Electronic Supplementary Information

Fluorinated aminoanthranilamides: Non-native amino acids for bringing proteomic approaches to charge-transfer systems

Jillian M. Larsen-Clinton,[a] Eli M. Espinoza,[b] Maximillian F. Mayther,[b] John Clark,[a] Christina Tao,[a] Duoduo Bao,[a] Christa M. Larino,[a] Michelle Wurch,[ac] Stephanie Lara[a] and Valentine I. Vullev*[abcd]

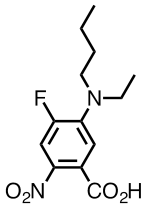
[a] Department of Bioengineering, [b] Department of Chemistry, [c] Materials Science and Engineering Program, and [d] Department of Biochemistry, University of California, Riverside, CA 92507, USA.

1. Materials

1.1. General methods

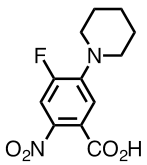
All chemicals were used as received unless otherwise specified. The reported ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and NOESY spectra were recorded on a 400 MHz spectrometer. ^{1}H chemical shifts (δ) are reported in ppm relative to $CHCl_3$ in $CDCl_3$ (δ = 7.24 ppm) and DMSO-d_5 in DMSO-d_6 (δ = 2.50 ppm); ^{13}C δ are reported in ppm relative to $CDCl_3$ (δ = 77.23 ppm) and DMSO-d_6 (δ = 39.51 ppm); and ^{19}F δ are reported in ppm relative to an internal standard, trifluorotoluene ($C_6H_5CF_3$, δ = –63.90 ppm), that was added in mM quantities only to samples for ^{19}F NMR analyses. Data for ^{1}H NMR and ^{19}F NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet/quintet, h = hextet/sextet, e = eptet(from επτά)/heptet, m = multiplet), and coupling constants. All ^{13}C NMR spectra were recorded with complete proton decoupling; nevertheless, fluorine causes splitting in the signals of five of the aromatic carbons (all but the one *para* to the fluorine), and sometime of the two carbons directly attached to the amine nitrogen (*ortho* to the fluorine) due to inherent long-range ^{19}F-^{13}C coupling.[1] High-resolution mass spectrometry (HRMS) was performed using Agilent LCTOF (6200) mass spectrometer (Agilent Technologies, Santa Clara, CA). Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 60 Å, 32−63 μm silica gel.

1.2. Synthesis of the 5-amino-4-fluoro-2-nitrobensoic acid derivatives, $(NO_2)Aa(CO_2H)$ ((*i*), Scheme 1)

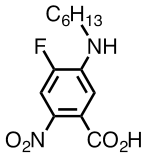


5-(butyl(ethyl)amino)-4-fluoro-2-nitrobenzoic acid, $(NO_2)Feb(CO_2H)$ *(microwave procedure).* 4,5-Difluoro-2-nitrobenzoic acid, DFNBA (203 mg, 1 mmol) and *N*-ethylbutylamine (820 μl, 6 mmol) were slowly mixed in a glass microwave vial, forming a dark brown solution. The mixture was microwaved for 70 min at 80 watts, 80 °C. The progress of the reaction was monitored using TLC, and in some occasions, using ^{19}F NMR. After cooling to room temperature, the mixture was diluted with DCM (50 ml) and washed with 2% HCl (3×50 ml). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to produce 273 mg yellow powder (0.96 mmol, 96% yield) of $(NO_2)Feb(CO_2H)$. ^{1}H-NMR ($CDCl_3$) δ/ppm: 10.36 (1H, $s_{(broad)}$), 7.75 (1H, d, J = 14.4 Hz), 6.84 (1H, d, J = 8.6 Hz), 3.46 (2H, q, J = 6.2 Hz), 3.37 (2H, t, J = 7.3 Hz), 1.61 (2H, p, J = 7.7 Hz), 1.35 (2H, h, 7.5 Hz), 1.23 (3H, t, J = 7.0 Hz), 0.95 (3H, t, J = 7.3 Hz)); ^{13}C-NMR ($CDCl_3$) δ/ppm: 172.17, 150.56 (d, J = 252 Hz), 142.49 (d, J = 7.4 Hz), 134.67 (d, J = 8.8 Hz), 126.73, 115.0 (d, J = 5.9 Hz), 114.64 (d, J = 29.0 Hz), 52.28 (d, J = 5.9 Hz), 47.52 (d, J = 5.9 Hz), 30.36, 20.32, 14.05, 13.30; HRMS *m/z* calculated for $C_{13}H_{18}FN_2O_4^+$ $(M+H)^+$ 285.1251, found 285.1245 $(M+H)^+$.

