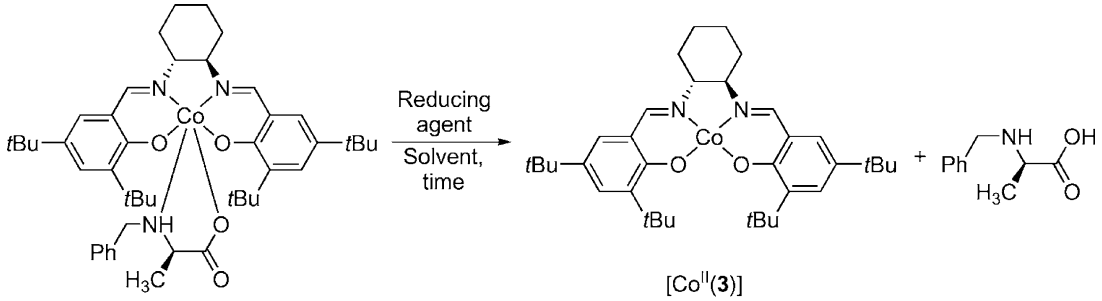
balt (III)/amino acid complexes were isolated form the organic phase in near-quantitative yields. The enantioselectivities with the unsubstituted and methyl-substituted salen ligands were only moderate (*ee* 54–55%, entries 4, 5) and practically equivalent to those obtained with the cobalt(II) derivatives (*ee* 39–56%, entries 1, 2). The striking result came from the *tert*-butyl-substituted complex [Co^{III}(**3**)(OAc)], which gave a remarkable 93% *ee* (entry 6). In addition, similar results (*ee* 85–88%, entries 8, 9) were obtained when the extractions were conducted with cobalt(III) complexes [Co^{III}(**3**)(X)] bearing alternative, less coordinating counterions (X = OTf, PF_6; Table 1, entries 8, 9). The substantial independence of the results from the type of counterion present in the complexes [Co^{III}(**3**)(X)] can be easily understood invoking a thermodynamic control for the entire process (see section below: Mechanistic Studies).

Reductive Cleavage

In order to realize a satisfactory separation protocol by liquid-liquid extraction, an efficient counter-extraction step is necessary. The counter-extraction, besides releasing quantitatively the complexed amino acid, should lead to a substantial recovery of the chiral transition metal selector, to be reused in a subsequent extraction cycle. Initial attempts to replace the amino acid in the cobalt(III) complex with acetate or other anions were not successful, despite the use of a huge excess of the corresponding acid or salt. In the original Fujii protocol,[14] the release of the amino acid was obtained with a $NaBH_4$ reductive cleavage, which gave a quantitative release of the amino acid but, in our hands, a very poor recovery of the reduced [Co^{II}(**3**)] complex (≤10%, Table 2, entry 1). Several other reducing agents were also tested, and are listed in Table 2.

No reaction took place with several water-soluble reducing agents such as ferrous chloride, sodium thiosulfate and sodium sulfide (Table 2, entries 2–4). Sodium dithionite is a versatile and cheap reagent, which has been employed to reduce a variety of organic compounds containing a range of unsaturated oxygen and nitrogen functionalities,[24] and has also been known as a reducing agents for transition metal complexes including cobalt(III) complexes.[25] It has also been reported that dithionite dissociates into the $SO_2^{-\cdot}$ radical anion which is the real reducing agent and that this radical reacts with oxygen.[26]

Table 2. Reductive cleavage of the [Co^{III}(**3**)(*N*-Bn-Ala)] complex with various reducing agents.



Entry	Reducing agent	Solvent	Time	% Recovery of the chiral selector[a]
1	$NaBH_4$ (1 equiv.)	MeOH	24 h	10
2	$Fe^{II}Cl_2$ (10 equiv.)	water/DCM	24 h	–
3	$Na_2S_2O_3$ (10 equiv.)	water/DCM	24 h	–
4	Na_2S (10 equiv.)	water/DCM	24 h	–
5	$Na_2S_2O_4$ (10 equiv.)	water/DCM	16 h	78
6	L-ascorbic acid (1.5 equiv.)	MeOH	30 min	75

[a] Recovered as Co^{II}(**3**) complex.

We found that by vigorously mixing a solution of the [Co^{III}(**3**)(*N*-Bn-Ala)] complex in dichloromethane with an aqueous solution containing excess sodium dithionite (10 equiv.), the bound *N*-benzylalanine was cleaved from the complex and recovered in the aqueous phase in quantitative yield (Table 2, entry 5). The *ee* value of this counter-extracted *N*-benzylalanine was consistent with the expected value based on the *ee* value of the *N*-benzylalanine which remained in the aqueous phase after the initial extraction step. Furthermore, cobalt(III) was reduced back to cobalt(II) during the cleavage of complex [Co^{III}(**3**)(*N*-Bn-Ala)]; after separation, the pure [Co^{II}(**3**)] complex could be isolated from the organic phase by simple evaporation of dichloromethane, trituration in methanol and filtration (78% recovered yield). The drawback of this methodology resides in the excess of sodium dithionite necessary to maximize the recovery of the cobalt(II) complex and in the yield of the recovered [Co^{II}(**3**)] complex, which, besides not being quantitative is not always reproducible.

An alternative reagent which has been used for the reduction of Co^{III} complexes containing ligand **3**,[17b,27] is L-ascorbic acid. In this case, the reaction was performed in methanol using 1.5 equiv. of L-ascorbic acid at room temperature. Under these conditions, [Co^{II}(**3**)] precipitated from methanol and was isolated virtually pure by simple filtration (75% recovered yield; Table 2, entry 6). The methanolic filtrate was then applied to a Dowex 50W-X8 resin, to eliminate dehydroascorbic acid and excess L-ascorbic acid, and eluted with 2 M ammonia to give pure *N*-benzylalanine in quantitative yield. Although the [Co^{II}(**3**)] recovered yield is comparable to the dithionite procedure, this methodology is cleaner and more reproducible.

The recovered [Co^{II}(**3**)] was then reoxidized with air in the presence of acetic acid, and the cobalt(III) acetate thus formed was reused in a second extraction with no loss of activity or enantioselectivity (Table 1, entry 7).

The significance of the substituents on the salen ligand is further apparent when the counter-extraction of complexed *N*-benzylalanine from ternary complex [Co^{III}-(**1**–**3**)(*N*-Bn-Ala)] is examined. Complexes [Co^{III}(**1**)(*N*-Bn-Ala)] and [Co^{III}(**2**)(*N*-Bn-Ala)] were not reduced by either dithionite or L-ascorbic acid (or may have been reduced but were immediately reoxidized by air in the presence of the released amino acid), and the only way to release the complexed amino acid was to use $NaBH_4$ as originally reported by Fujii,[14] albeit with a very poor recovery of the cobalt(II) selector.

In conclusion, the use of complex [Co^{III}(**3**)(OAc)] in the biphasic extraction procedure is particularly attractive over the complexes derived from other salen ligands (**1**, **2**). In fact: i) the enantioselectivities obtained with this complex are definitely higher; ii) [Co^{II}(**3**)] is not oxidized by air in the presence of the amino acid; iii) [Co^{III}(**3**)(*N*-Bn-Ala)] can be reduced back to [Co^{II}(**3**)], in good yield and with quantitative release of the complexed amino acid derivative; iv) an extraction/reductive-cleavage cycle can be realized; v) although one equivalent of chiral selector is necessary to resolve the racemic amino acid, its good recovered yield makes the cycle virtually "catalytic" in the chiral metal complex.

Substrate Scope: N-Benzyl Amino Acids

The scope of this methodology has been investigated with particular interest in adapting the procedure to substrates of varying hydrophilicity relative to *N*-benzylalanine. The results of extractions of a range of racemic *N*-benzyl amino acids (*N*-Bn-AA) using complex [Co^{III}(**3**)(OAc)] are summarized in Table 3. Although all the screening was conducted on a relatively small scale (typically 0.15–0.60 mmol of racemic *N*-benzyl amino acid), larger scale experiments (5.0 mmol) gave consistent and reproducible results.

