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Synthesis of Racemic δ,δ-Dimethylproline Derivatives

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ABSTRACT

A versatile methodology for the preparation of racemic δ,δ-dimethylproline derivatives has been developed. Methyl *N*-Boc-δ,δ-dimethylprolinate was synthesized from a β-amino acid in six steps and 55% overall yield. The route is amenable for the preparation of a broad range of δ,δ-disubstituted prolines starting from the adequate β-amino acids. In addition, one of the intermediate compounds in the synthetic route has been used for the preparation of a δ,δ-dimethylproline decorated at the β position with a phenyl substituent. This has been achieved by coupling of phenylboronic acid with a vinyl triflate, generated under regiochemical control, followed by stereoselective hydrogenation.

INTRODUCTION

Proline is the only coded amino acid that exhibits restricted conformational flexibility due to its cyclic structure. This unique structural feature explains its tendency to act as a β-turn inductor in peptides and proteins[1] as well as its ability to form *cis* peptide bonds.[2] These properties are at the basis of the important role of proline in biology. Indeed, peptide turns are known to be propitious sites for molecular recognition,[3] and the *cis*/*trans* isomerization of prolyl peptide bonds is considered to be a molecular switch controlling important biological processes.[4]

Among proline analogues,[5] those incorporating substituents at the δ carbon are receiving increasing attention. Such analogues are able to stabilize the *cis* state of the peptide bond involving the pyrrolidine nitrogen.[6] The *cis*/*trans* equilibrium of prolyl peptide bonds is governed by the different steric interactions established between the acyl substituent at the nitrogen atom and the α or δ positions of the pyrrolidine ring (Fig. 1). In the case of proline, the α is more sterically crowded than the δ position and therefore the *trans* prolyl amide geometry is preferred. Increasing the steric hindrance around C^{δ} translates into a higher preference of the prolyl peptide bond to accommodate the *cis* arrangement. Thus, δ,δ-dimethylproline was reported to generate peptides with the prolyl amide bond locked in the *cis* geometry (Fig. 1).[7] Accordingly, it is being used as a probe to explore the three-

dimensional structure and folding pathways of therapeutic proteins,[4d,7,8] the mechanism of receptors of importance in neuroscience,[9] and the bioactive conformations of peptides.[7,10]

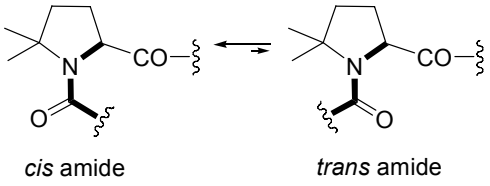
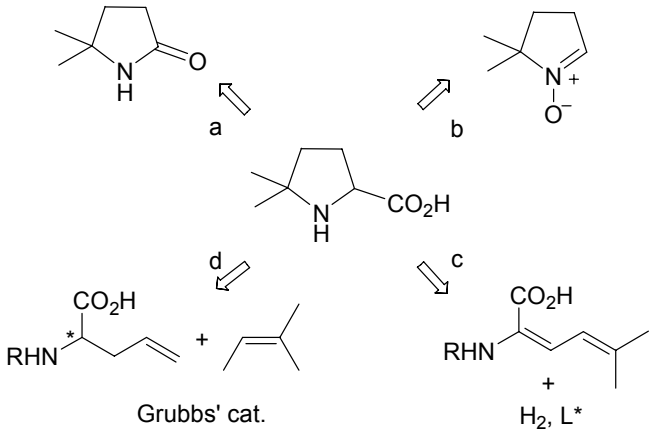


Figure 1. *Cis*-*trans* isomerism of the amide bond involving the pyrrolidine nitrogen when δ,δ-dimethylproline is incorporated into a peptide chain.

For example, the rational replacement of a proline residue in bovine pancreatic ribonuclease A by δ,δ-dimethylproline led to faster folding and enhanced conformational stability of this protein.[8] Additionally, δ,δ-dimethylproline has been shown to be an excellent probe to build an experimental model of ion-channel gating for the 5-HT_3 receptor, which belongs to a cys-loop superfamily of neurotransmitter-gated ion channels.[9] The mutagenesis of a proline residue in a receptor loop with different analogues revealed that only modified prolines favouring the *cis* amide geometry produced functional channels.[9d] Remarkably, a ≈60-fold increase in receptor activation was achieved with δ,δ-dimethylproline.[9d] Accordingly, the authors proposed that *trans*-to-*cis* isomerization of a single proline residue provides the switch to interconvert the closed and open states of the channel. Moreover, some applications of δ,δ-dimethylproline in the design of bioactive compounds for the treatment of pain,[11] neurodegenerative disorders,[12] or bacterial infections[13] have been reported.

