Syntheses of Proline Analogues as Potential Mechanism-Based Inhibitors of Proline Dehydrogenase: 4-Methylene-L-, (*E*)- and (*Z*)-4-(Fluoromethylene)-L-, *cis*- and *trans*-5-Ethynyl-(±)-, and *cis*- and *trans*-5-Vinyl-L-proline

Franco Manfré,† Jean-Marc Kern,‡ and Jean-François Biellmann*,†

Laboratoire de Chimie Organique Biologique, URA-CNRS 31 and Laboratoire de Chimie Organo-Minérale, URA-CNRS 422, Faculté de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, 67008 Strasbourg Cedex, France

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Proline dehydrogenase is an enzyme involved in the energetic processes required for flight in certain insects including the tse-tse fly. Proline analogues were designed for the inhibition of this enzyme. For this purpose 4-methylene-L-proline and (*E*)- and (*Z*)-4-(fluoromethylene)-L-proline were prepared from *trans*-4-hydroxyl-L-proline through the following sequence: protection at the nitrogen with a *tert*-butoxycarbonyl and at the caboxylic acid as methyl ester, oxidation of the hydroxyl group to a ketone, Wittig reaction, and removal of the protecting groups. The *cis*- and *trans*-5-ethynyl-(±)-proline was prepared from *N*-(*tert*-butoxycarbonyl)-3-(trimethylsilyl)-2-propynylamine whose dianion reacted with 1,2-epoxy 4-bromobutane. Cyclization in the presence of trifluoroacetic acid, oxidation of the primary alcohol to acid, and removal of the protecting groups gave *cis*- and *trans*-5-ethynyl-(±)-proline. Stereoselectivity was observed in the reaction of the dianion of 3-(trimethylsilyl)-2-propynylamine with 1,2-epoxy-4-bromobutane. The *cis*- and *trans*-5-vinyl-L-prolines were prepared from *N*-(methoxycarbonyl)-5-methoxy-L-proline ester by reaction with bis(trimethylsilyl)acetylene in the presence of titanium tetrachloride followed by sequential removal of the protecting groups and by reduction. The cis and trans configuration of the 5-vinyl-L-proline obtained was established by comparison of the NMR spectrum of the *trans*-*N*-tosyl-5-vinyl-(±)-proline methyl ester with the published spectrum of the trans derivative.

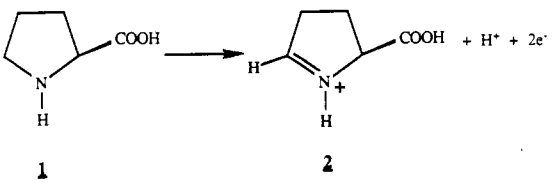
Proline dehydrogenase (E.C. 1.5.99.8) also known as proline oxidase is involved in the metabolic pathway from proline to glutamic acid.[1] The first step is the oxidation of proline 1 to Δ^1-pyrroline-5-carboxylic acid (2) (Scheme I). This enzyme has been found in mammals, bacteria, and insects such as, most interestingly, the tse-tse fly *Glossina morsitans*.[2] In the tse-tse fly, proline is the sole energy source for the flight.[2] Proline dehydrogenase is the first enzyme involved in this pathway. Inhibition of this enzyme may therefore be a strategy to control this insect.

A number of inhibitors of proline dehydrogenase from various sources have been mentioned in the literature. Inhibitors of the respiratory chain act upon the proline dehydrogenase, most likely through interaction with cytochrome.[3] 3,4-Dehydro-L-proline (3) is a competitive inhibitor of proline dehydrogenase from rat liver, and pyrrole-2-carboxylic acid is a noncompetitive inhibitor.[4]

We decided to prepare analogues of proline incorporating structural elements which could lead to mechanism-based inhibition. The rationale was that during the enzyme action on the substrate an electrophile and a nucleophile are produced and they could react together leading to irreversible inhibition, in the same way that a number of flavine-dependent enzymes react with unsaturated substrate analogue.[5-7] This rationale remains true even if the cofactor is not a flavine, so that the synthesis of proline analogues could be undertaken without knowledge of the cofactor of the insect enzyme. In analogy to the inhibition of related enzymes, the strategy was to introduce a double or triple bond on the proline skeleton, for instance, the products 3 (a competitive inhibitor of rat liver enzyme[4]), 4, 5 and 6, and 7 and 8, which could, after oxidation, react with a nucleophile of the enzyme.

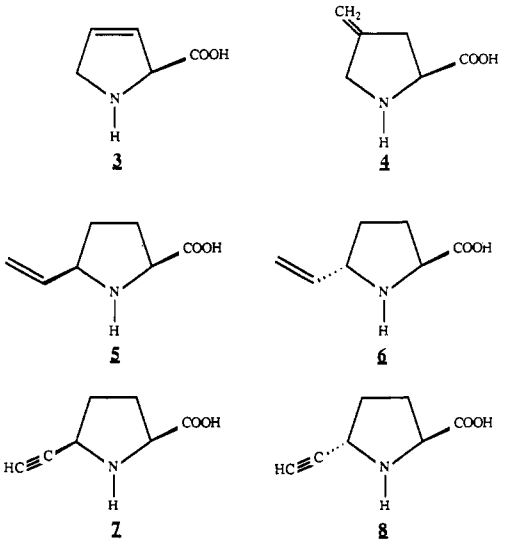
The synthesis of 4-methylene-L-proline 4 and the monofluoroanalogues (*E*)-14 and (*Z*)-15 will be described starting from *trans*-4-hydroxy-L-proline (9).

Scheme I



1 2

Chart I



3 4 5 6 7 8

For the vinylic and ethynyl analogues, cis and trans isomers had to be prepared since the stereochemistry of the enzymatic reaction (is the *pro-S* or *pro-H* hydrogen

* To whom correspondence should be addressed.
† Laboratoire de Chimie Organique Biologique.
‡ Laboratoire de Chimie Organo-Minérale.

