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DIASTEREOSELECTIVE HYDROGENATION OF MONODEHYDRO ENKEPHALINS CONTROLLED BY CHIRAL RHODIUM CATALYSTS

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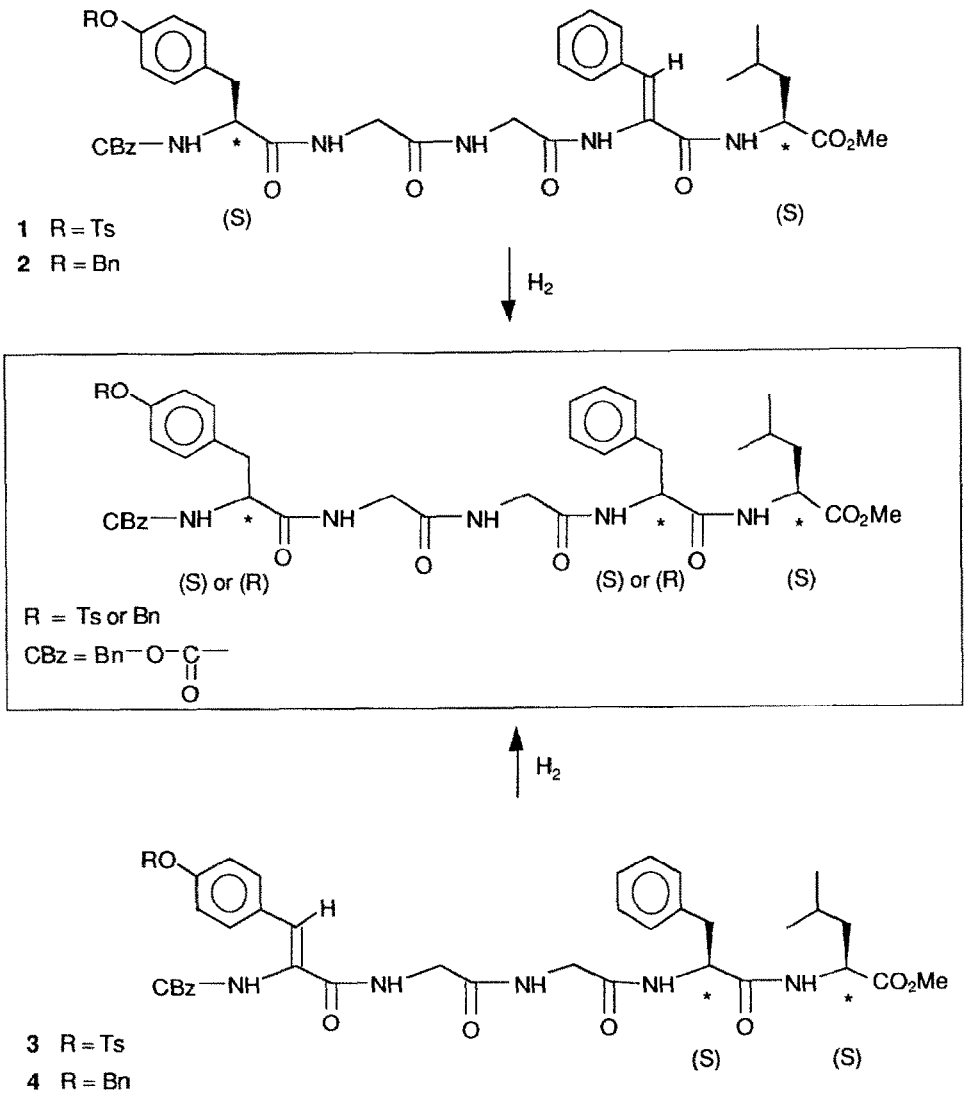
Abstract :
Protected (Z)dehydrophenylalanyl-Leu-enkephalin, (Z)dehydrotyrosyl-Leu-enkephalin and (Z)dehydrotyrosyl-(R)Ala^2-Leu-enkephalin, have been synthesized. These compounds have been hydrogenated to give protected Leu-enkephalins in the presence of various chiral rhodium complexes. Deprotection of the product gave Leu-enkephalins or epimers, ytterbium in liquid ammonia allows smooth deprotection of NHCBz or OTs groups on small amounts of peptides. Strong stereocontrol could be achieved by suitable choice of the chiral catalyst. This method has good potential for stereospecific labelling of enkephalins and other small peptides.

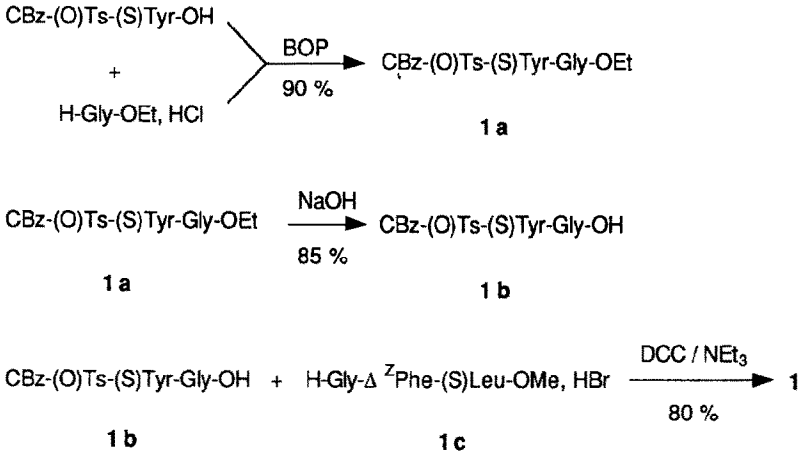
Asymmetric catalysis can apply to the formation of an asymmetric center in an already chiral molecule. A double asymmetric induction will occur[1], one is external (catalyst) and the other is internal (chiral backbone of the substrate). An interesting case happens when the external asymmetric induction is the predominant factor in the mismatched as well as in the matched pair[2]. The "catalyst control" allows us to obtain, at will, one given diastereomer of the product. This has not been investigated very much in catalytic homogeneous hydrogenation apart from the case of some dehydropeptides[3-9], including a bisdehydroenkephalin analog[8b] or of some allylic alcohols[10]. We wish to report the application of this concept to the synthesis of Leu-enkephalin and some of its epimers.

We previously published a preliminary report on the preparation of CBz-(O)Ts-(S)Tyr-Gly$_2$-Δ^ZPhe-(S)Leu-OMe **1**[9] and CBz-(O)Ts-Δ^ZTyr-Gly$_2$-(S)Phe-(S)Leu-OMe **3**[12]. The stereochemistry of asymmetric hydrogenation of these compounds was studied, however fully deprotected pentapeptides could not be easily obtained because of the presence of the O-tosyl moiety. We recently prepared the two dehydro-Leu-enkephalins **2** and **4** with an O-benzyl protection for the phenolic hydroxyl in the tyrosyl moiety. We will give here a full account on the synthesis of the various prepared dehydroenkephalins as well as the results of hydrogenation of these dehydropeptides in the presence of chiral rhodium complexes.



