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Diastereoselective Deuteration of (*Z*)-α,β-Dehydrotryptophanyl-Containing Biological Peptides Controlled by Chiral Rhodium Catalysts.

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Abstract: The regio- and stereoselective labelling (^{2}H, ^{3}H) of tryptophanyl containing biological peptides catalysed by chiral rhodium complexes is described. The Δ^ZTrp-residue is directly produced in the peptide by a catalytic process, involving the (*S*)-tryptophan 2',3'-oxydase from *Chromobacterium violaceum*. Two biological dehydropentapeptides, pGlu-His-Δ^ZTrp-Ser-Tyr and Boc-βAla-Δ^ZTrp-Met-Asp-Phe-NH_2, have been thus synthesized. Asymmetric deuteration of these compounds was then investigated in the presence of various rhodium catalysts. High stereocontrol was achieved by suitable choice of the chiral ligands. This strategy offers new possibilities for the asymmetric synthesis of labelled (^{2}H, ^{3}H) peptides without ultimate deprotection step.

We have previously proposed a novel approach to the diastereoselective labelling (^{2}H, ^{3}H) of peptides by a two-step procedure involving the enzymatic α,β-dehydrogenation of a tryptophan (Trp) side chain, followed by the asymmetric reduction of the double bond in the presence of tritium or deuterium gas using chiral rhodium complexes (Scheme 1).[1,2] This procedure that leads to a regio- and stereoselective labelling of peptide molecules, could be highly required to investigated dynamic features of protein-ligand relationships by ^{3}H NMR.[3] The (Z)-α,β-dehydrogenation reaction is realized by *S*-tryptophan 2',3'-oxidase (LTO) from *Chromobacterium violaceum* (ATCC 12472) which is highly specific for (*S*)-Trp residues.[4] Considering the second step, the reduction of (*Z*)-α,β-dehydropeptides catalyzed by chiral rhodium complexes offers the best route to the asymmetric syntheses of labelled peptides.[5] This chemo-enzymic strategy was first applied to the asymmetric deuteration and tritiation of *N*-acetyl-Δ^Ztryptophanamide[1,2] and Ac-Δ^ZTrp-(*S*)-Phe-OMe.[2] We showed that the reduction of these compounds catalysed by the cationic rhodium (*R,R*)-dipamp complex gave the corresponding (*S*)-isomers with high stereoselectivities. The objectives are now : (i) to check this approach especially on Δ^ZTrp-containing biological peptides, and (ii) to find chiral catalysts overthrowing the effect of the chiral centers of the substrate.

We describe here a full account of our research on the asymmetric deuteration of two biological pentapeptide hormones, the N-terminal fragment of the gonadotropin releasing-hormone (LH-RH[1-5]) of sequence pGlu-His-Trp-Ser-Tyr and pentagastrin (Boc-βAla-Trp-Met-Asp-Phe-NH_2), an active fragment of the gastrin. The results will be discussed in order to assess both the efficiency of various chiral diphosphine ligands and the effect of pressure on the asymmetric induction. Deuteration was used in the subsequent experiments as a model for the tritiation reaction.

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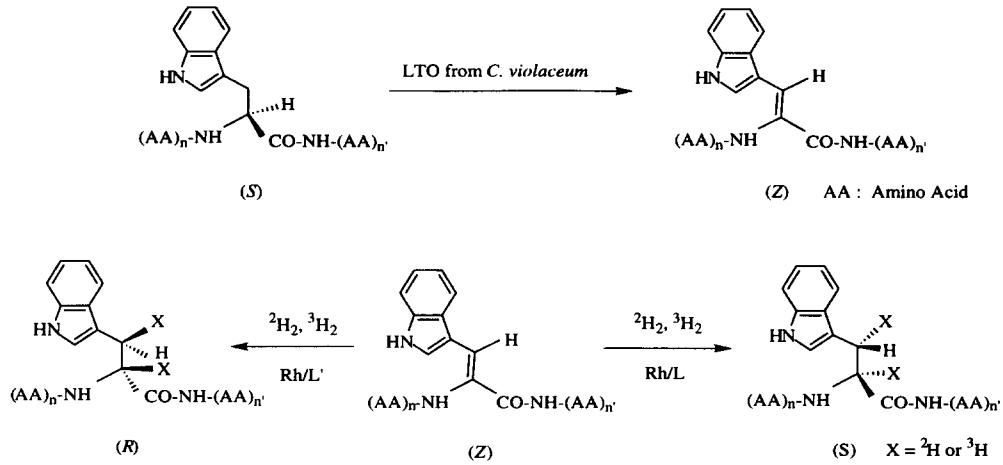
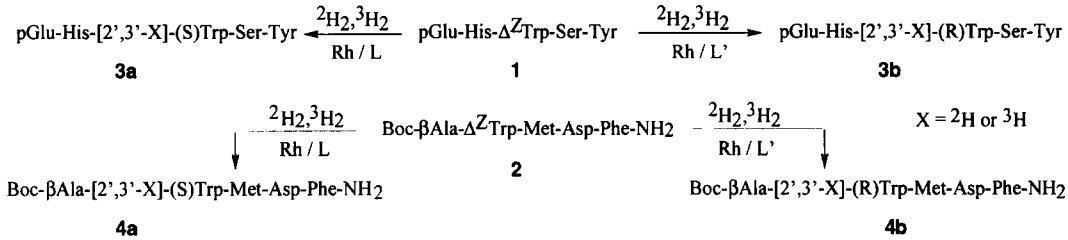