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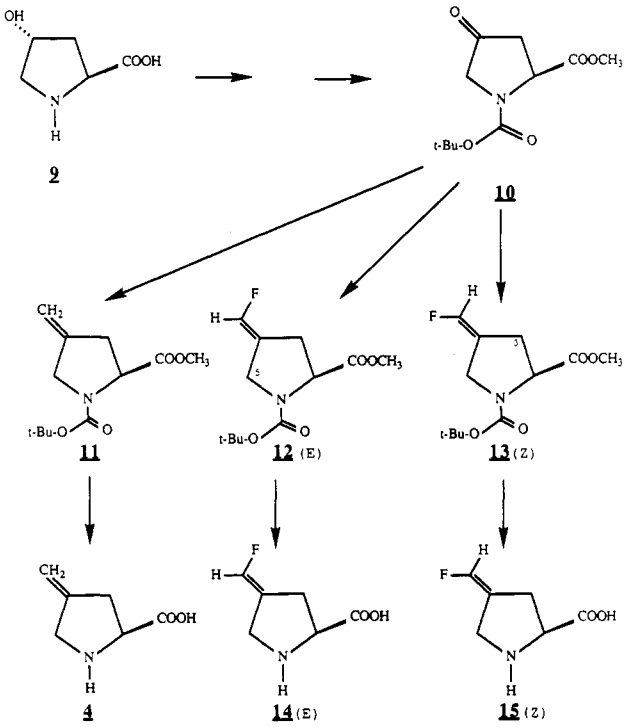
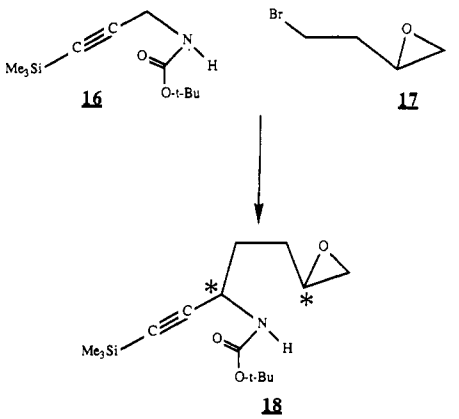
Scheme II



Scheme III



removed by proline dehydrogenase?) remains to be determined. The possibility of functionalizing the methylene group at the 5-position of proline, as known **25** → **26**, provided an entry to the optically active proline derivates, *cis*- and *trans*-5-vinyl-L-proline (**5**) and (**6**).

Synthesis of 4-Methylene-L-proline (4). D,L-4-Methyleneproline has been isolated from the plant *Eriobotrya japonica*.[8] A synthesis of D,L-4-methyleneproline starting from 1,3-dichloroisobutene and diethyl aminomalonate has been published.[9] We decided to prepare **4** optically pure starting from *trans*-4-hydroxy-L-proline (**9**). The Wittig reaction of ketone **10**[10] with the ylide derived from methyltriphenylphosphonium bromide gave the methylene compound **11** in a yield of 66%. Acid hydrolysis of the methyl ester and acid removal of the N-protecting group gave 4-methylene-L-proline (**4**).

Synthesis of 4-(Fluoromethylene)-L-prolines 14 and 15. The ylide derived from (fluoromethyl)triphenylphosphonium tetrafluoroborate[11] was generated at −78 °C with butyllithium, and the Wittig reaction with ketone **10** was carried out at −78 °C. Both isomers **12** and **13** were obtained in a total yield of 50% in a ratio of 4/5. The less abundant isomer was eluted first during chromatography on silica gel. The determination of the stereochemistry of the two isomers was done by the NOE effect. Irradiation of the olefinic proton at 6.6 ppm gave an enhancement of the signal at 4. 1 ppm, attributed to the hydrogen at C-5, for the isomer eluted first. The *E* stereochemistry as in **12** was attributed to this isomer. The isomer eluted second (mp 62–63 °C) showed in its ^{1}H NMR spectrum an NOE effect by irradiation of the olefinic proton at 6.5 ppm, on the signal at 2.6 ppm attributed to one of the hydrogens at the 3-position. This compound is therefore *Z* isomer **13**. The protecting groups were then removed as for compound **11**.

Synthesis of *cis*- and *trans*-D,L-5-Ethynylproline (7 and 8). Most currently, methods for synthesis of 2,5-disubstituted pyrrolidines are not compatible with the presence of a triple bond.[12–18] We chose to synthesize the pyrrolidine by a new method: first, formation of the bond between the C-4 and C-5 carbon atoms and then afterwards formation of the nitrogen–C-2–carbon bond.

The N-1–C-5 unit was selected as the silyl carbamate **16** the dianion of which has been used in a number of syntheses of substituted propargyl amines.[19] At first we tried to use *tert*-butyl 2,4-dibromobutyrate as the reagent for the C-2–C-4 part with the dianion derived from **16**, but this did not give any pyrrolidinic product. We then turned to the bromo epoxide **17** prepared from 4-bromo-1-butene.[20] This epoxide was unstable on distillation and was used directly after epoxidation.

The dianion was generated at −78 °C from **16** using LDA in the presence of tetramethylethylenediamine, and after reaction with bromide **17** the epoxide carbamate **18** was isolated in a yield of 60% beside an allenic product whose structure was not established. The NMR spectrum of the epoxide carbamate **18** in $CDCl_3$ showed no evidence of the presence of two isomers, and on chromatography we had no evidence for any heterogeneity. However, in hexadeuteriobenzene, the ^{1}H NMR spectrum (400-MHz) of carbamate **18** showed two signals for the trimethylsilyl group in 0.23 and 0.24 ppm and for the *tert*-butyl group at 1.49 and 1.50 ppm in a ratio of 7:3. So this alkylation of the dianion derived from **16** to a diastereoselective reaction. The effect of experimental conditions on the ratio of both isomers like addition of HMPT, lithium bromide, and ratio of reactants had only a slight effect.

The preference of the (*RS*,*SR*) isomer **20** is probably due to the interaction of the oxirane with the lithium cation in the aggregate. The effect of HMPT reducing such an interaction is to lower markedly the stereoselectivity (from de of 40–50% to 10%). This effect of the stereochemistry of the epoxide on the preference for the carbanion face which is attacked is noteworthy.