The extraction of water-soluble *N*-benzylthreonine and *N*-benzylserine proceeded in high yield (94% for *N*-Bn-Thr and 90% for *N*-Bn-Ser) and high *ee* values (96% and 90%), thus leaving uncomplexed (*S*)-*N*-Bn-amino acids in the aqueous phase (Table 3, entries 1 and 2). Extraction of racemic mixtures of *N*-benzylvaline, *N*-benzylleucine and *N*-benzylphenylalanine were also investigated (Table 3, entries 3–5). These substrates, with increased lipophilicity compared to *N*-benzylalanine, are essentially insoluble in both neutral water and dichloromethane. In the cases of *N*-benzylvaline and *N*-benzylleucine, however, all of the substrate

Table 3. Resolution of racemic *N*-Bn amino acids using [Co^{III}(**3**)(OAc)] at 10 °C.

Entry	Substrate	Method[a]	Product	Equiv. extracted	% *ee* (absol. configuration)
1	*N*-Bn-Thr	A	[Co^{III}(**3**)(*N*-Bn-Thr)]	0.94	96 (*R*)[b]
2	*N*-Bn-Ser	A	[Co^{III}(**3**)(*N*-Bn-Ser)]	0.90	90 (*S*)[c]
3	*N*-Bn-Val	A	[Co^{III}(**3**)(*N*-Bn-Val)]	0.98	90 (*S*)[c]
4	*N*-Bn-Leu	A	[Co^{III}(**3**)(*N*-Bn-Leu)]	0.99	99 (*S*)[c]
5	*N*-Bn-Phe	B	[Co^{III}(**3**)(*N*-Bn-Phe)]	0.99	93 (*S*)[c]
6	*N*-Bn-Val	C	[Co^{III}(**3**)(*N*-Bn-Val)]	0.99	94 (*S*)[c]
7	*N*-Bn-Phe	C	[Co^{III}(**3**)(*N*-Bn-Phe)]	0.98	93 (*S*)[c]
8	*N*-Bn-Ala	C	[Co^{III}(**3**)(*N*-Bn-Ala)]	0.98	16 (*S*)[c]
9	*N*-Bn-Ala	C	[Co^{III}(**3**)(*N*-Bn-Ala)]	0.98[d]	66 (*S*)[c]

[a] Method A: biphasic water/dichloromethane extraction. Method B: biphasic water/dichloromethane treatment and recovery of the uncomplexed amino acid by filtration. Method C: stirring a suspension of the racemic *N*-Bn amino acid with a dichloromethane solution of [Co^{III}(**3**)(OAc)] and recovery of the uncomplexed amino acid by filtration. [b] Determined by chiral HPLC analysis on *N*-Bn-Thr, following treatment of [Co^{III}(**3**)(*N*-Bn-Thr)] with aqueous sodium dithionite. [c] Determined on the uncomplexed *N*-Bn amino acid by chiral HPLC analysis. [d] Performed at –10 °C; 72 hours were necessary, compared to 24 h in all other cases.

was drawn into solution to form two clear phases over the course of the extraction. One equivalent was extracted into the dichloromethane phase to form the complexes [Co^{III}-(**3**)(*N*-Bn-AA)], while the second equivalent was dissolved in the aqueous phase. The enantiomeric excesses were determined by HPLC analysis of the aqueous phase and were found to be 90% and 99% in favor of (*S*)-*N*-benzylvaline and (*S*)-*N*-benzylleucine, respectively (Table 3, entries 3, 4). The extraction of *N*-Bn-phenylalanine under the same conditions also proceeded in high yield, with one equivalent of substrate extracted into the organic phase through formation of complex [Co^{III}(**3**)(*N*-Bn-Phe)] (Table 3, entry 5). The unbound substrate, (*S*)-*N*-benzylphenylalanine, remained as a suspension and could be isolated by filtration or dissolved into the aqueous phase by treatment with NaOH. Again, a high *ee* was observed (93% *ee*). In all the above mentioned cases, reductive cleavage of the complexes [Co^{III}(**3**)(*N*-Bn-AA)] with either aqueous sodium dithionite or L-ascorbic acid released the opposite enantiomer in quantitative yield and with consistent *ee* values.

Resolution of racemic *N*-Bn amino acids using only dichloromethane and no water was also attempted (Table 3, entries 6–9, Method C). In all cases, one equivalent of the substrate was complexed by [Co^{III}(**3**)(OAc)] and dissolved into the dichloromethane phase, while the uncomplexed amino acid could be isolated simply by filtration. Enantiomeric excesses of 94% and 93% were observed for the recovered *N*-benzylvaline and *N*-benzylphenylalanine, respectively (Table 3, entries 6, 7). Thus, method C represents a more practical procedure for less hydrophilic amino acids with no loss of enantioselectivity. Conversely and surprisingly, *N*-benzylalanine showed only poor enantioselectivity by this method (Table 3, entry 8), which could be somewhat improved by lowering the temperature, with an *ee* of 66% obtained at –10 °C (Table 3, entry 9).

Substrate Scope: Nitrogen Substitution

Since good to excellent enantioselectivities were obtained in the extraction of all the *N*-benzyl amino acids tested, irrespective of their side-chain (see Table 3), we decided to explore the scope of our method with reference to the nitrogen substitution. On one side, this investigation might lead to the use of more practical α-amino acid derivatives (e.g. carbamates), while on the other side it might shed new light on the role of the α-amino acid nitrogen substituents (compared to the original H and Bn) in promoting the stereoselective formation of the cobalt(III) complexes. Carbamate protected amino acids were initially tested (*t*Boc-Ala and Cbz-Ala), but unfortunately no complex formation was observed and the substrates were completely extracted into the organic phase due to their increased lipophilicity. Since the presence of a basic nitrogen atom is apparently necessary, several racemic *N*-mono- and *N*,*N*-disubstituted alanine and phenylalanine derivatives were prepared and tested in the extraction/resolution experiments using [Co^{III}(**3**)(OAc)]. In addition, unsubstituted phenylalanine and proline were also included in this screening. The extraction protocol was essentially identical to that described above for the *N*-benzyl amino acids: two equivalents of the racemic compound were dissolved or suspended in water (depending on their hydrophilicity) and thoroughly mixed with a dichloromethane solution containing one equivalent of [Co^{III}(**3**)(OAc)] at 10 °C for 24 h. The results are summarized in Table 4.

In the extraction of *N*-unsubstituted (Table 4, entry 1) and *N*-monosubstituted (entries 2, 3, 6, 7, 11) α-amino acids, the corresponding Co^{III} complexes were formed and characterized. In particular, in the case of phenylalanine (entry 1), this amino acid was extracted in high yield (0.98 equiv. extracted into the organic phase) but with poor enantioselectivity (*ee* 10%, by chiral HPLC). A similar result was observed for proline (entry 2): low enantioselectivity (*ee* 10–12%) and a slightly reduced extraction yield (0.85 equiv. extracted into the organic phase). In both cases, the complexes between the amino acid and chiral selector were fully characterized by ^{1}H NMR, ^{13}C-NMR and HR-MS spectroscopy. Additionally, in the NMR spectra of both [Co^{III}(**3**)(Phe)] and [Co^{III}(**3**)(Pro)] two diastereomeric complexes were clearly detectable, with a ratio in complete agreement with the *ee* of the unbound amino acid determined in the aqueous phase. In the extraction of racemic

Table 4. Extraction of racemic *N*-unsubstituted, *N*-mono- and *N*,*N*-disubstituted α-amino acids using [Co^{III}(**3**)(OAc)] at 10 °C.

