Asymmetric Synthesis of a New Helix-Forming β-Amino Acid: \textit{trans}-4-Aminopiperidine-3-carboxylic Acid


Keywords: Asymmetric synthesis / Amino acids / Helical structures

We report a synthesis of a protected derivative of \textit{trans}-4-aminopiperidine-3-carboxylic acid (APiC). The route provides either enantiomer. All intermediates are purified by crystallization, and large-scale preparation is therefore possible. An analogous route provides either enantiomer of \textit{trans}-2-aminocyclohexanecarboxylic acid (ACHC). We have previously shown that β-peptide oligomers containing ACHC adopt a helical conformation defined by 14-membered C=O(i)−H−N(i + 2) hydrogen bonds ("14-helix"). Here we show that APiC residues can be incorporated into the 14-helix. (© Wiley-VCH Verlag GmbH & Co. KGaA, 69451 Weinheim, Germany, 2003)

Introduction

Unnatural oligomers with a strong tendency to adopt specific and predictable conformations in solution ("foldamers") have been the subject of extensive investigation in recent years.\cite{1} β-Amino acid oligomers ("β-peptides") have been particularly well studied in this regard as β-peptides can adopt several distinct folding patterns if residue substitution patterns are chosen appropriately.\cite{2} Constrained β-amino acid residues, such as those in which the Cα−Cβ bond is part of a small ring, lead to extremely stable conformations. We have shown, for example, that β-peptides containing as few as six residues adopt either of two helical conformations in aqueous solution depending on the type of residue constraint.\cite{3,4} A five-membered ring constraint [as in \textit{trans}-2-aminocyclopentanecarboxylic acid (ACPC)] gives rise to a 12-helix, defined by 12-membered ring C=O(i)−H−N(i + 3) hydrogen bonds,\cite{3} while a six-membered ring constraint [as in \textit{trans}-2-aminocyclohexanecarboxylic acid (ACHC)] leads to a 14-helix, defined by 14-membered ring C=O(i)−H−N(i + 2) hydrogen bonds.\cite{4} In contrast, conventional peptides (α-amino acid residues) do not form helices at such short lengths, nor do most β-peptides comprised of unconstrained β-amino acid residues.\cite{5}

Here we report a short and enantioselective synthesis of \textit{trans}-4-aminopiperidine-3-carboxylic acid (APiC), a new β-amino acid with a six-membered ring constraint. The route employs α-methylbenzylamine as a chiral auxiliary and nitrogen source, which allows one to generate either enantiomer of APiC. No chromatography is required; intermediates are purified by crystallization. Thus, this method is amenable to large-scale preparations. A similar synthetic route provides access to either enantiomer of ACHC, again without chromatography. We show that a hexa-β-peptide containing both ACHC and APiC forms a 14-helix in aqueous solution.

Results and Discussion

Synthesis

Our synthetic approach (Scheme 1) follows the strategy we have previously employed for protected β-amino acids with five-membered ring constraints, including ACPC\cite{6} and a pyrrolidine analogue.\cite{7} This approach was inspired by the asymmetric synthesis of \textit{cis}-2-aminocyclohexanecarboxylic acid reported by Wu et al.\cite{8} The route to Fmoc-APiC (Boc) \textit{(4)} starts with commercially available β-oxoester \textit{1}, which bears a benzyl group on the ring nitrogen atom. Hydrogenolytic removal of the benzyl group and reaction with di-tert-butyl dicarbonate provided the Boc-protected β-oxo ester \textit{1}, which was allowed to react with \textit{(R)(+)-α-methylbenzylamine}\cite{9} in refluxing benzene containing a catalytic amount of \textit{p}-toluenesulfonic acid to form enamine \textit{2}. The enamine was then allowed to react with NaBH₄ in isobutyric acid. \textit{1}H NMR analysis indicated that this reduction generated a 4:1 mixture of the \textit{cis} diastereomers of the expected β-amino ester. This mixture was treated with sodium ethoxide in ethanol, causing epimerization to the \textit{trans}-β-amino esters. \textit{1}H NMR analysis of the product mixture suggested a 2.5:1 preference for \textit{trans} \textit{vs. cis} diastereomers. A single \textit{trans} diastereomer was isolated from this mixture following a two-stage crystallization protocol. The crude diastereomeric mixture was dissolved in diethyl ether, and 4 N HCl in dioxane was added slowly. Adding hexanes and storing this solution at 0 °C led to the precipitation of

