

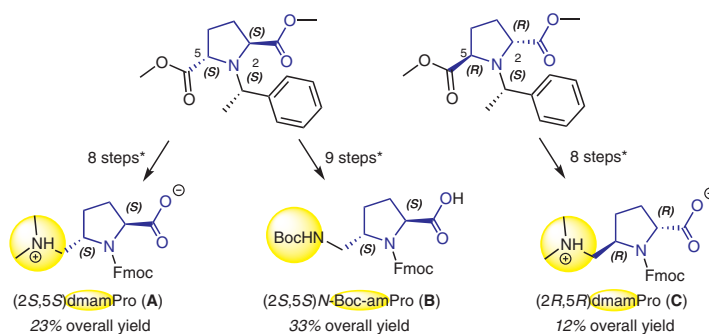
Synthesis of Fmoc- and Boc-Protected (2*S*,5*S*)- and (2*R*,5*R*)-5-Aminomethylprolines

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* only two purifications by column chromatography required

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Abstract The proline derivatives (2*S*,5*S*)dmamPro, (2*S*,5*S*)*N*-Boc-amPro and (2*R*,5*R*)dmamPro are useful building blocks for peptides since they allow conformational fixation of peptidyl-CO-N-prolyl bonds in the unusual *cis* conformation. The stereoselective syntheses of these dimethylaminomethyl-prolines is achieved from literature-known precursors with overall yields of 23% [over 8 steps for (2*S*,5*S*)dmamPro], 33% [over 9 steps for (2*S*,5*S*)*N*-Boc-amPro] and 12% [over 8 steps for (2*R*,5*R*)dmamPro]. The applicability of (2*S*,5*S*)*N*-Boc-amPro in peptide synthesis is demonstrated through the preparation of an Fmoc-Val-amPro-OMe dipeptide.

Key words proline, conformation, switchable, amides, peptides

Proline's unique cyclic structure combined with the resulting conformational restraints enables this amino acid to play an important role in the folding and stability of many proteins and peptides.^{1–4} In addition, when incorporated in a peptide, proline is the only protein amino acid featuring a

tertiary amide, which basically suggests that the *cis* as well as the *trans* rotamer of the amide should be observed.^{5–7} Astonishingly, only very few prolines (ca. 10%) incorporated in native peptides and proteins do actually prefer the *cis* conformation of their peptidyl-CO-N-prolyl bond.⁷ Synthetic studies on how to influence the stability and the folding properties of peptides and proteins have so far mainly been carried out with 3- and 4-substituted proline derivatives **1–4**,⁸ in which the additional substituents mainly exert strong effects on the conformation of the pyrrolidine ring (Figure 1, grey background). Among the broad variety of 3- and 4-substituted prolines,^{9,10} the 4-substituted derivatives **2–4** represent the more frequent approach and such compounds have often been employed to tune the properties of collagen¹¹ and other biomolecules.¹²

In contrast to the conformational effects on the pyrrolidine core observed for prolines **1–4**, an additional substituent(s) at the 5-position of proline, as in compounds **5–7**, can be useful to impact the conformation of the CO-N-prolyl bond.^{13–16} A *cis* to *trans* ratio of 66:34 was observed for

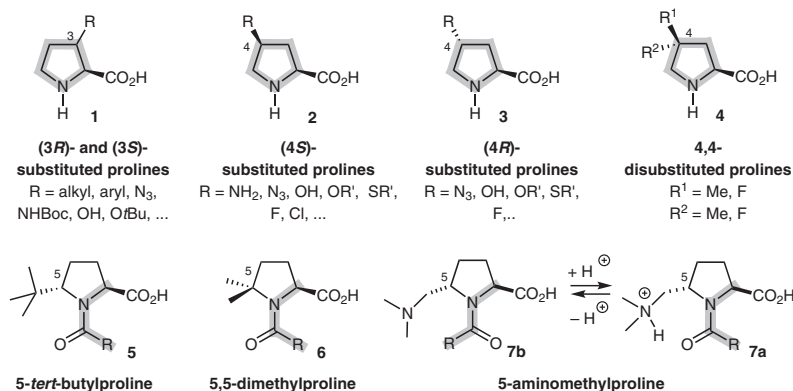


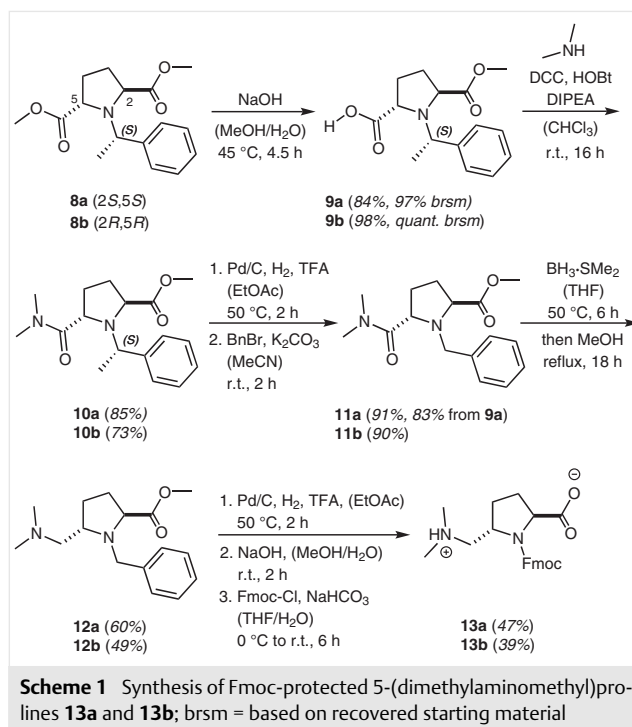
Figure 1 Examples of 3-, 4- and 5-substituted proline derivatives

the (5*S*)-*tert*-butyl derivative **5**,¹⁷ whilst 5,5-dimethyl substitution, as in proline **6**, led to an even higher prevalence of the *cis* rotamer of 90% in water.^{18–20} Further studies including the incorporation of 5,5-dimethylproline in peptides demonstrated the high potential of such derivatives in biological and medicinal applications.²¹ In a recent study on the influence of positive charges on the conformation of amide bonds, it was found that protonated 5-aminomethylprolines **7a** represent a valuable alternative to known 5-substituted proline derivatives, favoring a *cis* conformation of the prolyl amide.^{22,23} Besides a strong conformational fixation observed for **7a** in a range of solvents (87% to >95% *cis* rotamer), the charge-induced effect is pH-dependent and it can thus be used to reversibly favor either the *cis* rotamer **7a** or the *trans* rotamer **7b** through the addition of acid or base.²² Against the background that these initial studies on the conformational fixation of prolylamide **7a** were carried out with racemic 2,5-*trans*-configured proline derivatives, we now describe stereoselective syntheses of *trans*-configured 5-aminomethylprolines.²⁴ The suitability of the newly obtained Fmoc-protected acids for incorporation into peptides is further demonstrated with the synthesis of a Fmoc-L-Val-(2*S*,5*S*)amPro-OMe dipeptide.

The chiral, enantiomerically pure pyrrolidines **8a** and **8b** used as starting materials (Scheme 1) were readily available from adipic acid dichloride via dibromination, esterification and cyclization with (*S*)-1-phenylethylamine.²⁵ Following a procedure reported by Yamamoto,^{25b} the separation of the diastereoisomers **8a** and **8b** was achieved through a combination of crystallization and column chromatography.

The selective monohydrolysis of **8a** has been reported in the literature using 1.7 equivalents of sodium hydroxide in a methanol/water mixture at 20 °C to give **9a** in 79% yield after three days.²⁶ In this work, the single hydrolysis could be achieved in a much shorter reaction time of 4.5 hours with 1.5 equivalents of sodium hydroxide at a slightly elevated temperature of 45 °C. Unreacted **8a** was separated from **9a** by column chromatography or, more conveniently for larger scale reactions, via stepwise extraction at accurately defined pH values. *N,N'*-Dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) were chosen for the synthesis of amide **10a** from acid **9a** and dimethylamine due to the later on simple removal of *N,N'*-dicyclohexylurea through crystallization (Scheme 1). The purification of amide **10a** was possible via column chromatography or extraction, which is again favorable for larger reaction scales.

The subsequent change of the protecting group from (*S*)- α -methylbenzyl to benzyl was necessary since the originally intended reduction of the amide in **10a** with borane-dimethyl sulfide²⁷ led to a number of side reactions including demethylation at the amino group. In various attempts, suitable conditions for selective reduction of **10a** could neither be found with the borane-dimethyl sulfide nor with



the borane–THF adduct.²⁸ The (*S*)- α -methylbenzyl group of **10a** was thus cleaved through hydrogenation with palladium on charcoal (1 bar H₂), and alkylation with benzyl bromide gave **11a** in high yield without purification of the intermediate (Scheme 1).