Entry	Substrate	Product	Equiv. extracted	% *ee* (absol. configuration) uncomplexed[a]	% *ee* (absol. configuration) released[b]
1	Phe[c]	[Co^{III}(**3**)(Phe)]	0.98	10 (*S*)	10 (*R*)
2	Pro[c]	[Co^{III}(**3**)(Pro)]	0.85	10 (*S*)	12 (*R*)
3	*N*-Me-Phe[c]	[Co^{III}(**3**)(*N*-Me-Phe)]	0.97	20 (*S*)	20 (*R*)
4	*N*,*N*-Me_2-Phe[c]	[Co^{III}(**3**)(*N*-Me_2-Phe)]	0.66	50 (*S*)	100 (*R*)
5	*N*-Bn-*N*-Me-Phe[d]	–	0.90	0	–
6	*N*-Bn-Phe[d]	[Co^{III}(**3**)(*N*-Bn-Phe)]	0.99	93 (*S*)	93 (*R*)
7	*N*-Me-Ala[c]	[Co^{III}(**3**)(*N*-Me-Ala)]	0.20	0	0
8	*N*,*N*-Me_2-Ala[c]	–	0.20	0	–
9	*N*-Bn-*N*-Me-Ala[c]	–	0.60	0	–
10	*N*,*N*-Bn_2-Ala[d]	–	1.10	0	–
11	*N*-Bn-Ala[c]	[Co^{III}(**3**)(*N*-Bn-Ala)]	0.99	93 (*S*)	93 (*R*)

[a] Determined on the uncomplexed amino acid by chiral HPLC analysis. [b] Determined by chiral HPLC on the released amino acid after treatment of the cobalt(III) complex with L-ascorbic acid or sodium dithionite. [c] Method A: biphasic water/dichloromethane extraction. [d] Method B: biphasic water/dichloromethane extraction and recovery of the uncomplexed amino acid by filtration.

N-Me-Phe (entry 3), almost one equivalent was transferred into the organic phase with formation of the cobalt complex, and again with a modest enantioselectivity (20% *ee*). In the case of the more hydrophilic *N*-Me-Ala (entry 7), a poor extraction yield (0.20 equiv. extracted into the organic phase) and no enantioselectivity were observed. In all the above mentioned cases, reductive cleavage of the complexes with either aqueous sodium dithionite or L-ascorbic acid released the opposite enantiomer in quantitative yield and with consistent *ee* values.

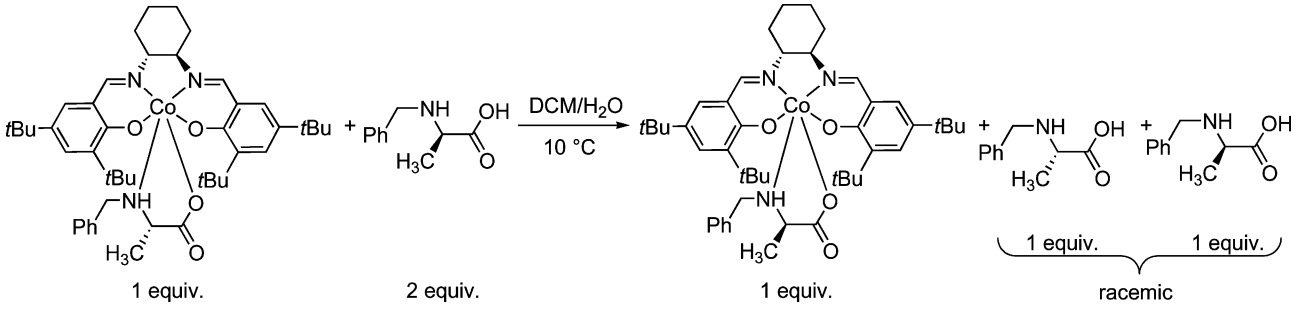
A completely different behavior was observed in the case of *N*,*N*-disubstituted derivatives, namely: *N*-Bn-*N*-Me-Phe (entry 5), *N*,*N*-Me_2-Ala (entry 8), *N*-Bn-*N*-Me-Ala (entry 9), *N*,*N*-Bn_2-Ala (entry 10). In no case was the formation of the complex observed, by analysis (NMR and HR-MS) of the organic phase after extraction and evaporation. All these *N*,*N*-disubstituted derivatives were extracted into the organic layer by simple partitioning between water and dichloromethane.

A strikingly different behavior was observed when *N*,*N*-dimethylphenylalanine was extracted under the same conditions (entry 4). This *N*,*N*-disubstituted amino acid, unlike the others discussed above, was extracted to a lesser extent into the organic phase (0.66 equiv.) leaving the unbound (*S*)-enantiomer in the water phase with a 50% *ee*. The NMR and HR-MS(ESI) analysis of organic residue obtained after evaporation of the solvent revealed that the complex [Co^{III}(**3**)(*N*,*N*-Me_2-Phe)] was actually formed. Cleavage of the complex with aqueous sodium dithionite released enantiomerically pure (*R*)-*N*,*N*-Me_2-Phe (100% *ee*). The smaller *ee* value of the unbound (*S*)-enantiomer is fully consistent with the reduced extraction yield (66%).

In conclusion, these results indicate that the presence of a single benzyl group on the amino acid nitrogen is important to obtain high enantioselectivity in the extraction process. Apparently, benzyl is the most suitable *N*-substituent to be accommodated in the chiral environment of the metal complex (see sections below: Modeling Studies and Spectroscopic Studies). *N*-unsubstituted and *N*-monosubstituted amino acids where the substituent is small (e.g. methyl) react with the chiral selector and form a complex; however, the small substituent does not provide sufficient steric interactions for chiral discrimination. On the contrary, *N*,*N*-disubstituted amino acids are too bulky and this prevents the formation of the complex. The abnormal behavior of *N*,*N*-dimethylphenylalanine remains without a reasonable explanation.

Mechanistic Studies

The kinetic vs. thermodynamic nature of the resolution process was then investigated. To this purpose and for running spectroscopic studies of the complexes in solution (see below the section on Spectroscopic Studies), it was necessary to obtain the two diastereomerically pure complexes, containing either enantiomer of the substrate. Therefore, [Co^{III}(**3**)(OAc)] was separately reacted with either enantiomer of *N*-Bn-Ala, and the two diastereomeric complexes were isolated. In particular, the complex [Co^{III}(**3**)(*R*-*N*-Bn-Ala)] was prepared by extraction of two equivalents of pure (*R*)-*N*-Bn-Ala (the enantiomer preferably bound to the chiral selector) with one equivalent of [Co^{III}(**3**)(OAc)] in water/dichloromethane; the complex was readily formed and isolated from the organic phase in quantitative yield. The other complex, [Co^{III}(**3**)(*S*-*N*-Bn-Ala)], was obtained under forcing conditions: 10 equiv. of (*S*)-*N*-Bn-Ala (the enantiomer preferably unbound to the chiral selector) were reacted for a prolonged time (144 h) with one equivalent of [Co^{III}(**3**)(OAc)] in water/dichloromethane. With the "mismatched" diastereomerically pure complex [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] in hands, the key equilibration experiment was performed. Two equivalents of (*R*)-*N*-Bn-Ala (the enantiomer which is preferentially bound to cobalt) were reacted, in a biphasic extraction protocol, with one quivalent of complex [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] (Scheme 3). After 16 h mixing, the two phases were separated and the enantiomeric excess of *N*-benzylalanine in the aqueous phase determined. A racemic mixture was found in the aqueous phase, indicating that an equilibration of the "mismatched" complex had taken place with formation of the thermodynamically more stable complex [Co^{III}(**3**)(*R*-*N*-Bn-Ala)], containing the "matched" enantiomer. This enantiomeric exchange shows that the liquid-liquid extraction with [Co^{III}(**3**)(OAc)] is an equilibrium process operating under thermodynamic control.[28]



Scheme 3. Transformation of [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] into [Co^{III}(**3**)(*R*-*N*-Bn-Ala)].

