Recl. Trav. Chim. Pays-Bas **114**, 231–238 (1995)
SSDI 0165-0513(95)00035-6

0165-0513/95/4/5 231–08$9.50

Synthesis of dipeptides containing α-substituted amino acids; their use as chiral ligands in Lewis-acid-catalyzed reactions

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(Received January 26, 1995)

Abstract. L-α-Methylphenylalanine, obtained by enzymatic resolution of the corresponding racemic amide with amidase from *Ochrobactrum anthropi* NCIMB 40321, was used in the synthesis of dipeptides containing α-substituted amino acids. The 2-hydroxynaphthalene-1-carboxaldehyde Schiff bases of the dipeptides (**1** and **2**) were tested as Ti(IV) and Al(III) complexes in asymmetric Lewis-acid-catalyzed reactions. Only the Al(III) complex of **2** showed moderate enantioselectivity in the cyanation reaction of benzaldehyde with TMS-CN.

Introduction

The use of α-substituted amino acids instead of α-hydrogen containing amino acids can be very convenient for many reasons. Especially their higher resistance to racemization can be of advantage. Moreover, their derivatives are very often crystalline, and thus easy to purify.
α-Substituted amino acids can be found in nature: for example, 2-aminoisobutyric acid [a] (Aib) and isovaline are present in peptaibol antibiotics[1].
The presence of α-substituted amino acids in peptides results in some conformational modifications, which are now under investigation[2]. For example, those peptides tend to form 3_{10}-helixes instead of α-helixes and, because of the steric hindrance, the conformational freedom is reduced. The presence of α-substituted amino acids stabilizes the peptide-bonds, making them less sensitive to enzymatic hydrolysis[3].
Especially α-methyl substituted amino acids are now of interest for the agrochemical and pharmaceutical industry[4]. Many routes to enantiomerically pure α-substituted amino acids are therefore under development[5].
Natural α-amino acids form a versatile pool of chirality which is often used in asymmetric synthesis[6]. *Inoue*[7] and *Oguni*[8] have shown that Schiff bases of peptides and amino alcohols are suitable ligands for complexation of Ti(IV) and Al(III)ions. These complexes are used in the asymmetric Lewis acid-mediated cyanation of aldehydes. With the dipeptides used in this reaction (see Figure 1), asymmetric inductions of up to 90% enantiomeric excess have been obtained (using L-Val-L-Trp-OCH_3 or L-Val-L-Phe-OCH_3).[7]
This interesting application prompted us to investigate the use of modified peptides bearing α-substituted amino acids instead of natural L-amino acids. In addition, we were also interested in the possibility to use these peptides [via Schiff base transformation and complexation with Ti(IV) and Al(III) salts] as inductors of asymmetry in other organic reactions like carbonyl-ene reactions and Diels–Alder cycloadditions.
For this reason we carried out the synthesis of the ligands **1** and **2**, containing one and two L-α-methylphenylalanines, respectively.

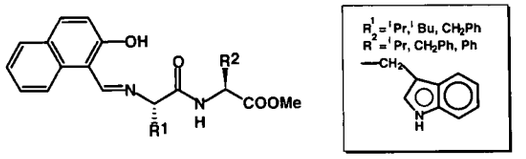


Figure 1.

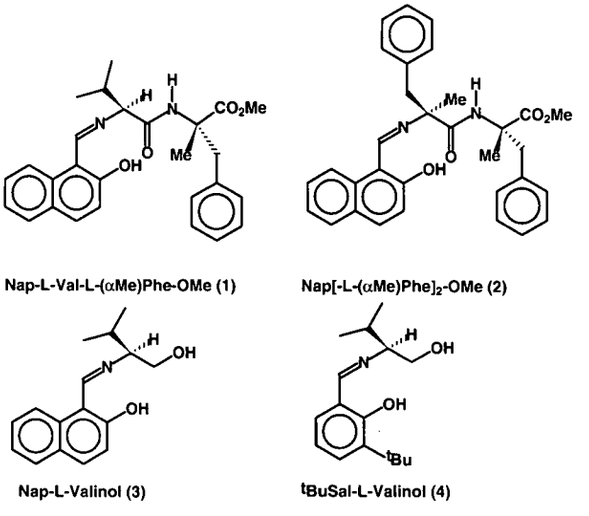
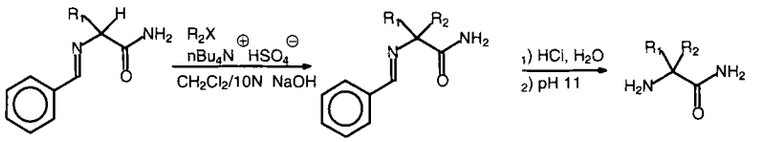
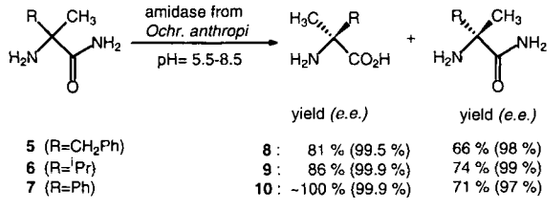


Figure 2.

[a] IUPAC name 2-amino-2-methylpropanoic acid



Scheme 1.



Scheme 2.

In addition ligand **3** was prepared from valinol [b] and compared with ligand **4**, previously described by *Oguni*[8] and used in the cyanation reaction of aldehydes.

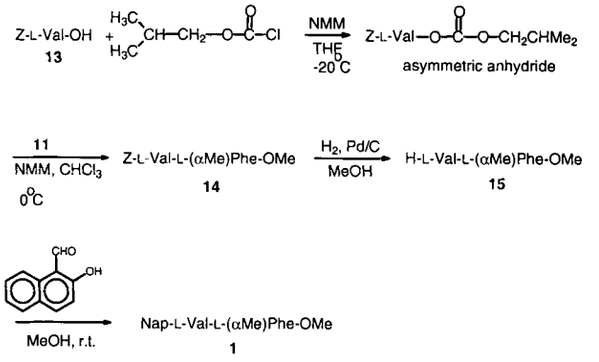
Synthesis and resolution of α-substituted amino acids

For the preparation of the desired dipeptides we needed L-α-methylphenylalanine [H-L-(αMe)Phe-OH, **8**] as starting material. It was therefore necessary to resolve the corresponding amide H-D,L-(αMe)Phe-NH_2 (**5**). Later on we also undertook the resolution of α-methylvalinamide [H-D,L-(αMe)Val-NH_2 (**6**)] and α-methylphenylglycinamide [H-D,L-(αMe)Phg-NH_2 (**7**)]. Briefly, the racemic amide can be easily obtained by the Strecker synthesis in good yields. The racemate of H-(αMe)Val-NH_2 was synthesized in this way in 80% yield as previously descibed[9]. Another way to synthesize the starting amide is by phase transfer catalyzed alkylation (Scheme 1)[10]. *N*-Benzylidene-α-amino acid amides, available from benzaldehyde and α-amino acid amides, are alkylated in CH_2Cl_2/10N NaOH under phase-transfer conditions using tetrabutylammonium hydrogen sulfate as a catalyst. H-D,L-(αMe)Phe-NH_2 (**5**) and H-D,L-(αMe)Phg-NH_2 (**7**) were synthesized in this way in 55% and 45% yields, respectively.

The resolution of the D,L-α-substituted amino acid amides was accomplished by enzymatic hydrolysis. In addition to the enzymatic resolution using an amino acid amidase from *Mycobacterium neoaurum ATCC 25695*[11], we recently described the use of an amidase from *Ochrobactrum anthropi NCIMB 40321* for the resolution of α-substituted amino acid amides[12]. We found that this enzyme was able to hydrolyse α-hydrogen and α-substituted amino acid amides, as well as α-hydroxy-amides and *N*-hydroxy-α-amino acid amides with both high activity and high enantioselectivity.

The resolution of H-D,L-(αMe)Phe-NH_2 (**5**), H-D,L-(αMe)Val-NH_2 (**6**) and H-D,L-(αMe)Phg-NH_2 (**7**) (both as a free amide and as a hydrochloric salt) was performed via the *Ochrobactrum anthropi* amidase method. The enzymatic hydrolysis was carried out with a 10% aqueous solution of the substrate (either the free amide or the hydrochloride salt), at pH 5.5–8.5 (depending on the solubility of the substrate) and with ratios of wet bacteria/substrate ranging from 1/3 to 1/10, at 37°C. The reaction was usually stopped at 50% conversion by acidification of the reaction mixture and centrifugation of the cells.

[b] IUPAC name: 2-amino-3-methylbutan-1-ol



Scheme 3.

