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

Amelie L. Bartuschat
Nina Hegmann
Markus R. Heinrich*

Department of Chemistry and Pharmacy, Pharmaceutical Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Nikolaus-Fiebiger-Str. 10, 91058 Erlangen, Germany
markus.heinrich@fau.de

Synthesis

A. L. Bartuschat et al.

Paper

Received: 11.09.2018
Accepted: 17.09.2018
Published online: 25.10.2018
DOI: 10.1055/s-0037-1610304; Art ID: ss-2018-t0612-op

Abstract The proline derivatives (2S,5S)-dmamPro, (2S,5S)-N-Boc-am-Pro and (2R,5R)-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 (2S,5S)-dmamPro), 33% (over 9 steps for (2S,5S)-N-Boc-am-Pro) and 12% (over 8 steps for (2R,5R)-dmamPro). The applicability of (2S,5S)-N-Boc-am-Pro in peptide synthesis is demonstrated through the preparation of an Fmoc-Val-am-Pro-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 1–4,5 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 prolines9,10 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 (S)-\textit{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-aminoethylprolines \(7a\) 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-aminoethylprolines.\(^{24}\) The suitability of the newly obtained Fmoc-protected acids for incorporation into peptides is further demonstrated with the synthesis of a Fmoc-L-Val-(2S,5S)-jamPro-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)-\textit{\textalpha{}}-methylbenzyl to benzyl was necessary since the originally intended reduction of the amide in \(10a\) with borane–\textit{\textalpha{}}-dimethyl sulfide\(^{27}\) 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–\textit{\textalpha{}}-dimethyl sulfide nor with the borane–THF adduct.\(^{28}\) The (S)-\textit{\textalpha{}}-methylbenzyl group of \(10a\) was thus cleaved through hydrogenation with palladium on charcoal (1 bar \(H_2\)), 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 amine of \(11a\) were removed through heating in refluxing methanol (Scheme 1).\(^{27c,29}\) 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-fluorenylmethoxy carbonyl (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 moieties were 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 (2R,5R)-configured diester 8b. In this sequence, a clean reduction of the amide functionality, removal of complexed borane, and subsequent Boc protection gave 14a. The exchange 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.

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)-1H-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.
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-amiPro-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 starting material, were prepared according to ref. 25b. The analytical data obtained were in agreement with those reported in the above starting material (% brsm).

For the synthesis of the (2R,5R)-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%, 97% brsm) as a white solid. R₉ = 0.7 (ethyl acetate) [UV, KMnO₄]; [α]D²⁴ = +108 (c 0.5, CHCl₃).

IR (NaCl): 2979, 2956, 2881, 1733, 1436, 1374, 1310, 1207, 1166, 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), 2.92 (dd, δ = 1.3 Hz, δ = 11.0 Hz, 1 H), 3.63 (d, δ = 7.3 Hz, 1 H), 3.57 (m, 1 H), 2.16–2.02 (m, 2 H), 1.88–1.80 (m, 1 H), 1.42–1.38 (m, 1 H), 0.83 (t, δ = 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.


Methyl (2S,5S)-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 monooester 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, KMnO_4]; [α]_D^24 +86.9 (c 0.5, CHCl_3).

HRMS (EI): m/z 277.1909.

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

1H NMR (600 MHz, CDCl_3): 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), 3.74 (s, 3 H), 2.81 (s, 3 H), 2.63–2.55 (m, 1 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), 0.91 (t, J = 7.1 Hz, 3 H).

13C NMR (151 MHz, CDCl_3): δ = 175.3, 173.3, 129.1, 128.1, 127.1, 63.9, 59.9, 53.9, 51.6, 36.8, 35.5, 28.4, 28.1.


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

Methyl (2R,5R)-5-(dimethylcarbamoyl)pyrrolidine-2-carboxylate (11b) For the synthesis of the (2R,5R)-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, KMnO_4]; [α]_D^24 +42.6 (c 0.5, CHCl_3).

IR (NaCl): 2952, 2850, 1733, 1390, 1291, 128.1, 127.1, 63.9, 59.9, 53.9, 51.6, 36.8, 35.5, 28.4, 28.1.