Despite its remarkable value, only a few synthetic strategies have been developed for the preparation of δ,δ-dimethylproline (Scheme 1). Some procedures make use of dimethyl-substituted pyrrolidine precursors where the carboxylic acid moiety is further installed, thus providing access to the racemic amino acid. In particular, the pyrrolidone depicted in Scheme 1 (route a) was reported to undergo selective reduction to form a Δ^1-pyrroline that was cyanated and hydrolyzed to the corresponding proline.[14] Alternatively, the substituted nitrone in route b underwent acid-catalyzed addition of cyanide to yield the *N*-hydroxy nitrile, which was further hydrolyzed and reduced to the amino acid,[15] with concomitant generation of a byproduct arising from an acid-catalyzed methyl migration from the δ carbon to the pyrrolidine nitrogen.[10b,12] The racemic δ,δ-dimethylproline obtained in this manner was

chemically resolved[7] by fractional crystallization of diastereoisomeric tartrates. The resolution process delivered δ,δ-dimethyl-L-proline with >98% enantiomeric purity. Additionally, the synthesis of enantioenriched δ,δ-dimethylproline has been achieved through an intramolecular cyclization procedure that involves N–C^{δ} bond formation.[16] Specifically, a chiral prenylglycine intermediate generated from two different precursors (Scheme 1, routes c and d) rendered δ,δ-dimethylproline in 98% ee upon heating under acidic conditions.[16]



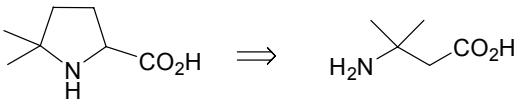
Scheme 1. Reported strategies for the synthesis of δ,δ-dimethylproline.

Herein we report an alternative preparation of δ,δ-dimethylproline through an intramolecular cyclization procedure involving formation of the N–C^{α} bond and making use of a β-amino acid as the starting material.

RESULTS AND DISCUSSION

We have recently shown that a β-amino acid can be used as suitable precursor to build the proline skeleton. Specifically, the proline analogue bearing a phenyl substituent attached to the pyrrolidine β carbon was prepared from β-alanine in a highly efficient manner.[17] The starting β-amino acid was progressed to an α-diazo-β-keto ester derivative that underwent an intramolecular N–H insertion reaction upon generation of a metal carbenoid. The resulting oxoproline was further modified at the β carbon to incorporate the desired aromatic functionality.[17]

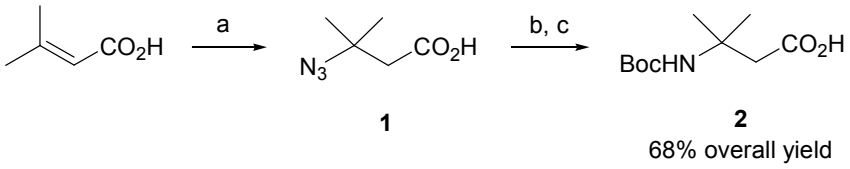
We decided to explore whether δ,δ-dimethylproline could be synthesized by applying the aforementioned approach. In this case, the required β-amino acid (3-amino-3-methylbutanoic acid, Scheme 2) is not as readily available as β-alanine.



Scheme 2. Structure of the β-amino acid selected as a precursor of δ,δ-dimethylproline.

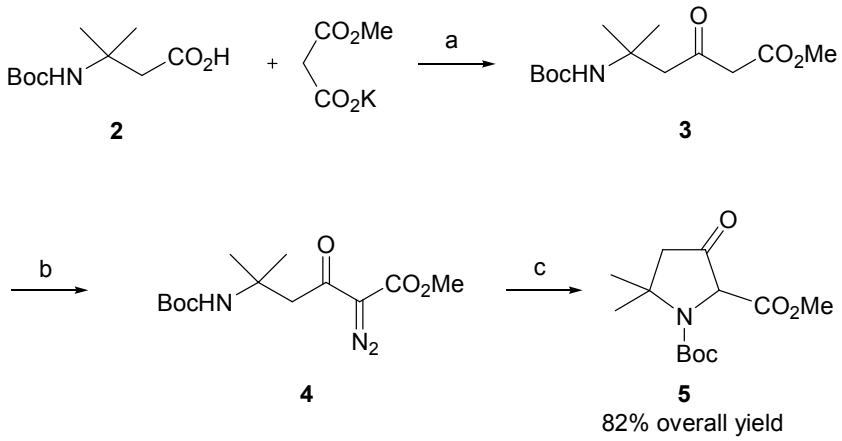
This compound belongs to the family of $\beta^{3,3}$-amino acids since it exhibits a geminal substitution pattern at the β carbon (C3). $\beta^{3,3}$-Amino acids are targets of interest for the preparation of structurally defined oligomers as well as building blocks for bioactive molecules. Therefore, a variety of methods have been described for their preparation.[18]

The synthesis of 3-amino-3-methylbutanoic acid protected with a *tert*-butoxycarbonyl (Boc) group at the amino function could be attempted by homologation of α-aminoisobutyric acid (Aib). However, the generation and rearrangement of the diazoketones implicated in the homologation of α,α-disubstituted α-amino acids have been reported to proceed with poor yields.[18] For this reason, we undertook an alternative three-step procedure (Scheme 3) that started with the conjugate addition of sodium azide to 3-methyl-2-butenoic acid under acidic conditions.[19] Reduction of the azido group in **1** was then accomplished by hydrogenation at atmospheric pressure using palladium on carbon as the catalyst.[19] The introduction of the Boc protecting group at the amino functionality was carried out by treatment of the crude β-amino acid with di-*tert*-butyl dicarbonate under standard conditions. In this way, multigram quantities of **2** were prepared in 68% overall yield without purification of intermediate compounds.