In order to separate the L-amino acid from the D-amino amide, three different systems were used. Because of the low solubility of H-(αMe)Phe-NH_2 at basic pH's, the separation was performed via extraction of the D-amide **5** with an organic solvent and the L-acid **8** was subsequently recovered from the water layer after neutralization and evaporation of the solvent. For H-(αMe)Phg-NH_2 direct separation of the L-acid **10** and the D-amide **7** by extraction was not successful. For this reason first the benzaldehyde Schiff base of the D-amide was formed before extraction. This method, however, is rather laborious since (complete) Schiff base formation of α-substituted amino acid amides takes several days. For H-(αMe)Val-NH_2 (**6**) the separation of the L-acid **9** and the D-amide **6** was performed using a strongly basic ionic exchange resin (DOWEX 1X8).

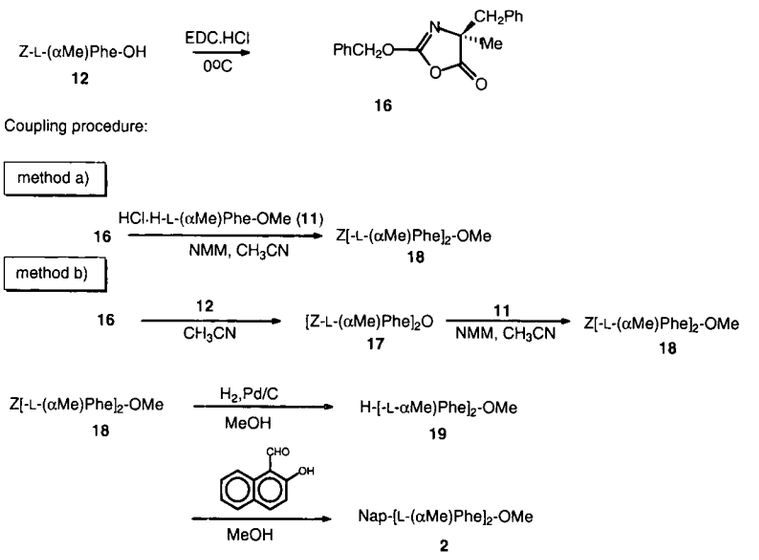
For the resolution of all three amino acid amides, *O. anthropi* was fully stereospecific. The L-amino acids were always isolated with an enantiomeric excess (*ee*) higher than 99.5%, and the remaining D-amino acid amides had enantiomeric excesses of 97–99%. The enzyme activity is reasonable even at a pH much lower than the optimum value (pH 8.0). For example with mandelic amide [c] approximately 50% of the maximum activity is preserved at pH 6.0. It is possible to resolve either the hydrochloride salt or the free amide without any difference in the resulting enantiomeric excess. The presence of bulky groups on the α-carbon (^{i}Pr or Ph versus CH_2Ph) makes the enzymatic reaction 2–3 times slower.

Synthesis of the peptides

The intermediates for the synthesis of the dipeptides, the *N*-(benzyloxycarbonyl (Z-) derivatives Z-L-Val-OH[13] (**13**), Z-L-(αMe)Phe-OH[14] (**11**) and the methyl ester H-L-(αMe)Phe-OMe[15] (**12**) were prepared as descibed in the literature and used for the synthesis of Nap-L-Val-L-(αMe)Phe-OMe [d] (**1**) as descibed in Scheme 3 and Nap [-L-(αMe)Phe]$_2$-OMe (**2**) (Scheme 4). The coupling be-

[c] IUPAC name: 2-hydroxybenzeneacetamide

[d] Nap = (2-hydroxy-1-naphthyl)methylene



Scheme 4.

tween Z-L-Val-OH and HCl.H-L-(αMe)Phe-OMe was performed via the formation of the asymmetric anhydride of Z-L-Val-OH with isobutyl chloroformate and *N*-methylmorpholine (NMM), and subsequent coupling to the protected dipeptide **14** in 75% overall yield. The Z group was removed via catalytic hydrogenation (10% Pd on charcoal) and the corresponding Schiff base **1** was synthesized in 71% yield from the dipeptide **15** and an excess of 2-hydroxynaphthalene-1-carboxaldehyde in methanol.

The synthesis of the [L-(αMe)Phe]$_2$-containing ligand **2** was performed in slightly modified way because coupling via the mixed anhydride is not possible. On the other hand, α-substituted amino acids can be transformed into the corresponding oxazolones without any risk of racemization.

Treatment of Z-L-(αMe)Phe-OH (**12**) with ethyl-[2-(dimethylamino)ethyl]carbodiimide · HCl (EDC · HCl) for 3 minutes gave the activated oxazolone **16** in approximately 60% yield (Scheme 4). During the oxazolone formation some symmetric anhydride **17** (32%) was formed by reaction of the oxazolone with unreacted Z-L-(αMe)Phe-OH (**12**). Since both the oxazolone and the symmetric anhydride can be used in the dipeptide coupling reaction, these compounds were not separated. The oxazolone could also be completely transferred into the symmetric anhydride **17** with an additional equivalent of Z-L-(αMe)Phe-OH (**11**) in 93% yield (Scheme 4, method *b*).

The peptide coupling via method *b* yielded **18** in 63% yield *versus* 56% yield for the coupling by method *a*. However, since the coupling of the symmetric anhydride (method *b*) is more sluggish than with the oxazolone (method *a*), and an additional equivalent of Z-L-(αMe) Phe-OH is needed, the direct coupling via the oxaxolone is preferred. The synthesis of ligand **2** was completed as described for ligand **1** by hydrogenation of the Z group and Schiff base formation in 95% and 89%, respectively. Both, **1** and **2** are stable, bright yellow crystalline compounds which very often were regenerated after the asymmetric syntheses (*vide infra*).

Nap-L-Valinol (**3**) and ^{t}BuSal-L-Valinol [e,8] (**4**) were prepared from commercially available L-valinol and an excess of the aldehyde in methanol at room temperature.

Complex formation and asymmetric cyanation reactions

Using the model peptides, modified to the corresponding 2-naphthol Schiff bases: Nap-L-Val-L-(αMe)Phe-OMe (**1**) and Nap[-L-(αMe)Phe]$_2$-OMe (**2**), complexes were formed with Ti(IV) and Al(III) ions. The synthesis of the metallic complexes was performed as follows:

- Ti complexes: Ti(OR)$_4$ or TiCl$_2$(O^{i}Pr)$_2$[16] and the ligand were dissolved in CH_2Cl_2 and stirred at room temperature for 1 hour.
- Al complexes: $AlMe_3$ and the ligand were dissolved in toluene and stirred at room temperature for 2 hours.

The mode of complexation was studied with ^{1}H-NMR spectroscopy on the complex between **2** and TiCl$_2$(O^{i}Pr)$_2$. Comparision between the free ligand **2** and the complex revealed chemical shifts for the -CH=N- and the N–H protons (8.7 *vs.* 8.5 ppm, and 6.5 *vs.* 8.0 ppm, respectively), whereas the naphthyl-OH signal (14.5 ppm for the free ligand) disappeared on complexation. From the ^{1}H-NMR spectrum and ^{13}C-NMR spectrum it can be concluded that Ti(IV)-bound isopropoxide as well as free isopropanol was present. From this we conclude that coordination between the ligand and Ti(IV) is formed by the O, N and O atoms of the -OH, -CH=N- and the NH–C=O groups, respectively, as depicted in Figure 3. The remaining three ligands may be either alkoxide and/or chloride. We first examined the Lewis acid capacity of the Ti(IV) complexes in the Diels–Alder reaction and the oxoacetate-ene reaction. Both reactions are catalyzed by

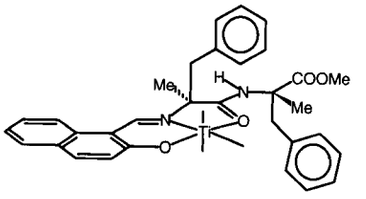


Figure 3.

[e] ^{t}BuSal = 3-*tert*-butyl-2-hydroxybenzylidene

Table I Asymmetric Lewis acid catalyzed synthesis of mandelonitril from TMS-CN and benzaldehyde.