\[\text{trans}-4\text{-Amino Acid:}\]

a white crystalline solid. Recrystallization of the precipitated solid from acetonitrile provided the pure diastereomer 3 (≥ 99% de according to NMR analysis) in 16% yield from crude 2. The absolute configuration of 3 prepared using (S)-(-)-α-methylbenzylamine was established by removal of the Boc group and conversion into the bis(HCl) salt 5, for which a crystal structure was obtained (Figure 1). The desired Fmoc-β-amino acid 4, obtained from 3 by the final three steps indicated in Scheme 1, was crystallized with more than 99% ee, as demonstrated by chiral HPLC analysis of the corresponding dinitrophenyl derivative 6.

Figure 1. Ball-and-stick representation of the conformation determined crystallographically for 5 in the solid state (H atoms not shown)

An analogous route (Scheme 2) provided enantiomerically pure Fmoc-ACHC (11) from the commercially available β-oxo ester 7. Reduction of enamine 8 yielded predominantly the cis-β-amino esters, and the desired diastereomer 9 was isolated by crystallization of the HBr salt, recrystallization from acetonitrile, and a base wash.[8] Base-catalyzed epimerization provided a diastereomeric mixture with a 4:1 trans/cis ratio. Pure diastereomer 10 was obtained in greater than 99% de by a two-stage crystallization protocol; the overall yield from crude 8 was 25%. The absolute configuration of Fmoc-ACHC (11) obtained from (R)-(-)-α-methylbenzylamine was established as (R,R) by comparing the optical rotation with (R,R)-Fmoc-ACHC obtained by a route that involves an enzymatic desymmetrization.[10] The new route provides access to either enantiomer of Fmoc-ACHC, since either enantiomer of α-methylbenzylamine can be used. The enantiomeric excess of 11 was shown to be greater 99% by chiral HPLC analysis of the corresponding dinitrophenyl derivative 12. This synthetic protocol represents a substantial improvement in terms of efficiency relative to earlier routes to enantiomerically pure ACHC derivatives.[10,11]

Oligomer Conformational Analysis

The ability of the APiC residue to support 14-helix formation was evaluated by preparing a hexamer (13) in which (R,R)-ACHC and (R,R)-APiC residues alternate. Circular dichroism (CD) in the far-UV region has been used for low-resolution characterization of β-peptide secondary structure,[2b] and we applied this method to 13. Figure 2 compares the CD spectra of the homochiral hexa-β-peptide 13 in methanol and in aqueous buffer. Methanol strongly promotes helix formation in β-peptides constructed from flexible residues (no ring constraints), while folding in water is generally more difficult to achieve.[5a] The β-peptide 13 displays a maximum near 217 nm in both solvents, consistent with at least partial population of the 14-helical conformation. The maxima in these two solvents have very similar intensities, which suggests that the 14-helix is populated to similar extents in water and methanol. This similarity indicates that APiC is a strong promoter of 14-helicity in aqueous solution.
**Conclusion**

Cyclically constrained β-amino acids are very important building blocks for the creation of short β-peptides that adopt defined conformations in aqueous solution. Here we identify a new preorganized residue, APiC, that can confer aqueous solubility and we provide a short, practical and scalable synthetic route that leads to either enantiomer. In addition, we have extended this route to generate a practical synthesis of enantiomerically pure ACHC. (After our work was completed Berkessel et al. reported an alternative and very practical synthesis of ACHC, in either enantiomeric form.[12]) These building blocks should further the exploration of 14-helical β-peptides, and they should be useful for a variety of other applications.

Figure 2. Circular dichroism spectra of hexamer 13 in 10 mM aqueous Tris, pH = 7 (solid line) and in methanol (dashed line)

**Experimental Section**

**General Procedures:** Melting points were determined with a capillary melting point apparatus and are uncorrected. Optical rotations were measured using sodium light (D line, 589.3 nm). Benzene and toluene were distilled from sodium/benzophenone ketyl under N₂ and ethanol from sodium/diethyl phthalate. Unless otherwise noted, all other commercially available reagents and solvents were purchased from Aldrich and used without further purification, except for 4 N HCl in dioxane, which was purchased from Pierce, and Fmoc-OSu, which was purchased from Advanced ChemTech. Analytical thin-layer chromatography (TLC) was carried out on Whatman TLC plates precoated with silica gel 60 (250 μm layer thickness). Visualization was accomplished using either a UV lamp or phosphomolybdic acid (PMA) stain (10% phosphomolybdic acid in ethanol). Column chromatography was performed on EM Science silica gel 60 (230–400 mesh). Solvent mixtures used for TLC and column chromatography are reported in v/v ratios. Diastereomeric excesses were determined using 1H NMR spectroscopy. Enantiomeric excesses were determined using chiral HPLC.