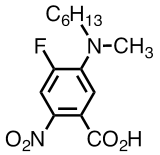
(scaled-up, conventional-heating procedure) 8.16 g of DFNBA (40.2 mmol) was placed in a glass pressure tube. While purging with argon, 16.6 ml of *N*-Ethylbutylamine (121 mmol) was added to the pressure tube. The pressure tube was sealed with a screw cap and immersed in an oil bath with a temperature regulator. The temperature was raised to 110 °C and kept for 20 hrs. The reaction mixture was allowed to cool to room temperature, diluted with 5% HCl, and extracted with DCM. The organic layers were combined, dried over anhydrous Na_2SO_4, and the solvents were evaporated *in vacuo* to afford 11.1 g light brown solid (39 mmol, 97% yield) of (NO_2)Feb(CO_2H), confirmed with NMR (Fig. 2c) and HRMS. For further purification, 5.2 g of the crude solid was placed in a round bottom flask; DCM (15 ml) was added to it and the mixture was sonicated for 5 minutes until becoming clear. The solution was diluted with 300 ml hexanes and kept in a refrigerator overnight, which led to the formation of yellow crystalline precipitate. The mixture was allowed to reach room temperature and the precipitate was collected using vacuum filtration and dried to afford 4.46 g light brown solid.



4-fluoro-2-nitro-5-(piperidin-1-yl)benzoic acid, (NO_2)Fpi(CO_2H). DFNBA (203 mg, 1 mmol) and piperidine (590 µl, 6 mmol) were slowly mixed in a glass microwave vial, forming a dark brown solution. The mixture was microwaved for 1 h at 80 watts, 80 °C. The progress of the reaction was monitored using TLC, and in some occasions, using ^{19}F NMR. After cooling to room temperature, the mixture was diluted with DCM (50 ml) and washed with 2% HCl (3×50 ml). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to produce a dark yellow oil. Purification was performed using flash chromatography: stationary phase – silica gel; eluent gradient – from 100% hexanes to 80% hexanes / 20% ethyl acetate. After the 80%/20% ratio was reached, 1% acetic acid was added to the solvent mixture to elute the product. The collected fraction was concentrated *in vacuo*, washed with dionized water (50 ml), dried over anhydrous Na_2SO_4 and the rest of the organic solvents were removed *in vacuo* to afforded 260 mg yellow powder (0.97 mmol, 97% yield) of (NO_2)Fpi(CO_2H). ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.72 (1H, d, *J* = 12.9 Hz), 7.04 (1H, d, *J* = 8.2 Hz), 3.28 (4H, t, *J* = 5.4 Hz), 1.72 (4H, m), 1.65 (2H, m); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 170.79, 153.20 (d, *J* = 253 Hz), 145.06 (d, *J* = 7.2 Hz), 137.83 (d, *J* = 8.4 Hz), 126.26 (d, *J* = 3.6 Hz), 117.67 (d, *J* = 5.4 Hz), 113.56 (d, *J* = 27.6 Hz), 50.99 (d, *J* = 5.4 Hz), 25.76, 24.04; HRMS *m/z* calculated for $C_{12}H_{14}FN_2O_4^+$ $(M+H)^+$ 269.0938, found 269.0926 $(M+H)^+$.

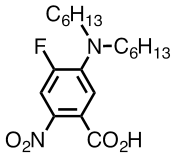


4-fluoro-5-(hexylamino)-2-nitrobenzoic acid, (NO_2)Fhx(CO_2H). DFNBA (203 mg, 1 mmol) and 1-hexylamine (790 µl, 6 mmol) were slowly mixed in a glass microwave vial, forming a dark brown solution. The mixture was microwaved for 1 h at 80 watts, 80 °C. The progress of the reaction was monitored using TLC. After cooling to room temperature, the mixture was diluted with DCM (50 ml) and washed with 2% HCl (3×50 ml). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to produce a dark orange-red oil. Recrystalization from hexanes resulted in 200 mg orange solid (0.70 mmol, 70% yield) of (NO_2)Fhx(CO_2H). ^{1}H-NMR (DMSO-d_6) *δ*/ppm: 7.86 (1H, d, *J* = 12.1 Hz), 7.20 (1H, t, *J* = 5.0 Hz), 6.78 (1H, d, *J* = 8.6 Hz), 3.23 (2H, t, *J* = 6.6 Hz), 1.55 (2H, p, *J* = 7.0 Hz), 1.29 (6H, m), 0.86 (3H, t, *J* = 6.6 Hz); ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.78 (1H, d, *J* = 11.4 Hz), 6.77 (1H, d, *J* = 7.9 Hz), 3.26 (2H, t, *J* = 7.2 Hz), 1.68 (2H, p, *J* = 7.3 Hz), 1.40 (2H, p, *J* = 7.5 Hz), 1.33 (4H, m), 0.89 (3H, t, *J* = 7.0 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 171.7, 149.44 (d, *J* = 246 Hz), 142.17 (d, *J* = 11.8 Hz), 134.17 (d, *J* = 7.6 Hz), 128.78, 111.87 (d, *J* = 24.0 Hz), 109.55 (d, *J* = 4.5 Hz), 43.42, 31.62, 29.16, 26.78, 22.75, 14.19; HRMS *m/z* calculated for $C_{13}H_{16}FN_2O_4^-$ $(M-H)^-$ 283.1094, found 283.1089 $(M-H)^-$.



