Efficient resolution of racemic *N*-benzyl β^3-amino acids by iterative liquid–liquid extraction with a chiral (salen)cobalt(III) complex as enantioselective selector†

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The efficient (up to 93% ee) resolution of racemic *N*-benzyl β^3-amino acids has been achieved by an iterative (two cycle) liquid–liquid extraction process using a lipophilic chiral (salen)cobalt(III) complex [Co^{III}(**1**)(OAc)]. As a result of the resolution by extraction, one enantiomer of the *N*-benzyl β^3-amino acid predominated in the aqueous phase, while the other enantiomer was driven into the organic phase by complexation to cobalt. The complexed amino acid was then quantitatively released into an aqueous phase, by a reductive (Co^{III} → Co^{II}) counter-extraction using L-ascorbic acid. The reductive cleavage allowed for the recovery of the cobalt(II) selector in up to 90% yield (easily re-oxidable to Co^{III} with air/AcOH).

1. Introduction

β-Peptides are appealing substrates due to their stability to proteolitic enzymes and their ability to form specific folded structures and mimic α-peptides in peptide–protein and protein–protein interactions.‡[1] For these reasons, the practical production of enantiopure β-amino acids is an important and challenging endeavour for the chemical community. Numerous methodologies for the enantioselective synthesis of these compounds have recently emerged, which often require multistep processes and are usually rather limited in scope.[1b,2] On the other hand, the large-scale separation of racemic mixtures of β-amino acids has been essentially restricted to enzymatic resolution processes (using lipases, aminoacylases, *etc.*),[3] chiral HPLC separation being confined to analytical purposes.[4]

An alternative and promising methodology is the enantiomeric separation of racemic mixtures of hydrophilic substrates by liquid–liquid extraction, where one enantiomer is driven into the organic phase by selective coordination to a hydrophobic selector (*e.g.* a chiral metal complex), leaving the uncomplexed enantiomer in the aqueous phase.[5] One of the attractions of this method is that it circumvents the use of excessive solids handling that is associated with classical resolution *via* crystallization of diastereomeric salts: on production scale this is often the slowest step in the process. Following the above concepts, we have recently disclosed the resolution of racemic *N*-benzyl α-amino acids by liquid–liquid extraction, using a lipophilic chiral (salen)cobalt(III) complex [Co^{III}(**1**)(OAc)] (ligand **1** = *N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamine), in excellent yield and enantioselectivity.[6] As a result of the resolution by extraction, one enantiomer of the *N*-benzyl α-amino acid predominated in the aqueous phase, while the other enantiomer was driven into the organic phase by complexation to cobalt. The complexed amino acid was then released by a reductive (Co^{III} → Co^{II}) counter-extraction into an aqueous phase. The original chiral complex [Co^{III}(**1**)(OAc)] could be restored and reused with essentially no loss of reactivity and selectivity.

2. Results and discussion

Herein, we report on the highly enantioselective separation of racemic *N*-benzyl β^3-amino acids by iterative liquid–liquid extraction using [Co^{III}(**1**)(OAc)]. A racemic mixture of *N*-benzyl β^3-homophenylglycine (*N*-Bn-β^3-hPhg) (2 equiv) was dissolved in water and added to a dichloromethane solution of [Co^{III}(**1**)(OAc)] (1 equiv) at 10 °C (Scheme 1).§ The biphasic mixture was thoroughly stirred, then the two phases were separated. Practically one equivalent (≥0.95 equiv) of *N*-benzyl β^3-homophenylglycine was driven into the organic phase by complexation to the chiral selector, and the unbound *N*-benzyl β^3-homophenylglycine present in the aqueous phase (≥0.95 equiv) was analysed by chiral HPLC, showing a 55% enantiomeric excess (ee) in favour of the *R* enantiomer. By evaporation of the dichloromethane phase, a brown-green solid [Co^{III}(**1**)(*N*-Bn-β^3-hPhg)] was isolated and characterised by FT-IR, HRMS, ^{1}H- and ^{13}C-NMR spectroscopy.

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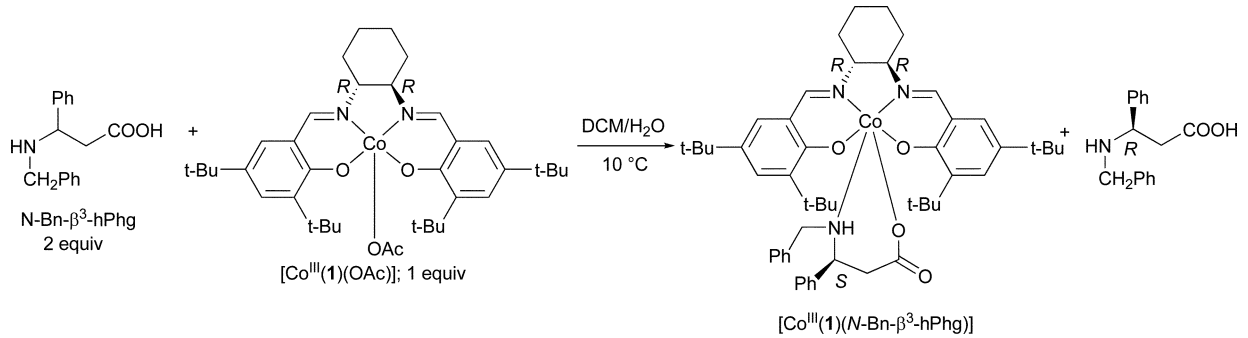
‡ Recently, a β^3-dodecapeptide has been prepared and its secondary and tertiary structures studied. The oligomer forms a 3$_{14}$-helix secondary structure (characteristic of β^3-amino acids) and crystallizes as an octamer which is best described as a pair of tetrameric "hands", see ref. 15. β^3-Amino acids are also efficient enantioselective catalysts for the aldol and Mannich reactions, see ref. 16.

§ We have observed a moderate effect of the temperature in this extraction and the best enantiomeric excesses were obtained operating between 5 and 10 °C (lower temperatures are not possible because of the solidification of the reaction mixture).



