Pergamon

Tetrahedron 56 (2000) 4473–4477

TETRAHEDRON

Asymmetric Reduction of (*Z*)-α,β-Dehydrotryptophanyl-containing Biological Peptides with Sulfur Functionality. Influence of Substrate on Stereoselectivity

Akli Hammadi,*,† Hubert Lam, Muriel Gondry, André Ménez and Roger Genet

CEA/Saclay, Département d'Ingénierie et d'Etudes des Protéines, F91191 Gif-sur-Yvette, France

Received 24 February 2000; accepted 26 April 2000

Abstract—Two (*Z*)-α,β-dehydropentapeptides, Boc-βAla-Δ^Z Trp-Met-Asp-Phe-NH_2 **1** and Boc-βAlaΔ^Z-Trp-NLeu-Asp-Phe-NH_2 **2**, have been synthesized by enzymatic α,β-dehydrogenation, and then submitted to rhodium-catalysed asymmetric hydrogenation. The influence of the S-atom on the rate and stereoselectivity of the reduction have been investigated.

Introduction

Asymmetric hydrogenation of α,β-dehydroamino acids with chiral rhodium catalysts has been widely studied leading to high enantioselectivities (e.e. up to 95%).[1] An obvious development of homogeneous hydrogenation has been the asymmetric reduction of more complex prochiral substrates such as α,β-dehydrodipeptides,[2] building blocks for synthesis of enkephalins,[3] and α,β-dehydroenkephalin analogs, which were realized with very high catalytic activity and stereoselectivity.[4] In spite of this success, examples of an asymmetric reduction of a α,β-dehydroamino acid with sulfur functionality, which are wide-spread among biological peptides, are rarely mentioned.[5] The well-known affinity of rhodium atom to sulfur atom[6] as well as the fact that the sulfur compounds can induce poisoning of homogeneous Rh^I-diphosphine catalysts,[7] could explain the reluctance to apply the methodology to this kind of substrates.

In our previous study[8] we reported about 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. These experiments demonstrate that the asymmetric reduction of (*Z*)-α,β-dehydrotryptophanyl-containing peptides produced by (L)-tryptophan 2′,3′-oxidase (LTO) is an efficient approach to the regio- and stereoselective labelling of biological peptides. We also found that the asymmetric reduction of Boc-βAla-Δ^ZTrp-Met-Asp-Phe-NH_2 **1** led to low 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. One of the questions arising was thus to determine whether the transition state could be sensible to electronic as well as steric factors induced by the substrate structure. Or more precisely; How the sulfur atom in the methionyl residue (Met) could influence the enantioselectivity of the asymmetric reduction?

The aim of the present work is thus to provide experimental evidence for this assumption. Replacement of Met in **1** by its steric isostere Norleucine (Nleu), which is commonly used in the literature,[9] offers a convenient and powerful tool to probe the effects of the methionyl residue on the rate and stereoselectivity of the reduction reaction. For comparison purpose, Boc-βAla-Δ^ZTrp-Met-Asp-Phe-NH_2 **1** (($\Delta^Z Trp^2$-Met^3)-PG) and Boc-βAla-Δ^ZTrp-NLeu-Asp-Phe-NH_2 **2** (($\Delta^Z Trp^2$-$NLeu^3$)-PG) were tested here as substrates in the rhodium-catalyzed asymmetric reduction.

