

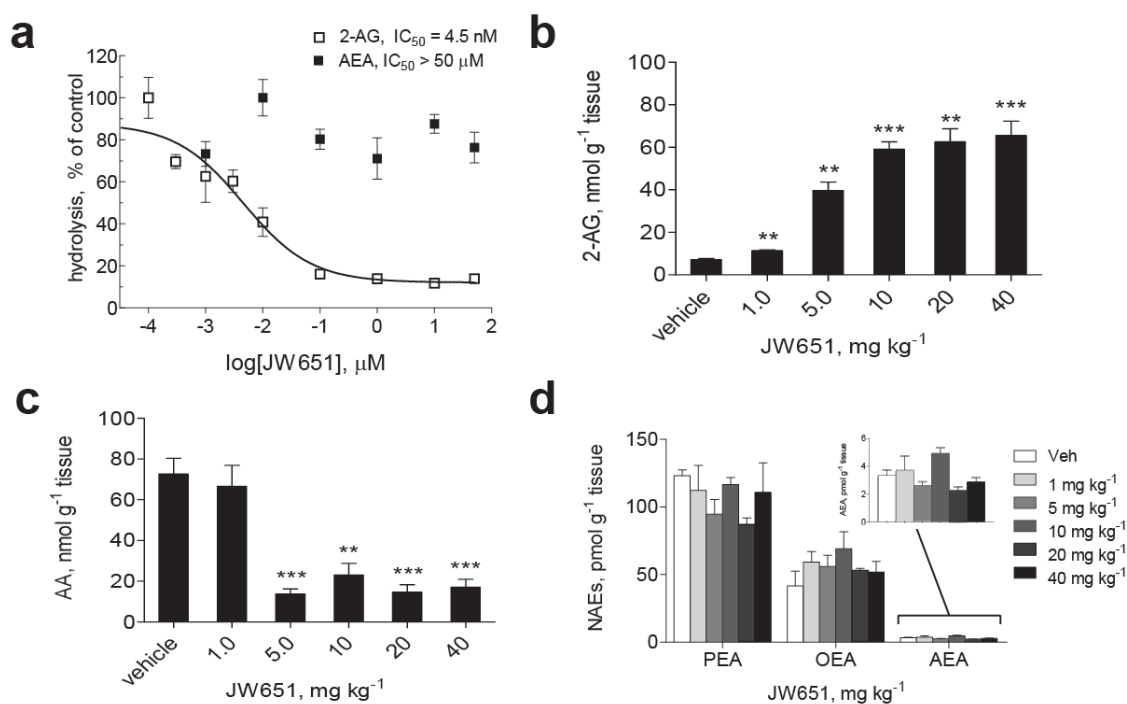
SUPPORTING INFORMATION

PROTEOME-WIDE REACTIVITY PROFILING IDENTIFIES DIVERSE CARBAMATE CHEMOTYPES TUNED FOR SERINE HYDROLASE INHIBITION

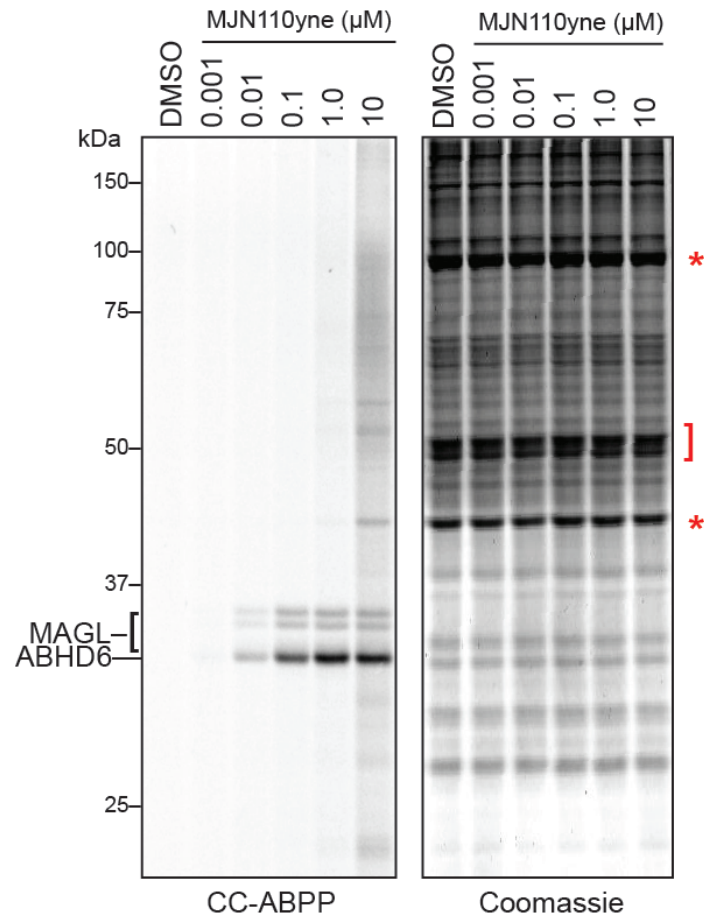
Jae Won Chang, Armand B. Cognetta, III, Micah J. Niphakis, Benjamin F. Cravatt

The Skaggs Institute for Chemical Biology and Department of Chemical Physiology, The Scripps Research Institute, La Jolla, California, USA.

SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1. Profiling JW651 *in vitro* and *in vivo* activity using metabolomics. (A) Blockade of MAGL and FAAH by JW651 in mouse brain proteomes as measured by hydrolysis of 2-AG and AEA, respectively. Data are presented as mean \pm SEM of three independent experiments. Brain lipid profile for 2-AG (B), AA (C), and NAE's (D) across the indicated dose range of JW651 (p.o.). Data are presented as mean \pm SEM ($n = 3$ mice per group). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ for vehicle-treated versus inhibitor-treated mice.



Supplementary Figure S2. Full *in vitro* CC-ABPP gel of MJN110yne (left) aligned with Coomassie staining of the same gel (right). Asterisks and bracket mark abundant proteins that exhibit evidence of low-level carbamate probe labeling *in vitro* (also see **Figure 4**).

Supplementary Table S1. Gel-based IC₅₀ values for various carbamate chemotypes against mouse MAGL, ABHD6 and FAAH.

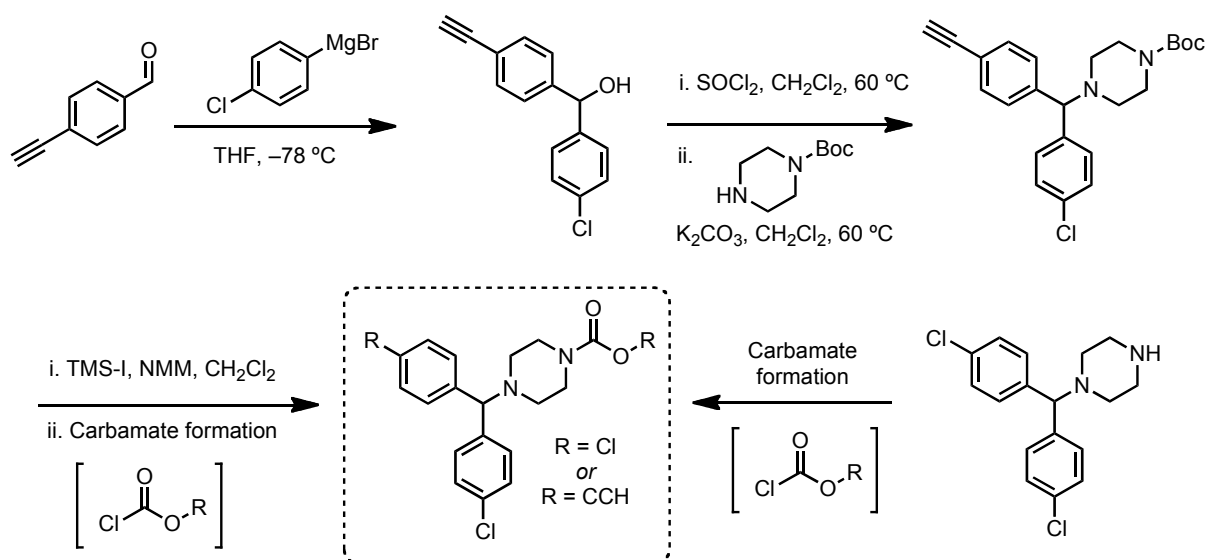
Inhibitor	Chemotype ^c	Inhibitor IC ₅₀ (95% CI) ^a		
		mMAGL	mABHD6	mFAAH
KML29 ^b	HFIP	15 (11 –21)	4,870 (4,120 –5,760)	> 50, 000
JW843	Ph	3,170 (1890 – 5340)	13,400 (10,300 – 17,500)	>100,000
JW842	PNP	357 (287 – 445)	26,100 (13,400 – 50,800)	>100,000
JW814	TFE	2,710 (1,940 – 3,790)	>100,000	>100,000
JW651	HFIP	38 (23 – 64)	10,380 (4,612 – 23,400)	>100,000
MJN110	NHS	9.5 (5.7 –15.8)	260 (174 – 394)	>100,000

^a IC₅₀ values were determined using gel-based competitive ABPP by quantifying reductions of FP-Rh labeling from gel images following preincubation of mouse brain proteomes with each inhibitor at a concentration range of 1–100,000 nM. IC₅₀ values are reported as the mean from three independent experiments with the 95% confidence intervals listed in parentheses. ^b Previously measured IC₅₀ values.⁽¹⁸⁾ ^c HFIP = hexafluoroisopropyl, Ph = phenyl, PNP = *p*-nitrophenyl, TFE = trifluoroethyl, NHS = *N*-hydroxysuccinimidyl.

