Facile Synthesis of DL-4,4-Difluoroornithine, DL-4,4-Difluoroglutamine, and γ-DL-4,4-Difluoroglutamyl-Containing Peptides: Regiospecific Addition of Nucleophiles to *N*-Cbz-di-*tert*-butyl-DL-4,4-difluoroglutamate

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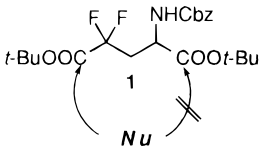
The reaction of several nucleophiles with *N*-Cbz-protected di-*tert*-butyl-DL-4,4-difluoroglutamate was investigated as an approach to the synthesis of various fluorinated amino acids and peptides. The γ-carboxyl group is highly activated by the two adjacent fluorine atoms, and nucleophilic reactions occurred exclusively at that carbonyl carbon. Further transformations of the reaction products resulted in the synthesis of DL-4,4-difluoroornithine, DL-4,4-difluoroglutamine, and γ-DL-4,4-difluoroglutamyl-containing dipeptides.

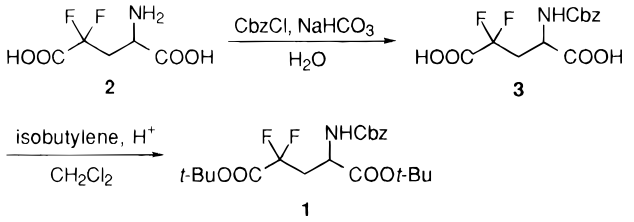
Introduction

Fluorinated amino acids and peptides are an important class of unnatural molecules which have been successfully used in a wide variety of biological applications.[1−4] These compounds owe their unique biological properties to the profound electron-withdrawing effect caused by fluorine atom(s) that occurs without significant steric consequence. We have been investigating folate and antifolate analogs in which the glutamate is replaced by several fluorinated glutamates. These fluorinated analogs have been useful tools for studying the effects of folylpolyglutamates on folate-dependent enzymes involved in a variety of critical one-carbon biochemistry reactions.[5,6] In the course of our studies on the chemistry of fluorinated glutamates, we became interested in the use of these compounds as intermediates in the synthesis of other fluorinated molecules of biological importance. In this report, we describe the reaction of the fully protected derivative of DL-4,4-difluoroglutamate (**1**) with various nucleophiles (H^-, NH_3, RNH_2, or OH^-). The



reactions proceed in a regiospecific manner at the carbonyl carbon of the γ-ester. The high regiospecificity is hypothesized to result from the two adjacent fluorine atoms that preferentially activate the γ-ester. Further chemical transformation of the primary products resulted in the facile synthesis of difluoromethylene-containing molecules such as DL-4,4-difluoroornithine, DL-4,4-difluoroglutamine, and γ-DL-4,4-difluoroglutamyl-containing peptides. All of the fluorinated analogs are of interest as probes for studying the mechanism of various enzymes that are involved in important biological processes. Specifically, DL-4,4-difluoroornithine is a potential alternate substrate for ornithine decarboxylase. The product of that enzyme-catalyzed decarboxylation, 2,2-difluoroputrescine, has been shown to act *in vivo* as a substrate for two (aminopropyl)transferases, spermidine synthase and spermine synthase, leading to difluoropolyamines as possible tumor markers.[7] DL-4,4-Difluoroglutamine could be a useful compound for mechanistic studies of glutamine-dependent amidotransferases.[8] Similarly, 4,4-difluoroglutamyl γ-glutamates and other related peptides are of interest to us in further mechanistic studies of the enzyme γ-glutamyl hydrolase.[9]

Scheme 1



Results and Discussion

We have previously reported the facile synthesis of DL-4,4-difluoroglutamic acid (**2**).[10] Diester **1** was obtained from **2** by first protecting the α-amino group as the *N*-(benzyloxycarbonyl) derivative, **3**, followed by esterification with isobutylene (Scheme 1).

Reduction of **1** with $NaBH_4$ proceeds exclusively at the γ-*tert*-butyl ester group to afford alcohol **4** in 81% yield

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(10) Tsukamoto, T.; Kitazume, T.; McGuire, J. J.; Coward, J. K. *J. Med. Chem.* **1996**, *39*, 66−72.