Results and Discussion

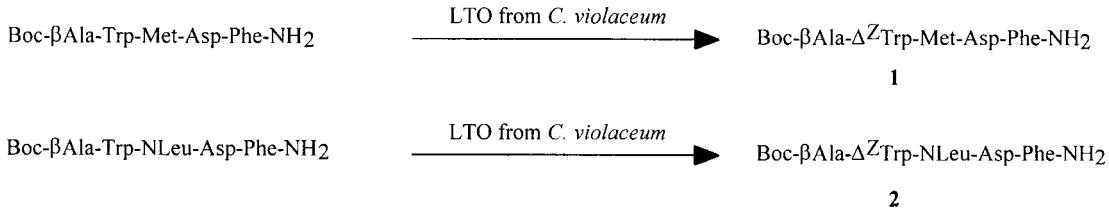
The ($\Delta^Z Trp^2$-Met^3)-PG **1** and ($\Delta^Z Trp^2$-$NLeu^3$)-PG **2** were synthesized by enzymatic α,β-dehydrogenation of Boc-βAla-Trp-Met-Asp-Phe-NH_2 and Boc-βAla-Trp-NLeu-Asp-Phe-NH_2, respectively (Scheme 1). The (*Z*)-α,β-dehydrogenation reaction was realized by *S*-tryptophan 2′,3′-oxidase (LTO) from *Chromobacterium violaceum* (ATCC 12472) which is highly specific for (*S*)-Trp residues.[10] The

Keywords: asymmetric reduction; α,β-dehydropeptide; rhodium catalyst; stereoselectivity.

* Corresponding author. Tel.: +33-1-69-086308; fax: +33-1-69-085753; e-mail: akli.hammadi@cea.fr

† Present address: CEA/Saclay, Institut National des Sciences et Techniques Nucléaires, Unité d'Enseignement Radioprotection, Biologie et Médecine, F91191 Gif-sur-Yvette, France.