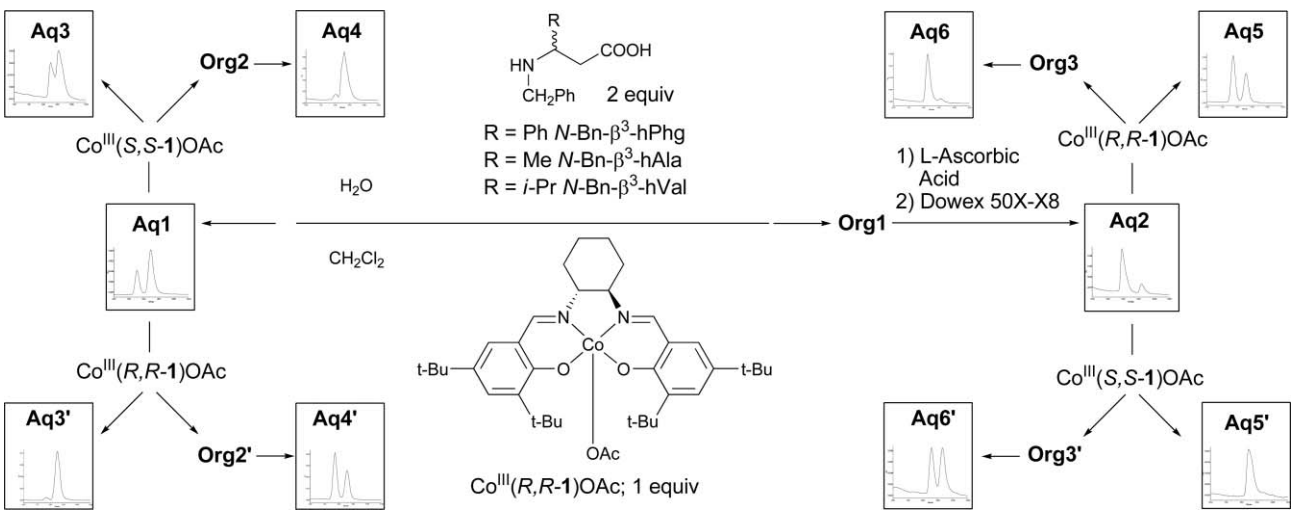
Scheme 1 Resolution of a racemic mixture of *N*-benzyl β³-homophenylglycine by liquid–liquid extraction using the chiral complex [Co^{III}(**1**)(OAc)].

Unfortunately, no conclusive evidence regarding the diastereomeric composition of the complex could be obtained from the ^{1}H- and ^{13}C-NMR spectra (see also the ESI†).

The release of *N*-benzyl β³-homophenylglycine from the cobalt(III) complex was then studied. In the case of *N*-benzyl α-amino acids,[6] a reductive cleavage with aqueous sodium dithionite (10 equiv) had been used. This caused the reduction of cobalt and formation of complex [Co^{II}(**1**)] with concurrent release of the bound *N*-benzyl α-amino acid into the aqueous solution. While this methodology allowed for the quantitative recovery of the released amino acid, the yield of the reduced cobalt complex was generally moderate (≤78%) and not always reproducible. Many other reducing agents were then investigated and it was found that L-ascorbic acid was definitely more efficient and reliable.[7] In fact, reaction of complex [Co^{III}(**1**)(*N*-Bn-β³-hPhg)] with an equimolar amount of L-ascorbic acid in methanol for 15 min caused precipitation of the red [Co^{II}(**1**)] complex, which was isolated by filtration. In this way, up to 90% recovery of the cobalt(II) complex was obtained (the average recovery yields were in the range 85–90%). 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*-benzyl β³-homophenylglycine in quantitative yield and 58% ee (in favour of the *S* enantiomer). From the above discussion it is evident that, while the separation yield is satisfactory (≥95%), the ee's are only moderate. To improve the enantiomeric excess to a preparatively useful level, we decided to iterate the extraction protocol once.

After the first separation step, which was performed using 1 equiv of the chiral selector [Co^{III}(*R*,*R*-**1**)(OAc)] and 2 equiv of the racemic β-amino acids (see above), two alternative paths were followed (Scheme 2). In the first procedure (see Scheme 2, upper part, and Table 1), the aqueous phase (Aq1), which is enriched in the *R* enantiomer (ee 55%), was treated with 0.5 equiv of [Co^{III}(*S*,*S*-**1**)(OAc)] in order to complex the more abundant enantiomer. In this way 0.48 equiv of *N*-Bn-β³-hPhg with 16% ee were found in the aqueous phase (Aq3), while [Co^{III}(*S*,*S*-**1**)(*N*-Bn-β³-hPhg)] was isolated from the organic phase (Org2), which, after cleavage with L-ascorbic acid and resin purification, gave 0.47 equiv of (*R*)-*N*-Bn-β³-hPhg in 90% ee (Aq4).

The organic phase (Org1) was treated with L-ascorbic acid and Dowex 50W-X8, to release the complexed amino acid; in this way 0.95 equiv of *N*-benzyl β³-homophenylglycine (ee = 58% in favour of the *S* enantiomer) was isolated (Aq2). Reaction with 0.5 equiv of [Co^{III}(*R*,*R*-**1**)(OAc)] yielded an aqueous phase (Aq5) containing 0.48 equiv *N*-benzyl-β³-homophenylglycine (16% ee)



Scheme 2 Iterative extraction of *N*-Bn-β-amino acids with [Co^{III}(**1**)OAc]. In the upper part of the scheme the aqueous phases Aq1 and Aq2 are reacted with the enantiomer of the cobalt complex which is more selective for the major enantiomer; in the lower part of the scheme the minor enantiomer of the mixture is preferentially complexed. The HPLC traces in the scheme refer to the iterative extraction of *N*-Bn-β³-hPhg (see the ESI†).

Table 1 Iterative extraction of *N*-Bn-β³-hPhg using [Co^{III}(**1**)OAc]

Aqueous phase[a]	Selector (equiv)	*N*-Bn-β³-hPhg (equiv)	% Extract. yield	% Selector recovery[b]	% ee[c,d] (abs. conf.)
Aq1	[Co(*R*,*R*-**1**)(OAc)] (1.0)	0.97[e]			55 (*R*)
Aq2		0.95[f]	95[f]	90	58 (*S*)
Aq3	[Co(*S*,*S*-**1**)(OAc)] (0.5)	0.48[e]			16 (*R*)
Aq4		0.47[f]	**94**[f]	**82**	**90** (***R***)
Aq5	[Co(*R*,*R*-**1**)(OAc)] (0.5)	0.48[e]			16 (*S*)
Aq6		0.47[f]	**94**[f]	**85**	**90** (***S***)
Aq3′	[Co(*R*,*R*-**1**)(OAc)] (0.5)	0.48[e]			**93** (***R***)
Aq4′		0.47[f]	93[f]	84	10 (*S*)
Aq5′	[Co(*S*,*S*-**1**)(OAc)] (0.5)	0.47[e]			**93** (***S***)
Aq6′		0.46[f]	92[f]	87	5 (*R*)

[a] See Scheme 2 for the origin of the aqueous phases. [b] Based on the yield of [Co^{II}(**1**)] after reductive cleavage with L-ascorbic acid. [c] Determined by chiral HPLC analysis using a Chirobiotic T column. [d] The absolute configuration was determined by comparison of the optical rotation with reported values (see the Experimental section). [e] The equiv were calculated after evaporation of the aqueous phase. [f] The equiv and yield were calculated after cleavage of the cobalt(III) complex with L-ascorbic acid and purification on the Dowex 50W-X8 resin.

and the organic phase (Org3) which, after the usual work up (Aq6), gave 0.47 equiv *N*-benzyl β³-homophenylglycine in 90% ee (in favour of the *S* enantiomer). The aqueous phases Aq3 and Aq5 could be combined (to yield *ca.* 1 equiv of almost *rac*-*N*-benzyl β³-homophenylglycine) and recycled into the feeding phase of the process. In addition, the cobalt selector (in the form of the reduced Co^{2+} species) was recovered (85–90%), oxidised (air/AcOH) and re-used.