Modeling Studies

In order to rationalize the high enantioselectivity observed in the extraction experiments, we tried to obtain the crystal structure of at least one [Co^{III}(**3**)(*N*-Bn-AA)] complex. Unfortunately, despite several attempts and the use of different solvent combinations (e.g. toluene, methanol/water), crystals suitable for X-ray determination could not be grown.

Several crystal structures of cobalt(III) complexes containing ligand **3** have been reported in the literature. In these structures, ligand **3** adopts an equatorial disposition around an octahedral Co^{III} ion, with two apical coordination sites,[29] or in alternative, pseudo square-pyramidal structures are also found, with the cobalt ion located slightly above the plane of the salen ligand in the direction of the axial ligand.[30] Only monodentate ancillary ligands are represented in these structures.

Fujii and co-workers reported two X-ray structures of cobalt(III) complexes containing an achiral α-methylsalen (α-Me-salen) ligand and L-*N*-benzylalanine[31] (Figure 2) or L-*N*-benzylisoleucine.[32]

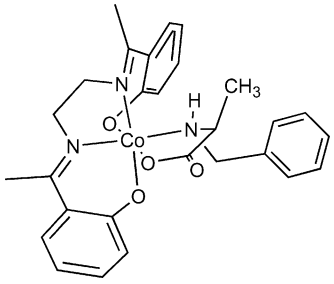


Figure 2. Crystal structure of Co(α-Me-salen)(L-*N*-Bn-Ala).[31]

In both cases, the salen ligand is in a *cis*-β-folded arrangement around the octahedral cobalt ion, being the remaining two *cis* coordination sites occupied by the *N*-benzyl amino acid. The amino acid coordination takes place so that a meridional arrangement of the three oxygen and three nitrogen atoms is obtained (meridional N_3O_3 structure). In addition, the absolute configuration of the additional stereogenic elements present in the molecule (the octahedral cobalt and the tetrasubstituted nitrogen of alanine) can be deduced from that of the coordinated amino acid (*S*), and resulted Λ and *R*, respectively. In summary, the structure reported in Figure 2 can be classified as follows: meridional N_3O_3, Λ (cobalt), *S* (carbon stereocenter), *R* (nitrogen stereocenter). In addition the same authors have also prepared a series of cobalt(III) complexes containing ligand (*S*,*S*)-**1** and an unsubstituted amino acid, and demonstrated that the (*S*,*S*)-enantiomer of ligand **1** imparts a Λ-configuration to the octahedral cobalt complex, irrespective of the absolute configuration of the amino acids and also that the Λ complex of ligand (*S*,*S*)-**1** shows a modest selectivity for the L-amino acid.[33]

Based on the crystal structure of Co^{III}(α-Me-salen)(L-*N*-Bn-Ala),[31] a computer model for complex [Co^{III}(*R*,*R*-**3**)(*N*-Bn-Ala)] was created. This structure was subsequently altered systematically to obtain a full range of possible diastereomers. The absolute configuration of ligand **3** (*R*,*R*) and its coordination mode to cobalt were retained. Modifications consisted of varying (*i*) the *R*/*S* configuration of the amino acid carbon, (*ii*) the *R*/*S* configuration of the amino acid nitrogen (nitrogen bound to cobalt cannot change stereochemistry through pyramidal inversion), (*iii*) the coordination mode to cobalt of the amino acid (with the nitrogen and oxygen atoms either facial or meridional), giving a total of 8 diastereomers. Conformational search and Molecular Mechanics calculations (MM+) were performed within each diastereomer, using HyperChem.[34] Low energy conformers were fully optimized using the DFT method[35] at the B3LYP[36]/LAV3P*[37] level of theory. Relative energies of the optimized conformers of the various diastereomers were used as input for a Boltzmann distribution of their population at the temperature of the experiment (283 K). Results for the three lowest energy structures, with a population of at least 1%, are given in Table 5.

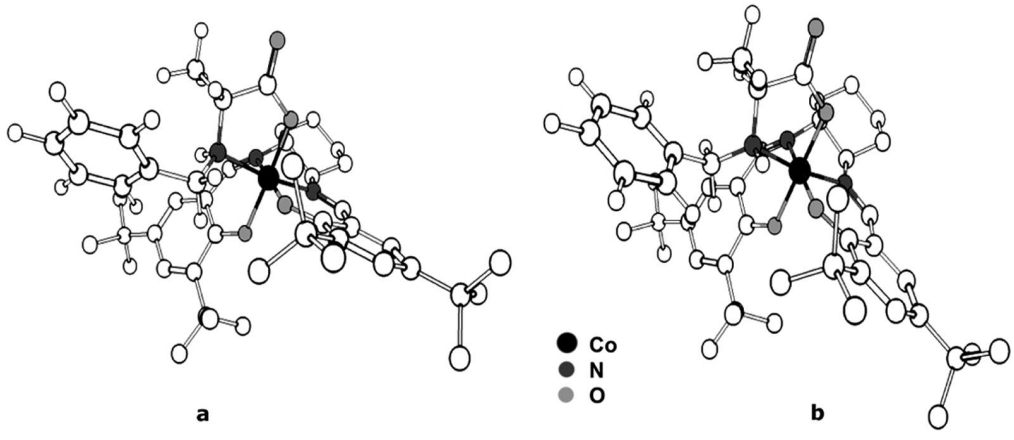


Figure 3. Optimized structures of [Co^{III}(*R*,*R*-**3**)(*N*-Bn-Ala)], hydrogens of ligand **3** omitted for clarity.

Table 5. Relative energies and population of diastereomers of [Co^{III}(*R*,*R*-**3**)(*N*-Bn-Ala)].

Entry	Configuration	Relative energy (kcal/mol)	Boltzmann population (%)	Structure in Figure 3
1	Δ, mer-N_3O_3, *R*, *S*	0.00	94.7	**a**
2	Δ, mer-N_3O_3, *S*, *R*	1.78	4.0	**b**
3	Δ, mer-N_3O_3, *R*, *R*	2.45	1.2	–

The lowest energy structure (structure a in Figure 3) has a meridional N_3O_3, Δ (cobalt), *R* (carbon stereocenter), *S* (nitrogen stereocenter) configuration, imparted by the (*R*,*R*) ligand **3**. The second lowest energy structure (structure b in Figure 3) has a meridional N_3O_3, Δ (cobalt), *S* (carbon stereocenter), *R* (nitrogen stereocenter) configuration.

The Boltzmann distribution reported in Table 5 corresponds to a 96:4 *R*/*S* enantiomeric ratio for the *N*-Bn-Ala carbon stereocenter, which nicely fits with the experimental 93% *ee* value (see Table 1, entries 6, 7). Our mechanistic studies (see the section above) show that the liquid-liquid extraction with [Co^{III}(**3**)(OAc)] is an equilibrium process operating under thermodynamic control, thus justifying the use of a Boltzmann distribution to calculate the diastereomeric ratio.

Spectroscopic Studies

To gain a deeper insight into the structure of the cobalt(III) complexes, the pure complexes [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] and [Co^{III}(**3**)(*R*-*N*-Bn-Ala)] were extensively characterized by NMR, IR, and HR-MS analyses. In particular, examination of the ^{1}H-NMR and COSY spectra of the "matched" complex [Co^{III}(**3**)(*R*-*N*-Bn-Ala)] (Figure 4, see the Supporting Information for the complete set of spectra) revealed that in $CDCl_3$ the PhCH_AH_BNH signals appear at δ = 4.64 ppm (H_A, J_{AB} = 13.6 Hz, J_{CH-NH} = 12.0 Hz) and 4.15 ppm (H_B, J_{AB} = 13.6 Hz). One of these two protons (H_A) is shifted strongly downfield in comparison to the CH_2Ph protons of non complexed (*R*)-*N*-Bn-Ala (AB system, ν_A = 4.25, ν_B = 4.23, J_{AB} = 12.8 Hz), which suggests its co-planarity with one of the aromatic rings of the chiral ligand (deshielding cone). The protons in the *ortho* positions of the *N*-*Bn* moiety are particularly shielded (δ = 6.64 ppm), which is probably due to their orientation with respect to the aromatic rings of the salen ligand.