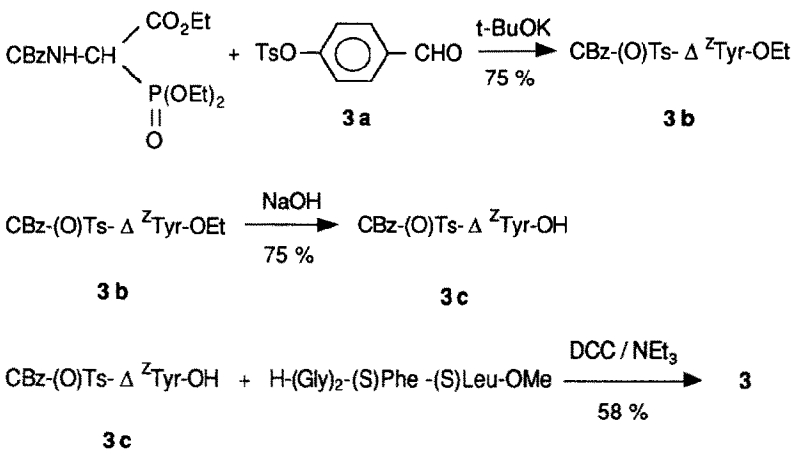
Synthesis of protected monodehydroenkephalins

CBz-(O)Ts-(S)Tyr-Gly2-Δ^{Z}Phe-(S)Leu-OMe **1** was prepared as described in Scheme 1, by a coupling reaction between CBz-(O)Ts-(S)Tyr-Gly-OH **1b** and H-Gly-Δ^{Z}Phe-(S)Leu-OMe, HBr **1c**. The synthesis of CBz-(O)Ts-(S)Tyr-OH is described in ref. 29. The unsaturated fragment **1c** was synthesized by CBz-Gly-Δ^{Z}Phe-OH azlactone opening with (S)leucine methyl ester, as described in ref. 24.



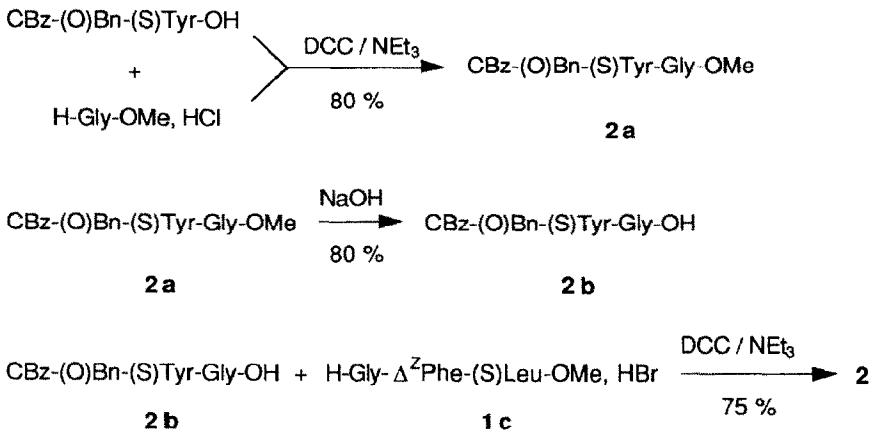
Scheme 1 *Synthesis of protected monodehydrophenylalanyl-Leu-enkephalin* **1**

CBz-(O)Ts-Δ^{Z}Tyr-Gly$_2$-(S)Phe-(S)Leu-OMe **3** was prepared according to Scheme 2. CBz-(O)Ts-Δ^{Z}Tyr-OH **3c** was obtained by action of the Schmidt reagent[11] on tosyl parahydroxybenzaldehyde **3a**.

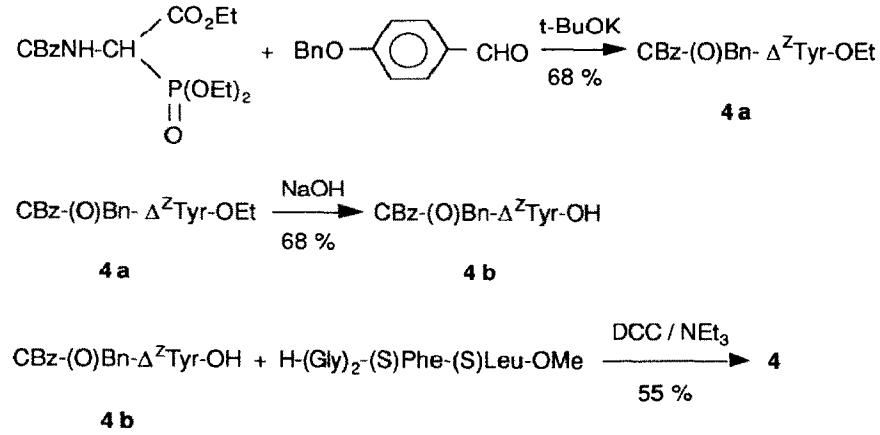


Scheme 2 *Synthesis of protected monodehydrotyrosyl-Leu-enkephalin* **3**

The two protected monodehydroenkephalins, CBz-(O)Bn-(S)Tyr-Gly_2-Δ^ZPhe-(S)Leu-OMe **2** and CBz-(O)Bn-Δ^ZTyr-Gly_2-(S)Phe-(S)Leu-OMe **4** were prepared by the condensation of CBz-(O)Bn-(S)Tyr-Gly-OH **2b** with H-Gly-Δ^ZPhe-(S)Leu-OMe **1c** (Scheme 3) and of CBz-(O)Bn-Δ^ZTyr-OH **4b** with H-Gly_2-(S)Phe-(S)Leu-OMe (Scheme 4). The synthesis of fragments CBz-(O)Bn-(S)Tyr-OH, and H-Gly-Δ^ZPhe-(S)Leu-OMe **1c** are described in refs. 25 and 24.