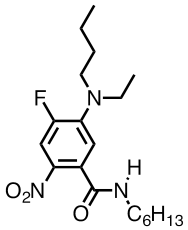
4-fluoro-5-(hexyl(methyl)amino)-2-nitrobenzoic acid, (NO_2)Fmx(CO_2H). Employing the procedure for (NO_2)Fpi(CO_2H), while using *N*-hexylmethylamine (910 µl, 6 mmol) instead of piperidine, resulted in 290 mg yellow powder (0.97 mmol, 97 %

yield) of (NO_2)Fmx(CO_2H). ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.75 (1H, d, *J* = 14.4 Hz), 6.83 (1H, d, *J* = 8.2 Hz), 3.43 (2H, t, *J* = 7.8 Hz), 3.08 (3H, d, *J* = 2 Hz), 1.61 (2H, p, *J* = 6.8 Hz), 1.29 (6H, s), 0.87 (3H, t, *J* = 6.6 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 172.36, 150.69 (d, *J* = 250 Hz), 143.61 (d, *J* = 7.4 Hz), 134.98 (d, *J* = 8.1 Hz), 126.71, 115.01 (d, *J* = 5.2 Hz), 114.22 (d, *J* = 28.0 Hz), 55.01 (d, *J* = 7.4 Hz), 40.29, 31.67, 27.97, 26.60, 22.72, 14.13; HRMS m/z calculated for $C_{14}N_2O_4FH_{20}^+$ $(M+H)^+$ 299.1407, found 299.1401 $(M+H)^+$.

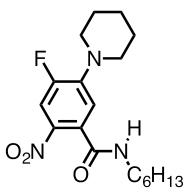


5-(dihexylamino)-4-fluoro-2-nitrobenzoic acid, (NO$_2$)Fdx(CO$_2$H). Employing the procedure for (NO_2)Fpi(CO_2H), while using dihexylamine (1.4 ml, 6 mmol) instead of piperidine, 4-hour microwave heating, and a gradient to 80% ethyl acetate instead of 20% for the flash chromatography, resulted in 290 mg yellow powder (0.79 mmol, 79% yield) of (NO_2)Fdx(CO_2H). ^{1}H-NMR (DMSO-d_6) *δ*/ppm: 7.87 (1H, d, *J* = 14.8 Hz), 6.89 (1H, d, *J* = 9.0 Hz), 3.39 (4H, t, *J* = 7.4 Hz), 1.53 (4H, p, *J* = 6.8 Hz), 1.26 (12H, m), 0.85 (6H, t, *J* = 6.6 Hz); ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 11.15 (1H, $s_{(broad)}$), 7.74 (1H, d, *J* = 14.0 Hz), 6.82 (1H, d, *J* = 8.5Hz), 3.37 (4H, t, *J* = 7.7 Hz), 1.60 (4H, p, *J* = 7.1 Hz), 1.29 (12H, m), 0.88 (6H, t, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 172.34, 150.53 (d, 251 Hz), 142.60 (d, 8.8 Hz), 134.63 (d, 8.8 Hz), 126.81, 115.13 (d, 5.9 Hz), 114.65 (d, 24.5 Hz), 53.17 (d, 5.9 Hz), 31.10, 28.12, 26.74, 22.78, 14.19; HRMS m/z calculated for $C_{19}H_{30}FN_2O_4^+$ $(M+H)^+$ 369.2190, found 369.2184 $(M+H)^+$.

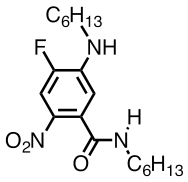
1.3. Synthesis of the 5-amino-4-fluoro-*N*-hexyl-2-nitrobenzamide derivatives, (NO$_2$)Aa ((*ii*), Scheme 1)