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00329-X

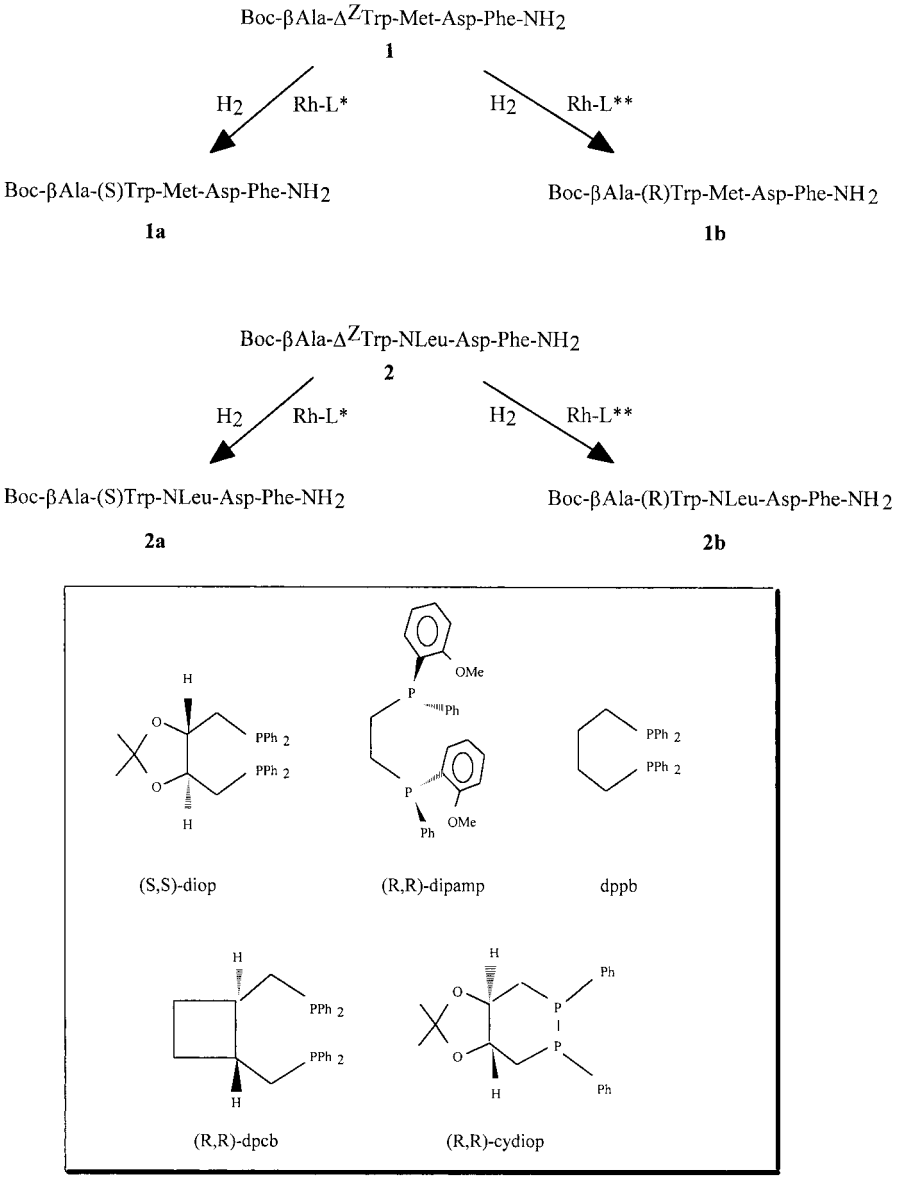


Scheme 1.

α,β-dehydropentapeptides **1** and **2** were obtained in quantitative yield and purified by HPLC.

Asymmetric micro-hydrogenation under pressure of **1** and **2** (6 μmol) were carried out in methanol by using cationic complexes [Rh (*R*,*R*)-dpcb (COD)]$^+$ PF_6^-,[11] [Rh (*S*,*S*)-diop (COD)]$^+$ PF_6^-,[12] [Rh (*R*,*R*)-dipamp (COD)]$^+$ BF_4^-,[13] and [Rh (*S*,*S*)-cydiop (COD)]$^+$ PF_6^-[14] (Scheme 2). The main results are listed in Table 1.

In the presence of the Rh/(*R*,*R*)-dipamp catalyst, the hydrogenation of **1** in methanol at room temperature under an initial hydrogen pressure of 5 atm. gave Boc-βAla-(S)Trp-Met-Asp-Phe-NH_2 **1a** in low diastereoselectivity (26% d.e., entry 1). Regarding the diastereoselectivity dependency on hydrogen pressure, it is interesting to observe that an increase in pressure from 5 to 15 atm. in the presence of (*R*,*R*)-dipamp abolished the stereoselectivity (entries 1 and 2).



Scheme 2.

Table 1. Asymmetric reduction of (Δ^{Z}Trp^{2}-Met^{3})-pentagastrin **1** and (Δ^{Z}Trp^{2}-NLeu^{3})-pentagastrin **2** catalysed by chiral rhodium complexes ([substrate]/[Rh]=1.5; [substrate]=0.006 M in methanol. Reaction performed at room temperature)

Entry	Substrate	Catalyst (ligand)[a]	Pressure (atm)	Time (h)	Yield (%)	*S*/*R*[b]	d.e. (%)
1	**1**	[Rh/(*R*,*R*)-dipamp]	5	20	69	63/37	26
2	**1**	[Rh/(*R*,*R*)-dipamp]	15	20	80	50/50	0
3	**1**	[Rh/(*S*,*S*)-diop]	5	20	73	28/72	44
4	**1**	[Rh/(*R*,*R*)-dpcb]	5	20	68	10/90	80
5	**1**	[Rh/(*R*,*R*)-cydiop]	5	18	99	34/66	32
6	**2**	[Rh/(*R*,*R*)-dipamp]	5	20	99	99.5/0.5	99
7	**2**	[Rh/(*R*,*R*)-dipamp]	11	20	99	99.5/0.5	99
8	**2**	[Rh/(*S*,*S*)-diop]	5	20	75	68/32	36
9	**2**	[Rh/(*R*,*R*)-dpcb]	5	24	99	88/12	76
10	**2**	[Rh/(*R*,*R*)-cydiop]	5	90	94	43/57	14

[a] The ligands are: (*R*,*R*)-dipamp: 1,2-bis[(2-methoxyphenyl)phosphino]-ethane; (*S*,*S*)-diop: 4,5-bis[diphenylphosphino]-2,2-dimethyl-1,3-dioxolane; (*R*,*R*)-dpcb: trans-1,2-bis[diphenylphosphinomethyl]-cyclobutane.
[b] Measured by HPLC. The configuration of the new asymmetric center was assigned by HPLC as compared to reference compounds.

