Supporting Information

For

New Fluorescent Substrate Enables Quantitative and High-Throughput Examination of Vesicular Monoamine Transporter 2 (VMAT2)

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Part I: Structure and Synthesis of FFN206

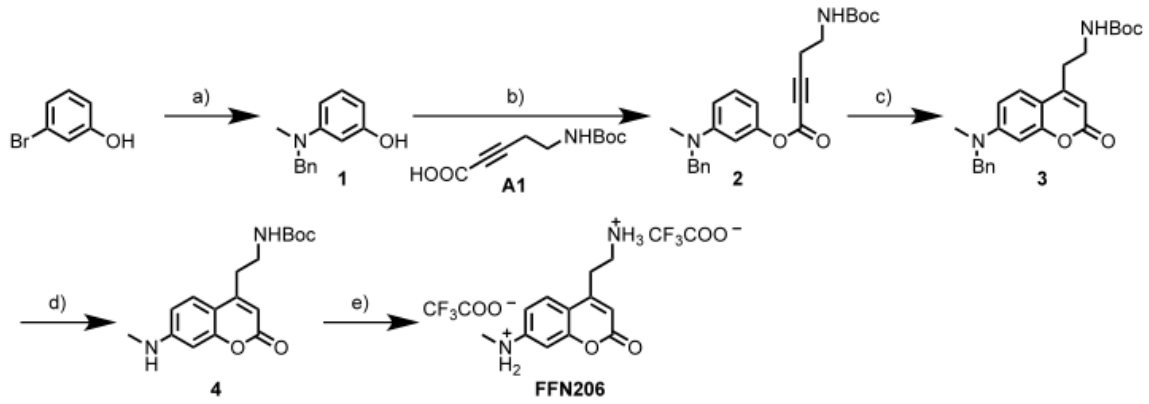
General

Unless otherwise noted, all chemicals were purchased from commercial companies and used without further purification. Nuclear Magnetic Resonance spectra were recorded at room temperature on Bruker 300, 400 MHz Fourier transform NMR spectrometers. Proton chemical shifts δ are expressed in parts per million (ppm) and are referenced to residual proton in the NMR solvent ($CDCl_3$, δ = 7.24 ppm; CD_3OD, δ = 3.30 ppm; DMSO-*d6*, δ = 2.49 ppm; D_2O, δ = 4.79 ppm). Data for ^1H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br. = broad peak), and coupling constant in Hz. Carbon chemical shifts are referenced to the carbon resonance of the NMR solvent ($CDCl_3$, δ = 77.0 ppm; CD_3OD, δ = 49.0 ppm; DMSO-*d6*, δ = 39.5 ppm). Fluorine chemical shifts were referenced to $BF_3{\cdot}OEt_2$ (δ=0 ppm). Mass spectra were recorded on a JEOL LCmate instrument (ionization mode: $APCI^+$).

Preparative HPLC was performed with a Waters 600 Controller on a Vydac C_{18} Protein & Peptide column; fractions were detected with a Waters 2487 Dual Wavelength Absorbance Detector (λ = 254 nm) and collected with a Waters 2767 Sample Manager. Data was analyzed using OpenLynx and GraphPad software. Gradient mobile phase of solvents A and B were used (A = HPLC grade acetonitrile or methanol; B = deionized water (Millipore Simplicity 185, 18.2 MΩ) containing 0.1 % (v/v) trifluoroacetic acid (TFA). Analytical HPLC was performed on a Phenomenex reverse phase column (Prodigy 5 micron ODS3 100A 250 x 4.6 mm) using isocratic mobile phase. All probes

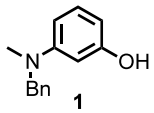
were purified by preparative HPLC in the final stage before lyophilized to give TFA salts as nice solid. The content of TFA in salts was determined by ^{1}H-NMR. The fluorine signal of TFA appears at δ = -76.02 ppm. In most cases, TFA salts of the probe was directly used in physical property measurements and cell assays. The synthesis of the key acid building block A1 was reported earlier.[1]