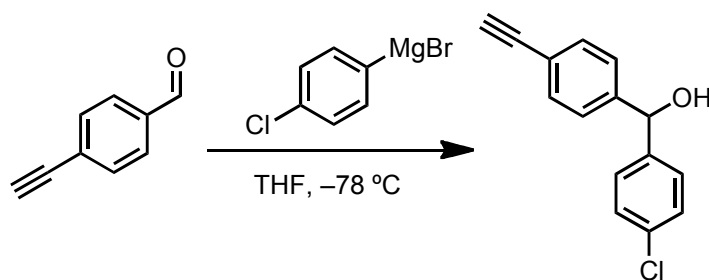
SYNTHETIC METHODS

General Information: All commercially available chemicals were obtained from Aldrich, Acros, Fisher, Fluka, or Maybridge and were used without further purification, except where noted. Dry solvents were obtained by passing these through activated alumina columns. All reactions were carried out under an inert nitrogen atmosphere using oven-baked glassware unless otherwise noted. Flash chromatography was performed using 230-400 mesh silica gel 60. NMR spectra were generated on Varian 400 MHz or Bruker 500 MHz instruments. Chemical shifts were recorded in ppm relative to tetramethylsilane (TMS) with multiplicities given as s (singlet), bs (broad singlet), d (doublet), t (triplet), dt (doublet of triplets), q (quadruplet), qd (quadruplet of doublets), m (multiplet).

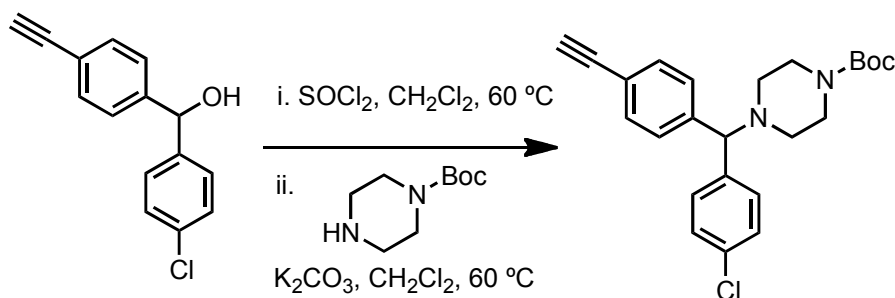
SYNTHESIS OF CLICKABLE PROBES



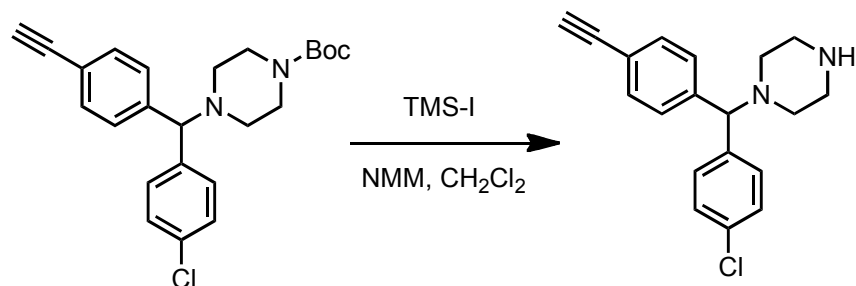
Scheme S1. Synthesis of parent (R = Cl) and clickable (R = CCH) probes.



(4-chlorophenyl)(4-ethynylphenyl)methanol. To a stirring solution of 4-ethynylbenzaldehyde (685 mg, 5.3 mmol) in dry THF (20 mL) at $-78\text{ }^{\circ}\text{C}$ under N_2 was added 4-chlorophenyl)magnesium bromide (10.5 mL, 10.5 mmol, 1.0 M in EtO_2). After 4 h, The reaction was quenched with saturated aqueous NaHCO_3 (20 mL) and the aqueous layer extracted with CH_2Cl_2 ($3 \times 20\text{ mL}$). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was purified by flash chromatography (5–15% EtOAc /hexanes) to give (4-chlorophenyl)(4-ethynylphenyl)methanol as a white solid (1.1 g, 87%): $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.46 (d, $J = 8\text{ Hz}$, 2H), 7.32 – 7.27 (m, 6H), 5.81 (s, 1H), 3.06 (s, 1H), 2.22 (s, 1H); HRMS (ESI+) m/z calc'd for $[\text{M}-\text{H}_2\text{O}]^+$ $\text{C}_{15}\text{H}_{11}\text{ClO}$: 225.0482, found 225.0480.