Following an alternative path (see Scheme 2, lower part, and Table 1), the aqueous phase (Aq1) was extracted with 0.5 equiv of [Co^{III}(*R*,*R*-**1**)(OAc)]; in this way, the minor component of the mixture was complexed and the *R*-enantiomer (0.48 equiv) was obtained uncomplexed in the aqueous phase (Aq3′) in 93% ee. The organic phase (Org2′), treated in sequence with L-ascorbic acid and Dowex W50-X8, yielded 0.47 equiv of *N*-Bn-β³-hPhg in 10% ee in favour of the *S*-enantiomer (Aq4′). The aqueous phase (Aq2) was subjected to extraction with [Co^{III}(*S*,*S*-**1**)(OAc)]: the aqueous phase (Aq5′) gave 0.47 equiv of *N*-Bn-β³-hPhg in 93% ee for the *S*-enantiomer, while the organic phase (Org3′), after the usual treatment, gave 0.46 equiv of *N*-Bn-β³-hPhg in 5% ee (Aq6′). The organic phases Org2′ and Org3′ could be combined, treated in sequence with L-ascorbic acid and Dowex W50-X8, to yield approximately 1 equiv of almost *rac*-*N*-benzyl β³-homophenylglycine, which could be recycled into the feeding phase of the process.

Due to the high extraction yields of the complexation steps and release of *N*-Bn-β³-hPhg from the cobalt(III) complexes in both schemes, the two methodologies are essentially equivalent in terms of yields, separation efficiency and ease of operation.

The scope of the resolution by extraction, following the procedure reported in Scheme 2, was then investigated with two other *N*-benzyl β³-amino acids: *N*-benzyl β³-homoalanine (*N*-Bn-β³-hAla, Table 2) and *N*-benzyl β³-homovaline (*N*-Bn-β³-hVal, Table 3). The two cycle approach resulted in an efficient resolution protocol (90% ee for *N*-Bn-β³-hAla; 93% ee for *N*-Bn-β³-hVal), although the extraction yields in both cases were poorer than those reported in Table 1 for *N*-Bn-β³-hPhg.

Conclusions

We have performed an efficient resolution of racemic *N*-benzyl β³-amino acids by an iterative (two cycle) liquid–liquid extraction using the chiral complex [Co^{III}(**1**)(OAc)]. As a result of the first resolution by extraction, one enantiomer predominated in the aqueous phase, while the other enantiomer was driven into the organic phase by complexation to cobalt. Two alternative procedures may be followed for improving the moderate ee's resulting from the first extraction: (a) enrichment of the major enantiomer through a second complexation to the appropriate chiral selector, or (b) depletion of the minor enantiomer through a second complexation

Table 2 Iterative extraction of *N*-Bn-β³-hAla using [Co^{III}(**1**)OAc]

Aqueous phase[a]	Selector (equiv)	*N*-Bn-β³-hAla (equiv)	% Extract. yield	% Selector recovery[b]	% ee[c,d] (abs. conf.)
Aq1	[Co(*R*,*R*-**1**)(OAc)] (1.0)	1.41[e]			25 (*S*)
Aq2		0.59[f]	59[f]	84	75 (*R*)
Aq3	[Co(*S*,*S*-**1**)(OAc)] (0.7)	0.99[e]			5 (*S*)
Aq4		0.31[f]	**44**[f]	**85**	**88** (***S***)
Aq5	[Co(*R*,*R*-**1**)(OAc)] (0.3)	0.38[e]			65 (*R*)
Aq6		0.17[f]	**58**[f]	**83**	**90** (***R***)
Aq3′	[Co(*R*,*R*-**1**)(OAc)] (0.7)	0.97[e]			36 (*S*)
Aq4′		0.34[f]	49[f]	82	75 (*R*)
Aq5′	[Co(*S*,*S*-**1**)(OAc)] (0.3)	0.39[e]			**83** (***R***)
Aq6′		0.16[f]	52[f]	84	51 (*S*)

[a] See Scheme 2 for the origin of the aqueous phases. [b] Based on the yield of [Co^{II}(**1**)] after reductive cleavage with L-ascorbic acid. [c] Determined by chiral HPLC analysis using a Chirobiotic T column. [d] The absolute configuration was determined by comparison of the optical rotation with reported values (see the Experimental section). [e] The equiv were calculated after evaporation of the aqueous phase. [f] The equiv and yield were calculated after cleavage of the cobalt(III) complex with L-ascorbic acid and purification on the Dowex 50W-X8 resin.

Table 3 Iterative extraction of *N*-Bn-β³-hVal using [Co^{III}(**1**)OAc]

Aqueous phase[a]	Selector (equiv)	*N*-Bn-β³-hVal (equiv)	% Extract. yield	% Selector recovery[b]	% ee[c,d]
Aq1	[Co(*R*,*R*-**1**)(OAc)] (1.0)	1.25[e]			36 (+)
Aq2		0.66[f]	66[f]	80	76 (−)
Aq3	[Co(*S*,*S*-**1**)(OAc)] (0.63)	0.72[e]			10 (−)
Aq4		0.43[f]	**68**[f]	**83**	**90 (+)**
Aq5	[Co(*R*,*R*-**1**)(OAc)] (0.33)	0.42[e]			64 (−)
Aq6		0.21[f]	**64**[f]	**80**	**93 (−)**
Aq3′	[Co(*R*,*R*-**1**)(OAc)] (0.63)	0.74[e]			80 (+)
Aq4′		0.42[f]	67[f]	82	40 (−)
Aq5′	[Co(*S*,*S*-**1**)(OAc)] (0.33)	0.40[e]			**92 (−)**
Aq6′		0.20[f]	61[f]	85	5 (−)

[a] See Scheme 2 for the origin of the aqueous phases. [b] Based on the yield of [Co^{II}(**1**)] after reductive cleavage with L-ascorbic acid. [c] Determined by chiral HPLC analysis using Chirobiotic T column. [d] The sign of the optical rotation is reported in brackets. [e] The equiv were calculated after evaporation of the aqueous phase. [f] The equiv and yield were calculated after cleavage of the cobalt(III) complex with L-ascorbic acid and purification on the Dowex 50W-X8 resin.

to the appropriate selector. The complexed amino acid were quantitatively released by a reductive (Co^{III} → Co^{II}) cleavage, using L-ascorbic acid as the reducing agent. The release of the complexed *N*-Bn-β³-AA by reductive cleavage allowed for the recovery of the cobalt(II) selector in up to 90% yield (easily re-oxidable to Co^{III} with air/AcOH), thus rendering this process virtually catalytic in the chiral cobalt complex. We are also actively investigating the origin of the observed selectivity which, upon preliminary inspection, appears to be governed by the relative thermodynamic stability of the diastereomeric cobalt(III) *N*-benzyl β³-amino acid complexes, rather than by a kinetic preference for their formation.