Overall, amide **11a** could be obtained in three steps from **9a** (83%), requiring no chromatography and only one crystallization step to remove *N,N'*-dicyclohexylurea from **10a**.

The selective reduction of the amide in **11a** was then achieved with borane–dimethyl sulfide under strictly anhydrous conditions.²⁷ Borane species coordinated to the aminoalkylproline **12a** were removed through heating in refluxing methanol (Scheme 1).^{27b,c,29} In this particular step, complete consumption of the starting material **11a** was crucial since the separation of **12a** from unreacted starting material **11a** through column chromatography turned out to be laborious.

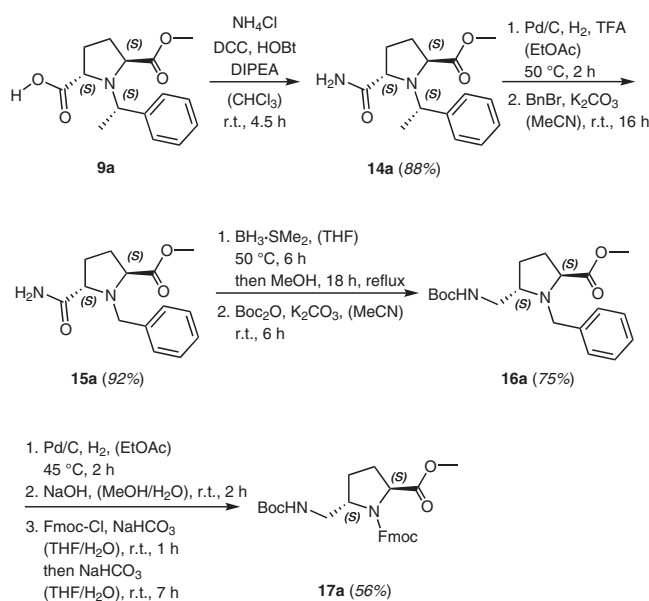
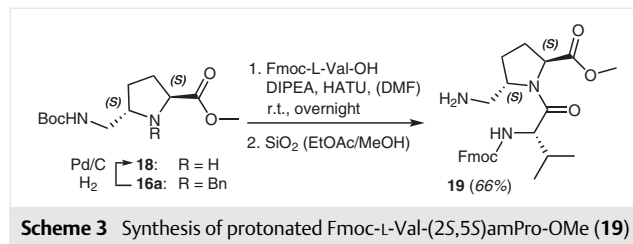
Deprotection of **12a** and attachment of the 9-fluorenyl-methoxycarbonyl (Fmoc) group was conducted in three steps. First, the cleavage of the benzyl protecting group was achieved via hydrogenation with palladium on charcoal in the presence of trifluoroacetic acid. Secondly, the ester moiety was hydrolyzed with 10 equivalents of sodium hydroxide in aqueous methanol, and the reaction mixture was then neutralized and concentrated in vacuo. In the third step, attachment of the Fmoc protecting group was performed under slightly alkaline conditions using sodium bicarbonate in aqueous tetrahydrofuran (Scheme 1). After quenching with trifluoroacetic acid, the Fmoc-protected

proline **13a** could be purified by HPLC. From reactions on larger scales, **13a** was obtained in good purity only by purification through extraction (see the Supporting Information for NMR spectra). After completion of the synthesis of **13a**, the synthetic strategy was transferred to the preparation of **13b** from the (2*R*,5*R*)-configured diester **8b**. In this sequence, a clean reduction of the amide **11a** to **12a** again required a prior change of the protecting group to benzyl. The yields for the single reaction steps were in most cases similar to those reached in the preparation of **13a** from **8a**, thus indicating the reproducibility of the sequence. The lower outcome for the final steps from **11b** to **13b** can be attributed to the smaller reaction scale which complicates especially the borane reduction. Determination of the optical purity of the target compounds **13a** and **13b** by chiral HPLC gave ee values of more than 95%.

To enable further synthetic options, and to allow conformational fixation as a result of acid-induced deprotection, a *N*-Boc protected 5-aminomethylproline derivative was prepared (Scheme 2). Starting from acid **9a**, coupling with ammonia provided amide **14a**. The exchange of the chiral auxiliary for the benzyl protecting group proceeded as described for **10a** (see Scheme 1). Only the benzylation step required a longer reaction time to give **15a**. Selective reduction of the amide functionality, removal of complexed borane, and subsequent Boc protection gave **16a**. In the next steps, the benzyl protecting group was cleaved by hydrogenolysis and the ester was hydrolyzed. As the protection with Fmoc chloride led to an unexpected twofold attachment of the Fmoc group including the formation of a mixed anhydride from the carboxylic acid, this final transformation was combined with a mild alkaline hydrolysis to

provide **17a**. After the final step, the orthogonally protected proline derivative was purified via extraction to give **17a** in 95% purity according to ¹H NMR analysis.³⁰

Finally, the applicability of the new building blocks for peptide synthesis was demonstrated by coupling the *N*-Boc-aminomethylproline methyl ester **18** to *N*-Fmoc-protected valine. The building block **18** was obtained in quantitative yield via hydrogenation of **16a**. Coupling of **18** and Fmoc-L-Val-OH could be achieved using HATU {1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate} and *N,N*-diisopropylethylamine (DIPEA) in dry DMF. HPLC monitoring of the reaction course indicated a conversion of **18** to the dipeptide of 66% without formation of detectable side products (Scheme 3). Work-up and purification by column chromatography finally gave the dipeptide **19**; during this step cleavage of the Boc group occurred since a polar solvent mixture and twofold chromatography on silica had to be used for purification. The fact that **19** showed only one set of signals when analyzed by ¹H and ¹³C NMR under acidic conditions demonstrates that the conformational fixation is also effective for this particular type of *N*-prolyl dipeptide.



Scheme 2 Synthesis of *N*-Boc-aminomethylproline **17a**

In summary, the synthesis of three new 5-(aminomethyl)proline building blocks has been achieved from literature-known precursors. The eight to nine step sequences could be performed with good to high yields for all single steps. Suitability for larger reaction scales is shown since only two purifications by column chromatography are required within each synthetic pathway. The starting materials **8a** and **8b** were available in two steps. All three 5-(aminomethyl)prolines were prepared in Fmoc-protected form to allow direct use in peptide synthesis, which has been demonstrated through incorporation of **18** into the Fmoc-Val-amPro-OMe dipeptide **19**.

Solvents and reagents were obtained from commercial sources and used as received. Analytical TLC was carried out on Merck silica gel plates using shortwave (254 nm) UV light, KMnO₄ [3 g KMnO₄, 20 g potassium carbonate, 5 mL aqueous sodium hydroxide (5% w/w) in 300 mL H₂O] or ninhydrin (200 mg ninhydrin in 100 mL ethanol) to visualize components. Merck silica gel (40–63 μm) was used for flash column chromatography. Yields were calculated in percent (%) based on the employed starting material or in percent based on recovered starting material (% *brsm*). Compounds **8a** and **8b**, which were used as starting materials, were prepared according to ref. 25b. The analytical data obtained were in agreement with those reported in the above reference. Melting points were recorded using a MPM-H2 instrument. Optical rotations were obtained using a Perkin–Elmer 241 polarimeter. IR spectra were recorded as a substance film on a sodium chloride crystal using a Jasco FT/IR 410 spectrometer. ¹H and ¹³C NMR spectra were recorded on Bruker Avance 600 (¹H: 600 MHz, ¹³C: 151 MHz), Bruker Avance 360 (¹H: 360 MHz, ¹³C: 91 MHz) and Bruker Avance 400 (¹H: 400 MHz, ¹³C: 101 MHz) spectrometers. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz). ¹H NMR measurements are referenced to TMS (0 ppm) or the signals for the residual protons of the used solvent: CDCl₃ (7.26 ppm), CD₃CN (1.94 ppm) or D₂O (4.79 ppm). For ¹³C NMR measurements, CDCl₃ and CD₃CN were used as solvents using CHCl₃ (77.0 ppm) or CD₃CN (1.32 ppm) as standards. The following abbreviations are used for the description of signals: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and br s (broad signal). Mass spectra were recorded using electron impact (EI) or electrospray ionization (ESI). A JOEL GC mate II GC-MS-System, Bruker Daltonik microTOF II, and Bruker Daltonik maXis 4G were used for HRMS measurements. High-performance liquid chromatography (HPLC) was performed on a Varian 940 C8 column (21.2 × 150 mm × 5 μm) with PDA (photodiode array) detection (solvent A: 0.1% CF₃CO₂H in water; solvent B: acetonitrile; flow rate: 20 mL/min). HPLC-MS was performed using an Agilent 1100 HPLC-MS with a Zorbax XDB-C8 Column (2.1 × 50 mm × 2.6 μm) with PDA detection at 254 nm and on-line ESI mass spectrometry detection (solvent A: 0.1% CF₃CO₂H in water; solvent B: acetonitrile containing 0.1% CF₃CO₂H; flow rate: 0.5 mL/min. Method A: gradient A/B: 90%/10% → 10%/90% over 20 min; method B: gradient A/B: 100%/0% → 10%/90% acetonitrile over 24 min). Chiral HPLC was performed using a Chiralpak IC column (4.6 × 250 mm × 5 μm) with UV detection at 254 nm (method A: solvent A: hexane; solvent B: ethanol containing 0.1% ethylenediamine; flow rate: 0.7 mL/min, A/B: 80%/20% isocratic; method B: solvent A: hexane; solvent B: isopropanol containing 0.1% ethylenediamine; flow rate: 0.7 mL/min, A/B: 80%/20% isocratic).

