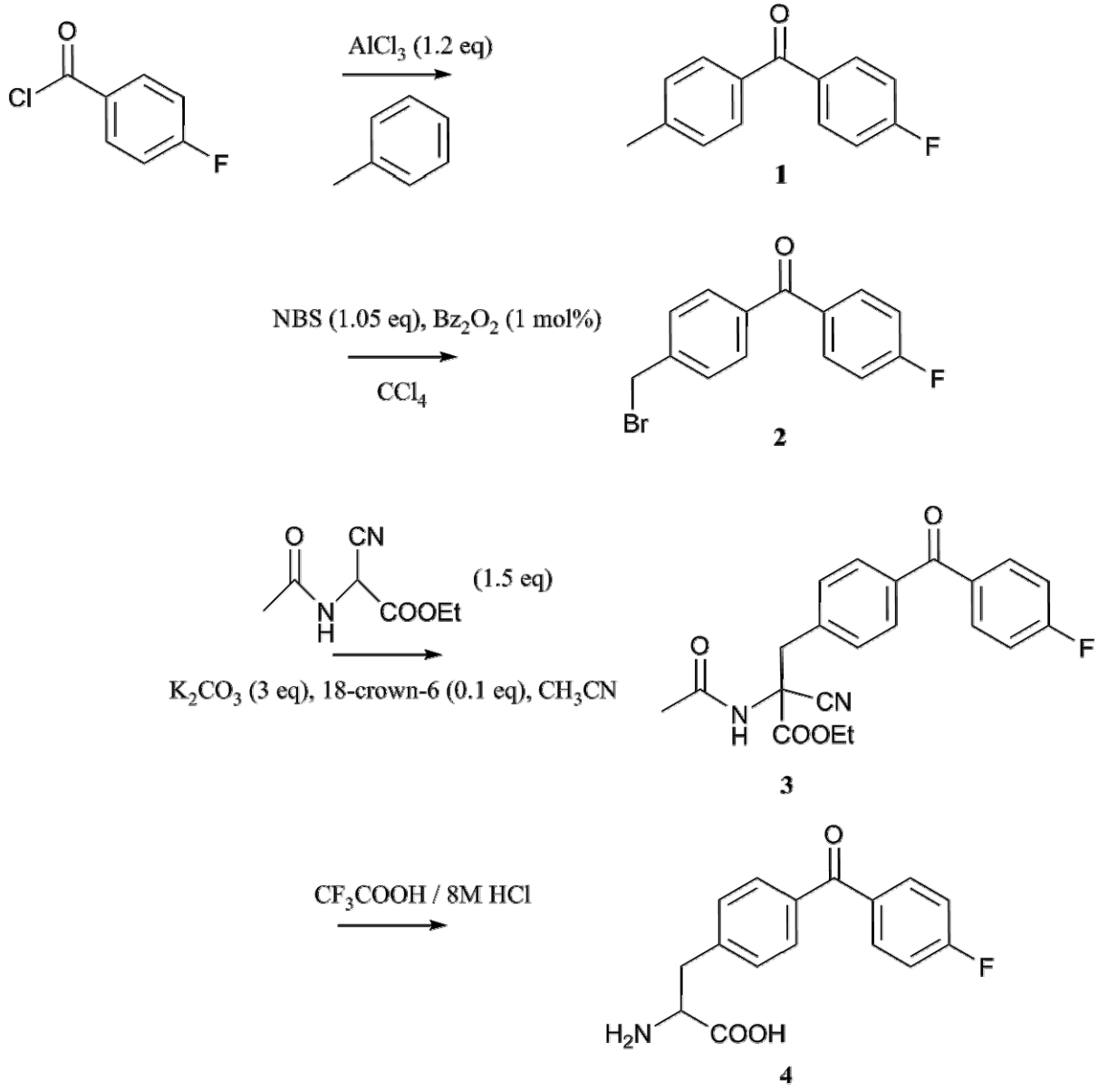
Supplemental Information

Synthesis of 4'-fluoro-*p*-benzoyl-*DL*-phenylalanine (4F-Bpa)



4-methyl-4'-fluoro-benzophenone (1). Aluminum chloride (3.74 g, 28 mmol) was slowly added to a stirred solution of 4-fluorobenzoyl chloride (3.42 g, 21.6 mmol) in toluene (60 mL). The solution was stirred at rt under argon for 1.5 h, then 5 mL of water was added dropwise to quench the reaction. The solution was further stirred for an additional 15 min before being washed with water (2 x 50 mL), 10% $NaHCO_3$ (2 x 50 mL), and brine (2 x 50 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered through a celite cake, and rotary evaporated, then dried under vacuum to obtain a yellow solid. The crude product was recrystallized from hexane and dried under vacuum to obtain a white solid (2.70 g, 62 % yield). ^{1}H NMR($CDCl_3$,

