Electronic Supplementary Material (ESI) for Chemical Science
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Supporting Information

Clickable, photoreactive inhibitors to probe the active site microenvironment of fatty acid amide hydrolase

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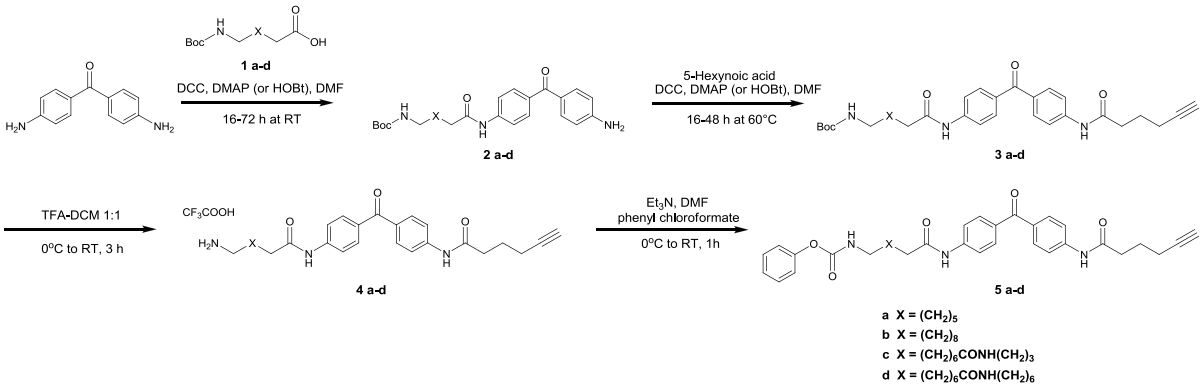
Synthesis of probes 5a-d:



Scheme S1. Synthesis of carbamate benzophenone probes **5a-d**. DCC = N,N'-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, HOBt = hydroxybenzotriazole, DMF = dimethylformamide, TFA-DCM = trifluoroacetic acid – dichloromethane, Et_3N = triethylamine.

General methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), and used without further purification. All reactions were carried out under a nitrogen atmosphere with dry solvents using anhydrous conditions, unless otherwise noted. Dry solvents were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and flash chromatography was performed using ICN silica gel (particle size 0.032 - 0.063 mm). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV-400 (400 MHz) and Bruker DRX-500 (500 MHz) spectrometers, respectively, and spectra are reported in parts per million (δ) relative to the residual solvent peak. Mass spectra were acquired with an ESI-TOF (high accuracy) system at the Scripps Center for Mass Spectrometry (La Jolla, CA).

Synthesis of 2a: 4,4'-Diaminobenzophenone (300 mg, 1.4 mmol) was dissolved in DMF (2 ml) followed by addition of DCC (700 mg, 3.4 mmol) and DMAP (35 mg, 0.28 mmol). To this mixture was added **1a** (8-[Boc-amino]octanoic acid) (730 mg, 2.8 mmol) in DMF (2 ml) and the reaction was stirred at rt 16 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% → 1.5% → 2% methanol:$CHCl_3$) giving compound **2a** (214 mg, 34%) as a yellow solid. R_f 0.74 (25% hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 8.8 Hz, 2H), 4.51 (s, 1H), 4.11 (s, 2H), 3.49 (s, 1H), 3.12 (bs, 2H), 2.39 (t, *J* = 7.6 Hz, 2H), 1.78 – 1.70 (m, 2H), 1.57 (bs, 2H), 1.44 (s, 9H), 1.41 – 1.35 (m, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{26}H_{35}N_3O_4$: 454.2700; found 454.2704 $[M + H]^+$.

Synthesis of 3a: 5-Hexynoic acid (103 mg, 0.92 mmol) was dissolved in DMF (3.5 ml) followed by addition of DCC (190 mg, 0.92 mmol) and DMAP (18 mg, 0.15 mmol). To this mixture was added **2a** (346 mg, 0.76 mmol) and the reaction was stirred at 60°C for 16 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% → 1.5% methanol:$CHCl_3$) giving compound **3a** (74 mg, 18%) as a yellow solid. R_f 0.60 (33% hexanes:EtOAc). ^{1}H NMR (400 MHz,

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$CDCl_3$): δ 7.80 – 7.63 (m, 8H), 4.53 (s, 1H), 4.12 (s, 1H), 3.49 (s, 1H), 3.12 (bs, 2H), 2.57 (t, *J* = 7.2 Hz, 1H), 2.41 – 2.33 (m, 4H), 2.03 – 1.95 (m, 4H), 1.78 – 1.71 (m, 2H), 1.57 (s, 2H), 1.44 (s, 9H), 1.41 – 1.35 (m, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{32}H_{41}N_3O_5$: 570.2938; found 570.2939 $[M + Na]^+$.