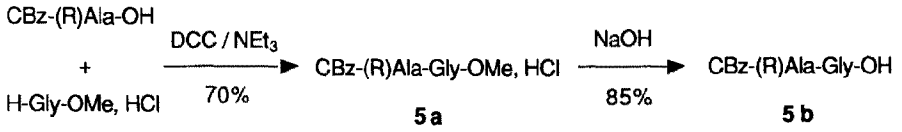


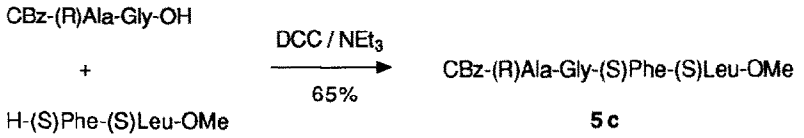
Scheme 3 *Synthesis of protected monodehydrophenylalanyl-Leu-enkephalin* **2**

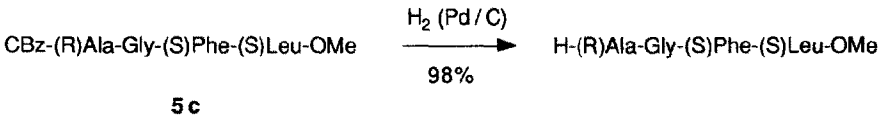


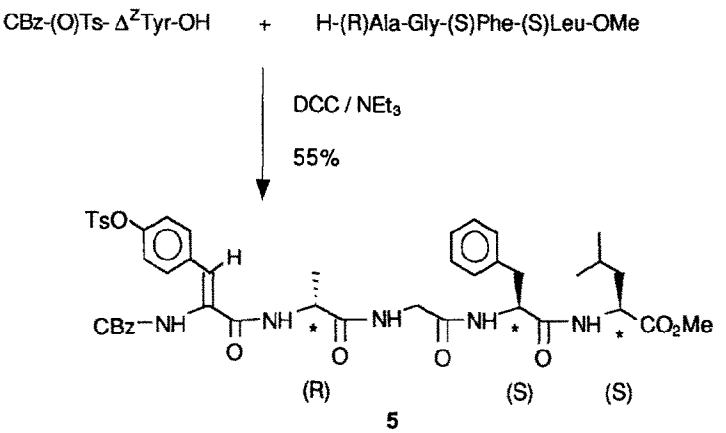
Scheme 4 *Synthesis of protected monodehydrotyrosyl-Leu-enkephalin* **4**

Finally CBz-(O)Ts-Δ^ZTyr-(R)Ala-Gly-(S)Phe-(S)Leu-OMe **5** was obtained by coupling CBz-(O)Ts-Δ^ZTyr-OH and H-(R)Ala-Gly-(S)Phe-(S)Leu-OMe (Scheme 5).









Scheme 5 *Synthesis of protected monodehydrotyrosyl-(R)Ala*^{2}*-Leu-enkephalin* **5**

All compounds have been fully characterized by ^1H nmr, elemental analysis, hplc (Zorbax ODS, MeOH/H_2O). The unsaturated building blocks having a double bond of Z configuration, double bond has Z configuration in the synthesized dehydroenkephalins.

Catalytic homogeneous hydrogenation

Asymmetric hydrogenation of **1-5** was performed in methanol in the presence of rhodium complexes containing various chiral diphosphines such as (S,S)diop[13], (S,S)bppm[14] or (R,R)dipamp[15]. The following catalysts were used : [Rh dipamp (COD)]$^+$ BF_4^-, "Rh Cl diop" and "Rh Cl bppm". The last two complexes were prepared *in situ* by addition of two equivalents of the chiral diphosphine to one equivalent of the dimeric complex [Rh Cl (COD)]$_2$. With Rh/diop and Rh/bppm catalysts some hydrogenations could be performed under one bar of hydrogen. With Rh/dipamp catalyst hydrogenations occured between 10 to 18 bar in order to avoid slow reactions. Conversions were always quantitative, as measured by ^1H nmr or hplc of the crude product. The mixture of epimeric products was analyzed by hplc (Zorbax ODS). Results were confirmed by cleavage of the pentapeptide and measurement of the enantiomeric excess of tyrosine or phenylalanine by chiral glc (XE60-(S)Val-(S)Phenylethylamide phase, derivatization into N-trifluoroacetyl amino acids isopropyl esters). Acidic cleavage and derivatization procedures were performed on 2 mg of crude pentapeptide in a one-pot procedure without isolation of compounds. In the case of hydrogenation of O-tosyl-dehydrotyrosyl-Leu-enkephalin **3** a special procedure is needed for deprotection of tyrosine phenolic group (see below).

Removing of protective groups

Deprotection of the pentapeptide products of hydrogenation was performed in the following way :

i) Saponification of the C-terminal methyl ester.
ii) Hydrogenolysis of the CBz and Bn groups (H_2, Pd/C 5%).

When the phenolic OH in tyrosyl fragment is protected as a tosylate, it is quite difficult to regenerate the free hydroxyl with integral retention of the pentapeptide structure. For example reduction by sodium in liquid ammonia is satisfactory for the conversion of (O)Ts-tyrosine into tyrosine, but the reaction is difficult to control for small scale reduction of various (O)Ts-Tyr-enkephalins. Naphthalene sodium in THF[16] was investigated as a reducing agent. It afforded good results in the transformation of CBz-(O)Ts-Tyr-OH into tyrosine or of CBz-(O)Ts-(S)Tyr-Gly$_2$-OH into H-Tyr-Gly$_2$-OH. However it was not successful with protected enkephalins, presumably because of their low solubility in THF. For that reason we set up a reagent able to work for the reductive deprotection of small amounts of peptides, avoiding undesirable peptide bond cleavages.

Ytterbium metal has a high atomic weight (173, compared to 23 for sodium) and is quite inert towards moisture and air. It is easy to handle in small quantities. White described its reducing properties in liquid ammonia and t-butanol[17]. Birch type reductions and triple bond reductions were observed. In order to avoid Birch reduction of aromatic rings we investigated the use of ytterbium in liquid ammonia in the absence of alcohols for the cleavage of benzyloxycarbonyl and tosyl groups. We obtained a quantitative deprotection of CBz-(O)Ts-(S)Tyr-OH and CBz-(O)Ts-(S)Tyr-Gly$_2$-OH, working on 0.15 mmol of peptide, with no racemization of the tyrosine. This procedure has been applied to the deprotection of CBz-(O)Ts-Tyr-Gly$_2$-Phe-(S)Leu-OH and

CBz-(O)Ts-Tyr-(R)Ala-Gly-(S)Phe-(S)Leu-OH, giving the corresponding enkephalins in good yields.

Results of asymmetric hydrogenation

As already found in asymmetric hydrogenation of dehydropeptides[3-11], (R,R)dipamp is a superior catalyst for diastereoselective reduction of dehydroenkephalins. (R,R)dipamp led to a predominance of (S) configuration, diastereomeric ratio up to 98:2 could be achieved in formation of (S)tyrosyl or (S)phenylalanyl moiety (Table 1, entries 1-4). (S,S)bppm gave a reversal of stereochemistry, with formation of (R)phenylalanyl and (R)tyrosyl fragments with diastereomeric ratio up to 90:10 and 80:20 respectively (entries 5-8). There is clearly a good "catalyst control" with (R,R)dipamp and (S,S)bppm catalysts, with stereochemical results ((S) and (R) configurations respectively) similar to asymmetric hydrogenation of N-acetyl-dehydrotyrosine or N-acetyl-dehydrophenylalanine derivatives[18]. We did not perform additional experiments with (S,S)dipamp and (R,R)bppm [19] which are not easily available although their synthesis has been described[20, 21]. Diop ((R,R) or (S,S) configuration) gave weakly stereoselective catalysts in the present case. Upon hydrogenation with (S,S)diop, the dehydrophenylalanyl-Leu-enkephalin **1** provides a 60:40 mixture of two diastereomers in favor of the (S)phenylalanyl fragment (entry 11).