However, the results obtained with **2** were quite different. Indeed, using the cationic complex [Rh (*R*,*R*)-dipamp (COD)]$^{+}$ BF_4^- under a hydrogen pressure of 5 atm, higher chiral recognition and quantitative reduction were found (99% d.e., 99% yield, entry 6). In this latter case, in contrast to **1**, the stereoselectivity showed no dependency at all in the range of 5–11 atm. (99% d.e., entries 6 and 7). This first set of data suggests that the steric and electronic character of S-atom in **1** also has significant influence upon the stereoselectivity and the relative rate of the reaction.

Moreover, the stereoselectivities exhibited in the reduction of **1** using (*S*,*S*)-diop and (*R*,*R*)-dpcb as chiral ligands were moderate (44 and 80% d.e., respectively, in favor of the *R*-tryptophan isomer, entries 3 and 4), and the direction of asymmetric inductions were opposite to that observed for **2** (36 and 76% d.e., respectively, in favor of the *S*-tryptophan isomer, entries 8 and 9). Together, these results confirmed the determining effect of methionyl residue on the stereoselectivity of reduction. The S-atom could possibly serve as an additional rhodium ligand and, hence, affect the mode of the enantioface selection of olefinic moiety by the chiral Rh complex.

However, the asymmetric reduction of **1** and **2** catalyzed by [Rh/(*R*,*R*)-cydiop] afforded in both cases ((*R*)-Trp)-pentagastrine in modest diastereoselectivity (32 and 14%, respectively, entries 5 and 10) whatever the structure of the α,β-dehydropentapeptide, suggesting that the double asymmetric induction depends on catalyst-substrate combination.

In order to identify the simple asymmetric induction caused by the methionyl residue, hydrogenations of (*Z*)-α,β-dehydropeptides **1** and **2** were performed with achiral catalysts, [Rh/dppb] and PdO (Table 2).

In contrast to the low selectivities obtained by hydrogenation of linear (*Z*)-α,β-dehydropeptides over heterogeneous catalysts (0–20% d.e.)[15], we showed that the reduction of **1** catalysed by PdO could be performed under a hydrogen pressure of 15 atm. with an acceptable diastereoselectivity (52% d.e., entry 2). Studying the reduction of **2**, then we observed that no asymmetric induction arose from the utilization of PdO under a pressure of 5 atm. (entry 4). Furthermore, the hydrogenation of the (*Z*)-α,β-dehydropeptide **1** occured under a pressure of 15 atm. in order to avoid a slow reaction. These results indicate that the presence of (*S*)-methionine in the peptide could be an important factor affecting both the reduction rate and the stereoselectivity. A pausible explanation could be that a significant attractive interaction between sulfur in (*S*)-methionine moiety and PdO, could increase the rigidity of the (*Z*)-α,β-dehydropentapeptide and, hence, improve optical yields.

We then proceeded to the reduction of (*Z*)-α,β-dehydropeptides **1** and **2** in the presence of an achiral rhodium complexe ([Rh/dppb]) and showed in both cases a clear, although opposite, asymmetric induction: 26% in favour of the (*R*)-tryptophan isomer with **1** (entry 1) and 6% in favour of the (*S*)-tryptophan isomer with **2** (entry 3). The finding of a more stereoselective hydrogenation of **1** than of **2** makes the suggestion of special coordination of sulfur to rhodium which modify the course of the reaction. Futhermore, the lack of selectivity achieved in the reduction of **2** could be interpreted by the exclusive coordination of the *N*-acyl-(*Z*)-α,β-dehydrotryptophan moiety with the rhodium complex.