Scheme 3. Synthesis of the β-amino acid **2**. Reagents and conditions: a) NaN_3, H_2O/AcOH, 95 ºC; b) H_2, Pd/C, EtOAc, r.t.; c) Boc_2O, KOH, dioxane/H_2O, r.t.

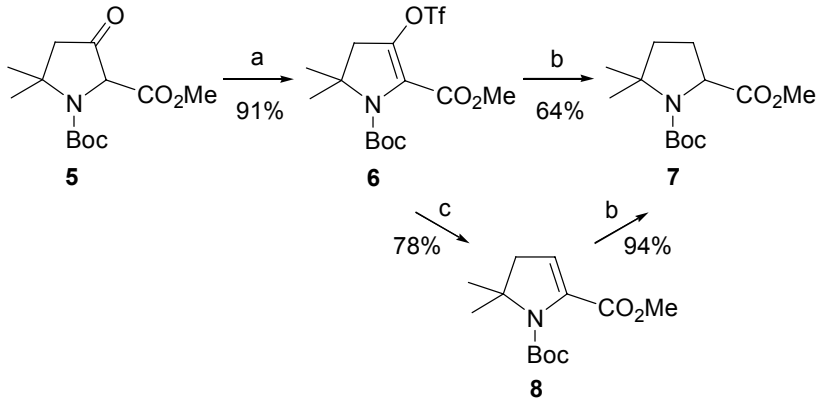
Once the precursor β-amino acid was obtained, it was converted to the β-keto ester **3** (Scheme 4).[17] Thus, the carbonyl group in **2** was activated with *N*,*N*'-carbonyldiimidazole and the resulting imidazolide was treated with a magnesium enolate generated from the potassium salt of monomethyl malonate. The nucleophilic addition provided **3** after decarboxylation occurring spontaneously. Next, the crude β-keto ester **3** was submitted to a diazo-transfer reaction by treatment with 4-acetamidobenzenesulfonyl azide.[20] The resulting α-diazo β-keto ester **4** successfully underwent decomposition of the diazo group upon treatment with rhodium diacetate in toluene at 85–90 ºC.[21] The intramolecular N–H insertion of the metal carbenoid generated *in situ* cleanly delivered δ,δ-dimethylketoproline **5**, which was readily purified by column chromatography. The overall sequence in Scheme 4 provided this ketoproline in 82% yield from the starting β-amino acid **2**.



Scheme 4. Synthesis of δ,δ-dimethylketoproline **5** from the β-amino acid **2**. Reagents and conditions: a) *N*,*N*'-carbonyldiimidazole, $MgCl_2$, THF, r.t.; b) 4-acetamidobenzenesulfonyl azide, Et_3N, CH_3CN, r.t.; c) $Rh_2(OAc)_4$, toluene, 85–90 ºC.

It should be mentioned that an alternative synthesis of a similar ketoproline, with *N*-acetyl protection, has previously been reported.[22] The strategy described by Sato et al. involved an intramolecular Dieckman condensation reaction with the corresponding $\beta^{3,3}$-amino methyl ester. However, the process suffered from a low overall yield (26% yield of *N*-acetylated ketoproline from the β-amino ester). Therefore, the sequence reported herein (Scheme 4) and involving the intramolecular N–H insertion of a metal carbenoid as the cyclization step represents a substantial improvement, in terms of efficiency, in comparison to the previous procedure.[22]

After preparation of δ,δ-dimethylketoproline **5**, we addressed its transformation into the desired δ,δ-dimethylproline derivative (Scheme 5). Firstly, a vinyl triflate was generated through deprotonation of **5** with potassium hexamethyldisilazide at room temperature, followed by enolate trapping with *N*-(5-chloro-2-pyridyl)triflimide.[23] This combination of base and triflating agent, which proved optimal for achieving regiocontrol when working with 3-oxoproline,[17] rendered **6** in high yield as a single regioisomer.



Scheme 5. Synthesis of methyl *N*-Boc-δ,δ-dimethylprolinate (**7**) from δ,δ-dimethylketoproline **5**. Reagents and conditions: a) KHMDS, *N*-(5-chloro-2-pyridyl)triflimide, THF, r.t.; b) H_2, Pd/C, MeOH, r.t.; c) $Pd(PPh_3)_4$, Et_3SiH, LiCl, Et_3N, DMF, 60 ºC.