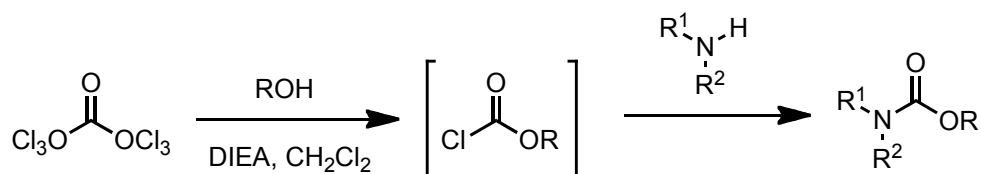


***tert*-butyl 4-((4-chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate.** To a stirring solution of (4-chlorophenyl)(4-ethynylphenyl)methanol (301 mg, 1.2 mmol) in dry CH₂Cl₂ (5 mL) at room temperature under N₂ was added thionylchloride (1.0 mL, 12.4 mmol). After stirred at 65 °C for overnight, the reaction was quenched with saturated aqueous NaHCO₃ (20 mL) and the aqueous layer extracted with CH₂Cl₂ (3×10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was used without further purification. To a solution of 1-chloro-4-(chloro(4-ethynylphenyl)methyl)benzene in CH₂Cl₂ (12 mL) was added *tert*-butyl piperazine-1-carboxylate (693 mg, 3.7 mmol) and K₂CO₃ (857 mg, 6.2 mmol) at room temperature. After stirred at 65 °C for overnight, the reaction was quenched with saturated aqueous NaHCO₃ (20 mL) and the aqueous layer extracted with CH₂Cl₂ (3×10 mL). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The mixture was purified by flash chromatography (5–15% EtOAc/hexanes) to give *tert*-butyl 4-((4-chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate as a white solid (370 mg, 73%): ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, *J* = 8 Hz, 2H), 7.34 – 7.24 (m, 6H), 4.21 (s, 1H), 3.42 – 3.40 (m, 4H), 3.03 (s, 1H), 2.32 – 2.30 (m, 4H), 1.43 (s, 9H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₄H₂₇ClN₂O₂: 411.1834, found 411.1836.

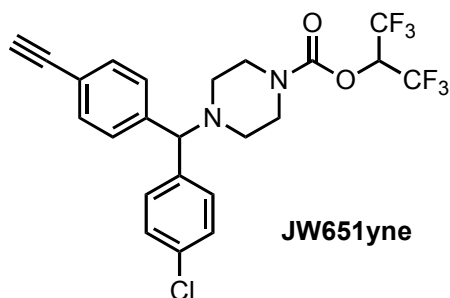


1-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine: To a solution of Boc-protected amine (72 mg, 0.17 mmol) in dry CH₂Cl₂ (8 mL) was added, *N*-methylmorpholine (96 μL, 0.87 mmol) and iodotrimethylsilane (88 mg, 0.43 mmol). After TLC indicated complete consumption of the starting material, the mixture was poured onto a saturated solution of NaHCO₃ and the product was extracted with CH₂Cl₂ (3x). The combined organic layers were dried over Na₂SO₄ and concentrated under reduced pressure to provide the crude product, which was used in the next step without further purification.

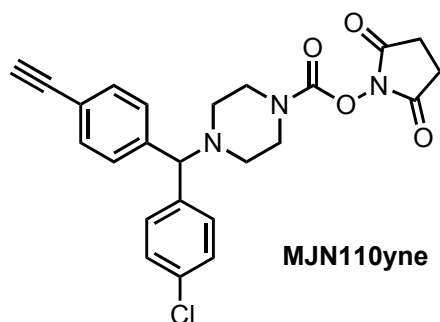
General Method for Carbamate Formation



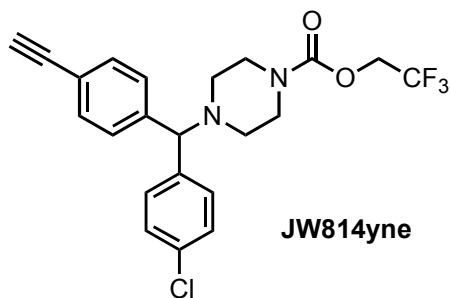
To a stirring solution of triphosgene (0.3 equiv) in CH_2Cl_2 (0.5 mL) at 0 °C was added the indicated alcohol (1.0 equiv) followed by *N,N*-diisopropylethylamine (2.0 equiv). After stirring for 2 h at room temperature, the secondary amine (1.0 equiv) was added as a solution in CH_2Cl_2 (1 mL) and stirred for another 2 h. The mixture was concentrated under reduced pressure and purified by SiO_2 flash chromatography (EtOAc/hexanes) to provide the pure carbamate.



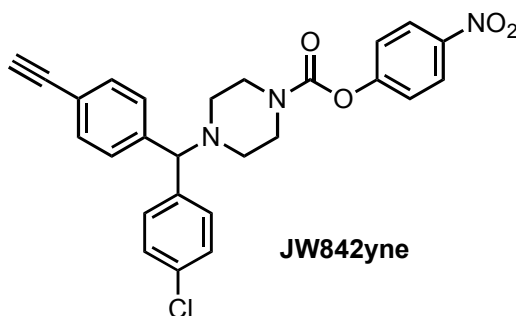
1,1,1,3,3,3-Hexafluoropropan-2-yl 4-((4-chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate (JW651yne). The title compound was prepared from 1-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine (54 mg, 0.175 mmol) and hexafluoroisopropanol (29 mg, 0.175 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO_2 flash chromatography (5% EtOAc/hexanes) to provide the title compound (78 mg, 88 %) as a white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.42 (d, J = 8.4 Hz, 2H), 7.34 – 7.26 (m, 6H), 5.75 (septet, J = 6.4 Hz, 1H), 4.24 (s, 1H), 3.55 – 3.54 (m, 4H), 3.04 (s, 1H), 2.40 – 2.37 (m, 4H); HRMS (ESI+) m/z calc'd for $[\text{M}+\text{H}]^+$ $\text{C}_{23}\text{H}_{19}\text{ClF}_6\text{N}_2\text{O}_2$: 505.1112, found 505.1116.