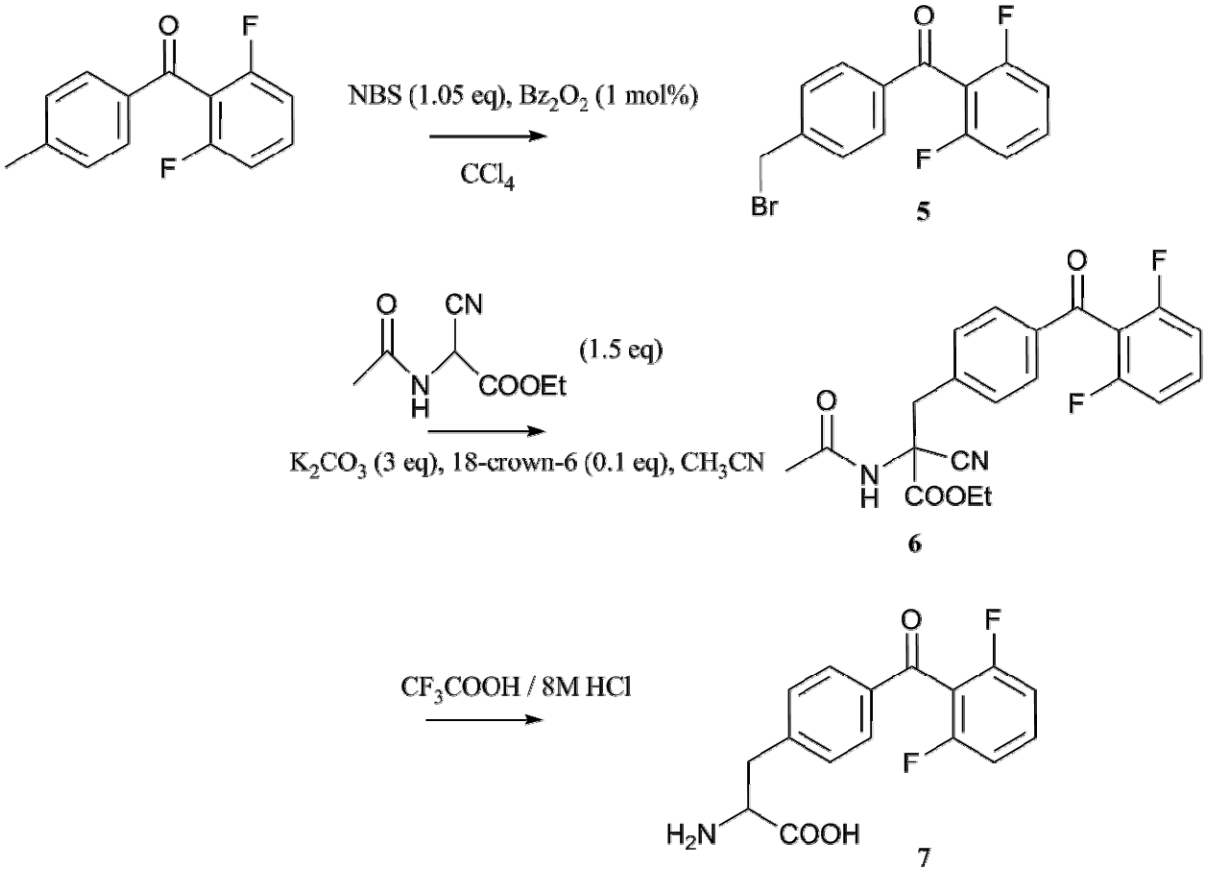
500 MHz): δ7.80 (dd, aromatic, 2 H), δ7.67 (d, aromatic, 2 H), δ7.26 (d, aromatic, 2 H), δ7.15 (t, aromatic, 2 H), δ2.42 (s, methyl, 3 H). ^{13}C NMR: carbonyl (δ195.0), aromatic (δ166.2, 164.2, 143.3, 134.7, 132.2, 130.1, 129.0, 115.3), alkyl (δ21.6). ^{19}F-NMR: δ-109.1 ppm.

4-bromomethyl-4'-fluorobenzophenone (2). A mixture of **1** (2.0 g, 9.9 mmol), recrystallized N-bromosuccinimide (1.524 g, 9.9 mmol), and benzoyl peroxide (170 mg) in CCl_4 (20 ml) was refluxed at ~150 °C under argon for 3 h. The hot solution was filtered and rinsed with hot CCl_4 (20 ml) until the crystals became colorless. The filtrate was cooled to rt and filtered again to completely remove residual succinimide. The filtrate was rotary evaporated and dried under vacuum. The crude product was recrystallized from hexane and dried under vacuum (43% yield). Rf = 0.25 (hexane: EtOAc; 85:15). ^{1}H-NMR($CDCl_3$, 500 MHz): δ7.85 (dd, aromatic, 2 H), δ7.75 (d, aromatic, 2 H), δ7.51 (d, aromatic, 2 H), δ7.17 (dd, aromatic, 2 H), δ4.52 (s, methyl, 3 H). ^{13}C NMR ($CDCl_3$): carbonyl (δ195.0), aromatic (δ166.5, 164.5, 142.5, 138.5, 133.4, 130.4, 129.2, 115.8, 115.5), alkyl (δ31.1).

Ethyl α-acetamido-α-cyano-β-(4'-fluoro-4-benzophenone)-DL-propionate (3). A mixture of **2** (600 mg, 2.13 mmol), ethyl acetamidocyanoacetate (545 mg, 3.2 mmol), 18-crown-6 (56 mg), and K_2CO_3 (883 mg, 6.4 mmol) in CH_3CN (8 ml) was stirred at rt under argon for 3 h. The solution was filtered through a celite cake, concentrated, and dried under vacuum. The crude product was washed with hexane to remove brown impurities and dried under vacuum (56% yield). Rf = 0.15 (hexane: EtOAc; 50:50). ^{1}H-NMR($CDCl_3$, 500 MHz): δ7.85 (dd, aromatic, 2 H), δ7.75 (d, aromatic, 2 H), δ7.38 (d, aromatic, 2 H), δ7.18 (dd, aromatic, 2 H), δ4.25 – 4.15 (m, 2 H, methylene of ethyl ester), δ 3.60 and δ 3.50 (dd, 2 H, hydrogens on C_β), δ 3.62 (s, 3 H, methyl), δ 1.22 (doublet of triplets, 3 H, ester methyl). ^{13}C-NMR($CDCl_3$): carbonyl (δ194.7, 170.5, 165.7), (137.3, 136.7, 133.4, 132.6, 132.5, 130.3, 130.1, 116.3, 115.7, 115.5, 70.0, 58.0, 22.4, 13.8).

