A Simple and Inexpensive Synthesis of 4-(Aminomethyl)-L-phenylalanine

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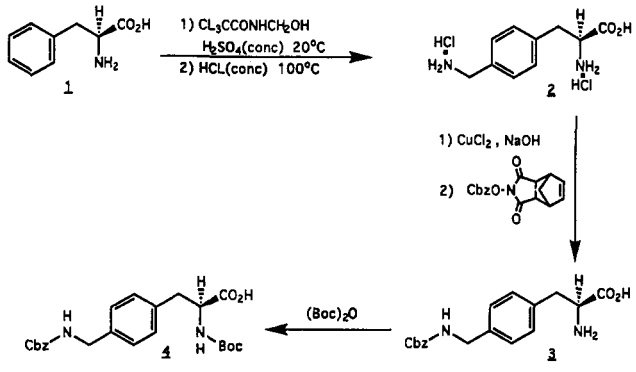
Synthesis of 4-(aminomethyl)-L-phenylalanine (2, AMF), a constrained lysine analog, or its N-protected derivatives was first reported in 1985[1] and subsequently in 1989[2] and 1990.[3] Unfortunately, the first preparation would require a resolution while the latter two are published in journals not readily accessible by the chemical community at large and none of these were amenable to large-scale preparation (>25 g) of 1. The most recently reported synthesis of AMF,[4] although slightly more efficient than the present case, suffers from the use of expensive reagents. Here we wish to report a new, simple, and inexpensive procedure to prepare AMF in two steps from L-Phe as well as procedures for subsequently converting AMF to N-protected derivatives 3 and 4 without racemization at the asymmetric center.

The key step, introduction of the 4-[[N-(trichloroacetyl)-amino]methyl] group is accomplished readily via acid-catalyzed, nuclear amidoalkylation (Tscherniac–Einhorn)[5] of L-Phe and is followed by acid hydrolysis to yield AMF dihydrochloride in modest yield (~30%) but in a high state of chiral purity. Dilution of the concentrated sulfuric acid used in the amidoalkylation step with acetic acid offered no advantage and, when the concentration of sulfuric acid fell below 50%, no alkylation occurred. Replacement of the N-hydroxytrichloroacetamide reagent with the more conventional monochloro analog gave no isolable amide intermediate on neutralization of the first step.



The selectively N-protected derivatives 3 and 4 were prepared using appropriately modified procedures for N^{ω}-p-toluenesulfonylation of L-lysine followed by N^{α}-*tert*-butyloxycarbonylation as described by Bodanszky.[6]

The present method constitutes a simple and inexpensive large-scale synthesis of this unnatural amino acid without the use of toxic or hard-to-handle reagents and is free of racemization.

Experimental Section

4-(Aminomethyl)-L-phenylalanine Dihydrochloride (2). Phenylalanine (1, 60 g, 0.36 mol) was added in portions to concentrated H_2SO_4 (200 mL) maintaining the temperature at 20–25 °C. *N*-(Hydroxymethyl)trichloroacetamide (71 g, 0.394 mol) was added in portions while maintaining the temperature at 20–25 °C. The cooling bath was removed and the light-brown cloudy solution was stirred at room temperature for 1 h. The reaction mixture was added to ice (3.5 L) and the pH was adjusted to ~5.5 with 8 N NaOH solution while maintaining the quench temperature at 15–20 °C. The white solid was filtered off and washed with H_2O,[7] dried, and then dissolved in hot concentrated HCl (100 mL).[8] The resulting solution was heated for 16 h at the reflux temperature, cooled to 20–25 °C, and filtered through a medium porosity sintered glass funnel to remove solid Na_2SO_4. The filter cake was washed with concentrated HCl (2 × 25 mL) and the combined filtrates are concentrated. The residue was dissolved in hot concentrated HCl (80 mL) and the resulting solution was cooled to −20 °C then filtered. The product was washed with cold (−20 °C) concentrated HCl (2 × 15 mL), dried, washed with acetone (2 × 30 mL), and dried to yield 2 (30.1 g, 31.3% yield): mp 271 °C dec with an HPLC purity exceeding 96%; ^{1}H NMR (D_2O, 3-(trimethylsilyl)propanesulfonic acid sodium salt as reference) δ 3.28 (1H, d, d, J = 11, 6 Hz), 3.39 (2H, d, d, J = 11, 6 Hz), 4.20 (2H, s), 4.37 (1H, t, J = 6 Hz), 7.41 (1H, d, J = 7 Hz), 7.48 (1H, d, J = 7 Hz). Anal. Calcd for $C_{10}H_{14}N_2O_4 \cdot 2HCl \cdot H_2O$: C, 42.12; H, 6.36; N, 9.82. Found: C, 41.80; H, 6.33; N, 9.70.