The substitution of a glycyl residue by (R)alanyl did not much change the stereochemical course of asymmetric hydrogenations with (R,R)dipamp and (S,S)bppm catalysts, good "catalyst control" has also been observed (entries 9 and 10).

(R,R)dipamp is the best ligand for formation of (S) configuration in hydrogenation of protected dehydroenkephalins. We tested a potential use for the preparation of labelled enkephalins. For this purpose we selected the dehydrophenylalanyl enkephalin **2** and studied its deuterogenation in methanol in the presence of [Rh (R,R)dipamp (COD)]$^+$ catalyst. The reaction is complete after 48 h under 10 bar with quantitative formation of CBz-(O)Bn-(S)Tyr-Gly$_2$-(S)(2,3-^{2}H)Phe-(S)Leu-OMe. There is a clean incorporation of two deuterium atoms (and no exchange with proton from methanol). The diastereomeric ratio is very high (>99:1), higher than with hydrogen (entries 2 and 12).

Fully deprotected enkephalin is easily prepared by saponification of methyl ester followed by removal of CBz and Bn groups catalyzed by Pd/C. The final product coming from hydrogenation catalyzed by (R,R)dipamp complex is identical to an authentic natural enkephalin (^{1}H NMR and hplc on ODS column).

Table 1 Asymmetric hydrogenation of protected monodehydroenkephalins

Entry	Substrate[a]	Catalyst[b]	H_2 pressure (bar)[c]	Diastereomer ratio[d] S:R[e]
1	**1**	[Rh (R,R)dipamp COD]$^+$	10	96.5:3.5
2	**2**	" "	10	>98:<2
3	**3**	" "	18	91:9
4	**4**	" "	10	93:7
5	**1**	Rh Cl (S,S)bppm	1	16:84
6	**2**	" "	10	9:91
7	**3**	" "	18	20:80
8	**4**	" "	10	25:75
9	**5**	[Rh (R,R)dipamp COD]$^+$	10	89:11
10	**5**	Rh Cl (S,S)bppm	10	12:88
11	**1**	Rh Cl (S,S)diop	1	60:40
12	**2**	[Rh (R,R)dipamp COD]$^+$	10[f]	99.5:0.5

a) **1** : CBz-(O)Ts-(S)Tyr-(Gly)$_2$-Δ^ZPhe-(S)Leu-OMe.
2 : CBz-(O)Bn-(S)Tyr-(Gly)$_2$-Δ^ZPhe-(S)Leu-OMe.
3 : CBz-(O)Ts-Δ^ZTyr-(Gly)$_2$-(S)Phe-(S)Leu-OMe.
4 : CBz-(O)Bn-Δ^ZTyr-(Gly)$_2$-(S)Phe-(S)Leu-OMe.
5 : CBz-(O)Ts-Δ^ZTyr-(R)Ala-Gly-(S)Phe-(S)Leu-OMe.

b) Catalyst prepared *in situ* from [Rh Cl (COD)]$_2$ and (S,S)bppm (1:2) or [Rh Cl (COD)]$_2$ and (S,S)diop (1:2). [substrate]/[complex] = 4, [substrate] = 0.028 M in methanol. Reaction performed at room temperature.

c) Quantitative conversion (measured by ^{1}H nmr and hplc) after 48 h. Isolated yield is close to 90% after flash-chromatography on silica gel (ethyl acetate/cyclohexane = 93/7).

d) Measured by hplc (Zorbax ODS) in entries 1, 2, 5, 6, 9, 10, 11, 12. Measured by chiral glc (XE60-(S)Val-(S)Phenylethylamide phase) after acidic cleavage and derivatization entries 3, 4, 7, 8.

e) Configuration at the new asymmetric center, assigned by hplc or by chiral glc, by comparison to reference compounds.

f) Reaction performed with D_2 instead of H_2. [substrate]/[complex] = 4, [substrate] = 0.024 M.

Conclusion

Various monodehydroenkephalins were prepared, protected by standard groups such as benzyloxycarbonyl (CBz, nitrogen protection) and benzyl group (phenolic hydroxyl protection). These compounds were good precursors for the synthesis of enkephalins with (S) or (R) configuration at the tyrosyl or phenylalanyl moiety, additionally labelled at these asymmetric centres[31]. Homogeneous hydrogenation (or deuterogenation) has been performed with a very high diastereoselectivity with rhodium catalysts, dipamp being the most efficient chiral ligand. It has been shown that asymmetric tritiation of dehydro amino acids by rhodium complexes with chiral diphosphine ligands can be applied to the synthesis of labeled amino acids[22]. These experiments should be extended without problems to synthesis of stereospecifically tritiated enkephalins.

Acknowlegments

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Experimental

Apparatus

Melting points were determined on a Reichert microscope equipped with heating-block, and are not corrected. ^{1}H-NMR spectra were recorded on a Bruker AM 250 (250 MHz) or a Bruker AM 200 (200 MHz) with tetramethylsilane as internal standard. Mass spectra were obtained using a Riber-Mag R 10-10 instrument, electroimpact ionisation at 70 eV. Optical rotations were measured in a 1 dm cell at 20°C (± 2°C) using a Perkin Elmer Polarimeter 241. Microanalyses were performed by the Service de Microanalyse du CNRS, Gif-sur-Yvette. Analytical thin layer chromatography was performed on Merck Kieselgel 60 F_{254}. Silica gel 60 (230-400 mesh) was used for column chromatography. Hplc analyses were performed on a 250 x 4.5 mm Zorbax ODS column, with a duPont 8800 isocratic pump and a Isco 1840 UV detector, eluents were methanol-water mixtures. Chiral gas chromatography of N-trifluoroacetyl aminoacids isopropyl esters were performed in a 50 m x 0.25 mm capillary column coated with 0.1 µm XE60-(S)Val-(S)Phenylethylamide phase (Chrompack), in a 2150 Erba Science chromatograph with a flame ionization detector, hydrogen was used as the vector gas. Hydrogenation solvents were distilled before use, dried following literature methods and kept under an inert atmosphere.

Chemicals

N,N-Dicyclohexylcarbodiimide (DCC), N-benzyloxycarbonyl-(R)alanine and glycine methyl ester hydrochloride were used as purchased. Benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) was prepared by a reported method[26]. (S,S)bppm[14], (S,S)diop[13] were prepared as described in the literature.

