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# An Efficient Route to Either Enantiomer of Orthogonally Protected *trans*-3-Aminopyrrolidine-4-carboxylic Acid

Hee-Seung Lee, Paul R. LePlae, Emilie A. Porter, and Samuel H. Gellman\*

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706-1396

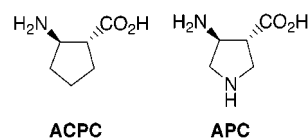
gellman@chem.wisc.edu

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Oligo- $\beta$ -amino acids (" $\beta$ -peptides") and other oligomers with discrete and predictable folding propensities ("foldamers") are subjects of increasing attention.<sup>1</sup> Recent work has established that short  $\beta$ -peptides ( $\leq$  six residues) containing conformationally restrained residues display well-defined conformations in aqueous solution.<sup>2</sup> In addition,  $\beta$ -peptides have been shown to display interesting biological activities.<sup>3</sup> Exploration of the structural and functional properties of  $\beta$ -peptides requires the availability of a wide range of enantiomerically pure  $\beta$ -amino acids. Despite the extensive effort that has been devoted to  $\beta$ -amino acid synthesis,<sup>4</sup> however, many substitution patterns, particularly those that provide conformational constraint, are not readily accessible.

We have shown that homooligomers of enantiomerically pure *trans*-2-aminocyclopentanecarboxylic acid (ACPC) form a helix defined by 12-membered ring hydrogen bonds between backbone amide groups ("12-helix").<sup>5</sup> This finding prompted us to seek ACPC analogues that bear an additional point of functionalization on the five-membered ring. *trans*-3-Aminopyrrolidine-4-carboxylic acid (APC) fulfills this need. We have recently shown that short  $\beta$ -peptides constructed from APC and ACPC adopt the 12-helical conformation in aqueous solution<sup>2b</sup> and that a 17-residue  $\beta$ -peptide composed of these two residues displays antimicrobial activity.<sup>3c</sup> Here

we report an improved synthesis of APC that is amenable to large-scale preparation.



The new route is summarized in Scheme 1. Our original route,<sup>2b</sup> like the new one, started from the known  $\beta$ -ketoester **1**.<sup>6</sup> In the original route, reduction followed by elimination produced the  $\alpha,\beta$ -unsaturated ester, and Michael addition of enantiomerically pure  $\alpha$ -methylbenzylamine then yielded a mixture of the four diastereomeric  $\beta$ -aminoesters. The desired isomer was isolated in 13% yield after tedious column chromatography.<sup>2b</sup> This original route was serviceable for initial evaluation of APC as a  $\beta$ -peptide building block, but this route was not efficient enough for thorough exploration of the structures and properties of APC-containing  $\beta$ -peptides.

The new route (Scheme 1) has been streamlined by reducing the number of chemical operations and by eliminating the need for chromatographic separations. Development of this route was inspired by an asymmetric synthesis of *cis*-2-aminocyclohexanecarboxylic acid reported by Xu et al.<sup>7</sup>  $\beta$ -Ketoester **1** is allowed to react with (*R*)- $\alpha$ -methylbenzylamine in the presence of acetic acid, and the resulting enamine is reduced in situ with NaBH<sub>3</sub>CN.<sup>8</sup> This reduction produces a mixture of four diastereomeric  $\beta$ -aminoesters in which **3** is the major product, and we have found a two-step crystallization protocol that allows isolation of hydrochloride salt **2** in diastereomerically pure form. The crude  $\beta$ -aminoester mixture is dissolved in ethyl acetate and converted to a mixture of hydrochloride salts by treatment with 4 N HCl in dioxane. A single *trans* isomer crystallizes in relatively pure form after this treatment ( $\geq 98\%$  *de*), although there is contamination from the other *trans* isomer. Recrystallization from acetonitrile yields a very pure form of **2** ( $\geq 99\%$  *de*) in 38% overall yield from **1**. When (*R*)- $\alpha$ -methylbenzylamine is used, the purified  $\beta$ -aminoester hydrochloride is spectroscopically identical to material previously identified by crystal structure determination as the diastereomer shown in Scheme 1. Thus, use of (*R*)- $\alpha$ -methylbenzylamine leads ultimately to a protected form of (3*S*,4*R*)-*trans*-3-aminopyrrolidine-4-carboxylic acid.<sup>9</sup>

