4-Aminopiperidine-4-carboxylic Acid: A Cyclic α,α-Disubstituted Amino Acid for Preparation of Water-Soluble Highly Helical Peptides

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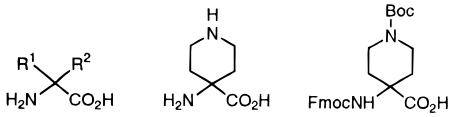
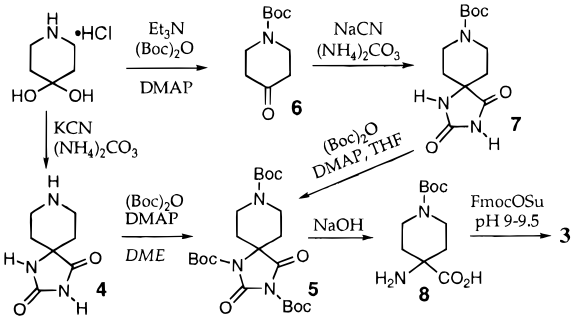
α,α-Disubstituted amino acids (ααAAs) (**1**, R^1, R^2 = alkyl; ≠ H) are now popular replacements for proteinogenic amino acids in peptides because of their strong secondary structure-promoting effects[2] and the increased proteolytic stability of the resulting peptides.[3] The prototypical ααAA, α-aminoisobutyric acid (Aib or α-Me-Ala, **1**, R^1, $R^2 = CH_3$), first found naturally in fungal peptides,[4] is the most widely studied.[5] Many other ααAAs[6] have been incorporated and studied in peptides; however, all these ααAAs have been hydrophobic. As a result, structural investigations of peptides with large percentages of ααAAs have been limited to the solid-state or spectroscopic studies (CD, NMR, IR) in organic solution.[5−7]



We are interested in water-soluble peptides containing high percentages of ααAAs both for their potential bioactivity[8] and as molecular design tools and catalysts. While several groups have synthesized polar ααAAs,[9] to date there has been no report of their incorporation into peptides or the preparation of protected derivatives suitable for peptide synthesis. We have designed an achiral "cationic" ααAA, 4-aminopiperidine-4-carboxylic acid (**2**, H-Pip-OH),[10] which has a γ-nitrogen that will be protonated under the usual conditions of peptide structure studies (pH < 9). We report an efficient synthesis of N^{α}-Fmoc-N^{γ}-Boc-protected 4-aminopiperidine-4-carboxylic acid [Fmoc-Pip(Boc)-OH, **3**] and the incorporation of Pip residues into peptides using derivative **3** and Fmoc solid-phase synthesis methods.

The synthesis of **3** (Scheme 1) starts with formation of hydantoin **4** from 4-piperidone by a Bucherer−Bergs

Scheme 1



procedure.[11] To obtain a protected derivative suitable for solid-phase Fmoc strategy, we envisioned protecting all the nitrogen functionalities of hydantoin **4** with Boc groups and then unmasking the α-amino and α-acid moieties by mild treatment with hydroxide,[12] which would leave the N^{γ}-position still protected with a Boc group. This strategy avoids separate steps such as copper complex formation[13] often required to differentially protect the α-amine and side-chain groups in trifunctional amino acids like lysine. Early attempts to form the tris-Boc hydantoin **5** in THF were unsuccessful because of the low solubility of hydantoin **4**. Other solvents such as DMF or DMSO required heating to initiate reaction, which led to low yields of colored products. As an interim solution, piperidone was protected with a Boc group (**6**) and converted to the N^{γ}-Boc-hydantoin **7**, which has greatly improved solubility and is easily converted to tris-Boc hydantoin **5** quantitatively in THF (40% yield from 4-piperidone). Ultimately, we found conditions that allowed direct conversion of hydantoin **4** to tris-Boc hydantoin **5** using 1,2-dimethoxyethane (DME) as solvent at room temperature producing **5** in excellent yield (72% from 4-piperidone).

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(14) While Rebek and co-workers (ref 12) have clearly demonstrated the usefulness of the mild basic hydrolysis of di-Boc-hydantoins for preparation of amino acids with sensitive side-chain protection, no mechanism for this cleavage has been proposed. The essentially quantitative formation of Boc_2NH, *not tert*-butylcarbamate ($BocNH_2$), has profound implications for this highly useful reaction.
(15) Compound **3** was prepared from isolated **8** by several different methods in 70−80% yield (for example, see ref 8), but the one-pot procedure conversion of **5** to **8** to **3** was faster and gave analytically pure product directly (no chromatographic purifications required).