Synthesis of dehydropeptide **1** (Scheme 1)

N-Benzyloxycarbonyl-O-tosyl-(S)tyrosyl-glycine ethyl ester **1a**

Glycine ethyl ester hydrochloride (0.40 g, 3 mmol), BOP (1.90 g) and N,N-diisopropylethylamine (1.38 g) were added to a solution of N-benzyloxycarbonyl-O-tosyl-(S)tyrosine[29] (2.0 g, 3.5 mmol) in CH_2Cl_2 (11 ml). The reaction mixture was then stirred at room temperature for 16 h. The solvent was evaporated and the solid thus obtained was recrystallized from water. The white crystals were washed with 1 N aqueous HCl; water; 5% $NaHCO_3$; water and dried (P_2O_5-vacuum). The product was purified by filtration through silica gel (eluent : ethyl acetate). Removal of the solvent afforded **1a**, which was recrystallized from water-methanol to give 1.17 g (90%) of **1a** as a white solid (m.p. 60-61°C).

$[\alpha]_D$ = -14.3 (c 2.0, ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ : 1.2 (3H, t, 8Hz); 2.4 (3H, s); 3.0 (2H, m); 3.9 (2H, m); 4.2 (2H, q, 8Hz); 4.4 (1H, m); 5.0 (2H, s); 6.9 (2H, d, 8Hz); 7.3 (2H, d, 8Hz); 7.4 (7H, m); 7.7 (2H, d, 8Hz). Analysis; Found : C 59.14%; H 5.44%; N 4.87%; S 6.03%. $C_{18}H_{30}N_2O_8S$ requires : C 60.42%; H 5.44%; N 5.03%; S 5.76%.

N-Benzyloxycarbonyl-O-tosyl-(S)tyrosyl-glycine **1b**

To a solution of N-benzyloxycarbonyl-O-tosyl-(S)tyrosyl-glycine ethyl ester **1a** (1.1 g, 2.0 mmol) in acetone (15 ml) and water (8 ml) was added a solution of 1 N NaOH (2.2 ml). After 2h at room temperature, 1.5 ml of 2 N HCl were added and the precipitated white solid was collected. The crude product thus obtained was recrystallized from ethyl acetate-cyclohexane to give 0.67 g (85%) of **1b** as a white solid (m.p. 140-141°C).

$[\alpha]_D$ = -17.5 (c 1.0, CH_3OH). ^{1}H-NMR ($CDCl_3$-CD_3OD, 2-1) δ : 2.4 (3H, s); 3.0 (2H, m); 3.9 (2H, m); 4.5 (1H, m); 5.1 (2H, s); 6.9 (2H, d, 8Hz); 7.3 (2H, d, 8Hz); 7.4 (7H, m); 7.7 (2H, d, 8Hz). Analysis; Found : C 57.60%; H 4.98%; N 5.06%; S 6.15%. $C_{16}H_{16}N_2O_8S$ requires : C 59.08%; H 4.96%; N 5.30%; S 6.07%.

N-Benzyloxycarbonyl-O-tosyl-(S)tyrosyl-glycyl-glycyl-(Z)dehydrophenylalanyl-(S)leucine methyl ester **1**

N-Benzyloxycarbonyl-glycyl-(Z)dehydrophenylalanyl-(S)leucine methyl ester[24] (0.96 g, 2 mmol) was suspended in 2 ml of HBr in acetic acid (33%) and the mixture allowed to stand at 25°C for 20 min. The hydrobromide of glycyl-(Z)dehydrophenylalanyl-(S)leucine methyl ester was precipitated from the reaction mixture with ether (12 ml), collected by filtration and washed with ether to give 0.77 g (90%) of product **1c** which was pure enough for the subsequent reactions.

^{1}H-NMR (CD_3OD) δ : 1.0 (6H, m); 1.7 (3H, m); 3.8 (3H, s); 3.9 (2H, s); 4.5 (1H, m); 7.3 (1H, s); 7.4 (5H, m).

To a solution of N-deprotected dehydrotripeptide **1c** (0.64 g, 1.5 mmol) in CH_2Cl_2 (10 ml), were added N-methyl morpholine (190 ml, 1.7 mmol) and **1b** (0.80 g, 1.5 mmol) at 0°C. DCC (0.31 g, 1.5 mmol) was added and the solution stirred at room temperature for 18 h. The solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, washed with 1 N aqueous HCl, water, 1 N aqueous $NaHCO_3$ and water. The organic layer was separated and dried, and the solvent evaporated under reduced pressure. The crude product thus obtained was recrystallized from ethyl acetate-cyclohexane to give 1.03 g (80%) of **1** as a white solid (m.p. 128-129°C).

$[\alpha]_D$ = - 18.1 (c 1.0, CH_3OH). ^{1}H-NMR ($CDCl_3$-CD_3OD, 2-1) δ : 1.0 (6H, m); 1.5 (1H, m); 1.6 (2H, m); 2.4 (3H, s); 3.1 (2H, m); 3.6 (3H, s); 3.8 (2H, s); 3.9 (2H, s); 4.4 (1H, m); 4.6 (1H, m); 5.1 (2H, s); 7.1 (4H, m); 7.4 (9H, m); 7.5 (5H,m). Analysis; Found : C 60.52%; H 5.92%; N 8.10%; S 3.72%. $C_{44}H_{49}N_5O_{11}S$ requires C 61.60%; H 5.76%; N 8.16%; S 3.74%.

Synthesis of dehydropeptide **2** (Scheme 3)

N-Benzyloxycarbonyl-O-benzyl-(S)tyrosyl-glycine methyl ester **2a**

To a mixture of N-benzyloxycarbonyl-O-benzyl-(S)tyrosine[25] (2.0 g, 4.9 mmol), glycine methyl ester hydrochloride (0.62 g, 4.9 mmol) and triethylamine (0.50 g, 4.9 mmol) in CH_2Cl_2 (11 ml) was added DCC (1.0 g, 4.9 mmol) at 0°C. The solution was stirred for 3 h at 0°C and 15h at room temperature, the mixture was filtered and concentrated. The resultant oil was dissolved in ethyl acetate, filtered and evaporated. The crude product thus obtained was recrystallized from ethyl acetate-cyclohexane to afford 1.87 g (80%) of **2a** as a white solid (m.p. 129-130°C).

$[\alpha]_D$ = 3.2 (c 1.08, $CHCl_3$). ^{1}H-NMR (CD_3COCD_3) δ : 3.0 (2H, m); 3.7 (3H, s); 4.0 (2H, m); 4.4 (1H, m); 5.0 (2H, s); 5.1 (2H, s); 6.9 (2H, d, 8.5Hz); 7.1 (2H, d, 8.5Hz); 7.4 (10H, m). Analysis; Found : C 67.98%; H 6.05%; N 6.02%. $C_{27}H_{28}N_2O_6$ requires : C 68.07%; H 5.88%; N 5.88%.