Entry	Lewis acid (mol%)	Solvent	Time (h)	Temp. (°C)	Yield (%)	*ee* (%)
1	Nap[L-(αMe)Phe]$_2$-OMe/Ti(OEt)$_4$ (20)	CH_2Cl_2	20	−78	47	< 5
2	Nap[L-(αMe)Phe]$_2$-OMe/$AlMe_3$ (20)	toluene	132	−40	48	23
3	Nap[L-(αMe)Phe]$_2$-OMe/$AlMe_3$ (100)	toluene	140	−45	68	< 5
4	Nap[L-(αMe)Phe]$_2$-OMe/$AlMe_3$ (100)	toluene	95	−78	± 100	10
5	^{t}BuSal-L-Valinol/Ti(O^{i}Pr)$_4$ (22)[8]	CH_2Cl_2	40	−78	45	85

Lewis-acids and many asymmetric examples have been described in literature[16–18].In the reaction of cyclopentadiene and propenal or 3-buten-2-one with complexes of ligand **1–3** and $Ti(OR)_4$ or $TiCl_2(O^iPr)_2$ under different conditions (Scheme 5), no asymmetric induction was observed. Only the *endo / exo* ratio (appr. 85/15) was slightly influenced in comparision to the uncatalyzed reaction. With 3-(2-butenoyl)oxazolidin-2-one as a dienophile no reaction was observed with the chiral complexes, although reaction with $TiCl_2(O^iPr)_2$ as Lewis acid occurred.

The asymmetric oxoacetate-ene reaction was tested with isopropenylbenzene and methyl oxoacetate[19] (Scheme 6). Although the reaction was catalyzed by the chiral Ti(IV) complexes, only low chemical and optical yields were obtained. For instance, reaction with 10 mol% of the complex of $TiCl_2(O^iPr)_2$ and ligand **2** in dichloromethane at −30°C gave product with an *ee* of 10%.

After these discouraging results in both reactions we decided to concentrate our research on the cyanation reaction of benzaldehyde with trimethylsilyl cyanide (TMS-CN) (Scheme 7).

Many chiral metal complexes are known to catalyze this reaction in excellent *ee*'s[20,21].

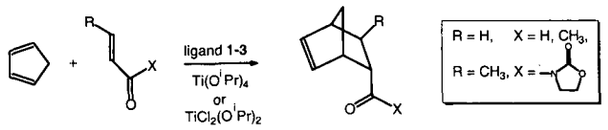
For example, using $TiCl_2(O^iPr)_2$ in combination with diol ligands derived from tartaric acid, *ee*'s of up to 96% are obtained[21]. *Inoue* and co-workers have demonstrated that complexes made of $Ti(O^iPr)_4$ and chiral Schiff bases (e.g. Nap-L-Val-L-Phe-OMe) catalyze the reaction in Scheme 7. The obtained level of *ee* was rather poor (19%).

Therefore we tried to run the reaction using a catalytic amount of a complex made from $Ti(OEt)_4$ and Nap[-L-(αMe)Phe]$_2$-OMe (**2**) in order to see if the use of another titanium alkoxide and a different peptide in respect to the ones used by *Inoue* would induce any variation to the enantioselectivity.

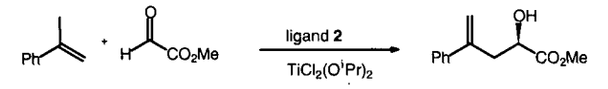
The general reaction procedure was as follows: to the solution of the chiral complex in CH_2Cl_2 or toluene, previously cooled to −78°C, trimethylsilyl cyanide and freshly distilled benzaldehyde were added in a ratio 3/1 respectively. The chiral complex was used in different percentages with respect to the aldehyde. The solution was warmed to the reaction temperature and the mixture was stirred under an inert atmosphere for the indicated time (see Table I). Acidic work up and purification by column chromatography yielded mandelonitrile. The results and the reaction conditions are reported in Table I. The results demonstrate that although the Ti(IV) complexes act as a catalyst it was not an asymmetric inductor (entry 1).

Because *Inoue* had already reported that complexes of $AlMe_3$ and Nap-dipeptide were more suitable as asymmetric inductors in the investigated reaction, we decided to try as well this kind of complexes first in a catalytic and then in an equimolar amount (entries 2–4). The best results were obtained with a catalytic amount (20 mol%) of the complex with ligand **2**. The mandelonitrile was obtained in 47% yield and 23% *ee* (*R* isomer) (entry 2). Surprisingly, the use of an equimolar amount of catalyst at −45°C improved the yield but the product was almost racemic. Lowering of the reaction temperature only improved the yield.

At this point, in order to verify our results, we tried to reproduce the experiment reported by *Oguni*[8] who ran the same reaction using a complex of $Ti(OiPr)_4$ and ^{t}BuSal-L-Valinol (**4**) (entry 5). Our result was completely in line with the one reported in literature (67% yield, *ee* 85% (*R* isomer)).



Scheme 5.

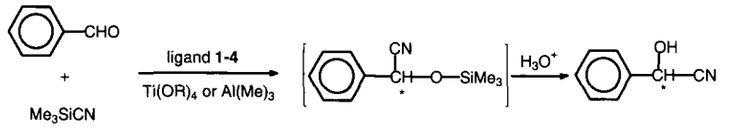


Scheme 6.

Discussion

Enantiomerically pure α-substituted amino acids are readily available by enzymatic resolution of the corresponding amides by enzymatic resolution with the amidase from *Ochrobactrum anthropi*. Because the amidase is fully stereospecific, both the D-amide as well as the L-acid can be obtained in nearly 100% enantiomeric excess. From these α-substituted amino acids dipeptides were prepared using conventional peptide coupling reaction. In addition coupling procedures can be used which for α-hydrogen-containing amino acids would lead to racemization, *i.e.* via the oxazolone. In general reaction times for disubstituted amino acids are longer than for the natural amino acids. On the other hand, the prepared dipeptide compounds **1** and **2** were not very susceptible to hydrolysis, and they were easily regained from the Lewis-acid-catalyzed reactions after column chromatography.

Analyzing the results in the Lewis-acid-catalyzed reactions we can conclude that complexes of titanium alkox-



Scheme 7.

ides and the Schiff-base-modified peptides are ineffective asymmetric inductors for the reaction under examination. This is probably not related to the kind of alkoxy group or to the amino acids present in the dipeptide.

The results obtained with $AlMe_3$ as a metal ligand in the cyanation reactio of benzaldehyde are more encouraging. Surprisingly, on analysis of our results, it seems that the catalytic reaction (Table I, entry 2) gives a better *ee* than the equimolar one (entries 3 and 4). In contrast *Inoue* et al.[7] used a equimolar amount of the peptide complexes to obtain the highest *ee*'s. Only with an alternative ligand (Nap-L-Val-NH-*cyclo*hexyl) were catalytic amounts of catalyst used. Since no information exists about the nature of complexation during the catalytic reaction, it is not easy to find an explanation for this observation. It is, however, possible to compare the result obtained in entry 2 with the one obtained by *Inoue et al.* using Nap-L-Phe-L-Phe-OMe as an Al(III) ligand. In the latter case the mandelonitrile was obtained with 100% yield and 34% *ee* (R isomer). From this we conclude that the exchange of α-hydrogen amino acids for the corresponding α-substituted amino acids in the ligands seems to have a negative influence on the enantioselectivity. Therefore, we think that the difference in size between the two amino acid substituents plays an important role: it might be possible that the use of α-substituted amino acids, in which a more profound difference in size between the substituents is present, could give better results.

Experimental part

General

Infrared spectra were obtained using a Perkin-Elmer 1600 series FT-IR. ^{1}H NMR spectra were determined in $CDCl_3$ (unless otherwise specified) using a Bruker ACF 200 (200 MHz) spectrometer. ^{13}C NMR (50.31 MHz) spectra were determined on the same apparatus in $CDCl_3$ (unless otherwise indicated). Chemical shifts (δ) are given in ppm downfield from internal tetramethylsilane (TMS) (For D_2O the deuterium lock signal was used as the reference). Optical rotations were measured on a Perkin-Elmer 241 spectrophotometer. R_f values were obtained by using thin-layer chromatography (TLC) on silica gel-coated plates (Merck silica gel 60 F_{254}). R_{fA}, R_{fB}, R_{fC} were measured in eluent A ($CHCl_3/CH_3OH/NH_4OH$, 60/45/20), eluent B (^{t}BuOH/HCOOH/H_2O, 75/15/10) and eluent C (n-BuOH/HOAc/AcOEt/H_2O, 1/1/1/1), respectively. Column chromatography refers to flash chromatography using Merck silica gel 60 (230–400 mesh). Melting points are uncorrected. The enantiomeric excesses (*ee*'s) of amino acids were determined by HPLC analysis using a Nucleosil 120-S-C_{18} (25 cm × 0.4 cm) (flux 1 ml/min, *T* 40°C, fluorimetric detection (λ_{exc} 338 nm, λ_{em} = 415 nm), eluents: (50 mM NaOAc/HOAc buffer pH 6.00)/methanol, 65/35).

3-*tert*-Butyl-2-hydroxybenzaldehyde and (*S*)-2-[(3-*tert*-butyl-2-hydroxybenzylidene)amino]-3-methyl-butan-1-ol (^{t}BuSal-L-Valinol **IV**)[8] and $TiCl_2(O^iPr)_2$[16] were prepared according to the literature procedure.