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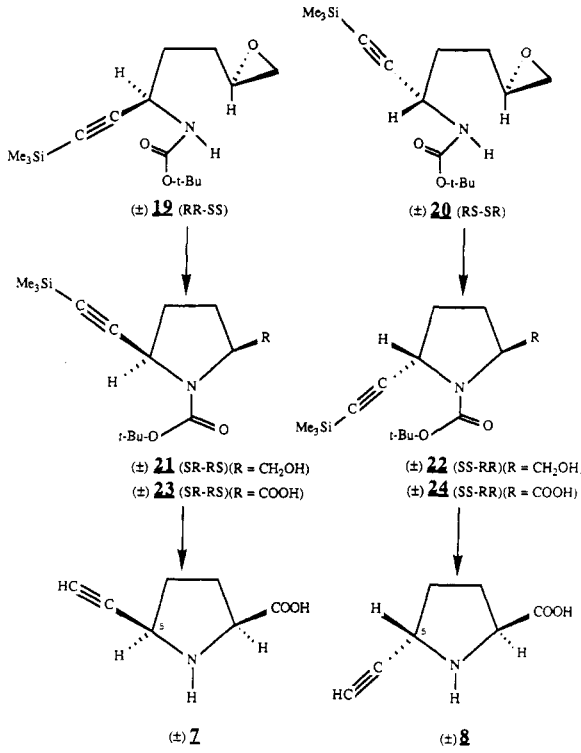
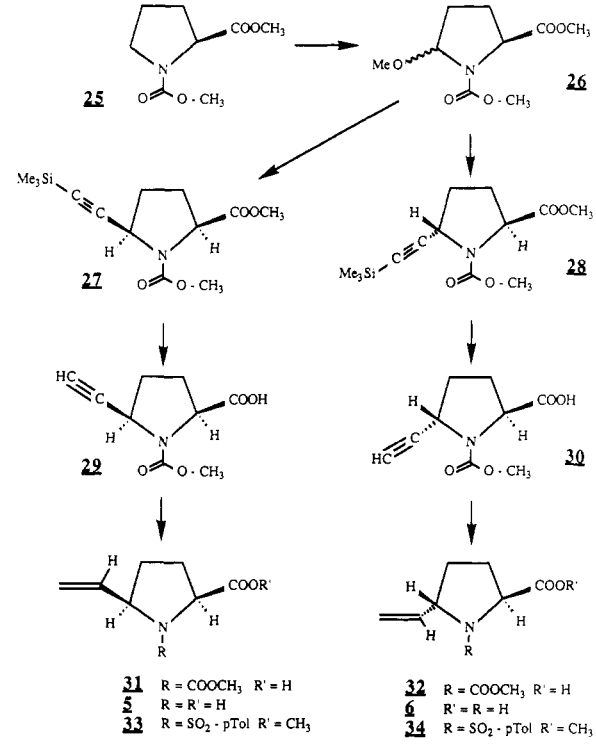
Scheme IV



Scheme V



Since we were unable to separate the epoxide carbamates **19** and **20**, further reaction was carried out on the isomer mixture. The cyclization was first performed at 65 °C for 20 h using activated silica gel suspended in toluene.[21-23] The two pyrrolidines *cis*-**21** and *trans*-**22** were obtained in a yield of 52% and in the ratio **21**(cis)/**22**(trans) (1/9). A higher temperature lowered the yield. No piperidine was detected so the exo tetragonal cyclization is preferred.[24,25]

The cis/trans ratio did not correspond to the epoxide carbamate isomer ratio. The steric crowding of the *tert*-butoxycarbamate group could be the cause of this, and its removal before cyclization was tried. However, instead of the removal of the protecting group, trifluoroacetic acid at 0 °C in methylene chloride cyclized the carbamates **19** and **20** to the pyrrolidines **21** and **22** in a yield of 80% with an isomer ratio **21**(cis)/**22**(trans) (15/85). These experiments were carried out with the **19**/**20** mixtures (30:70) obtained with 3 equiv of TMEDA and 1 equiv of bromoepoxide. The mixtures of carbamates **19** and **20** obtained under different experimental conditions were then cyclized with trifluoroacetic acid under the same experimental conditions, and the cis/trans ratios obtained were in agreement with a cyclization of one of the isomers **20** (*RS*/*SR*) to the *trans*-**22** in an almost quantitative yield and a cyclization of the other isomer **19** (*RR*/*SS*) to the *cis*-**21** in a yield of 40%. Probably degradation of the minor epoxide proceeded as fast as the cyclization. Indeed, for the cyclization of the minor isomer **19**, there is more steric crowding than for the cyclization of isomer **20**.

Jones oxidation[26] of both alcohols **21** and **22** gave the acids **23** and **24** in a yield of 90% and 80%. The protecting groups were removed and the cis and trans (±) acids **7** and **8** were obtained. The stereochemistry at C-5 will be discussed later.

Synthesis of *cis*- and *trans*-5-Vinyl-L-proline (5 and 6). Electrochemical oxidation of amides[27] is easy to perform in good to high yields[27-32] and is in general a regiospecific reaction.[33] For instance, the methoxylation at the 5-position of properly protected proline derivate **25** has been described[34-36] and the mixture of both isomers **26** (cis/trans (1:1)) has been obtained in a total yield of 87%.

The methoxy amides react in the presence of Lewis acid with nucleophiles to form, for instance, a C–C bond.[33] We used this route to prepare *cis*- and *trans*-5-vinyl-L-proline (**5** and **6**) whose stereochemistry at position C-5 was determined by chemical correlation.

The protected L-proline **25** was converted to the methoxylated product **26** as described.[34-36] The highest yield (90%) was obtained when 2.5 F/mol of electricity was consumed. The product was purified by filtration on silica gel or distillation.

The introduction of an ethynyl chain was achieved by treating the methoxy amide **26** with bis(trimethylsilyl)-acetylene in the presence of a Lewis acid.[27-29] Titanium tetrachloride as catalyst gave in a yield of 61% both *cis*-**27** and *trans*-**28** isomers (4:3 ratio). The removal of the tri-

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methylsilyl group with tetrabutylammonium fluoride gave the carbamate esters, base treatment of which gave the free carbamate acids **29** and **30**. However, the carbamate group in products **29** and **30** could not removed. Various methods with trimethylsilyl iodide gave complex products of undetermined structure, and lithium *n*-propylthiolate in HMPT[37,38] was not effective in this case.