N-Benzyloxycarbonyl-O-benzyl-(S)tyrosyl-glycine **2b**

Prepared in the same way as **1b** with 80% yield (m.p. 165-166°C).

$[\alpha]_D$ =-16.5 (c 1.0, DMF). ^{1}H-NMR ($CDCl_3$-CD_3OD, 2-1) δ : 2.8 (1H, m); 3.1 (1H, m); 3.9 (2H, s); 4.4 (1H, m); 5.0 (4H, m); 6.9 (2H, d, 8.5Hz); 7.1 (2H, d, 8.5Hz); 7.3 (10H, m).

N-Benzyloxycarbonyl-O-benzyl-(S)tyrosyl-glycyl-glycyl-(Z)dehydrophenylalanyl-(S)leucine methyl ester **2**

The coupling reaction was performed using the same conditions described for **1**. Starting from glycyl-(Z)dehydrophenylalanyl-(S)leucine methyl ester hydrobromide **1c** (0.55 g, 1.3 mmol) and **2b** (0.60 g, 1.3 mmol), 0.77 g (75%) of **2** were obtained (m.p. 91-93°C).

$[\alpha]_D$ = -9.9 (c 1.0, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ : 0.9 (6H, m); 1.6 (3H, m); 2.9-3.2 (2H, m); 3.7 (3H, s); 3.9 (2H, s); 4.0 (2H, s); 4.4 (1H, m); 4.7 (1H, m); 6.8 (2H, d, 8Hz); 7.0 (2H, d, 8Hz); 7.3 (10H, m). Analysis; Found : C 66.72%; H 6.14%; N 8.12 %. $C_{44}H_{48}N_5O_9$ requires : C 66.84%; H 6.08%; N 8.86%.

Synthesis of dehydropeptide **3** (Scheme 2)

4-Tosyloxybenzaldehyde **3a**

A solution of 4-hydroxybenzaldehyde (1.06 g, 10 mmol), p-toluenesulfonyl chloride (1.91 g, 10 mmol) and triethylamine (2 g, 20 mmol) in 20 ml of CH_2Cl_2 was stirred at room temperature for 2 h. The reaction mixture was then washed with 1 N aqueous HCl and with water, dried over $MgSO_4$ and evaporated. The residual solid was recrystallized from ethyl acetate-cyclohexane to give 2.27 g (83%) of 4-tosyloxybenzaldehyde as a white solid (m.p. 72-74°C).

^{1}H-NMR ($CDCl_3$) δ : 2.50 (3H, s); 7.30 (2H, d, 8.5Hz); 7.65 (2H, d, 8.5Hz); 7.70 (2H, d, 8.5Hz); 8.00 (2H, d, 8.5Hz); 10,00 (1H, s). Analysis; Found : C 60.67%; H 4.30%; O 22.79%. $C_{14}H_{10}O_4S$ requires : C 60.42%; H 4.35%; O 22.99%.

N-Benzyloxycarbonyl-O-tosyl-(Z)dehydrotyrosine ethyl ester **3b**

Ethyl 2-benzyloxycarbonylamino-2-(diethoxyphosphinyl)-acetate[11] (3.73 g, 10 mmol) in CH_2Cl_2 (2 ml) was added to a solution of potassium t-butoxide (1.12 g, 10 mmol) in CH_2Cl_2 (2 ml) at -60°C under argon. After 10 min, a solution of 4-tosyloxybenzaldehyde (2.76 g, 10 mmol) in CH_2Cl_2 was added under argon. The temperature was allowed to rise to room temperature over a 3 h period. The solvent was then removed under reduced pressure. The residue was dissolved in ethyl acetate (50 ml), washed with a solution of ammonium chloride and then water. The organic phase was dried over

anhydrous $MgSO_4$, filtered and evaporated. Crystallization from ethyl acetate-cyclohexane, afforded 3.7 g (75%) of **3b** as a white solid (m.p. 98-99°C).
^{1}H-NMR ($CDCl_3$) δ : 1.3 (3H, t, 8Hz); 2.4 (3H, s); 4.3 (2H, q, 8Hz); 5.1 (2H, s); 6.4 (1H, s); 7.0 (2H, d, 8.5Hz); 7.3 (2H, d, 8.5Hz); 7.4 (7H, m); 7.7 (2H, d, 8.5Hz). Analysis; Found : C 62.49%; H 4.86%; N 2.88%; S 6.49%. $C_{26}H_{23}NO_7S$ requires : C 62.76%; H 5.06%; N 2.82%; S 6.44%.

N-Benzyloxycarbonyl-O-tosyl-(Z)dehydrotyrosine **3c**
Compound **3b** (2.97 g, 6 mmol) was dissolved in acetone (12 ml) and 1 N aqueous NaOH (6 ml) was added with stirring. The mixture was stirred for 3 h at room temperature. The resultant solution was than diluted with ethyl acetate and 2 N aqueous HCl (4 ml). The organic layer was dried over anhydrous $MgSO_4$ and the solvent evaporated. The residue was diluted in methanol, activated carbon was added and the mixture was stirred, filtered and then concentrated under vacuum to afforded 2.1 g (75%) of **3c** as a white solid (m.p. 118-120°C).
^{1}H-NMR ($CDCl_3$) δ : 2.4 (3H, s); 5.1 (2H, s); 7.0 (2H, d, 8.5Hz); 7.3 (2H, d, 8.5Hz); 7.4 (7H, m); 7.7 (2H, d, 8.5Hz). Analysis; Found : C 60.49%; H 4.51%; N 2.94%; S 6.85%. $C_{24}H_{21}NO_7S$ requires : C 61.40%; H 4.51%; N 2.98%; S 6.83%.

N-Benzyloxycarbonyl-O-tosyl-(Z)dehydrotyrosyl-glycyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester **3**
A solution of N-benzyloxycarbonyl-glycyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester[23] (4.27 g, 10 mmol) in methanol (25 ml) was hydrogenated under atmospheric pressure with palladium on activated charcoal (10% Pd) (0.45 g). The reaction was shaken overnight. The catalyst was then filtered and the solvent was evaporated under reduced pressure to afford 3.86 g (95%) of glycyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester as a colourless oil.
^{1}H-NMR ($CDCl_3$) δ : 0.75 (3H, m); 0.85 (3H, m); 1.55 (1H, m); 1.60 (2H, m); 3.00 (2H, m); 3.70 (3H, s); 3.85 (2H, s); 3.95 (2H, s); 4.50 (1H, m); 4.65 (1H, m); 7.00 (5H, m).