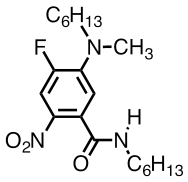
5-(butyl(ethyl)amino)-4-fluoro-N-hexyl-2-nitrobenzamide, (NO$_2$)Feb. (NO_2)Feb(CO_2H) (142 mg, 0.5 mmol) was placed in a baked round bottom flask with a stir bar, and blanked with N_2. Anhydrous DCM (3.5 ml) and 5 drops of amine-free dry DMF were added, and the reaction was cooled down in a dry ice/acetone bath. While stirring, oxalyl chloride (130 µl, 1.5 mmol) was added drop-wise and allowed to react for 30 min. The progress of the reaction was monitored using TLC, i.e., a drop of the reaction was quenched with dry methanol to form methyl ester that shows distinctly different R_f values than the starting material. After the completion of the reaction, the mixture was concentrated *in vacuo* followed by resuspension in dry DCM (3×2.5 ml) and dried *in vacuo*. 1-Hexylamine (460 µl, 3.5 mmol) dissolved in dry DCM (3.5 ml) was blanketed with N_2 and cooled in a dry ice/acetone bath. The carboxylic chloride was suspended in 5 ml dry DCM and added drop-wise to the cold amine solution. The reaction mixture was allowed to slowly reach room temperature. The progress of the reaction was monitored with TLC. Upon completion, the reaction mixture was dissolved in 45 ml DCM, washed with 2% HCl (3×25 ml), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to afford dark yellow solid. Purification using flash chromatography (stationary phase: silica gel; eluent gradient: from 100 % hexanes to 60 % hexanes / 40% ethyl acetate) produced 180 mg yellow solid (0.48 mmol, 96%) of (NO_2)Feb. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.77 (1H, d, *J* = 14.8 Hz), 6.61 (1H, d, *J* = 8.8 Hz), 5.72 (1H, t, *J* = 5.5 Hz), 3.41 (4H, m), 3.33 (2H, t, *J* = 7.8 Hz), 1.58 (4H, h, *J* = 7.1 Hz), 1.31 (8H, m), 1.19 (3H, t, *J* = 7.1 Hz), 0.92 (3H, t, *J* = 7.3 Hz), 0.87 (3H, t, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 167.37, 149.93 (d, *J* = 248 Hz), 142.70 (d, *J* = 7.4 Hz), 133.57 (d, *J* = 7.4 Hz), 132.2, 114.67 (d, *J* = 5.9 Hz), 114.43, 52.24 (d, *J* = 5.9 Hz), 47.49 (d, *J* = 5.9 Hz), 40.56, 31.66, 30.31, 29.31, 26.80, 22.76, 20.29, 14.21, 14.02, 13.30; HRMS *m/z* calculated for $C_{19}H_{31}FN_3O_3^+$ $(M+H)^+$ 368.2349, found 368.2344 $(M+H)^+$.



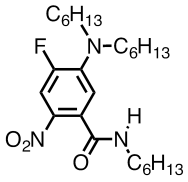
4-fluoro-N-hexyl-2-nitro-5-(piperidin-1-yl)benzamide, (NO_2)Fpi. Employing the procedure for (NO_2)Feb, while starting with (NO_2)Fpi(CO_2H) (134 mg, 0.5 mmol), and using 7 ml dry DCM for making the acyl chloride, and a flash chromatography gradient to hexanes to 80 % hexanes / 20% ethyl acetate, resulted in 160 mg yellow solid (0.45 mmol, 89%) of (NO_2)Fpi. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.75 (1H, d, *J* = 13.4 Hz), 6.78 (1H, d, *J* = 8.3 Hz), 5.81 (1H, t, *J* = 5.5 Hz), 3.37 (2H, q, *J* = 6.9 Hz), 3.25 (4H, t, *J* = 5.4 Hz), 1.72 (4H, m), 1.62 (4H, m), 1.32 (6H, m), 0.86 (3H, t, *J* = 6.4 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 167.11, 152.52 (d, *J* = 251 Hz), 145.73 (d, *J* = 5.9 Hz), 136.25 (d, *J* = 5.9 Hz), 117.16, 113.90, 113.64, 51.06 (d, *J* = 5.9 Hz), 40.59, 31.64, 28.30, 26.60, 25.92, 24.22, 22.75, 14.19; HRMS *m/z* calculated for $C_{18}H_{27}FN_3O_3^+$ $(M+H)^+$ 352.2036, found 352.2031 $(M+H)^+$.



4-fluoro-N-hexyl-5-(hexylamino)-2-nitrobenzamide, (NO_2)Fhx. Employing the procedure for (NO_2)Feb, while starting with (NO_2)Fhx(CO_2H) (142 mg, 0.5 mmol), and using 7 ml dry DCM for making the acyl chloride, 760 µl 1-hexylamine (5.75 mmol), and a flash chromatography gradient to hexanes to 70 % hexanes / 30% ethyl acetate, resulted in 95 mg yellow solid (0.46 mmol, 92%) of (NO_2)Fhx. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.78 (1H, d, *J* = 9.2 Hz), 7.41 (1H, t, *J* = 4.9 Hz), 7.32 (1H, d, *J* = 7.0 Hz), 6.26 (1H, d, *J* = 4.5 Hz), 3.30 (2H, t, *J* = 6.9 Hz), 3.10 (2H, t, *J* = 7.0 Hz), 1.55 (2H, h, *J* = 7.2 Hz), 1.44 (2H, p, *J* = 6.4 Hz), 1.28 (12H, m), 0.82 (6H, t, *J* = 7.0 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 165.12, 157.38 (d, *J* = 254 Hz), 144.89 (d, *J* = 5.9 Hz), 136.42 (d, *J* = 14.7 Hz), 130.50, 127.89, 112.95 (d, *J* = 26.5 Hz), 52.00, 39.74, 31.70, 31.55, 31.47, 29.21, 26.68, 26.39, 22.67, 22.61, 14.12, 14.04; HRMS *m/z* calculated for $C_{19}H_{29}FN_3O_3^-$ $(M-H)^-$ 366.2193, found 366.2198 $(M-H)^-$.