Experimental

General remarks

Proton NMR spectra were recorded on a spectrometer operating at 400.13 MHz. Proton chemical shifts are reported in ppm (δ) with the solvent reference relative to tetramethylsilane (TMS) employed as the internal standard (D_2O δ 4.70 ppm, $CDCl_3$ δ 7.26 ppm). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, dd = doublet of doublets. Carbon NMR spectra were recorded on a 400 spectrometer operating at 100.56 MHz, with complete proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ 77.0). Infrared spectra were recorded on a standard FT-IR; peaks are reported in cm^{-1}. Optical rotation values were measured on an automatic polarimeter with a 1 dm cell at the sodium D line. HPLC determination of the enantiomeric excesses was carried out on a Waters 515 HPLC machine equipped with a Waters 996 PAD diode array detector (detection wavelength 254 nm) using a chiral stationary phase (ASTEC Chirobiotic T chiral column, 250 × 4.6 mm). High resolution mass spectra (HRMS) were performed on a hybrid quadrupole time of flight mass spectrometer equipped with an ESI ion source. A Reserpine solution 100 pg μl^{-1} (about 100 count s^{-1}), 0.1% HCOOH–CH_3CN 1 : 1, was used as reference compound (Lock Mass). All commercially available reagents were used as received. Benzylamine was distilled under nitrogen immediately prior to use, and pyridine was dried by distillation over CaH_2. (*R*,*R*)-*N*,*N*′-Bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II), (*R*,*R*)-[Co^{II}(**1**)], and (*S*,*S*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II), (*S*,*S*)-[Co^{II}(**1**)], were purchased from Sigma-Aldrich. Extractions were performed using HPLC grade water and dichloromethane.

Synthesis of (*rac*)-3-benzylamino-3-phenylpropionic acid (*N*-benzyl-β³-homophenylglycine, *N*-Bn-β³-hPhg)

(*rac*)-*N*-Benzyl-β³-homophenylglycine was prepared according to the method reported by Nagao *et al.* as follows.[8] Cinnamic acid (2.96 g, 20 mmol) and benzylamine (2.14 g, 20 mmol) were added to dry pyridine (15 mL). The mixture was heated to 120–130 °C under nitrogen and stirred for 1 h; the solvent was evaporated under reduced pressure to afford a crude crystalline product. Re-crystallization from methanol–water gave the desired product (3.07 g, yield: 60%). ^{1}H-NMR (D_2O–NaOH): δ = 7.45–7.12 (m, 10H, Ar-*H*), 3.96 (t, J = 7.4 Hz, 1H, C*H*N), 3.52–3.44 (AB system, J_{AB} = 13.2 Hz, δ_A = 3.50, δ_B = 3.46, 2H, CH_2Ph), 2.65 (dd, J = 14.2 Hz, J = 6.7 Hz, 1H, CH*H*), 2.45 (dd, J = 14.2 Hz, J = 8.1 Hz, 1H, C*H*H) ppm. ^{13}C-NMR (D_2O–NaOH): δ = 180.4 (*C*O), 142.7 (Ar-*C*), 139.9 (Ar-*C*), 129.3 (Ar-*C*H), 129.3 (Ar-*C*H), 129.2 (Ar-*C*H), 128.4 (Ar-*C*H), 128.3 (Ar-*C*H), 127.9 (Ar-*C*H), 60.1 (*C*HN), 50.9 (*C*H_2N), 45.8 (*C*H_2) ppm. FT-IR (Nujol): ν = 2854, 1469, 1375 cm^{-1}. HRMS (ESI): calcd. for $C_{16}H_{18}N_1O_2$ 256.1332 [M + H]$^+$; found: 256.1331. HPLC conditions: Chirobiotic T (250 × 4.6 mm) 90 : 10 CH_3OH–H_2O, 1.0 mL min^{-1}, *S* enantiomer t_r = 8.2 min, *R* enantiomer t_r = 9.2 min.

Synthesis of (*rac*)-3-benzylaminobutyric acid (*N*-benzyl-β³-homoalanine, *N*-Bn-β³-hAla)

(*rac*)-*N*-Benzyl-β³-homoalanine was prepared according to the method reported by Zilkha and Rivlin as follows.[9] To a solution of crotonic acid (0.43 g, 5.0 mmol) in dry pyridine (15 mL) was added benzylamine (0.53 g, 5 mmol). The mixture was heated at 120–130 °C under an argon atmosphere for 1.5 h. On cooling, white (*rac*)-*N*-benzyl-β³-homoalanine crystallized, which was filtered and washed with acetone to give the desired product. Yield: 0.83 g (86%). ^{1}H-NMR (D_2O): δ = 7.46–7.38 (m, 5H, Ar-*H*), 4.24–4.14 (AB system, J_{AB} = 13.2 Hz, δ_A = 4.21, δ_B = 4.15, 2H, CH_2Ph), 3.55–3.43 (m, 1H, C*H*N), 2.49 (d, J = 6.5 Hz, 2H, CH_2), 1.32

(d, $J = 6.6$ Hz, 3H, CH_3) ppm. ^{13}C-NMR (D_2O–NaOH): $\delta =$ 178.3 (*C*O), 131.6 (Ar-*C*), 129.9 (Ar-*C*H), 129.8 (Ar-*C*H), 129.7 (Ar-*C*H), 51.9 (*C*HN), 48.4 (*C*H_2N), 39.2 (*C*H_2), 16.3 (*C*H_3) ppm. FT-IR (Nujol): $\nu = 2921$, 1457, 1376 cm^{-1}. HRMS (ESI): calcd. for $C_{11}H_{16}N_1O_2$ 194.1176 [M + H]$^+$; found: 194.1173. HPLC conditions: Chirobiotic T (250 × 4.6 mm) CH_3OH, 0.4 mL min^{-1}, *R* enantiomer $t_r = 34.6$ min, *S* enantiomer $t_r = 38.6$ min.