Synthesis of FFN206



a) MeNHBn, LiHMDS, $Pd_2(dba)_3$/JohnPhos, THF, 65 °C; b) **A1**, DIC, DMAP, DCM, 0 °C; c) $PtCl_4$, DCE/dioxane, 80 °C; d) H_2 (53 PSI), MeOH, RT; e) TFA, DCM, RT (HPLC purification)

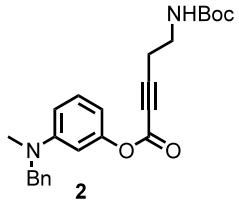
Scheme S1: Synthesis of FFN206



1

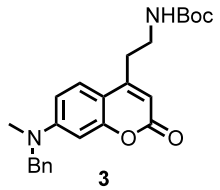
To a dried round bottom flask was added 3-bromophenol (865 mg, 5 mmol), *N*-methyl benzylamine (774 uL, 727 mg, 6 mmol), 2-(dicyclohexylphosphino)biphenyl (42 mg, 0.12 mmol) and $Pd_2(dba)_2$ (46 mg, 0.05 mmol, 1 mol%) consecutively. Under argon protection, LiHMDS (1.0 M in THF, 11 mL, 11 mmol) was added to the reaction mixture slowly with magnetic stirring. The flask was then sealed with a rubber septum and heated to 65 °C for 24 hrs. Upon cooling, $NaHCO_3$ (2.0 g, solid) was added to quench the reaction and the mixture was allowed to pass through a segment of silica gel column (~2 cm). The crude product was washed down

with ethyl acetate and the solution was concentrated *in vacuo*. The residue was purified by flash chromatography (EtOAc-hexane, 1:9) to afford compound **1** (849 mg, 80 %) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36-7.26 (m, 5H), 7.12 (t, 1H, J = 8.0 Hz), 6.42 (dd, 1H, J = 8.0, 2.0 Hz), 6.31-6.27 (m, 2H), 5.84 (br. s, 1H), 4.54 (s, 2H), 3.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.3, 151.1, 138.5, 130.0, 128.4, 126.8, 126.7, 105.3, 103.7, 99.6, 56.3, 38.3; LRMS (APCI$^+$): Calculated for $C_{14}H_{15}NO$ 213.1, measured 214.2 (***M***H$^+$).



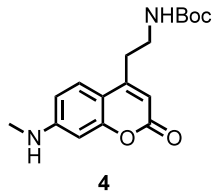
2

A round bottom flask was charged with aminophenol **1** (125mg, 0.59 mmol), carboxylic acid **A1** (150 mg, 0.70 mmol) and DMAP (18 mg, 0.15 mmol). Under argon protection, dichloromethane (6 ml) was added to dissolve the solid mixture. The reaction mixture was then cooled down in an ice-water bath. Diisopropylcarbodiimide (136 uL, 111 mg, 0.88 mmol) was added slowly through a syringe into the solution. The reaction mixture was allowed to react at 0 °C for 5 hr until its completion. The reaction mixture was directly loaded on a silica gel column. Flash chromatography (EtOAc-hexane, 1:2) afforded compound **2** as colorless oil (207 mg, 87 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37-7.20 (m, 6H), 6.66-6.63 (m, 1H), 6.51 (d, 1H, J = 7.8 Hz), 6.50 (s, 1H), 4.97 (br. s, 1H), 4.56 (s, 2H), 3.39-3.34 (m, 2H), 3.04 (s, 3H), 2.61 (t, 2H, J = 6.4 Hz), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.5, 151.8, 151.2, 150.9, 138.2, 129.8, 128.6, 126.9, 126.5, 110.2, 108.9, 104.9, 88.8, 79.7, 73.8, 56.3, 38.5, 28.3, 20.4; LRMS (APCI$^+$): Calculated for $C_{24}H_{28}N_2O_4$ 408.2, measured 408.9 (***M***H$^+$).