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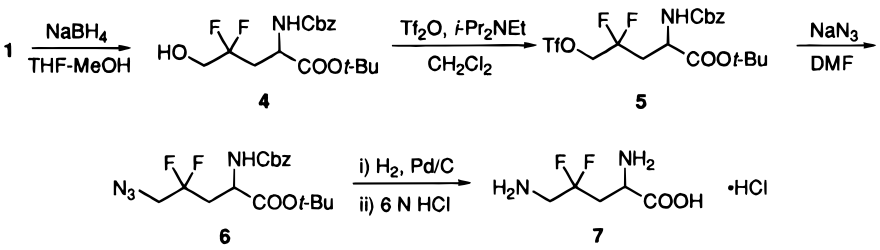
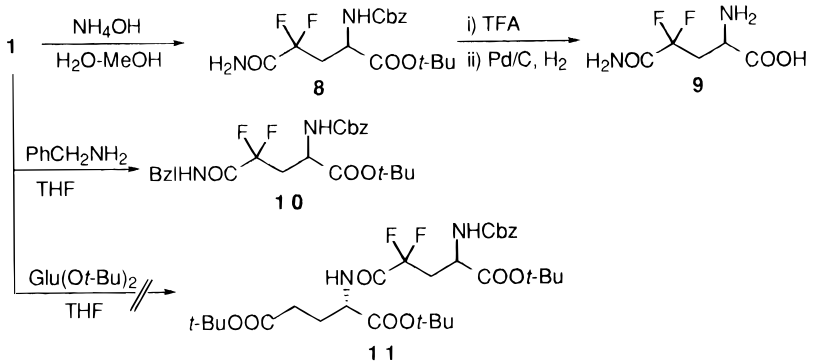
Scheme 2



Scheme 3

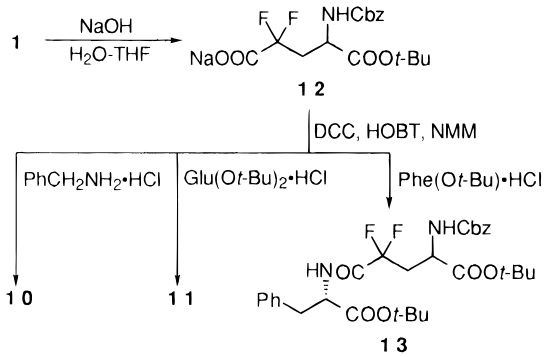


(Scheme 2). Although it is well known that methyl or ethyl esters of fluorinated carboxylic acid are readily converted into the corresponding primary alcohols by $NaBH_4$,[11,12] there is no such precedent for the bulky *tert*-butyl esters. The results indicate a significant electronic effect at the carbonyl of the γ-carboxylic acid ester due to the two adjacent fluorine atoms. Azide **6** was obtained from alcohol **4** by first converting **4** into triflate **5**, followed by treatment with NaN_3 (Scheme 2). Catalytic hydrogenation of **6** and subsequent hydrolysis afforded DL-4,4-difluoroornithine (**7**) in 37% overall yield from **6**.

The same regiospecificity also has been observed in aminolysis reactions. Aminolysis of **1** with aqueous ammonia took place exclusively at the γ-ester to afford the corresponding amide **8** in 80% yield (Scheme 3). DL-4,4-Difluoroglutamine (**9**) was obtained by first treating **8** with trifluoroacetic acid, followed by catalytic hydrogenation. Using a nonaqueous solvent for solubility reasons, reaction of **1** with benzylamine was more sluggish; higher temperature and longer reaction time were required for the formation of *N*-benzylamide **10**.[13] The low reactivity observed with this primary amine apparently reflects the intolerance of the aminolysis reaction to nonpolar solvents and/or sterically hindered nucleophiles. Indeed, an even more sterically hindered amine, di-*tert*-butyl glutamate, was unreactive in the aminolysis reaction, and none of the desired dipeptide **11** was formed under a variety of conditions investigated, including the addition of bases such as excess glutamate, Et_3N, and DMAP (Sibley, R. N. unpublished results).

NaOH was also found to be an effective nucleophile and hydrolyzed only the γ-ester of **1** to give half ester **12** (Scheme 4). Subsequent DCC-mediated coupling to benzylamine afforded **10** in 89% overall yield. A significant advantage of the stepwise formation of amides through **12** over the previously mentioned direct aminolysis reactions (Scheme 3) is the tolerance of the coupling reaction to sterically hindered amines. In fact, coupling of **12** to di-*tert*-butyl glutamate proceeds smoothly to give 93% yield of dipeptide **11**, a compound that could not be obtained via the direct aminolysis reaction described above. The fluorinated dipeptide **11** is a useful precursor for the (bio)synthesis of fluorinated analogs of folate and antifolate poly-γ-glutamates.[9] Coupling of **12** to phenylalanine *tert*-butyl ester was also successful, affording a 91% yield of fluorinated dipeptide **13**, thus demonstrating the viability of the method for the synthesis of 4,4-difluoroglutamyl-containing peptides.