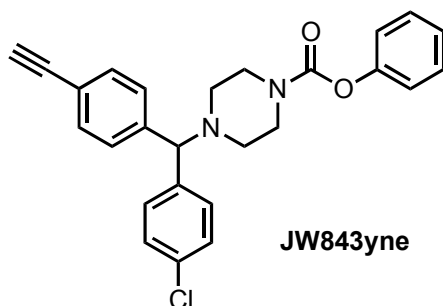
2,5-Dioxopyrrolidin-1-yl 4-((4-chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate (MJN110yne). The title compound was prepared from 1-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine (31 mg, 0.099 mmol) and *N*-hydroxysuccinimide (25 mg, 0.099 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO_2 flash chromatography (30% EtOAc/hexanes) to provide the title compound (32 mg, 71 %) as a white solid: ^1H NMR (400 MHz, CDCl_3) δ 7.41 (d, J = 7.8 Hz, 2H), 7.36 – 7.30 (m, 4H), 7.26 (d, J = 6.3 Hz, 2H), 4.25 (s, 1H), 3.63 – 3.52 (m, 4H), 3.03 (s, 1H), 2.80 (s, 4H), 2.43 – 2.38 (m, 4H); HRMS (ESI+) m/z calc'd for $[\text{M}+\text{H}]^+$ $\text{C}_{24}\text{H}_{22}\text{ClN}_3\text{O}_4$: 452.1372, found 452.1372.



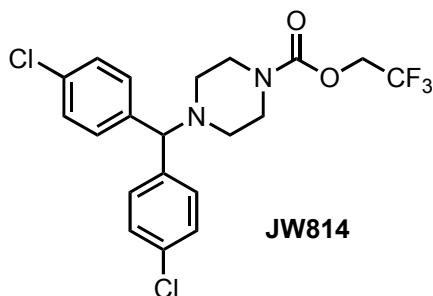
2,2,2-Trifluoroethyl 4-((4-chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate (JW814yne). The title compound was prepared from 1-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine (24 mg, 0.080 mmol) and 2,2,2-trifluoroethanol (8 mg, 0.080 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (10% EtOAc/hexanes) to provide the title compound (28 mg, 79 %) as a colorless liquid: ¹H NMR (400 MHz, CDCl₃) δ 7.42 (d, *J* = 7.9 Hz, 2H), 7.37 - 7.31 (m, 4H), 7.26 (d, *J* = 8.3 Hz, 2H), 4.47 (q, *J* = 4 Hz, 2H), 4.24 (s, 1H), 3.52 - 3.50 (m, 4H), 3.05 (s, 1H), 2.40 - 2.36 (m, 4H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₂H₂₀ClF₃N₂O₂: 437.1238, found 437.1239.



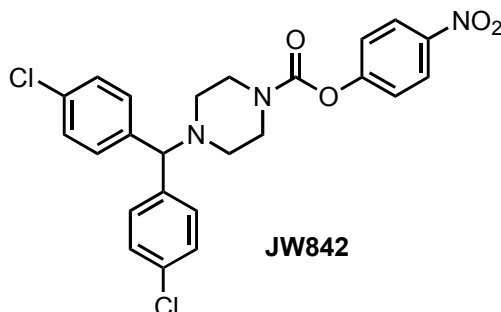
4-Nitrophenyl 4-((4-chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate (JW842yne). The title compound was prepared from 1-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine (25 mg, 0.082 mmol) and *p*-nitrophenol (16 mg, 0.082 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (10% EtOAc/hexanes) to provide the title compound (32 mg, 81 %) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.24 (d, *J* = 8.2 Hz, 2H), 7.44 (d, *J* = 7.6 Hz, 2H), 7.39 - 7.31 (m, 4H), 7.29 - 7.26 (m, 4H), 4.29 (s, 1H), 3.67 - 3.59 (m, 4H), 3.06 (s, 1H), 2.45 - 2.42 (m, 4H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₆H₂₂ClN₃O₄: 476.1373, found 476.1368.



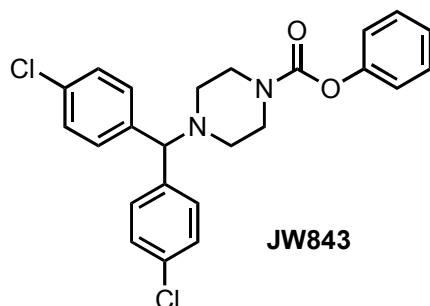
4-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine-1-carboxylate (JW843yne). The title compound was prepared from 1-((4-Chlorophenyl)(4-ethynylphenyl)methyl)piperazine (25 mg, 0.081 mmol) and phenol (21 mg, 0.081 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (10% EtOAc/hexanes) to provide the title compound (30 mg, 91 %) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 8.1 Hz, 2H), 7.37 - 7.33 (m, 6H), 7.28 - 7.25 (m, 2H), 7.18 (t, *J* = 7.3 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 2H), 4.27 (s, 1H), 3.62 - 3.57 (m, 4H), 3.05 (s, 1H), 2.43 - 2.41 (m, 4H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₆H₂₃ClN₂O₂: 431.1521, found 431.1518.