Synthesis of 4a: 3a (72 mg, 0.13 mmol) was dissolved in ice-cold mixture of TFA (1.5 ml) and DCM (1.5 ml), the reaction was stirred at rt for 3 h and concentrated. The residue was triturated with ice-cold ether to give **4a** (70 mg, 98%) as a yellow solid. ^{1}H NMR (400 MHz, MeOD): δ 7.76 (s, 8H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 1H), 2.45 (t, *J* = 7.3 Hz 2H), 2.33 – 2.28 (m, 4H), 1.92 (p, *J* = 7.2 Hz, 2H), 1.76 – 1.65 (m, 4H), 1.45 (s, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{27}H_{33}N_3O_3$: 448.2595; found 448.2587 $[M + H]^+$.

Synthesis of 5a: **4a** (70 mg, 0.125 mM) was dissolved in DMF (1 ml) followed by addition of Et_3N (21 µl, 0.15 mmol) at 0 °C. To this mixture was added phenyl chloroformate (19 µl, 0.15 mmol) and the reaction was stirred at rt for 1 h. The reaction mixture was diluted with ethyl acetate (30 ml), washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% methanol:$CHCl_3$) giving compound **5a** (7.6 mg, 11%) as a white solid. R_f 0.56 (33% hexanes:EtOAc). ^{1}H NMR (400 MHz, MeOD): δ 7.77 (s, 8H), 7.38 – 7.09 (m, 5H), 3.2 (t, *J* = 6.8 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 1H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.33 – 2.28 (m, 4H), 1.92 (p, *J* = 7.2 Hz, 2H), 1.76 (bs, 2H), 1.59 (bs, 2H), 1.45 (bs, 6H). ^{13}C NMR (500 MHz, MeOD): δ 196.77, 175.17, 174.23, 157.49, 152.90, 144.41, 144.39, 134.21, 132.40, 130.44, 126.37, 122.93, 120.22, 84.25, 70.49, 42.06, 38.20, 36.82, 30.85, 30.34, 30.19, 29.92, 27.80, 26.80, 25.64, 18.78 ppm. HRMS (ESI-TOF high acc): *m/z* calcd for $C_{34}H_{37}N_3O_5$: 568.2806; found 568.2814 $[M + H]^+$.

Synthesis of 2b: 4,4'-Diaminobenzophenone (300 mg, 1.41 mmol) was dissolved in DMF (5 ml) followed by addition of DCC (700 mg, 3.4 mmol) and DMAP (35 mg, 0.28 mmol). To this mixture was added **1b** (11-[Boc-amino]undecanoic acid) (852 mg, 2.82 mmol) very slowly and the reaction was stirred at rt for 22 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% → 1.5% methanol:$CHCl_3$) giving compound **2b** (613 mg, 88%) as a yellow solid. R_f 0.71 (25% hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, *J* = 8.7 Hz, 2H), 7.69 (d, *J* = 8.7 Hz, 2H), 7.62 (d, *J* = 8.7 Hz, 2H), 6.67 (d, *J* = 8.8 Hz, 2H), 4.50 (s, 1H), 4.12 (s, 2H), 3.49 (s, 1H), 3.10 (bs, 2H), 2.37 (t, *J* = 8 Hz, 2H), 1.78 – 1.70 (m, 2H), 1.58 (bs, 2H), 1.44 (s, 9H), 1.36 – 1.28 (m, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{29}H_{41}N_3O_4$: 496.3170; found 496.3154 $[M + H]^+$.