Scheme 4



In summary, we have evaluated the use of **1** as an intermediate for the synthesis of several 4,4-difluoro amino acids and found that regiospecific nucleophilic addition to **1** allows facile access to various difluoromethylene-containing compounds. Biological studies with the new compounds described in this paper are currently in progress, and the results from these experiments will be reported in future publications.

Experimental Section

NMR chemical shift data are reported in parts per million (ppm) downfield from tetramethylsilane (internal standard for ^1H and ^{13}C) or trifluoroacetic acid (external standard for ^{19}F).

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NMR spectral data for **11** and **13** reflect the fact that the products are mixtures of two diastereomers. Where distinguishable, the resonances attributed to the same atom of each diastereomer are reported as a pair; e.g., δ 26.8 and 26.9. Di-*tert*-butyl L-glutamate hydrochloride and L-phenylalanine *tert*-butyl ester hydrochloride were obtained from BACHEM Bioscience Inc. (Philadelphia, PA). DL-4,4-Difluoroglutamic acid was synthesized as recently reported.[10]

***N*-(Benzyloxycarbonyl)-DL-4,4-difluoroglutamic Acid (3).** To a solution of $NaHCO_3$ (504 mg, 6.0 mmol) in H_2O (15 mL) was added DL-4,4-difluoroglutamic acid **2** (250 mg, 1.37 mmol) and benzyl chloroformate (372 mg, 2.18 mmol) at 0 °C, and the mixture was stirred at ambient temperature for 9 h. Ether (15 mL) was then added to the reaction mixture. The aqueous layer was separated and poured into ethyl acetate (15 mL) and then diluted with 3 N HCl (10 mL) at 0 °C. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 15 mL). The combined extracts were dried over Na_2SO_4 and concentrated. The resulting product was crystallized from chloroform to give 369 mg of **3** (85% yield) as a white solid: mp 140−141 °C; R_f 0.74 (*i*-PrOH/H_2O, 7:3); IR (KBr) 3331, 3029, 1736, 1710, 1693, 1533 cm^{-1}; ^{1}H NMR (CD_3OD) δ 2.5−2.9 (m, 2 H), 4.50 (dd, J = 3.5, 9.7 Hz, 1 H), 4.96 (s, 2 H), 7.3−7.5 (m, 5 H); ^{13}C NMR (CD_3OD) δ 36.8 (t, J = 24.0 Hz), 50.0 (t, J = 4.5 Hz), 67.7, 116.5 (t, J = 249 Hz), 128.7 (2 C), 128.9, 129.4 (2 C), 138.0, 158.3, 166.4 (t, J = 31.7 Hz), 173.9; ^{19}F NMR (CD_3OD) δ −30.8 (dt, J = 261, 17.3 Hz, 1 F), −29.6 (dt, J = 261, 14.4 Hz, 1 F); MS (EI) *m*/*e* (rel intensity) 317 (MNH_4^+, 69.9), 108 (68.5), 91 (100); HRMS (EI) *m*/*e* calcd for $C_{13}H_{13}F_2NO_6$ (MNH_4^+) 317.0711, found 317.0727. Anal. Calcd for $C_{13}H_{13}F_2NO_6$: C, 49.22; H, 4.13; N, 4.42. Found: C, 48.87; H, 4.22; N, 4.48.