(2*S*,5*S*)-5-(Methoxycarbonyl)-1-[(*S*)-1-phenylethyl]pyrrolidine-2-carboxylic Acid (**9a**)

Sodium hydroxide (2 M, 14.3 mL) was added to **8a** (5.53 g, 19.0 mmol) in water (58 mL) and methanol (143 mL) and was stirred for 4.5 h at 45 °C. Water (35 mL) was added and the mixture was extracted with ethyl acetate (1 × 200 mL then 1 × 75 mL). The combined organic phases were dried over sodium sulfate and the solvent was removed under reduced pressure. Column chromatography (silica gel, hexane/ethyl acetate = 4:1 → 100% ethyl acetate → ethyl acetate/methanol = 9:1) allowed recovery of unreacted **8a** (710 mg, 2.45 mmol). The aqueous phase of the previous extraction was adjusted to a pH value of 3 with hydrochloric acid (3 M), supersaturated with sodium chloride and extracted with ethyl acetate (4 × 75 mL). The combined organic phases were dried over sodium sulfate and the solvent was removed under reduced pressure to give title compound **9a** (4.43 g, 16.0 mmol, 84%, 97% *brsm*) as a white solid.

Mp 107.4 °C; *R*_f = 0.7 (ethyl acetate) [UV, KMnO₄]; [α]²⁴₅₈₉ –110.7 (c 0.5, CHCl₃).

IR (NaCl): 2977, 2956, 2881, 1733, 1456, 1374, 1310, 1207, 1166, 767, 704 cm⁻¹.

¹H NMR (360 MHz, CDCl₃): δ = 7.36–7.22 (m, 5 H), 4.20 (q, *J* = 6.7 Hz, 1 H), 3.92 (dd, *J* = 1.3 Hz, *J* = 11.0 Hz, 1 H), 3.63 (d, *J* = 7.3 Hz, 1 H), 3.57 (s, 3 H), 2.66–2.51 (m, 1 H), 2.16–2.02 (m, 2 H), 1.88–1.80 (m, 1 H), 1.38 (d, *J* = 6.8 Hz, 3 H).

¹³C NMR (91 MHz, CDCl₃): δ = 176.1, 172.8, 142.5, 128.8, 128.0, 127.1, 63.9, 63.4, 60.1, 51.6, 29.8, 28.8, 22.9.

HRMS (ESI): *m/z* [M + H]⁺ calcd for C₁₅H₂₀NO₄: 278.1387; found: 278.1391.

(2*R*,5*R*)-5-(Methoxycarbonyl)-1-[(*S*)-1-phenylethyl]pyrrolidine-2-carboxylic Acid (**9b**)

For the synthesis of the (2*R*,5*R*)-isomer **9b**, starting material **8b** (413 mg, 1.42 mmol) was treated as described above to give the title compound **9b** (390 mg, 1.40 mmol, 98%, *quant. brsm*) as a white solid.

*R*_f = 0.7 (ethyl acetate) [UV, KMnO₄]; [α]²⁴₅₈₉ +10.8 (c 0.5, CHCl₃).

IR (NaCl): 2974, 2952, 1734, 1617, 1456, 1436, 1375, 1306, 1282, 1200, 1166, 1060, 763, 702 cm⁻¹.

¹H NMR (360 MHz, CDCl₃): δ = 7.33 (d, *J* = 7.2 Hz, 2 H), 7.29 (t, *J* = 7.4 Hz, 2 H), 7.25 (t, *J* = 7.2 Hz, 1 H), 4.10 (d, *J* = 5.7 Hz, 2 H), 3.87 (d, *J* = 9.1 Hz, 1 H), 3.60 (s, 3 H), 2.52–2.45 (m, 1 H), 2.28–2.21 (m, 1 H), 1.93–1.89 (m, 2 H), 1.46 (d, *J* = 6.4 Hz, 3 H).

¹³C NMR (91 MHz, CDCl₃): δ = 175.8, 173.6, 141.8, 128.5, 128.4, 128.3, 63.6, 63.4, 60.0, 51.7, 29.4, 29.1, 21.3.

HRMS (ESI): *m/z* [M + Na]⁺ calcd for C₁₅H₁₉NNaO₄: 300.1206; found: 300.1210.

Methyl (2*S*,5*S*)-5-(Dimethylcarbamoyl)-1-[(*S*)-1-phenylethyl]pyrrolidine-2-carboxylate (**10a**)

Diisopropylethylamine (1.00 mL, 0.75 g, 5.82 mmol), 1-hydroxybenzotriazole (0.79 g, 5.82 mmol) and *N,N'*-dicyclohexylcarbodiimide (1.64 g, 7.94 mmol) were added to a solution of monoester **9a** (1.47 g, 5.29 mmol) in dry chloroform (20.7 mL) under an argon atmosphere. Dimethylamine (2 M in dry tetrahydrofuran, 5.29 mL, 10.6 mmol) was added dropwise and the resulting mixture was stirred for 16 h overnight at room temperature. Subsequently, water (90 mL) and a saturated aqueous solution of sodium carbonate (10 mL) were added and the mixture was extracted with chloroform (4 × 40 mL). The combined organic phases were washed with a saturated aqueous solution of sodium chloride and dried over sodium sulfate. The solvent was re-

moved under reduced pressure. The crude mixture was redissolved in ethyl acetate and kept at 4 °C overnight. After removal of the white precipitate by filtration (containing mainly *N,N'*-dicyclohexylurea) and washing of the filter cake with small amounts of ethyl acetate, the filtrate was concentrated under reduced pressure. The crude product was further purified by column chromatography (silica gel, hexane/ethyl acetate = 2:1 → 1:1). The title compound **10a** (1.37 g, 4.52 mmol, 85%) was obtained as a white solid.

Mp 69.1 °C; R_f = 0.2 (1:1 hexane/ethyl acetate) [UV, KMnO₄]; [α]₅₈₉²⁴ –86.9 (c 0.5, CHCl₃).

IR (NaCl): 2949, 2359, 2355, 1750, 1730, 1645, 1494, 1455, 1259, 1192, 1152, 1057, 705 cm⁻¹.

¹H NMR (600 MHz, CDCl₃): δ = 7.38–7.34 (m, 2 H), 7.29 (t, J = 7.6 Hz, 2 H), 7.21 (t, J = 7.4 Hz, 1 H), 4.07–3.93 (m, 3 H), 3.73 (s, 3 H), 2.81 (s, 3 H), 2.63–2.55 (m, 1 H), 2.35 (s, 3 H), 2.24–2.15 (m, 1 H), 1.87–1.80 (m, 1 H), 1.65 (dd, J = 7.9 Hz, J = 12.1 Hz, 1 H), 1.26 (d, J = 4.5 Hz, 3 H).

¹³C NMR (151 MHz, CDCl₃): δ = 177.7, 173.8, 145.3, 128.2, 127.7, 127.1, 63.3, 60.5, 60.1, 51.7, 26.5, 35.4, 29.9, 28.5, 23.4.

HRMS (ESI): m/z [M + H]⁺ calcd for C₁₇H₂₅N₂O₃: 305.1860; found: 305.1857.

Methyl (2*R*,5*R*)-5-(Dimethylcarbamoyl)-1-[(*S*)-1-phenylethyl]pyrrolidine-2-carboxylate (**10b**)

For the synthesis of the (2*R*,5*R*)-isomer **10b**, starting material **9b** (356 g, 1.30 mmol) was treated as described above to give the title compound **10b** (287 mg, 0.94 mmol, 73%) as a white solid.

Mp 111.3 °C; R_f = 0.4 (ethyl acetate) [UV, KMnO₄]; [α]₅₈₉²⁴ +42.6 (c 0.5, CHCl₃).

IR (NaCl): 3559, 3463, 2970, 2949, 1734, 1647, 1494, 1455, 1434, 1398, 1366, 1320, 1280, 1195, 1167, 1130, 1090, 1059, 767, 703 cm⁻¹.