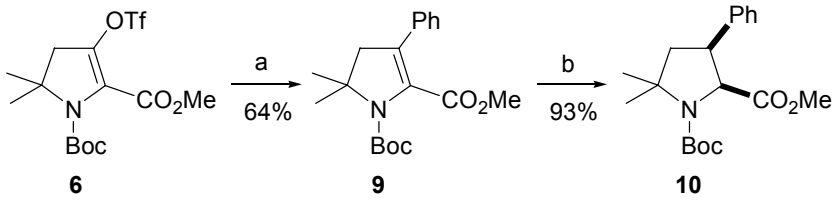
Although vinyl triflate **6** proved stable when stored at –20 ºC, it was submitted to hydrogenation right after its chromatographic purification. Thus, it was reduced to an alkane under an atmospheric pressure of hydrogen gas in the presence of palladium on carbon (Scheme 5, step b). This transformation, which has been proposed[24] to occur through a concerted hydrogenolysis of the C–O bond by PdH_2 species followed by hydrogenation of the alkene, provided **7** in 64% yield when working on about a gram scale. However, concomitant deprotection of the amino group occurs as the reaction progresses and the isolation of **7** becomes troublesome when scaling-up. In order to circumvent this inconvenience, we undertook reduction of **6** through a two-step procedure that involved palladium-catalyzed reductive removal of the triflate employing triethylsilane as a hydride source,[25] followed by hydrogenation of the resulting Δ^2-pyrroline **8** (Scheme 5, steps c/b). This procedure cleanly furnished **7** in 73% yield from **6** without encountering difficulties for its isolation. Consequently, these transformations seem preferable for the preparation of **7**.

Therefore, we have developed a methodology that renders racemic methyl *N*-Boc-δ,δ-dimethylprolinate (**7**) in 55% overall yield through a six-step sequence that uses a β-amino acid as the starting material. The procedure seems to be a well suited approach for the preparation of a broader range of δ,δ-disubstituted prolines since the required β-amino acids should be accessible through a wide variety of methodologies.[18]

In addition, the synthetic route developed provides access to valuable intermediate compounds, namely vinyl triflate **6** and Δ^2-pyrroline **8**. Thus, the latter could be used for the preparation of enantiopure or enantioenriched δ,δ-dimethylproline derivatives by means of asymmetric catalytic hydrogenations, as already tested for a 2,3-dehydroprolinate.[26] Moreover, both **8** and **6** could act as precursors of δ,δ-dimethylproline derivatives functionalized at the β position. Specifically, vinyl triflate **6** and Δ^2-pyrroline **8** should be suitable substrates to carry out cross-coupling reactions and 1,4-conjugate additions, respectively. In fact, analogous compounds derived from proline have been successfully employed, by us[17] and other authors,[27] in the synthesis of β-substituted prolines following these strategies. The β-functionalization of δ,δ-dimethylproline is expected to render analogues able to combine the propensity of the parent amino acid to lock the prolyl amide bond in the *cis* geometry with the presence of new side-chains. Such analogues are anticipated to be highly valuable for the development of pharmacologically active compounds. To date, only few attempts have been made to access δ,δ-dimethylproline analogues with additional substituent at C^{β}.[28]

We exemplified the value of these precursors (**6**, **8**) in the synthesis of β-substituted δ,δ-dimethylproline derivatives using vinyl triflate **6**. Specifically, we carried out the preparation of the analogue that bears a phenyl substituent attached to the β carbon, given the demonstrated utility of β-phenylproline in improving the pharmacological profile of biologically active peptides.[29] The incorporation of the aromatic substituent into the δ,δ-dimethylproline scaffold was achieved by means of a palladium-mediated cross-coupling reaction (Scheme 6).[30] Treatment of vinyl triflate **6** with phenylboronic acid in the presence of potassium carbonate and a catalytic amount of [1,1-bis(diphenylphosphino)ferrocene]dichloropalladium (II) rendered Δ^2-pyrroline **9** in moderate yield. The presence of a double bond connecting the α and β carbons in **9** ensured the *cis* relative disposition of substituents upon reduction of the pyrroline. Thus, catalytic

hydrogenation of **9** under standard conditions furnished **10** in high yield. This result emphasizes the importance of the regioselectivity previously attained in the generation of vinyl triflate **6** (Scheme 5), which was not significant for the preparation of the parent δ,δ-dimethylproline but becomes a key issue for the synthesis of β-substituted analogues under complete stereocontrol. A copper-mediated 1,4 addition of a phenyl Grignard reagent to **8** would most probably render **10** as *cis*/*trans* mixture of isomers, as observed in the case of the Δ^2-pyrroline derived from proline.[27]



Scheme 6. Synthesis of the *cis* β-substituted δ,δ-dimethylproline analogue **10**. Reagents and conditions: a) $PhB(OH)_2$, $PdCl_2(dppf)$, K_2CO_3, toluene/MeOH, 80 ºC; b) H_2, Pd/C, MeOH, r.t.