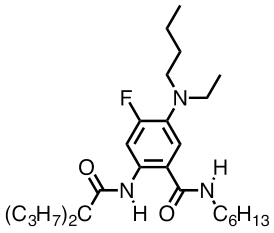


4-fluoro-N-hexyl-5-(hexyl(methyl)amino)-2-nitrobenzamide, (NO_2)Fmx. Employing the procedure for (NO_2)Feb, while starting with (NO_2)Fmx(CO_2H) (285 mg, 1 mmol), and using 170 µl oxalyl chloride (2 mmol) and 7 ml dry DCM for making the acyl chloride, and 620 µl 1-hexylamine (4.7 mmol) with 5 ml dry DCM for the amide coupling, resulted in 324 mg yellow solid (0.84 mmol, 84%) of (NO_2)Fmx. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.75 (1H, d, *J* = 14.8 Hz), 6.59 (1H, d, *J* = 8.6 Hz), 6.09 (1H, t, *J* = 5.5 Hz), 3.36 (4H, m), 3.01 (3H, d, *J* = 2.0 Hz), 1.57 (4H, p, *J* = 7.2 Hz), 1.28 (12H, m), 0.83 (6H, t, *J* = 6.8 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 167.32, 150.11 (d, *J* = 259 Hz), 143.90 (d, *J* = 7.4 Hz), 134.02, 132.12, 114.74 (d, *J* = 4.6 Hz), 114.25 (d, *J* = 27.5 Hz), 55.07 (d, *J* = 8.1 Hz), 40.60, 40.34, 31.72, 31.67, 29.33, 28.02, 26.83, 26.65, 22.77, 14.22; HRMS *m/z* calculated for $C_{20}H_{34}FN_3O_3^+$ $(M+H)^+$ 383.2584, found 383.2532 $(M+H)^+$.

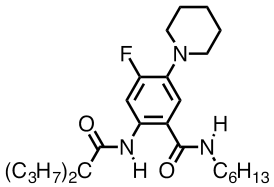


5-(dihexylamino)-4-fluoro-N-hexyl-2-nitrobenzamide. (NO_2)Fdx. Employing the procedure for (NO_2)Feb, while starting with (NO_2)Fdx(CO_2H) (250 mg, 0.67 mmol), and using 260 µl oxalyl chloride (3 mmol) and 7 ml dry DCM for making the acyl chloride, 930 µl 1-hexylamine (7 mmol) with 7 ml dry DCM for the amide coupling, and a flash chromatography gradient to hexanes to 80 % hexanes / 20% ethyl acetate, resulted in 264 mg yellow solid (0.59 mmol, 87%) of (NO_2)Fdx. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 7.79 (1H, d, *J* = 14.9 Hz), 6.60 (1H, d, *J* = 8.7 Hz), 5.63 (1H, t, *J* = 5.6 Hz), 3.42 (2H, q, *J* = 6.8 Hz), 3.33 (4H, t, *J* = 7.5 Hz), 1.59 (6H, m), 1.30 (18H, m), 0.87 (9H, t, *J* = 6.8 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 167.40, 149.79 (d, *J* = 248 Hz), 142.78 (d, *J* = 6.6 Hz), 133.35 (d, *J* = 7.7 Hz), 132.16, 114.68, 114.40, 53.16 (d, *J* = 5.6 Hz), 40.51, 31.67, 29.30, 28.08, 26.80, 26.72, 22.76, 14.20, 14.16; HRMS *m/z* calculated for $C_{25}H_{43}FN_3O_3^+$ (M+H) 452.3288, found 452.3250 $(M+H)^+$.

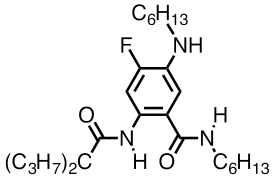
1.4. Synthesis of the 5-amino-4-fluoro-*N*-hexyl-2-(alkaneamido)benzamide derivatives, Aa ((*iii*) and (*iv*), Scheme 1)