¹H NMR (600 MHz, CDCl₃): δ = 7.35 (d, J = 6.6 Hz, 2 H), 7.26 (t, J = 7.6 Hz, 2 H), 7.18 (t, J = 7.2 Hz, 1 H), 4.35 (d, J = 5.4 Hz, 1 H), 4.16 (d, J = 5.2 Hz, 1 H), 3.99 (d, J = 8.4 Hz, 1 H), 3.36 (s, 3 H), 2.83 (s, 3 H), 2.79 (s, 3 H), 2.52–2.41 (m, 1 H), 1.80–1.71 (m, 2 H), 1.33 (d, J = 5.7 Hz, 3 H).

¹³C NMR (91 MHz, CDCl₃): δ = 175.6, 173.6, 143.0, 128.0, 127.4, 126.8, 63.7, 59.8, 59.5, 50.7, 36.4, 35.0, 28.9, 28.4, 22.0.

HRMS (EI): m/z [M + Na]⁺ calcd for C₁₇H₂₄N₂NaO₃: 327.1680; found: 327.1672.

Methyl (2*S*,5*S*)-1-Benzyl-5-(dimethylcarbamoyl)pyrrolidine-2-carboxylate (**11a**)

A mixture of **10a** (609 mg, 2.00 mmol), palladium on carbon (10% w/w, 120 mg, 0.11 mmol) and trifluoroacetic acid (3.90 mL) in dry ethyl acetate (39 mL) was stirred under a hydrogen atmosphere (1 bar) at 50 °C for 2 h. The reaction course was monitored via TLC. When the reaction was finished, the mixture was filtered over Celite® and the filter cake was washed with ethyl acetate. The solvent was removed under reduced pressure and the crude product was dissolved in acetonitrile (19.5 mL). Potassium carbonate (1.35 g, 9.77 mmol) and benzyl bromide (0.70 mL, 1.01 g, 5.89 mmol) were added and the reaction mixture was stirred for 2 h. Subsequently, water (40 mL) was added and the aqueous layer was extracted with ethyl acetate (4 × 40 mL). The combined organic phases were washed with a saturated aqueous solution of sodium chloride and dried over sodium sulfate. The solvent was removed under reduced pressure and the crude

product was purified via column chromatography (silica, hexane/ethyl acetate = 1:1) to give the title compound **11a** (527 mg, 1.82 mmol, 91%) as a colorless oil.

R_f = 0.3 (hexane/ethyl acetate = 1:2) [UV, KMnO₄]; [α]₅₈₉²⁴ –84.5 (c 0.5, CHCl₃).

IR (NaCl): 2952, 2850, 1733, 1645, 1495, 1454, 1399, 1265, 1200, 1127, 748, 702 cm⁻¹.

¹H NMR (600 MHz, CDCl₃): δ = 7.36 (d, J = 7.2 Hz, 2 H), 7.29 (t, J = 7.2 Hz, 2 H), 7.22 (t, J = 7.2 Hz, 1 H), 4.13–3.94 (m, 3 H), 3.78 (d, J = 12.5 Hz, 1 H), 3.67 (s, 3 H), 2.89 (s, 3 H), 2.70 (s, 3 H), 2.42 (qd, J = 9.3 Hz, J = 12.4 Hz, 1 H), 2.30–2.20 (m, 1 H), 1.99 (t, J = 9.1 Hz, 1 H), 1.78 (tdd, J = 2.8 Hz, J = 9.4 Hz, J = 12.1 Hz, 1 H).

¹³C NMR (151 MHz, CDCl₃): δ = 175.3, 173.3, 139.0, 129.1, 128.1, 127.1, 63.9, 59.9, 53.9, 51.6, 36.8, 35.5, 28.4, 28.1.

HRMS (EI): m/z [M + H]⁺ calcd for C₁₆H₂₃N₂O₃: 291.1703; found: 291.1697.

The ¹H NMR and ¹³C NMR data are in agreement with those previously reported for the racemic compound.²²

Methyl (2*R*,5*R*)-1-Benzyl-5-(dimethylcarbamoyl)pyrrolidine-2-carboxylate (**11b**)

For the synthesis of the (2*R*,5*R*)-isomer **11b**, starting material **10b** (243 mg, 0.80 mmol) was treated as described above to give the title compound **11b** (209 mg, 0.72 mmol, 90%) as a colorless oil. The spectroscopic data obtained are in agreement with the data described above.

[α]₅₈₉²⁴ +90.0 (c 0.5, CHCl₃).

Methyl (2*S*,5*S*)-1-Benzyl-5-[(dimethylamino)methyl]pyrrolidine-2-carboxylate (**12a**)

Borane dimethyl sulfide (2 M in dry tetrahydrofuran, 4.00 mL) was added to a solution of amide **11a** (801 mg, 2.76 mmol) in dry tetrahydrofuran (40 mL) under an argon atmosphere and the reaction mixture was stirred for 6 h at 50 °C. Subsequently, dry methanol (35 mL) was added carefully and the mixture was stirred at 75 °C for 18 h. The reaction course was monitored by TLC. After the reaction was finished, the solvent was removed under reduced pressure and the crude product was purified via column chromatography (silica gel, deactivated with triethylamine, hexane/ethyl acetate = 1:1). Title compound **12a** (485 mg, 1.66 mmol, 60%) was obtained as a clear viscous oil.

R_f = 0.1 (hexane/ethyl acetate = 1:2) [UV, KMnO₄]; [α]₅₈₉²⁴ –164.2 (c 0.5, CHCl₃).

IR (NaCl): 2948, 2817, 2764, 2364, 1732, 1454, 1196, 1159, 1036, 996, 845, 745, 700 cm⁻¹.

¹H NMR (360 MHz, CDCl₃, TFA salt): δ = 7.32–7.18 (m, 5 H), 4.07 (d, J = 13.7 Hz, 1 H), 3.74 (d, J = 13.7 Hz, 1 H), 3.64 (s, 3 H), 3.59 (d, J = 7.9 Hz, 1 H), 3.40–3.33 (m, 1 H), 2.36 (dd, J = 3.8 Hz, J = 12.2 Hz, 1 H), 2.30–2.17 (m, 8 H), 2.12–2.00 (m, 1 H), 1.83–1.73 (m, 2 H).

¹³C NMR (151 MHz, CDCl₃, TFA salt): δ = 174.7, 139.8, 128.5, 128.2, 126.9, 65.2, 63.0, 59.6, 53.3, 51.0, 46.2, 28.9, 27.9.

HRMS (EI): m/z [M + H]⁺ calcd for C₁₆H₂₅N₂O₂: 277.1911; found: 277.1909.

The ¹H NMR and ¹³C NMR data are in agreement with those previously reported for the racemic compound.²²

Methyl (2*R*,5*R*)-1-Benzyl-5-[(dimethylamino)methyl]pyrrolidine-2-carboxylate (12*b*)

For the synthesis of the (2*R*,5*R*)-isomer **12b**, starting material **11b** (174.2 mg, 0.60 mmol) was treated as described above to give the title compound **12b** (81.7 mg, 0.29 mmol, 49%) as a colorless oil. The spectroscopic data obtained are in agreement with the data described above.

$[\alpha]_{589}^{24} +166.0$ (c 0.5, CHCl₃).

(2*S*,5*S*)-1-[(9*H*-Fluoren-9-yl)methoxy]carbonyl]-5-[(dimethylammonio)methyl]pyrrolidine-2-carboxylate (13*a*)

A mixture of amine **12a** (276 mg, 1.00 mmol), palladium on carbon (10% w/w, 100 mg, 0.1 mmol) and trifluoroacetic acid (0.46 mL, 0.68 mmol) in dry ethyl acetate (9.5 mL) was stirred for 2.5 h at 50 °C under a hydrogen atmosphere (1 bar). The reaction course was monitored by TLC. When the reaction was finished, the mixture was filtered over glass wool and the filter bed was washed with ethyl acetate. The solvent was removed under reduced pressure and the obtained residue was dissolved in methanol (36 mL). After addition of sodium hydroxide (2 M, 5.60 mL), the mixture was stirred for 1 h at 30 °C. The pH value was adjusted to a value of 7 and the solvent was removed under reduced pressure. The obtained residue was dissolved in acetonitrile and the remaining solid was filtered off. The filter cake was washed with acetonitrile and the solvent was removed under reduced pressure. The residue was dissolved in tetrahydrofuran (21 mL) and water (6 mL). Sodium bicarbonate (168 mg, 2.00 mmol) and 9-fluorenylmethoxycarbonyl chloride (388 mg, 1.50 mmol) were added and the mixture was stirred for 2.5 h at 30 °C. Trifluoroacetic acid (1 mL) was added and the mixture was extracted with ethyl acetate (1 × 20 mL). After addition of hexane (30 mL), the organic phase was washed with water (4 × 30 mL). The combined aqueous phases were extracted with ethyl acetate (7 × 50 mL) and the completion of the extraction was monitored by TLC. The solvent was concentrated under reduced pressure and trifluoroacetic acid (1 mL) was added. After washing with water, the solvent was removed under reduced pressure to give the title compound **13a** (186 mg, 0.47 mmol, 47%) as a white solid. The compound was further purified via HPLC (gradient A/B: min 0–5: 90%/10%; min 5–7: 90%/10% → 60%/40%; min 7–21: 60%/40% → 50%/50%; min 21–22: 50%/50% → 10%/90%; min 22–28: 10%/90%; min 28–29: 10%/90% → 90%/10%; min 29–33: 90%/10%; $t_R = 16.3$ min).