The new route is completed by alkaline ester hydrolysis, hydrogenolytic removal of the  $\alpha$ -methylbenzyl group, and Fmoc protection of the resulting amino group. These three steps can be performed in rapid succession, and the

(1) (a) Seebach, D.; Matthews, J. L. *J. Chem. Soc., Chem. Commun.* **1997**, 2015. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (c) DeGrado, W. F.; Schneider, J. P.; Hamuro, Y. *J. Peptide Res.* **1999**, *54*, 206. (d) Gademann, K.; Hintermann, T.; Schreiber, J. V. *Curr. Med. Chem.* **1999**, *6*, 905. (e) Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530. (f) Stigers, K. D.; Soth, M. J.; Nowick, J. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 714. (g) Barron, A. E.; Zuckermann, R. N. *Curr. Opin. Chem. Biol.* **1999**, *3*, 681.

(2) (a) Appella, D. H.; Barchi, J. J.; Durell, S.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309. (b) Wang, X.; Espinosa, J. F.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 4821.

(3) (a) Werder, M.; Hausre, H.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1999**, *82*, 1774. (b) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1999**, *121*, 12200. (c) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565.

(4) For recent literature on  $\beta$ -amino acid synthesis, see: Juaristi, E.; López-Ruiz, H. *Curr. Med. Chem.* **1999**, *6*, 983. Juaristi, E. *Enantioselective synthesis of  $\beta$ -amino acids*; Wiley-VCH: New York, 1997. Davis, F. A.; Reddy, G. V.; Liang, C.-H. *Tetrahedron Lett.* **1997**, *38*, 5139. Ishitani, H.; Ueno, M.; Kobayashi, S. *J. Am. Chem. Soc.* **1997**, *119*, 7153. Sibi, M. P.; Shay, J. J.; Liu, M.; Jasperse, C. P. *J. Am. Chem. Soc.* **1998**, *120*, 6615. Tang, T. P.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 12. Zhu, G.; Chen, Z.; Zhang, X. *J. Org. Chem.* **1999**, *64*, 6907. Dexter, C. S.; Jackson, R. F. W. *J. Org. Chem.* **1999**, *64*, 7579.

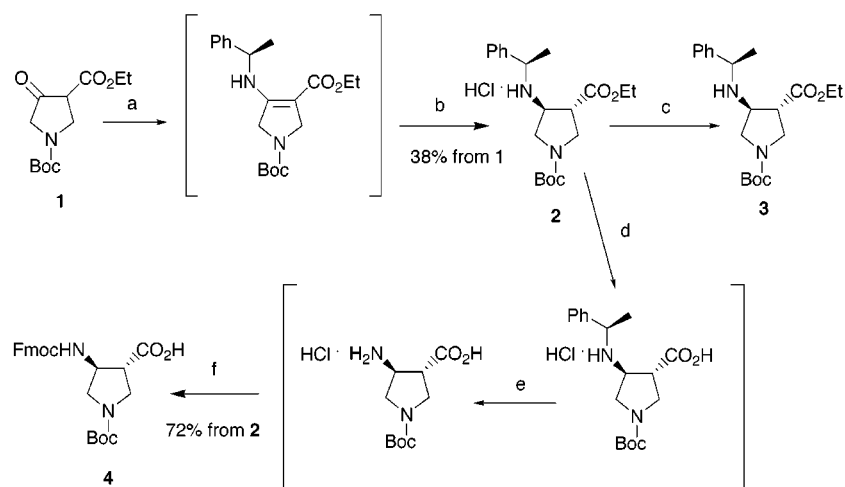
(5) (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Huang, X.; Barchi, J. J.; Powell, D. R.; Gellman, S. H. *Nature* **1997**, *387*, 381. (b) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 7574. (c) Barchi, J. J.; Huang, X.; Appella, D. H.; Christianson, L. A.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* **2000**, *122*, 2711.

(6) Blake, J.; Willson, C. D.; Rapoport, H. *J. Am. Chem. Soc.* **1964**, *86*, 5293.

(7) Xu, D.; Prasad, K.; Repic, O.; Blacklock, T. J. *Tetrahedron: Asymmetry* **1997**, *8*, 1445. For thorough reviews on the use of  $\alpha$ -methylbenzylamine in the preparation of enantiomerically pure molecules, see: Juaristi, E.; Escalante, J.; León-Romo, J. L.; Reyes, A. *Tetrahedron: Asymmetry* **1998**, *9*, 715. Juaristi, E.; León-Romo, J. L.; Reyes, A.; Escalante, J. *Tetrahedron: Asymmetry* **1999**, *10*, 2441.