Synthesis of (*rac*)-3-benzylamino-4-methylpentanoic acid (*N*-benzyl-β^3-homovaline, *N*-Bn-β^3-hVal)

N-Benzyl-β^3-homovaline was prepared according to the method reported by Williams *et al.* as follows.[10] Ethyl isobutyrylacetate (400 μL, 2.4 mmol, 1.0 equiv) was dissolved in benzene (4 mL) and treated with benzylamine (276 μL, 2.4 mmol, 1.0 equiv). The mixture was heated at reflux under nitrogen with azeotropic water removal (Dean–Stark apparatus) for 8 h. The solvent was then evaporated, the residue dissolved in acetic acid (4 mL) and treated with sodium cyanoborohydride (702 mg, 10.8 mmol, 4.5 equiv). The mixture was stirred at room temperature for 2 h. The solvent was then evaporated, the residue taken up in ether and washed with 1 N NaOH and brine. The ethereal solution was dried over Na_2SO_4 and evaporated to give *rac*-ethyl-3-benzylamino-4-methylpentanoate as an oil (506 mg, 82% yield over two steps). This compound was dissolved in 2 M KOH–methanol (1.8 mL, 3.6 mmol, 1.5 equiv) and the mixture was stirred overnight at room temperature. The resulting mixture was acidified to pH 5 with HCl–MeOH (about 1.8 mL used). Diethyl ether was added and the precipitate was filtered off. The filtrate was evaporated to afford *N*-benzyl-β^3-homovaline as a hygroscopic solid. The solid was dissolved in water and purified on a Dowex 50W-X8 resin, eluting with 2 M NH_4OH, to give pure *N*-benzyl-β^3-homovaline (382 mg, 84% yield). ^{1}H-NMR (D_2O): $\delta = 7.42$ (s, 5H, Ar-*H*), 4.20 (s, 2H, CH_2Ph), 3.30–3.25 (m, 1H, C*H*N), 2.51 (dd, $J =$ 16.8 Hz, $J = 4.8$ Hz, 1H, CH*H*), 2.36 (dd, $J = 16.8$ Hz, $J =$ 7.6 Hz, 1H, C*H*H), 2.13–2.08 (m, 1H, C*H*Me$_2$), 0.91 (d, $J =$ 6.8 Hz, 3H, CH_3), 0.87 (d, $J = 6.8$ Hz, 3H, CH_3) ppm. ^{13}C-NMR (D_2O): $\delta = 178.7$ (*C*O), 131.3 (Ar-*C*), 129.6 (Ar-*C*H), 129.5 (Ar-*C*H), 129.3 (Ar-*C*H), 60.5 (*C*HN), 48.4 (*C*H_2N), 32.4 (*C*H_2), 28.2 (*C*HMe$_2$), 18.3 (*C*H_3), 15.8 (*C*H_3) ppm. FT-IR (Nujol): $\nu = 1632$, 1562 cm^{-1}. HRMS (ESI): calcd. for $C_{13}H_{19}N_1O_2Na$ 244.1308 [M + Na]$^+$; found: 244.1306. HPLC conditions: Chirobiotic T (250 × 4.6 mm) 90 : 10 CH_3OH–H_2O, 1.0 mL min^{-1}, (+) enantiomer $t_r =$ 7.7 min, (−) enantiomer $t_r = 9.1$ min.

Synthesis of (*R*,*R*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(III) acetate, (*R*,*R*)-[Co^{III}(**1**)(OAc)]

(*R*,*R*)-[Co^{III}(**1**)(OAc)] was prepared according to a literature procedure, with slight modifications.[11] Glacial acetic acid (0.35 mL, 6.0 mmol) was added to a stirred solution of [Co^{II}(**1**)] (180.9 mg, 0.30 mmol) in toluene (20 mL) under air. An immediate color change from bright red to brown was observed. The solution was stirred for a further 30 min before volatiles were removed under reduced pressure to leave (*R*,*R*)-[Co^{III}(**1**)(OAc)] as a brown powder, which was used directly in subsequent extractions without further purification. Yield: 194.6 mg (98%). Spectroscopic data were consistent with those previously reported.[12]

Synthesis of (*S*,*S*)-*N*,*N*′-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediaminocobalt(III) acetate, (*S*,*S*)-[Co^{III}(**1**)(OAc)]

See the procedure above as for the synthesis of (*R*,*R*)-[Co^{III}(**1**)(OAc)].

Procedure for the extraction of *N*-Bn-β^3-hPhg

To a solution of (*R*,*R*)-[Co^{III}(**1**)(OAc)] (99.3 mg, 0.15 mmol) in dichloromethane (40 mL) at 10 °C in a 250 mL round-bottom flask, was added a pre-cooled (10 °C) solution of racemic *N*-Bn-β^3-hPhg (76.8 mg, 0.30 mmol, 2 equiv) in H_2O (80 mL). The biphasic mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separatory funnel, the organic phase (Org1) removed and the aqueous phase washed once with dichloromethane (10 mL). A small aliquot (*ca.* 100 μL) of the aqueous phase was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase (Aq1) was then evaporated under reduced pressure to give *N*-Bn-β^3-hPhg as a white powder (37.2 mg, yield: 97%, 55% ee in favour of *R* enantiomer). The combined dichloromethane extracts were washed once with H_2O (10 mL). Volatiles were removed under reduced pressure to leave the complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β^3-hPhg)] as a green-brown powder. This was dissolved in methanol (10 mL) and ascorbic acid (26.4 mg, 0.15 mmol) was added.[7] The mixture was stirred vigorously for 15 min until a red precipitate was formed ([Co^{II}(**1**)]). The red product was filtered, washed with methanol (10 mL) and dried under vacuum to obtain [Co^{II}(**1**)] as a red powder (81.4 mg, yield: 90%). HRMS (ESI): calcd. for $C_{36}H_{52}N_2O_2Co$ 603.3360 [M]$^+$; found: 603.3332. The combined pale yellow methanolic filtrates were purified using Dowex 50W-X8 resin (pre-washed with 1 M NaOH, water, 1 M HCl and water) and the amino acid was eluted using 2 M ammonia to obtain *N*-Bn-β^3-hPhg as white powder (36.5 mg, yield: 95%, 58% ee in favour of *S* enantiomer).

Procedure for the iterative extraction of *N*-Bn-β^3-hPhg (Scheme 2, upper part)

To a solution of (*R*,*R*)-[Co^{III}(**1**)(OAc)] (49.6 mg, 0.075 mmol) in dichloromethane (20 mL) at 10 °C in a 100 mL round-bottom flask, was added a pre-cooled (10 °C) solution of racemic *N*-Bn-β^3-hPhg (38.6 mg, 0.15 mmol, 2 equiv) in H_2O (40 mL). The biphasic mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separatory funnel, the organic phase (Org1) removed and the aqueous phase (Aq1) washed once with dichloromethane (10 mL). The combined dichloromethane extracts were washed once with H_2O (10 mL).