Trimethylsilyl cyanide was purchased from Janssen and used without further purification. Benzaldehyde was freshly distilled before use (b.p. 70–72°C, 32mbar).

General procedure for the enzymatic resolution of α-substituted amino acid amides (α-methylphenylalaninamide (**5**) *is used as a typical example)*

H-D,L-(αMe)Phe-NH_2 (49.7 g, 278 mmol) was dissolved in 450 ml of water, the pH was regulated at 5.6 with 4N HCl and 10 g of wet *Ochrobactrum anthropi* NCIMB 40321 were added. The resulting mixture was shaken at 37°C/200 rpm and the reaction was followed measuring the NH_3 evolved by means of an ammonia-sensitive electrode. At approx. 50% conversion (48–90 h), the solution was acidified to pH 2.16 with 2N HCl and the cells were separated by centrifugation (15 min, 9000 · g).

Separation of H-L-(αMe)Phe-OH (**8**) *and H-D-(αMe)Phe-NH₂* (**5**)

After sedimentation of the cells, the supernatant was basified to pH 9 using 4N NaOH solution and H-D-(αMe)Phe-NH_2 was extracted with $CHCl_3$. The organic layer was washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure, giving the free amide. The water layer was neutralized to pH 7.2 using 4N HCl solution and partially concentrated under reduced pressure giving the free acid after filtration.

H-D-(αMe)Phe-NH_2: Yield: 16.4 g (66%); m.p. 122.5–123.5°C; $[\alpha]_D^{20}$ +68.2° (*c* 0.51, $CHCl_3$); *ee* 98%; ^{1}H-NMR δ: 7.40–7.15 (m + br, 6H, Ar–H and CONH); 5.92 (br, 1H, CONH); 3.35 and 2.65 (AB system, *J* 13.3 Hz, 2H, CH_2); 1.4 (2s, 5H, αCH_3 and NH_2). ^{13}C-NMR δ: 180.53 (s, CO); 137.44 (s, *ipso*-C); 131.06–127.43 (5d, Ar–CH); 59.02 (s, αC), 47.15 (t, CH_2); 28.47 (q, CH_3). R_{fA} 0.90, R_{fB} 0.32, R_{fC} 0.53.

H-L-(αMe)Phe-OH: Yield 20.2 g (81%); m.p. 285.7°C; $[\alpha]_D^{20}$ −19.0° (*c* 1.0, H_2O); *ee* > 99.5%. ^{1}H-NMR (D_2O) δ: 7.35 (m, 3H, *m*- and *p*-H); 7.25 (m, 2H, *o*-H); 3.28 and 2.96 (AB system, 2H, CH_2); 1.50 (s, 3H, CH_3). ^{13}C-NMR (D_2O) δ: 176.67 (s, CO_2H); 134.74 (s, *ipso*-C); 130.49–128.34 (3d, phenyl-CH); 62.66 (s, αC); 43.17 (t, CH_2); 22.87 (q, CH_3). R_{fA} 0.79, R_{fB} 0.37, R_{fC} 0.54.

Separation of H-L-(αMe)Val-OH (**9**) *and H-D-(αMe)Val-NH₂* (**6**)

After centrifugation of the cells, the supernatant was eluted on basic ionic exchange column (DOWEX 1X8). H-D-(αMe)Val-NH_2 was eluted by washing the column with water. H-L-(αMe)Val-OH was then eluted by washing the column with 2N acetic acid. H-D-(αMe)Val-NH_2 and H-L-(αMe)Val-OH were recovered by concentration of the corresponding eluted liquids.

H-D-(αMe)Val-NH_2. Yield 74%; m.p. 73.5–78.5°C; $[\alpha]_D^{20}$ +68.8° (*c* 0.55, $CHCl_3$); *ee* 99%. ^{1}H-NMR δ: 7.42 and 5.85 (2 br s, 2H, $CONH_2$); 2.23 (hept, 1H, βCH); 1.25 (s, 3H, βCH_3); 1.1–1.3 (m, 2H, NH_2); 0.95–0.80 (dd, 6H, $2\gamma CH_3$). ^{13}C-NMR δ: 179.79 (s, $CONH_2$); 59.37 (s, αC); 33.12 (d, βCH); 24.81 (q, βCH_3); 16.53 and 14.74 (2s, 2 γCH_3). R_{fA} 0.75, R_{fB} 0.25, R_{fC} 0.4.

H-L-(αMe)Val-OH. Yield 86%; m.p. > 300°C; $[\alpha]_D^{20}$ −3.98° (*c* 1.0, H_2O); *ee* 99.9%. ^{1}H-NMR (D_2O) δ: 2.11 (hept, 1H, βCH); 1.43 (s, 3H, βCH_3); 0.95 (t, 6H, $2\gamma CH_3$). ^{13}C-NMR (D_2O) δ: 177.25 (s, CO_2H); 65.35 (s, αC); 33.86 (d, βCH); 20.54 (q, βCH_3); 17.03 and 15.71 (2q, $2\gamma CH_3$). R_{fA} 0.40, R_{fB} 0.25, R_{fC} 0.35.

Separation of H-L-(αMe)Phg-OH (**10**) *and H-D-(αMe)Phg-NH₂* (**7**)

After centrifugation of the cells, the supernatant was basified to pH 9 using a 4N NaOH solution and the amide was extracted with CH_2Cl_2. The free amide was recovered after evaporation of the solvent. Because the water layer still contained the amide, it was treated with benzaldehyde (0.5 eq.) and the solution was stirred, after basification to pH 10 with a 4N NaOH solution, for 5 days at r.t. The Schiff base was extracted from the water layer with EtOAc. The water layer was neutralized and concentrated under reduced pressure to give the free amino acid. The organic phase was concentrated and 37% HCl was added (9.5 mmol). The mixture was stirred for one night. HCl · H-D-(αMe)Phg-NH_2 was extracted from the organic layer with 2N HCl and the water layer was concentrated under reduced pressure. HCl · H-D-(αMe)Phg-NH_2 was crystallized from $MeOH/Et_2O$.

H-D-(αMe)Phg-NH_2. Yield 71%; m.p. 111.5–112.5°C; $[\alpha]_D^{20}$ −3.75° (*c* 0.56, $CHCl_3$); *ee* 97%. ^{1}H-NMR δ: 7.6–7.2 (m, 5H, phenyl-H); 7.09 and 5.50 (2br s, 2H, $CONH_2$); 1.85 (br, 2H, NH_2); 1.75 (s, 3H, CH_3). ^{13}C-NMR δ: 178.12 (s, $CONH_2$); 143.54 (s, *ipso*-C); 127.70–124.48 (3d, phenyl-CH); 59.40 (s, αC); 27.31 (q, CH_3). R_{fA} 0.95, R_{fB} 0.30, R_{fC} 0.45.

H-L-(αMe)Phg-OH. Quantitative yield; m.p. > 300°C; *ee* 99.9%. ^{1}H-NMR (D_2O) δ: 7.45 (m, 5H, phenyl-H); 1.9 (s, 3H, CH_3). ^{13}C-NMR δ: 177.20 (s, CO_2H); 138.09 (s, *ipso*-C); 129.62 and 126.32 (d, phenyl-CH); 63.46 (s, αC); 21.73 (q, CH_3). R_{fA} 0.85, R_{fB} 0.30, R_{fC} 0.35.

HCl · H-D-(αMe)Phg-NH_2: m.p. > 300°C. ^{1}H-NMR (D_2O) δ: 7.55 (m, 5H, phenyl-H); 2.12 (s, 3H, CH_3).

Synthesis of HCl · H-L-(αMe)Phe-OMe (**11**)

To a solution of $SOCl_2$ (0.81 ml, 11.1 mmol) in MeOH (16 ml) at −25°C, H-L-(αMe)Phe-OMe (1.73 g, 9.65 mmol) was added. The solution was refluxed 20 h and more $SOCl_2$ was added during this period (3.75 ml, 8.19 mmol) after previous cooling of the solution at 0°C. The solvent was removed under reduced pressure and the product was crystallized from $MeOH/Et_2O$; yield 1.89 g, 85%; m.p. 108.5–110.0°C; $[\alpha]_D^{20}$ +8.5° (*c* 0.53, MeOH). ^{1}H-NMR (D_2O) δ: 7.41–7.17 (2m, 5H, phenyl-H); 3.83 (s, 3H, OMe); 3.37 and 3.13 (2d, *J* 14 Hz, 2H, βCH_2); 1.66 (s, 3H, CH_3). ^{13}C-NMR (D_2O) δ: 172,19 (s, CO_2); 132.98 (s, *ipso*-C); 130.20–128.52 (3d, phenyl-CH); 61.20 (s, αC); 53.94 (q, OMe); 42.66 (t, βCH_2); 21.66 (q, CH_3). R_{fB} 0.47, R_{fC} 0.56.