**Enamine 2:** Pd/C (10%, 2.40 g) and ammonium formate (15.7 g, 249 mmol) were added to a clear solution of ethyl N-benzyl-4-oxo-piperidine-3-carboxylate hydrochloride (15.3 g, 51.4 mmol) in absolute ethanol (480 mL) under nitrogen. The mixture was refluxed for 1 h. The cooled solution was filtered through Celite and washed with ethanol. The filtrate was concentrated to obtain a white solid (9.85 g, 92%). This solid was taken up in chloroform (85 mL), and a solution of NaHCO₃ (4.17 g, 49.6 mmol) in water (80 mL) and NaCl (8.33 g, 143 mmol) were added. A solution of di-tert-butyl dicarbonate (10.4 g, 47.5 mmol) in chloroform (30 mL) was slowly added at room temperature (15 min), and the mixture was then refluxed for 15 h. The organic layer was separated, and the aqueous phase was extracted with chloroform (3 × 50 mL). The combined organic layers were dried with Na₂SO₄, and the solvent was evaporated to obtain a white solid (9.85 g, 92%). This solid was taken up in chloroform (85 mL), and a solution of NaHCO₃ (4.17 g, 49.6 mmol) in water (80 mL) and NaCl (8.33 g, 143 mmol) were added. A solution of di-tert-butyl dicarbonate (10.4 g, 47.5 mmol) in chloroform (30 mL) was slowly added at room temperature (15 min), and the mixture was then refluxed for 15 h. The organic layer was separated, and the aqueous phase was extracted with chloroform (3 × 50 mL). The combined organic layers were dried with Na₂SO₄, and the solvent was evaporated to obtain a yellow solid (R₂ = 0.29, hexane/ethyl acetate, 7:1). A stirred solution of this solid, (R)-(+)-(S)-α-methylbenzylamine (6.05 g, 49.9 mmol) and a catalytic amount of p-toluenesulfonic acid (451 mg, 2.37 mmol, 5 mol%) in 150 mL of dry benzene was
refluxed under nitrogen with continuous removal of water by using a Dean–Stark trap for 7 h. The cooled reaction mixture was washed twice with saturated aqueous NaHCO₃ (2 × 100 mL). The organic layer was dried with Na₂SO₄. After removal of the solvent, the yellow oily residue was filtered through a pad of silica gel (CH₂Cl₂, washing until the filtrate became colorless; a yellow color remained on the silica gel). The filtrate was concentrated to obtain 13.7 g of enamine 2 as a pale yellow oil (71% over three steps): Rᵣ = 0.31, hexane/ethyl acetate, 6:1. HNMR (300 MHz, CDCl₃): δ = 9.25 (d, J = 7.3 Hz, 1 H), 7.35–7.22 (m, 5 H), 4.60 (quint, J = 6.7 Hz, 1 H), 4.19 (q, J = 7.0 Hz, 2 H), 4.07 (s, 2 H), 3.46–3.38 (m, 1 H), 3.33–3.26 (m, 1 H), 2.43–2.35 (m, 1 H), 2.09–1.99 (m, 1 H), 1.50 (d, J = 6.7 Hz, 3 H), 1.43 (s, 9 H), 1.29 (t, J = 7.0 Hz, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 168.77 (C), 156.91 (C), 154.33 (C), 145.00 (C), 128.28 (CH), 126.85 (CH), 125.62 (CH), 88.35 (C), 79.37 (C), 58.73 (CH₃), 52.02 (CH), 41.21 (CH₃), 39.67 (CH₂), 28.16 (CH₃), 26.02 (CH₃), 24.98 (CH₃), 14.34 (CH₃) ppm. MS-ESI: m/z = 375.2 [M + H]⁺, 397.2 [M + Na⁺], 771.4 [2 M + Na⁺].