Processing of the aqueous phase (Aq1). The aqueous phases were combined, concentrated to 40 mL and extracted again with (*S*,*S*)-[Co^{III}(**1**)(OAc)] (24.8 mg, 0.0375 mmol, 0.5 equiv) in dichloromethane (20 mL) for 24 h at 10 °C. The biphasic mixture was transferred to a separatory funnel, the organic phase (Org2) removed and the aqueous phase (Aq3) washed once with dichloromethane (10 mL). The organic phase (Org2) was washed with water (10 mL). The aqueous phases were combined and a small aliquot (*ca.* 100 μl) was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase was evaporated to dryness to obtain

N-Bn-β³-hPhg as white powder (9.1 mg, yield: 95%, 16% ee in favour of *R* enantiomer). The organic phase (Org2) was evaporated to dryness under vacuum to yield a brown-green powder. This was dissolved in methanol (10 mL) and treated with ascorbic acid (6.6 mg, 0.0375 mmol) for 15 min, until a red precipitate of [Co^{II}(**1**)] was formed, which was filtered, washed with 10 mL methanol and dried (18.2 mg, yield: 82%). The combined pale yellow methanolic filtrates were purified on Dowex 50W-X8 resin to obtain (*R*)-*N*-Bn-β³-hPhg as a white powder. Yield: 9.0 mg (94%).

Processing of the organic phase (Org1). Volatiles were removed under reduced pressure to leave the complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hPhg)] as a green-brown powder. This was dissolved in methanol (10 mL) and ascorbic acid (13.2 mg, 0.075 mmol) was added. The mixture was stirred vigorously for 15 min until a red precipitate of [Co^{II}(**1**)] was formed, which was filtered, washed with 10 mL methanol and dried (40.7 mg, yield: 90%). The combined pale yellow methanolic filtrates were purified on a Dowex 50W-X8 resin. The white crude solid (18.2 mg, yield: 94%) was dissolved in water (40 mL, Aq2), filtered and submitted to a second extraction with (*R*,*R*)-[Co^{III}(**1**)(OAc)] (24.8 mg 0.075 mmol) in dichloromethane (20 mL) for 24 hours at 10 °C with vigorous stirring. The biphasic mixture was then transferred to a separatory funnel, the organic phase (Org3) removed and the aqueous phase (Aq5) washed once with dichloromethane (10 mL). The organic phase (Org3) was washed with water (10 mL). The aqueous phases were combined and a small aliquot (*ca.* 100 μL) was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase (Aq5) was evaporated to dryness to obtain *N*-Bn-β³-hPhg as white powder (8.7 mg, yield: 95%, 16% ee in favour of *S* enantiomer). The organic phase (Org3) was washed with water (10 mL), and evaporated to dryness under vacuum to yield a brown-green powder. This was dissolved in methanol (10 mL) and treated with ascorbic acid (6.6 mg, 0.037 mmol) for 15 minutes, until a red precipitate of [Co^{II}(**1**)] was formed, washed with 10 mL methanol and dried (19.0 mg, yield: 85%). The combined pale yellow methanolic filtrates were purified on Dowex 50W-X8 resin to obtain (*S*)-*N*-Bn-β³-hPhg as a white powder. Yield: 8.6 mg (94%).

Procedure for the iterative extraction of *N*-Bn-β³-hPhg (Scheme 2, lower part)

To a solution of (*R*,*R*)-[Co^{III}(**1**)(OAc)] (49.6 mg, 0.075 mmol) in dichloromethane (20 mL) at 10 °C in a 100 mL round-bottom flask, was added a pre-cooled (10 °C) solution of racemic *N*-Bn-β³-hPhg (38.6 mg, 0.15 mmol, 2 equiv) in H_2O (40 mL). The biphasic mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separatory funnel, the organic phase (Org1) removed and the aqueous phase (Aq1) washed once with dichloromethane (10 mL). The combined dichloromethane extracts were washed once with H_2O (10 mL).

Processing of the aqueous phase (Aq1). The aqueous phases were combined, concentrated to 40 mL and extracted again with (*R*,*R*)-[Co^{III}(**1**)(OAc)] (24.8 mg, 0.037 mmol, 0.5 equiv) in dichloromethane (20 mL) for 24 h at 10 °C. The biphasic mixture was transferred to a separatory funnel, the organic phase (Org2′) removed and the aqueous phase (Aq3′) washed once with dichloromethane (10 mL). The combined dichloromethane extracts were washed once with H_2O (10 mL). Volatiles were removed under reduced pressure to leave the complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hPhg)] as a green-brown powder. This was dissolved in methanol (10 mL) and ascorbic acid (6.6 mg, 0.037 mmol) was added. The mixture was stirred vigorously for 15 min until a red precipitate was formed ([Co^{II}(**1**)]), which was filtered, washed with 10 mL methanol and dried (18.9 mg, yield: 84%). The combined pale yellow methanolic filtrates were purified on Dowex 50W-X8 resin to obtain *N*-Bn-β³-hPhg as a white powder (8.9 mg, yield: 93%, 10% ee in favour of *S* enantiomer). The aqueous phases (Aq3′) were combined and a small aliquot (*ca.* 100 μL) was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase was then evaporated under reduced pressure to give (*R*)-*N*-Bn-β³-hPhg as a white powder. Yield: 9.3 mg (96%). $[\alpha]_D^{24} = +52.1$ ($c = 0.93$ in MeOH), lit.[13] $[\alpha]_D^{24} = +56.3$ ($c = 1$ in MeOH), (*R*)-enantiomer.

Processing of the organic phase (Org1). Volatiles were removed under reduced pressure to leave the complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hPhg)] as a green-brown powder. This was dissolved in methanol (10 mL) and ascorbic acid (13.2 mg, 0.075 mmol) was added. The mixture was stirred vigorously for 15 min until a red precipitate of [Co^{II}(**1**)] was formed, which was filtered, washed with methanol (10 mL) and dried (40.6 mg, yield: 90%). The combined pale yellow methanolic filtrates were purified on Dowex 50W-X8 resin. The crude white solid (18.2 mg, yield: 95%) was dissolved in water (40 mL, Aq2), filtered and submitted to a second extraction with (*S*,*S*)-[Co^{III}(**1**)(OAc)] (24.8 mg, 0.037 mmol, 0.5 equiv) in dichloromethane (20 mL) for 24 h, at 10 °C, with vigorous stirring. The biphasic mixture was then transferred to a separatory funnel, the organic phase (Org3′) removed and the aqueous phase (Aq5′) washed once with dichloromethane (10 mL). After washing the organic phase with water (10 mL), volatiles were removed under reduced pressure to leave the complex (*S*,*S*)-[Co^{III}(**1**)(*N*-Bn-β³-hPhg)] as a green-brown powder. This was dissolved in methanol (10 mL) and ascorbic acid (6.6 mg, 0.037 mmol) was added. The mixture was stirred vigorously for 15 min until a red precipitate of [Co^{II}(**1**)] was formed, which was washed with 10 mL methanol and dried (19.5 mg, yield: 87%). The combined pale yellow methanolic filtrates were purified on Dowex 50W-X8 resin to obtain *N*-Bn-β³-hPhg as a white powder (8.3 mg, yield: 92%, 5% ee in favour of *R* enantiomer, Aq6′). The aqueous phases (Aq5′) were combined and a small aliquot (*ca.* 100 μL) was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase was then evaporated under reduced pressure to give (*S*)-*N*-Bn-β³-hPhg as a white powder. Yield: 8.4 mg (93%).