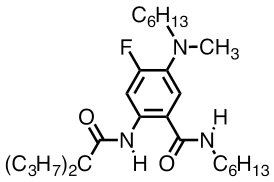
5-(butyl(ethyl)amino)-4-fluoro-N-hexyl-2-(2-propylpentanamido)benzamide, Feb. (NO_2)Feb (165 mg, 0.44 mmol) was suspended in ethyl acetate with 40 mg Pd/C. The mixture was stirred overnight under a hydrogen atmosphere at room temperature. The completion of the reduction led to a color change from yellow to colorless and appearance of blue fluorescence, which was monitored using TLC. Pd/C was filtered out and the ethyl acetate removed *in vacuo.* The solid was resuspended in dry DCM (5 ml), blanked with continuous flow of nitrogen and pyridine (2 ml) was added. The mixture was placed in a dry ice/acetone bath for 10 minutes and 2,2-di-*n*-propylacetyl chloride (150 µl, 0.875 mmol) was added drop-wise while stirring. The reaction was allowed to reach room temperature and upon completion, as monitored using TLC, diluted in 25 ml DCM and washed with 2% HCl (2×25) and Brine (25 ml). The organic layer was collected, dried over Na_2SO_4, concentrated *in vacuo*. Purification using flash chromatography, stationary phase: silica gel; eluent gradient: from 100 % hexanes to 80 % hexanes / 20% ethyl acetate afforded 14 mg (0.04 mmol, 9%) of Feb. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 11.01 (1H, s), 8.38 (1H, d, J = 14.8 Hz), 7.12 (1H, d, J = 7.8 Hz), 6.16 (1H, s), 3.39 (2H, q, J_1 = 6.7 Hz), 3.13 (2H, q, J_1 = 7.0 Hz), 3.05 (2H, t, J_1 = 7.8 Hz), 2.26 (1H, e, J = 4.7 Hz), 1.60 (4H, m), 1.43 (4H, m), 1.38 (8H, m), 1.25 (4H, m), 1.02 (3H, t, J = 7.0), 0.87 (9H, t, J = 7.0 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 175.55, 168.70, 158.93 (d, J = 250 Hz), 136.31 (d, J = 11.0 Hz), 131.91 (d, J = 7.7 Hz), 122.74 (d, 5.8 Hz), 116.93, 110.34 (d, J = 26.9 Hz), 53.03, 49.33, 48.13, 40.38, 35.51, 31.70, 29.71, 29.55, 26.86, 22.75, 20.96, 20.79, 14.30, 14.19; HRMS *m/z* calculated for $C_{27}H_{47}FN_3O_2^+$ $(M+H)^+$ 464.3652, found 464.3709 $(M+H)^+$.



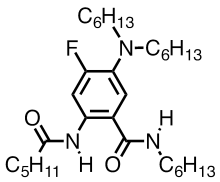
4-fluoro-N-hexyl-5-(piperidin-1-yl)-2-(2-propylpentanamido)benzamide (Fpi). Employing the procedure for Feb, while starting with (NO_2)Fpi (150 mg, 0.43 mmol) and using 110 µl 2,2-di-*n*-propylacetyl chloride (1.3 mmol), resulted in 65 mg (0.15 mmol, 36%) of Fpi. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 10.93 (1H, s), 8.39 (1H, d, J = 15.2 Hz), 7.02 (1H, d, J = 8.4 Hz), 6.29 (1H, t, J = 5.6 Hz), 3.39 (2H, td, J_1 = 7.2 Hz, J_2 = 5.8 Hz), 2.97 (4H, t, J = 5.2 Hz), 2.25 (1H, e, J = 4.9 Hz), 1.72 (4H, p, J = 5.2 Hz), 1.61 (6H, m), 1.43 (4H, m), 1.32 (8H, m), 0.88 (9H, td, J_1 = 7.2 Hz, J_2 = 4.7 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 175.42, 168.87, 157.93 (d, J = 251 Hz), 136.65 (d, J = 8.8 Hz), 135.28 (d, J = 14.7 Hz), 117.54 (d, 5.9 Hz), 117.22, 110.26 (d, J = 29.5 Hz), 52.50, 49.30, 40.35, 35.51, 31.67, 29.71, 26.83, 26.22, 24.25, 22.72, 20.93, 14.28, 14.19; HRMS *m/z* calculated for $C_{26}H4_3FN_3O_2^+$ $(M+H)^+$ 448.3339, found 448.3383 $(M+H)^+$.



4-fluoro-N-hexyl-5-(hexylamino)-2-(2-propylpentanamido)benzamide (Fhx). Employing the procedure for Feb, while starting with (NO_2)Fhx (70 mg, 0.19 mmol) and using 50 µl 2,2-di-*n*-propylacetyl chloride (0.3 mmol), resulted in 65 mg (0.14 mmol, 74%) of Fhx. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 11.50 (1H, s), 8.60 (1H, d, J = 12.9 Hz), 7.46 (1H, d, J = 7.5 Hz), 7.23 (1H, t, J = 5.7 Hz), 6.72 (1H, q, J = 4.1 Hz), 3.29 (2H, m), 3.08 (2H, m), 2.31 (1H, e, J = 4.8 Hz), 1.57 (4H, m), 1.39 (4H, m), 1.29 (8H, m), 1.22 (8H, m), 0.88 (12H, t, J = 7.1 Hz); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 176.86, 167.85, 162.03 (d, J = 198 Hz), 141.25 (d, J = 11.80 Hz), 127.73, 123.55 (d, J = 14.74 Hz), 116.76, 108.78 (d, J = 28.0), 51.12, 40.44, 39.68, 35.43, 31.68, 31.63, 31.51, 29.49, 26.86, 26.56, 22.74, 22.68, 20.92, 14.27; HRMS *m/z* calculated for $C_{27}H_{46}FN_3O_2^-$ $(M-H)^-$ 462.3501, found 462.3526 $(M-H)^-$.