3

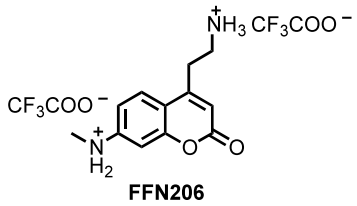
To a round bottom flask was added ester **2** (207 mg, 0.51 mmol), $PtCl_4$ (8 mg, 0.025 mmol) and dichloroethane-dioxane (1:1, 12 mL). The flask was sealed with a Teflon septum cap and heated in oil bath (80 °C) for 2 hrs. Upon removal of volatiles *in vacuo*, the residue was loaded on a silica gel column. Flash chromatography with (EtOAc-hexane, 2:3) afforded compound **3** as a yellowish solid (118 mg, 57 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50 (d, 1H, J = 8.8 Hz), 7.35-7.25 (m, 3H), 7.18 (d, 2H, J = 6.8 Hz), 6.67 (dd, 1H, J = 8.8, 2.4 Hz), 6.57 (d, 1H, J = 2.4 Hz), 5.96 (s, 1H), 4.86 (br. s, 1H), 4.63 (s, 2H), 3.45-3.32 (m, 2H), 3.15 (s, 3H), 2.89 (t, 2H, J = 2.8 Hz), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.8, 156.0, 155.7, 153.6, 152.2, 137.2, 128.7, 127.2, 126.3, 125.3, 109.1, 109.0, 108.8, 98.5, 79.5, 56.0, 39.6, 38.9, 32.3, 28.3; LRMS (APCI$^+$): Calculated for $C_{24}H_{28}N_2O_4$ 408.2, measured 408.9 ($\boldsymbol{M}H^+$).



4

To a glass pressure tube was added compound **3** (118 mg, 0.289 mmol), 10% Pd/C catalyst (27 mg), methanol (8 mL) and 4 drops of 1 N HCl. The tube was sealed and exposed to H_2 (53 psi) with constant magnetic stirring at RT. After 12-15 min until completion, the reaction mixture was neutralized with solid $NaHCO_3$, filtered and concentrated. The residue was loaded on a silica gel column. Flash chromatography with (EtOAc-hexane, 2:3) afforded yellowish solid compound **4** (75 mg, 82 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.41 (d, 1H, J = 8.8 Hz), 6.48 (dd, 1H, J = 8.8, 2.4 Hz), 6.41 (d, 1H, J = 2.4 Hz), 5.92 (s, 1H), 4.77 (br. s, 1H), 4.45 (br. s, 1H), 3.41-3.37 (m, 2H), 2.87-2.86 (m, 5H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.8, 156.3, 155.8, 153.9, 152.6, 125.3, 110.3, 109.3, 108.9, 97.8,

79.6, 39.6, 32.5, 30.1, 28.3; LRMS (APCI$^+$): Calculated for $C_{17}H_{22}N_2O_4$ 318.2, measured 319.0 (***M***H$^+$).