Table 2. Asymmetric reduction of (Δ^{Z}Trp ^{2}-Met^{3})-pentagastrin **1** and (Δ^{Z}Trp^{2}-NLeu^{3})-pentagastrin **2** catalyzed by PdO or [Rh/dppb] (All reactions were run with 6 μmol of substrate and 4.5 mg of PdO or 4 μmol of [Rh/dppb] in methanol. [Substrate]/[Rh]=1.5; [substrate]=0.006 M in methanol. Reaction performed at room temperature)

Entry	Substrate	Catalyst	Pressure (atm)	Time (h)	Yield (%)	*S*/*R*[a]	d.e. (%)
1	**1**	[Rh/dppb]	5	24	85	37/63	26
2	**1**	PdO	15	20	80	24/76	52
3	**2**	[Rh/dppb]	5	43	85	53/47	6
4	**2**	PdO	5	20	95	50/50	0

[a] Measured by HPLC. The configuration of the new asymmetric center was assigned by HPLC as compared to reference compounds.

In this case, the rest of the molecule is located in the outer sphere of the coordination site. This may be the reason why a low-asymmetric induction was observed with **2** (entry 3).

Conclusion

In order to ascertain the putative role of the methionyl residue in the asymmetric reduction of the ($\Delta^{Z}Trp^{2}$-Met^{3})-pentagastrine **1**, the hydrogenation of two (*Z*)-α,β-dehydropentapeptides **1** and **2** were performed with achiral and chiral catalysts. We showed that a (*Z*)-α,β-dehydropeptide with sulfur functionality can be asymmetrically hydrogenated in the presence of homogeneous Rh^{I}-diphosphine complexes or PdO without poisoning these catalysts. However, the presence of (*S*)-methionine in the (ΔTrp)-pentagastrine **1** appears to have a strong influence upon the way of asymmetric induction, and the effects on the stereoselectivity of the reaction remain unpredictable. Indeed, the asymmetric reduction of Boc-βAla-Δ^{Z}Trp-Met-Asp-Phe-NH_2 **1** catalysed by [Rh (*R*,*R*)-dipamp (COD)]$^{+}$ BF_4^{-} led to low diastereomeric excesses (0–26% d.e. in favor of the *S*-tryptophan isomer), whereas the asymmetric hydrogenation of Boc-βAla-Δ^{Z}Trp-NLeu-Asp-Phe-NH_2 **2** with the same catalytic system proceeded with extremely high stereoselectivity (up to 99%).

The results obtained with **2** constituted a quasi-theoretical example for the stereoselective hydrogenation of dehydropeptides, suggesting that asymmetric induction is mainly determined by only steric repulsion between the substrate and the ligand coordinated to the rhodium species. In contrast, the asymmetric hydrogenation of ($\Delta^{Z}Trp^{2}$-Met^{3})-pentagastrine **1** involves electronic interaction between rhodium and sulfur in methionine moiety as well as steric interactions between the chiral diphosphine and the substrate. Furthermore, the asymmetric induction arises from the electronic environment due to *S*-atom rather than the catalyst structure.

In order to improve the regio- and stereoselective reduction (hydrogenation, deuteration or tritiation) of biological peptides of higher molecular weight, the effects of heteroatoms, especially sulfur, on the stereoselectivity of the reaction should be taken into account.

Experimental

General experimental procedures

HPLC analyses were performed on a Waters 600 chromatographic system (Vidac 18C column 250×4.6 mm^2) supplied with a Waters 996 photodiode array detector. Ultraviolet spectra were recorded with a Beckman DU-70 spectrophotometer. Products were analyzed either by chemical ionization-mass spectrometry (Nermag R 10-10). Methanol was freshly distilled, dried following literature method and stored under argon. All experiments with organometallic elements were performed using standard Schlenck techniques.