**HCl Salt 3:** Sodium borohydride (3.60 g, 95.2 mmol) was added portionwise under N₂ at 0 °C to isobutyric acid (60.0 mL, 647 mmol). This mixture was further stirred at room temperature for 0.5 h and then 10 mL of absolute toluene was added and the mixture was cooled to 0 °C. A solution of enamine 2 (11.8 g, 31.6 mmol) in dry toluene (40 mL) was added dropwise under N₂ at 0 °C for 1 h, then 10 mL of MeOH. The mixture was refluxed for 2 h. After the reaction was complete (disappearance of starting material, as monitored by TLC), the cooled solution was filtered through Celite, and the filtrate was concentrated in vacuo to give 2.07 g of 2. ¹H NMR of the corresponding free amine indicated the diastereomeric excess to be greater than 99%. M. p. 197–198 °C. [α]β₉ = +11.7 (c = 1.03, MeOH). ¹H NMR (300 MHz, CDCl₃): δ = 10.20 (br. s, 1 H), 9.92 (br. s, 1 H), 7.79–7.76 (m, 2 H), 7.44–7.40 (m, 3 H), 4.60 (br. s, 1 H), 4.27 (q, J = 7.2 Hz, 2 H), 3.87 (br. d, J = 11.8 Hz, 1 H), 3.28–3.19 (m, 2 H), 3.00–2.78 (m, 1 H), 2.54 (br. s, 1 H), 2.06–1.93 (m, 4 H), 1.74–1.60 (m, 1 H), 1.44–1.30 (m, 13 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 171.30 (C), 153.67 (C), 136.10 (C), 129.24 (CH), 129.02 (CH), 128.34 (CH), 80.05 (C), 61.63 (CH₃), 59.78 (CH), 54.86 (CH), 44.74 (CH), 44.12 (CH₂), 41.70 (CH₂), 28.56 (CH₃), 27.99 (CH₃), 20.46 (CH₃), 13.88 (CH₃) ppm. MS-ESI: m/z = 377.2 [M – Cl]⁺, 399.2 [M – HCl + Na⁺], 775.4 [2 M – HCl + Na⁺].

(R,R)-Fmoc-APIC(Boc) (4): Compound 3 (1.89 g, 4.58 mmol) was dissolved in THF/EtOH/H₂O, 2:1:1 (100 mL), and this clear solution was cooled to 0 °C. LiOH·H₂O (1.02 g, 24.3 mmol) was added in 10 mL of H₂O, and the mixture was stirred at 0 °C for 1 h. The solution was removed under reduced pressure to give a white solid (Rᵣ = 0.30, CH₂Cl₂/MeOH, 10:1). Pd/C (10%, 1.1 g) and ammonium formate (2.38 g, 37.7 mmol) were added under N₂ and ammonium formate (2.38 g, 37.7 mmol) was added under N₂ at room temperature to a turbid solution of this white solid in 200 mL of MeOH. The mixture was refluxed for 2 h. After the reaction was complete, the mixture was concentrated in vacuo to give a white solid. This solid was dissolved in acetone/H₂O, 2:1 (200 mL), cooled to 0 °C, and Fmoc-OSu (1.53 g, 4.53 mmol) was added under N₂ at room temperature overnight. The acetone was removed under reduced pressure. The aqueous residue was diluted with H₂O (50 mL), stirred for 1 h at room temperature with diethyl ether (200 mL), and the layers were separated. The organic phase was washed with saturated aqueous NaHCO₃ (3 × 100 mL). All the aqueous phases were combined, acidified with 1 N aqueous HCl, and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with MgSO₄ and concentrated under reduced pressure to give a white salt. The crude product was purified by crystallization from refluxing chloroform (100 mL) after careful addition of MeOH (ca. 3 mL) until all the solid had dissolved. For complete precipitation, hexane was added to the cold reaction mixture until the solution became turbid. After storage at 0 °C overnight, 1.92 g (90%) of 4 was obtained as a white solid. M. p. 202–203 °C. Rᵣ = 0.32, CH₂Cl₂/MeOH, 8:1. [α]β₉ = −4.9 (c = 0.51, MeOH). ¹H NMR (300 MHz, CDCl₃/CDOD): δ = 7.77 (d, J = 7.4 Hz, 2 H), 7.61 (d, J = 7.5 Hz, 2 H), 7.42–7.29 (m, 4 H), 4.36–4.34 (m, 2 H), 4.24–4.19 (m, 2 H), 4.06–4.02 (m, 1 H), 3.92–3.85 (m, 1 H), 3.03–2.85 (m, 2 H), 2.47–2.41 (m, 1 H), 2.01–1.97 (m, 1 H), 1.47 (s, 10 H) ppm. ¹³C NMR (75 MHz, CDCl₃/CDOD): δ = 173.44 (C), 156.04 (C), 154.46 (C), 143.49 (C), 140.95 (C), 127.33 (CH), 126.72 (CH), 124.68 (CH), 119.53 (CH), 108.25 (C), 66.35 (CH₃), 49.96 (CH), 47.26 (CH), 46.81 (CH), 44.59 (CH₃), 41.87 (CH₃), 30.87 (CH₃), 27.79 (CH₃) ppm. MS-ESI: m/z = 243.1 [M – Fmoc]⁻, 465.2 [M – H]⁻, 931.3 [2 M – H]⁻. (S,S)-Fmoc-APIC was prepared by starting with (S)-(−)-a-methylbenzylamine. [α]β₉ = +5.0 (c = 0.51, MeOH).