(8) Attempts to reduce the enamine with NaBH<sub>4</sub> were unsuccessful. Reduction of a related enamine with NaB(OAc)<sub>3</sub>H provides predominantly the *cis*  $\beta$ -amino ester: Cimarelli, C.; Palmieri, G. *J. Org. Chem.* **1996**, *61*, 5557.

(9) The stereochemical nomenclature was ambiguous in our previous description of APC synthesis (ref 2b), but the structures drawn in that paper are stereochemically accurate.

Scheme 1<sup>a</sup>

<sup>a</sup> Reagents and conditions: (a) (*R*)-(+)- $\alpha$ -methylbenzylamine, AcOH, EtOH, rt; (b) NaBH<sub>3</sub>CN, 75 °C and then 4 N HCl in dioxane, EtOAc, 0 °C; (c) saturated Na<sub>2</sub>CO<sub>3</sub>, EtOAc; (d) LiOH·H<sub>2</sub>O, THF/CH<sub>3</sub>OH/H<sub>2</sub>O (6/3/1), 0 °C and then acidic workup; (e) H<sub>2</sub> (45 psi), 10% Pd-C, 95% EtOH; (f) Fmoc-OSu, NaHCO<sub>3</sub>, acetone/H<sub>2</sub>O (2/1), 0 °C to rt.

final product can be purified by crystallization from *n*-heptane/ethyl acetate. The protection pattern of di-amino acid derivative **4** is suitable for Fmoc-based synthesis of the  $\beta$ -peptide backbone on a solid support, with deprotection of the pyrrolidine ring nitrogens upon acidolytic cleavage from the resin. The synthetic route outlined in Scheme 1 is amenable to multigram synthesis of  $\beta$ -peptide building block **4** from **1** in 1 week. We are currently exploring the attachment of functionally diverse side chains to the pyrrolidine nitrogen atom.

## Experimental Section

**General Procedures.** Melting points were determined on a capillary melting point apparatus and were uncorrected. Optical rotations were measured using sodium light (D line, 589.3 nm). THF was distilled from sodium benzophenone ketyl under N<sub>2</sub>. 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  $\mu$ m layer thickness). Visualization was accomplished using either a UV lamp, potassium permanganate stain (2 g of KMnO<sub>4</sub>, 13.3 g of K<sub>2</sub>CO<sub>3</sub>, 3.3 mL of 5% (w/w) NaOH, 200 mL of H<sub>2</sub>O), 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.

**(3*S*,4*R*)-1-(*N*-tert-Butoxycarbonyl)-3-[(1*R*)-phenylethylamino]-4-ethoxycarbonylpyrrolidine Hydrochloride (**2**).** To a stirred solution of  $\beta$ -ketoester **1**<sup>6</sup> (16.0 g, 62.3 mmol) in absolute ethanol (250 mL) under N<sub>2</sub> were added (*R*)-(+)- $\alpha$ -methylbenzylamine (16.0 mL, 124.5 mmol) and glacial acetic acid (7.1 mL, 124.5 mmol) to obtain a cloudy solution. The reaction mixture was stirred at room temperature until the formation of the enamine was complete (3 h, monitored by TLC, product *R*<sub>f</sub> 0.55, 7:3 hexane/ethyl acetate). Sodium cyanoborohydride (16.5 g, 249.2 mmol) was then added to the reaction mixture at room temperature, and the resulting solution was heated to 75 °C and stirred for 14 h under N<sub>2</sub>. (This reaction must be carefully monitored by TLC; disappearance of the enamine indicates completion of reaction. Higher temperature and/or longer reaction time leads to formation of an  $\alpha,\beta$ -unsaturated ester side product.) The ethanol was removed via rotary evaporation in a well-ventilated hood (CAUTION-possible HCN evolution!). Water (250 mL) was added. The mixture was extracted three