Synthesis of 3b: 5-Hexynoic acid (68 mg, 0.61 mmol) was dissolved in DMF (2 ml) followed by addition of DCC (150 mg, 0.73 mmol) and DMAP (15 mg, 0.12 mmol). To this mixture was added **2b** (300 mg, 0.61 mmol) and the reaction was stirred at 60°C for 24 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% methanol:$CHCl_3$) giving compound **3b** (118 mg, 34%) as a yellow solid. R_f 0.60 (33% hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 – 7.63 (m, 8H), 4.53 (s, 1H), 4.14 (s, 1H), 3.5 (s, 1H)3.11 (bs, 2H), 2.58 (t, *J* = 7.2 Hz, 1H), 2.41 – 2.33 (m, 4H), 2.03 – 1.95 (m, 4H), 1.78 – 1.71 (m, 2H), 1.57 (s, 2H), 1.45 (s, 9H), 1.36 – 1.28 (m, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{35}H_{47}N_3O_5$: 590.3588; found 590.3593 $[M + H]^+$.

Synthesis of 4b: **3b** (115 mg, 0.195 mmol) was dissolved in ice-cold mixture of TFA (1.6 ml) and DCM (1.6 ml), the reaction was stirred at rt for 3 h and concentrated. The residue was triturated with ice-cold ether to give **4b** (117 mg, 100%) as a yellow solid. ^{1}H NMR (400 MHz, MeOD): δ 7.76 (s, 8H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.58 (t, *J* = 7.4 Hz, 1H), 2.43 (t, *J* = 7.6 Hz, 2H), 2.33 – 2.29 (m, 4H), 1.92 (p, *J* = 7.2 Hz, 2H), 1.76 – 1.62 (m, 4H), 1.38 (s, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{30}H_{39}N_3O_3$: 490.3064; found 490.3064 $[M + H]^+$.

Synthesis of 5b: **4b** (117 mg, 0.194 mM) was dissolved in DMF (0.8 ml) followed by addition of Et_3N (32 µl, 0.233 mM) at 0 °C. To this mixture was added phenyl chloroformate (29 µl, 0.233 nM) and the reaction was stirred at rt for 1 h. The reaction mixture was diluted with ethyl acetate (25 ml), washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 1% methanol:$CHCl_3$) giving compound **5b** (38 mg, 32%) as a white solid. R_f 0.7 (33% hexanes:EtOAc). ^{1}H NMR (400 MHz, MeOD): δ 7.76 (s, 8H), 7.38 – 7.08 (m, 5H), 3.18 (t, *J* = 6.8 Hz, 2H), 2.58 (t, *J* = 7.6 Hz, 1H), 2.43 (t, *J* = 7.4 Hz, 2H), 2.33 – 2.30 (m, 4H), 1.92 (p, *J* = 7.2 Hz, 2H), 1.73 (bs, 2H), 1.57 (bs, 2H), 1.37 (bs, 12H). ^{13}C NMR (500 MHz, MeOD): δ 196.79, 175.22, 174.24, 157.44, 152.91, 144.42, 144.38, 132.40, 130.45, 130.44, 126.36, 122.92, 120.23, 84.26, 70.47, 42.13, 38.20, 36.82, 30.90, 30.72, 30.64, 30.54, 30.48, 30.43, 27.98, 26.90, 25.64, 18.78 ppm. HRMS (ESI-TOF high acc): *m/z* calcd for $C_{37}H_{43}N_3O_5$: 610.3275; found 610.3265 $[M + H]^+$.

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Synthesis of 1c: Boc-8-Aminocaprylic acid (2.7 g, 10.4 mmol) and N-hydroxysuccinimide (1.2 g, 10.4 mmol) were dissolved in DCM (55 ml). To this mixture was added DCC (3.2 g, 15.5 mmol) and the reaction mixture was stirred at rt for 16 h. The reaction was filtered and the filtrate was diluted with DCM, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated giving Boc-8-Aminocaprylic acid N-hydroxysuccinimide ester (3.5 g, 94%) as a light pink solid. R_f 0.55 (50% hexanes:EtOAc) ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.51 (s, 1H), 3.10 (bs, 2H), 2.84 (s, 4H), 2.60 (t, *J* = 7.4 Hz, 2H), 1.93 – 1.90 (m, 2H), 1.78 - 1.70 (m, 2H), 1.44 (s, 9H), 1.34 – 1.27 (m, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{17}H_{28}N_2O_6$: 357.2020; found 357.2011 [*M* + H]$^+$.