Chiral HPLC Assay of 6 and ent-6: Compound 4 was converted into 6 by esterification, Fmoc deprotection, and reaction with 2,4-dinitrofluorobenzene. Similarly, (S,S)-Fmoc-APIC was derivatized to obtain ent-6. Racemic 6 in hexanes-2-propanol, 9:1 (15 μL of a 1.0 mg/mL solution) was injected onto a CHIRALCEL OD col-

umn (4.6 × 250 mm; Daicel Chemical Ind., Ltd.). Elution with hexanes/2-propanol, 1:1 at 0.6 ml/min resulted in separation of the enantiomers (6: t_{f} = 19.7 min; ent-6: t_{f} = 32.4 min). Solutions of 6 and ent-6 (1.0 mg/mL) were similarly analyzed, and integration of peak areas showed greater than 99% ee for both enantiomers.

Enamine 8: A stirred solution of (R)-(-)-α-methylbenzylamine (8.01 g, 66.1 mmol), ethyl 2-oxocyclohexanecarboxylate (11.0 g, 64.7 mmol) and a catalytic amount of p-toluenesulfonic acid (5.3 mL, 25.8 mmol) was added dropwise with vigorous stirring. A voluminous white precipitate formed during the addition. The mixture was stored at 0 °C overnight for complete precipitation, the cooled solution was filtered through asbestos and washed with hexane/ethyl acetate, 4:1. The filtrate was concentrated in vacuo and the resulting yellow oily residue was fractionally distilled to give 15.4 g (87%) of 8 as a pale yellow oil. Bp. 150–155 °C, 0.6 Torr. R_{f} = 0.31, hexane/ethyl acetate, 20:1. 1^H NMR (300 MHz, CDCl₃): δ = 9.42 (d, J = 7.5 Hz, 1 H), 7.36–7.22 (m, 5 H), 4.64 (qunt, J = 7.2 Hz, 1 H), 4.18 (dq, J = 1.0 Hz, 7.2 Hz, 2 H), 2.93–2.25 (m, 3 H), 1.98–1.91 (m, 1 H), 1.54–1.47 (m, 1.7 H), 1.31 (t, J = 7.2 Hz, 3 H) ppm. 13C NMR (75 MHz, CDCl₃): δ = 174.62 (C), 146.70 (C), 136.59 (C), 128.93 (CH), 128.93 (CH), 128.40 (CH), 61.13 (CH₂), 59.44 (CH₂), 53.55 (CH), 44.71 (CH), 30.04 (CH₂), 25.67 (CH₂), 24.79 (CH₂), 23.46 (CH₂), 22.99 (CH₂), 14.53 (CH₃) ppm. MS-ESI: m/z = 276.1 [M + Na]⁺, 298.1 [M + Na]⁺, 573.2 [2 M + Na]⁺.