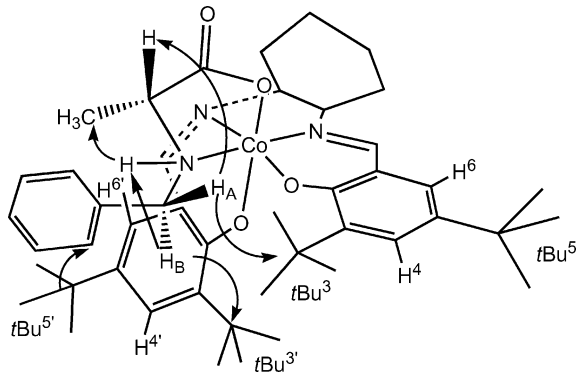


Figure 4. NOE contacts in "matched" complex [Co^{III}(**3**)(*R*-*N*-Bn-Ala)].

Non-bonding interactions in metal complexes are generally well investigated by NMR techniques and in particular by determining the NOE contacts of the relevant protons. The NOESY spectrum of [Co^{III}(**3**)(*R*-*N*-Bn-Ala)] (Figure 4, see the Supporting Information for the complete set of spectra) revealed the that two protons H_A and H_B strongly interact through space with the *tert*-butyl groups of the ligand. In particular, proton H_A gives a cross-peak with *t*Bu^3, and proton H_B with *t*Bu$^{3'}$. A NOE contact is also clearly visible between the protons in the *ortho* positions of the *N*-Bn moiety and the *t*Bu$^{5'}$. Additional NOE contacts are observed between the NH of (*R*)-*N*-Bn-Ala and both the alanine CH_3 group and H_B. On the other hand, the α-C–H of (*R*)-*N*-Bn-Ala is in NOE contact with H_A but not with the N–H. This suggests that the N–H is pointing in the opposite direction with respect to the α-C–H. Therefore, being the nitrogen atom locked in the coordination to cobalt, its absolute configuration can be determined as *S*. The NOESY spectrum also reveals that the alanine CH_3 group does not interact with any part of the salen ligand, nor with the benzyl group of (*R*)-*N*-Bn-Ala. Hence, it looks that (*R*)-*N*-Bn-Ala is nicely accommodated in the "binding pocket" of the chiral cobalt complex. The distances between the protons for which we could detect NOE contacts were measured using the calculated lowest energy conformation for the following complex: (*R*,*R*)-**3** (salen ligand), meridional N_3O_3, Δ (cobalt), *R* (alanine carbon stereocenter), *S* (alanine nitrogen stereocenter), see structure a in Figure 3 and the modeling section above. A good agreement was found between the calculated structure and the experimental data:[38] H_A–H(*t*Bu^3) 2.2 Å (a strong NOE contact is observed), H_B–H(*t*Bu$^{3'}$) 2.3 Å (a strong NOE contact is observed), H_{ortho}–H(*t*Bu$^{5'}$) 3.1 Å (a medium NOE contact is observed), H_{NH}–H_B 2.4 Å (a strong NOE contact is observed), H_{NH}–H_{CH3} 2.4 Å (a strong NOE contact is observed), H_A–$H_{C\alpha}$ 2.3 Å (a strong NOE contact is observed).

The ^{1}H-NMR spectrum of the "mismatched" complex [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] (Figure 5, see the Supporting Information for the complete set of spectra) revealed that in $CDCl_3$ the PhCH_AH_BNH signals appear at δ = 3.29 ppm (H_A) and 3.92 ppm (H_B). One of those two protons (H_A) is strongly upfield in comparison to the CH_2Ph protons of non complexed (*S*)-*N*-Bn-Ala (AB system, ν_A = 4.25, ν_B = 4.23, J_{AB} = 12.8 Hz), which suggests that its position is above/below one of the aromatic rings of the chiral ligand.

The NOESY spectrum of [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] shows that the two protons H_A and H_B of (*S*)-*N*-Bn-Ala fail to interact through space with any group of the chiral ligand (Figure 5). On the other hand, the alanine CH_3 group shows two spatial interactions [which are not seen in the case of the "matched" complex with (*R*)-*N*-Bn-Ala, see discussion above] with the aromatic *ortho* protons of the *N*-benzyl substituent, and with *t*Bu^3 of the salen ligand. An

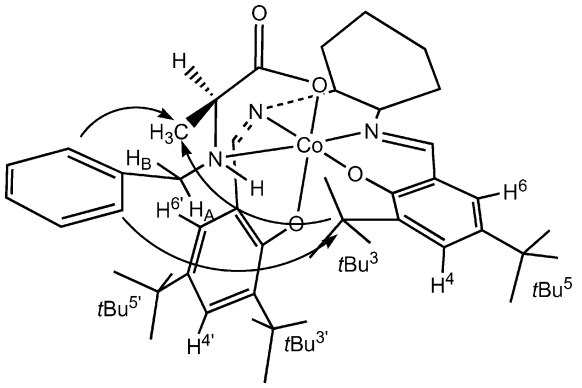


Figure 5. NOE contacts in "mismatched" complex [Co^{III}(**3**)(*S*-*N*-Bn-Ala)].

additional interaction is observed between the aromatic *ortho* protons of the *N*-benzyl substituent and tBu$_3$ of the salen ligand. The distances between the protons for which we could detect NOE contacts were measured using the calculated lowest energy conformation for the following complex: (*R*,*R*)-**3** (salen ligand), meridional N_3O_3, Δ (cobalt), *S* (alanine carbon stereocenter), *R* (alanine nitrogen stereocenter), see structure b in Figure 3 and the modeling section above. A good agreement was found between the calculated structure and the experimental data:[38] H_{ortho}–H_{CH3} 3.1 Å (a medium NOE contact is observed); H_{CH3}–H(tBu^3) 2.8 Å (a medium NOE contact is observed); H_{ortho}–H(tBu^3) 2.4 Å (a strong NOE contact is observed).

From the analysis of the NOE contacts it appears that (*R*)-*N*-Bn-Ala and (*S*)-*N*-Bn-Ala are oriented in a completely different manner in the respective complexes. (*R*)-*N*-Bn-Ala is nicely accommodated in the "binding pocket" of the "matched" chiral cobalt complex and the alanine CH_3 group does not interact with any part of the salen ligand, nor with the *N*-benzyl group. On the contrary, in the "mismatched" chiral cobalt complex, the (*S*)-*N*-Bn-Ala CH_3 group shows spatial interactions with both the *N*-benzyl group and with the tBu group of the salen ligand.

In order to further prove the role of the *N*H-benzyl group in the chiral recognition, we investigated the cobalt(III) complexes of unsubstituted phenylalanine. Racemic phenylalanine is extracted in high yield but with very poor enantioselectivity (*ee* 10%, see Table 4, entry 1). The ^{1}H-NMR and ^{13}C-NMR spectra of the complex obtained from the extraction of the racemic mixture clearly show the presence of two diastereomeric species in an almost equal ratio (55:45). The pure complexes [Co^{III}(**3**)(*R*-Phe)] and [Co^{III}(**3**)-(*S*-Phe)] were then synthesized by separately reacting [Co^{III}(**3**)(OAc)] with either enantiomer of phenylalanine (water/dichloromethane, 24 h). By examination of the NOESY spectra of both these complexes, no spatial interactions between the protons of the bound amino acid and the chiral selector could be observed (see the Supporting Information for the complete set of spectra). This observation further supports the crucial role of the *N*H-benzyl group in the recognition/resolution process (see also the section above: Nitrogen Substitution).