Determination of Chiral Purity of 2. Individual samples of 2 and racemic AMF were dissolved in reaction buffer EtOH/H_2O/Et_3N (6:1:1) at a concentration of 1 mg/mL. Two hundred microliters of each sample was added to individual samples of 10 μL of (*R*)-(+)-α-methylbenzyl isothiocyanate and allowed to react for 30 min before removal of buffer under vacuum at room temperature. A 1-mL aliquot of H_2O/CH_3CN (1:1) was added, the solution was extracted with *n*-BuCl (4 mL), and the H_2O layer was injected onto the HPLC. HPLC assay: column = Hypersil ODS 4.6 × 250 mm. Mobile Phase Program: gradient from 40% of 0.02M NH_4OAc (pH 5.0 with HOAc) buffer and 60% MeOH to 65% buffer and 35% MeOH over 45 min at 50 °C. Flow rate: 2 mL/min. Detection = UV at 260 nM. Retention time: 13.20 min for L isomer (2) and 14.24 min for D isomer. Chiral purity of 2 was shown to be equivalent to that of the starting Phe used (>99%).

4-[[N-(Benzyloxycarbonyl)amino]methyl]-L-phenylalanine (3). A solution of 2 (22.8 g, 0.085 mol) and $CuCl_2 \cdot 2H_2O$ (10.2 g, 0.060 mol) in H_2O (200 mL) was adjusted to a pH of 10.0 with 10% (w/v) NaOH solution and stirred for ~18 h at 20–25 °C. A solution of *N*-[(benzyloxycarbonyl)oxy]-5-norbornene-2,3-dicarboximide (27.5 g, 0.088 mol) in CH_3CN (120 mL) was added over 15 min while maintaining the pH at 9.0 ± 0.2 by the simultaneous addition of 10% (w/v) NaOH solution. After completion of the addition, the pH was stabilized at 9.0 ± 0.2 and stirred for ~18 h at 20–25 °C and then filtered. The filter cake was washed with H_2O and EtOAc (3 × 200 mL) and then dried. The resulting solid was suspended in HOAc (160 mL), and concentrated HCl was added to dissolve the solid. After 10 min the solution was then added to ice–water (3 L) and the pH was adjusted to 1.7 with concentrated HCl. The mixture was filtered and the filter cake was dried to yield 20.0 g of 3. The pH of the

(1) Nutt, R. F., Curley, P. E., Pitzenberger, S. M., Freidinger, R. M., Saperstein, R., Veber, D. F. *Peptides: Structure and Function*; Proceedings of the Ninth American Peptide Symposium, 1985; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.; p 441.
(2) He, B., Liu, K., Xiao, S. *Chin. Sci. Bull* **1989**, *34*, 997.
(3) Liu, K., He, B., Xiao, S. *Yingyong Huaxue* **1990**, *7*, 72.
(4) Hartman, G. D., Halczenko, W. *Synth. Commun.* **1991**, *21*, 2103.
(5) Zaugg, H. E. *Synthesis* **1984**, 85 and refs cited therein.
(6) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*, Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, 1984, pp 66 and 20, respectively.
(7) Water solubility of intermediate amide was determined to be 6.53 mg/mL.
(8) Since frothing was observed on dissolution in the HCl, use of a larger than normal flask is recommended.

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filtrate was adjusted from 1.4 to 1.7 with solid NaOH to give an additional 3.0 g (total yield 82.4%) of **3**.