Synthesis of Z-L-(αMe)Phe-OH (**12**)

To a solution of H-L-(αMe)Phe-OH (7.01 g, 39 mmol) in 2N NaOH (19.5 ml) and acetone at 0°C a solution of Z-Cl (6.3 ml, 43 mmol) in acetone was added dropwise in 1 h. The pH was kept between 10 and 11 by addition of 2N NaOH. The mixture was stirred at r.t. for 12 h. The acetone was removed under reduced pressure and the Z-Cl excess was extracted with Et_2O. The aqueous phase was acidified to pH 3 with 2N HCl and the product was extracted with EtOAc. This was washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The product was obtained as an oil; yield 10.56 g, 86.2%; $[\alpha]_D^{20}$ −20.0° (*c* 0.52, MeOH).
^{1}H-NMR δ: 7.36 (m, 5H, Z-phenyl-H); 7.23–6.96 [m, 5H, (αMe) Phe-phenyl-H]; 5.44 (s, 1H, NH); 5.23–5.07 [2d, 2H, Z-CH_2]; 3.43–3.21 (2d, 2H, (αMe)Phe-βCH_2); 1.66 (s, 3H, CH_3).
^{13}C-NMR δ: 178.28 (s, CO_2H); 154.90 (s, OCONH); 135.77 [s, Z and/or (αMe)Phe-*ipso*-C] 130.04–126.99 [d, Z and (αMe)Phe-phenyl CH]; 66.65 (t, Z-CH_2); 60.44 (s, αC); 41.37 [t, (αMe)Phe-βCH_2]; 23.58 (s, CH_3). R_{fA} 0.75, R_{fC} 0.79.

Synthesis of Z-L-Val-OH (**13**)

This compound was synthesized according to the procedure described for Z-L-(αMe)Phe-OH; yield 4.26 g, 54.3%; m.p. 59.5–60.5°C; $[\alpha]_D^{20}$ −5.3° (*c* 0.49, MeOH). ^{1}H-NMR δ: 9.4 (br s, 1H, OH); 7.5–7.3 (m, 5H, Z-phenyl-H); 6.03 + 5.27 (d, 1H, Val-NH, rotamers); 5.08 (s, 2H, Z-CH_2); 4.34 + 4.20 (dd, 1H, $^\alpha$CH, ^{1}J 4.4 = Hz, ^{2}J 8.5 Hz); 2.23 (m, 1H, βCH); 1.30 and 0.94 (d, 3H, 2 γCH_3). ^{13}C-NMR δ: 178.30 (s, COO); 157,95 (s, OCONH); 137.59 (s, Z-*ipso*-C); 130.04–129.64 (d, Z-phenyl CH); 68.72 (t, Z-CH_2); 60.36 (d, αCH); 32.53 (d, βCH); 20.48 and 18.83 (q, 2 γCH_3). R_{fA} 0.88, R_{fB} 0.90, R_{fC} 0.1.

Synthesis of the oxazolone **16** *from Z-L-(αMe)Phe-OH*

To a solution of Z-L-(αMe)Phe-OH (2.99 g, 9.55 mmol) in CH_3CN (15 ml), HCl.EDC (Et-N=C=N-CH_2-CH_2-NMe_2) was added (2.01 g, 10.4 mmol) at 0°C. The reaction was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the residue dissolved in EtOAc (60 ml), washed with 0.5M citric acid, water, 5% $NaHCO_3$, water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The product was a 2/1 mixture of oxazolone from Z-L-(αMe)Phe-OH and $[Z\text{-L-}(\alpha Me)Phe]_2O$, and was obtained as an oil; yield oxazolone + $[Z\text{-L-}(\alpha Me)Phe]_2O$ (2/1) 92%.
^{1}H-NMR δ: 7.4–7.0 [m, 10 H, Z and (αMe)Phe-phenyl-H]; 5.28 and 5.25 (2d, *J* 19 Hz, 2H, Z-CH_2); 3.09 and 3.01 [2d, *J* 13.4 Hz, 2H, (αMe)Phe-CH_2]; 1.54 (s, 3H, CH_3). ^{13}C-NMR δ: 179.66 (s, COO); 158.68 (s, C=N); 136.47 and 135.96 (2s, *ipso*-C); 132.24–129.99 (6d, phenyl-CH); 73.33 (t, Z-CH_2); 62.36 (s, αC); 46.26 [t, (αMe)Phe-CH_2]; 26.03 (q, CH_3). R_{fB} 0.79, R_{fC} 0.89.

Synthesis of $[Z\text{-L-}(\alpha Me)Phe]_2O$ (**17**)

To a solution of oxazolone from Z-L-(αMe)Phe-OH (2.46 g, 8.33 mmol) in CH_3CN, Z-L-(αMe)Phe-OH was added (2.99 g, 9.55 mmol) and the mixture was stirred at room temperature. After 48 h, the solvent was removed under reduced pressure, EtOAc was added (120 ml) and the organic layer washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The product was obtained as an oil; yield 4.72 g, 93%; $[\alpha]_D^{20}$ −46.7° (*c* 0.54, AcOEt). IR (neat) cm^{-1}: 3403.9, 3336.1, 1820.2, 1782.6, 1719.1, 1497.0, 1454.3. ^{1}H-NMR δ: 7.4–6.9 [m, 10H, Z and (αMe)Phe-phenyl-H]; 5.60 (s, 2H, Z-CH_2); 4.92 (s, 1H, NH); 3.3–3.1 [2d, 2H, (αMe)Phe-CH_2]; 1.40 (s, 3H, CH_3). ^{13}C-NMR δ: 168.42 (s, COO); 154.83 (s, OCONH); 134.92 and 130.02 (2s, *ipso*-C); 130.42–127.21 (6d, phenyl-CH); 66.92 (t, Z-CH_2); 60.56 (s, αC); 41.11 [t, (αMe) Phe-CH_2]; 22.55 (q, CH_3). R_f 0.56 (EtOAc/petroleum-ether-(40–60°C) 2/7).

Synthesis of Z-L-Val-L-(αMe)Phe-OMe (**14**)

To a solution of Z-L-Val-OH (1.66 g, 6.61 mmol) and *N*-methylmorpholine (NMM, 0.73 ml, 6.59 mmol) in THF (20 ml) at −20°C, isobutyl chloroformate (0.94 ml, 7.25 mmol) was added, followed, after 10 min, by a suspension of HCl·H-L-(αMe)Phe-OMe (1.517 g, 6.60 mmol) and NMM (0.725 ml, 6.59 mmol) in $CHCl_3$ (15 ml) at 0°C. After the reaction mixture was stirred 65 h at room temperature, the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed with 0.5M citric acid, water, 5% $NaHCO_3$, water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (*n*-hexane/EtOAc 3/1); yield 2.11 g, 74.8%; m.p. 82–82.8°C; $[\alpha]_D^{20}$ −48.0° (*c* 0.5, MeOH). ^{1}H-NMR δ: 7.40 (m, 5H, Z-phenyl-H); 7.2 [m, 3H, (αMe)Phe *m*- and *p*-H]; 7.0 [m, 2H, (αMe)Phe *o*-H]; 6.48 [s, 1H, (αMe)Phe-NH]; 5.42 (d, 1H, Val-NH); 5.10 (s, 2H, Z-CH_2); 3.91 (m, 1H, Val-αCH); 3.7 (s, 3H, OMe); 3.5–3.1 [AB system, 2H, (αMe)Phe-CH_2]; 2.12 (m, 1H, Val-βCH); 1.59 (s, 3H, αCH_3)); 1.0–0.80 (dd, 6H, Val-γCH_3). ^{13}C-NMR δ: 173.82 and 170.45 (2s, COO and CONH); 153.21 (s, OCONH); 135.84 (s, *ipso*-C); 129.74–127.11 [6d, Z-and (αMe)Phe-phenyl CH]; 67.01 (t, Z-CH_2); 61.37 [s, (αMe)Phe-αC]; 60.56 (d, Val-αCH); 52.65 (q, OMe); 41.77 [t, (αMe)Phe-CH_2); 31.08 (d, Val-βCH_2); 23.02 [q, (αMe)Phe-CH_3]; 19.13 and 17.70 (2q, Val-γCH_3).

Synthesis of $Z[\text{-L-}(\alpha Me)Phe]_2\text{-OMe}$ (**18**)

Method a. To a suspension of HCl·H-(αMe)Phe-OMe (3.22 g, 14.0 mmol) and NMM (1.54 ml, 14.0 mmol) in CH_3CN, a mixture of oxazolone **16** from Z-L-(αMe)Phe-OH (11.1 mmol) and $(Z\text{-L-}(\alpha Me)Phe)_2O$ (2.96 mmol) was added and the mixture was stirred at room temperature for 3 days and then refluxed for 24 h.