Summary and Conclusions

In conclusion, we have developed a novel approach to the resolution of racemic *N*-benzyl α-amino acids in excellent yields and enantiomeric excesses by using the lipophilic chiral salen–cobalt(III) complex [Co^{III}(**3**)(OAc)]. The complexed amino acid can then be released by a reductive ($Co^{III} \rightarrow Co^{II}$) counter-extraction with aqueous sodium dithionite or L-ascorbic acid. The original chiral cobalt(III) complex can be regenerated and reused with essentially no loss of reactivity and selectivity. Investigation on the nitrogen substitution indicates that the presence of a single benzyl group on the amino acid nitrogen is important to obtain high enantioselectivity in the extraction process. The kinetic vs. thermodynamic nature of the resolution process was also investigated with an enantiomeric exchange experiment, which shows that the liquid-liquid extraction with [Co^{III}(**3**)(OAc)] is an equilibrium process operating under thermodynamic control. In the absence of a suitable crystal structure of the [Co^{III}(**3**)(*N*-Bn-AA)] complexes, computational and spectroscopic studies were used to investigate how the *N*-benzyl α-amino acids are accommodated in the "binding pocket" of the chiral cobalt complex.

Experimental Section

General Procedure for the Extraction of Hydrophilic *N*-Benzyl Amino Acids Using Complex [Co^{III}(3)(OAc)] (Method A): The following procedure for the extraction of *N*-Bn-Ala is typical of hydrophilic *N*-benzyl amino acids. To a solution of the cobalt complex [Co^{III}(**3**)(OAc)] (0.198 g, 0.300 mmol) in dichloromethane (40 mL) at 10 °C in a 100 mL round-bottom flask, was added a precooled (10 °C) solution of racemic *N*-benzylalanine (0.108 g, 2 equiv., 0.600 mmol) in H_2O (30 mL). The biphasic mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separating funnel, the organic phase removed and the aqueous phase washed once with dichloromethane (10 mL). The combined dichloromethane extracts were washed once with H_2O (10 mL) before volatile components were removed under reduced pressure to leave complex [Co^{III}(**3**)(*N*-Bn-Ala)] as a green powder (0.233 g, yield 99%). The aqueous phases were combined and an aliquot removed which was filtered through micropore filters before the *ee* (93%) was determined by chiral HPLC: Chirobiotic R (50 × 4.6 mm) column (9:1 H_2O/CH_3CN); 1.0 mL/min; (*S*) enantiomer, 96.5%, t_R = 1.141 min; (*R*) enantiomer, 3.5%, t_R = 2.754 min. The aqueous phase was evaporated to leave *N*-benzylalanine as a white powder (0.053 g, yield 99%).

General Procedure for the Extraction of Hydrophobic *N*-Benzyl Amino Acids Using Complex [Co^{III}(3)(OAc)] (Method B): The following procedure for the extraction of *N*-Bn-Phe is typical of hydrophobic *N*-benzyl amino acids. To a solution of [Co^{III}(**3**)-(OAc)] complex (0.090 g, 0.150 mmol) in dichloromethane (20 mL) at 10 °C in a 50 mL round-bottom flask, was added H_2O (15 mL) and racemic *N*-benzylphenylalanine (0.076 g, 2 equiv., 0.300 mmol). The mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separating funnel, the organic phase removed and the aqueous phase washed once with dichloromethane (10 mL). The combined dichloromethane extracts were washed once with H_2O (10 mL) before volatiles were removed under reduced pressure to leave the complex [Co^{III}(**3**)(*N*-Bn-Phe)] as a dark green powder (0.127 g, 99% yield). The aqueous phase was filtered

to recover *N*-benzylphenylalanine as a white powder which was washed with dichloromethane (5 mL), water (5 mL) and acetone (5 mL), then dried in vacuo (0.037 g, 99% yield). The *ee* (93%) of recovered *N*-Bn-Phe was determined by chiral HPLC: Chirobiotic R (50 × 4.6 mm) column (9:1 H_2O/CH_3CN); 1.0 mL/min; (*S*) enantiomer, 96.5%, t_R = 1.824 min; (*R*) enantiomer, 3.5%, t_R = 2.776 min.

General Procedure for the Extraction of Hydrophobic *N*-Benzyl Amino Acids Using Complex [Co^{III}(3)(OAc)] without Water (Method C): The following procedure for the extraction of *N*-Bn-Val is typical of hydrophobic *N*-benzyl amino acids. To a stirred, cooled (10 °C) solution of [Co^{III}(**3**)(OAc)] complex (0.045 g, 0.075 mmol) in dichloromethane (10 mL) was added powdered racemic *N*-benzylvaline (0.031 g, 2 equiv., 0.150 mmol). The mixture was stirred at 10 °C for 24 h, then filtered to recover uncomplexed *N*-Bn-Val as a white powder, which was washed with dichloromethane (5 mL) and dried (0.015 g, 99% yield). The *ee* (94%) of recovered *N*-Bn-Val was determined by chiral HPLC: Chirobiotic R (50 × 4.6 mm) column (9:1 H_2O/CH_3CN); 1.0 mL/min; (*S*) enantiomer, 97%, t_R = 1.175 min; (*R*) enantiomer, 3%, t_R = 2.199 min. The dichloromethane phase was concentrated under reduced pressure to leave the complex [Co^{III}(**3**)(*N*-Bn-Val)] as a dark green powder (0.060 g, 99% yield).

General Procedure for the Reductive Cleavage of Complexes [Co^{III}(3)(*N*-Bn-AA)] Using Sodium Dithionite ($Na_2S_2O_4$): The following procedure for the reductive counter-extraction of complex [Co^{III}(**3**)(*N*-Bn-Ala)] is typical. To a solution of [Co^{III}(**3**)(*N*-Bn-Ala)] (0.050 g, 0.064 mmol) in dichloromethane (20 mL) was added a solution of sodium dithionite (0.110 g, 0.640 mmol, 10 equiv.) in H_2O (10 mL). The mixture was stirred vigorously for 5 h (or until the dichloromethane phase became bright red). The organic phase was separated, washed with H_2O (5 mL) and then volatiles were removed under reduced pressure. The resulting residue was suspended in MeOH (5 mL) and filtered to collect [Co^{II}(**3**)] as a red powder (0.029 g, 78% yield). HRMS (ESI): calcd. for $C_{36}H_{52}N_2O_2Co$ 603.3360 $[M]^+$; found: 603.3332. $C_{36}H_{52}CoN_2O_2$ (M_W = 603.76): C 71.62, H 8.68, N 4.64; found: C 71.41, H 8.71, N 4.61. An aliquot was removed from the aqueous phase, filtered through micropore filters and the *ee* (93.5%) of the counter-extracted *N*-benzylalanine was determined by chiral HPLC as described above. The aqueous phase was purified on Dowex 50W-X8 resin (pre-washed with 1 M NaOH, water, 1 M HCl and water). Elution with water and then with 2 M ammonia gave *N*-Bn-Ala as a white powder (11.2 mg, 98% yield).

General Procedure for the Reductive Cleavage of Complexes [Co^{III}(3)(N-Bn-AA)] Using L-Ascorbic Acid: The following procedure for the reductive counter-extraction of complex [Co^{III}(**3**)(*N*-Bn-Ala)] is typical. To a solution of [Co^{III}(**3**)(*N*-Bn-Ala)] (0.90 g, 0.115 mmol) in methanol (15 mL) L-ascorbic acid was added in small portions (0.030 g, 0.172 mmol, 1.5 equiv.) while stirring. The mixture was stirred vigorously for 15 min. A red precipitate was formed, which was filtered, washed with 10 mL methanol and dried under reduced pressure to give [Co^{II}(**3**)] as a red powder (0.052 g, 75% yield). The combined pale yellow methanolic filtrates were purified on Dowex 50W-X8 resin (pre-washed with 1 M NaOH, water, 1 M HCl and water). Elution with water and then with 2 M ammonia gave *N*-Bn-Ala as a white powder (20.4 mg, 99% yield). The *ee* (93.5%) of the counter-extracted *N*-benzylalanine was determined by chiral HPLC as described above.