(*S*,*S*)-[Co^{III}(1)(*N*-Bn-β³-hPhg)]. ^{1}H-NMR ($CDCl_3$): $\delta = 7.64$ (s, 1H), 7.46 (d, $J = 2.8$ Hz, 1H), 7.40–7.27 (m, 7H), 7.08 (d, $J = 2.4$ Hz, 1H), 6.98 (d, $J = 2.4$ Hz, 1H), 6.95 (d, $J = 7.2$ Hz, 2H), 6.59–6.55 (m, 2H), 6.27 (s, 1H), 5.18 (d, $J = 10.6$ Hz, 1H), 4.71–4.55 (m, 2H), 4.21–4.10 (m, 1H), 3.87 (s, 1H), 3.10 (dd, $J = 17.6$ Hz, $J = 7.4$ Hz, 1H), 3.00–2.91 (m, 1H), 2.78–2.67 (m, 2H), 1.93 (d, $J = 10.4$ Hz, 2H), 1.87–1.80 (m, 1H) 1.77–1.70 (m, 1H), 1.61 (s, 9H), 1.48–1.43 (m, 1H), 1.45 (s, 9H), 1.36 (s, 9H), 1.32–1.22 (m, 2H), 1.09 (s, 9H) ppm. ^{13}C-NMR ($CDCl_3$): $\delta = 175.0$, 163.8, 162.7, 161.0, 158.6, 144.8, 140.7, 140.3, 137.8, 136.7, 134.7, 130.3, 129.9, 129.4, 129.2, 128.3, 128.0, 127.7, 122.5, 120.4, 117.3, 75.5, 70.5, 55.3, 54.5, 35.7, 35.6, 34.0, 33.7, 32.9, 31.6, 31.5, 30.2, 29.7,

28.8, 25.0, 23.9 ppm. FT-IR (Nujol): ν = 3375, 3173, 2903, 2850, 1714, 1461, 1376, 1306, 1167, 1155, 970 cm^{-1}. HRMS (ESI): calcd. for $C_{52}H_{68}N_3O_4Co$ 858.4615 $[M + H]^+$; found: 858.4685.

Procedure for the extraction of *N*-Bn-β³-hAla

To a solution of (*R*,*R*)-[Co^{III}(**1**)(OAc)] (99.3 mg, 0.15 mmol) in dichloromethane (20 mL) at 10 °C in a 100 mL round-bottom flask, was added a pre-cooled (10 °C) solution of racemic *N*-Bn-β³-hAla (58.0 mg, 0.30 mmol, 2 equiv) in H_2O (15 mL). The biphasic mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separatory funnel, the organic phase (Org1) removed and the aqueous phase washed once with dichloromethane (10 mL). A small aliquot (*ca.* 100 μL) of the aqueous phase was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase (Aq1) was then evaporated under reduced pressure to give *N*-Bn-β³-hAla as a white powder (40.6 mg, 25% ee in favour of *S* enantiomer). The combined dichloromethane extracts (Org1) were washed once with H_2O (10 mL). Volatiles were removed under reduced pressure to leave the complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hAla)] as a brown powder, which was characterised by HRMS (ESI): calcd. for $C_{47}H_{66}N_3O_4Co$ 818.4277 $[M + Na]^+$; found: 818.4289. (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hAla)] was dissolved in methanol (10 mL) and ascorbic acid (26.4 mg, 0.15 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (76 mg, yield: 84%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and pure *N*-Bn-β³-hAla was obtained as a white powder (17.0 mg, yield: 59%, 75% ee in favour of the *R* enantiomer).

Procedure for the iterative extraction of *N*-Bn-β³-hAla (Scheme 2, upper part)

Processing of the aqueous phase (Aq1). Aq1 was extracted again with (*S*,*S*)-[Co^{III}(**1**)(OAc)] (69.5 mg, 0.105 mmol). Aq 3: *N*-Bn-β³-hAla was obtained as a white powder (28.8 mg, 5% ee in favour of *S* enantiomer). Org2: complex (*S*,*S*)-[Co^{III}(**1**)(*N*-Bn-β³-hAla)] was obtained as a brown powder, dissolved in methanol (10 mL) and ascorbic acid (18.4 mg, 0.105 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (53.8 mg, yield: 85%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hAla was obtained as a white powder (9.0 mg, yield: 44%, 88% ee in favour of *S* enantiomer).

Processing of the organic phase (Org1). Pure *N*-Bn-β³-hAla, obtained as a white powder (17.0 mg, yield: 59%, 75% ee in favour of the *R* enantiomer) from the reductive cleavage and work-up of Org1, was dissolved in water (15 mL, Aq2), filtered and submitted to a second extraction with (*R*,*R*)-[Co^{III}(**1**)(OAc)] (29.8 mg, 0.045 mmol). Aq 5: *N*-Bn-β³-hAla was obtained as a white powder (11.0 mg, 65% ee in favour of *R* enantiomer). Org3: (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hAla)] was obtained as a brown powder, which was dissolved in methanol (10 mL) and ascorbic acid (7.9 mg, 0.045 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (22.5 mg, yield: 83%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hAla was obtained as white powder (5.0 mg, yield: 58.0%, 90% ee in favour of *R* enantiomer).

Procedure for the iterative extraction of *N*-Bn-β³-hAla (Scheme 2, lower part)

To a solution of (*R*,*R*)-[Co^{III}(**1**)(OAc)] (99.3 mg, 0.15 mmol) in dichloromethane (20 mL) at 10 °C in a 100 mL round-bottom flask, was added a pre-cooled (10 °C) solution of racemic *N*-Bn-β³-hAla (58.0 mg, 0.30 mmol, 2 equiv) in H_2O (15 mL). The organic phase (Org1) and the aqueous phase (Aq1) were obtained.

Processing of the aqueous phase (Aq1). Aq1 was extracted again with (*R*,*R*)-[Co^{III}(**1**)(OAc)] (69.5 mg, 0.105 mmol). Aq 3′: *N*-Bn-β³-hAla was obtained as a white powder (28 mg, 36% ee in favour of *S* enantiomer). Org2′: (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hAla)] was obtained as a brown powder, which was dissolved in methanol (10 mL) and ascorbic acid (18.4 mg, 0.105 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (51.8 mg, yield: 82%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hAla was obtained as white powder (10 mg, yield: 49%, 75% ee in favour of *R* enantiomer).