The crude N-deprotected tetrapeptide (1.3 g, 3 mmol) was dissolved in a mixture of CH_2Cl_2 (20 ml) and DMF (20 ml). Then N-methyl morpholine (0.35 ml, 3 mmol), dehydro-α-amino acid **3c** (1.4 g, 3 mmol) and DCC (0.6 g, 3 mmol) were added at 0°C. The mixture was stirred for 2 h at 0°C and 16 h at 25°C. The solution was then filtered and concentrated. The resultant oil was dissolved in ethyl acetate and filtered. The solution was washed with 1 N aqueous HCl, water, 5% $NaHCO_3$, dried over $MgSO_4$, and concentrated to give the crude product. Purification by flash chromatography (elution with ethyl acetate-methanol, 93/7) afforded 1.49 g (58%) of **3** as a white solid (m.p. 88-89°C).
$[\alpha]_D$ = 7.1 (c 1.0, CH_3OH). ^{1}H-NMR ($CDCl_3$-CD_3OD, 2-1) δ : 0.8 (3H, m); 0.9 (3H, m); 1.5 (1H, m); 1.6 (2H, m); 2.4 (3H, s); 3.1 (2H, m); 3.6 (3H, s); 3.8 (2H, s); 3.9 (2H, s); 4.4 (1H, m); 4.5 (1H, m); 5.1 (2H, s); 7.0 (2H, d, 8.5Hz); 7.2 (5H, m); 7.4 (9H, m); 7.7 (2H, d, 8.5Hz). Analysis; Found : C 60.22%; H 5.98%; N 7.53%; S 3.57%. $C_{44}H_{49}N_5O_{11}S$ requires : C 61.60%; H 5.76%; N 8.16%; S 3.74%.

Synthesis of dehydropeptide **4** (Scheme 4)

N-Benzyloxycarbonyl-O-benzyl-(Z)dehydrotyrosine ethyl ester **4a**
The reaction was performed using the same conditions described for **3b**. Starting from ethyl 2-benzyloxycarbonylamino-2-(diethoxyphosphinyl)-acetate (3.1 g, 8.3 mmol) and 4-benzyloxybenzaldehyde (1.76 g, 8.3 mmol), 2.4 g (68%) of **4a** were obtained (m.p. 111-112°C).

^{1}H-NMR ($CDCl_3$) δ : 1.3 (3H, t, 8Hz); 4.2 (2H, q, 8Hz); 5.1 (2H, s); 5.2 (2H, s); 6.9 (2H, d, 8Hz); 7.2-7.5 (10H, m); 7.6 (2H, d, 8Hz). Analysis: Found : C 72.39%; H 6.09%; N 3.21%. $C_{26}H_{25}NO_5$ requires : C 72.39%; H 5.57%; N 3.25%.

N-Benzyloxycarbonyl-O-benzyl-(Z)dehydrotyrosine **4b**
To a solution of N-benzyloxycarbonyl-O-benzyl-(Z)dehydrotyrosine ethyl ester **4a** (1.1 g, 2.6 mmol) in acetone (15 ml) and water (8 ml) was added a solution of 1N aqueous NaOH (2.8 ml). The reaction mixture was stirred for 2h at room temperature. Then 3 ml of 1N aqueous HCl were added and a precipitate was formed. The white solid was recrystallized from ethyl acetate-cyclohexane to give 0.71 g (68%) of **4b** (m.p. 150-151°C).
^{1}H-NMR ($CDCl_3$) δ : 5.10 (2H, s); 5.20 (2H, s); 6.50 (1H, s); 6.95 (2H, d, 8.5Hz); 7.3-7.4 (m, 5H); 7.5 (2H, d, 8.5Hz). Analysis: Found : C 71.47%; H 5.02%; N 3.37%. $C_{24}H_{21}NO_5$ requires : C 71.46%; H 5.21%; N 3.47%.

N-Benzyloxycarbonyl-O-benzyl-(Z)dehydrotyrosyl-glycyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester **4**
The reaction was performed using the same conditions described for **3**. Starting from glycyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester (1.1 g, 2.65 mmol), triethylamine (0.27 g, 2.65 mmol), N-benzyloxycarbonyl-O-benzyl-(Z)dehydrotyrosine **4b** (1.06 g, 2.63 mmol) and DCC (0.54 g, 2.63 mmol). The mixture was stirred overnight at room temperature, the precipitated urea was filtered off and the filtrate was evaporated to give an oil. Chilled water (100 ml) was added to the mixture and the precipitated yellow solid collected. The resultant crude product was dissolved in ethyl acetate, the solution was then filtered and the solvent was evaporated under vacuum. The crude product thus obtained was recrystallized from ethyl acetate-cyclohexane to afford 1.14 g (55%) of **4** as a white solid (m.p. 124-126°C).
$[\alpha]_D$ = 6.7 (c 1.0, CH_3OH). ^{1}H-NMR ($CDCl_3$-CD_3OD, 1-1) δ : 0.9 (6H, m); 1.5 (1H, m); 1.6 (2H, m); 3.1 (2H, m); 3.7 (3H, s); 3.8 (2H, m); 3.9 (2H, m); 4.4 (1H, m); 4.6 (1H, m); 5.1 (2H, s); 5.2 (2H, s); 6.9 (2H, d, 8.5Hz); 7.2 (5H, m); 7.4 (12H, m). Analysis; Found : C 66.78%; H 6.31%; N 8.80%. $C_{44}H_{48}N_5O_9$ requires : C 66.84%; H 6.08%; N 8.86%.

Synthesis of dehydropeptide **5** (Scheme 5)

N-Benzyloxycarbonyl-(R)alanyl-glycine methyl ester **5a**
Prepared by the condensation of N-benzyloxycarbonyl-(R)alanine (2.0 g, 9 mmol) with glycine methyl ester hydrochloride (1.1 g, 9 mmol) using DCC (1.1 g, 9 mmol) in the same conditions described for **2a**. The crude product thus obtained was recrystallized from ethyl acetate-cyclohexane to give 1.85 g (70%) of **5a** as a white solid (m.p. 94-96°C).
$[\alpha]_D$ = 52.8 (c 0.25, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ : 1.4 (3H, d, 7.5Hz); 3.7 (3H, s); 4.0 (2H, d, 5Hz); 4.3 (1H, m); 5.1 (2H, s); 5.4 (1H, m); 6.7 (1H, m); 7.4 (5H, s). Analysis; Found C 57.18%; H 6.22%; N 9.62%. $C_{14}H_{18}N_2O_5$ requires C 57.14%; H 6.12%; N 9.52%.

N-Benzyloxycarbonyl-(R)alanyl-glycine **5b**.
The reaction was performed in the same way as **1b**. Starting from N-benzyloxycarbonyl-(R)alanyl-glycine methyl ester **5a** (1.8 g, 6 mmol), 1.4 g (85%) of **5b** were obtained (m.p. 130°C).
$[\alpha]_D$ = 46.5 (c 0.25, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ : 1.4 (3H, m); 4.0 (2H, s); 4.3 (1H, m); 5.1 (2H, s); 7.4 (5H, s).