times with diethyl ether. The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub>, and concentrated to give a colorless oil. The oil was applied to a plug of silica gel and washed with 2:1 hexane/ethyl acetate. The filtrate was concentrated to obtain a colorless oil. The oil was dissolved in ethyl acetate (250 mL), and 4 N HCl in dioxane (15.6 mL) was added dropwise at room temperature. The resulting solution was cooled to 0 °C and allowed to stand for 3 h at 0 °C. A precipitate formed during this time. The solid was filtered and washed two times with 100 mL portions of ethyl acetate to provide the desired material in 42% crude yield ( $\geq 98\%$  *de*) from **1**. The diastereomeric excess was determined by GC-MS (0.25 mm  $\times$  12.0 m Supelco SPB-1 fused silica capillary column; temperature gradient = 150 °C, 2 min; 150–220 °C, 10 °C/min; 220–250 °C, 0.9 °C/min; 250–280 °C, 10 °C/min; 280 °C, 1 min; injector temperature = 280 °C; detector temperature = 300 °C; flow rate = 1.2 mL/min); the (3*S*,4*R*)-trans isomer had a retention time of 13.3 min, and the (3*R*,4*S*)-trans isomer had a retention time of 13.0 min). This crude product could be purified by recrystallization from acetonitrile. The solid was suspended in acetonitrile (200 mL) and heated to reflux for 1 h. The mixture was then cooled to 0 °C for 3 h. The resulting precipitate was isolated by filtration and washed two times with 30 mL portions of acetonitrile. The solid was further dried under vacuum to give 9.4 g of **2** as a white crystalline solid ( $\geq 99.0\%$  *de*, 38% yield from **1**): mp 190–191 °C;  $[\alpha]_D^{23} = 4.8$  (*c* 1.05, MeOH); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz, 24 °C)  $\delta$  10.12–9.75 (br, 2H), 7.70–7.28 (m, 5H), 4.54 (m, 1H), 4.05 (q, *J*<sub>HH</sub> = 7.2 Hz, 2H), 3.80–3.59 (m, 3H), 3.54–3.22 (m, 3H), 1.62 (d, *J*<sub>HH</sub> = 6.3 Hz, 3H), 1.37 (s, 9H), 1.13 (t, *J*<sub>HH</sub> = 7.2 Hz, 3H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz, 60 °C)  $\delta$  10.22 (br s, 2H), 7.66–7.65 (m, 2H), 7.42–7.83 (m, 3H), 4.48 (m, 1H), 4.05 (q, *J*<sub>HH</sub> = 7.0 Hz, 2H), 3.78–3.68 (m, 3H), 3.54–3.35 (m, 3H), 1.67 (d, *J*<sub>HH</sub> = 7.0 Hz, 3H), 1.36 (s, 9H), 1.13 (t, *J*<sub>HH</sub> = 7.0 Hz, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125.7 MHz, 60 °C)  $\delta$  170.20, 152.70, 136.54, 128.68, 128.58, 127.90, 78.95, 60.92, 56.61, 56.00, 47.86, 46.69, 44.70, 27.82, 19.76, 13.50.

**(3*S*,4*R*)-1-(*N*-tert-Butoxycarbonyl)-3-[(1*R*)-phenylethylamino]-4-ethoxycarbonylpyrrolidine (**3**).** A sample of compound **2** was mixed with excess saturated Na<sub>2</sub>CO<sub>3</sub> solution, extracted into ethyl acetate, dried over MgSO<sub>4</sub>, and concentrated in vacuo: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.34–7.16 (m, 5H), 4.22–4.07 (m, 2H), 3.80 (q, *J*<sub>HH</sub> = 6.0 Hz, 1H), 3.71–3.53 (m, 1H), 3.53–3.22 (m, 3H), 2.94–2.78 (m, 2H), 1.39 (d, rotamer, 9H), 1.32 (d, *J*<sub>HH</sub> = 6.3 Hz, 3H), 1.29–1.18 (m, 3H); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz, 60 °C)  $\delta$  7.35–7.25 (m, 3H), 7.22–7.18 (m, 2H), 4.12–4.05 (m, 2H), 3.78 (q, *J*<sub>HH</sub> = 7.0 Hz, 1H), 3.54 (m, 1H), 3.36 (m, 1H), 3.24 (m, 2H), 3.00–2.90 (m, 2H), 1.36 (s, 9H), 1.24 (d, *J*<sub>HH</sub> = 6.5 Hz, 3H), 1.54 (t, *J*<sub>HH</sub> = 7.0 Hz, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125.7 MHz, 24 °C)  $\delta$  172.57, 172.43, 153.25, 153.14, 145.90, 128.11, 126.60, 126.51, 78.31, 60.28, 58.84, 58.02, 55.61,

51.39, 51.17, 48.53, 47.79, 46.70, 39.33, 24.56, 13.86;  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 125.7 MHz, 60 °C)  $\delta$  172.09, 153.02, 145.72, 127.79, 126.27, 126.21, 78.06, 59.94, 58.43, 55.50, 51.19, 48.28, 46.52, 27.83, 24.12, 13.57; MS-MALDI  $m/z$  363.3 (M + H).