$R_f = 0.5$ (acetonitrile + 1% TFA) [UV, ninhydrin]; $[\alpha]_{589}^{24} -43.9$ (c 0.5, CHCl₃).

IR (NaCl): 3059, 2961, 2891, 2713, 2607, 2501, 1699, 1451, 1415, 1343, 1268, 1198, 1131, 761, 740, 721 cm⁻¹.

¹H NMR (600 MHz, CD₃CN, TFA salt): $\delta = 7.87$ – 7.80 (m, 2.0 H), 7.67 – 7.60 (m, 2.0 H), 7.47 – 7.40 (m, 2.0 H), 7.40 – 7.32 (m, 2.0 H), 4.85 (dd, $J = 4.9$ Hz, $J = 11.3$ Hz, 0.1 H), 4.69 (dd, $J = 4.5$ Hz, $J = 11.3$ Hz, 0.1 H), 4.44 (dd, $J = 6.4$ Hz, $J = 10.6$ Hz, 0.9 H), 4.40–4.36 (m, 1.8 H), 4.32 (dd, $J = 1.8$ Hz, $J = 8.3$ Hz, 0.9 H), 4.24 (t, $J = 6.6$ Hz, 1.0 H), 4.15 (d, $J = 8.3$ Hz, 0.1 H), 3.91–3.87 (m, 0.1 H), 3.26 (ddd, $J = 1.8$ Hz, $J = 9.8$ Hz, $J = 13.2$ Hz, 0.9 H), 3.07 (ddd, $J = 2.2$ Hz, $J = 8.5$ Hz, $J = 13.5$ Hz, 0.9 H), 2.96 (d, $J = 5.1$ Hz, 2.7 H), 2.82 (d, $J = 5.1$ Hz, 2.7 H), 2.76–2.72 (m, 0.1 H), 2.58–2.54 (m, 0.1 H), 2.52 (d, $J = 5.1$ Hz, 0.3 H), 2.38 (d, $J = 5.1$ Hz, 0.3 H), 2.32–2.12 (m, 2.0 H), 2.08–2.00 (m, 1.0 H), 1.70–1.66 (m, 1.0 H).

¹³C NMR (151 MHz, CDCl₃, TFA salt): $\delta = 173.2$, 158.4, 154.9, 144.9, 144.6, 142.3, 142.2, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 126.1, 125.9, 125.7, 121.11, 121.08, 69.4, 67.0, 65.0, 61.0, 60.6, 60.4, 55.1, 53.6, 48.3, 47.8, 46.1, 45.1, 43.8, 43.7, 29.2, 28.7, 28.0, 27.6.

HRMS (ESI): m/z [M + H]⁺ calcd for C₂₃H₂₇N₂O₄: 395.1965; found: 395.1961.

HPLC-MS (ESI): Method A, $t_R = 6.71$ min, $m/z = 395$ [M + H]⁺; chiral HPLC: Method A, $t_R = 14.79$ min.

(2*R*,5*R*)-1-[(9*H*-Fluoren-9-yl)methoxy]carbonyl]-5-[(dimethylammonio)methyl]pyrrolidine-2-carboxylate (13*b*)

For the synthesis of (2*R*,5*R*)-isomer **13b**, starting material **12b** (50.9 mg, 0.18 mmol) was treated as described above to give the title compound **13b** (27.7 mg, 0.07 mmol, 39%) as a white solid. The spectroscopic data obtained are in agreement with the data described above.

$[\alpha]_{589}^{24} +47.3$ (c 0.25, CHCl₃).

HPLC-MS (ESI): Method A, $t_R = 6.89$ min, $m/z = 395$ [M + H]⁺; chiral HPLC: Method A, $t_R = 25.93$ min.

Methyl (2*S*,5*S*)-5-Carbamoyl-1-[(*S*)-1-phenylethyl]pyrrolidine-2-carboxylate (14*a*)

Diisopropylethylamine (11.5 mL, 8.71 g, 67.4 mmol), 1-hydroxybenzotriazole (2.19 g, 16.2 mmol) and *N,N'*-dicyclohexylcarbodiimide (5.57 g, 27.0 mmol) were added to a solution of monoester **9a** (3.74 g, 13.5 mmol) in dry chloroform (54 mL) under an argon atmosphere. Ammonium chloride (2.16 g, 40.4 mmol) was added and the mixture was stirred for 4.5 h at room temperature. Subsequently, hexane (200 mL) was added and the mixture was extracted with hydrochloric acid (3 M, 5 × 100 mL). The combined aqueous phases were washed with hexane (100 mL) and the pH of the aqueous phase was carefully adjusted to a value of 9 by addition of potassium carbonate under vigorous stirring. The aqueous phase was extracted with ethyl acetate (5 × 200 mL), washed with a saturated aqueous solution of sodium chloride and dried over sodium sulfate. The solvent was removed under reduced pressure and the title compound **14a** was obtained as a white solid which was used in the next step without further purification. Mp 183.3 °C; $R_f = 0.3$ (ethyl acetate) [UV, KMnO₄]; $[\alpha]_{589}^{24} -129.1$ (c 0.5, CHCl₃).

IR (NaCl): 3458, 3235, 3179, 2992, 1730, 1684, 1666, 1454, 1436, 1391, 1376, 1202, 1162, 1140, 991, 766, 702, 612 cm⁻¹.

¹H NMR (360 MHz, CDCl₃): $\delta = 7.34$ – 7.20 (m, 5 H), 7.06 (br s, 1 H), 5.73 (br s, 1 H), 4.09 (q, $J = 6.7$ Hz, 1 H), 3.78 (dd, $J = 1.9$ Hz, $J = 11.0$ Hz, 1 H), 3.56 (d, $J = 7.6$ Hz, 1 H), 3.54 (s, 3 H), 2.57 (ddt, $J = 7.3$ Hz, $J = 11.1$ Hz, $J = 13.0$ Hz, 1 H), 2.08 (tt, $J = 7.7$ Hz, $J = 12.7$ Hz, 1 H), 1.96 (ddd, $J = 2.0$ Hz, $J = 7.8$ Hz, $J = 12.8$ Hz, 1 H), 1.78 (dd, $J = 7.6$ Hz, $J = 12.8$ Hz, 1 H), 1.36 (d, $J = 6.7$ Hz, 3 H).

¹³C NMR (91 MHz, CDCl₃): $\delta = 179.9$, 173.5, 143.9, 128.6, 127.6, 127.2, 64.5, 64.2, 60.4, 51.2, 30.4, 28.8, 23.5.

HRMS (ESI): m/z [M + Na]⁺ calcd for C₁₅H₂₀N₂NaO₃: 299.1366; found: 299.1369.

Methyl (2*S*,5*S*)-1-Benzyl-5-carbamoylpyrrolidine-2-carboxylate (15*a*)

A mixture of **14a** (max. 13.5 mmol), palladium on carbon (10% w/w, 450 mg, 0.42 mmol) and trifluoroacetic acid (4.50 mL) in dry ethyl acetate (45 mL) was stirred for 2 h at 45 °C under a hydrogen atmosphere. The reaction course was monitored by TLC. When the reaction was finished the mixture was filtered over Celite® and washed with ethyl acetate. The solvent was removed under reduced pressure and the obtained residue was dissolved in acetonitrile (67.5 mL). Potassium carbonate (14.9 g, 108 mmol) and benzyl bromide (8.02 mL, 11.5 g, 67.5 mmol) were added and the mixture was stirred at 45 °C for 16 h. Subsequently, hexane (150 mL) was added and the mixture

was extracted with water (5 × 75 mL). The combined aqueous phases were washed with hexane (20 mL) and the pH was adjusted to a value of 11 by addition of potassium carbonate under vigorous stirring. The aqueous phase was extracted with ethyl acetate (5 × 250 mL), dried over sodium sulfate and the solvent was removed under reduced pressure. The title compound **15a** (2.59 g, 9.46 mmol, 70% from **9a**) was obtained as a white solid and was used for the next step without further purification.

Mp 136.7 °C; R_f = 0.3 (ethyl acetate) [UV, KMnO₄]; [α]₅₈₉²⁴ –121.1 (c 0.5, CHCl₃).

IR (NaCl): 3437, 3183, 2945, 2883, 2362, 2333, 1733, 1679, 1453, 1359, 1207, 1158, 754 cm⁻¹.