To a mixture of Boc-8-Aminocaprylic acid N-hydroxysuccinimide ester (1.35 g, 3.79 mmol) and 5-aminovaleric acid (440 mg, 3.79 mmol) in DMF (20 ml) was added Et_3N (1.58 ml, 11.4 mmol) and reaction was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (70 ml), washed with 5% aqueous HCl, dried over Na_2SO_4 and concentrated. Recrystallization from 50% hexanes:EtOAc gave compound **1c** (668 mg, 49%) as a white solid. R_f 0.2 (25% hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.76 (s, 1H), 4.65 (s, 1H), 3.45 (s, 1H), 3.28 (bs, 2H), 3.11 – 3.08 (m, 2H), 2.37 (t, *J* = 6.3 Hz, 2H), 2.18 (m, 2H), 1.76 – 1.56 (m, 8H), 1.44 (s, 9H), 1.42 – 1.26 (m, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{18}H_{34}N_2O_5$: 359.2540; found 359.2542 [*M* + H]$^+$.

Synthesis of 2c: 4,4'-Diaminobenzophenone (178 mg, 0.837 mmol) was added to stirred mixture of SS-084 (300 mg, 0.837 mmmol), DCC (170 mg, 0.873 mmol) and HOBt (113 mg, 0.837 mmol) in DMF (3 ml) and the reaction was stirred at rt for 72 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated giving compound **2c** (449 mg, 97%) as a yellow solid. R_f 0.13 (17% hexanes:EtOAc). ^{1}H NMR (400 MHz, MeOD): δ 7.78 – 7.53 (m, 6H), 6.68 (d, *J* = 8.6 Hz, 2H), 3.26 – 3.21 (m, 2H), 3.02 – 2.99 (m, 2H), 2.45 (t, *J* = 7.4 Hz, 2H), 2.19 (t, *J* = 7.5 Hz, 2H), 1.78 – 1.65 (m, 2H), 1.63 – 1.56 (m, 6H), 1.43 (s, 9H), 1.33 (s, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{31}H_{44}N_4O_5$: 553.3384; found 553.3388 [*M* + H]$^+$.

Synthesis of 3c: 5-Hexynoic acid (90 mg, 0.80 mmol) was dissolved in DMF (2 ml) followed by addition of DCC (166 mg, 0.80 mmol) and HOBt (11 mg, 0.80 mmol). To this mixture was added **2c** (445 mg, 0.80 mmol) in DMF (1ml) and the reaction was stirred at 60°C for 48 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% → 2% methanol:$CHCl_3$) giving compound **3c** (219 mg, 42%) as a yellow solid. R_f 0.42 (15% methanol:$CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 – 7.64 (m, 8H), 6.0 (s, 1H), 4.65 (bs, 1H), 4.18 (s, 1H), 3.49 (s, 1H), 3.35 – 3.30 (m, 2H), 3.10 (bs, 2H), 2.57 (t, *J* = 7.3 Hz, 1H), 2.48 – 2.44 (m, 2H), 2.33 (td, *J* = 2.6, 4.2 Hz, 2H), 2.21 – 2.17 (m, 2H), 2.01 – 1.93 (m, 4H), 1.74 – 1.54 (m, 8H), 1.43 (s, 9H), 1.29 (s, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{37}H_{50}N_4O_6$: 647.3803; found 647.3802 [*M* + H]$^+$.

Synthesis of 4c: **3c** (218 mg, 0.337 mmol) was dissolved in ice-cold mixture of TFA (1.5 ml) and DCM (1.5 ml), the reaction was stirred at rt for 3 h and concentrated. The residue was triturated with ice-cold ether to give **4c** (202 mg, 91%) as a yellow solid. ^{1}H NMR (400 MHz, MeOD): δ 7.75 (s, 8H), 3.23 (t, *J* = 6.8 Hz, 2H), 2.92 – 2.87 (m, 2H), 2.89 (t, *J* = 7.8 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 1H), 2.47 – 2.43 (m, 2H), 2.32 – 2.28 (m, 4H), 2.21 – 2.16 (m, 2H), 1.91 (p, *J* = 7.1 Hz, 2H), 1.77 – 1.70 (m, 2H), 1.65 – 1.55 (m, 6), 1.37 (s, 6H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{32}H_{42}N_4O_4$: 547.3279; found 547.3279 [*M* + H]$^+$.