Therefore, the ethynyl group was converted to the vinyl group by catalytic hydrogenation of the carbamate esters of **29** and of **30** to give esters of **31** and of **32**. Saponification of the ester and removal of the carbamate by trimethylsilyl iodide generated in situ gave *cis*- and *trans*-5-vinyl-L-proline (**5** and **6**).

NMR did not allow the unambiguous determination of the configuration of the substituent at position C-5. We therefore had to rely on a correlation. The related *N*-(*p*-toluenesulfonyl)proline esters have been prepared and their relative configurations established by iodolactonization. The two isomers differed in their NMR spectra, especially at the vinylic proton.[39] We therefore prepared the *N*-sulfonyl derivatives **33** and **34** by removal of the carbamate group from products **31** and **32** followed by reaction with *p*-toluenesulfonyl chloride. The NMR spectra of the tosylamides **33** and **34** were compared to the published spectra of the cis isomer.[39] The vinylic proton shifts in the cis isomer **33** (mp 86–89 °C) were at 5.09, 5.36, and 5.78 ppm and in the *trans*-**34** isomer at 5.09 (2 H) and 5.60 ppm (1 H).

For the racemic series described above chemical transformation of (±)-**24** the major isomer gave the *trans*-*N*-sulfonamide ester (±)-**34** whose relative configuration was attributed on the basis of its NMR spectrum as for **34** obtained above.

Experimental Section

All the experiments sensitive to oxygen were performed under argon. Tetrahydrofuran was distilled from sodium–benzophenone. Ether and methylene chloride were dried over calcium hydride. The commercial solution of butyllithium was titrated with diphenylacetic acid.[40] When an extraction was performed, the organic layer was washed with water and brine and dried over anhydrous magnesium sulfate.

(2*S*)-Methyl *N*-(*tert*-Butoxycarbonyl)-4-methylenepyrrolidine-2-carboxylate (11). Methyltriphenylphosphonium bromide (4.8 g) was added with stirring at 3 °C to a suspension of potassium *tert*-butoxide (1.41 g) in anhydrous ether (100 mL). After 15 min, a solution of (2*S*)-methyl *N*-(*tert*-butoxycarbonyl)-4-oxopyrrolidinecarboxylate **10** (2.2 g)[10] in ether (30 mL) was added. After 3 h at 35 °C, a saturated solution of NH4Cl (50 mL) was added at 5 °C. The organic layer was separated and the aqueous phase extracted with ether (3 × 50 mL). Removal of the solvent gave an oil (4.3 g) which was chromatographed on silica gel. The product **11** was eluted with hexane/ethyl acetate (85:15) as an oil (66%). ^{1}H NMR ($CDCl_3$): δ 1.44 and 1.48 (2s, 9 H); 2.55–2.72 (m, 1 H); 2.85–3.07 (m, 1 H); 3.72 (s, 3 H); 4.03–4.14 (m, 2 H); 4.35–4.56 (m, 1 H); 4.95–5.15 (m, 2 H). $[\alpha]^{25}_D$: −23.2° (*c* = 1.1; EtOH). Found: C, 59.7; H, 8.0; N, 5.7.

(2*S*)-4-Methylenepyrrolidine-2-carboxylic Acid (4). The hydrolysis of the methyl ester was performed first. 2 N sodium hydroxide (1 mL) was added to a solution of (2*S*)-methyl *N*-(*tert*-butoxycarbonyl)-4-methylenepyrrolidine-2-carboxylate (**11**) (0.28 g) in dioxane (7 mL) and water (3 mL). After 3 h at 25 °C, the reaction mixture was extracted with ether (2 × 10 mL). The aqueous phase was acidified (to pH 2) at 5 °C with 0.1 N hydrochloric acid and extracted with ethyl acetate (3 × 40 mL). The oil obtained (0.26 g) was used without purification for the next step. A solution of trifluoroacetic acid (3.3 mL) in methylene chloride (CH_2Cl_2) (3.3 mL) was slowly added at 0 °C to a solution of the oil obtained (0.26 g). After 1.5 h at 20 °C, the solvents were removed under vacuum. The product **4** was chromatographed on silica gel (50 g, chloroform–ethanol) and was crystallized in 95% ethanol. Overall yield of **4** and **13**: 71%. Mp: 243–245 °C dec. ^{1}H NMR (D_2O): δ 2.42–2.55 (m, 1 H); 2.7–2.83 (m, 1 H); 3.67–3.8 (m, 2 H); 3.98 (dd, 1 H, *J* = 7.2, 9.0 Hz); 4.9–4.96 (m, 2 H). $[\alpha]^{25}_D$: −50.9° (*c* = 0.44; H_2O). Found: C, 56.8; H, 7.3; N, 10.8.

(*E*)- and (*Z*)-(2*S*)-Methyl *N*-(*tert*-Butoxycarbonyl)-4-(fluoromethylene)pyrrolidine-2-carboxylate (12 and 13). A solution of butyllithium in hexane (1.25 M; 3.3 mL) was added at −78 °C to a suspension of (fluoromethyl)triphenylphosphonium tetrafluoroborate (2.4 g) in THF (25 mL). After 1 h at −78 °C, a solution of (2*S*)-methyl *N*-(*tert*-butoxycarbonyl)-4-oxopyrrolidine-2-carboxylate (**10**) (0.5 g) in THF (10 mL) was added at −78 °C. After 3 h at −78 °C, the temperature was gradually raised to 25 °C, and after 12 h a saturated ammonium sulfate solution (30 mL) was added. The mixture was extracted with ethyl acetate (3 × 50 mL). After removal of the solvent the resulting oil was chromatographed on silica gel (50 g; hexane/ethyl acetate (95:5)). The *E* isomer **12** (120 mg) was eluted first as an oil and *Z* isomer **13** (150 mg) later. The *Z* isomer **13** crystallized as needles from hexane. Yield of *E* and *Z* isomers: 50%.

***E* Isomer 12.** ^{1}H NMR ($CDCl_3$): δ 1.42; 1.47 (9 H); 2.75–3.02 (m, 2 H); 3.74 (s, 3 H); 4.04–4.14 (2 H); 4.4–4.6 (m, 1 H); 6.63 (s, 1 H, J_{CHF} = 80.3 Hz). $[\alpha]^{19}_D$: −24.6° (*c* = 0.6; EtOH). Found: C, 55.2; H, 6.9; N, 5.0.