***N*-(Benzyloxycarbonyl)-DL-4,4-difluoroglutamic Acid, Di-*tert*-butyl Ester (1).** To a solution of **3** (2.025 g, 6.4 mmol) in dry CH_2Cl_2 (90 mL) were added concd sulfuric acid (0.5 mL) and then isobutylene (30 mL), condensed in a pressure bottle at −30 °C. The bottle was closed, and the mixture was stirred at rt for 5 days. The reaction mixture was again cooled to −30 °C, diluted with ethyl acetate (100 mL), and poured into 20% aqueous K_2CO_3 solution (100 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined extracts were dried over $MgSO_4$ and the solvent evaporated. The resulting oil was purified by silica gel chromatography (hexane/EtOAc, 4:1) to give 2.433 g of **1** (89% yield) as a colorless oil: R_f 0.44 (hexane/EtOAc, 4:1); IR (neat) 3360, 2981, 2938, 1724, 1525 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9 H), 1.49 (s, 9 H), 2.55 (dq, J = 14.8, 6.6 Hz, 1 H), 2.69 (dq, J = 14.8, 4.9 Hz, 1 H), 4.47 (dt, J = 5.3, 7.3 Hz, 1 H), 5.10 (s, 2 H), 5.34 (d, J = 7.9 Hz, 1 H), 7.3−7.4 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 27.8 (3 C), 28.0 (3 C), 36.3 (t, J = 23.8 Hz), 50.0, 67.3, 83.1, 85.1, 115.0 (t, J = 252 Hz), 128.3 (2 C), 128.4, 128.7 (2 C), 136.3, 155.8, 162.6 (t, J = 31.6 Hz), 169.6; ^{19}F NMR ($CDCl_3$) δ −28.3 (dt, J = 266, 16.2 Hz, 1 F), −27.7 (dt, J = 266, 15.2 Hz, 1 F); MS (CI) *m*/*e* (rel intensity) 447 (MNH_4^+, 100), 391 (65.9), 335 (35.1); HRMS (CI) *m*/*e* calcd for $C_{21}H_{29}F_2NO_6NH_4$ (MNH_4^+) 447.2307, found 447.2304. Anal. Calcd for $C_{21}H_{29}F_2NO_6$: C, 58.73; H, 6.81; N, 3.26. Found: C, 58.72; H, 6.58; N, 3.60.

***N*-(Benzyloxycarbonyl)-2-amino-4,4-difluoro-5-hydroxypentanoic Acid, *tert*-Butyl Ester (4).** $NaBH_4$ (61 mg, 1.6 mmol) was added to a solution of **1** (351 mg, 0.82 mmol) in THF/methanol (9:1, 7.0 mL) at 0 °C, and the mixture was stirred at rt for 12 h. The mixture was then diluted with ethyl acetate (10 mL) and poured into 1 N HCl (10 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined extracts were dried over $MgSO_4$, and the solvent was evaporated. The resulting crude oil was purified by silica gel chromatography to give 240 mg of **4** (81%) as a colorless oil: R_f 0.33 (hexane/EtOAc, 2:1); IR (neat) 3395, 2981, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9 H), 2.3−2.6 (m, 3 H), 3.75 (t, J = 12.8 Hz, 2 H), 4.47 (br m, 1 H), 5.12 (s, 2 H), 5.61 (d, J = 8.0 Hz, 1 H), 7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 27.9 (3 C), 35.4 (t, J = 24.0 Hz), 50.2, 64.0 (t, J = 32.0 Hz), 67.2, 83.0, 122.6 (t, J = 244 Hz), 128.2 (2 C), 128.3, 128.6 (2 C), 136.3, 156.1, 170.4; ^{19}F NMR ($CDCl_3$) δ −29.1 (m, 2 F); MS (CI) *m*/*e* (rel intensity) 377 (MNH_4^+, 31.9), 321 (47.4), 303 (61.2), 269 (100); HRMS (CI) *m*/*e* calcd for $C_{17}H_{23}F_2NO_5NH_4$ (MNH_4^+) 377.1888, found 377.1885. Anal. Calcd for $C_{17}H_{23}F_2NO_5$: C, 56.82; H, 6.45; N, 3.90. Found: C, 56.75; H, 6.41; N, 3.78.

***N*-(Benzyloxycarbonyl)-2-amino-4,4-difluoro-5-(trifluoromethanesulfoxy)pentanoic Acid, *tert*-Butyl Ester (5).** To a solution of **4** (609 mg, 1.70 mmol) in dry CH_2Cl_2 (10 mL) at −30 °C was added diisopropylethylamine (575 μL, 3.3 mmol) followed by the addition of trifluoromethanesulfonic anhydride (336 μL, 2.0 mmol). The mixture was stirred at rt for 45 min and then diluted with ether (25 mL) and H_2O (25 mL). The organic layer was separated, and the aqueous layer was extracted with ether (2 × 25 mL). The combined extracts were dried over $MgSO_4$ and evaporated to give 932 mg of **5** as a colorless oil. The crude product was used for the next reaction without further purification: R_f 0.67 (hexane/EtOAc, 2:1); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 2.3−2.7 (m, 2 H), 4.4−4.6 (m, 3 H), 5.09 (s, 2 H), 5.61 (d, J = 7.9 Hz, 1 H), 7.31 (m, 5 H).