4'-fluoro-p-benzoyl-phenylalanine (4). A mixture of **3** (0.40 g) and 30 ml of a 1:1 solution of 8 M HCl and CF_3COOH was refluxed with stirring at 180 °C for 24 hours. The reaction mixture was lyophilized to collect the crude product. Lingering trifluoroacetate ion was exchanged for chloride ion by suspending the crude product in ~ 20 mL 1 M HCl and lyophilizing the resulting solution. The product was dissolved in 1 M NaOH and filtered. The clear solution was adjusted to a pH of 7 by addition of 1 M HCl and the precipitate was collected and washed with cold water (2 x 10 mL). The remaining solid was dried under vacuum to obtain ~ 180 mg of a white powder. ^{1}H-NMR DMSO: δ7.8 (dd, aromatic, 2 H), δ7.6 (d, aromatic, 2 H), δ7.43 (d, aromatic, 2 H), δ7.38 (dd, aromatic, 2 H), δ3.44 (bs, 2 H, NH_2), δ3.45 (dd, 1 H, $CHCH_ACH_B$), δ3.25 (dd, 1 H, $CHCH_ACH_B$), δ3.0 (m, 1 H, $CHCH_2$). ^{19}F-NMR: δ-109.27 ppm.). MS ES (cal 287.0) pos. ion 288.1, neg. ion 286.1.

Synthesis of 2',6'-difluoro-*p*-benzoyl-*DL*-phenylalanine (2,6dF-Bpa)



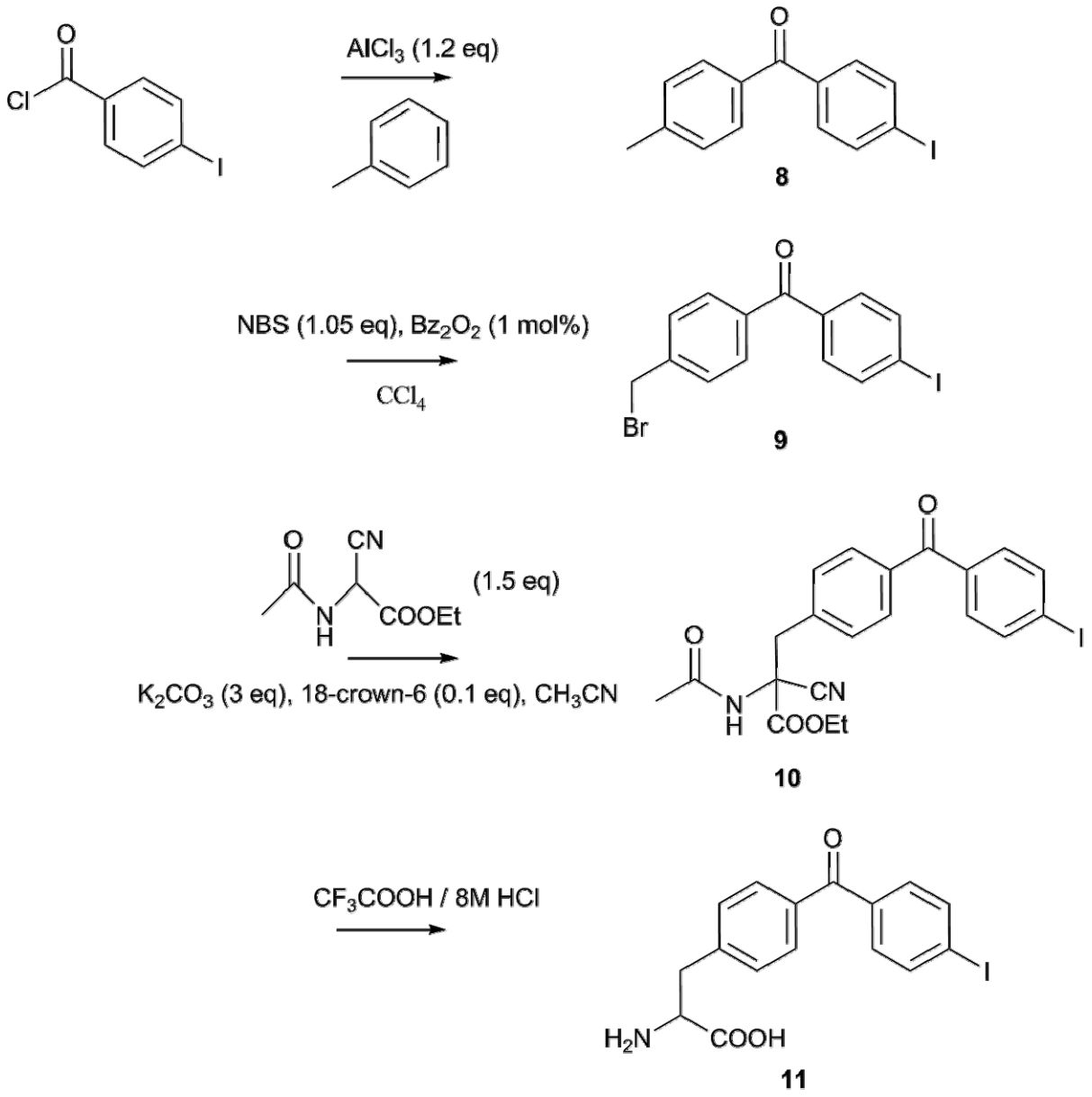
4-bromomethyl-2',6'-difluorobenzophenone (5). A mixture of 4-methyl-2',6'-difluorobenzophenone (233 mg, 1 mmol, Asiatech, Inc.), recrystallized N-bromosuccinimide (178 mg, 1 mmol), and benzoyl peroxide (17 mg) in CCl_4 (3 ml) was refluxed at ~150 °C under argon for 3 h. The hot solution was filtered and rinsed with hot CCl_4 (20 ml) until the crystals became colorless. The filtrate was cooled to rt and filtered again to completely remove residual succinimide. The filtrate was rotary evaporated and dried under vacuum. The crude product was purified by column chromatography on a silica gel column (13 cm X 55 mm) eluted with hexane:EtOAc (85:15) and then rotary evaporated and dried under vacuum (55% yield). Rf = 0.3 (hexane:EtOAc; 85:15). ^{1}H-NMR($CDCl_3$, 500 MHz): δ7.84 (d, 2 H, aromatic), δ7.50 (d, 2 H, aromatic), δ7.46 (t, 1 H, aromatic), δ7.01 (m, 2 H, aromatic), δ4.51 (s, 2 H). ^{13}C-NMR($CDCl_3$): carbonyl (δ188.1), aromatic (δ160.8, 158.8, 143.9, 136.6, 132.0, 130.0, 129.4, 111.9), alkyl bromide (δ31.8).