***Z* Isomer 13.** Mp: 62–63 °C (from hexane). ^{1}H NMR ($CDCl_3$): δ 1.44; 1.49 (s, 9 H); 2.54–2.61 (m, 1 H); 2.74–2.95 (m, 1 H); 3.74 (s, 3 H); 4.10–4.32 (m, 2 H); 4.4–4.6 (m, 1 H); 6.51 (d, 1 H, J_{CHF} = 82.0 Hz). $[\alpha]^{19}_D$: −33.9° (*c* = 1.3; EtOH). Found: C, 55.4; H, 7.0; N, 5.1.

(*E*)- and (*Z*)-(2*S*)-*N*-(*tert*-Butoxycarbonyl)-4-(fluoromethylene)pyrrolidine-2-carboxylic Acid. The same procedure as for (2*S*)-methyl *N*-(*tert*-butoxycarbonyl)-4-methylene-2-pyrrolidinecarboxylate (**11**) was used for *E* and *Z* isomers **12** and **13**.

The *E* isomer was an oil (yield 91%). ^{1}H NMR ($CDCl_3$): δ 1.43; 1.48 (s, 9 H); 2.75–3.05 (m, 2 H); 4.04–4.14 (m, 2 H); 4.40–4.60 (m, 1 H); 6.63 (d, 1 H; J_{CHF} = 82.3 Hz).

The *Z* isomer was an oil (yield 80%). ^{1}H NMR ($CDCl_3$): δ 1.45; 1.49 (s, 9 H); 2.6–3.1 (m, 2 H); 4.1–4.35 (m, 2 H); 4.38–4.68 (m, 1 H); 6.55 (d, 1 H; J_{CHF} = 82.4 Hz).

(*E*)- and (*Z*)-(2*S*)-4-(Fluoromethylene)pyrrolidine-carboxylic Acid (14 and 15). The same procedure for the removal of the *N*-*tert*-butoxylcarbonyl group as for (*S*)-*N*-(*tert*-butoxycarbonyl)-4-methylenepyrrolidine-2-carboxylic acid was used.

E isomer **14** was recrystallized from methanol/ether (1:1) (yield 64%). Mp: 215–220 °C dec. ^{1}H NMR (CD_3OD): δ 2.8–3.22 (m, 2 H); 3.83–4.09 (m, 2 H); 4.18 (dd, 1 H, *J* = 7.0, 9.1 Hz); 6.87 (d, 1 H, J_{CHF} = 81.5 Hz). Found: C, 49.7; H, 5.7.

Z isomer **15** recrystallized from methanol/ether (yield 45%). Mp: 180–185 °C dec. ^{1}H NMR (CD_3OD): δ 2.7–3.05 (m, 2 H); 3.9–4.2 (m, 3 H); 6.81 (d, 1 H, J_{CHF} = 81.7 Hz). Found: C, 47.7; H, 5.5 ($C_6H_8F_1O_2 + {}^1/_2H_2O$: C, 46.7; H, 5.8).

6,7-Epoxy-3-[(*tert*-butoxycarbonyl)amino]-1-(trimethylsilyl)-1-heptyne (18). At −78 °C, a solution of butyllithium in hexane (40.9 mL; 1.4 M) was added to a solution of diisopropylamine (37 mmol) and tetramethylethylenediamine (57 mmol) in THF (150 mL). After 10 min, a solution of *N*-(*tert*-butoxycarbonyl)-3-(trimethylsilyl)-2-propynylamine (**16**) (4.34 g)[19] in THF (50 mL) was added. After 1.5 h at −78 °C, a solution of 1,2-epoxy-4-bromobutane (**17**) (21 mmol) in THF (50 mL) was added in 30 min. After 30 min, a solution of acetic acid (3.5 mL) in water (100 mL) was added and then ether (150 mL). The temperature was raised to 20 °C. The organic phase was washed with water and brine. After removal of the solvent, chromatography on silica gel (300 g; hexane/ethylacetate (9:1)) was performed. The product **18** (3.4 g) was isolated as an oil (yield: 60%). ^{1}H NMR ($CDCl_3$): δ 0.15 (s, 9 H); 1.45 (s, 9 H); 1.6–1.73 (m, 2 H); 1.75–1.87 (m, 2 H); 2.52 (dd, 1 H; *J* = 5.0, 2.6 Hz); 2.78 (dd, 1 H; *J* = 5.0, 4.4 Hz); 2.9–3.0 (m, 1 H); 4.4–4.5 (m, 1 H); 4.7–4.85

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(m, 1 H). In C_6D_6, see text. Found: C, 60.5; H, 9.1; N, 4.6.

Cyclization of the Epoxy Carbamate 18 to *N*-(*tert*-Butoxycarbonyl)-2-(hydroxymethyl)-5-[2-(trimethylsilyl)ethynyl]pyrrolidine (*cis*-21 and *trans*-22). Method A. The silica (Merck 7734) was heated at 140 °C for 24 h under vacuum. A solution of 6.7-epoxy-3-[(*tert*-butoxycarbonyl)amino]-1-(trimethylsilyl)-1-heptyne (**18**) (1.3 g) in toluene (75 mL) was heated at 65 °C for 20 h in the presence of the activated silica gel (6.5 g). Water (20 mL) was added, and the solution was refluxed for 1 h and after cooling filtered over Celite. The organic phase was separated and the aqueous phase extracted with ether. After evaporation of the solvents, chromatography on silica gel (60 g; hexane/ethyl acetate (85:15)) was performed on the oil (1.2 g). The cis product **21** (70 mg) was eluted first, the *trans*-**22** (610 mg) later. Total yield: 52%.

Method B. A solution of trifluoroacetic acid (50 mL) in methylene chloride (150 mL) was added dropwise at 0 °C to a solution of the epoxycarbamate **18** (5.4 g) in CH_2Cl_2 (500 mL). After 1 h at 0 °C, 4 N sodium hydroxide was added to give basic pH. After extraction, the same separation as in method A was applied.