Synthesis of 5c: **4c** (200 mg, 0.30 mM) was dissolved in DMF (2 ml) followed by addition of Et_3N (84 µl, 0.60 mmol) at 0 °C. To this mixture was added phenyl chloroformate (46 µl, 0.36 mmol) and the reaction was stirred at rt for 1 h. The reaction mixture was diluted with ethyl acetate (30 ml), washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% → 2% methanol:$CHCl_3$) giving compound **5c** (32 mg, 16%) as a white solid. R_f 0.48 (15% methanol:$CHCl_3$). ^{1}H NMR (400 MHz, MeOD): δ 7.76 (s, 8H), 7.45 – 7.07 (m, 5H), 3.23 (t, *J* = 6.8 Hz, 2H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 1H), 2.45 (t, *J* = 7.3 Hz, 2H), 2.34 – 2.27 (m, 4H), 2.22 – 2.17 (m, 2H), 1.91 (p, *J* = 7.2 Hz, 2H), 1.78 – 1.70 (m, 2H), 1.67 – 1.53 (m, 6), 1.43 – 1.30 (m, 6H). ^{13}C NMR (500 MHz, MeOD): δ 196.76, 176.48, 174.74, 174.23, 157.42, 152.89, 144.48, 144.38, 134.21, 132.40, 130.44, 126.37, 122.93, 120.22, 84.26, 70.49, 42.08, 40.02, 37.65, 37.25, 36.82, 30.84, 30.29, 30.16, 30.10, 27.83, 27.12, 25.66, 24.11, 18.78 ppm. HRMS (ESI-TOF high acc): *m/z* calcd for $C_{39}H_{46}N_4O_6$: 667.3490; found 667.3495 [*M* + H]$^+$.

Synthesis of 1d: To a mixture of boc-8-Aminocaprylic acid N-hydroxysuccinimide ester (1.35 g, 3.79 mmol) and 8-aminocaprylic acid (600 mg, 3.79 mmol) in DMF (20 ml) was added Et_3N (1.58 ml, 11.4 mmol) and reaction was stirred at rt for 16 h. The reaction mixture was diluted with EtOAc (70 ml), washed with 5%

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aqueous HCl, dried over Na_2SO_4 and concentrated. Recrystallization from 50% hexanes:EtOAc gave compound **1d** (700 mg, 46%) as a white solid. R_f 0.36 (33% hexanes:EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.52 (s, 1H), 4.58 (s, 1H), 3.45 (s, 1H), 3.27 – 3.22 (m, 2H), 3.09 (bs, 2H), 2.33 (t, *J* = 7.1 Hz, 2H), 2.16 (m, 2H), 1.72 – 1.56 (m, 8H), 1.44 (s, 9H), 1.38 – 1.24 (m, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{21}H_{40}N_2O_5$: 401.3010; found 401.3020 [*M* + H]$^+$.

Synthesis of 2d: 4,4'-Diaminobenzophenone (369 mg, 1.74 mmol) was added to stirred mixture of **1d** (697 mg, 1.74 mmol), DCC (359 mg, 1.74 mmol) and HOBt (235 mg, 1.74 mmol) in DMF (4 ml) and the reaction was stirred at rt for 72 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated giving compound **2d** (720 mg, 70%) as a yellow solid. R_f 0.23 (17% hexanes:EtOAc). ^{1}H NMR (400 MHz, MeOD): δ 7.76 – 7.54 (m, 6H), 6.70 (d, *J* = 8.6 Hz, 2H), 3.20 – 3.15 (m, 2H), 3.02 – 3.00 (m, 2H), 2.43 (t, *J* = 7.4 Hz, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 1.74 – 1.71 (m, 2H), 1.61 – 1.58 (m, 2H), 1.55 – 1.52 (m, 4H), 1.44 (s, 9H), 1.33 (s, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{34}H_{50}N_4O_5$: 595.3854; found 595.3851 [*M* + H]$^+$.