Ethyl α-acetamido-α-cyano-β-(2',4'-difluoro-4-benzophenone)-DL-propionate (6). A mixture of **5** (172 mg, 0.55 mmol), ethyl acetamidocyanoacetate (142 mg, 0.83 mmol), 18-crown-6 (15.8 mg, 0.06 mmol), and K_2CO_3 (231 mg, 1.67 mmol) in CH_3CN (8 ml) was stirred at rt under argon for 3 h. The solution was filtered through a celite cake, concentrated, and dried under vacuum. The crude product was washed with hexane to remove brown impurities and dried under vacuum (56% yield). Rf = 0.15 (hexane:EtOAc; 50:50). ^{1}H-NMR($CDCl_3$, 500 MHz): δ7.83 (d, 2H, aromatic), δ 7.48 (t, 1 H, aromatic), δ 7.41 (d, 2 H, aromatic), δ 7.01 (d, 2

H, aromatic), δ 6.90 (s, 1 H, –NH), 4.30 – 4.05 (m, 2 H, methylene of ethyl ester), δ 3.60 and δ 3.50 (dd, 2 H, hydrogens on C_β), δ 2.05 (s, 3 H, methyl of ketone), δ 1.22 (doublet of triplets, 3 H, ester methyl). ^{13}C-NMR($CDCl_3$): carbonyl (δ188.6, 170.1, 165.3), aromatic (160.7, 158.7, 138.4, 136.7, 132.3, 130.6, 130.0, 111.9), aromatic C–F (δ77.0), alkyl (δ64.0, 57.4, 41.3, 22.6, 13.7).

2',6'-difluoro-*p*-benzoyl-phenylalanine (7). A mixture of **6** (0.12 g) and 10 ml of a 1:1 solution of 8 M HCl and CF_3COOH was refluxed with stirring at 150 °C for 24 hours. The reaction mixture was lyophilized to collect the crude product. Lingering trifluoroacetate ion was exchanged for chloride ion by suspending the crude product in ~ 20 mL 1 M HCl and lyophilizing the resulting solution. The product was dissolved in 1 M NaOH and filtered. The clear solution was adjusted to a pH of 7 by addition of 1 M HCl and the precipitate was collected by centrifugation and washed with cold water (2 x 10 mL). The remaining white solid was dried under vacuum to obtain ~ 50 mg of a white powder. ^{1}H-NMR: δ7.71 (d, 2 H, aromatic), δ7.67 (t, 1 H, aromatic), δ7.47 (d, 2 H, aromatic), δ7.29 (t, 2 H, aromatic), δ3.44 (dd, 3 H, NH_3^+), δ 3.31 (m, 1 H, $CHCH_2$), δ3.21 (dd, 1 H, $CHCH_ACH_B$), δ2.95 (dd, 1 H, $CHCH_ACH_B$). ^{13}C-NMR: carbonyl (δ188.2), carboxylic (δ168.8), aromatic (161.2, 159.2, 157.7, 145.8, 134.4, 130.4, 129.3, 112.3), alkyl (δ55.0). MS ES (cal 305.0) pos. ion 306.1, neg. ion 304.1.

Synthesis of 4'-iodo-*p*-benzoyl-*DL*-phenylalanine (4I-Bpa)