Enzymatically prepared 1 and 2

(L)-Tryptophan 2′,3′-oxidase was purified from *C. Violaceum* (ATCC 12472) according to Genet and coworkers.[10] The enzymatically prepared α,β-dehydrotryptophanyl-peptides **1** and **2** were obtained by incubation of the corresponding pentapeptides (Boc-βAla-Trp-Met-Asp-Phe-NH_2 and Boc-βAla-Trp-NLeu-Asp-Phe-NH_2, 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**. The α,β-dehydropeptides **1** and **2** showed characteristic absorption peaks at λ_{max}=337 nm and λ_{max}=338 nm, respectively.

Chromatographic procedure for the purification of 1 and 2

The dehydropentapeptides were purified on reverse-phase chromatography (Vydac 18 C column; eluent, 35% acetonitril in water; flow rate, 1 ml min^{-1}; UV detection at 280 and 330 nm). Retention times were: 12.1 min for **1** and 34.1 min for **2**. Evaporation of the solvent afforded 7.6 mg (75%) of **1** and 8.1 mg (80%) of **2** as a yellow oil. The dehydropeptides **1** and **2** were then characterized by mass spectrometry (*m*/*z*=767, MH^{+} and *m*/*z*=749, MH^{+}, respectively).

Preparation of the in situ neutral complex [Rh/dppb]

[Rh Cl dppb] was prepared in situ, by reaction of [Rh Cl (COD)]$_2$ with diphosphine in degassed methanol. Typically, [Rh Cl (COD)]$_2$ (2 μmol) and dppb (4 μmol) were dissolved in 1 ml of methanol under argon. The solution was then stirred for 30 min. The complex thus obtained was ready to use for asymmetric hydrogenation and was introduced into the hydrogenation flask by means of a syringe. Any contact with air was avoided.

Hydrogenation, general procedure

The high pressure reductions are run using an automatic gas transfer unit[16] supplied with a liquid helium cryostat and fitted with an inlet for introduction of the solvent. This cryostat is used to bring the hydrogen 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 hydrogen can be compressed by heating in a very small volume (ca 1 ml). Using this equipment, it is possible to rise the gas pressure to about 30 atm.

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 hydrogen. The reductions were run under the reaction conditions given in Tables 1 and 2. The solvent was removed under reduced pressure. Conversion rates were determined by HPLC analysis.

Identification of diastereomers

The stereoselectivity was mesured by HPLC with a Vydac 18C column (250×4.6 mm^2) using acetonitril-water (35/65) eluent. The flow rate was 1 ml min^{-1} with UV detection at 280 nm. The resultant diastereomers **1a**, **1b**, **2a** and **2b** were characterized as compared to reference compounds.

References

1. (a) Fryzuk, M. D.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6262–6267. (b) Caplar, V.; Comisso, G.; Sunjic, V. *Synthesis* **1981**, 85–116. (c) Kagan, H. B. *Comprehensive Organometallic Chemistry;* Wilkinson, G., Ed.; Pergamon Press: Oxford, 1982; Volume 8, 463. (d) Knowles, W. S. *Acc. Chem. Res.* **1983**, *16*, 106–111. (e) Apsimon, J. W.; Collier, T. L. *Tetrahedron* **1986**, *42*, 5154–5257. (f) Ojima, I.; Clos, N.; Bastos, C. *Tetrahedron* **1989**, *45*, 6901–6939. (f) Ohkuma, T.; Doucet, H.; Pham, T.; Mikami, K.; Korenaga, T.; Terada, M.; Noyori, R. *J. Am. Chem. Soc.* **1998**, *120*, 1086–1087. (g) Brown, J. M. In *Comprehensive Asymmetric Catalysis*; Jacobsen, E. N., Pfaltz, A., Yamamoto, H., Eds.; Springer: Berlin, 1999; Vol. I, 122.

