Tetrahedron Vol. 38, No. 1, pp. 119 to 123, 1982
Printed in Great Britain.

0040-4020/82/010119-05$03.00/0

ASYMMETRIC TRITIATION OF *N*-ACETYL DEHYDROPHENYLALANYL-(*S*) PHENYLALANINE (METHYLESTER) CATALYZED WITH A RHODIUM (+) DIOP COMPLEX

H. LEVINE-PINTO*, J. L. MORGAT and P. FROMAGEOT
Service de Biochimie, Département de Biologie, CEN Saclay, 91191 Gif-sur-Yvette Cédex, France

and

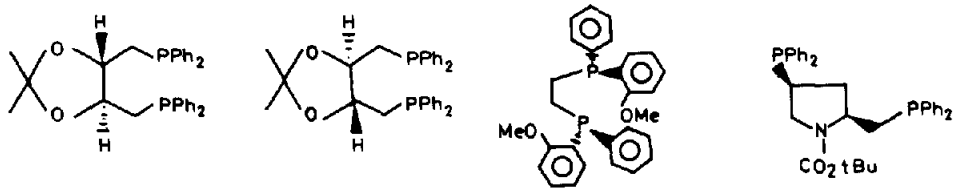
D. MEYER, J. C. POULIN and H. B. KAGAN
Laboratoire de Synthèse Asymétrique, Associé au CNRS (LA 255) Université Paris-Sud, 91405 Orsay, France

(*Received in France* 27 *May* 1981)

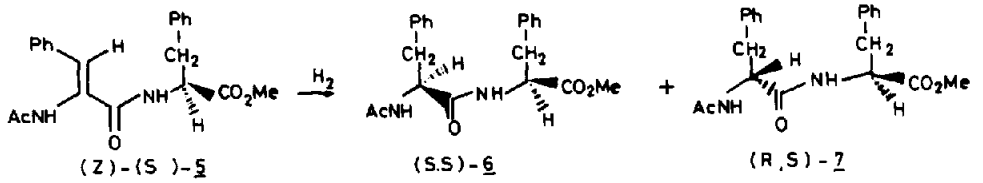
Abstract—Rhodium-(+)diop complex catalyzes the stereoselective addition of two tritium atoms in Ac-ΔPhe-(*S*) PheOMe (diop stands for isopropylidene - 2,3 - dihydroxy - 2,3 - bis - diphenylphosphino - 1,4 - butane). Tritiated Ac(*S*) Phe-(*S*) PheOMe and Ac-(*R*) Phe-(*S*) PheOMe were obtained with a theoretical specific radioactivity. Each diastereoisomer was isolated in a pure state, their ^{3}H-nmr spectra indicated the ratio and the sites (C_α–C_β) of ^{3}H labelling. ^{3}H–^{3}H and ^{1}H–^{1}H coupling constants used together allowed the unequivocal assignment of the three staggered rotamers around C_α–C_β in the N-terminal phenylalanine moiety. The scope of the reaction for selective preparation of tritiated dipeptides is discussed.

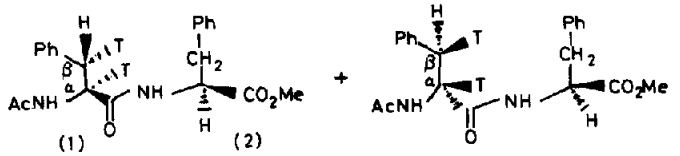
Highly active homogeneous rhodium catalysts were discovered by Wilkinson in 1966.[1] Since then, many efficient chiral diphosphines have been used in order to perform various asymmetric reductions (for some reviews, see Ref. 2).

We have recently demonstrated that some dipeptides can be easily prepared by asymmetric reduction of the corresponding monodehydropeptides[3] by using chiral catalysts $RhClL_2$ or $[RhCODL_2]^+$ (in which COD stands for 1,5-cyclooctadiene and L_2 is a chiral diphosphine such as (+)diop **1**, (−)diop **2**,[4] dipamp **3**[5] or bppm **4**[6]). For example, Ac-Δ^z Phe-(*S*) PheOMe **5** was reduced in high yield into a mixture of Ac-(*S*) Phe-(*S*) PheOMe **6** and Ac-(*R*) Phe-(*S*) PheOMe **7** with the following



Scheme I





Scheme II

stereoselectivities:

(+)diop:**6**/**7** = 95/5
(−)diop:**6**/**7** = 10/90
dipamp:**6**/**7** = ≥95/≤5
bppm:**6**/**7** = ≤5/≥95.