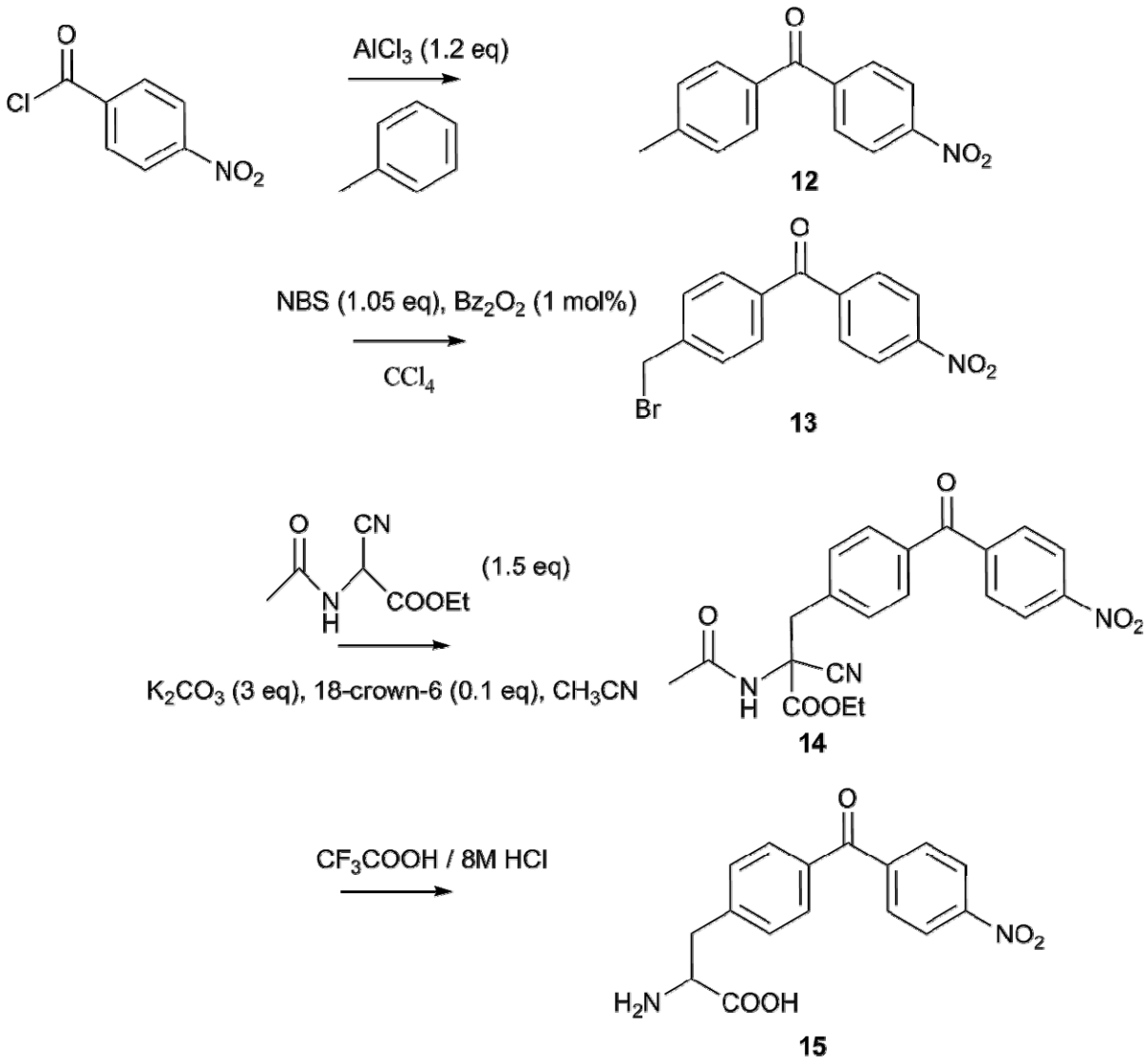
4-methyl-4'-iodo-benzophenone (8). Aluminum chloride (3.070 g, 23.02 mmol) was slowly added to a stirred solution of 4'-iodobenzoyl chloride (4.961 g, 18.61 mmol) in toluene (50 mL). The solution was stirred at rt under argon for 1 h, then water was added dropwise to quench the reaction. The solution was stirred for an additional 15 minutes before being washed with water (2 x 50 mL), 10% $NaHCO_3$ (2 x 50 mL), and brine (2 x 50 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered through a celite cake, and rotary evaporated, and dried under vacuum to obtain a white solid. The crude product was recrystallized from hexane and dried under vacuum to obtain a white solid (4.362 g, 13.5 mmol, 72.7% yield). Rf = 0.5 (hexane:EtOAc; 85:15). ^{1}H-NMR($CDCl_3$, 500 MHz): δ7.82 (d, 2 H, aromatic), δ7.66 (d, 2 H, aromatic), δ7.48 (d, 2 H, aromatic), δ7.26 (d, 2 H, aromatic), δ2.42 (s, 3 H, methyl).

4-bromomethyl-4'-iodobenzophenone (9). A mixture of **8** (3.096 g, 9.61 mmol), recrystallized N-bromosuccinimide (1.799 g, 10.10 mmol), and benzoyl peroxide (25 mg, 0.10 mmol) in CCl_4 (50 ml) was refluxed at ~150^oC under argon for 3.5 h. The hot solution was filtered and rinsed with hot CCl_4 (20 ml) until the crystals became colorless. The filtrate was cooled to rt and filtered again to completely remove residual succinimide. The filtrate was rotary evaporated and dried under vacuum. The crude product was recrystallized from hexane and dried under vacuum to obtain a purplish white solid (2.736 g, 6.823 mmol, 70.9% yield). Rf = 0.4 (hexane:EtOAc; 85:15). ^{1}H-NMR($CDCl_3$, 500 MHz): δ7.84 (d, 2 H, aromatic), δ7.50 (d, 2 H, aromatic), δ7.49 (d, 2 H, aromatic), δ7.52 (d, 2 H, aromatic), δ4.51 (s, 2 H).

Ethyl α-acetamido-α-cyano-β-(4'-iodo-4-benzophenone)-DL-propionate (10). A mixture of **9** (916 mg, 2.28 mmol), ethyl acetamidocyanoacetate (668 mg, 3.88 mmol), 18-crown-6 (70 mg), and K_2CO_3 (1.09 g) in CH_3CN (25 ml) was stirred at rt under argon for 3 h. The solution was filtered through a celite cake, concentrated, and dried under vacuum.