Computational Procedure: Each diastereomer was subjected to the conformational search using MM+ molecular mechanics in HyperChem.[34] The following three dihedral angles were varied using a conformational search procedure implemented in the program: C(α-amino acid) – N(amino acid) – C(benzyl methylene) – C(*ipso* phenyl), N(amino acid) – C(benzyl methylene) – C(*ipso* phenyl) – C(*ortho* phenyl), and one angle in the cyclohexane ring (with ring constraint). After removing duplicate entries, conformers were fully optimized at the B3LYP[36]/LAV3P*[37] level of theory, using Jaguar[39] with default convergence criteria. Degenerate conformers were discarded. Final electronic energies were then used as input for the Boltzmann population analysis at 283 K. See Supporting Information for more details.

Supporting Information (see also the footnote on the first page of this article): Synthesis of ligands (*R*,*R*)-**1** and (*R*,*R*)-**2**, synthesis of cobalt complexes [Co^{II}(**1**–**2**)] and [Co^{III}(**1**–**3**)(OAc)], synthesis of *N*-substituted amino acids; determination of enantiomeric excesses by HPLC; transformation of [Co^{III}(**3**)(*S*-*N*-Bn-Ala)] into [Co^{III}(**3**)(*R*-*N*-Bn-Ala)]; extraction of *N*-benzyl alanine using complex [Co^{II}(**1**–**2**)] and [Co^{III}(**1**–**2**)(OAc)]; experimental procedures using complex [Co^{III}(**3**)(OAc)], structural characterization and NMR spectra of complexes [Co^{III}(**3**)(*N*-Bn-AA)]; computational methods.

Acknowledgments

We thank the European Commission for financial support and for fellowships to P. D. (Marie Curie postdoctoral fellowship MEIF-CT-2005-006253), T. B. R. (postdoctoral fellowship IHP Network "Enantioselective Recognition" HPRN-CT-2001-00182), M. K. (predoctoral fellowship RTN Network "REVCAT" MRTN-CT-2006-035866). C. G. gratefully acknowledges Merck Research Laboratories for the Merck's Academic Development Program Award.

[1] a) E. L. Eliel, S. H. Wilen, *Stereochemistry of organic compounds*, Wiley, New York, **1994**, p. 374–381; b) D. Kozma (Ed.), *CRC handbook of optical resolution* via *diastereomeric salt formation*, CRC Press, Boca Raton, FL, **2004**, p. 9–40.

[2] a) W. H. Pirkle, B. C. Hamper in *Preparative liquid chromatography, Journal of chromatography library*, vol. 38, Elsevier, Amsterdam, **1987**, p. 238–283; b) S. G. Allenmark, *Chromatographic enantioseparations, methods and applications*, 2nd ed., Ellis Horwood Ltd., Chichester, **1991**, p. 90–141; c) E. Francotte, *J. Chromatogr. A* **1994**, *666*, 565–601; d) S. Ahuja, *Chiral separations: applications and technology*, ACS, New York, **1996**; e) S. Andersson, S. G. Allenmark, *J. Biochem. Bioph. Methods* **2002**, *54*, 11–23; f) G. B. Cox (Ed.), *Preparative Enantioselective Chromatography*, Blackwell Publishing, Oxford, **2005**.

[3] a) J. B. Jones, J. F. Beck in *Applications of biochemical systems in organic chemistry*, part I, Wiley, New York, **1976**, p. 107–401; b) E. Santaniello, P. Ferraboschi, P. Grisenti, A. Manzocchi, *Chem. Rev.* **1992**, *92*, 1071–1140, and references cited therein; c) R. D. Schmid, R. Verger, *Angew. Chem. Int. Ed.* **1998**, *37*, 1608–1633; d) T. Miyzawa, *Amino Acids* **1999**, *16*, 191–213, and references cited therein; e) G. Carrea, S. Riva, *Angew. Chem. Int. Ed.* **2000**, *39*, 2226–2254, and references cited therein; f) A. Liljeblad, L. T. Kanerva, *Tetrahedron* **2006**, *62*, 5831–5854.

[4] For an excellent review covering simulated moving bed (SMB) chromatography and applications of SMB to the separation of enantiomers, see: M. Schulte, J. Strube, *J. Chromatogr. A* **2001**, *906*, 399–416, and references cited therein.

[5] W. H. Pirkle, W. E. Bowen, *Tetrahedron: Asymmetry* **1994**, *5*, 773–776.

[6] a) T. Takeuchi, R. Horikawa, T. Tanimura, *Anal. Chem.* **1984**, *56*, 1152–1155; b) T. Takeuci, R. Horikawa, T. Tanimura, Y.

Kabasawa, *Sep. Sci. Technol.* **1990**, *25*, 941–951; c) H. Nishizawa, K. Tahara, A. Hayashida, Y. Abe, *Anal. Sci.* **1993**, *9*, 611–615; d) H. Tsukube, J. Uenishi, T. Kanatani, H. Itoh, O. Yonemitsu, *Chem. Commun.* **1996**, 477–478; e) H. Tsukube, S. Shinoda, J. Uenishi, T. Kanatani, H. Itoh, M. Shiode, T. Iwachido, O. Yonemitsu, *Inorg. Chem.* **1998**, *37*, 1585–1591; f) S. E. Snyder, J. R. Carey, W. H. Pirkle, *Tetrahedron* **2005**, *61*, 7562–7567, and references cited therein; g) K. Tang, Y. Chen, K. Huang, J. Liu, *Tetrahedron: Asymmetry* **2007**, *18*, 2399–2408.

[7] a) X. X. Zhang, J. S. Bradshaw, R. M. Izatt, *Chem. Rev.* **1997**, *97*, 3313–3361; b) P. Breccia, M. Van Gool, R. Perez-Fernandez, S. Martin-Santamaria, F. Gago, P. Prados, J. De Mendoza, *J. Am. Chem. Soc.* **2003**, *125*, 8270–8284; c) for the use of chiral selectors involving a combination of hydrogen bonding and π–π interactions, see ref.[6f]

[8] a) P. D. Knight, P. Scott, *Coord. Chem. Rev.* **2003**, *242*, 125, and references cited therein; b) H.-J. Kim, R. Asif, D. S. Chung, J.-I. Hong, *Tetrahedron Lett.* **2003**, *44*, 4335–4338; c) H.-J. Kim, W. Kim, A. J. Lough, B. M. Kim, J. Chin, *J. Am. Chem. Soc.* **2005**, *127*, 16776–16777.

[9] a) R. R. Fenton, F. S. Stephens, R. S. Vagg, P. A. Williams, *Inorg. Chim. Acta* **1995**, *236*, 109–115; b) J. Chin, S. S. Lee, K. J. Lee, S. Park, D. H. Kim, *Nature* **1999**, *401*, 254–257.

[10] a) W. Linder, J. N. LePage, G. Davies, D. E. Seitz, B. L. Karger, *J. Chromatogr. A* **1979**, *185*, 323–344; b) E. Gil-Av, A. Tishbee, P. E. Hare, *J. Am. Chem. Soc.* **1980**, *102*, 5115–5117; c) E. Armani, L. Barazzoni, A. Dossena, R. Marchelli, *J. Chromatogr. A* **1988**, *441*, 287–298, and references cited therein; d) T. Arai, H. Koike, K. Hirota, H. Oizumi, *J. Chromatogr. A* **1988**, *448*, 439–444; e) N. Ôi, H. Kitahara, R. Kira, *J. Chromatogr. A* **1992**, *592*, 291–296; f) N. Ôi, H. Kitahara, F. Aoki, *J. Chromatogr. A* **1995**, *707*, 380–383; g) G. Galaverna, R. Corradini, A. Dossena, E. Chiavaro, R. Marchelli, F. Dallavalle, G. Folesani, *J. Chromatogr. A* **1998**, *829*, 101–113.