***N*-(Benzyloxycarbonyl)-2-amino-5-azido-4,4-difluoropentanoic Acid, *tert*-Butyl Ester (6).** To a solution of **5** (932 mg) in dry DMF (7 mL) was added NaN_3 (332 mg, 5.1 mmol) at 0 °C, and the mixture was stirred at 40 °C for 90 min. The reaction mixture was diluted with ether (20 mL) and poured into water (20 mL). The organic layer was separated, and the aqueous layer was extracted with ether (2 × 20 mL). The combined extracts were dried over $MgSO_4$ and evaporated. The resulting product was purified by silica gel chromatography (hexane/EtOAc, 4:1) to give 529 mg of **6** (81% for two steps) as a colorless oil: R_f 0.68 (hexane/EtOAc, 2:1); IR (neat) 3353, 2981, 2109, 1729, 1518 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9 H), 2.43 (dq, J = 6.7, 15.8 Hz, 1 H), 2.56 (dq, J = 4.5, 15.8 Hz, 1 H), 3.52 (t, J = 13.1 Hz, 2 H), 4.48 (dt, J = 5.2, 7.0 Hz, 1 H), 5.13 (s, 2 H), 5.48 (d, J = 8.0 Hz, 1 H), 7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 28.0 (3 C), 36.0 (t, J = 23.5 Hz), 50.2, 54.2 (t, J = 30.6 Hz), 67.3, 83.2, 122.2 (t, J = 245 Hz), 128.3 (2 C), 128.4, 128.7 (2 C), 136.3, 155.9, 169.7; ^{19}F NMR ($CDCl_3$) δ −23.9 (quint, J = 13.5 Hz, 2 F); MS (CI) *m*/*e* (rel intensity) 402 (MNH_4^+, 28.8), 357 (53.7), 346 (100), 329 (15.5), 301 (59.7); HRMS (CI) *m*/*e* calcd for $C_{17}H_{22}F_2N_4O_4NH_4$ (MNH_4^+) 402.1953, found 402.1951. Anal. Calcd for $C_{17}H_{22}F_2N_4O_4$: C, 53.12; H, 5.77; N, 14.58. Found: C, 53.22; H, 5.97; N, 14.25.

DL-4,4-Difluoroornithine•HCl (7). To a solution of **6** (448 mg, 1.17 mmol) in dry ethanol (20 mL) was added 10% Pd/C (25 mg), and the suspension was shaken under a hydrogen atmosphere (50 psi) for 36 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The resulting oil (398 mg) was dissolved in 6 N HCl (5 mL), and the solution was heated at 90 °C for 8 h. The solvent was evaporated, and the crude, gummy product was dissolved in dry ethanol (3 mL). Propylene oxide (one drop) was added to the solution to give a white precipitate. The white solid was recrystallized from ethanol/H_2O to give 96 mg of **7** (37% yield) as a white solid: mp 185−187 °C; R_f 0.46 (2-propanol/30% NH_3, 7:3); IR (KBr) 3500, 3431, 2088, 1638, 1588 cm^{-1}; ^{1}H NMR (D_2O) δ 2.63 (ddt, J = 16.2, 26.0, 8.5 Hz, 1 H), 2.81 (dddd, J = 3.6, 10.3, 16.2, 28.1 Hz, 1 H), 3.55−3.75 (m, 2 H), 4.10 (dd, J = 3.6, 8.5 Hz, 1 H); ^{13}C NMR (D_2O) δ 35.5 (t, J = 22.8 Hz), 43.6 (t, J = 25.2 Hz), 49.3, 120.9 (t, J = 244 Hz), 172.5; ^{19}F NMR (D_2O) δ −28.5 (dm, J = 243 Hz, 1 F), −29.9 (dm, J = 243 Hz, 1 F); MS (EI) *m*/*e* (rel intensity) 150 ($M^+ - H_2O$, 29.2), 122 (17.2), 106 (25.5), 92 (100); MS (CI) *m*/*e* (rel intensity) 151 ($MH^+ - H_2O$, 100), 131 (26.6), 103 (55.8). Anal. Calcd for $C_5H_{10}F_2N_2O_2$•HCl•0.5H_2O: C, 28.11; H, 5.66; N, 13.11. Found: C, 28.17; H, 5.36; N, 13.02.