cis-β-Amino Ester 9: Sodium borohydride (2.45 g, 64.8 mmol) was added portionwise under N₂ at 0 °C to isobutyric acid (40.0 mL, 431 mmol). This mixture was further stirred at room temperature for 0.5 h and then cooled to 0 °C. A solution of 8 (5.89 g, 21.6 mmol) in dry toluene (24.0 mL) was added dropwise under N₂ at 0 °C. The mixture was stirred at 0 °C for 1 h and then another portion of sodium borohydride (0.50 g, 13.2 mmol) was added. The reaction mixture was stirred at 0 °C for another 2 h. When the reaction was complete, 100 mL of water was added carefully, and the reaction mixture was stirred at room temperature for 10 min. Afterwards, the mixture was brought to pH = 10 with 3 N NaOH and extracted with EtOAc (3 × 150 mL). The combined organic layers were dried with MgSO₄ and concentrated under reduced pressure. The resulting oil could be purified by recrystallization from acetonitrile. The cold solution was filtered through wool and cooled to 0 °C overnight. The resulting precipitate was isolated and washed three times with 10-mL portions of cold acetonitrile. The mother liquor and the washings were combined and reduced to about half volume to obtain a second crop. The combined crops were further dried under vacuum to give 3.44 g of 10 as a white crystalline solid (51% yield from 9). 1H NMR spectroscopy of the corresponding free amine indicated the diastereomeric excess to be greater than 99%. M.p. 211–212 °C. [α]_D = −37.5 (c = 1.05, MeOH). 1H NMR (300 MHz, CDCl₃): δ = 9.76 (br s, 1 H), 7.80–7.77 (m, 2 H), 7.47–7.38 (m, 3 H), 4.59 (q, J = 7.0 Hz, 1 H), 4.34–4.18 (m, 2 H), 3.19–3.06 (m, 2 H), 2.22–2.18 (m, 1 H), 1.93–1.60 (m, 8 H), 1.34–1.24 (m, 5 H), 1.03–0.90 (m, 1 H) ppm. 13C NMR (75 MHz, CDCl₃): δ = 174.10 (C), 136.59 (C), 128.93 (CH), 128.83 (CH), 128.40 (CH), 61.13 (CH₂), 59.44 (CH₂), 56.48 (CH), 45.87 (CH), 29.95 (CH₂), 29.95 (CH₂), 24.79 (CH₂), 23.91 (CH₂), 23.69 (CH₂), 20.48 (CH₃), 13.93 (CH₃) ppm. MS-ESI: m/z = 276.1 [M + Cl]⁺, 298.2 [M + HCl + Na]⁺.

(R,R)-Fmoc-ACHC (11): Compound 10 (2.80 g, 8.98 mmol) was dissolved in THF/MeOH/H₂O, 2:1:1 (100 mL), and the clear solution was cooled to 0 °C. LiOH·H₂O (1.89 g, 45.1 mmol), dissolved in 10 mL of H₂O, was added. The mixture was stirred at 0 °C for 36 h. The solvent was removed under reduced pressure to obtain a white solid (R = 0.31, CHCl₃/MeOH, 8:1). Pd/C (10% v/v) and ammonium formate (2.83 g, 44.9 mmol) were added under N₂ at room temperature to a turbid solution of this white solid in 200 mL of MeOH. The mixture was refluxed for 2 h. After the reaction was complete, the crude solution was filtered through Celite, and the filtrate was concentrated to obtain a solid white. This solid was dissolved in acetone/H₂O, 2:1 (100 mL), cooled to 0 °C, and Fmoc-OSu (3.03 g, 8.98 mmol) and NaHCO₃ (7.32 g, 87.1 mmol) were added. The turbid reaction mixture was stirred at 0 °C for 1 h and was then stirred at room temperature overnight. The acetone was removed under reduced pressure. The aqueous residue was diluted.
with H₂O (50 mL), stirred for 1 h at room temperature with diethyl ether (200 mL), and the layers were separated. The diethyl ether layer was washed with saturated aqueous NaHCO₃ (3 × 100 mL). The aqueous layers were combined, acidified with 1 N aqueous HCl, and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried with MgSO₄ and concentrated to give a white solid. This crude product was purified by crystallization from refluxing chloroform (300 mL) with careful addition of MeOH (ca. 10 mL) until all solid had dissolved. For complete precipitation, hexane was added to the cooled solution until the solution became turbid. After storage at 0 °C overnight, 2.52 g (77%) of 11 was obtained as a white solid. M.p. 206–207 °C. δRₛ = 0.44, CH₂Cl₂/MeOH, 10:1. [α]₁₂ = −36.4 (c = 0.50, acetone). ¹H NMR (300 MHz, CDCl₃/CD₃OD): δ = 7.77 (d, J = 7.3 Hz, 2 H), 7.62 (d, J = 7.0 Hz, 2 H), 7.42–7.29 (m, 4 H), 4.33–4.19 (m, 3 H), 3.73–3.67 (m, 1 H), 2.35–2.28 (m, 1 H), 2.02–1.98 (m, 2 H), 1.78–1.73 (m, 2 H), 1.63–1.51 (m, 1 H), 1.41–1.22 (m, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃/CD₃OD): δ = 180.61 (C), 160.16 (C), 147.69 (C), 147.53 (C), 144.92 (C), 131.26 (C), 130.67 (C), 128.69 (CH), 123.47 (CH), 70.23 (CH₂), 55.12 (CH), 52.45 (CH), 50.84 (CH), 36.27 (CH₂), 32.73 (CH₂), 28.35 (CH₃), 28.11 (CH₂) ppm. MS-ESI: m/z = 388.1 [M + Na⁺]⁺, 753.2 [2 M + Na⁺]⁺. (S,S)-Fmoc-ACHC was prepared by starting with (S)-(−)-α-methylbenzylamine. [α]₁₂° = +36.7 (c = 0.52, acetone).