Accordingly, **6** (and **8**) could be seen as suitable precursors to generate proline analogues that stabilize the *cis* geometry of the prolyl amide bond while incorporating additional functionality at C^{β}.[31]

CONCLUSIONS

A simple route for the preparation of methyl *N*-Boc-δ,δ-dimethylprolinate in racemic form has been developed. The procedure involves the construction of the pyrrolidine ring through an intramolecular N–C^{α} bond cyclization reaction, and makes use of a β-amino acid as the starting material. The methodology is adequate to be extended to the preparation of other δ,δ-disubstituted prolines by using different starting β-amino acids. In addition, it provides access to valuable intermediates for the preparation of δ,δ-dimethylproline derivatives functionalized at C^{β}. In particular, the *cis* stereoisomer of methyl *N*-Boc-β-phenyl-δ,δ-dimethylprolinate was obtained through a cross-coupling reaccion on a vinyl triflate intermediate generated under regioselective control. Such δ,δ-disubstituted prolines functionalized at the β position combine the ability to stabilize the *cis* geometry of the prolyl amide bond with the presence of

additional side-chain functionality. Efforts to obtain the pure enantiomers of δ,δ-dimethylproline by HPLC resolution on a chiral column are underway.[32]

EXPERIMENTAL SECTION

General

All reagents were used as received from commercial suppliers without further purification. Thin-layer chromatography (TLC) was performed on Macherey–Nagel Polygram® SIL G/UV_{254} precoated silica gel polyester plates. The products were visualized by exposure to UV light, iodine vapour, or submersion in ninhydrin stain or ethanolic solution of phosphomolybdic acid. Column chromatography was performed using 60 M (0.04–0.063 mm) silica gel from Macherey–Nagel. Melting points were determined on a Gallenkamp apparatus. IR spectra were registered on a Nicolet Avatar 360 FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 or AV-500 instrument at room temperature using the residual solvent signal as the internal standard; chemical shifts (δ) are expressed in parts per million and coupling constants (*J*) in hertz. High-resolution mass spectra were obtained on a Bruker Microtof-Q spectrometer.

***N*-(*tert*-butoxycarbonyl)-3-amino-3-methylbutanoic acid (2):**

A solution of sodium azide (13.00 g, 199.97 mmol) in water (25 mL) was added dropwise by syringe to a solution of 3-methyl-2-butenoic acid (5.00 g, 49.94 mmol) in acetic acid (13 mL). After stirring the mixture for 1 h, the temperature was raised to 95 ºC and the solution was stirred for an additional 2 days. The reaction mixture was diluted with water and extracted with diethyl ether (5 × 50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The solvent was concentrated in vacuo to afford a white solid (7.10 g) that was used without purification. A mixture of the solid and 10% Pd/C (700 mg) in ethyl acetate (100 mL) was stirred 24 h at room temperature under an atmospheric pressure of hydrogen gas. The catalyst was filtered off washing with ethyl acetate (100 mL). The resulting solution was washed with water (100 mL) and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The aqueous phase was lyophilized to afford 3-amino-3-methylbutanoic acid (4.80 g, 40.97 mmol) as a white solid. The crude compound was dissolved in dioxane (95 mL) and di-*tert*-butyl dicarbonate (17.80 g, 81.94 mmol) was added. The resulting mixture was treated with 1 M aqueous potassium hydroxide (42 mL) and stirred for 36 h. The reaction was diluted

with water and basified with lithium hydroxide. After extraction with diethyl ether (3 × 50 mL), the aqueous phase was acidified by addition of 2 M HCl solution, and extracted several times with ethyl acetate. The organic solvent was evaporated to dryness to afford pure **2** as a white solid (7.42 g, 34.15 mmol, 68% overall yield); m.p. 98 ºC. IR (nujol): ν = 3313, 3260, 3100–2500, 1701, 1652 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.39 (s, 6H, C^{β}-CH_3), 1.44 (br. s, 9H, *t*Bu-CH_3), 2.72 (br. s, 2H, H^{α}), 4.96 (br. s, 1H, N*H*Boc), 10.08 (br. s, 1H, CO_2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.88 (C^{β}-CH_3), 28.50 (*t*Bu-CH_3), 44.33 (C^{α}), 51.24 (C^{β}), 79.74 (*t*Bu-C), 155.14 (*t*BuO*C*O), 176.50 (CO_2H). HRMS (ESI): calcd. for $C_{10}H_{19}NNaO_4$ $[M+Na]^+$ 240.1206; found 240.1213.

Methyl *N*-(*tert*-butoxycarbonyl)-5,5-dimethyl-3-oxopyrrolidine-2-carboxylate (5):
A solution of *N*-(*tert*-butoxycarbonyl)-3-amino-3-methylbutanoic acid (6.00 g, 27.62 mmol) in anhydrous tetrahydrofuran (120 mL) kept under an argon atmosphere was treated with *N*,*N*'-carbonyldiimidazole (5.40 g, 33.30 mmol). After stirring at room temperature for 1 h, previously mixed magnesium chloride (2.00 g, 21.00 mmol) and monomethyl monopotassium malonate (6.50 g, 41.62 mmol) were added. The resulting mixture was stirred at room temperature for an additional 18 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (100 mL) and washed with 5% aqueous $KHSO_4$ (2 × 50 mL), 5% aqueous $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford a pale orange oil (7.50 g, 27.43 mmol). The crude compound was dissolved in anhydrous acetonitrile (100 mL), under an argon atmosphere, and 4-acetamidobenzenesulfonyl azide (6.90 g, 28.72 mmol) was added in one portion, followed by triethylamine (10.90 mL, 78.15 mmol). The resulting mixture was stirred at room temperature for 2 h. The solvent was concentrated in vacuo and the residue was dissolved in diethyl ether (100 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), saturated aqueous NH_4Cl (2 × 50 mL) and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure to afford an oil (7.80 g, 26.06 mmol) that was taken on without purification. It was dissolved in anhydrous toluene (300 mL), under an argon atmosphere, and $Rh_2(OAc)_4$ (56 mg, 0.127 mmol) was added. The reaction mixture was heated at 85–90 ºC for approximately 40 min in a preheated oil bath. The solvent was evaporated to dryness and the residue was purified by column chromatography (eluent: hexanes/ethyl acetate 2:1) to afford pure **5** as a white solid (6.15 g, 22.67 mmol, 82% overall yield). m.p. 112 ºC. IR (nujol): ν = 1767, 1744, 1714 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): (2:1