In these cases the chiral catalyst can clearly control the stereochemistry of the reaction to a large degree. Similar results were also observed by two Japanese groups.[7,8] The great interest in highly tritiated peptides for biological studies has prompted us to study stereospecific tritiation of the double bond in dehydropeptides.[9] *N*-acetyl-dehydrophenylalanyl-(*S*) phenylalanylmethyl ester was taken as a model and (+)diop **1** as the chiral ligand of the catalyst.

EXPERIMENTAL

Chemicals and solvents. Synthesis of chemicals and purification of solvents are described elsewhere.[3] Pure tritium gas was purchased from Commissariat à l'Energie Atomique (France).

Apparatus. The automatic gas transfer unit used for catalytic tritiation has been previously described.[10] The tritiation vial was adapted to experimental conditions (Fig. 1). It contained separately the rhodium complex precursor and the chiral ligand. Nitrogen and solvents were injected through the lateral septum. Before each introduction of gas (nitrogen or tritium) a vacuum of 10^{-4} Torr was reached.

Tritiation procedure. The tritiation vial was connected to the tritiation apparatus. The catalyst was prepared *in situ* under nitrogen by mixing 3 μmoles of $(Rh(C_2H_4)_2Cl)_2$ and 5 μmoles of (+)-diop in benzene, then the dehydropeptide **5** (2 mg, i.e. 5.5 μmoles) dissolved in methanol was added. The solution was frozen in liquid nitrogen. Tritium gas was introduced and compressed to 1.3 bars. After thawing, the reaction mixture was kept at 20° and magnetically stirred for 2 hr. Then, the labile tritium atoms were exchanged by successive flash evaporations with 150 ml of methanol.

Separation and identification of the dipeptide diastereoisomers. The tritiation mixture was easily purified by chromatography. The identification of diastereoisomers: Ac-(*S*) Phe-(*S*) PheOMe **8** and Ac-(*R*) Phe-(*S*) PheOMe **9** was based upon their physical characteristics (UV absorption, *Rf* values and circular dichroism). The following solvent systems were used for tlc and hplc: chloroform–dioxane (4:1), diisopropylether–acetonitrile (2:1). The hplc column was packed with silicagel Lichroprep Si 60 (5–20 μm) purchased by Merck (Germany). The hplc apparatus was a Chromatem 380 equipped with an Altex detector (Touzart–Matignon). Peptide weight determinations were carried out after acidic hydrolysis with an autoanalyser TSM (Technicon). Radioactive countings were determined in a liquid scintillation Counter SL 30 (Intertechnique).

NMR analysis of the diastereoisomers. Tritium NMR spectra were recorded at 106 MHz with a WP-100 Brucker spectrometer operating in the Fourier transform mode. The tritium distribution was estimated by integration of signals. Coupling constants were determinate on spectra with and without proton decoupling. Proton NMR spectra were recorded at 250 MHz on a Cameca TSN 250 spectrometer also operating in the Fourier transform mode.

RESULTS

The tritiated diastereoisomers **8** and **9** were separated from the parent dehydrocompound and from the catalyst and their stereochemistry was established as indicated in the experimental section. Tritiation of the dehydropeptide Ac-Δ^zPhe-(*S*) PheOMe **5** in presence of the *in situ* chiral Rh(+)diop catalyst yields a high ratio of (*S,S*) **8**/(*R,S*)/**9** up to 88/12. This stereoselectivity is close to the one observed previously in asymmetric hydrogenation of **5**.[3] The mean specific radioactivity is found to be slightly different in the two products: (*S,S*) **8**:57 Ci/mmole and (*R,S*) **9**:53 Ci/mmole and is close to the theoretical value (58 Ci/mmole).

Each diastereoisomer was examined by ^3H NMR spectroscopy (Fig. 2). This analysis shows that in **8** both α and β sites are identically labelled whereas in **9** the small deficiency at C_α could be explained by a $^3H/H$ exchange with the protic solvent. However, it could well not be a simple exchange through enolisation at C_α in **9** if the mechanism is similar to one proposed to explain the stereospecific H/D exchange observed in the D_2 addition on (*Z*)-acetyldehydro-phenylalanine carried out in methanol solution in presence of a rhodium-diop catalyst.[11] It was proposed that the insertion of the C=C double bond on the Rh–D bond must occur in such a way that rhodium is placed at C_α.