Synthesis of 3d: 5-Hexynoic acid (132 mg, 1.18 mmol) was dissolved in DMF (2 ml) followed by addition of DCC (243 mg, 1.18 mmol) and HOBt (159 mg, 1.18 mmol). To this mixture was added **2d** (700 mg, 1.18 mmol) in DMF (1ml) and the reaction was stirred at 60°C for 48 h. The reaction was filtered and the filtrate was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, 10 % aqueous citric acid and brine, dried over Na_2SO_4 and concentrated giving compound **3d** (523 mg, 64%) as a light brown solid. R_f 0.62 (15% methanol:$CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 – 7.64 (m, 8H), 5.65 (s, 1H), 4.61 (s, 1H), 4.11 (s, 1H), 3.49 (s, 1H), 3.28 – 3.24 (m, 2H), 3.08 (bs, 2H), 2.57 (t, *J* = 7.3 Hz, 1H), 2.42 – 2.32 (m, 4H), 2.18 – 2.14 (m, 2H), 2.03 – 1.91 (m, 4H), 1.75 – 1.54 (m, 8H), 1.44 (s, 9H), 1.28 (s, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{40}H_{56}N_4O_6$: 689.4272; found 689.4268 [*M* + H]$^+$.

Synthesis of 4d: **3d** (523 mg, 0.760 mmol) was dissolved in ice-cold mixture of TFA (1.5 ml) and DCM (1.5 ml), the reaction was stirred at rt for 3 h and concentrated. The residue was triturated with ice-cold ether to give **4d** (533 mg, 100%) as a yellow solid. ^{1}H NMR (400 MHz, MeOD): δ 7.74 (s, 8H), 3.15 (t, *J* = 7.0 Hz, 2H), 2.92 – 2.87 (m, 2H), 2.55 (t, *J* = 7.4 Hz, 1H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.31 – 2.27 (m, 4H), 2.18 – 2.14 (m, 2H), 1.89 (p, *J* = 7.1 Hz, 2H), 1.72 – 1.68 (m, 2H), 1.65 – 1.56 (m, 4), 1.53 – 1.46 (m, 2), 1.42 – 1.30 (m, 12H). HRMS (ESI-TOF high acc): *m/z* calcd for $C_{35}H_{48}N_4O_4$: 589.3748; found 589.3752 [*M* + H]$^+$.

Synthesis of 5d: **4d** (533 mg, 0.758 mM) was dissolved in DMF (3 ml) followed by addition of Et_3N (211 µl, 1.52 mmol) at 0 °C. To this mixture was added phenyl chloroformate (115 µl, 0.910 mmol) and the reaction was stirred at rt for 1 h. The reaction mixture was diluted with ethyl acetate (30 ml), washed with H_2O and brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0% → 0.5% → 1% → 2% → 3% methanol:$CHCl_3$) giving compound **5d** (29 mg, 6%) as a white solid. R_f 0.17 (33% hexanes:EtOAc). ^{1}H NMR (400 MHz, MeOD): δ 7.76 (s, 8H), 7.46 – 7.08 (m, 5H), 3.28 (t, J = 6.8 Hz, 2H), 3.17 (t, *J* = 6.8 Hz, 2H), 2.57 (t, *J* = 7.4 Hz, 1H), 2.45 – 2.41 (m, 2H), 2.33 – 2.27 (m, 4H), 2.22 – 2.17 (m, 2H), 1.92 (p, *J* = 7.0 Hz, 2H), 1.73 (bs, 2H), 1.65 – 1.46 (m, 6), 1.40 – 1.31 (m, 12H). ^{13}C NMR (500 MHz, MeOD): δ 196.76, 176.36, 175.13, 174.22, 157.42, 152.89, 144.42, 144.40, 134.22, 132.40, 130.44, 126.37, 122.93, 120.22, 84.26, 70.48, 42.09, 40.40, 38.19, 37.25, 36.82, 30.85, 30.49, 30.33, 30.18, 27.92, 27.85, 27.15, 26.79, 25.62, 18.77 ppm. HRMS (ESI-TOF high acc): *m/z* calcd for $C_{42}H_{52}N_4O_6$: 709.3959; found 709.3958 [*M* + H]$^+$.