**(3*S*,4*R*)-1-(*N*-*tert*-Butoxycarbonyl)-3-(9*H*-fluoren-9-yl-methoxycarbonylamino)-4-hydroxycarbonylpyrrolidine (4).** Compound **2** (1.39 g, 3.49 mmol) was dissolved in THF/MeOH/H<sub>2</sub>O (6/3/1, v/v/v, 40 mL), and the solution was cooled to 0 °C. LiOH·H<sub>2</sub>O (732 mg, 17.4 mmol) was added. The mixture was stirred at 0 °C for 3 h. Aqueous HCl (1 N, 18 mL) was added at 0 °C. The solvent was then removed on a vacuum rotary evaporator to give a white solid ( $R_f$  0.32, 1:9 MeOH/CH<sub>2</sub>Cl<sub>2</sub>). The white solid was dissolved in 150 mL of 95% ethanol in a hydrogenation flask. Pd-C (10%, 1.1 g) was added. The resulting mixture was shaken under H<sub>2</sub> (45 psi) for 24 h. After the reaction was complete (disappearance of starting material, as monitored by TLC), the mixture was filtered through Celite, and the filtrate was concentrated to obtain a white solid. This solid was dissolved in acetone/H<sub>2</sub>O (2/1, v/v, 150 mL) and cooled to 0 °C, and Fmoc-Osu (1.53 g, 4.54 mmol) and NaHCO<sub>3</sub> (2.93 g, 34.9 mmol) were added. The reaction mixture was stirred at 0 °C for 1 h and then allowed to stir at room temperature overnight. Water (50 mL) was added. The acetone was removed under reduced pressure. The aqueous layer was stirred for 1 h with diethyl ether (50 mL), the layers were separated, and the aqueous layer was acidified with 1 N aqueous HCl, extracted with ethyl acetate, dried over MgSO<sub>4</sub>, and concentrated to give a foamy solid. The crude product was purified by crystallization from *n*-heptane/ethyl

acetate to afford 1.13 g (72%) of **4** as a white solid: mp 113–115 °C;  $[\alpha]^{23}_D = -18.3$  (*c* 1.2, MeOH);  $^1\text{H}$  NMR (CD<sub>3</sub>OD, 300 MHz)  $\delta$  7.77 (d,  $J_{\text{HH}} = 7.2$  Hz, 2H), 7.62 (d,  $J_{\text{HH}} = 7.5$  Hz, 2H), 7.37 (t,  $J_{\text{HH}} = 7.2$  Hz, 2H), 7.28 (t,  $J_{\text{HH}} = 7.2$  Hz, 2H), 4.48–4.27 (m, 3H), 4.18 (t,  $J_{\text{HH}} = 7.2$  Hz, 1H), 3.72–3.46 (m, 3H), 3.14 (m, 1H), 2.88 (m, 1H), 1.44 (s, 9H);  $^{13}\text{C}$  NMR (CD<sub>3</sub>OD, 75.4 MHz, 24 °C)  $\delta$  174.63, 158.13, 156.02, 145.24, 145.16, 142.56, 128.74, 128.11, 126.17, 126.10, 120.90, 81.29, 67.77, 54.73, 54.06, 51.73, 51.20, 28.70;  $^{13}\text{C}$  NMR (CD<sub>3</sub>OD, 125.7 MHz, 50 °C)  $\delta$  158.20, 156.11, 145.28, 145.22, 142.56, 128.71, 128.09, 126.06, 120.87, 81.12, 67.81, 55.10, 51.61, 28.77; MS-MALDI  $m/z$  475.3 (M + Na), 491.2 (M + K).

The enantiomer of **4** was prepared by starting with (*S*)-(-)- $\alpha$ -methylbenzylamine:  $[\alpha]^{23}_D = +18.3$  (*c* 1.2, MeOH).

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**Supporting Information Available:**  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and mass spectrometry data for **2–4**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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