When the life time of this alkylrhodium intermediate is high enough an exchange mechanism may occur between the last deuterium on rhodium and hydrogen of the methanol. Then the reductive elimination, which occurs with retention of configuration necessarily introduces H at C_α with the same stereochemistry that deuterium would take.[11] This explanation is also supported by the recent isolation of alkyl rhodium with rhodium at the C_α position of α-aminoacid precursors.[12,13] We could not firmly establish if there is stereospecific formation of

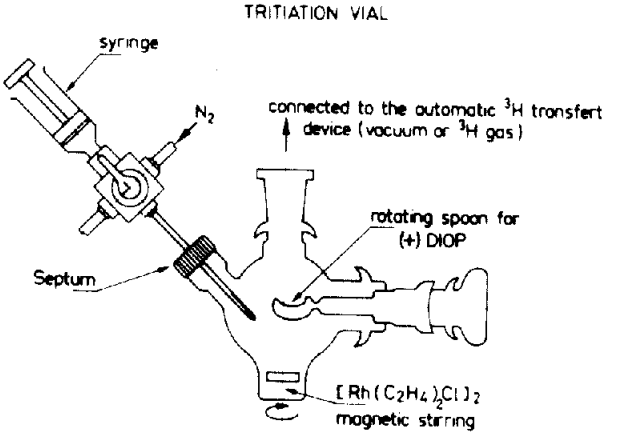


Fig. 1. Tritiation vial.

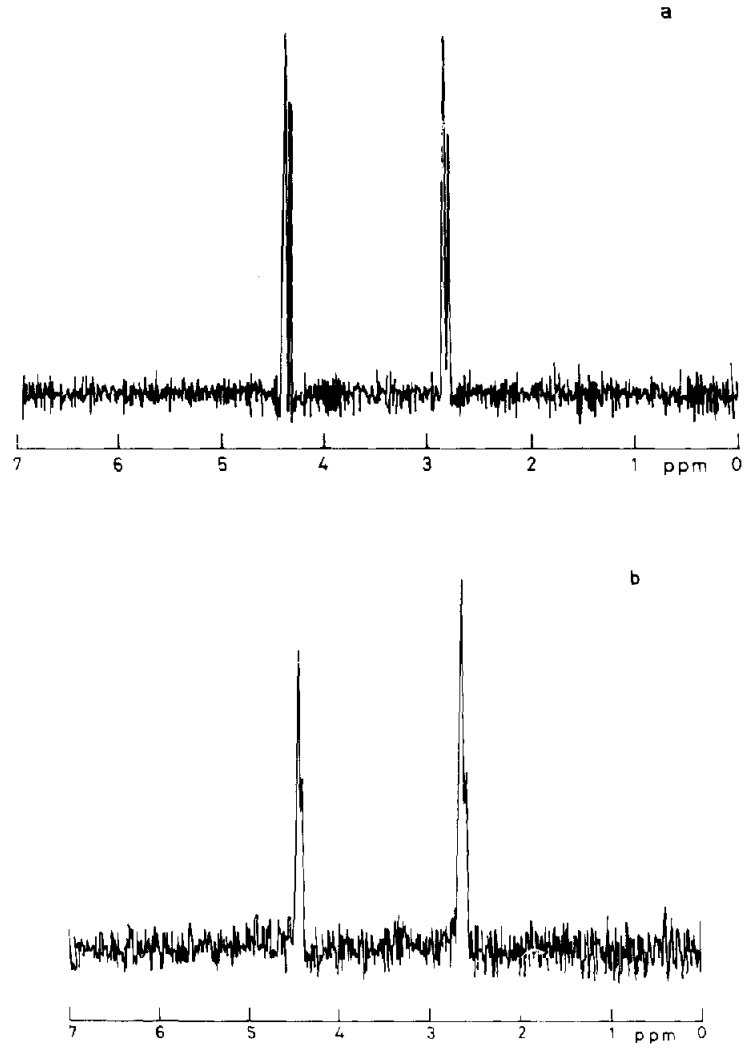
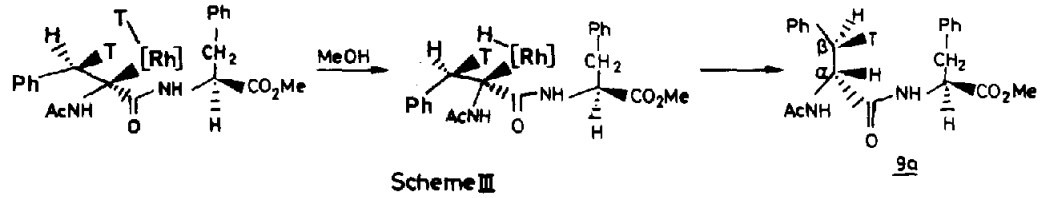