Chiral HPLC Assay of 12 and ent-12: Compound 11 was converted into 12 by esterification, Fmoc deprotection, and reaction with 2,4-dinitrofluorobenzene. Similarly, (S,S)-Fmoc-ACHC was derivatized to obtain ent-12. Racemic 12 in hexanes/2-propanol, 9:1 (15 µL of a 1.0 mg/mL solution) was injected onto a CHIRALCEL OD column (4.6 × 250 mm; Daicel Chemical Ind., Ltd.). Elution with hexanes/2-propanol, 1:1 at 1.0 mL/min resulted in separation of the enantiomers (12: tᵣ = 26.0 min; ent-12: tᵣ = 29.3 min). Solutions of 12 and ent-12 (1.0 mg/mL) were similarly analyzed, and integration of peak areas showed greater than 99% ee for both enantiomers.

Synthesis and Purification of 13: β-Peptide 13 was synthesized from Fmoc-ACHC and Fmoc-APiC(Boc) on a 25-µmol scale by standard methods on Rink amide AM resin (Applied Biosystems), using a Synergy automated synthesizer (Applied Biosystems). Amino acid (3 equiv.), HBTrU (3 equiv.), and DIEA (6 equiv.) were used in each coupling cycle. Coupling cycles were 120 min in duration, and piperidine deprotection cycles were approximately 60 min in duration (actual deprotection time was regulated automatically by monitoring of the conductivity trace). Residues 3–6 were double coupled. The resin-bound β-peptide was cleaved from the solid support and deprotected simultaneously by using 2 mL of trifluoroacetic acid/H₂O, 19:5 and stirring for 4 h. β-Peptide 13 was precipitated by concentration of the deprotection solution followed by addition of a large excess of cold anhydrous diethyl ether; the precipitate was collected by centrifugation. The β-peptide was purified by reversed-phase HPLC on a C₁₈-silica semi-preparative column (5 µm; 10 mm × 250 mm; Vydac). The column was eluted with a gradient of acetonitrile in water (4–32%; 0.1% trifluoroacetic acid in each) at a flow rate of 3 mL/min. Purified 13 (15.5 mg, 51%) was shown to be greater than 96% homogeneous by HPLC on a C₁₈-silica reversed-phase analytical column (5 µm; 4 mm × 250 mm; Vydac), and its mass was confirmed by mass spectrometry (MALDI-TOF-MS: calcd. for C₁₀₂H₁₉₀N₁₀O₁₁ [M] 770.5, found 770.9 [M + H⁺], 792.9 [M + Na⁺], 808.9 [M + K⁺]). The β-peptide concentration for all experiments was determined from the weight of the lyophilized 13 calculated as the TFA salt (assuming association of one molecule of TFA per cationic residue).

CD Spectroscopy: Circular dichroism (CD) spectra were obtained with an Aviv instrument at 25 °C using a quartz cell with a 1-mm path length, between 190 and 260 nm. β-peptide 13 was analyzed at 0.01 mM in methanol or Tris buffer (10 mM, pH = 7.4). The data were normalized for β-peptide concentration and number of residues (i.e., the vertical axis in CD plots is mean residue ellipticity).

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