4'-fluoro-p-benzoyl-phenylalanine (11). The crude product **10** and 50 ml of a 1:1 solution of 8 M HCl and CF_3COOH was refluxed with stirring at ~150 °C for 24 hours. The lingering CF_3COOH was removed by blowing air into the reaction flask with stirring. The reaction mixture was lyophilized to collect the crude product. Lingering trifluoroacetate ion was exchanged for chloride ion by suspending the crude product in ~ 20 mL 1 M HCl and lyophilizing the resulting solution. The product was dissolved by the dropwise addition of 8 M NaOH to pH ~10. The solution was centrifuged to remove insoluble organics, and the clear solution was adjusted to pH ~7 by dropwise addition of 2 M HCl and the precipitate was collected and washed with cold water (2 x 10 mL). The remaining solid was dried under vacuum to obtain a white powder. ^{1}H-NMR DMSO: δ7.96 (d, aromatic, 2 H), δ7.65 (d, aromatic, 2 H), δ7.47 (dd, aromatic, 2 H), δ7.28 (bs, 2 H, NH_2), δ3.43 (dd, 1 H, $CHCH_ACH_B$), δ3.22 (dd, 1 H, $CHCH_ACH_B$), δ3.0 (m, 1 H, $CHCH_2$).

Synthesis of 4'-nitro-*p*-benzoyl-DL-phenylalanine (4nitro-Bpa)



4-methyl-4'-nitro-benzophenone (12). Aluminum chloride (12.37 g, 92.78 mmol) was slowly added to a stirring solution of 4'-nitrobenzoyl chloride (14.13 g, 76.18 mmol) in toluene (75 mL). The solution was stirred at rt under argon for 1 h, then water was added dropwise to quench the reaction. The solution was further stirred for an additional 15 minutes before being washed with water (2 x 50 mL), 10% $NaHCO_3$ (2 x 50 mL), and brine (2 x 50 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered through a celite cake, and rotary evaporated, and dried under vacuum to obtain a yellow solid. The crude product was recrystallized from hexane and dried under vacuum to obtain a yellow solid (12.97 g, 53.8 mmol, 70.9% yield). Rf = 0.4 (hexane:EtOAc; 85:15). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ8.29 (d, 2 H, aromatic), δ7.88 (d, 2 H, aromatic), δ7.88 (d, 2 H, aromatic), δ7.29 (d, 2 H, aromatic), δ2.44 (s, 3 H, methyl).

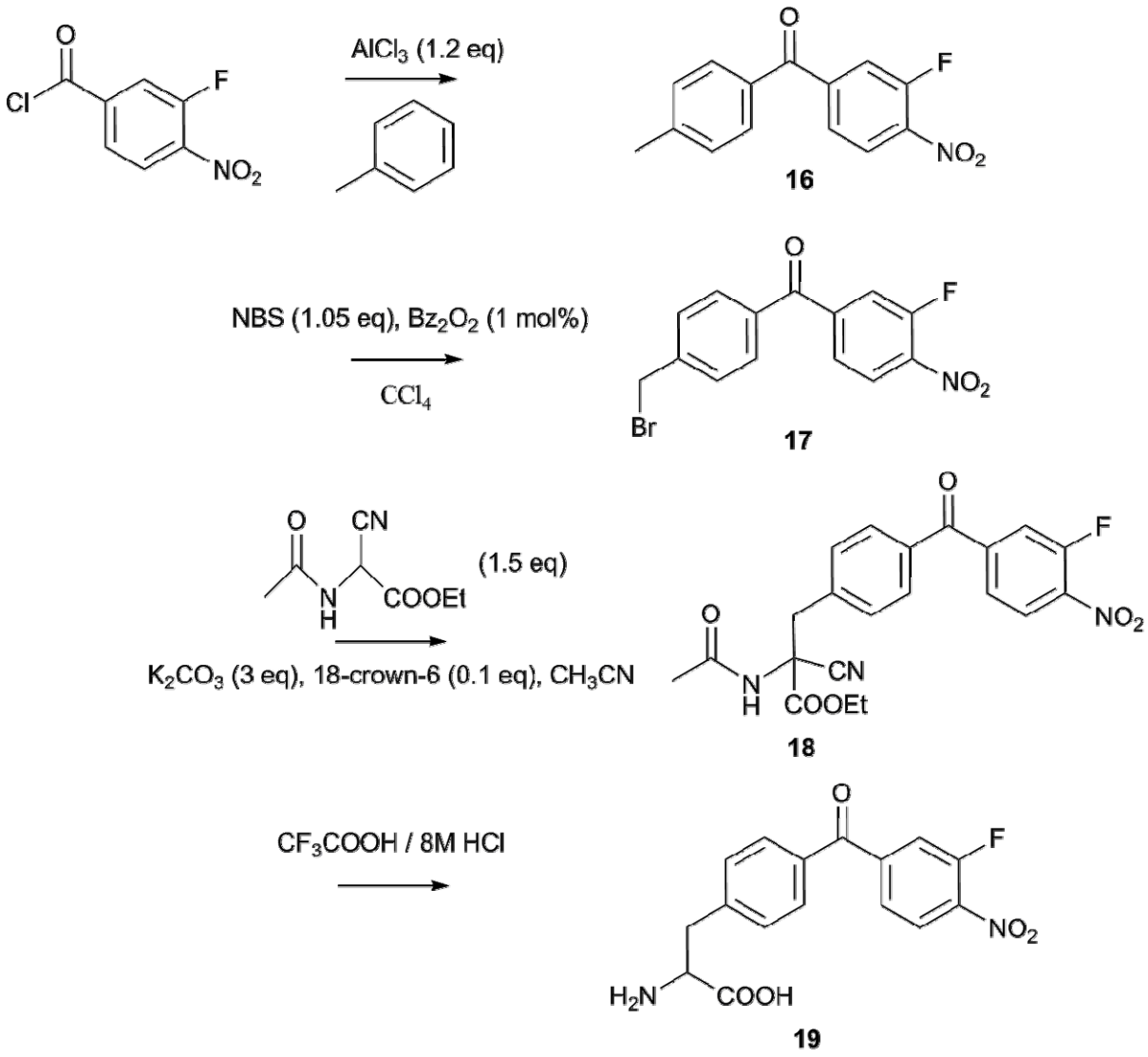
4-bromomethyl-4'-nitrobenzophenone (13). A mixture of **12** (4.497 g, 18.66 mmol), recrystallized N-bromosuccinimide (3.487 g, 19.59 mmol), benzoyl peroxide (46.7 mg, 0.191 mmol) in CCl_4 (50 ml) was refluxed at ~150 °C under argon for 4 h. The hot solution was