Fig. 2. ^{3}H NMR spectra of a) **8** and b) **9** recorded in DMSO d_6 (20 mCi/100 μl). Chemical shifts are given from TMS used as internal reference.

monotritiated **9a** as depicted in Scheme 3 or if epimer at C_α is also present (as predicted by an enolisation mechanism). The tritium distribution was 54% and 46% on C_β and C_α respectively (tritium loss: 8%). If some exchange with epimerization occured at C_α, minor additional tritium signal at C_β should appear on the spectrum. Unfortunately the accuracy of detection is limiting and a definitive conclusion cannot be given.

The coupling constants allow us to calculate the fractional population of rotamers defined around C_α–C_β bond (Fig. 3). However tritium–tritium vicinal coupling constants have to be previously transformed to their corresponding proton–proton vicinal coupling constants by using the ratio: 106 MHz (^{3}H)/100 MHz (^{1}H) = 1.06 as a correcting factor. The obtained values are $^3J_{H_\alpha H_\beta}$ = 4.60 Hz (for **8**) and 3.90 Hz (for **9**) (Table 1). Estimation of rotamer fractions from these coupling constant values is performed by using the classical set of values given by Pachler $J_g = 2.56$ Hz and $J_t = 13.6$ Hz.[14,16] Since reduction corresponds to a *cis*-addition,[11, 17] calculations from $^3J_{H_\alpha H_\beta}$ lead to the fractions of rotamer II in the case of the *S*-residue (**8**) and rotamer III in the case of



Scheme III

Mechanism of stereospecific T/H exchange

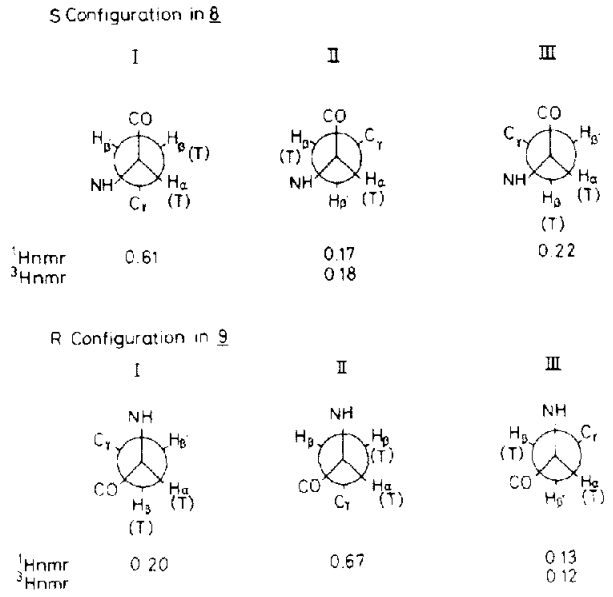


Fig. 3. Rotamer population of **8** and **9** around C_α–C_β axis of phenylalanyl residue N-terminus.

Table 1. ^{3}H NMR and ^{1}H NMR data for the dipeptides (*S,S*) **8** an (*R, S*) **9**.

Dipeptide	δT_α (ppm)	δT_β (ppm)	$^3J_{T_\alpha T_\beta}$ (Hz)	$^3J_{H_\alpha H_\beta}$ (calculated) (Hz)	$^3J_{H_\alpha H_\beta}$ (Hz)	$^3J_{H_\alpha H_{\beta'}}$ (Hz)
(S,S) 8	4.30	2.80	4.90	4.60	4.40	9.3
(R,S) 9	4.40	2.75	4.15	3.90	4.0	10

the *R*-residue (**9**) (Fig. 3). These are of 0.18 (rotamer II) and 0.12 (rotamer III) respectively. Therefore it appears that rotamer II in *S*-phenylalanyl residue (gauche conformation between phenyl and carbonyl groups) is only weakly populated as was already found for *N*-acetylphenylalanine (14%).[11] In the case of the *R*-phenylalanyl residue it is the rotamer III which is now very weakly populated. ^{1}H NMR spectra allow us to further estimate the distribution of the three staggered rotamers in the hydrogenated **8** and **9** dipeptides. Between the two α proton-β protons coupling constants $^3J_{\alpha\beta}$ and $^3J_{\alpha\beta'}$ measured in the *N*-terminal phenyl-alanyl moiety in compounds **6** and **7**, only $^3J_{\alpha\beta}$ is nearly identical to the unique value obtained from the tritiated compound.