Scheme 1

The (Δ^ZTrp)-LH-RH(1-5) **1** of sequence pGlu-His-Δ^ZTrp-Ser-Tyr and the (Δ^ZTrp)-pentagastrin **2** (Boc-βAla-Δ^ZTrp-Met-Asp-Phe-NH_2) were synthesized by enzymatic α,β-dehydrogenation of pGlu-His-Trp-Ser-Tyr and Boc-βAla-Trp-Met-Asp-Phe-NH_2, respectively. The α,β-dehydropentapeptides **1** and **2** were obtained in quantitative yield, purified by HPLC and checked by mass spectrometry.[6]

The high pressure reductions are run using an automatic gas transfer unit[7] supplied with a liquid helium cryostat and fitted with an inlet for introduction of the solvent. This cryostat is used to bring the deuterium or the tritium at the solid state (at 4°K) in a thin capillary tube. The 1-ml reactor is connected close to the capillary tube so that the deuterium or the tritium can be compressed by heating in a very small volume (ca 1 ml). Using this equipment, it is now possible to rise the gas pressure to about 30 atm. Asymmetric micro-deuteration of **1** and **2** were carried out by using cationic complexes [Rh (*R,R*)-dpcb (COD)]$^+$ PF_6^-,[8] [Rh (*S,S*)-diop (COD)]$^+$ PF_6^-,[9] and [Rh (*R,R*)-dipamp (COD)]$^+$ BF_4^-[10] (Scheme 2).[11] Results obtained under different reaction conditions are summarized in Table 1.



Scheme 2

In the first case, with (*R,R*)-dipamp ligand, the deuteration of **1** in methanol at room temperature and 5 atm of deuterium gave pGlu-His-(*S*)-Trp-Ser-Tyr **3a** in modest yield (45%) with high diastereoselectivity (99% d.e., entry 1). Increasing the deuterium pressure from 5 to 15 atm allowed us to reduce the reaction

time and to improve the yield (entry 2). Regarding the diastereoselectivity dependence on deuterium pressure, it is interesting to observe that the stereoselectivity shows no dependence at all in the range of 5 to 15 atm (99% d.e., entries 1 and 2). pGlu-His-(*R*)-Trp-Ser-Tyr **3b** with 30% d.e. was obtained using [Rh (*R,R*)-dpcb (COD)]$^+$ PF_6^- in methanol at room temperature and 18 atm of deuterium (entry 3). Using PdO under a pressure of 5 atm, no diastereomeric excess could be measured (entry 4). The results obtained with **1** show that the newly created chiral center is mainly determined by the catalyst.

In contrast to (Δ^ZTrp)-LH-RH(1,5) **1**, the asymmetric reduction of (Δ^ZTrp)-pentagastrin **2** catalysed by [Rh (*R,R*)-dipamp (COD)]$^+$ BF_4^- led to lower diastereomeric excesses (entries 5 and 6). On the other hand, it was found that the deuterium pressure exerts a significant influence on the stereoselectivity. Increasing deuterium pressure from 5 up to 15 atm led to a decrease of selectivity (26% and 0% d.e. respectively, entries 5 and 6). Surprisingly, the stereoselectivity exhibited in the reaction using (*S,S*)-diop as chiral ligand was moderate, and the direction of asymmetric induction was opposite to that observed for (*Z*)-α,β-dehydroamino acids[9] and (*Z*)-α,β-dehydropeptides.[12] Since a large effect can be observed on the asymmetric induction by [Rh/(*S,S*)-diop] due to the chiral centers in **2**, we estimated the simple asymmetric induction caused by the chiral centers in the substrate with the use of an achiral catalyst, PdO. In contrast to previous results concerning hydrogenation of linear (*Z*)-α,β-dehydropeptides using heterogeneous catalyst (0-20% d.e.)[13] and to the low selectivity obtained with **1** (0% d.e., entry 4), PdO afforded ((*R*)-Trp)-pentagastrin **4b** in acceptable stereoselectivity (52% d.e., entry 9).