filtered and rinsed with hot CCl_4 (20 ml) until the crystals became colorless. The filtrate was cooled to rt and filtered again to completely remove residual succinimide. The filtrate was rotary evaporated and dried under vacuum. The crude product was recrystallized from hexane and dried under vacuum to obtain a yellow solid (5.079 g, 15.87 mmol, 85.0% yield). Rf = 0.3 (hexane:EtOAc; 85:15). ^{1}H-NMR ($CDCl_3$, 500 MHz): δ8.34 (d, 2 H, aromatic), δ7.93 (d, 2 H, aromatic), δ7.79 (d, 2 H, aromatic), δ7.52 (d, 2 H, aromatic), δ4.52 (s, 2 H).

Ethyl α-acetamido-α-cyano-β-(4'-nitro-4-benzophenone)-DL-propionate (14). A mixture of **13** (3.95 g, 12.35 mmol), ethyl acetamidocyanoacetate (3.16 g, 18.58 mmol), 18-crown-6 (327 mg, 1.23 mmol), and K_2CO_3 (5.17 g, 37.4 mmol) in CH_3CN (50 mL) was stirred at rt under argon for 14 h. The solution was filtered through a celite cake, concentrated, and dried under vacuum to obtain a greenish-black crude product (3.36 g). Rf = 0.15 (hexane:EtOAc; 50:50).

4'-nitro-*p*-benzoyl-DL-phenylalanine (15). A mixture of crude protected **14** and 50 ml of a 1:1 solution of 8 M HCl and CF_3COOH was refluxed with stirring at 160 °C for 24 h. The reaction mixture was lyophilized to collect the crude product. Lingering trifluoroacetate ion was exchanged for chloride ion by suspending the crude product in ~ 50 mL 1 M HCl and lyophilizing the resulting solution. The product was dissolved in 50 mL of distilled water and dissolved by dropwise addition of 10% NaOH. The mixture was centrifuged to separate the free amino acid in the supernatant from the water-insoluble organic compounds. The pH of the solution was then adjusted to ~6 by the addition of 1 M HCl, and the precipitate was collected by centrifugation, washed with cold water (2 x 10 mL), and dried under high vacuum to obtain a yellow powder (2.93 g, 9.33 mmol, 75.5%). ^{1}H-NMR DMSO: δ7.92 (dd, aromatic, 2 H), δ7.3 (d, aromatic, 2 H), δ7.58 (d, aromatic, 2 H), δ7.54 (dd, aromatic, 2 H), δ8.5 (bs, 2 H, NH_2), δ 3.27 (dd, 1 H, $CHCH_ACH_B$), δ3.14 (dd, 1 H, $CHCH_ACH_B$), δ4.25 (m, 1 H, $CHCH_2$).

<u>Synthesis of 4'-nitro, 3'-fluoro-*p*-benzoyl-*DL* –phenylalanine (3F-4nitro-Bpa)</u>



4-methyl-4'-nitro-3'-fluoro-benzophenone (16). Aluminum chloride (0.90 g, 6.8 mmol) was added into a stirred solution of toluene (15 mL) and 4-nitro-3-fluorobenzoyl chloride (1.0 g, 5.2 mmol). The solution was stirred at rt under argon for 1 h, then 5 mL of water was added dropwise to quench the reaction. The solution was further stirred for an additional 15 minutes before being washed with water (2 x 50 mL), 10% $NaHCO_3$ (2 x 50 mL), and brine (2 x 50 mL). The organic phase was dried with anhydrous $MgSO_4$, filtered through a celite cake, and rotary evaporated, and dried under vacuum to obtain a yellow solid. The crude product was dissolved in a minimum amount of CH_2Cl_2 and hexane was added dropwise until precipitation occurred. The solution was then heated until precipitate redissolved, then cooled to rt and subsequently chilled to -20 °C. The crystals were triturated with cold hexane to obtain a yellow solid (1.10 g, 81 % yield). ^{1}H NMR($CDCl_3$, 500 MHz): δ8.15 (dd, aromatic, 2 H), δ7.71 (d, aromatic, 2 H), δ7.68 (m, aromatic, 1 H), δ7.34 (d, aromatic, 2 H), δ2.44 (s, methyl, 3 H). ^{13}C NMR: carbonyl (δ 193.0), aromatic (δ156.2, 154.2, 145.0, 144.5, 133.0, 130.5, 129.5, 126.1, 125.5, 121.5, 115.3), alkyl (δ22.2).

4-bromomethyl-4'nitro-3'-fluorobenzophenone (17). A mixture of **16** (1.1 g, 4.2 mmol), recrystallized N-bromosuccinimide (0.654 g), and benzoyl peroxide (60 mg) in CCl_4 (20 ml) was refluxed at ~150 °C under argon for 3 hours. The hot solution was filtered and rinsed with hot CCl_4 (20 ml) until the crystals became colorless. The filtrate was cooled to rt and filtered again to completely remove residual succinimide. The filtrate was rotary evaporated and dried under vacuum. The crude product was recrystallized from hexane and dried under vacuum (58% yield). ^{1}H NMR($CDCl_3$, 500 MHz): δ8.18 (dd, aromatic, 2 H), δ7.78 (d, aromatic, 2 H), δ7.68 (m, aromatic, 1 H), δ7.56 (d, aromatic, 2 H), δ4,57 (s, methyl, 3 H). ^{13}C NMR($CDCl_3$): carbonyl (δ192.4), aromatic (δ156.2, 154.0, 146.7, 144.5, 144.4, 143.7, 143.6, 135.0, 133.0 130.5, 126.5, 125.5, 119.7, 119.4), alkyl (δ31.7). ^{19}F-NMR: δ-118.5 ppm.