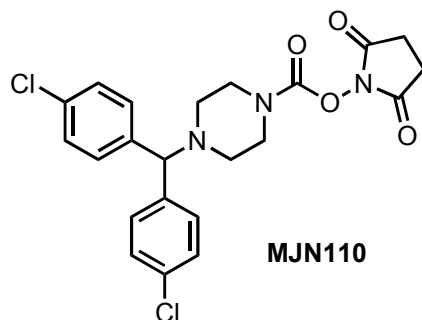
2,2,2-Trifluoroethyl 4-(bis(4-chlorophenyl)methyl)piperazine-1-carboxylate (JW814). The title compound was prepared from 1-(bis(4-chlorophenyl)methyl)piperazine (22 mg, 0.069 mmol) and 2,2,2-trifluoroethanol (7 mg, 0.069 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (10% EtOAc/hexanes) to provide the title compound (23 mg, 73 %) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.32 – 7.25 (m, 8H), 4.44 (q, *J* = 8.4 Hz, 2H), 4.20 (s, 1H), 3.52 – 3.45 (m, 4H), 2.48 – 2.36 (m, 4H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₀H₁₉Cl₂F₃N₂O₂: 447.0848, found 447.0845.



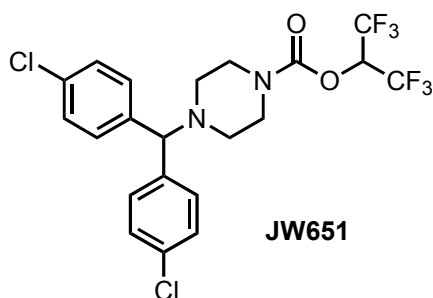
4-Nitrophenyl 4-(bis(4-chlorophenyl)methyl)piperazine-1-carboxylate (JW842). The title compound was prepared from 1-(bis(4-chlorophenyl)methyl)piperazine (21 mg, 0.066 mmol) and *p*-nitrophenol (13 mg, 0.066 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (5% EtOAc/hexanes) to provide the title compound (26 mg, 80 %) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.24 (d, *J* = 8.5 Hz, 2H), 7.37 – 7.18 (m, 10H), 4.27 (s, 1H), 3.66 – 3.58 (m, 4H), 2.45 – 2.42 (m, 4H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₄H₂₁Cl₂N₃O₄: 486.0982, found 486.0980.



Phenyl 4-(bis(4-chlorophenyl)methyl)piperazine-1-carboxylate (JW843). The title compound was prepared from 1-(bis(4-chlorophenyl)methyl)piperazine (31 mg, 0.097 mmol) and phenol (25 mg, 0.097 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (10% EtOAc/hexanes) to provide the title compound (33 mg, 77 %) as a colorless liquid: ¹H NMR (400 MHz, CDCl₃) δ 7.44 – 7.40 (m, 2H), 7.36 – 7.16 (m, 11H), 4.26 (s, 1H), 3.66 – 3.57 (m, 4H), 2.43 – 2.41 (m, 4H); HRMS (ESI+) *m/z* calc'd for [M+H]⁺ C₂₄H₂₂Cl₂N₂O₂: 441.1131, found 441.1131.