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To reveal the amino acid functionality, fully protected **5** is treated with hydroxide to produce N^{γ}-Boc-4-aminopiperidine-4-carboxylic acid (**8**) with concomitant formation of di-*tert*-butyl imidodicarbonate (Boc_2NH).[14] Isolated yields of N^{γ}-Boc-protected **8** were often low and contaminated with salts, so a one-pot procedure for conversion of tri-Boc hydantoin **5** to **3** was developed. Thus, the above cleavage reaction containing **8** is extracted with ether to remove Boc_2NH and then Fmoc-OSu was added along with NaOH to keep the pH ~9, giving **3** in 63% overall yield from 4-piperidone.[15,16]

With Fmoc-Pip(Boc)-OH (**3**) in hand, we prepared three peptides by automated solid-phase Fmoc chemistry on PAL-PEG-PS support:[17] H-Tyr-Pip-Aib-Phe-Leu-NH_2 (**9**) and the two sequence permutation isomers H-Aib-Aib-Pip-Lys-Aib-Aib-Pip-Lys-Aib-Aib-NH_2 (**10**) and H-Pip-Aib-Aib-Lys-Aib-Aib-Lys-Aib-Aib-Pip-NH_2 (**11**). Peptide **9**, a Pip analog of a test peptide used by Carpino,[18] was synthesized with HATU *in situ* activation.[18] Crude peptide **9** was 80% pure by reversed-phase HPLC analysis. Peptides **10** and **11** were prepared in automated fashion using preformed acid fluorides.[19] This method worked well for peptide **10**,[8] producing material that was 36% pure by reversed-phase HPLC; however, the synthesis of peptide **11** failed to give full-length product. This was traced to problems with the initial three *C*-terminal ααAA couplings, perhaps due to steric crowding around the PAL-linker/resin attachment point. This problem was alleviated by coupling the first three amino acid fluorides in *refluxing* CH_2Cl_2 for extended periods. The rest of the synthesis went smoothly with standard automated acid fluoride couplings, giving a crude peptide **11** that was 78% pure by HPLC. All three peptides **9**−**11** were readily purified to homogeneity by preparative HPLC.

Peptides **9**−**11** were readily soluble in water without any organic modifier present. Circular dichroism (CD) studies of the 10-mer peptides **10** and **11** in the presence of SDS micelles showed very high helicity. Peptide **10**, recently reported to have activity against intracellular pathogens,[8] showed a CD indicative of 43% α-helix,[8] consistent with its amphipathic design. Peptide **11**, designed to be an amphipathic 3_{10}-helix, also showed a highly helical structure in the presence of SDS micelles, but in contrast had a unique CD spectum with a strong negative ellipticity at 206 nm (−5000 θ) and a low 222/206 intensity ratio (0.32), which is strongly indicative of a 3_{10}-helix.[7b] Detailed studies of solvent effects on the solution structures of peptides **10** and **11** and related sequences will be reported elsewhere.[20]

We have reported the first suitably protected "polar" ααAA derivative, Fmoc-Pip(Boc)-OH (**3**), that can be readily incorporated into peptides. Access to polar peptides with large percentages of ααAAs should provide fertile ground for the continuing debate of the relative stabilities of 3_{10}- and α-helices in aqueous media.[21]

Acknowledgment. We are grateful to Dr. Atsuo Kuki for helpful discussions at the early stages of this work. We thank the National Science Foundation, the Lousiana Education Quality Support Fund (LEQSF) and the National Institutes of Health for support of this research via an NSF/EPSCoR grant [(RII/EPSCoR)-LEQSF(1992−1996)-ADP-01], an NSF grant (CHE-9500992, to R.P.H.), and an NIH grant (GM 42101, to Prof. Mary D. Barkley and M.L.M.). We are grateful also to LEQSF for a Board of Regents Fellowship to C.L.W. and to the NSF REU Program (CHE-9424021) for a summer stipend to R.L.G.

Supporting Information Available: Futher details on the synthesis and characterization of **3**−**11** (6 pages).