mixture of rotamers) δ = 1.39 (br. s, 6H, *t*Bu-CH_3) overlapped with 1.46–1.72 (m, 9H, *t*Bu-CH_3 and C^{δ}-CH_3), 2.51–2.73 (m, 2H, H^{γ}), 3.79 (s, 3H, OCH_3), 4.58 and 4.66 (two br. s, 1H, H^{α}). ^{13}C NMR (100 MHz, $CDCl_3$): (duplicate signals are observed for most carbons; asterisks indicate those corresponding to the minor rotamer) δ = 26.84, 27.48 and 27.87* (C^{δ}-CH_3), 28.30/28.52* (*t*Bu-CH_3), 53.01/53.16* (OCH_3), 53.34/53.96* (C^{γ}), 58.72*/59.22 (C^{δ}), 68.28/68.42* (C^{α}), 80.87/81.58* (*t*Bu-C), 152.59/154.33* (*t*BuO*C*O), 166.92*/167.25 (*C*O_2CH_3), 203.03*/203.72 (C^{β}=O). HRMS (ESI): calcd. for $C_{13}H_{21}NNaO_5$ $[M+Na]^+$ 294.1312; found 294.1301.

Methyl *N*-(*tert*-butoxycarbonyl)-3-trifluoromethanesulfonyl-5,5-dimethyl-Δ^2-pyrroline-2-carboxylate (6):

A 0.5 M solution of KHMDS in toluene (12.4 mL, 6.20 mmol) was added by syringe to a stirred solution of **5** (1.40 g, 5.16 mmol) in anhydrous tetrahydrofuran (16 mL) at room temperature. After 40 min stirring, *N*-(5-chloro-2-pyridyl)triflimide (2.43 g, 6.20 mmol) was added and the resulting solution was allowed to stir for an additional 4 h. The solvent was evaporated to dryness and the residue was purified by column chromatography (eluent: hexanes/ethyl acetate 5:1) to afford pure **6** as an oil that solidified in the freezer (1.90 g, 4.70 mmol, 91% yield). IR (neat): ν = 1750, 1716, 1429, 1387, 1370 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 (s, 9H, *t*Bu-CH_3), 1.56 (s, 6H, C^{δ}-CH_3), 2.82 (s, 2H, H^{γ}), 3.85 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.30 (C^{δ}-CH_3), 28.19 (*t*Bu-CH_3), 45.40 (C^{γ}), 52.68 (OCH_3), 64.64 (C^{δ}), 82.38 (*t*Bu-C), 118.41 (q, *J* = 320,4 Hz, CF_3), 128.38 (C^{α}), 133.55 (C^{β}), 150.97 (*t*BuO*C*O), 160.03 (*C*O_2CH_3). HRMS (ESI): calcd. for $C_{14}H_{20}F_3NNaO_7S$ $[M+Na]^+$ 426.0805; found 426.0795.

Methyl *N*-(*tert*-butoxycarbonyl)-5,5-dimethylpyrrolidine-2-carboxylate (7):

Procedure A:

A mixture of **6** (1.90 g, 4.70 mmol) and 10% Pd/C (0.19 g) in methanol (40 mL) was stirred 18 h at room temperature under an atmospheric pressure of hydrogen gas. The catalyst was filtered off and washed with methanol. The solvent was concentrated in vacuo and the crude was purified by column chromatography (eluent: hexanes/diethyl ether 5:1) to afford **7** as an oil (0.77 g, 3.00 mmol, 64% yield).

Procedure B:

A mixture of **8** (100 mg, 0.39 mmol) and 10% Pd/C (10 mg) in methanol (10 mL) was stirred overnight at room temperature under an atmospheric pressure of hydrogen gas. The catalyst was filtered off and washed with methanol. The solvent was concentrated in vacuo and the crude was purified by column chromatography (eluent: hexanes/ethyl acetate 5:1) to afford **7** as an oil (94 mg, 0.37 mmol, 94% yield). IR (neat): ν = 1752, 1706, 1686 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): (1.2:1 mixture of rotamers) δ = 1.31 and 1.38 (two s, 3H, C^{δ}-CH_3), 1.39 and 1.48 (two s, 9H, *t*Bu-CH_3), 1.49 and 1.53 (two s, 3H, C^{δ}-CH_3), 1.69–1.80 (m, 1H, H^{γ}), 1.81–1.99 (m, 2H, H^{β} and H^{γ}), 2.06–2.20 (m, 1H, H^{β}), 3.71 and 3.72 (two s, 3H, OCH_3), 4.30 (dd, *J* = 8.8, 3.3 Hz, 0.54H, H^{α}), 4.41 (dd, *J* = 8.7, 2.9 Hz, 0.46H, H^{α}). ^{13}C NMR (125 MHz, $CDCl_3$): (duplicate signals are observed for all carbons) δ = 26.07/26.92 (C^{δ}-CH_3), 26.34/26.90 (C^{γ}), 26.46/27.58 (C^{δ}-CH_3), 28.51/28.66 (*t*Bu-CH_3), 40.19/41.02 (C^{β}), 52.00/52.16 (OCH_3), 60.90/61.67 (C^{δ}), 61.51/61.52 (C^{α}), 79.52/80.05 (*t*Bu-C), 152.75/154.48 (*t*BuO*C*O), 173.85/174.25 (*C*O_2CH_3). HRMS (ESI): calcd. for $C_{13}H_{23}NNaO_4$ $[M+Na]^+$ 280.1519; found 280.1528.

Methyl *N*-(*tert*-butoxycarbonyl)-5,5-dimethyl-Δ^2-pyrroline-2-carboxylate (8):
Triethyl silyl hydride (0.55 mL, 3.45 mmol) and triethylamine (0.97 mL, 6.95 mmol) were added dropwise to a solution of **6** (0.70 g, 1.74 mmol), lithium chloride (221 mg, 5.21 mmol) and $Pd(PPh_3)_4$ (27 mg, 0.17 mmol) in anhydrous DMF (28 mL). The reaction mixture was stirred at 60 ºC for 1 h, after which the solvent was removed under reduced pressure. The solid residue was purified by column chromatography (eluent: hexanes/ethyl acetate 5:1) to afford pure **8** as an oil (347 mg, 1.36 mmol, 78% yield). IR (neat): ν = 1741, 1705, 1627 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.43 (s, 9H, *t*Bu-CH_3), 1.47 (s, 6H, C^{δ}-CH_3), 2.52 (d, *J* = 3.0 Hz, 2H, H^{γ}), 3.78 (s, 3H, OCH_3), 5.43 (t, *J* = 3.0 Hz, 1H, H^{β}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.28 (C^{δ}-CH_3), 28.27 (*t*Bu-CH_3), 45.82 (C^{γ}), 52.09 (OCH_3), 64.81 (C^{δ}), 80.92 (*t*Bu-C), 113.36 (C^{β}), 136.33 (C^{α}), 151.46 (*t*BuO*C*O), 163.42 (*C*O_2CH_3). HRMS (ESI): calcd. for $C_{13}H_{21}NO_4$ $[M+H]^+$ 256.1543, found 256.1538.

Methyl *N*-(*tert*-butoxycarbonyl)-3-phenyl-5,5-dimethyl-Δ^2-pyrroline-2-carboxylate (9):
To a solution of **6** (350 mg, 0.87 mmol) and phenylboronic acid (212 mg, 1.74 mmol) in a 10:1 mixture of toluene/methanol (5.5 mL), potassium carbonate (180 mg, 1.30 mmol) and [1,1-bis(diphenylphosphino)ferrocene] dichloropalladium (II) (32 mg, 0.044 mmol) were

added. The reaction mixture was stirred at 80 ºC for 1 h. The solvent was concentrated in vacuo and the resulting residue was purified by silica gel column chromatography (eluent: hexanes/ethyl acetate 5:1) to afford pure **9** as a white solid (185 mg, 0.56 mmol, 64% yield). m.p 108 ºC. IR (nujol): ν = 1734, 1697 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.50 (s, 9H, *t*Bu-CH_3), 1.59 (s, 6H, C^{δ}-CH_3), 2.93 (s, 2H, H^{γ}), 3.82 (s, 3H, OCH_3), 7.20–7.41 (m, 5H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 27.29 (C^{δ}-CH_3), 28.42 (*t*Bu-CH_3), 49.41 (C^{γ}), 52.46 (OCH_3), 63.23 (C^{δ}), 81.44 (*t*Bu-C), 126.48 (Ar), 127.38 (Ar), 128.49 (Ar), 128.58 (C^{β}), 129.86 (C^{α}), 134.16 (Ar), 151.26 (*t*BuO*C*O), 164.75 (*C*O_2CH_3). HRMS (ESI): calcd. for $C_{19}H_{25}NO_4$ $[M+H]^+$ 332.1856; found 332.1837; calcd. for $C_{19}H_{25}NNaO_4$ $[M+Na]^+$ 354.1676; found 354.1660.