[11] a) P. Scrimin, U. Tonellato, N. Zanta, *Tetrahedron Lett.* **1988**, *29*, 4967–4970; b) P. Scrimin, P. Tecilla, U. Tonellato, *Tetrahedron* **1995**, *51*, 217–230; c) P. J. Pickering, J. B. Chaudhuri, *J. Membr. Sci.* **1997**, *127*, 115–130; d) B. Baragaña, A. G. Blackburn, P. Breccia, A. P. Davis, J. de Mendoza, J. M. Padrón-Carrillo, P. Prados, J. Riedner, J. G. de Vries, *Chem. Eur. J.* **2002**, *8*, 2931–2936; e) J. D. Clark, B. Han, A. S. Bhown, S. R. Wickramasinghe, *Sep. Purif. Technol.* **2005**, *42*, 201–211.

[12] T. J. M. De Bruin, A. T. M. Marcelis, H. Zuilhof, L. M. Rodenburg, H. A. G. Niederländer, A. Koudijs, P. E. M. Overdevest, A. van der Padt, E. J. R. Sudhölter, *Chirality* **2000**, *12*, 627–636.

[13] A. L. Creagh, B. B. E. Hasenack, A. van der Padt, E. J. R. Sudholter, K. van't Riet, *Biotechnol. Bioeng.* **1994**, *44*, 690–698.

[14] Y. Fujii, M. Matsufuru, A. Saito, S. Tsuchiya, *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2029–2038.

[15] The opposite enantiomer (*S*,*S*) of ligand **1** was actually used in Fujii's work: treating [Co^{II}(*ent*-**1**)] with 2 equiv. of a racemic mixture of *N*-Bn-Ala in 5:1 MeOH/H_2O/air, followed by $CHCl_3$/H_2O extraction, led to enantiomeric excesses of 94% (*S*) and 93% (*R*) for the complexed ([Co^{III}(*ent*-**1**)(*N*-Bn-Ala)]) and the uncomplexed amino acid, respectively (see ref.[14]).

[16] J. R. Larrow, E. N. Jacobsen, *Top. Organomet. Chem.* **2004**, *6*, 123–152, and references cited therein.

[17] a) T. B. Reeve, J.-P. Cros, C. Gennari, U. Piarulli, J. G. de Vries, *Angew. Chem. Int. Ed.* **2006**, *45*, 2449–2453; b) for the extension of this method to the resolution of racemic *N*-benzyl β^3-amino acids, see: P. Dzygiel, C. Monti, U. Piarulli, C. Gennari, *Org. Biomol. Chem.* **2007**, *5*, 3464–3471.

[18] a) For a single-step synthesis of cyclopropanols [EtMgBr, cat. Ti(O*i*Pr)$_4$], see: I. L. Lysenko, O. G. Kulinkovich, *Russ. J. Org. Chem.* **2001**, *37*, 1238–1243; b) for the preparation of enzyme inhibitors, see: C. T. Supuran, A. Scozzafava, *Eur. J. Pharm. Sci.* **2000**, *10*, 67–76; c) for the synthesis of 2,3,4-trisubstituted piperidines, see: S. Laschat, R. Fröhlich, B. Wibbeling, *J. Org. Chem.* **1996**, *61*, 2829–2838; d) for the synthesis of statine and hydroxymethylene dipeptide isosteres, see: A. McCluskey, J. Garner, D. J. Young, S. Caballero, *Tetrahedron Lett.* **2000**, *41*, 8147–8151.

[19] S. N. N. Babu, G. R. Srinivasa, D. C. Santhosh, D. C. Gowda, *J. Chem. Res. Synop.* **2004**, 66–67.

[20] T. V. Hansen, L. Skattebøl, *Tetrahedron Lett.* **2005**, *46*, 3829–3830.

[21] a) A. Nishinaga, K. Tajima, B. Speiser, E. Eichorn, A. Rieker, H. Ohya-Nishiguchi, K. Ishizu, *Chem. Lett.* **1991**, 1403–1406; b) M. Hirotsu, M. Kojima, K. Nakajima, S. Kashino, Y. Yoshikawa, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2549–2557.

[22] S. E. Schaus, B. D. Brandes, J. F. Larrow, M. Tokunage, K. B. Hansen, A. E. Gould, M. E. Furrow, E. N. Jacobsen, *J. Am. Chem. Soc.* **2002**, *124*, 1307–1315.

[23] The variation of other parameters (stirring speed, concentrations, type of reaction vessel) did not significantly affect the extraction results (yields and enantioselectivities). Once the extraction was complete, the enantiomeric excesses did not vary over additional time.

[24] a) M. Balasubramanian, J. G. Keay, in *Encyclopedia of Reagents for Organic Synthesis*, vol. 7 (Ed.: L. Paquette), Wiley, New York, **1995**, pp. 4554–4557; b) L. K. Sydnes, S. Elmi, P. Heggen, B. Holmelid, D. Malthe-Sørensen, *Synlett* **2007**, 1695–1698, and references cited therein.

[25] a) F. K. Yousafzai, R. R. Eady, *J. Biol. Chem.* **2002**, *37*, 34067–34073; b) D. A. Pierce, R. M. Hartshorn, A. M. Sargeson, *J. Chem. Soc. Dalton Trans.* **2002**, 1747–1752; E. V. Kudrik, S. V. Makarov, A. Zahl, R. van Eldik, *Inorg. Chem.* **2003**, *42*, 618–624.

[26] C. Creutz, N. Sutin, *Inorg. Chem.* **1974**, *13*, 2041–2043.

[27] J. F. Larrow, K. E. Hemberger, S. Jasmin, H. Kabir, P. Morel, *Tetrahedron: Asymmetry* **2003**, *14*, 3589–3592.

[28] A similar enantiomeric exchange occurring at high temperatures and leading to the thermodynamically most stable complex was recently described in the case of a ruthenium complex [$RuCl_2$(PP)(NN)], see: W. Baratta, G. Chelucci, E. Herdtweck, S. Magnolia, K. Siega, P. Rigo, *Angew. Chem. Int. Ed.* **2007**, *46*, 7651–7654.

[29] Y. L. Zhang, W. J. Ruan, X. J. Zhao, H. G. Wang, Z. A. Zhu, *Polyhedron* **2003**, *22*, 1535–1545.

[30] a) C. T. Cohen, C. M. Thomas, K. L. Peretti, E. B. Lobkovsky, G. W. Coates, *Dalton Trans.* **2006**, 237–249; b) J. J. Chapman, C. S. Day, M. E. Welker, *Eur. J. Org. Chem.* **2001**, 2273–2282.

[31] Y. Kushi, T. Tada, Y. Fujii, H. Yoneda, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 1834–1839.

[32] Y. Kushi, R. Tamura, M. Kuramoto, T. Yoshizawa, H. Yoneda, Y. Fujii, *J. Chem. Soc., Chem. Commun.* **1978**, 266–267.

[33] Y. Fujii, M. Sano, Y. Nakano, *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2609–2614.

[34] HyperChem™ Professional 7.51, *Hypercube*, Inc., 1115 NW 4th Street, Gainesville, Florida 32601, USA.

[35] a) R. G. Parr, W. Yang, *Density Functional Theory of Atoms and Molecules*, Oxford University Press, New York, **1989**; b) W. Koch, M. C. Holthausen, *A Chemist's Guide to Density Functional Theory*, Wiley-VCH, Weinheim, **2000**.

[36] Becke's three-parameter hybrid method using the LYP (C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785–789) correlation functional: A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648–5652.

[37] P. J. Hay, W. R. Wadt, *J. Chem. Phys.* **1985**, *82*, 270–283.

[38] In the case of NOE contacts involving groups (CH_3, *t*Bu), the shortest measured H–H distance is reported.

[39] *Jaguar*, version 6.0, Schrödinger, LLC, New York, NY, **2005**.

Received: November 21, 2007
Published Online: January 7, 2008