Cis product **21** (0.65 g) and *trans*-**22** (3.65 g) were obtained in a total yield of 80%.

***cis*-21.** Mp: 72–73 °C (from hexane). ^1H NMR ($CDCl_3$): δ 0.16 (s, 9 H); 1.49 (s, 9 H); 1.75–1.92 (1 H); 1.95–2.25 (3 H); 3.65–3.75 (2 H); 3.91–3.96 (1 H); 4.45–4.68 (1 H). Found: C, 60.4; H, 9.2; N, 4.7.

***trans*-22.** Mp: 63–65 °C (from hexane). ^1H NMR ($CDCl_3$): δ 0.15 (s, 9 H); 1.5 (s, 9 H); 1.63–1.74 (1 H); 1.91–1.97 (m, 1 H); 2.03–2.13 (m, 1 H); 2.23–2.31 (m, 1 H); 3.61 (d, 2 H; J = 6.0 Hz); 4.05–4.15 (1 H); 4.45 (d, 1 H, J = 7.1 Hz). Found: C, 60.7; H, 9.1; N, 4.8.

***cis*- and *trans*-*N*-(*tert*-Butoxycarbonyl)-5-[2-(trimethylsilyl)ethynyl]pyrrolidine-2-carboxylic Acid (23 and 24).** The Jones oxidation[26] was performed. A solution of cis or trans isomer **21** or **22** (700 mg) in acetone (50 mL) was added at −5 °C to a solution of Jones reagent (7.5 mL) in acetone (20 mL). Jones reagent was a solution of chromic trioxide (26.5 g), concentrated sulfuric acid (23 mL), and water (to a total vol of 100 mL). After 3 h at −5 °C, 2-propanol was added dropwise until the medium turned blue. Water was added, and extraction with methylene chloride was performed. After removal of the solvent, the acid was crystallized from hexane.

***cis*-23** (Yield 82%). Mp: 102–103 °C (hexane). ^1H NMR ($CDCl_3$): δ 0.16 (s, 9 H); 1.53 (s, 9 H); 1.95–2.25 (3 H); 2.35–2.65 (1 H); 4.3–4.5 (2 H). Found: C, 57.8; H, 8.1; N, 4.5.

***trans*-24** (Yield 91%). Mp: 133–136 °C (hexane). ^1H NMR ($CDCl_3$): δ 0.16 (s, 9 H); 1.42 and 1.52 (2s, 9 H); 2.0–2.4 (4 H); 4.31–4.73 (2 H). Found: C, 57.9; H, 8.3; N, 4.5.

***cis*- and *trans*-5-Ethynyl-(±)-proline (7 and 8).** A 1 M solution of sodium methoxide in methanol (5.2 mL) was added at 0 °C to a solution of *cis*- or *trans*-*N*-(*tert*-butoxycarbonyl)-5-[2-(trimethylsilyl)ethynyl]pyrrolidine-2-carboxylic acid (2.4 mmol, **23** or **24**) in methanol (30 mL). After 3 h at 20 °C, the reaction mixture was cooled to 0 °C and 2 N sulfuric acid was added to pH 2. Extraction with CH_2Cl_2 was performed. The organic phase was washed with water and brine and dried with $MgSO_4$. After removal of the solvent, the oil (0.5 g) was dissolved in CH_2Cl_2 (30 mL). At 0 °C, a solution of trifluoroacetic acid (30 mL) in CH_2Cl_2 (80 mL) was added. After 30 min at 0 °C, the reaction was left at 20 °C for 2 h. CH_2Cl_2 and trifluoroacetic acid were removed under vacuum, and chromatography on silica gel (40 g, eluent ethyl acetate/methanol) was performed. After removal of the solvents, the product was recrystallized from hot ethanol.

(±)-*cis*-7 (Yield 61%). Mp: 203–205 °C dec. ^1H NMR (CD_3OD): δ 1.95–2.16 (m, 1 H); 2.23–2.45 (m, 3 H); 3.29 (d, 1 H); 4.04 (dd, 1 H, J = 8.6, 6.6 Hz); 4.35–4.43 (m 1 H). Found: C, 60.5; H, 6.6; N, 10.2.

(±)-*trans*-8 (Yield 50%). Mp: 203–205 °C dec. ^1H NMR (CD_3OD): δ 2.0–2.81 (4 H); 3.29 (d, 1 H; J = 2.1 Hz); 4.09 (dd, 1 H, J = 6.5, 8.2 Hz); 4.47–4.54 (1 H). Found: C, 60.4; H, 6.7; N, 10.1.

***N*-(Methoxycarbonyl)-5-[2-(trimethylsilyl)ethynyl]-L-proline Methyl Ester (*cis*-27 and *trans*-28).** Titanium tetrachloride (0.2 mL) was added at 0 °C to a solution of the methoxycarbamate **26**[41] (0.4 g) in CH_2Cl_2 (10 mL). After 15 min, a solution of bis(trimethylsilyl)acetylene ((0.65 g) in CH_2Cl_2 (5 mL)) was added. After 30 min at 0 °C, the reaction was left at 20 °C for 7 h. Hydrolysis was performed at 0 °C by addition of a 5% solution of sodium bicarbonate in water (30 mL). The titanium hydroxyde was removed by filtration over Celite. After decanting the aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL) and ethyl acetate (2 × 30 mL). After drying over $MgSO_4$, the solvents were removed under vacuum and the oily product was chromatographed over silica gel (60 g; hexane–ethyl acetate). The trans isomer **28** was eluted first and the cis isomer **27** second. Total yield was 61%.

Cis Isomer 27. ^1H NMR ($CDCl_3$): δ 0.15 (s, 9 H); 2.02–2.35 (m, 4 H); 3.67; 3.70; 3.71; 3.74 (4s, 6 H in total); 4.26–4.42 (1 H); 4.52–4.75 (1 H). $[\alpha]^{20}_D$: +93° (c = 0.8; MeOH). Found: C, 55.5; H, 7.4; N, 4.9.

Trans Isomer 28. ^1H NMR ($CDCl_3$): δ 0.14 (s, 9 H); 1.99–2.23 (m, 4 H); 3.69; 3.71; 3.73; 3.76 (4s, 6 H in total); 4.36–4.48 (m, 1 H); 4.61–4.78 (m, 1 H). $[\alpha]^{25}_D$: −170° (c = 1.2; MeOH). Found: C, 55.0; H, 7.4; N, 4.8.