N-Benzyloxycarbonyl-(R)alanyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester **5c**

A solution of N-benzyloxycarbonyl-(S)phenylalanyl-(S)leucine methyl ester[27] (3.8 g, 9 mmol) in methanol (30 ml) was hydrogenated under atmospheric pressure with palladium on activated charcoal (10% Pd) (0.45 g) and 2 N aqueous HCl (46 ml). The reaction was shaken overnight, the catalyst was then filtered. The product was purified by filtration through activated carbon (eluent : methanol). Removal of the solvent afforded 2.8 g (95%) of S-phenylalanyl-(S)leucine methyl ester hydrochloride as a colourless oil.

^{1}H-NMR (CD_3OD) δ : 0.8 (6H, m); 1.5 (3H, m); 3.1 (2H, m); 3.55 (3H, s); 4.20 (1H, m); 4.40 (1H, m); 7.2 (5H, s).

The tetrapeptide **5c** was prepared by coupling of the dipeptide **5b** (1.12 g, 4 mmol) with (S)phenylalanyl-(S)leucine methyl ester hydrochloride (1.30 g, 4 mmol), using DCC (0.83 g, 4 mmol) as a coupling agent. The reaction was performed in the same way described for **1**. The crude product obtained was recrystallized from ethyl acetate-cyclohexane to afford 1.44 g (65%) of **5c** as a white solid (m.p. 123-124°C).

$[\alpha]_D$ = 5.2 (c 0.25, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ : 0.9 (6H, m); 1.3 (3H, m); 3.0 (2H, m); 3.7 (3H, s); 4.0 (2H, m); 4.4 (1H, m); 4.5 (1H, m); 4.9 (1H, m); 5.1 (2H, m); 6.0 (1H, m); 7.1 (5H, m); 7.3 (5H, m); 7.5 (1H, m). Analysis; Found : C 62.02%; H 6.91%; N 9.82%. $C_{29}H_{38}N_4O_7$ requires : C 62.82%; H 6.86%; N 10.12%.

N-Benzyloxycarbonyl-O-tosyl-(Z)dehydrotyrosyl-(R)alanyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester **5**

5c (0.70 g, 1.26 mmol) in methanol (5 ml) was hydrogenated under atmospheric pressure with palladium on activated charcoal (10% Pd) (0.08 g). The reaction was shaken overnight, and the catalyst was filtered. The solvent was then evaporated under reduced pressure to afford 0.52 g (98%) of (R)alanyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester as a colourless oil.

^{1}H-NMR ($CDCl_3$) δ : 0.9 (6H, m); 1.3 (3H, m); 1.5 (3H, m); 3.1 (2H, m); 3.7 (3H, s); 3.9 (2H, m); 4.5 (1H, m); 4.6 (1H, m); 7.3 (5H, s).

The dehydrotyrosylpeptide **5** was synthesized by coupling of the CBz-(O)Ts-(Z)dehydrotyrosine **3c** (0.61 g, 1.3 mmol) with (R)alanyl-glycyl-(S)phenylalanyl-(S)leucine methyl ester (0.53 g, 1.3 mmol), using DCC (0.27 g, 1.3 mmol) as the coupling agent. The reaction was performed in the same ways described for **1**. Purification by flash chromatography (elution with ethyl acetate-methanol, 93-7) afforded 0.62 g (55%) of **5**. Crystallization from ethyl acetate-cyclohexane gave **5** as a white solid (m.p. 93-95°C).

^{1}H-NMR ($CDCl_3$) δ : 0.9 (6H, m); 1.3 (3H, m); 1.5 (3H, m); 2.4 (3H, s); 3.0 (2H, m); 3.6 (3H, s); 3.9 (2H, m); 4.4 (2H, m); 4.7 (1H, m); 5.1 (2H, s); 6.9 (2H, d, 8Hz); 7.2 (5H, m); 7.4 (9H, m); 7.7 (2H, d, 8Hz). Analysis; Found : C 62.02%; H 6.09%; N 7.81%. $C_{45}H_{51}N_5O_{11}S$ requires : C 62.14%; H 5.87%; N 8.06%.

Preparation of chiral catalyst solution

[Rh Cl (S,S)diop] and [Rh Cl (S,S)bppm] were prepared *in situ* as described in refs. 13, by reaction of [Rh Cl $(C_2H_4)_2]_2$ or [Rh Cl (COD)]$_2$ with chiral diphosphine in degassed solvent. Typically, [Rh Cl (COD)]$_2$ (1.5×10^{-5} mol) and (S,S)bppm (3.0×10^{-5} mol) were dissolved in 5 ml of methanol under argon. The solution was stirred for 15 min and was introduced into the hydrogenation flask by means of a syringe. Any contact with air was avoided.

Hydrogenation procedure

For hydrogenation a flask with magnetic stirrer connected to a gas burette was used. Solid substrate (and catalyst, if solid : [Rh (R,R)dipamp COD]$^+$ BF_4^-) was introduced, air displaced by argon, degassed methanol (and catalyst, if in solution : [Rh Cl (S,S)diop] or [Rh Cl (S,S)bppm]) was added, argon displaced by hydrogen. The mixture was stirred under hydrogen for 48 h. High pressure experiments were performed in a 100 ml autoclave. The order of addition of reactants into the autoclave was substrate (and catalyst, if solid), hydrogen and methanol (and catalyst, if in solution).
Typically, the dehydrotyrosyl peptide **2** (150 mmol) was hydrogenated at 18 atm. in the presence of [Rh (R,R)dipamp COD]$^+$ BF_4^- (30 mmol) in 5 ml methanol at 18°C for 48 h. The solvent was then removed under reduced pressure. Purification by flash chromatography (elution with ethyl acetate-methanol, 93/7) afforded the protected Leu-enkephalin.

Identification of diastereomers

The stereoselectivity was measured by hplc or glc analysis.
Hplc analysis was performed on a Zorbax ODS 250 x 4,6 mm column using methanol-water (80/20) eluent. The flow rate was 1ml/min with UV detection at 230 nm. After hydrogenation, the protected Leu-enkephalin or (R)Ala^2-Leu-enkephalin were injected into the chromatographic system.
Glc analysis was performed according to the following procedure :

- -treatment of the polypeptide with ytterbium in liquid ammonia,
- -acidic hydrolysis[28] of the product thus obtained,
- -derivatization of the aminoacids into N-trifluoroacetyl isopropyl esters
- -and analysis by glc on a chiral stationary phase (Chrompack 50 m x 0.25 mm column coated with 0.1 µm of XE60-(S)Val-(S)phenylethylamide).

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