For the synthesis of the (2S,5R)-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]_D^24 +90.0 (c 0.5, CHCl_3).

Methyl (25S,5S)-1-Benzyl-5-[(dimethylcarbamoyl)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, KMnO_4]; [α]_D^24 −164.2 (c 0.5, CHCl_3).

IR (NaCl): 2948, 2817, 2764, 2364, 1723, 1454, 1195, 1159, 1036, 991, 845, 745, 700 cm⁻¹.

1H NMR (360 MHz, CDCl_3, 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, CDCl_3, 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.


The 1H NMR and 13C NMR data are in agreement with those previously reported for the racemic compound.23
Methyl (2R,5R)-1-Benzyl-5-[[dimethylamino)methyl]pyrrolidine-2-carboxylate (12b)

For the synthesis of the (2R,5R)-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.

\[ \delta_{13C} = 53.6, 48.3, 47.8, 46.1, 45.1, 43.8, 43.7, 29.2, 28.7, 28.0, 27.6. \]

IR (NaCl): 3059, 2961, 2891, 2713, 2607, 2501, 1699, 1451, 1415, 1391, 1376, 1202, 1162, 1140, 991, 766, 702, 612 cm\(^{-1}\).

HRMS (ESI): \[ m/z \text{ [M + H]}^+ \text{ calcd for C}_{27}H_{32}N_{2}O_{4}: 395.1965; \text{ found: 395.1961.} \]

HPLC-MS (ESI): Method A, \( t_\phi = 6.71 \text{ min, } m/z = 395 \text{ [M + H]}^+ \); chiral HPLC: Method A, \( t_\phi = 14.79 \text{ min} \).


For the synthesis of (2S,5S)-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.

\[ \delta_{13C} = 125.9, 125.7, 121.11, 121.08, 69.4, 67.0, 65.0, 61.0, 60.6, 60.4, 55.1, 53.6, 48.3, 47.8, 46.1, 45.1, 43.8, 43.7, 29.2, 28.7, 28.0, 27.6. \]

HRMS (ESI): \[ m/z \text{ [M + Na]}^+ \text{ calcd for C}_{15}H_{20}N_{2}NaO_{3}: 305.1010; \text{ found: 305.1000.} \]

HPLC-MS (ESI): Method A, \( t_\phi = 6.89 \text{ min, } m/z = 395 \text{ [M + Na]}^+ \); chiral HPLC: Method A, \( t_\phi = 25.93 \text{ min} \).

Methyl (25S,5S)-5-Carbamoyl-1-[(S)-1-phenylethyl]pyrrolidine-2-carboxylate (14a)

Diisopropylethylamine (11.5 mL, 8.71 g, 67.4 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 \text{ (ethyl acetate) [UV, KMnO}_4\]; [α]_{24}^{D} -129.1 \text{ (c 0.5, CHCl}_3\).}

IR (NaCl): 3458, 3235, 3179, 2992, 1720, 1684, 1668, 1454, 1436, 1391, 1376, 1202, 1162, 1140, 991, 766, 702, 612 cm\(^{-1}\).

\[ \text{H NMR (360 MHz, CDCl}_3\); \delta = 7.34–7.20 (m, 5 H), 7.06 (br s, 1 H), 5.73 (br s, 1 H), 4.09 (q, \text{J} = 6.7 \text{ Hz, 1 H}), 3.78 (dd, \text{J} = 1.9 \text{ Hz, J} = 11.0 \text{ Hz, 1 H}), 3.56 (d, \text{J} = 7.6 \text{ Hz, 1 H}), 3.54 (s, 3 H), 2.57 (dtt, \text{J} = 7.3 \text{ Hz, J} = 11.3 \text{ Hz, J} = 13.0 \text{ Hz, 1 H}), 2.08 (tt, \text{J} = 7.7 \text{ Hz, J} = 12.7 \text{ Hz, 1 H}), 1.96 (ddd, \text{J} = 2.0 \text{ Hz, J} = 7.8 \text{ Hz, J} = 12.8 \text{ Hz, 1 H}), 1.78 (dd, \text{J} = 7.6 \text{ Hz, J} = 12.8 \text{ Hz, 1 H}), 1.36 (d, \text{J} = 6.7 \text{ Hz, 3 H}). \]

\[ \text{13C NMR (91 MHz, CDCl}_3\); [α]_{24}^{D} = 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): \[ m/z \text{ [M + Na]}^+ \text{ calcd for C}_{18}H_{24}N_{2}NaO_{3}: 299.1366; \text{ found: 299.1369.} \]