Removal of the Trimethylsilyl Group of Compounds 27 and 28. To a solution of the methyl esters **27** or **28** (0.32 g) in THF (20 mL) was added at −20 °C a solution of tetrabutylammonium fluoride (0.33 g) in THF (20 mL). The temperature was raised to 0 °C over 40 min, and water (20 mL) followed by a saturated solution of NH_4Cl (30 mL) was added. The organic phase was collected, and the aqueous phase was extracted with ether (3 × 50 mL). After drying over $MgSO_4$, removal of the solvent gave an oil (0.26 g) which was filtered over silica gel (30 g) with hexane–ethyl acetate (8:2) as eluant. The *cis*-*N*-(methoxycarbonyl)-5-ethynyl-L-proline methyl ester crystallized at −15 °C from ether/hexane (1:3) (yield 95%). Mp: 59–60 °C. ^1H NMR ($CDCl_3$): δ 2.1–2.4 (m, 5 H); 3.70; 3.73; 3.74; 3.77 (4s, 6 H in total); 4.3–4.45 (m, 1 H); 4.58–4.8 (m, 1 H). $[\alpha]^{25}_D$: +39° (c = 2.3; MeOH). Found: C, 56.9; H, 6.2; N, 6.7.

Trans-*N*-(Methoxycarbonyl)-5-ethynyl-L-proline methyl ester crystallized from ether/hexane (1:3) at −15 °C (yield 97%). Mp: 77–78 °C. ^1H NMR ($CDCl_3$): δ 2.0–2.6 (m, 5 H); 3.7; 3.72; 3.73; 3.77 (4s, 6 H in total); 4.35–4.48 (m, 1 H); 4.63–4.78 (m, 1 H). $[\alpha]^{25}_D$: −162° (c = 0.6; MeOH). Found: C, 57.1; H, 6.1; N, 6.6.

Preparation of *N*-(Methoxycarbonyl)-5-ethynylproline (29 and 30). The methyl ester of the cis or trans isomer was saponified by alkaline hydrolysis. 1 N sodium hydroxide (1 mL) was added to the solution of the cis or trans ester (0.2 g) in dioxane–water (10 mL:10 mL) at 0 °C. After 3 h at 20 °C, ether was added and the pH of the aqueous phase was brought to a value of 2 by addition of 0.1 N hydrochloric acid and extracted with ethyl acetate. After drying over $MgSO_4$, solvent was evaporated and products were colorless oils.

Cis Isomer 29 (Yield 98%). ^1H NMR ($CDCl_3$): δ 2.1–2.45 (m, 4 H); 2.36 (d, 1 H; J = 2.1 Hz); 3.79 (s, 3 H); 4.3–4.45 (m, 1 H); 4.55–4.75 (m, 1 H).

Trans Isomer 30 (Yield 98%). ^1H NMR ($CDCl_3$): δ 2.0–2.65 (m, 5 H); 3.71; 3.77 (2s, 3 H); 4.40 (dd, 1 H; J = 8.8, 11.4 Hz); 4.59–4.78 (m, 1 H).

***N*-(Methoxycarbonyl)-5-vinyl-L-proline (*cis*-31 and *trans*-32) Methyl Esters.** A suspension of Lindlar catalyst (40 mg) in a solution of *N*-(methoxycarbonyl)-5-ethynyl-L-proline methyl ester cis or trans (0.21 g) in ethyl acetate (20 mL) was stirred in the presence of hydrogen at atmospheric pressure. After consumption of the stoichiometric amount (22.4 mL), the suspension was filtered and the solvent removed under vacuum. The oily product was chromatographed on silica gel (20 g) with hexane–ethyl acetate (9/1) as eluant. The products were colorless oils.

Cis Isomer Methyl Ester of 31 (Yield 85%). ^1H NMR ($CDCl_3$): δ 1.7–2.3 (m, 4 H); 3.7 (s, 3 H); 3.76 (s, 3 H); 4.3–4.5 (2 H); 5.1–5.25 (1 H); 5.3–5.48 (1 H); 5.7–5.97 (m, 1 H). $[\alpha]^{25}_D$: −62° (c = 2.3; MeOH). Found: C, 56.5; H, 7.4; N, 6.5.

Trans Isomer Methyl Ester of 32 (Yield 90%). ^1H NMR ($CDCl_3$): δ 1.68–1.75 (m, 1 H); 1.9–2.4 (m, 3 H); 3.64; 3.68 (2s, 3 H in total); 3.73; 3.75 (2s, 3 H in total); 4.35–4.4 (m, 1 H); 4.53–4.6 (m, 1 H); 5.01–5.18 (m, 2 H); 5.68–5.84 (m, 1 H). $[\alpha]^{25}_D$: −77° (c = 0.87; MeOH). Found: C, 56.0; H, 7.5; N, 6.5.

Hydrolysis of Methyl Esters of *cis*- and *trans*-*N*-(Methoxycarbonyl)-5-vinyl-L-proline. The same procedure as de-

scribed above for compounds **29** and **30** was applied. Removal of the N-protecting group was performed without further purification of the acids.

***cis*-N-(Methoxycarbonyl)-5-vinyl-L-proline (31).** ^{1}H NMR ($CDCl_3$): δ 1.7–2.35 (m, 4 H); 3.73 (s, 3 H); 4.3–4.5 (m, 2 H); 5.13 (d, 1 H, J = 10 Hz); 5.2–5.46 (m, 1 H); 5.72–5.98 (m, 1 H).

***trans*-*N*-(Methoxycarbonyl)-5-vinyl-L-proline (32).** ^{1}H NMR ($CDCl_3$); δ 1.7–1.8 (m, 1 H); 2.01–2.35 (m, 3 H); 3.69; 3.72 (2s, 3 H in total); 4.4–4.63 (m, 2 H); 5.0–5.17 (m, 2 H); 5.68–5.87 (m, 1 H).