Ethyl α-acetamido-α-cyano-β-(4'-nitro-3'-fluoro-4-benzophenone)-DL-propionate (18). A mixture of **17** (835 mg, 2.47 mmol), ethyl acetamidocyanoacetate (630 mg), 18-crown-6 (65 mg), and K_2CO_3 (1.02 mg) in CH_3CN (15 ml) was stirred at rt under argon for 3 h. The solution was filtered through a celite cake, concentrated, and dried under vacuum. The crude product was washed with hexane to remove brown impurities and dried under vacuum (95% yield). Rf = 0.05 (hexane:EtOAc; 50:50). ^{1}H-NMR($CDCl_3$, 500 MHz): δ8.18 (dd, aromatic, 2 H), δ7.78 (d, aromatic, 2 H), δ7.68 (m, aromatic, 1 H), δ7.41 (d, aromatic, 2 H), δ4.35 – 4.20 (m, 2 H, methylene of ethyl ester), δ 3.72 and δ 3.52 (dd, 2 H, hydrogens on C_β), δ 3.62 (s, 3 H, methyl), δ 1.32 (doublet of triplets, 3 H, ester methyl). ^{13}C-NMR($CDCl_3$): carbonyl (δ1942.5, 171.2, 170.0), (165.4, 136.7, 156.1, 154.0, 143.5, 138.1, 135.6, 130.4, 126.4, 125.5, 119.5, 115.7, 70.0, 60.4, 57.4, 22.7, 13.8). ^{19}F-NMR: δ-118.5 ppm.

4'-nitro-3'-fluoro-*p*-benzoyl-phenylalanine (19). A mixture of **18** (1.0 g) and 30 ml of a 1:1 solution of 8 M HCl and CF_3COOH was refluxed with stirring at 180 °C for 18 hours. The reaction mixture was lyophilized to collect the crude product. Lingering trifluoroacetate ion was exchanged for chloride ion by suspending the crude product in ~ 20 mL 1 M HCl and lyophilizing the resulting solution. The product was dissolved in 1 M NaOH and filtered. The clear solution was adjusted to a pH of 7 by addition of 1 M HCl and the precipitate was collected and washed twice with cold water. The remaining yellow solid was dried under vacuum to obtain ~ 260 mg of a yellow powder. ^{1}H-NMR DMSO: δ8.25 (t, aromatic, 1 H), δ7.8 (d, aromatic, 1 H), δ7.65 (m, aromatic, 3 H), δ7.38 (dd, aromatic, 2 H), δ3.55 (m, 1 H, $CHCH_2$), δ3.25 (dd, 1 H, $CHCH_ACH_B$), δ3.25 (dd, 1 H, $CHCH_ACH_B$). ^{13}C-NMR: δ192.9, 169.6, 155.3, 153.2, 144.2, 144.0, 138.8, 133.8, 130.0, 123.0, 126.7, 125.7, 119.0, 118.9, 55.0, 36.9. ^{19}F-NMR: δ-115.86 ppm. MS ES (cal 332.0) pos. ion 333.1, neg. ion 331.1.

Mutant Construction

Using a tRNA-synthetase (RS) gene-complementary primer containing a site mutation (fig. 1) and a downstream reverse-complimentary primer (*pDuleRev*) to the *pDule* vector (5' – GGTCGACGGCGCTATTCAG – 3'), the back portion of the mutant gene was amplified using PCR. Similarly, using the reverse compliment of the mutagenic primer and a downstream primer (*pDuleFor*) complimentary to the vector (5' – CGTCACTGCGTCTTTTACTG – 3'), the front portion of the mutant gene was amplified. The amplification products were combined and amplified using overlap PCR with *pDuleRev* and *pDuleFor*. The full length product and a pDule vector were each digested with NcoI and SacI restriction enzymes, then ligated together to

complete the construction of the new RS construct in *pDule* vector. Genes were sequenced after mutation to confirm success.

Figure 1. Sequences of mutagenic primers (5' – 3') used to construct synthetase mutants.

Synthetase	Forward Sequence	Reverse Compliment Sequence
Bpa RS A31V, T158S	*	*
Bpa RS T158S	GCAGGTTAATAGCAGTCATTATCTGGGCG	CGCCCAGATAATGACTGCTATTAACCTGC
Bpa RS L162A	CGAGTCATTATGCGGGCGTTGATG	CATCAACGCCCGCATAATGACTCG
Bpa RS V164A	CATTATCTGGGCGCGGATGTTGCAG	CTGCAACATCCGCGCCCAGATAATG
Nap RS V167A	GCGTTGATGTTGCGGTTGGAGGGATGG	CCATCCCTCCAACCGCAACATCAACGC
Nap RS L32A	GATGAAAAATCTGCTGCCATAGGTTTTGAACCAAGTGG	CCACTTGGTTCAAAACCTATGGCAGCAGATTTTTCATC
Nap RS L32G	GATGAAAAATCTGCTGGGATAGGTTTTGAAC	GTTCAAAACCTATCCCAGCAGATTTTTCATC
Nap RS L32V	GATGAAAAATCTGCTGTGATAGGTTTTGAACCAAGTGG	CCACTTGGTTCAAAACCTATCACAGCAGATTTTTCATC

* Mutation identified by sequencing on one of the T158S constructs.