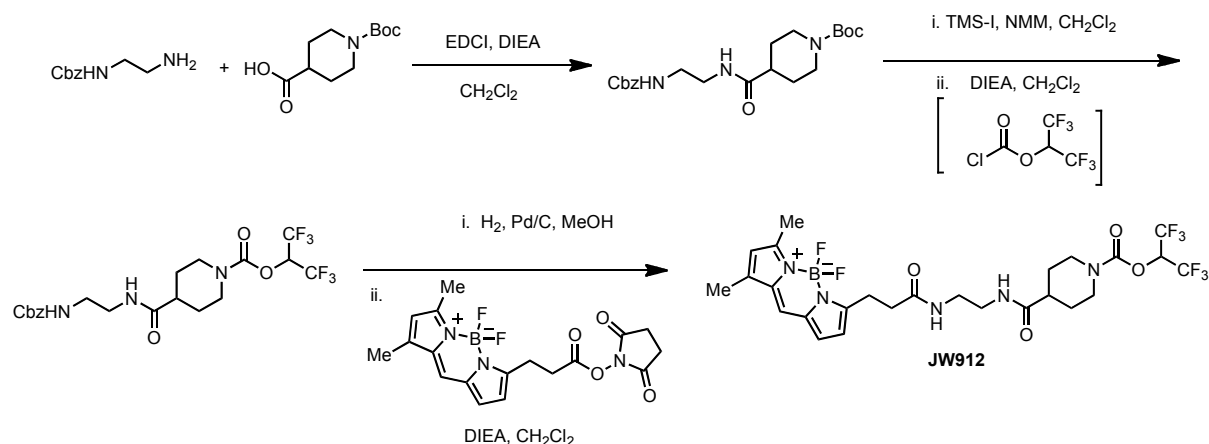


2,5-Dioxopyrrolidin-1-yl 4-(bis(4-chlorophenyl)methyl)piperazine-1-carboxylate (MJN110). To a stirring solution of *N,N'*-disuccinimidyl carbonate (130 mg, 0.50 mmol, 1.0 equiv) and *N*-methylmorpholine (0.16 mL, 1.5 mmol, 3.0 equiv) in dry CH₂Cl₂ (5.0 mL) was added 1-(bis(4-chlorophenyl)methyl)piperazine (0.50 mmol, 1.0 equiv). The reaction mixture was stirred at room temperature for 12 h. A stream of nitrogen was passed over the reaction mixture to remove the solvent and to the remaining residue was added EtOAc (20 mL). The resulting precipitate was filtered off and the filtrate was concentrated and purified by SiO₂ flash chromatography (50% EtOAc/hexanes) provided the title compound (180 mg, 78%) as a white solid: ¹H NMR (500 MHz, CDCl₃) δ 7.33 – 7.29 (m, 4H), 7.28 – 7.25 (m, 4H), 4.24 (s, 1H), 3.63 (bs, 2H), 3.51 (bs, 2H), 2.79 (s, 4H), 2.42 (d, *J* = 4.8 Hz, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 170.14, 150.70, 140.44, 133.60, 129.42, 74.76, 51.45, 51.38, 45.54, 45.04, 25.89; HRMS (ESI-TOF+) *m/z* calc'd for C₂₂H₂₁Cl₂N₃O₄ [M+H]⁺: 462.0987, found 462.0979.

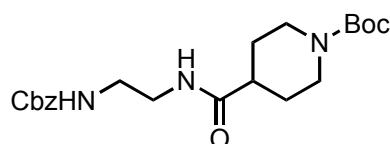


1,1,1,3,3,3-hexafluoropropan-2-yl 4-(bis(4-chlorophenyl)methyl)piperazine-1-carboxylate (JW651). The title compound was prepared from 1-(bis(4-chlorophenyl)methyl)piperazine (27 mg, 0.085 mmol) and hexafluoroisopropanol (14 mg, 0.085 mmol) according to the representative procedures for preparation of carbamates. The crude product was purified by SiO₂ flash chromatography (10% EtOAc/hexanes) to provide the title compound (39 mg, 88 %) as a white solid: ¹H NMR 400 MHz (CDCl₃) δ 7.29 (m, 8H), 5.72 (m, 1H), 4.23 (s, 1H), 3.55 (m, 4H), 2.38 (m, 4H); HRMS *m/z* calc'd for [M+H]⁺ C₂₁H₁₈Cl₂F₆N₂O₂: 515.0722, found 515.0725.

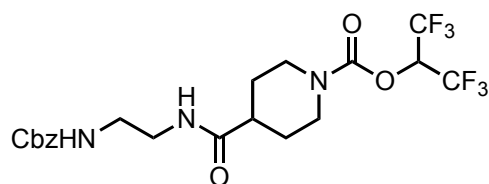
SYNTHESIS OF ACTIVITY-BASED IMAGING PROBE JW912



Scheme S2. Synthesis of JW912.

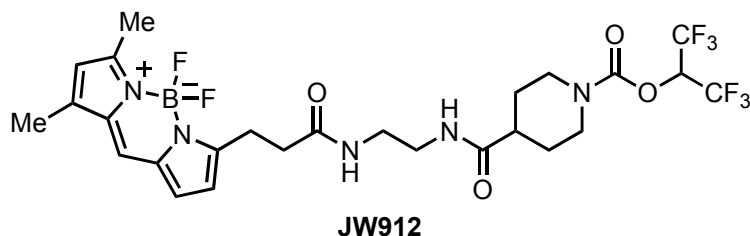


tert-butyl 4-((2-((benzyloxy)carbonyl)amino)ethyl)carbamoylpiperidine-1-carboxylate. To a solution of 1-(tert-butoxycarbonyl)piperidine-4-carboxylic acid (400 mg, 1.744 mmol) in dichloromethane (9.0 mL) was added benzyl (2-aminoethyl)carbamate (372 mg, 1.918 mmol), EDC (669 mg, 3.488 mmol), and DIEA (0.92 mL, 5.232 mmol) at room temperature. After stirring at r.t 12 hours, the reaction mixture treated with 15.0 mL of saturated aqueous NaHCO_3 solution. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over MgSO_4 , filtered and concentrated. The crude product was purified by flash column chromatography (10% EtOAc/hexanes) to afford title compound (623 mg, 88 % yield) as a white solid: $^1\text{H NMR}$ 400 MHz (CDCl_3) δ 7.39-7.31 (*m*, 5H), 6.19 (*bs*, 1H), 5.16 (*bs*, 1H), 5.10 (*s*, 2H), 4.21-4.07 (*m*, 2H), 3.41-3.34 (*m*, 4H), 2.71-2.67 (*m*, 2H), 2.20-2.14 (*m*, 1H), 1.76-1.73 (*m*, 2H), 1.58-1.52 (*m*, 2H), 1.46 (*s*, 9H); HRMS (*m/z*): $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5$, 406.2336; found, 406.2349.