4-fluoro-N-hexyl-5-(hexyl(methyl)amino)-2-(2-propylpentanamido)benzamide (Fmx). Employing the procedure for Feb, while starting with (NO_2)Fmx (310 mg, 0.81 mmol) and using 300 µl 2,2-di-*n*-propylacetyl chloride (1.75 mmol), resulted in 284 mg (0.60 mmol 74%) of Fmx. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 10.94 (1H, s), 8.36 (1H, d, *J* = 15.6 Hz), 7.07 (1H, d, *J* = 9.0 Hz), 6.29 (1H, t, *J* = 6.0 Hz), 3.38 (2H, q, *J* = 5.8 Hz), 3.04 (2H, t, *J* = 7.8 Hz), 2.78 (3H, s), 2.26 (1H, e, *J* = 4.8 Hz), 1.61 (4H, m), 1.42 (4H, m), 1.31 (8H, m), 1.24 (8H, m), 0.86 (12H, m); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 176.30, 166.93, 157.49 (d, *J* = 253 Hz), 143.05 (d, *J* = 12.16 Hz), 125,04, 121.06 (d, *J* = 10.1 Hz), 117.14, 110.13 (d, *J* = 26.5), 58.42 (d, *J* = 2.9), 49.43, 44.82, 40.60, 35.32, 31.62, 31.20, 29.37, 26.80, 26.17, 25.69, 22,67, 22.52, 20.87, 14.21, 14.16, 13.99; HRMS *m/z* calculated for $C_{28}H_{49}FN_3O_2^+$ $(M+H)^+$ 478.3809, found 478.3859, $(M+H)^+$.



5-(dihexylamino)-4-fluoro-2-hexanamido-N-hexylbenzamide (Fdx).[2,3] (NO_2)Fdx (70 mg, 0.155 mmol) was suspended in 2 ml of dimethoxyethane and $SnCl_2{\cdot}2H_20$ (210 mg, 0.93 mmol) was added. Under argon, the reaction was refluxed at 75 °C for 24 hours while stirring. The reduction led to a color change from yellow to colorless and a new fluorescent spot on TLC. Upon completion of the reduction, the reaction mixture was brought to room temperature. Hexanoic anhydride (54 µl, 0. 23 mmol) and triethylamine (33 µl, 0.23 mmol) were added and the reaction was stirred for 3 hours. The mixture was diluted with 10 ml DCM and washed with saturated aqueous solution of $NaHCO_3$ (3×10 ml). The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification using flash chromatography on silica gel (100% hexanes to 90% hexanes 10% ethyl acetate) produced 10 mg (0.02 mmol, 13%) Fdx. ^{1}H-NMR ($CDCl_3$) *δ*/ppm: 10.91 (1H, s), 8.33 (1H, d, *J* = 15.2 Hz), 6.96 (1H, d, *J* = 8.4 Hz), 6.11 (1H, t, *J* = 5.6 Hz), 3.38 (2H, q, *J* = 6.1 Hz), 3.03 (4H, t, *J* = 7.5 Hz), 2.33 (2H, t, *J* = 7.6 Hz), 1.68 (2H, p, *J* = 7.5 Hz), 1.59 (2H, p, *J* = 7.3 Hz), 1.38 (4H, m), 1.30 (10H, m), 1.22 (12H, m), 0.84 (12H, m); ^{13}C-NMR ($CDCl_3$) *δ*/ppm: 172.16, 168.80, 158.73 (d, *J* = 252 Hz), 135.01 (d, *J* = 12.3 Hz), 133.53 (d, *J* = 8.7 Hz), 120.30 (d, 5.5 Hz), 116.83, 110.32 (d, *J* = 27.9 Hz), 53.35, 40.28, 38.57, 31.88, 31.66, 31.56, 29.67, 26.99, 26.85, 25.47, 22.83, 22.76, 22.59, 14.20, 14.13; HRMS *m/z* calculated for $C_{31}H_{53}FN_3O_2^-$ $(M-H)^-$ 518.4116, found 518.4126 $(M-H)^-$.