***cis*- and *trans*-5-Vinyl-L-proline (5 and 6).** Sodium iodide (0.18 g) and trimethylsilyl chloride (0.235 mL) were added to a solution of *cis*- and *trans*-*N*-(methoxycarbonyl)-5-vinyl-L-proline (**31** or **32**) (0.2 g) in acetonitrile (20 mL). After reflux for 4 h in the dark, the solid was removed by filtration. Methanol (2 mL) was added, and after 10 min, the solvents were removed under vacuum. The oil was chromatographed on silica gel (15 g, chloroform–methanol). The products were crystallized from ethanol–hexane (8:2).

***cis*-5** (Yield 75–80%). Mp: 260–265 °C dec. ^{1}H NMR (CD_3OD): δ 1.68–1.93 (m, 1 H); 2.08–2.4 (m, 3 H); 3.98–4.16 (m, 2 H); 5.35–5.54 (m, 2 H); 5.93–6.12 (m, 1 H).

***trans*-6** (Yield 75–80%). Mp: 209–212 °C dec. ^{1}H NMR (CD_3OD): δ 1.8–2.54 (4 H); 4.04 (dd, 1 H; J = 7.8, 8.1 Hz); 4.21 (dd, 1 H, J = 7.4, 15.6 Hz); 5.39–5.56 (2 H); 5.88–6.02 (1 H).

(2*S*,5*S*)- or (2*S*,5*R*)-*N*-Tosyl-5-vinylproline Methyl Ester (33 and 34). The same procedure has been applied to both isomers of (2*S*, 5*R* or 5*S*)-*N*-(methoxycarbonyl)-5-vinyl-L-proline methyl ester of the acids **31** and **32**.

Iodotrimethylsilane (88 mL) was added to a solution of the methyl ester of acid **31** or **32** (110 mg) in chloroform (10 mL). After 18 h at reflux under argon, methanol (2 mL) was added at 0 °C and the solvents were removed under vacuum. The solid was dissolved in 0.1 N hydrochloric acid (30 mL) and washed with ether. The aqueous layer was treated at 0 °C with ammonia to pH 9 and extracted with ethyl acetate (3 × 20 mL). The amine (70 mg) was dissolved in pyridine (20 mL), and tosyl chloride (115 mg) was added at 0 °C. After 12 h at 20 °C, ethyl acetate (50 mL) was added and the organic phase was washed with 1 N hydrochloric acid (4 × 30 mL), water (2 × 20 mL), and brine. After drying over $MgSO_4$, the solvents were removed under vacuum and the product was chromatographed on silica gel (10 g, hexane/ethyl acetate (9:1)).

The (2*S*,5*R*)-**33** has a mp 86–89 °C (hexane/ether (1:1)) (yield 55%). ^{1}H NMR ($CDCl_3$): δ 1.74–2.07 (m, 4 H); 2.44 (s, 3 H); 3.75 (s, 3 H); 4.17–4.30 (m, 1 H); 4.35–4.43 (m, 1 H); 5.09 (ddd, 1 H; J = 10.3, 1.3, 1.3 Hz); 5.36 (ddd, 1 H; J = 17.0, 1.3, 1.3 Hz); 5.7–5.89 (m, 1 H); 7.29–7.33 (m, 2 H); 7.74–7.79 (m, 2 H). Found: C, 58.1; H, 6.2; N, 4.4.

The (2*S*,5*S*) isomer **34** was a colorless oil (yield 40%). ^{1}H NMR ($CDCl_3$): δ 1.66–1.77 (m, 1 H); 1.90–2.01 (m, 1 H); 2.14–2.42 (m, 2 H); 2.46 (s, 3 H); 3.71 (s, 3 H); 4.42–4.50 (m, 2 H); 4.99–5.19 (m, 2 H); 5.53–5.71 (m, 1 H); 7.25–7.30 (m, 2 H); 7.69–7.75 (m, 2 H).

(±)-*trans*-*N*-Tosyl-5-vinylproline Methyl Ester (34). The reduction of the (±)-*trans*-*N*-(*tert*-butoxycarbonyl)-5-ethynylpyrrolidine-2-carboxylic acid obtained from the major isomer (±)-**24** was performed as for **29** and **30**, and the crude acid was used for the next reaction as such.

(±)-*N*-(*tert*-Butoxycarbonyl)-5-vinylproline. ^{1}H NMR ($CDCl_3$): δ 1.41; 1.43 (2s, 9 H); 1.65–1.8 (m, 1 H); 1.95–2.35 (m, 3 H); 4.29–4.6 (m, 2 H); 5.0–5.16 (m, 2 H); 5.65–5.87 (m, 1 H); 8.0–8.5 (m, 1 H).

To a solution of this acid (60 mg) and of potassium carbonate (52 mg) in dimethylformamide (10 mL) was added at −5 °C methyl iodide (24 μL). After stirring for 4 h, the excess reagent and the solvent were removed under vacuum. Water (20 mL) was added, and the ester was extracted with ethyl acetate. The organic phase was washed with a 5% solution of sodium bicarbonate, water, and brine. This methyl ester [^{1}H NMR ($CDCl_3$): δ 1.40; 1.42 (2s, 9 H); 1.6–2.3 (m, 4 H); 3.71; 3.73 (2s, 3 H); 4.2–4.6 (m, 2 H); 5.0–5.16 (m, 2 H); 5.65–5.85 (m, 1 H)] was used for the next step.

A solution of trifluoroacetic acid (5 mL) in CH_2Cl_2 (5 mL) was added at 0 °C to a solution of the ester (55 mg) in CH_2Cl_2 (10 mL). After 2 h, the solvent and the acid were removed under vacuum. The residue was dissolved in 0.1 N hydrochloric acid (30 mL) and washed with ether. The aqueous phase was treated with ammonia at 0 °C until the pH was 9 and then extracted with ethyl acetate (3 × 20 mL). The amino ester (25 mg) was dissolved in pyridine (20 mL), and tosyl chloride (42 mg) was added at 0 °C. After 12 h at 20 °C, ether acetate (50 mL) was added and the organic phase was washed with 1 N hydrochloric acid, water, and brine. After drying over $MgSO_4$ and removal of solvent, the product was chromatographed on silica gel (10 g, hexane–ethyl acetate (9:1)). The product (±)-**34** (32 mg) was identified by comparison of its NMR spectrum with that of **34** obtained above.

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