Using (*R,R*)-dpcb as chiral ligand, we found a conversion of **2** to ((*R*)-Trp)-pentagastrin **4b** with 68% yield and high stereoselectivity (80% d.e., entry 8). The asymmetric reduction of **2** led to weaker diastereomeric excesses suggesting that a preferential conformation of the peptide in solution or a peculiar electronic environment due to the amino acid side chains could exert a great influence on the formation of the complex with the catalyst and, hence, on the stereoselectivity of the reaction.

Table 1. Asymmetric deuteration of (Δ^ZTrp)-LH-RH(1-5) **1** and (Δ^ZTrp)-pentagastrin **2**.[a]

Entry	Substrate	Catalyst	Pressure (atm)	Time (h)	Yield (%)	*S/R*[b]	d.e. (%)
1	**1**	[Rh/(*R,R*)-dipamp]	5	72	45	99.5/0.5	99
2	**1**	[Rh/(*R,R*)-dipamp]	15	20	72	99.5/0.5	99
3	**1**	[Rh/(*R,R*)-dpcb]	18	48	80	35/65	30
4	**1**	PdO	5	20	95	50/50	0
5	**2**	[Rh/(*R,R*)-dipamp]	5	20	69	63/37	26
6	**2**	[Rh/(*R,R*)-dipamp]	15	20	80	50/50	0
7	**2**	[Rh/(*S,S*)-diop]	5	20	73	28/72	44
8	**2**	[Rh/(*R,R*)-dpcb]	5	20	68	10/90	80
9	**2**	PdO	15	20	80	24/76	52

a) [substrate]/[Rh] = 1.5; [substrate] = 0.006 M in methanol. Reaction performed at room temperature. b) Measured by HPLC. The configuration of the new asymmetric center was assigned by HPLC as compared to reference compounds.

These two examples demonstrate that the asymmetric reduction of Δ^ZTrp-containing peptides produced by LTO is an efficient approach to the regio- and stereoselective labelling of biological peptides. This method allows flexible synthesis of optically pure *(R)*- or *(S)*-Trp moiety in the peptides with high diastereoisomeric excesses by suitable choice of the chiral catalyst and without previous protection step. The reduction pressure also appears to be one of the crucial factors, affecting both the reduction rate and the stereoselectivity. This pressure dependency is highly sensitive to the structure of substrates. These experiments should be extended to the asymmetric tritiation of pentapeptides **1** and **2**. The relative ease with which both antipodes of labelled dipeptides[3] and pentapeptides (**3** and **4**) are prepared with our strategy should allow the regio- and stereoselective tritiation of biological peptides of higher molecular weight.

References and notes

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6. **Enzymatically prepared 1 and 2** : LTO was purified from *C. Violaceum* (ATCC 12472) according to Genet and coworkers.[2] The enzymatically prepared α,β-dehydrotryptophanyl-peptides **1** and **2** were obtained by incubation of the corresponding pentapeptides (pentagastrin and fragment 1-5 of the luteinizing-hormone releasing-hormone respectively, 10 mg, 1 mM) with LTO (2.6 nM), in 50 mM succinate buffer, pH 5.6, containing 20 μg ml^{-1} catalase, for 20 h at 30 °C. The α,β-dehydrogenation reactions were followed spectrophotometrically by monitoring the UV-spectrum of **1** and **2** (λ_{max} = 337 nm).
   Chromatographic procedure for the purification of 1 and 2 : The dehydropentapeptides were purified on reverse-phase chromatography (Vydac column; eluent, 35% acetonitril in water; flow rate, 1 ml min^{-1}; UV detection at 280 and 330 nm). Retention times were : 34.0 min for **1** and 12.1 min for **2**. Evaporation of the solvent afforded 6.6 mg (66%) of **1** and 7.6 mg (75%) of **2** as a yellow oil. The dehydropeptides **1** and **2** were then characterized by mass spectrometry (m/z = 702, MH^+ and m/z = 767, MH^+ respectively).
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11. The solution of appropriate catalyst (4 μmol) in degassed methanol (1 ml) was added under argon to the dehydropentapeptide (6 μmol) in the hydrogenation flask which was connected to the reduction apparatus. The argon atmosphere was replaced with deuterium. The reductions were run under the reaction conditions given in the table 1. The stereoselectivity was measured by HPLC on a vydac column; eluent, 35% acetonitrile in water; flow rate, 1 ml min^{-1}; UV detection at 280 nm. The resultant diastereomers **3a**, **3b**, **4a** and **4b** were characterized as compared to reference compounds. Retention times were : 32.0 min for **3a**, 28.0 min for **3b**, 13.4 min for **4a** and 12.5 min for **4b**.
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