2. Methods

2.1. NMR analysis of the progress of the (NO_2)Aa(CO_2H) synthesis (Fig. 2a-d)

In addition to TLC, we used ^{19}F and ^{1}H NMR to monitor the progress of the nucleophilic aromatic substitution of DFNBA and the corresponding amine. For each sample, a drop of the stirred reaction mixture was transferred into an NMR tube and diluted with about 1 ml DMSO-d_6. To the samples for ^{19}F NMR, $C_6H_5CF_3$ was added for an internal reference. ^{19}F NMR spectra show if the starting material, DFNBA, or the product, (NO_2)Aa(CO_2H), is present. We could not observe extra ^{19}F signals at early time that we could ascribe to the fluoride replaced by the amine. Since we use glass vessels for the reactions, we believe that most likely Si from the class surface reacts with the produced F^-, capturing it and forming SiF_4, which is a gas. While for most of the amines, the reaction appeared to be completed within a few minutes of microwave radiation, we always observe traces (< 1%) of starting material, DFNBA, within the first 15 – 30 min. When conducting the same tests at room temperature (e.g., for pyridine and *N*-methylbutylamine as solvents), the reaction mixture contains undissolved solid material ascribed to DFNBA. NMR tests of the clear brown reaction mixture, however, tend to show only the presence of the product and the amine. It leads us to believe, that dissolving DFNBA in the amine is a rate-limiting process; and once the difluoro reagent is dissolved it undergoes relatively fast nucleophilic aromatic substitution. Indeed, overexposure of the DFNBA/amine mixture to microwave radiation leads to formation of side products. Conversely, separation of DFNBA from the formed (NO_2)Aa(CO_2H) tends to be challenging, even chromatographically. Most of the impurities from the prolonged reaction times proved to be separatable from the product. Therefore, we aimed at conditions where the removal of the starting material was practically complete without compromising the yields (NO_2)Aa(CO_2H) due to prolonged heating. *N*-ethylbutylamine and *N*-methylbutylamine prove most

promising as nucleophiles under neat (solvent-free) conditions where the final crude products are readily isolated in good purity without requiring column chromatography for their isolation.

2.2. Computational analysis (Fig. 3b)

The *N*-acylated Aa residues are modeled using density functional theory (DFT). For simplicity, the aliphatic chains are truncated to two carbons. The DFT calculations are performed at the B3LYP/6-311+G(d,p) level for the gas phase using Gaussian 09. Spin-unrestricted calculations are used for radical-cation (doublet state) modeling.

2.3. Electrochemical analysis (Fig. 3c, Table 1)

Cyclic voltammetry is conducted using Reference 600 Potentiostat/Galvanostat/ZRA (Gamry Instruments, PA, U.S.A.), connected to a three-electrode cell, at scan rates of 50 mV s^{-1}, as previously described.[4] Anhydrous solvents are employed for the sample preparation, with different concentrations of tetrabutylammonium hexafluorophosphate (NBu_4PF_6) as supporting electrolyte. Prior to recording the voltammograms, the samples are extensively purged with argon while maintaining constant volume by adding more of the anhydrous solvent. For each sample and each solvent, a set of voltammograms is recorded where the electrolyte concentration is increased from 25 mM to 200 mM in steps of 25 mM. The half-wave potentials, $E^{(1/2)}$, are determined from the midpoints between the anodic and cathodic peak potentials for reversible or quasireversible oxidation. The anodic and cathodic peak potentials are determined from the zero points of the first derivatives of the voltammograms, that is, the potentials where $\partial I/\partial E = 0$ at $\partial E/\partial t$ = constant.[2-5] To correct for potential drifts in the reference electrode (which is SCE, connected with the cell via a salt bridge), ferrocene was used as a standard ($E^{(1/2)} = 0.45 \pm 0.01$ V vs. SCE for MeCN, 100 mM NBu_4BF_4).[6] Voltammograms of the standard are recorded before and after each set of measurements. From the dependence of $E^{(1/2)}$ on the electrolyte concentration, the potential for each neat solvents are estimated from extrapolation to zero electrolyte concentration (Figure 2b).[2,6,7]

2.4. Optical spectroscopy and analysis (Fig. 3d)

Steady-state absorption spectra were recorded in a transmission mode using a JASCO V-670 spectrophotometer (Tokyo, Japan); and steady-state emission spectra were measured, also in a transmission mode, with a FluoroLog-3 spectrofluorometer (Horiba-Jobin-Yvon, Edison, NJ, USA) as previously reported.[8,9]

While the spectra were recorded as functions of wavelength, λ, they are plotted vs. energy, $\mathscr{E} = h\,c\,/\,\lambda$, where $h = 4.135667\times10^{-15}$ eV s, and $c = 2.9979\times10^{17}$ nm s^{-1}. The magnitudes of the emission spectra recorded as functions of wavelength were converted prior to plotting them against energy: $F(\mathscr{E}) = F(\lambda)\,\lambda^2$.[8,10] For estimating zero-to-zero energy, $\mathscr{E}_{00}$, of a conjugate we plot its absorption and fluorescence spectra on the same graph where the fluorescence maximum is adjusted to be equal to the maximum of the band at the red edge of the absorption spectrum, and $\mathscr{E}_{00}$ corresponds to the energy at which the thus normalized spectra cross (Fig. 3d). The Stokes' shift, $\Delta\mathscr{E}$, corresponds to the separation between the emission and absorption spectral maxima. For the fluorinated residues the red-edge absorption bands were not well resolved. Fitting them to a sum of Gaussian functions deconvoluted the absorption spectra and the red-edge components were used of the analyses.

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