1,1,1,3,3,3-hexafluoro-2-yl 4-((2-((benzyloxy) carbonyl)amino)ethyl) carbamoyl piperidine-1-carboxylate. To a solution of tert-butyl 4-((2-((benzyloxy)carbonyl)amino)ethyl)carbamoylpiperidine-1-carboxylate (353 mg, 0.871 mmol) in MeOH (4.3 mL) was added 4 M HCl in dioxane (4.0 mL) at 0 °C. After stirring at 0 °C 15 min, the solution was allowed to warm to room temperature and after sitting for a further period of 3 hours, the solvent was removed under reduced pressure. The reaction mixture treated with 10.0 mL of 0.5 M NH_3 in 1,4-dioxane. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over MgSO_4 , filtered and concentrated. The crude product was used without further purification. To a solution of carbamate intermediate (266 mg, 0.871 mmol) in dichloromethane (4.3 mL) was added triphosgene (117 mg, 0.396 mmol) and DIEA (0.70 mL, 3.958 mmol) at 0 °C. After stirring at 0 °C 1 hour, the reaction mixture was added 1,1,1,3,3,3-hexafluoro-2-propanol (133 mg, 0.792 mmol) at 0 °C. After stirring at r.t 12 hours, the reaction mixture treated with 5.0 mL of saturated aqueous NaHCO_3 solution. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over MgSO_4 , filtered and concentrated. The

crude product was purified by flash column chromatography (20% EtOAc/hexanes) to afford title compound (357 mg, 82 % yield) as a yellowish oil: $^1\text{H NMR}$ 400 MHz (CDCl_3) δ 7.39-7.31 (*m*, 5H), 6.36 (*bs*, 1H), 5.79-5.73 (*septet*, $J = 6.6$ Hz, 1H), 5.23 (*bs*, 1H), 5.10 (*s*, 2H), 4.19-4.09 (*m*, 2H), 3.41-3.31 (*m*, 4H), 3.00-2.88 (*m*, 2H), 2.29-2.21 (*m*, 1H), 1.85-1.72 (*m*, 2H), 1.69-1.61 (*m*, 2H); HRMS (m/z): $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{20}\text{H}_{23}\text{F}_6\text{N}_3\text{O}_5$, 500.1615; found, 500.1618.



5,5-difluoro-7-(3-((2-(1-(((1,1,1,3,3,3-hexafluoropropan-2-yl)oxy)carbonyl)piperidine-4-carboxamido)ethyl)amino)-3-oxopropyl)-1,3-dimethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (JW912). To a solution of 1,1,1,3,3,3-hexafluoropropan-2-yl 4-((2-(((benzyloxy)carbonyl)amino)ethyl)carbamoyl)piperidine-1-carboxylate (10 mg, 0.020 mmol) in MeOH (0.3 mL) was added activated Pd/C (2.5 mg, 25% wt) and $\text{H}_2(\text{g})$ at room temperature. After stirred at room temperature overnight, the reaction mixture was filtered and concentrated. The crude product was used without further purification. To a solution of the carbamate intermediate in dichloromethane (0.5 mL) was added 7-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)-5,5-difluoro-1,3-dimethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (5.0 mg, 0.013 mmol) and DIEA (4.0 mg, 0.039 mmol) at 0 °C. After stirring at room temperature for 8 hours, the reaction mixture was treated with 3.0 mL of saturated aqueous NaHCO_3 solution. The organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with brine, dried over MgSO_4 , filtered and concentrated. The crude product was purified by preparative TLC (50% EtOAc/hexanes) to afford title compound (6.7 mg, 56 % yield) as a red solid: $^1\text{H NMR}$ 400 MHz (CDCl_3) δ 7.10 (*s*, 1H), 6.89-6.88 (*d*, $J = 4$ Hz, 1H), 6.68-6.66 (*m*, 1H), 6.30-6.26 (*m*, 2H), 6.15 (*s*, 1H), 5.78-5.72 (*septet*, $J = 6.4$ Hz, 1H), 4.18-4.10 (*m*, 2H), 3.36-3.32 (*m*, 2H), 3.29-3.24 (*m*, 4H), 2.99-2.86 (*m*, 2H), 2.69-2.65 (*t*, $J = 7.6$ Hz, 2H), 2.56 (*s*, 3H), 2.25 (*s*, 3H), 1.87-1.82 (*m*, 2H), 1.71-1.62 (*m*, 3H); HRMS (m/z): $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{26}\text{H}_{30}\text{BF}_8\text{N}_5\text{O}_4$, 640.2336; found, 640.2335.

NMR SPECTRA