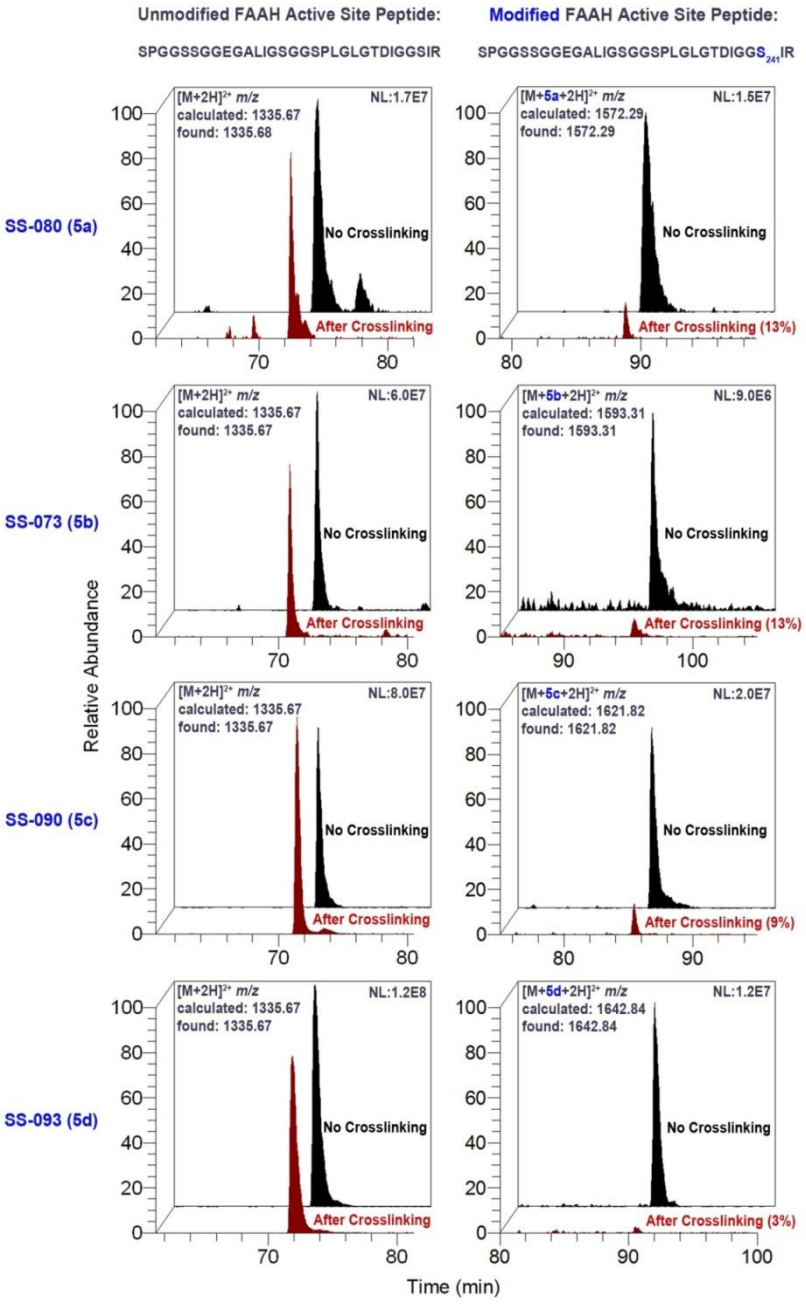

Figure S1. LC-MS analysis shows loss of probe-modified FAAH active site peptide following crosslinking for probes **5a-d**. Extracted ion chromatograms correspond to the unmodified (left column) and probe-labeled (right column) active site FAAH peptide before (black) and after (red) crosslinking. Relative peak area of modified peptide after crosslinking is indicated in parenthesis. Absolute scale of each chromatogram shown in upper right corner.

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Table S1. MS1 and MS2 analysis of FAAH active site peptide in crosslinked and noncrosslinked samples.