This example underlines the interest of using specific tritium labelling for eliminating the ambiguities of assignment of the β and β' protons in **6** and **7** ^{1}H NMR spectra and hence to the univocal estimation of I and II rotamers (in *S*-aminoacid) and II and III rotamers (in *R*-aminoacid). In addition, fractions of rotamers reported in Fig. 3 clearly indicate that rotamer I is by far the most populated in the *S*-residue (**8**) as found in *N*-acetyl phenylalanine[11] whereas in the *R*-residue (**9**) rotamer II predominates. In conclusion, homogeneous catalysis seems to be a worthwhile tool to prepare stereoselectively tritiated dipeptides. Considering the almost perfect stereoselectivity obtained by asymmetric reduction with dipamp **3** or bppm **4** as ligands[3,7] it becomes clear that tritiated dipeptides **8** and **9** can be prepared nearly pure by homogeneous asymmetric tritiation. The same conclusion can apply to the tritiation of phenylalanyl residue in other types of dipeptides or longer peptides. We are presently working to define the scope of the tritiation of small biological peptides containing the dehydrophenylalanyl residue or the dehydrotryptophanyl residue.

Acknowledgements—One of us (D. Meyer) thanks the Institut Français du Pétrole for a fellowship. We thank CNRS and CEA for their financial support and the Compagnie des Métaux Précieux for a loan of rhodium trichloride. We are grateful to Mr J. P. Beaucourt for ^{3}H NMR analysis (Service des Molécules Marquées, Département de Biologie, CEN Saclay) and Mr F. Toma for ^{1}H NMR analysis (Service de Biochimie, Département de Biologie, CEN Saclay).

REFERENCES

[1]J. A. Osborn, F. H. Jardine, J. F. Young and G. Wilkinson, *J. Chem. Soc., A* 1711 (1966).
[2a]H. B. Kagan and J. C. Fiaud, *Topics in Stereochemistry* (Edited by E. L. Eliel and A. N. Allinger) **10**, 175 (1978). [b]D. Valentine and J. W. Scott, *Synthesis* 329 (1978).
[3]D. Meyer, J. C. Poulin, H. B. Kagan, H. Levine-Pinto, J. L. Morgat and P. Fromageot, *J. Org. Chem.* **45**, 4680 (1980).
[4]H. B. Kagan and T. P. Dang, *J. Am. Chem. Soc.* **94**, 6429 (1972).

[5]W. S. Knowles, M. J. Sabacky, B. D. Vineyard and D. J. Weinkauff, *Ibid* **97**, 2569 (1975).
[6]K. Achiwa, *Ibid.* **96**, 8625 (1976).
[7]I. Ojima and T. Suzuki, *Tetrahedron Letters* 1239 (1980).
[8]K. Ohnuma, T. Ito and A. Nakamura, *Chem. Letters* 418 (1980).
[9]U. Schmidt, J. Häusler, E. Öhler and H. Poisel, *Progress in the Chemistry of Natural Products* (Edited by W. Herz, H. Grisebach and G. W. Kirby), **37**, 251 (1979).
[10]J. L. Morgat, J. Desmares and M. Cornu, *J. Label. Comp.* **XI**, 257 (1975).
[11]C. Detellier, G. Gelbard and H. B. Kagan, *J. Am. Chem. Soc.* **100**, 7556 (1978).
[12]A. S. C. Chan, J. J. Pluth and H. Halpern, *Ibid.* **102**, 5952 (1980).
[13]J. M. Brown, P. A. Chaloner, *J. Chem. Soc., Chem. Comm.* 344 (1980).
[14]K. G. R. Pachler, *Spectrochim. Acta* **19**, 2085 (1963).
[15]K. G. R. Pachler, *Ibid.* **20**, 581 (1964).
[16]J. Feeney, *J. Magn. Reson.* **21**, 473 (1976).
[17]K. E. Koenig and W. S. Knowles, *J. Am. Chem. Soc.* **100**, 7561 (1978).