Methyl *cis*-*N*-(*tert*-butoxycarbonyl)-3-phenyl-5,5-dimethylpyrrolidine-2-carboxylate (10):

A mixture of **9** (166 mg, 0.50 mmol) and 10% Pd/C (17 mg) in methanol (5 mL) was stirred overnight at room temperature under an atmospheric pressure of hydrogen gas. The catalyst was filtered off and washed with methanol. The solvent was concentrated in vacuo and the crude was purified by column chromatography (eluent: hexanes/ethyl acetate 5:1) to afford **10** as an oil (155 mg, 0.46 mmol, 93% yield). IR (neat): ν = 1745, 1705, 1684 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): (1.2:1 mixture of rotamers) δ = 1.37 and 1.49 (two s, 9H, *t*Bu-CH_3), 1.43 and 1.47 (two s, 3H, C^{δ}-CH_3), 1.67 and 1.73 (two s, 3H, C^{δ}-CH_3), 1.89–2.02 (m, 1H, H^{γ}), 2.48–2.73 (m, 1H, H^{γ}), 3.22 and 3.25 (two s, 3H, OCH_3), 3.61–3.79 (m, 1H, H^{β}), 4.53 (d, *J* = 8.8 Hz, 0.55H, H^{α}), 4.62 (d, *J* = 8.7 Hz, 0.45H, H^{α}), 7.16–7.36 (m, 5H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$): (duplicate signals are observed for all carbons) δ = 26.04/26.90 (C^{δ}-CH_3), 27.16/28.20 (C^{δ}-CH_3), 28.44/28.62 (*t*Bu-CH_3), 43.07/43.62 (C^{β}), 43.71/44.51 (C^{γ}), 51.20/51.34 (OCH_3), 60.52/61.18 (C^{δ}), 66.70/66.84 (C^{α}), 79.68/80.24 (*t*Bu-C), 127.45/127.51 (Ar), 127.96/128.00 (Ar), 128.38/128.41 (Ar), 136.74/136.77 (Ar), 152.47/154.26 (*t*BuO*C*O), 172.15/172.23 (*C*O_2CH_3). HRMS (ESI): calcd. for $C_{19}H_{27}NO_4$ $[M+H]^+$ 334.2013; found 334.1993; calcd. for $C_{19}H_{27}NNaO_4$ $[M+Na]^+$ 356.1832; found 356.1828.

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[31] We confirmed that the β-substituted analogue **10** exhibited a capacity to stabilize the *cis* state of the amide bond involving the pyrrolidine nitrogen. To this end, we synthesized *N*-acetyl derivatives of **7** and **10** by acidic treatment followed by reaction with acetic anhydride. Analysis of the two set of resonances in the ^{1}H-NMR spectra acquired in $CDCl_3$ revealed 67 and 70% *cis* amide ratio respectively, that is, a slightly superior tendency of the β-phenyl derivative to stabilize the *cis* amide geometry. It should be noted that the remaining ~30% *trans* amide bond is due to the small size of the methyl group in the *N*-acyl substituent. When δ,δ-dimethylproline is within a peptide chain an amino acid is linked to the pyrrolidine nitrogen (instead of the acetyl group) and the higher steric hindrance prevents the *trans* arrangement of the prolyl peptide bond.

[32] Analytical assays have shown that benzyl *N*-benzyloxycarbonyl-δ,δ-dimethylprolinate (obtained by deprotection and further protection of the amino and carboxylic acid groups in **7** following standard procedures) is well resolved by HPLC on the cellulose-derived column Chiralpak® IB (retention times of 4.6 and 6.7 min upon elution with *n*-hexane/2-propanol 95:5 on a 150 × 4.6 mm column at 1.0 mL/min rate). Optimization of these preliminary results (mainly by testing the effect of other mobile phases on enantioseparation) and subsequent extension to a preparative scale would provide access to significant amounts of enantiopure δ,δ-dimethylproline (both enantiomers). For previous works of our group describing the isolation of (multi)gram-quantities of enantiomerically pure amino acids by preparative HPLC resolution of racemic precursors, see: a) P. Fatás, A. M. Gil, M. I. Calaza, A. I. Jiménez, C. Cativiela, *Chirality*, in press (DOI: 10.1002/chir.22101); b) F. J. Sayago, M. J. Pueyo, M. I. Calaza, A. I. Jiménez, C. Cativiela, *Chirality* **2011**, *23*, 507–513; c) F. J. Sayago, A. I. Jiménez, C. Cativiela, *Tetrahedron: Asymmetry* **2007**, *18*, 2358–2364; d) S. Royo, A. I. Jiménez, C. Cativiela, *Tetrahedron: Asymmetry* **2006**, *17*, 2393–2400; e) M. Lasa, P. López, C. Cativiela, *Tetrahedron: Asymmetry* **2005**, *16*, 4022–4033; f) A. I. Jiménez, P. López, C. Cativiela, *Chirality* **2005**, *17*, 22–29; g) C. Cativiela, M. Lasa, P. López, *Tetrahedron: Asymmetry* **2005**, *16*, 2613–2623; h) A. M. Gil, E. Buñuel, P. López, C. Cativiela, *Tetrahedron: Asymmetry* **2004**, *15*, 811–819; i) C. Cativiela, P. López, M. Lasa, *Eur. J. Org. Chem.* **2004**, 3898–3908; j) M. Alías, M. P. López, C. Cativiela, *Tetrahedron* **2004**, *60*, 885–

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