Probe [Mass Mod]	Treatment	Peptide Modification Status	AUC	Ratio[1]	Sequest Search Results										
					FAAH active site peptide (* = probe modification assigned by Sequest)	ch	#	XCorr	DeltCN	Conf%	ObsM+H$^+$	CalcM+H$^+$	PPM	ZScore	Ion%
SS-080 (5a) [+473.232]	No Crosslinking	Unmod	7.52E+08	13%	K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	5	6.26	0.37	100%	2671.350	2670.333	5.1	8.7	48%
						3	2	6.71	0.16	100%	2671.351	2670.333	5.6	8.0	36%
		Mod	7.53E+08		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	7	5.09	0.54	100%	3143.577	3143.565	3.7	9.5	48%
						3	2	6.12	0.41	100%	3147.599	3143.565	6.6	7.5	33%
						4	1	3.45	0.19	100%	3147.597	3143.565	6.1	4.5	24%
	After Crosslinking	Unmod	4.06E+08		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	3	6.27	0.37	100%	2671.344	2670.333	2.8	8.6	48%
						3	1	6.36	0.11	100%	2671.351	2670.333	5.6	8.2	36%
		Mod	5.22E+07		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	2	5.07	0.47	100%	3144.590	3143.565	6.8	8.9	42%
						3	3	5.77	0.53	100%	3143.590	3143.565	7.9	10.0	36%
SS-073 (5b) [+515.279]	No Crosslinking	Unmod	1.54E+09	13%	K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	4	5.80	0.39	100%	2674.358	2670.333	4.2	6.6	45%
						3	1	6.67	0.50	100%	2671.348	2670.333	4.4	8.6	36%
		Mod	3.03E+08		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	4	5.62	0.48	100%	3186.613	3185.612	-0.6	8.8	45%
						3	2	6.05	0.42	100%	3187.645	3185.612	8.4	8.4	36%
						4	1	3.81	0.29	100%	3186.638	3185.612	7.3	5.3	24%
	After Crosslinking	Unmod	1.00E+09		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	4	5.34	0.42	100%	2670.353	2670.333	7.3	8.0	38%
						3	1	3.33	0.14	99%	2671.355	2670.333	7	4.5	23%
		Mod	2.62E+07		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	7	5.44	0.49	100%	3186.648	3185.612	10.1	9.3	43%
						3	3	6.32	0.50	100%	3186.657	3185.612	13.2	9.2	33%
SS-090 (5c) [+572.300]	No Crosslinking	Unmod	1.85E+09	9%	K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	4	5.10	0.52	100%	2670.334	2670.333	0.3	9.2	43%
						3	3	5.63	0.44	100%	2672.326	2670.333	-5.1	7.7	32%
		Mod	5.74E+08		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	6	4.95	0.56	100%	3243.638	3242.633	0.6	8.6	43%
						3	4	6.40	0.42	100%	3245.645	3242.633	0.7	7.8	31%
						4	1	2.86	0.09	100%	3243.632	3242.633	-1.3	3.4	22%
	After Crosslinking	Unmod	2.13E+09		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	4	6.50	0.49	100%	2671.330	2670.333	-2.3	9.0	50%
						3	3	4.82	0.46	100%	2671.341	2670.333	1.7	8.5	30%
		Mod	5.80E+07		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	3	5.19	0.54	100%	3243.632	3242.633	-1.3	9.2	42%
						3	1	4.12	0.36	100%	3242.639	3242.633	1.9	6.0	25%
SS-093 (5d) [+614.347]	No Crosslinking	Unmod	4.92E+09	3%	K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	21	6.57	0.50	100%	2671.330	2670.333	-2.4	9.4	48%
						3	3	6.45	0.51	100%	2671.332	2670.333	-1.5	9.0	38%
		Mod	3.80E+08		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	3	5.04	0.48	100%	3285.685	3284.680	0.5	8.8	42%
						3	4	6.49	0.43	100%	3285.689	3284.680	1.7	7.3	33%
	After Crosslinking	Unmod	3.81E+09		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGSIR.F	2	16	6.34	0.50	100%	2671.340	2670.333	1.5	9.0	48%
						3	2	5.13	0.48	100%	2672.339	2670.333	-0.3	8.0	33%
		Mod	9.10E+06		K.SPGGSSGGEGALIGSGGSPLGLGTDIGGS*IR.F	2	1	4.63	0.44	100%	3286.687	3284.680	0.1	7.8	38%
						3	1	3.46	0.07	100%	3288.691	3284.680	-0.8	3.7	19%

[1]Ratio = $(AUC_{mod}/AUC_{unmod})_{Crosslinked}/(AUC_{mod}/AUC_{unmod})_{NonCrosslinked}$