Method b. To a suspension of HCl·H-L-(αMe)Phe-OMe (1.44 g, 6.3 mmol) and NMM (0.70 ml, 6.37 mmol) in CH_3CN, $[Z\text{-L-}(\alpha Me)Phe]_2O$ (4.09 g, 6.7 mmol) was added and the mixture was stirred at room temperature for 4 days and then refluxed for 9 days. The precipitated salts were filtered off. The solvent was evaporated under reduced pressure, the residue dissolved in EtOAc and washed with 0.5M citric acid, water, 5% $NaHCO_3$, water, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (*n*-hexane/EtOAc 7/2); yield method (*a*) 3.83 g (white solid), 55.9%; method (*b*) 1.93 g, 62.6%; m.p.145.5–145.9°C; $[\alpha]_D^{20}$, −67.8° (*c* 0.54, MeOH). ^{1}H-NMR δ: 7.36 (m, 5H, Z-phenyl-H); 7.17 (m, 6H, (αMe)Phe 1 and 2 *m*- and *p*-H); 7.00 [m, 4H, (αMe)Phe 1 and 2 *o*-H]; 6.79 and 5.46 (2s, 2H, [αMe)Phe 1 and 2 NH]; 5.10 (s, 2H, Z-CH_2); 3.78 (s, 3H, OMe); 3.45 and 3.14 (AB system, *J* 13.6 Hz, 2H, (αMe)Phe 1 or 2 CH_2); 3.23 and 3.11 [AB system, *J* 13.6 Hz, 2H, (αMe)Phe 1 or 2 CH_2]; 1.61 and 1.39 [2s, 6H, (αMe)Phe 1 and 2 βCH_3]. ^{13}C-NMR δ: 172.85 and 170.90 (2s, COO and CONH); 153.38 (s, OCONH); 135.05 and 134.470 (2s, *ipso*-C); 128.95–125.46 [9d, 15 H, Z and (αMe)Phe 1 and 2 phenyl-CH); 65.07 (t, Z-CH_2); 59.77 and 58.85 [2s, (αMe)Phe 1 and 2 αC]; 40.60 and 40.18 [2t, (αMe)Phe 1 and 2 CH_2]; 22.26 and 21.19 (2q, [αMe)Phe 1 and 2 CH_3]. R_{fB} 0.82, R_{fC} 0.89.

Synthesis H-L-Val-L-(αMe)Phe-OMe (**15**)

Z-L-Val-L-(αMe)Phe-OMe (1.683 g, 3.94 mmol) was dissolved in 70 ml of MeOH and the Z group was removed by catalytic hydrogenation (0.2 g of 10% Pd–C, 1 atm H_2 pressure). The reaction was completed after 2 h. The catalyst was filtered off on a celite bed and the solvent was removed under reduced pressure. The product was obtained as an colourless oil; yield 1.16 g, 100%; $[\alpha]_D^{20}$ −7.7° (*c* 0.52, MeOH). ^{1}H-NMR δ: 7.8–7.7 (m, 3H, *m* and *p*-H); 7.70 [s, 1H, (αMe)Phe-NH]; 7.1–7.0 (m, 2H, *o*-H); 3.73 (s, 3H, OMe); 3.30 (s, 2H, CH_2); 3.25 (d, 1H, Val-αCH); 3.00 (s, 2H, Val-NH_2); 2.22 (m, 1H, Val-βCH); 1.55 [s, 3H, (αMe)Phe-CH_3]; 1.00 and 0.85 (2d, 6H, Val-CH_3). ^{13}C-NMR δ: 172.75 and 171.92 (2s, COO and CONH); 134.41 (s, *ipso*-C); 128.62–125.47 (3d, phenyl-CH): 58.57 (s, (αMe) Phe-αC); 58.45 (d, Val-αCH); 50.89 (q, OMe); 40.51 [t, (αMe)Phe-CH_2]; 29.32 (d, Val-βCH); 21.23, 17.87 and 14.82 [3q, (αCH_3)Phe and Val-CH_3].

Synthesis of $H[\text{-L-}(\alpha Me)Phe]_2\text{-OMe}$ (**19**)

The product was synthesized from $Z[\text{-L-}(\alpha Me)Phe]_2$-OMe according to the procedure described for H-L-Val-L-(αMe)Phe-OMe. It was obtained as an oil that solidified on standing; yield 2.73 g, 95%; $[\alpha]_D^{20}$ −40.2° (*c* 0.49, MeOH). ^{1}H-NMR δ: 8.03 (s, 1H, (αMe)Phe_2-NH); 7.23 (m, 6H, (αMe)Phe 1 and 2 *m*- and *p*-H); 6.94 [m, 4H, (αMe)Phe 1 and 2 *o*-H]; 3.71 (s, 3H, OMe); 3.31 and 3.18 [AB system, *J* 13.3 Hz, 2H, (αMe)Phe 1 or 2 CH_2]; 3.27 and 2.73 [AB system, *J* 12.9 Hz, 2H, (αMe)Phe 1 or 2 CH_2]; 1.76 (s, 2H, NH_2); 1.57 and 1.34 [2s, 6H, (αCH_3)Phe 1 and 2]. ^{13}C-NMR δ: 175.12 and 173.71 (2s, COO and CONH); 136.29 and 135.64 (2s, (αMe)Phe 1 and 2 *ipso*-C); 130.22–126.35 [6d, (αMe)Phe 1 and 2 phenyl-CH]; 59.71 and 57.98 [2s, (αMe)Phe 1 and 2 αC]; 51.88 (q, OMe); 45.45 and 41.52 [2t, (αMe)Phe 1 and 2 βCH_2]; 26.99 and 22.30 [2q, (αMe)Phe 1 and 2 CH_3]. R_{fB} 0.56, R_{fC} 0.64.

Synthesis of Nap-L-Val-L-(αMe)Phe-OMe (**1**)

H-L-Val-L-(αMe)Phe-OMe (1.13 g, 3.86 mmol) and 2-hydroxy-naphthalene-1-carboxadehyde (1.00 g, 5.79 mmol) were dissolved in MeOH (90 ml) and the solution was stirred at room temperature for 19 h.

The solvent was then removed under reduced pressure and the crude product was purified by column chromatography (n-hexane/EtOAc 5/2); yield 1.23 g (yellow crystals), 71.3%; m.p. 147.5–148°C; $[\alpha]_D^{20}$ +45.7° (*c* 0.49, MeOH). ^{1}H-NMR δ: 14.45 (br s, 1H, OH); 9.00 (s, 1H, CH=N); 8.25 (d, 1H, naphthyl-H4); 7.85 (d, 1H, naphthyl-H5 or -H8); 7.75 (dd, 1H, naphthyl-H5 or -H8); 7.55 (qd, 1H, naphthyl-H6 or -H7); 7.40 (qd, 1H, naphthyl-H6 or -H7); 7.15 (d, 1H, naphthyl-H3); 7.02 [m, 5H, (αMe)Phe-phenyl-H]; 6.45 (s, 1H, NH); 3.80 (s, 3H, OMe); 3.75 (dd, 1H, Val-αCH); 3.25 [s, 2H, (αMe)Phe-CH_2]; 2.50 (m, 1H, Val-βCH_2); 1.63 [s, 3H, (αMe)Phe-CH_3]; 0.95 (d, 6H, Val-CH_3). ^{13}C-NMR δ: 174.07 and 170.33 (s, COO and CONH); 167.08 (s, naphthyl-C2); 162.32 (d, CH=N); 136.05, 135.73 and 133.21 [s, naphthyl-C9 and -C10, (αMe)Phe *ipso*-C); 108.98 (s, naphthyl-C1); 78.17 (d, Val-αCH); 60.52 [s, (αMe)Phe-αC]; 52.83 (q, OMe); 42.38 [t, (αMe)Phe-CH_2]; 31.95 (d, Val-βCH); 23.36, 19.88 and 17.60 [3q, Val-CH_3 and (αMe)Phe-CH_3].