Processing of the organic phase (Org1). Pure *N*-Bn-β³-hAla, obtained as a white powder (17.0 mg, yield: 59%, 75% ee in favour of the *R* enantiomer) from the reductive cleavage and work-up of Org1, was dissolved in water (15 mL, Aq2), filtered and submitted to a second extraction with (*S*,*S*)-[Co^{III}(**1**)(OAc)] (29.8 mg, 0.045 mmol). Aq 5: *N*-Bn-β³-hAla was obtained as a white powder (11.5 mg, 83% ee in favour of *R* enantiomer; $[\alpha]_D^{24}$ = +18.0 (*c* 0.86 in H_2O); lit.[14] $[\alpha]_D^{24}$ = +22.5 (*c* 1.20 in H_2O), (*R*)-enantiomer). Org3: (*S*,*S*)-[Co^{III}(**1**)(*N*-Bn-β³-hAla)] was obtained as a brown powder, which was dissolved in methanol (10 mL) and ascorbic acid (7.9 mg, 0.045 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (22.5 mg, yield: 84%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hAla was obtained as white powder (4.5 mg, yield: 52.0%, 51% ee in favour of *S* enantiomer).

Procedure for the extraction of *N*-Bn-β³-hVal

The procedure used is similar to that described above for *N*-Bn-β³-hPhg. To a solution of (*R*,*R*)-[Co^{III}(**1**)(OAc)] (62.9 mg, 0.095 mmol) in dichloromethane (20 mL) at 10 °C in a 100 mL round-bottom flask, was added a pre-cooled (10 °C) solution of racemic *N*-Bn-β³-hVal (42 mg, 0.19 mmol, 2 equiv) in H_2O (15 mL). The biphasic mixture was stirred vigorously for 24 h at 10 °C, then transferred to a separatory funnel, the organic phase (Org1) removed and the aqueous phase washed once with dichloromethane (10 mL). A small aliquot (*ca.* 100 μL) of the aqueous phase was removed, filtered on a micropore filter, and the ee was determined by chiral HPLC as described above. The aqueous phase (Aq1) was then evaporated under reduced pressure to give *N*-Bn-β³-hVal as a white powder (26.2 mg, 36% ee in favour of (+) enantiomer). The combined dichloromethane extracts (Org1) were washed once with H_2O (10 mL). Volatiles were removed under reduced pressure to leave the complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hVal)] as a brown powder, which was characterised by HR-MS (ESI): calcd. for $C_{49}H_{71}N_3O_4Co$ 824.4771 $[M + H]^+$; found: 824.4771. (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hVal)] was dissolved in methanol (10 mL) and ascorbic acid (26.4 mg, 0.15 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (45.5 mg, yield: 80%). The methanolic filtrate was purified using

Dowex 50W-X8 resin, and pure *N*-Bn-β³-hVal was obtained as a white powder [13.9 mg, yield: 66%, 76% ee in favour of (−) enantiomer].

Procedure for the iterative extraction of *N*-Bn-β³-hVal (Scheme 2, upper part)

Processing of the aqueous phase (Aq1). Aq1 was extracted again with (*S*,*S*)-[Co^{III}(**1**)(OAc)] (39.7 mg, 0.06 mmol, 0.63 equiv). Aq 3: *N*-Bn-β³-hVal was obtained as a white powder (15 mg, 10% ee in favour of (−) enantiomer). Org2: complex (*S*,*S*)-[Co^{III}(**1**)(*N*-Bn-β³-hVal)] was obtained as a brown powder, dissolved in methanol (10 mL) and ascorbic acid (10.6 mg, 0.06 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (30.1 mg, yield: 83%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hVal was obtained as a white powder [9.0 mg, yield: 68%, $[\alpha]_D^{24} = +31.9$, *c* 0.78 in MeOH, 90% ee in favour of (+) enantiomer].

Processing of the organic phase (Org1). Pure *N*-Bn-β³-hVal, obtained as a white powder [13.9 mg, yield: 66%, 76% ee in favour of the (−) enantiomer] from the reductive cleavage and work-up of Org1, was dissolved in water (15 mL, Aq2), filtered and submitted to a second extraction with (*R*,*R*)-[Co^{III}(**1**)(OAc)] (21.2 mg, 0.032 mmol). Aq 5: *N*-Bn-β³-hVal was obtained as a white powder [9.0 mg, 64% ee in favour of (−) enantiomer]. Org3: (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hVal)] was obtained as a brown powder, which was dissolved in methanol (10 mL) and ascorbic acid (5.6 mg, 0.032 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (15.8 mg, yield: 80%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hVal was obtained as white powder [4.5 mg, yield: 64%, $[\alpha]_D^{24} = -32.0$, *c* 0.34 in MeOH, 93% ee in favour of (−) enantiomer].

Procedure for the iterative extraction of *N*-Bn-β³-hVal (Scheme 2, lower part)

Processing of the aqueous phase (Aq1). Aq1 was extracted again with (*R*,*R*)-[Co^{III}(**1**)(OAc)] (39.7 mg, 0.06 mmol, 0.63 equiv). Aq 3′: *N*-Bn-β³-hVal was obtained as a white powder (15.5 mg, $[\alpha]_D^{24} = +28.5$, *c* 0.80 in MeOH, 80% ee in favour of (+) enantiomer]. Org2′: complex (*R*,*R*)-[Co^{III}(**1**)(*N*-Bn-β³-hVal)] was obtained as a brown powder, dissolved in methanol (10 mL) and ascorbic acid (10.6 mg, 0.06 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (29.6 mg, yield: 82%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hVal was obtained as a white powder [8.9 mg, yield: 67%, 40% ee in favour of (−) enantiomer].

Processing of the organic phase (Org1). Pure *N*-Bn-β³-hVal, obtained as a white powder [13.9 mg, yield: 66%, 76% ee in favour of the (−) enantiomer] from the reductive cleavage and work-up of Org1, was dissolved in water (15 mL, Aq2), filtered and submitted to a second extraction with (*S*,*S*)-[Co^{III}(**1**)(OAc)] (21.2 mg, 0.032 mmol). Aq 5′: *N*-Bn-β³-hVal was obtained as a white powder [8.5 mg, $[\alpha]_D^{24} = -32.6$, *c* 0.51 in MeOH, 92% ee in favour of (−) enantiomer]. Org3′: (*S*,*S*)-[Co^{III}(**1**)(*N*-Bn-β³-hVal)] was obtained as a brown powder, which was dissolved in methanol (10 mL) and ascorbic acid (5.6 mg, 0.032 mmol) was added. [Co^{II}(**1**)] was obtained as a red powder (16.4 mg, yield: 85%). The methanolic filtrate was purified using Dowex 50W-X8 resin, and *N*-Bn-β³-hVal was obtained as white powder [4.4 mg, yield: 61%, 5% ee in favour of (−) enantiomer].

Attribution of the absolute configuration to *N*-Bn-β³-hVal: by analogy with the complexation selectivity shown for *N*-Bn-β³-hPhg and *N*-Bn-β³-hAla, we attribute to (+)-*N*-Bn-β³-hVal the (*R*) absolute configuration: $[\alpha]_D^{24} = +35.6$, *c* 0.80 in MeOH, 100% ee in favour of (*R*)-enantiomer.

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