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(16) **Piperidine-4-spiro-5′-hydantoin (4).** A solution of NaCN (17.0 g, 347 mmol) in H_2O (50 mL) added dropwise over 5 min to a solution of 4-piperidone monohydrate hydrochloride (25.0 g, 163 mmol) and $(NH_4)_2CO_3$ (chunks, 34.5 g, 359 mmol) in H_2O (90 mL) and CH_3OH (110 mL). An off-white precipitate began to form soon after addition was complete. The reaction flask was sealed and the suspension stirred at room temperature for an additional 2 days. The resultant light yellow precipitate was isolated by filtration and washed with small portions of H_2O until almost pure white: yield 24.0 g (87%); mp >300 °C. A second crop was obtained from the filtrate by evaporation of most of the solvent and dilution with H_2O (100 mL), filtration, and again repeated washings with H_2O: yield 1.5 g, mp >300 °C. Overall yield: 25.5 g (93%). ^{1}H NMR (250 MHz, CD_3SOCD_3) δ 10.75 (bs, 1H), 8.46 (s, 1H), 2.83 (app dt, $J \approx 13$, 4 Hz, 2H), 2.67 (app dt, $J \approx 12$, 2 Hz, 2H), 1.67 (app dt, $J \approx 12$, 4 Hz, 2H), 1.39−1.34 (m, 2H). **1-Boc-piperidine-4-spiro-5′-(1′,3′-bis-Boc)hydantoin (5).** In a flask fitted with an oil bubbler, piperidine hydantoin (**4**) (15.3 g, 90.5 mmol) was suspended in DME (450 mL), and Boc_2O (102 g, 468 mmol), DMAP (0.22 g, 1.64 mmol), and Et_3N (12.8 mL, 91.9 mmol) were added in succession. CO_2 evolution was vigorous at the initial addition of DMAP and continued at a steady pace (1 bubble/20−40 s). After 3 h, an additional portion of DMAP (0.2 g, 1.64 mmol) was added and the reaction mixture stirred for an additional 18 h. The mixture was concentrated under reduced pressure to yield a solid that was taken up in CH_2Cl_2 (500 mL), washed with 1 N HCl (2 × 75 mL), saturated aqueous Na_2CO_3 (1 × 100 mL), and brine (1 × 100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to yield a light creamy white solid (42.4 g, quantitative): mp 186−190 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.18−4.03 (dd, 1H), 3.38 (bt, 1H), 2.67 (dt, J = 5.3, 13.1 Hz, 1H), 1.77−1.71 (d, 1H), 1.57 (s, 9H), 1.52 (s, 9H), 1.46 (s, 9H). **1-Boc-piperidine-4-Fmoc-amino-4-carboxylic Acid (3).** 1 N NaOH (287 mL, 287 mmol) was added all at once to a suspension of tri-Boc-hydantoin **5** (15.0 g, 32.0 mmol) in DME (200 mL), resulting in a homogeneous solution. After 26 h, the resulting light yellow solution was extracted with Et_2O (3 × 75 mL) to remove Boc_2NH. The aqueous layer containing 4-amino-1-Boc-piperidine-4-carboxylic acid (**8**) from above was cooled in an ice bath and the pH adjusted to 9.5 with 12 N HCl. This precooled solution was added dropwise to a chilled mixture (ice bath) of Fmoc-OSu (16.0 g, 47.4 mmol) in DME (40 mL). A precipitate formed immediately, and the reaction mixture was allowed to warm to room temperature, keeping the pH at 9.0−9.5 by addition of 1 N NaOH; total reaction time 18 h. The DME was removed *in vacuo* (<40 °C), and the resultant aqueous layer was extracted with Et_2O (2 × 50 mL) to remove unreacted Fmoc-OSu. The aqueous fraction was chilled in an ice bath and adjusted to pH 4 with 12 N HCl and extracted with EtOAc (4 × 250 mL). The combined EtOAc layers were washed with 1 N HCl (100 mL) and brine (100 mL) and dried (Na_2SO_4) and the solvent removed *in vacuo* to yield a light yellow powder (10.8 g, 87%): mp 80−82 °C; ^{1}H NMR (200 MHz, CD_3SOCD_3) δ 8.30 (s, 1H), 7.85−7.96 (d, 2H), 7.70−7.80 (d, 2H), 7.22−7.54 (m, 4H), 4.19−4.32 (m, 3H), 3.55−3.76 (m, 2H), 2.90−3.09 (m, 2H), 1.91−2.15 (m, 2H), 1.65−1.89 (m, 2H), d 1.39 (s, 9H).

(17) **Peptide Synthesis.** Solid-phase peptide syntheses were performed on a PerSeptive Biosystems 9050 using Fmoc-PAL-PEG-PS resin (PerSeptive Biosystems, 0.15 mmol/g loading). Peptide **9** was prepared exactly according to the HATU protocol described by Carpino (ref 18) on a 0.1 mmol scale (660 mg resin) using HATU (0.4 mmol, 4 equiv), Fmoc-amino acid (0.4 mmol, 4 equiv), and DIEA (0.8 mmol, 8 equiv) in DMF (1.5 mL, 0.27 M amino acid). Syntheses of peptides **10** and **11** were performed using Fmoc-amino acid fluorides (prepared according to ref 19a) as suggested by Carpino (ref 19b) with protocols, cleavage and purification as described by us (ref 18), with the exception that deblocking was performed with DBU−piperidine−DMF (2:20:80; 1 × 1 min, 1 × 10 min). Also, in the case of H-Pip-Aib-Aib-Lys-Aib-Aib-Lys-Aib-Aib-Pip-NH_2 (**11**), the first three acid fluorides (1.6 mmol, 8 equiv) with DIEA (0.56 mL, 3.2 mmol, 2 equiv) were coupled off the machine onto the deblocked resin (1.34 g, 0.2 mmol) in *refluxing* CH_2Cl_2 (10 mL, 0.16 M of amino acid, 0.32 M DIEA) overnight. After the resin was washed with CH_2Cl_2 (4 × 30 mL), deblocking was performed with DBU−piperidine−DMF (2:20:80; 1 × 1 min, 1 × 10 min) and the resin washed with CH_2Cl_2 (5 × 30 s) and coupled to the next acid fluoride using the same method. After the third coupling, the resin was placed on the instrument and couplings, cleavage, and purification were accomplished by the standard methods.

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