***N*$^{\alpha}$-(Benzyloxycarbonyl)-DL-4,4-difluoroglutamine, α-*tert*-Butyl Ester (8).** Diester **1** (300 mg, 0.70 mmol) was dissolved in methanol (5 mL), and 30% aqueous ammonia (5 mL) was added to the solution. The mixture was stirred at ambient temperature for 7 h and evaporated to dryness. The resulting yellow solid was purified by chromatography on silica gel (hexane/EtOAc, 4:1) to give 210 mg of **8** (80% yield) as a white solid: mp 132−134 °C; R_f 0.42 (hexane/EtOAc, 1:1); IR (KBr) 3409, 3191, 2987, 1729, 1722, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9 H), 2.56 (dq, J = 8.5, 15.0 Hz, 1 H), 2.68 (dq, J = 4.3, 15.0 Hz, 1 H), 4.51 (dt, J = 4.2, 8.5 Hz, 1 H), 5.07

(d, J = 12.2 Hz, 1 H), 5.13 (d, J = 12.2 Hz, 1 H), 5.51 (d, J = 8.6 Hz, 1 H), 5.83 (s, 1 H), 6.40 (s, 1 H), 7.35 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 28.0 (3 C), 36.0 (t, J = 23.3 Hz), 50.0, 67.3, 83.3, 116.7 (t, J = 254 Hz), 128.2 (2 C), 128.3, 128.6 (2 C), 136.4, 156.0, 166.0 (t, J = 28.2 Hz), 169.6; ^{19}F NMR ($CDCl_3$) δ −28.6 (dt, J = 263, 15.5 Hz), −27.3 (dt, J = 263, 15.5 Hz); MS (CI) m/e (rel intensity) 390 (MNH_4^+, 17.3), 373 (MH^+, 27.0), 334 (100), 317 (66.6); HRMS (CI with NH_3) m/e calcd for $C_{17}H_{22}F_2N_2O_5H$ (MH^+) 373.1575, found 373.1591. Anal. Calcd for $C_{17}H_{22}F_2N_2O_5$: C, 54.83; H, 5.96; N, 7.52. Found: C, 54.55; H, 5.75; N, 7.47.

DL-4,4-Difluoroglutamine (9). Amide **8** (318 mg, 0.85 mmol) was dissolved in trifluoroacetic acid (5 mL) at 0 °C, and the solution was stirred at rt for 2 h. The solvent was evaporated, and the resulting white solid was dissolved in methanol (10 mL). Catalyst (10% Pd/C, 20 mg) was added to the solution, and the suspension was stirred under hydrogen (1 atm) at 0 °C for 1 h. The catalyst was removed by filtration, and the filtrate was evaporated to dryness to give a white solid. This was recrystallized from H_2O/methanol to give 74 mg of **9** (48% yield) as a white solid: mp 157−159 °C; R_f 0.63 (2-butanone/AcOH/H_2O, 20:5:8); IR (KBr) 3500, 3200−2500, 1701, 1657, 1595 cm^{-1}; ^{1}H NMR (D_2O) δ 2.66 (dddd, J = 9.2, 10.5, 16.2, 23.7 Hz, 1 H), 2.86 (dddd, J = 3.5, 13.0, 16.2, 25.1 Hz, 1 H), 4.05 (dd, J = 3.5, 9.0 Hz, 1 H); ^{13}C NMR (D_2O) δ 35.0 (t, J = 23.6 Hz), 49.4, 116.6 (t, J = 252 Hz), 167.3 (t, J = 29.8 Hz), 172.4; ^{19}F NMR (D_2O) δ −30.6 (ddd, J = 12.7, 24.9, 256 Hz, 1 F), −28.9 (ddd, J = 9.9, 25.9, 256 Hz, 1 F); MS (CI) m/e (rel intensity) 183 (MH^+, 83.7), 165 (47.6), 84 (100); HRMS (CI) m/e calcd for $C_5H_8F_2N_2O_3H$ (MH^+) 183.0581, found 183.0581. Anal. Calcd for $C_5H_8F_2N_2O_3$: C, 32.97; H, 4.43; N, 15.38. Found; C, 33.11; H, 4.43; N, 15.48.