¹H NMR (360 MHz, CDCl₃): δ = 7.35–7.27 (m, 3 H), 7.24–7.21 (m, 2 H), 6.90 (br s, 1 H), 5.61 (br s, 1 H), 3.92 (d, J = 13.1 Hz, 1 H), 3.84 (d, J = 13.1 Hz, 1 H), 3.77 (dd, J = 3.3 Hz, J = 10.9 Hz, 1 H), 3.74 (d, J = 7.7 Hz, 1 H), 3.69 (s, 3 H), 2.54 (dtd, J = 8.1 Hz, J = 10.9 Hz, J = 12.6 Hz, 1 H), 2.16–2.04 (m, 1 H), 2.00–1.85 (m, 2 H).

¹³C NMR (91 MHz, CDCl₃): δ = 177.7, 173.5, 138.0, 128.7, 128.6, 127.6, 65.8, 62.9, 54.2, 51.4, 29.4, 28.6.

HRMS (ESI): m/z [M + Na]⁺ calcd for C₁₄H₁₈N₂NaO₃; 285.1209; found: 285.1202.

Methyl (2*S*,5*S*)-1-Benzyl-5-[[*tert*-butoxycarbonyl]amino]methyl]pyrrolidine-2-carboxylate (**16a**)

Borane dimethyl sulfide (2 M in dry tetrahydrofuran, 2.73 mL, 5.55 mmol) was added dropwise to a solution of amide **15a** (393 mg, 1.50 mmol) in dry tetrahydrofuran (7.6 mL) under an argon atmosphere and the reaction mixture was stirred for 6 h at 50 °C. Subsequently, dry methanol (13 mL) was added dropwise and the reaction mixture was stirred for 18 h at 75 °C. The solvent was removed under reduced pressure and the obtained residue was dissolved in tetrahydrofuran (15 mL) and treated with potassium carbonate (212 mg, 2.00 mmol) and di-*tert*-butyldicarbonate (437 mg, 2.00 mmol) for 8.5 h. Water (50 mL) was added and the resulting mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with a saturated aqueous solution of sodium chloride and dried over sodium sulfate. The crude mixture was purified by column chromatography (silica gel, hexane/ethyl acetate = 9:1 → 4:1 → ethyl acetate) to give the title compound **16a** (392 g, 1.13 mmol, 75%) as a colorless oil.

R_f = 0.5 (hexane/ethyl acetate = 4:1) [UV, KMnO₄]; [α]₅₈₉²⁴ –110.3 (c 0.5, CHCl₃).

IR (NaCl): 3392, 2977, 2952, 1733, 1714, 1496, 1454, 1366, 1249, 1163, 743, 699 cm⁻¹.

¹H NMR (600 MHz, CDCl₃): δ = 7.38–7.36 (m, 1 H), 7.32–7.28 (m, 2 H), 7.26–7.22 (m, 2 H), 4.79 (br s, 1 H), 3.91 (d, J = 13.5 Hz, 1 H), 3.72 (d, J = 13.5 Hz, 1 H), 3.66 (s, 3 H), 3.63 (d, J = 8.2 Hz, 1 H), 3.45–3.39 (m, 1 H), 3.34 (dd, J = 8.3 Hz, J = 12.2 Hz, 1 H), 3.05 (d, J = 13.1 Hz, 1 H), 2.16 (qd, J = 9.4 Hz, J = 12.6 Hz, 1 H), 2.05–1.98 (m, 1 H), 1.78 (dd, J = 9.8 Hz, J = 11.9 Hz, 1 H), 1.74–1.68 (m, 1 H), 1.45 (s, 9 H).

¹³C NMR (91 MHz, CDCl₃): δ = 174.4, 156.4, 139.2, 128.5, 128.4, 127.1, 78.1, 63.3, 60.9, 52.9, 51.5, 42.1, 28.4, 28.1, 27.0.

HRMS (ESI): m/z [M + Na]⁺ calcd for C₁₉H₂₈NaN₂O₄; 371.1941; found: 371.1939.

(2*S*,5*S*)-1-[[*(9H*-Fluoren-9-yl)methoxy]carbonyl]-5-[[*tert*-butoxycarbonyl]amino]methyl]pyrrolidine-2-carboxylic Acid (**17a**)

A suspension of **16a** (912 mg, 2.62 mmol) and palladium on carbon (10% w/w, 524 mg, 0.49 mmol) in dry ethyl acetate (26 mL) was stirred at 45 °C for 2 h under a hydrogen atmosphere (1 bar). The reaction course was monitored by TLC. After the reaction was complete, the reaction mixture was filtered over Celite® and the filter cake was washed with ethyl acetate. The solvent was removed under reduced pressure and (2*S*,5*S*)-methyl 5-[[*tert*-butoxycarbonyl]amino]methyl]pyrrolidine-2-carboxylate (**18**) (*quant.*) was used without further purification. The obtained product (301 mg, 1.17 mmol) was dissolved in methanol (12 mL), sodium hydroxide (2 M, 5.83 mL) was added, and the reaction mixture was stirred for 2 h at room temperature. The reaction course was monitored via TLC. Subsequently, the pH was adjusted to a value of 8 by addition of hydrochloric acid (3 M) and a saturated aqueous solution of potassium carbonate. The solvent was removed under reduced pressure and the residue was dissolved in tetrahydrofuran (29 mL) and water (7.3 mL). Sodium bicarbonate (392 mg, 4.66 mmol) and 9-fluorenylmethoxycarbonyl chloride (453 mg, 1.75 mmol) were added and the reaction mixture was stirred for 1 h at room temperature. The reaction course was monitored by TLC. After extraction with ethyl acetate (2 × 50 mL), the combined organic phases were washed with a saturated solution of sodium bicarbonate, dried over sodium sulfate and the remaining aqueous phases were kept for further processing (see below). The solvent of the organic phase was removed under reduced pressure leading to a residue containing (2*S*,5*S*)-1-[[*(9H*-fluoren-9-yl)methoxy]carbonyl]-5-[[*tert*-butoxycarbonyl]amino]methyl]pyrrolidine-2-carboxylic [(9*H*-fluoren-9-yl)methyl-carbonic] anhydride. The residue containing the anhydride was dissolved in tetrahydrofuran (29 mL) and water (7.3 mL) and the mixture was stirred for 7 h at room temperature, while the reaction course was monitored by TLC. The solvent was removed under reduced pressure, the residue was dissolved in water (30 mL) and washed with methyl-*tert*-butyl ether (2 × 30 mL). The remaining aqueous phase was adjusted to a pH value of 2 with hydrochloric acid (3 M) and was extracted with ethyl acetate (4 × 40 mL). The combined ethyl acetate phases were dried over sodium sulfate and the solvent was removed under reduced pressure to give **17a** (52%). Subsequently, the previously kept combined aqueous phases (see above) were adjusted to a pH value of 2 with hydrochloric acid (3 M) and extracted with ethyl acetate (4 × 70 mL). The combined organic phases of this extraction were dried over sodium sulfate and the solvent was removed under reduced pressure to afford an additional amount of **17a** (27 mg, 0.06 mmol, 4%). The title compound **17a** (304 mg, 94% w/w, 0.61 mmol, 52%, in total 56%) was obtained as a white solid.

R_f = 0.3 (CH₂Cl₂/methanol, 19:1) [UV, ninhydrin].

IR (NaCl): 3354, 2976, 2360, 2341, 1699, 1520, 1450, 1415, 1365, 1340, 1247, 1166, 1128, 1089, 758, 741 cm⁻¹.

¹H NMR (600 MHz, CDCl₃): δ (mixture of rotamers, 1:1) = 7.76 (dd, J = 3.7 Hz, J = 7.4 Hz, 1 H), 7.73 (d, J = 7.5 Hz, 1 H), 7.59–7.52 (m, 2 H), 7.44–7.27 (m, 4 H), 5.10 (t, J = 5.9 Hz, 0.5 H), 4.72–4.65 (m, 1 H), 4.50–4.43 (m, 1 H), 4.32–4.28 (m, 0.5 H), 4.26–4.21 (m, 1 H), 4.15–4.11 (m, 0.5 H), 4.08–4.01 (m, 1 H), 3.47–3.40 (m, 0.5 H), 3.33–3.26 (m, 0.5 H), 3.26–3.13 (m, 0.5 H), 2.84–2.77 (m, 0.5 H), 2.63–2.57 (m, 0.5 H), 2.35–1.76 (m, 4 H), 1.44 (s, 4.5 H), 1.42 (s, 4.5 H).