Synthesis of Nap[-L-(αMe)Phe]$_2$-OMe (**2**)

The product was synthesized according to the procedure described for Nap-L-Val-L-(αMe)Phe-OMe. The crude was purified by column chromatography (n-hexane/EtOAc 5/1 to 3/1); yield 1.41 g (yellow crystals), 88.5%; m.p. 115.5–117.8°C; $[\alpha]_D^{20}$ −30.0° (*c* 0.50, MeOH). ^{1}H-NMR δ: 14.52 (br s, 1H, OH); 8.71 (s, 1H, CH=N); 7.79 (d, 1H, naphthyl-H4); 7.69 (dd, ^1J 7.8 Hz, ^2J 2 Hz, 1H, naphthyl-H5 or -H8); 7.66 (dd, ^1J 8.0 Hz, ^2J 2.0 Hz, 1H, naphthyl-H5 or -H8); 7.42 (qd, ^1J 9.0 Hz, ^2J 6.85 Hz, ^3J 1.5 Hz, 1H, naphthyl-H6 or -H7); 7.29 (m, 1H, naphthyl-H6 or H7); 7.25–7.10 [m, 5H, (αMe)Phe 1 or 2 phenyl-H]; 7.07 (d, *J* 9.18 Hz, 1H, naphthyl-H3); 7.0–6.8 [m, 5H, (αMe)Phe 1 or 2 phenyl-H]; 3.74 (s, 3H, OMe); 3.28 and 3.18 [AB system, *J* 13.4 Hz, 2H, (αMe)Phe 1 or 2 CH_2]; 3.16 [s, 2H, (αMe)Phe 1 or 2 CH_2]; 1.60 and 1.58 [2s, 3H + 3H, (αMe)Phe 1 and 2 CH_3]. ^{13}C-NMR δ: 171.63 and 170.63 (2s, COO and CONH); 167.55 (s, naphthyl-C2); 156.30 (d, CH=N); 134.41–116.83 [16d, naphthyl and (αMe)Phe 1 and 2 Ar-CH]; 133.30, 133.12 and 131.29 [3s, naphthyl-C9 and -C10 and (αMe)Phe 1 and 2 *ipso*-C); 106.12 (s, naphthyl-C1); 66.00 and 58.60 (2s, (αMe)Phe 1 and 2 αC); 50.62 (q, OMe); 44.03 and 40.91 [2t, (αMe)Phe 1 and 2 CH_2]; 21.11 and 20.04 [2q, (αMe)Phe 1 and 2 CH_3].

Synthesis of Nap-L-valinol (**3**)

The product was synthesized according to the procedure described for Z-L-Val-L-(αMe)Phe-OMe, starting from 1.46 g 2-hydroxynaphthalene-1-carboxaldehyde and 0.58 g H-L-valinol. The crude was purified by column chromatography (n-hexane/EtOAc 5/9); yield 1.36 g (yellow crystals), 93.4%; m.p. 121.5–122°C; $[\alpha]_D^{20}$ −79.4° (*c* 0.50, MeOH); ^{1}H NMR δ: 14.25 (br s, 1H, naphthyl-OH); 8.65 (s, 1H, CH=N); 7.74 (d, *J* 8.31 Hz, 1H, naphthyl-H4); 7.42–7.24 (m, 3H, naphthyl-H5, -H6 or -H7, -H8); 7.12 (ddd, ^1J 7.5 Hz, ^2J 6.5 Hz, ^3J 1 Hz, 1H, naphthyl-H6 or -H7); 6.65 (d, 1H, naphthyl-H3); 4.0–3.7 (m, 2H, valinol-CH_2); 3.32 (m, 1H, valinol-αCH); 2.00 (m, 1H, valinol-βCH); 1.65 (br s, 1H, valinol-OH); 1.00 and 1.05 (2d, 6H, valinol-γCH_3). ^{13}C-NMR δ: 178.62 (s, naphthyl-C2); 158.94 (d, CH = N); 138.08–118.01 (6d, naphthyl-CH); 134.18 and 126.03 (2s, naphthyl-C9 and -C10); 106.20 (s, naphthyl-C1); 72.24 (d, valinol-αCH); 64.17 (t, valinol-CH_2); 29.99 (d, valinol-βCH); 20.32 and 18.60 (2q, valinol-γCH_3).

General procedure for NMR study

Nap[-L-(αMe)Phe]$_2$-OMe (101.6 mg, 0.20 mmol) and $Ti(OiPr)_2Cl_2$ (0.20 ml of a 1.0M solution in $CDCl_3$, 0.20 mmol) were dissolved in 2 ml of $CDCl_3$ and 1.0 g of molecular sieves 4A were added. The solution was stirred 1 h at room temperature under nitrogen. The solution (1 ml) was then transferred into a NMR tube after the molecular sieves were filtered off and ^{1}H and ^{13}C-NMR were immediately taken.

Synthesis of 3-(2-butenoyl)oxazolidin-2-one

To a solution of oxazolidin-2-one (2.67 g, 30 mmol) in THF (100 ml) at −60°C, BuLi (1.6 M in hexane, 18.75 ml, 30 mmol) was added. After 15 min, 2-butenoyl chloride (3.52 ml, 33 mmol) was also added and the mixture was stirred at −60°C for 30 min, then at 0°C for additional 15 min. The solution was poured in 200 ml of saturated NH_4Cl and concentrated under reduced pressure. The aqueous layer was extracted with Et_2O, the combined extracted were concentrated and washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude was purified by column chromatography (*n*-hexane/EtOAc 2/1); yield 1.84 g, 40%; m.p. 39.8–40.2°C. ^{1}H-NMR δ: 7.35–7.05 (m, 2H, CH = CH); 4.45 (m, 2H, CH_2O); 4.05 (m, 2H, NCH_2); 2.00 (d, 3H, CH_3). ^{13}C-NMR δ: 166.23 (s, CHCON); 154.66 (s, NCOO); 147.80 (d, CH=CHCO); 122.56 (d, CH=CHCO); 63.19 (t, CH_2O); 43.77 (t, NCH_2); 19.59 (q, CH_3).

General procedure for the Diels–Alder reactions with propenal or but-3-en-2-one

$Ti(O^iPr)_4$ (290 mg, 1.0 mmol) and Nap[-L-(αMe)Phe]$_2$-OMe (508 mg, 1.0 mmol) were dissolved in CH_2Cl_2 (40 ml) and stirred at room temperature and under inert atmosphere for 1 h. The solution was cooled at −78°C and propenal (0.69 ml, 10 mmol) and cyclopentadiene (1.334 g, 20 mmol) were added. The solution was then stirred at −20°C for 20 h. The reaction mixture was poured in saturated $NaHCO_3$ (50 ml) and the product extracted with EtOAc (2×50 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure.

Bicyclo[2.2.1]hept-5-ene-2-carboxaldehyde. The *exo* and *endo* isomers have never been completely separated by column chromatography. Therefore the assignation of the signal is not completely certain, especially for the *exo* isomer.
Endo isomer: ^{1}H-NMR δ: 9.42 (d, 1H, CHO); 6.20 (dd, ^1J 5.66 Hz, ^2J 3.2 Hz, 1H, CH=CH); 6.01 (dd, ^1J 5.66 Hz, ^2J 2.71 Hz, 1H, CH=CH); 3.25 (m, 1H, CH–CH=CH); 3.0–2.8 (2m, 2H, CH–CH=CH + OC–CH–CH_2); 1.90 (ddd, ^1J 13.05 Hz, ^2J 9.45 Hz, ^3J 3.55 Hz, 1H, from OC–CH–CH_2); 1.5–1.2 (m, 3H, from OC–CH–CH_2 and CH–CH_2–CH). ^{13}C-NMR δ: 205.20 (d, CHO); 138.62 and 131.83 (2d, CH=CH); 52.23 (d, OC–CH–CH_2); 49.64 (t, CH–CH_2–CH); 45.02 and 42.75 (2d, CH–CH=CH–CH); 27.60 (t, OC–CH–CH_2).
Exo isomer. ^{1}H NMR δ: 9.81 (d, 1H, CHO); 6.15 and 6.00 (2dd, 2H, CH=CH); 3.10 and 2.95 (2m, 2H, CH–CH=CH–CH); 2.32 (m, 1H, OC–CH–CH_2); the assignation of the other signals is not certain.
Determination of enantiomeric excess (*ee*). The mixture of *exo* and *endo* isomers was previously derivatized with (2*R*,4*R*)-pentanediol to give the corresponding acetals, as follows: a mixture of the Diels–Alder adducts (0.1 mmol), (2*R*,4*R*)-pentanediol (0.14 mmol) and triethyl orthoformate (0.12 mmol) in dry benzene (2 ml) was stirred at room temperature for 1 h in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*TSA·H_2O, 1–2 mg). The mixture was poured into saturated $NaHCO_3$ (5 ml) and extracted with Et_2O (2×5 ml). The organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure giving the acetals in ~100% yield. The diastereoselectivity of of the acetals was determined by GLC analysis; column: 2,5-dipentyl-(3-trifluoroacetyl)-γ-cyclodextrine (30 m×0.25 mm), temperature: 60°C (5 min) → 1°C/min → 130°C (5 min), pressure: 50 kPa (N_2).