***N*$^\delta$-Benzyl-*N*$^\alpha$-(benzyloxycarbonyl)-DL-4,4-difluoroglutamine, α-*tert*-Butyl Ester (10). Method A: Reaction of 1 with Benzylamine.** To a solution of **1** (58 mg, 0.14 mmol) in dry THF (5 mL) was added benzylamine (210 mg, 2.0 mmol) at 0 °C, and the mixture was heated at reflux temperature for 18 h. The reaction mixture was diluted with ethyl acetate (10 mL) and poured into 0.5 N HCl (10 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined extracts were dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel chromatography to give 51 mg of **10** (80% yield) as a white solid: mp 86−88 °C; R_f 0.21 (hexane/EtOAc, 4:1); IR (KBr) 3325, 2981, 1750, 1730, 1708, 1680, 1553 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9 H), 2.60 (dq, J = 8.2 and 15.3 Hz, 1 H), 2.72 (dq, J = 4.7 and 15.3 Hz, 1 H), 4.34 (dd, J = 5.6 and 14.8 Hz, 1 H), 4.45 (dd, J = 5.9 and 14.8 Hz, 1 H), 4.5 (m, 1 H), 5.08 (s, 2 H), 5.50 (d, J = 8.5 Hz, 1 H), 6.7−6.8 (br, 1 H), 7.2−7.4 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 27.9 (3 C), 35.9 (t, J = 23.9 Hz), 43.6, 49.9, 67.0, 82.9, 116.8 (t, J = 254 Hz), 127.7, 127.8, 128.0, 128.1, 128.4, 128.8, 136.2, 136.6, 155.6, 163.4 (t, J = 28.4 Hz), 169.3; ^{19}F NMR ($CDCl_3$) δ −28.6 (dt, J = 262, 16.6 Hz, 1 F), −27.5 (dt, J = 262, 15.5 Hz, 1 F); MS (CI) m/e (rel intensity) 480 (MNH_4^+, 6.8), 463 (MH^+, 41.9), 407 (100), 363 (99.8); HRMS (CI) m/e calcd for $C_{24}H_{28}F_2N_2O_5H$ (MH^+) 463.2044, found 463.2042. Anal. Calcd for $C_{24}H_{28}F_2N_2O_5$: C, 62.33; H, 6.10; N, 6.06. Found: C, 62.50; H, 6.18; N, 6.05.

***N*-(Benzyloxycarbonyl)-DL-4,4-difluoroglutamic Acid α-*tert*-Butyl Ester, Sodium Salt (12).** To a solution of **1** (344 mg, 0.80 mmol) in THF (5 mL) was added 2 N NaOH (0.4 mL) at 0 °C, and the mixture was stirred at rt for 20 h. The solvent was evaporated, and the resulting oil was dissolved in distilled H_2O (3 mL). The solvent was removed by lyophilization to give 319 mg of **12** as a gummy semisolid. This product was used for the next reaction without further purification: R_f 0.70 (*i*-PrOH/H_2O, 7:3); ^{1}H NMR (DMSO) δ 1.38 (s, 9 H), 2.4−2.7 (m, 2 H), 4.18 (dt, J = 4.3 and 8.2 Hz, 1 H), 5.04 (s, 2 H). 7.3−7.5 (m, 5 H), 7.80 (d, J = 8.4 Hz, 1 H); ^{19}F NMR (DMSO) δ −28.9 to −28.0 (m, 2 F).

***N*$^\delta$-Benzyl-*N*$^\alpha$-(benzyloxycarbonyl)-DL-4,4-difluoroglutamine, α-*tert*-Butyl Ester (10). Method B. Reaction of 12 with Benzylamine Hydrochloride.** To a solution of **12** (99 mg, derived from 0.25 mmol of **1**) in dry CH_2Cl_2 (5 mL) were added benzylamine hydrochloride (53 mg, 0.37 mmol), *N*-methylmorpholine (17 μL, 0.12 mmol), HOBt (50 mg, 0.37 mmol), and DCC (76 mg, 0.37 mmol) at 0 °C. The mixture was allowed to warm to rt and stirred for 24 h. DCU was removed by filtration, the resulting filtrate was concentrated, and the residual crude product was purified by silica gel chromatography (CH_2Cl_2/EtOAc, 20:1) to give 102 mg of **10** (89% yield) as a white solid. This product is identical in all respects to the compound prepared by the direct aminolysis reaction described above (Method A).