¹³C NMR (151 MHz, CDCl₃): δ (mixture of rotamers, 1:1) = 175.6, 174.7, 156.4, 156.1, 155.4, 144.0, 143.8, 143.7, 143.6, 141.41, 141.36, 141.3, 127.84, 127.79, 127.7, 127.6, 127.4, 127.3, 127.1, 127.0, 124.9, 124.8, 124.6, 119.94, 119.91, 119.85, 79.5, 79.3, 67.3, 66.8, 59.6, 59.3, 58.9, 58.6, 47.3, 47.2, 43.7, 42.4, 28.8, 28.4, 27.4, 26.9, 26.7.

HRMS (ESI): m/z [M + H]⁺ calcd for C₂₆H₃₁N₂O₆: 467.2177; found: 467.2175.

Chiral HPLC: Method A: t_R = 30.49 min; Method B: t_R = 22.42 min.

Methyl (2S)-1-(((9H-Fluoren-9-yl)methoxy)carbonyl)-L-valyl)-5-(aminomethyl)pyrrolidine-2-carboxylate (**19**)

A suspension of **16a** (71.6 mg, 0.03 mmol) and palladium on carbon (10% w/w, 42 mg, 0.05 mmol) in dry ethyl acetate (5 mL) was stirred at 45 °C under a hydrogen atmosphere (1 bar). The reaction course was monitored by HPLC-MS. After the reaction was complete, the reaction mixture was filtered over Celite® and the filter cake was washed with ethyl acetate. The solvent was removed under reduced pressure and (2S,5S)-methyl 5-[[*tert*-butoxycarbonyl]amino]methyl]pyrrolidine-2-carboxylate (**18**) (*quant.*) was used without further purification.

¹H NMR (400 MHz, CDCl₃): δ = 4.99 (s, 1 H), 3.83–3.78 (m, 1 H), 3.74 (s, 3 H), 3.43–4.39 (m, 1 H), 3.24–3.17 (m, 1 H), 3.04–2.96 (m, 1 H), 2.22–2.10 (m, 2 H), 1.90–1.81 (m, 2 H), 1.45 (s, 9 H).

For the synthesis of **19**, compound **18** (20 mg, 0.08 mmol), Fmoc-L-Val-OH (102 mg, 0.3 mmol) and *N,N*-diisopropylethylamine (0.17 mL, 1.00 mmol) were dissolved in dry *N,N*-dimethylformamide (2 mL) before 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) (0.4 mmol, 152 mg) was added and the reaction mixture stirred overnight. The reaction course was monitored by HPLC. After 18 h, water was added and the reaction mixture was extracted with ethyl acetate (3 × 15 mL). The combined organic phases were washed with a saturated aqueous solution of sodium chloride and dried over sodium sulfate. Subsequently, the solvent was removed under reduced pressure. Prepurification by column chromatography on silica was conducted using hexane/ethyl acetate = 1:1 → ethyl acetate/methanol = 9:1, during which cleavage of the Boc group occurred on removal of the solvent. A second column chromatography (silica, hexane/ethyl acetate = 4:1) provided **19** (23.1 mg, 0.05 mmol, 66%) as a colorless oil.

R_f = 0.7 (hexane/ethyl acetate = 1:1) [UV, KMnO₄].

¹H NMR (400 MHz, CDCl₃, acidic): δ = 7.79 (d, J = 7.5 Hz, 2 H), 7.63 (dd, J = 2.4 Hz, J = 7.5 Hz, 2 H), 7.43 (t, J = 7.5 Hz, 2 H), 7.38–7.32 (m, 3 H), 5.38–5.28 (m, 1 H), 4.55–4.40 (m, 2 H), 4.34 (dd, J = 4.9 Hz, J = 9.1 Hz, 1 H), 4.26 (t, J = 7.0 Hz, 1 H), 3.78 (s, 3 H), 2.24–2.15 (m, 1 H), 1.76–1.52 (m, 3 H), 1.45–1.43 (m, 1 H), 1.29–1.23 (m, 1 H), 1.15–1.10 (m, 3 H), 1.00 (d, J = 6.9 Hz, 3 H), 0.94 (d, J = 6.9 Hz, 3 H).

¹³C NMR (151 MHz, CDCl₃, acidic): δ = 172.6 (C_q), 156.2 (C_q), 143.9 (C_q), 143.8 (2 × C_q), 141.3 (2 × C_q), 127.7 (2 × CH), 127.1 (2 × CH), 125.1 (2 × CH), 120.0 (2 × CH), 67.0 (CH₂), 59.5 (CH₂), 59.0 (CH), 52.2 (CH₃), 47.2 (2 × CH), 38.2 (2 × CH₂), 31.3 (CH), 31.2 (CH), 18.9 (CH₃), 17.6 (CH₃).

HRMS (ESI): m/z [M + H]⁺ calcd for C₂₇H₃₄N₃O₅: 480.2493; found: 480.2493.

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Supporting Information

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References

- Brandts, J. F.; Halvorson, H. R.; Brennan, M. *Biochemistry* **1975**, *14*, 4953.
- Andreotti, A. H. *Biochemistry* **2003**, *42*, 9515.
- Eckert, B. S.; Schmid, F.-X. *BioSpectrum* **2006**, *3*.
- Shoulders, M. D.; Raines, R. T. *Annu. Rev. Biochem.* **2009**, *78*, 929.
- Dorman, D. E.; Bovey, F. A. *J. Org. Chem.* **1973**, *38*, 2379.
- Cheng, H. N.; Bovey, F. A. *Biopolymers* **1977**, *16*, 1465.
- Fischer, S.; Dunbrack, R. L.; Karplus, M. *J. Am. Chem. Soc.* **1994**, *116*, 11931.
- For a review article, see: Mothes, C.; Caumes, C.; Guez, A.; Boulet, H.; Gendrineau, T.; Darses, S.; Delsuc, N.; Moumné, R.; Oswald, B.; Lequin, O.; Karoyan, P. *Molecules* **2013**, *18*, 2307.
- For syntheses of 3-substituted proline derivatives, see: (a) Perni, R. B.; Farmer, L. J.; Cottrell, K. M.; Court, J. J.; Courtney, L. F.; Deininger, D. D.; Gates, C. A.; Harbeson, S. L.; Kim, J. L.; Lin, C.; Lin, K.; Luong, Y.-P.; Maxwell, J. P.; Murcko, M. A.; Pitlik, J.; Rao, B. G.; Schairer, W. C.; Tung, R. D.; Van Drie, J. H.; Wilson, K.; Thomson, J. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1939. (b) Beausoleil, E.; Sharma, R.; Michnick, S. W.; Lubell, W. D. *J. Org. Chem.* **1998**, *63*, 6572. (c) Hodges, J. A.; Raines, R. T. *J. Am. Chem. Soc.* **2005**, *127*, 15923. (d) Jenkins, C. L.; Bretscher, L. E.; Guzei, I. A.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 6422. (e) Caumes, C.; Delsuc, N.; Azza, R. B.; Correia, I.; Chemla, F.; Ferreira, F.; Carlier, L.; Luna, A. P.; Moumné, R.; Lequin, O.; Karoyan, P. *New J. Chem.* **2013**, *37*, 1312.
- For syntheses of 4-substituted proline derivatives, see: (a) Doi, M.; Nishi, Y.; Kiritoshi, N.; Iwata, T.; Nago, M.; Nakano, H.; Uchiyama, S.; Nakazawa, T.; Wakamiya, T.; Kobayashi, Y. *Tetrahedron* **2002**, *58*, 8453. (b) Demange, L.; Ménez, A.; Dugave, C. *Tetrahedron Lett.* **1998**, *39*, 1169. (c) Pandey, A. K.; Ganguly, H. K.; Yap, G. P. A.; Zondlo, N. J. *Org. Biomol. Chem.* **2016**, *14*, 2327. (d) Owens, N. W.; Stetefeld, J.; Lattová, E.; Schweizer, F. *J. Am. Chem. Soc.* **2010**, *132*, 5036. (e) Klein, L. L.; Li, L.; Chen, H.-J.; Curty, C. B.; DeGoey, D. A.; Grampovnik, D. J.; Leone, C. L.; Thomas, S. A.; Yeung, C. M.; Funk, K. W.; Kishore, V.; Lundell, E. O.; Wodka, D.; Meulbroek, J. A.; Alder, J. D.; Nilius, A. M.; Lartey, P. A.; Plattner, J. J. *Bioorg. Med. Chem.* **2000**, *8*, 1677. (f) Jiang, J.; Yang, L.; Jin, Q.; Ma, W.; Moroder, L.; Dong, S. *Chem. Eur. J.* **2013**, *19*, 17679. (g) Zhang, Z.; Aerschot, A. V.; Hendrix, C.; Busson, R.; David, F.; Sandra, P.; Herdewijn, P. *Tetrahedron* **2000**, *56*, 2513. (h) Zondlo, N.; Pandey, A.; Tressler, C. WO2014127052A1, **2014**.
- (a) Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. T. *Nature* **1998**, *392*, 666. (b) Kotch, F. W.; Guzei, I. A.; Raines, R. T. *J. Am. Chem. Soc.* **2008**, *130*, 2952. (c) Erdmann, R. S.; Wennemers, H. *Angew. Chem. Int. Ed.* **2011**, *50*, 6835. (d) Shoulders, M. D.; Guzei, I. A.; Raines, R. T. *Biopolymers* **2008**, *89*, 443. (e) Shoulders, M. D.; Hodges, J. A.; Raines, R. T. *J. Am. Chem. Soc.* **2006**, *128*, 8112. (f) DeRider, M. L.; Wilkens, S. J.; Waddell, M. J.; Bretscher, L. E.; Weinhold, F.; Raines, R. T.; Markley, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 2497. (g) Jenkins, C. L.; McCloskey, A. I.; Guzei, I. A.; Eberhardt, E. S.; Raines, R. T. *Biopolymers* **2005**, *80*, 1. (h) Siebler, C.; Erdmann, R. S.; Wennemers, H. *Angew. Chem. Int. Ed.* **2014**, *53*, 10340. (i) Erdmann, R. S.; Wennemers, H. *J. Am. Chem. Soc.* **2010**, *132*, 13957.