The purity of **3** (93–6%, HPLC analysis) can be increased to >99% by introducing the BOC group and subsequent removal as follows. A solution of di-*tert*-butyl dicarbonate (16.9 g, 77.4 mmol) in *t*-BuOH (70.3 mL) was added to a solution of **3** (23.1 g, 70.3 mmol) in a mixture of *t*-BuOH (70.3 mL), H_2O (70.3 mL), and 1 N NaOH solution (70.3 mL), maintaining the reaction temperature at ~25 °C. The pH was maintained at 10.2 during and after the above addition by the simultaneous addition of 1 N NaOH solution. The mixture was stirred for ~2 h and filtered, and the filtrate was diluted with H_2O to a volume of 1 L. The filtrate was washed with pentane (3 × 250 mL) and Et_2O (3 × 250 mL) and then cooled to ~5 °C, and the pH was adjusted to 2.5 with 1 N $NaHSO_4$ solution. The acidified mixture was extracted with EtOAc (3 × 250 mL) and the combined organic layers were washed with saturated NaCl solution (3 × 100 mL) and then dried over anhydrous $MgSO_4$. The mixture was filtered and the filtrate was concentrated to a volume of 300 mL and then cooled to ~5 °C. Hydrogen chloride gas was bubbled into the solution while maintaining the temperature at <25 °C followed by degassing with N_2 for 30 min. The mixture was filtered and the filter cake was treated with Et_2O (500 mL) for 30 min and then air-dried to give 23.9 g of **3**. The solid was dissolved in CH_3OH (120 mL) and the resulting solution was filtered. Water (250 mL) was added to the filtrate to precipitate the product which was collected by filtration to give 19.74 g: mp 246 °C dec (85.5% purification yield, 70.5% overall yield) of **3**. Analysis for $C_{18}H_{20}N_2O_4$: Calcd C 65.84; H, 6.14; N 8.53. Found: C 65.72; H 6.32; N 8.64. MW = 328.36 The purity as determined by HPLC analysis is >99%. HPLC assay: column C18, 4.6 mm × 25 cm. Mobile Phase Program: gradient from 95% of 0.1% phosphoric acid buffer and 5% acetonitrile to 5% of 0.1% phosphoric acid buffer and 95% acetonitrile over 15 min. Flow rate: 2 mL/min. Detection: UV at 254 nM. Retention time: 7.2 min. ^1H NMR (D_2O/NaOD): δ 2.81 (1 H, m), 2.95 (1 H, m), 3.47 (1 H, t, J = 7 Hz), 4.28 (2 H, s), 5.11 (2 H, s), 7.22 (4 H, s), 7.41 (5 H, br s).

4-[[*N*-(Benzyloxycarbonyl)amino]methyl]-*N*-[[(1,1-dimethylethyl)oxy]carbonyl]-L-phenylalanine (4). A solution of di-*tert*-butyl dicarbonate (26.7 g, 0.122 mol)[9] in *t*-BuOH (111 mL) was added over 30 min to a mixture of **3** (36.5 g, 0.111 mol), *t*-BuOH (111 mL), H_2O (111 mL), and 1 N NaOH solution (111 mL) while maintaining the temperature at ~24 °C and the pH at 10.2 with the simultaneous addition of 1 N NaOH solution. The mixture was stirred for ~2 h while the pH was stabilized at 10.2. The mixture was filtered and the filtrate was diluted with H_2O to a volume of 1.5 L. The filtrate was washed with pentane (3 × 300 mL) and Et_2O (3 × 300 mL) and cooled to ~5 °C, and the pH was adjusted to 2.5 with 1 N $NaHSO_4$ solution. The mixture was extracted with EtOAc (3 × 300 mL), and the combined organic layers were washed with saturated NaCl solution (3 × 100 mL) then dried over anhydrous $MgSO_4$. The mixture was filtered, and the filtrate was concentrated, dissolved in EtOAc (300 mL),[10] washed with H_2O (3 × 100 mL), and dried over anhydrous $MgSO_4$. The mixture was filtered and the filtrate was concentrated and then dried under vacuum for 24 h to give 44.4 g (93.3% yield) of **4**. HPLC assay was performed as in **3**. Retention time: 10.2 min with purity >98.7%. ^1H NMR (CD_3OD): δ 1.37 (9 H, s), 2.89 (1 H, m), 3.11 (1 H, m), 4.26 (2 H, s), 4.30 (1 H, m), 5.08 (2 H, s), 7.19 (4 H, br s), 7.33 (5 H, m).

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(9) BOC-ON, [2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile], is an alternate reagent and may be used with appropriately modified reaction conditions.

(10) This procedure removes ~0.25 mol of t-BuOH contained in the product.