***N*$^\alpha$-[*N*-(Benzyloxycarbonyl)-DL-4,4-difluoroglutamyl]-γ-L-glutamic Acid, Tri-*tert*-butyl Ester (11).** Using L-glutamic acid di-*tert*-butyl ester hydrochloride (355 mg, 1.2 mmol) and **12** (319 mg, derived from 0.80 mmol of **1**), the same procedure as described above for the synthesis of **10** (method B) afforded 455 mg of **11** (93% yield) as a colorless oil: R_f 0.46 (CH_2Cl_2/EtOAc, 19:1); IR (neat) 3353, 2981, 2938, 1728, 1708, 1532 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 1.46 (2s, 18 H), 1.95−2.20 (m, 2 H), 2.20−2.40 (m, 2 H), 2.55−2.85 (m, 2 H), 4.40−4.45 (m, 1 H), 4.45−4.55 (m, 1 H), 5.10 (s, 2 H), 5.61 (d, J = 8.0 Hz, 1 H) and 5.71 (d, J = 8.0 Hz, 1 H), 7.24−7.38 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 26.8 and 26.9, 27.8 (3 C), 27.9 (3 C), 28.0 (3 C), 31.2 and 31.3, 35.6 (t, J = 24.0 Hz) and 35.8 (t, J = 24.0 Hz), 49.7, 52.5, 66.9, 81.0, 82.8, 82.9, 116.7 (t, J = 255 Hz), 128.0 (3 C), 128.4 (2 C), 136.0, 155.6 and 155.7, 163.4 (t, J = 27.3 Hz) and 163.6 (t, J = 30.6 Hz), 169.4, 169.8 and 169.9, 172.1; ^{19}F NMR ($CDCl_3$) δ −29.1 (dt, J = 261, 18.1 Hz, 1 F) and −28.1 (dt, J = 261, 16.4 Hz, 1 F), −27.4 (dt, J = 261, 16 Hz, 1 F) and −25.8 (dt, J = 261, 14.1 Hz, 1 F); MS (CI) m/e (rel intensity) 632 (MNH_4^+, 39.9), 615 (MH^+, 100), 559 (59.3), 503 (32.6); HRMS (CI) m/e calcd for $C_{30}H_{44}F_2N_2O_9H$ (MH^+) 615.3093, found 615.3094. Anal. Calcd for $C_{30}H_{44}F_2N_2O_9$: C, 58.62; H, 7.21; N, 4.56. Found: C, 58.61; H, 7.18; N, 4.53.

***N*$^\alpha$-[*N*-(Benzyloxycarbonyl)-DL-4,4-difluoroglutamyl]-γ-L-phenylalanine, Di-*tert*-butyl Ester (13).** Using L-phenylalanine *tert*-butyl ester hydrochloride (182 mg, 0.71 mmol) and **12** (189 mg, derived from 0.47 mmol of **1**), the same procedure as described above for the synthesis of **10** (method B) afforded 250 mg of **13** (91% yield) as a colorless oil: R_f 0.54 (CH_2Cl_2/EtOAc, 19:1); IR (neat) 3346, 2981, 2938, 1743, 1701, 1532 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9 H) and 1.39 (s, 9 H), 1.44 (s, 9 H), 2.5−2.8 (m, 2 H), 3.05−3.15 (m, 2 H), 4.40−4.55 (m, 1 H), 4.65−4.75 (m, 1 H), 5.07 (s, 2 H), 5.53 (d, J = 8.8 Hz, 1 H) and 5.65 (d, J = 8.0 Hz, 1 H), 6.95 (d, J = 5.8 Hz, 1 H) and 7.03 (d, J = 7.3 Hz, 1 H), 7.10−7.35 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 27.9 (3 C), 28.0 (3 C), 35.6 (t, J = 23.2 Hz) and 35.8 (t, J = 23.7 Hz), 37.8, 49.8 and 49.9, 53.8, 67.1, 83.0, 83.1, 116.8 (t, J = 254 Hz), 127.3, 128.2, 128.2 (2 C), 128.6 (4 C), 129.6 (2 C), 135.6, 136.3 and 136.4, 155.8 and 155.9, 163.1 (t, J = 29.0 Hz) and 163.2 (t, J = 28.5 Hz), 169.6, 169.7; ^{19}F NMR ($CDCl_3$) δ −29.5 (dt, J = 261, 17.0 Hz, 1 F) and −28.8 (dt, J = 261, 15.7 Hz, 1 F), −28.1 (dt, J = 261, 17.0 Hz, 1 F) and −27.1 (dt, J = 261, 14.5 Hz, 1 F); MS (CI) m/e (rel intensity) 594 (MNH_4^+, 14.9), 577 (MH^+, 52.4), 555 (10.0), 521 (39.0), 465 (100); HRMS (CI) m/e calcd for $C_{30}H_{38}F_2N_2O_7H$ (MH^+) 577.2725, found 577.2738. Anal. Calcd for $C_{30}H_{38}F_2N_2O_7 \cdot 0.4H_2O$: C, 61.72; H, 6.70; N, 4.80. Found: C, 61.76; H, 6.70; N, 4.85.

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