- (12) (a) Golbik, R.; Yu, C.; Weyher-Stingl, E.; Huber, R.; Moroder, L.; Budisa, N.; Schiene-Fischer, C. *Biochemistry* **2005**, *44*, 16026. (b) Boulègue, C.; Milbradt, A. G.; Renner, C.; Moroder, L. *J. Mol. Biol.* **2006**, *358*, 846. (c) Steiner, T.; Hess, P.; Bae, J. H.; Wilttschi, B.; Moroder, L.; Budisa, N. *PLoS ONE* **2008**, *3*, e1680. (d) Heindl, C.; Hübner, H.; Gmeiner, P. *Tetrahedron: Asymmetry* **2003**, *14*, 3153. (e) Renner, C.; Alefelder, S.; Bae, J. H.; Budisa, N.; Huber, R.; Moroder, L. *Angew. Chem. Int. Ed.* **2001**, *40*, 923. (f) Cadamuro, S. A.; Reichold, R.; Kusebauch, U.; Musiol, H.-J.; Renner, C.; Tavan, P.; Moroder, L. *Angew. Chem. Int. Ed.* **2008**, *47*, 2143. (g) Kuemin, M.; Nagel, Y. A.; Schweizer, S.; Monnard, F. W.; Ochsenfeld, C.; Wennemers, H. *Angew. Chem. Int. Ed.* **2010**, *49*, 6324. (h) Naduthambi, D.; Zondlo, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 12430.
- (13) (a) Teixidó, M.; Zurita, E.; Mendieta, L.; Oller-Salvia, B.; Prades, R.; Tarragó, T.; Giral, E. *Biopolymers* **2013**, *100*, 662. (b) Talbot, A.; Maltais, R.; Kenmogne, L. C.; Roy, J.; Poirier, D. *Steroids* **2016**, *107*, 55.
- (14) Nagaike, F.; Onuma, Y.; Kanazawa, C.; Hojo, H.; Ueki, A.; Nakahara, Y.; Nakahara, Y. *Org. Lett.* **2006**, *8*, 4465.
- (15) Johannesson, P.; Lindeberg, G.; Tong, W.; Gogoll, A.; Synnergren, B.; Nyberg, F.; Karlén, A.; Hallberg, A. *J. Med. Chem.* **1999**, *42*, 4524.
- (16) (a) Sunilkumar, G.; Nagamani, D.; Argade, N. P.; Ganesh, K. N. *Synthesis* **2003**, 2304. (b) Kamijo, S.; Hoshikawa, T.; Inoue, M. *Org. Lett.* **2011**, *13*, 5928.
- (17) Beausoleil, E.; Lubell, W. D. *J. Am. Chem. Soc.* **1996**, *118*, 12902.
- (18) (a) Magaard, V. W.; Sanchez, R. M.; Bean, J. W.; Moore, M. L. *Tetrahedron Lett.* **1993**, *34*, 381. (b) Alonso De Diego, S. A.; Muñoz, P.; González-Muñiz, R.; Herranz, R.; Martín-Martínez, M.; Cenarruzabeitia, E.; Frechilla, D.; Del Río, J.; Luisa Jimeno, M.; Teresa García-López, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2279.
- (19) (a) Melis, C.; Bussi, G.; Lummis, S. C. R.; Molteni, C. *J. Phys. Chem. B* **2009**, *113*, 12148. (b) Rodríguez, I.; Calaza, M. I.; Cativiela, C. *Eur. J. Org. Chem.* **2013**, 1093. (c) Hinderaker, M. P.; Raines, R. T. *Protein Sci.* **2003**, *12*, 1188.
- (20) For related, ring-annulated derivatives, see: Aillard, B.; Kilburn, J. D.; Blydes, J. P.; Tizzard, G. J.; Findlow, S.; Werner, J. M.; Bloodworth, S. *Org. Biomol. Chem.* **2015**, *13*, 4562.
- (21) (a) An, S. S. A.; Lester, C. C.; Peng, J.-L.; Li, Y.-J.; Rothwarf, D. M.; Welker, E.; Thannhauser, T. W.; Zhang, L. S.; Tam, J. P.; Scheraga, H. A. *J. Am. Chem. Soc.* **1999**, *121*, 11558. (b) Arnold, U.; Hinderaker, M. P.; Köditz, J.; Golbik, R.; Ulbrich-Hofmann, R.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 7500.
- (22) Bartuschat, A. L.; Wicht, K.; Heinrich, M. R. *Angew. Chem. Int. Ed.* **2015**, *54*, 10294.
- (23) For alternative effects leading to the conformational fixation of amide bonds, see: (a) Gorske, B. C.; Bastian, B. L.; Geske, G. D.; Blackwell, H. E. *J. Am. Chem. Soc.* **2007**, *129*, 8928. (b) Gorske, B. C.; Stringer, J. R.; Bastian, B. L.; Fowler, S. A.; Blackwell, H. E. *J. Am. Chem. Soc.* **2009**, *131*, 16555. (c) Caumes, C.; Roy, O.; Faure, S.; Taillefumier, C. *J. Am. Chem. Soc.* **2012**, *134*, 9553. (d) Szekely, T.; Caumes, C.; Roy, O.; Faure, S.; Taillefumier, C. *C. R. Chim.* **2013**, *16*, 318. (e) Moure, A.; Sanclimens, G.; Bujons, J.; Masip, I.; Alvarez-Larena, A.; Pérez-Payá, E.; Alfonso, I.; Messeguer, A. *Chem. Eur. J.* **2011**, *17*, 7927. (f) Stringer, J. R.; Crapster, J. A.; Guzei, I. A.; Blackwell, H. E. *J. Org. Chem.* **2010**, *75*, 6068. (g) Yamasaki, R.; Tanatani, A.; Azumaya, I.; Saito, S.; Yamaguchi, K.; Kagechika, H. *Org. Lett.* **2003**, *5*, 1265. (h) See also ref. 11a.
- (24) For related synthetic routes, see: (a) Kajino, H.; Nihei, S.; Ikeuchi, Y.; Miyamoto, H.; Numagami, E. EP2103619A1, **2009**. (b) Jain, S.; Sujatha, K.; Krishna, K. V. R.; Roy, R.; Singh, J.; Anand, N. *Tetrahedron* **1992**, *48*, 4985.
- (25) (a) Watson, H. A.; O'Neill, B. T. *J. Org. Chem.* **1990**, *55*, 2950. (b) Yamamoto, Y.; Hoshino, J.; Fujimoto, Y.; Ohmoto, J.; Sawada, S. *Synthesis* **1993**, 298. (c) Aggarwal, V. K.; Sandrinelli, F.; Charmant, J. P. H. *Tetrahedron: Asymmetry* **2002**, *13*, 87.
- (26) Gu, Q.; Jiang, L.-X.; Yuan, K.; Zhang, L.; Wu, X.-Y. *Synth. Commun.* **2008**, *38*, 4198.
- (27) (a) Brown, H. C.; Choi, Y. M.; Narasimhan, S. *J. Org. Chem.* **1982**, *47*, 3153. (b) Lehmann, L.; Friebe, M.; Brumby, T.; Sülzle, D.; Platzeck, J. 2004 WO 2004087656. (c) Burkholder, T. P.; Fuchs, P. L. *J. Am. Chem. Soc.* **1990**, *112*, 9601.
- (28) Roeske, R. W.; Weitl, F. L.; Prasad, K. U.; Thompson, R. M. *J. Org. Chem.* **1976**, *41*, 1260.
- (29) Dubowchik, G. M.; Firestone, R. A. *Tetrahedron Lett.* **1996**, *37*, 6465.
- (30) The optical purity of the Boc-protected derivative **17a** was determined by chiral HPLC using two different solvent systems.