Methyl (25S,5S)-1-Benzyl-5-carbamoylpyrrolidine-2-carboxylate (15a)

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. The mixture was stirred for 4.5 h at 30 °C. Trifluoroacetic acid (1.50 mL) and water (6 mL) 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 15a was obtained as a white solid which was used in the next step without further purification.

\[ \text{Mp 183.3 °C; [α]_{24}^{D} = 0.5 (acetonitrile + 1% TFA) [UV, ninhydrin]; [α]_{24}^{D} -129.1 (c 0.5, CHCl}_3\).} \]
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; \( R_3 = 0.3 \) (ethyl acetate) [UV, KMnO\(_4\)]; \( [\alpha]^{25}_{D} = 121.1 \) (c 0.5, CHCl\(_3\)).

IR (NaCl): 3437, 3183, 2945, 2883, 2362, 2333, 1733, 1679, 1453, 1413, 1384, 1365, 1251, 1165, 1125, 1087, 1035, 978, 958, 880, 841, 743, 699 cm\(^{-1}\).


\( \delta (\text{HRMS (ESI))}: 371.1939. \)

Methyl (25.55)-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 obtained residue was dissolved in tetrahydrofuran (50 mL) and treated with potassium carbonate (212 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 solution was removed under reduced pressure leading to a residue containing (25.55)-1-[[[9H-fluoren-9-yl]methoxycarbonyl]-5-[[([tert-butoxycarbonyl]amino)methyl]pyrrolidine-2-carboxylic acid (17a) (quant.)]. The reaction mixture was filtered over Celite\(^\text{\textregistered}\) and the filter cake was washed with ethyl acetate. The solvent was removed under reduced pressure and the residue was dissolved in methanol (12 mL), sodium hydroxide (2 M, 5.83 mmol) 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 (25.55)-1-[[[9H-fluoren-9-yl]methoxycarbonyl]-5-[[([tert-butoxycarbonyl]amino)methyl]pyrrolidine-2-carboxylic acid (17a) (72%). Subsequently, the previously kept combined aqueous phases (see above) were added and 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. 

\( R_3 = 0.3 \) (CH\(_2\)Cl\(_2\)/methanol, 19:1) [UV, ninhydrin].

| IR (KBr): 3545, 2976, 2365, 2306, 1693, 1529, 1450, 1364, 1326, 1242, 1154, 758, 753, 741 cm\(^{-1}\). |

HRMS (ESI): \( m/z \) [M + Na\(^+\)] calcd for C\(_{13}\)H\(_{17}\)Na\(_2\)O\(_4\): 371.1941; found: 371.1939. 

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\(^\text{\textregistered}\) and the filter cake was washed with ethyl acetate. The solvent was removed under reduced pressure and the resulting reaction mixture was quenched by adding sodium bicarbonate (5.72 g, 44 mmol) and aqueous hydrochloric acid (3 M). The resulting oil was extracted with diethyl ether (4 × 30 mL) and evaporated. The resulting residue was subjected to flash chromatography (silica gel, hexane/ethyl acetate = 9:1) to give the product (912 mg, 2.62 mmol, 75%) as a colorless oil.
HRMS (ESI): m/z [M + H]+ calcd for C_{20}H_{24}N_4O_6: 467.2177; found: 467.2175.

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

Supporting Information
Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1610304.

References
(6) Cheng, H. N.; Bovey, F. A. Biopolymers 1977, 16, 1465.


Funding Information
Deutsche Forschungsgemeinschaft HE5413/6-1

Acknowledgment
The authors are grateful for the support of this project by Karina Wicht, Laura Hofmann, Eva Gans, Jonas Ludwig, Jannis Beutel and Rebecca Hoffmann (FAU Erlangen-Nürnberg).


(30) The optical purity of the Boc-protected derivative 17a was determined by chiral HPLC using two different solvent systems.