1-(Bicyclo[2.2.1]hept-5-en-2-yl)ethanone (5-acetyl-2-norbornene).
Endo isomer: ^{1}H-NMR δ: 6.15 and 5.85 (2dd, 2H, CH=CH); 3.25 and 2.90 (2m, 2H, CH–CH=CH–CH); 2.98 (m, 1H, OC–CH–CH_2); 2.15 (s, 3H, CH_3); 1.75 (m, 1H, from OC–CH–CH_2); 1.51 (m, 2H, CH–CH_2–CH); 1.30 (m, 1H, from OC–CH–CH_2). ^{13}C-NMR δ: 209.25 (s, CO); 138.19 and 131.59 (2d, CH=CH); 52.69 (d, OC–CH–CH_2); 50.30 (t, CH–CH_2–CH); 46.20 and 43.03 (2d, CH–CH=CH–CH); 29.53 (q, CH_3); 27.77 (t, OC–CH–CH_2).
Exo isomer: ^{1}H-NMR δ: 6.15 (2dd, 2H, CH=CH); 3.01 and 2.89 (2m, 2H, CH–CH=CH–CH); 2.42 (m, 1H, OC–CH–CH_2); 2.23 (s, 3H, CH_3); 2.0–1.7 (m, 3H, OC–CH–CH_2 + CH–CH_2–CH); 1.29 (m, 1H, from CH–CH_2–CH).
ee determination. Via GLC analysis of the diastereomers; column: 2,5-dipentyl-3-(trifluoracetyl)-γ-cyclodextrine (30 m×0.25 mm), temperature: 110°C, pressure: 65 kPa (N_2).

General procedure for the Diels–Alder reaction with 3-(2-butenoyl)-oxazolidin-2-one

To a solution of $Ti(O^iPr)_2Cl_2$ (1.3 ml of a 0.3M solution in toluene, 0.39 mmol) in toluene (45 ml) molecular sieves 4A (2 g) and Nap[-L-(αMe)Phe]$_2$-OMe (200 mg, 0.39 mmol) were added and the solution was stirred at room temperature, under inert atmosphere for 1 h. The solution was then cooled at 0°C and cyclopentadiene (529 mg, 8 mmol) and 3-(2-butenoyl)oxazolidin-2-one (608 mg, 3.9 mmol) were added and the mixture was stirred at that temperature for 24 h. The solution was poured in 200 ml of phosphate buffer at pH 7, filtered and extracted many times with EtOAc (5×150 ml). The organic

phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure.

3-[(3-Methylbicyclo[2.2.1]hept-5-en-2-yl)carbonyl)]oxazolidin-2-one. *Endo* isomer. M.p. 98.5–99.5°C. ^{1}H-NMR δ: 6.40 and 5.75 (2dd, 1H + 1H, C*H*=C*H*); 4.38 and 3.95 (2m, 4H, N-CH_2-CH_2-O); 3.51 (dd,1H, OC-C*H*-CH-CH_3); 3.30 and 2.50 (2m, 2H, C*H*-CH=CH-C*H*); 2.2–2.0 (m, 1H, OC-CH-C*H*-CH_3); 1.68 and 1.53 (2m, 2H, CH-CH_2-CH); 1.12 (d, 3H, CH_3). ^{13}C-NMR δ: 174.86 (s, O*C*-CH-CH_3); 153.82 (s, N-*C*O-O); 140.14 and 131.37 (2d, *C*H=*C*H); 62.31 (t, N-CH_2-*C*H_2-O); 51.72, 49.95 and 47.90 (3d, OC-*C*H-CH-CH_3 and/or *C*H-CH=CH-*C*H); 47.57–43.44 (2t, N-*C*H_2-CH_2-O and CH-*C*H_2-CH); 36.90 (d, OC-CH-*C*H-CH_3); 20.84 (q, CH_3).
Exo isomer. ^{1}H-NMR δ: 6.29 and 6.15 (2dd, 2H, C*H*=C*H*); 4.40 and 4.05 (2m, 4H, N-CH_2-CH_2-O); 2.9–2.6 (m, 4H, C*H*-CH=CH-C*H* and OC-C*H*-C*H*-CH_3); 1.65 and 1.38 (2m, 2H, CH-CH_2-CH); 0.85 (d, 3H, CH_3). ^{13}C-NMR δ: 176.59 and 153.82 (2s, O*C*-CH-CH-CH_3 and N-*C*O-O); 137.92 and 136.56 (2d, *C*H=*C*H); 62.82 (t, N-CH_2-*C*H_2-O); 51.68 and 50.56 (2d, *C*H-CH=CH-*C*H); 48.54 (d, OC-*C*H-CH-CH_3); 47.69 and 44.09 (2t, N-*C*H_2-CH_2-O and CH-*C*H_2-CH); 38.40 (OC-CH-*C*H-CH_3); 19.84 (q, CH_3).

Synthesis of methyl oxoacetate

The product was obtained by vacuum distillation of methyl oxoacetate methyl hemiacetal (10.168 g, 84 mmol) over P_2O_5[19] (b.p. 64–67°C/20 mmHg) and it was collected at −78°C; yield 45%; ^{1}H-NMR δ: 9.4 (s, 1H, CHO); 3.95 (s, 3H, OMe). ^{13}C-NMR δ: 169.81 (s, COO); 93.30 (d, CHO); 55.41 (q, OMe).

General procedure for the oxoacetate-ene reaction

To a suspension of molecular sieves 4A (1.12 g) in CH_2Cl_2 (15 ml), $Ti(O^iPr)_2Cl_2$ (0.8 ml of a 0.3M toluene solution, 0.24 mmol) and Nap[-L-(αMe)Phe]$_2$-OMe (122 mg, 0.24 mmol) were added under stirring, at room temperature and under an inert atmosphere. After stirring for 1 h, the solution was cooled at −78°C, then freshly distilled methyl oxoacetate (209 mg, 2.4 mmol) and isopropenylbenzene (581 mg, 4.9 mmol) were added. The solution was stirred at −30°C for 6 h. The molecular sieves were filtered off and the mixture poured into a saturated $NaHCO_3$ solution (20 ml). The product was extracted with EtOAc (2×25 ml) and the combined organic layer washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure.

Methyl 2-hydroxy-4-phenylpent-4-enoate. Purified by column chromatography (*n*-hexane/EtOAc 3/1). ^{1}H-NMR δ: 7.45–7.20 (m, 5H, phenyl-H); 5.41 and 5.15 (2m, 2H, C=CH_2); 4.25 (m, 1H, CH-O); 3.58 (s, 3H, OMe); 3.03 (ddd, ^1J 14 Hz, ^2J 4.5 Hz, ^3J 1 Hz, 1H, CH_2 and br s, 1H, OH); 2.81 (ddd, ^1J 14 Hz, ^2J 7.7 Hz, ^1J 1 Hz, 1H, CH_2). ^{13}C-NMR δ: 173.26 (s, COO); 142.00 (s, *C*=CH_2); 138.71 (s, *ipso*-C); 126.82, 126.15 and 124.86 (3d, phenyl-CH); 114.65 (t, C=*C*H_2); 67.72 (d, CH-O); 50.63 (q, OMe); 38.92 (t, CH_2).
ee determination by HPLC, column: Chiraleel OB 250×4.6 mm; eluent: *n*-hexane/2-propanol 90/10; flow: 1.0 ml/min; detection: UV 210 nm; temperature: room temperature.

General synthesis of mandelonitrile (2-hydroxybenzeneacetonitrile)

$Ti(OEt)_4$ (83 mg, 0.36 mmol) and Nap[-L-(αMe)Phe]$_2$-OMe (183 mg, 0.36 mmol) were dissolved in toluene (8 ml) and stirred under inert atmosphere at room temperature for 1 h. The mixture was then cooled at −78°C and Me_3SiCN (0.75 ml, 5.5 mmol) and benzaldehyde (0.18 ml, 1.77 mmol) were added. The solution was stirred at that temperature for 20 h. The solution was then poured in 2N HCl (200 ml) and stirred for 2 h. The product was extracted with EtOAc (4×200 ml). The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure.

Mandelonitrile. The crude product was purified by column chromatography (n-hexane/EtOAc 5/1). ^{1}H-NMR δ: 5.52 (s, 1H, CH); 3.58 (br s, 1H, OH); 7.6–7.3 (m, 5H, phenyl-H). ^{13}C-NMR δ: 192.68 (s, CN); 129.07, 128.47 and 125.99 (3d, phenyl-CH); 118.32 (s, *ipso*-C); 62.74 (d, CH).
ee determination. Via HPLC analysis; column: Daicel Chiracel OD (250×4.6mm); eluent: *n*-hexane/2-propanol 90/10; flux: 0.5 ml/min; temperature: room temperature; detector: linear 203 UV. For yields and *ee*s see Table I.

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