2. (a) Ojima, I.; Suzuki, T. *Tetrahedron Lett.* **1980**, *21*, 1239–1242. (b) Ojima, I.; Kogure, T.; Yoda N.; Suzuki, T.; Yatabe, M.; Tanaka, T. *J. Org. Chem.* **1982**, *47*, 1329–1334. (c) Fuganti, C.; Grasselli, P.; Malpezzi, L. *J. Org. Chem.* **1986**, *51*, 1126–1128.

3. (a) Ojima, I.; Yoda N. *Tetrahedron Lett.* **1982**, *23*, 3913–3916. (b) Ojima, I.; Yoda N.; Yatabe, M.; Tanaka, T.; Kogure, T. *Tetrahedron* **1984**, *40*, 1255–1268. (c) Ojima, I.; Tsai, C.-Y.; Zhang, Z. *Tetrahedron Lett.* **1994**, *35*, 5785–5788.

4. (a) Nuzillard, J. M.; Poulin, J. C.; Kagan, H. B. *Tetrahedron Lett.* **1986**, *27*, 2993–2996. (b) Hammadi, A.; Nuzillard, J. M.; Poulin, J. C.; Kagan, H. B. *Tetrahedron: Asymmetry* **1992**, *3*, 1247–1262.

5. Ojima, I.; Yoda, N.; Yatabe, M.; Tanaka, T.; Kogure, T. *Tetrahedron* **1984**, *40*, 1255–1268.

6. (a) Sanger, A. R. *Can. J. Chem.* **1983**, *61*, 2214–2219. (b) Bonuzzi, C.; Bressan, M.; Morandini, F.; Morvillo, A. *Inorg. Chim. Acta* **1988**, *154*, 41–43. (c) Reetz, M. T.; Rudolph, J. *Tetrahedron Asymmetry* **1993**, *4*, 2405–2406.

7. Faller, J. W.; Parr, J. *J. Am. Chem. Soc.* **1993**, *115*, 804–805.

8. Hammadi, A.; Meunier, G.; Ménez, A.; Genet, R. *Tetrahedron Lett.* **1998**, *39*, 2955–2958.

9. (a) Marseigne, I.; Dor, A.; Bégué, D.; Reibaud, M.; Zundel, J. L.; Blanchard, J. C.; Pélaprat, D.; Roques, B. P.; *J. Med. Chem.* **1988**, *31*, 966–970. (b) Gilles, A.-M.; Malière, P.; Rose, T.; Sarfati, R.; Longin, R.; Meier, A.; Fermandjian, S.; Monnot, M.; Cohen, G. N.; Bârzu, O. *J. Biol. Chem.* **1988**, *263*, 8204–2009.

10. (a) Genet, R.; Denoyelle, C.; Ménez, A. *J. Biol. Chem.* **1994**, *269*, 18177–18184. (b) Genet, R.; Bénetti, P.-H.; Hammadi, A.; Ménez, A. *J. Biol. Chem.* **1995**, *270*, 23540–23545. (c) Hammadi, A.; Ménez, A.; Genet, R. *Tetrahedron Lett.* **1996**, *19*, 3309–3312.

11. Aviron-Violet, P.; Colleuille, Y.; Varagnat, J. *J. Mol. Catal.* **1979**, *5*, 41–50.

12. Kagan, H. B.; Dang, T. P. *J. Am. Chem. Soc.* **1972**, *94*, 6429–6433.

13. Vineyard, B. D.; Knowles, W. S.; Sabacky, M. J.; Bachman, G. L.; Weinkauff, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 5946–5952.

14. Zhang, S. Y.; Yemul, S.; Kagan, H. B.; Stern, R.; Commereuc, D.; Chauvin, Y. *Tetrahedron Lett.* **1981**, *22*, 3955–3958.

15. Takasaki, M.; Harada, K. *J. Chem. Soc., Chem. Commun.* **1987**, 571–573.

16. Morgat, J. L.; Desmares, J.; Cornu, M. *J. Labelled Compds.* **1975**, *XI*, 257–264.