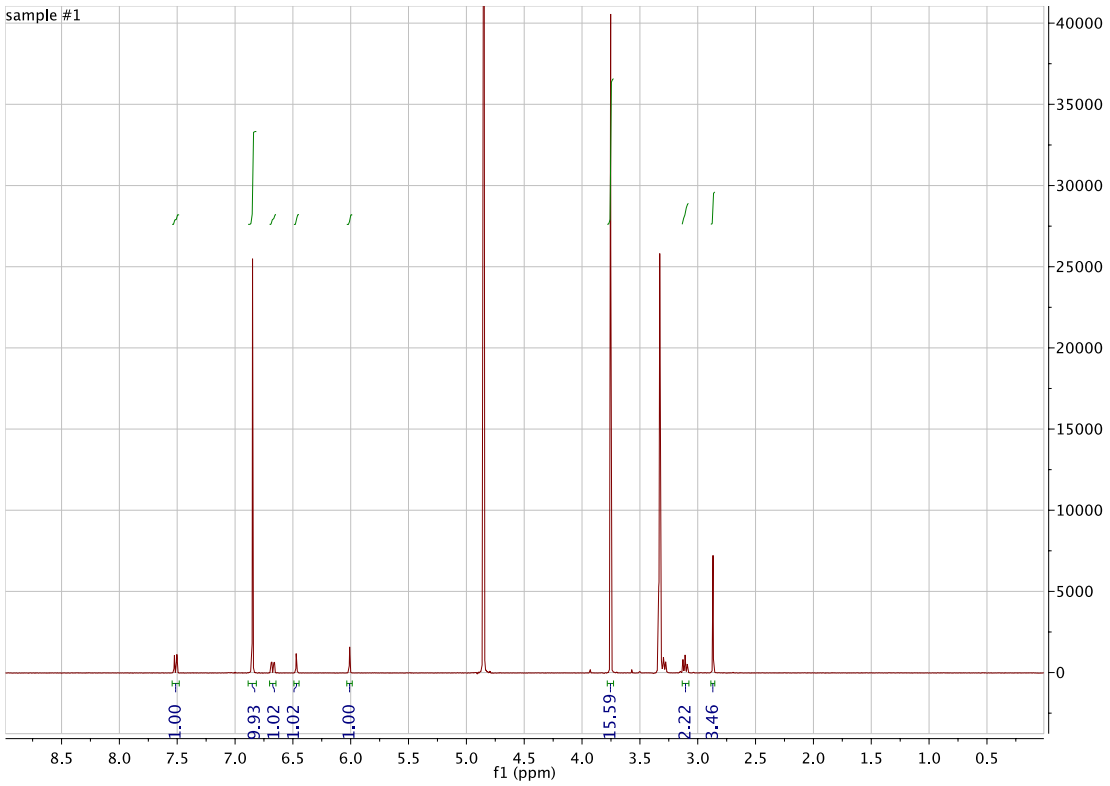
FFN206

To a solution of coumarin **4** (75 mg, 0.24 mmol) in DCM (2 mL) was added TFA (2 mL) slowly at RT. The reaction mixture was stirred for 10 min at RT at which time the starting material had been fully consumed. The solvent was removed *in vacuo* and the residue was purified by reverse phase HPLC (TFA/MeCN/H_2O). The fractions containing the product were pooled and evaporated. The residue was dissolved in water and lyophilized to afford **FFN206** as a yellow salt (77 mg with 2 eq TFA/FFN206, 73 %). ^{1}H NMR (CD_3OD, 300 MHz): δ 7.49 (d, 1H, *J* = 8.7 Hz), 6.64 (dd, 1H, *J* = 9.0 Hz, 2.4 Hz), 6.41 (d, 1H, *J* = 2.4 Hz), 5.98 (s, 1H), 3.27 (t, 2H, *J* = 7.8 Hz), 3.08 (t, 1H, *J* = 8.1 Hz), 2.83 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz): δ 164.1, 158.0, 155.6, 154.1, 126.2, 112.1, 109.0, 108.7, 97.6, 39.5, 30.4, 29.8; LRMS (APCI$^+$): Calculated for $C_{12}H_{14}N_2O_2$ 218.1, measured 219.2 (***M***H$^+$).

Determination of FFN206·TFA Molecular Weight:

The molecular weight of FFN206·TFA was determined by ^{1}H NMR spectroscopy using 1,4-dimethoxybenzene (DMB) as an internal standard. FFN206·TFA and DMB were weighed out on a microbalance and transferred to a vial. The mixture was then dissolved in CD_3OD and transferred to a NMR tube. The ^{1}H NMR spectrum was recorded on a Bruker 400 MHz NMR instrument (NMR probe was tuned prior to measurement). The molecular weight was calculated from the ratio of integral intensities of aromatic-coumarin protons to the aromatic protons of DMB. As a control, the ratio of the methyl protons of FFN206·TFA to methoxy protons of DMB was also evaluated. Both

approaches afford similar results. Three independent samples were examined, and results were consistent and reproducible. The molecular weight was determined from the mean of three independent samples to be 449.9 g.mol^{-1} and corresponds to FFN206 associating with two equivalents of TFA (calculated molecular weight for FFN206 with two molecules of TFA = 446.1 g.mol^{-1}). A representative spectrum from one analysis is displayed below.



Part II: Photophysical Characterization

Ultraviolet (UV) absorption spectra of FFN206 were measured on a Molecular Devices SpectraMax Plus 384 UV-Visible spectrophotometer equipped with SOFTMAX software. The reported extinction coefficient is the average of triplicate measurements of the lowest energy wavelength transition at three different concentrations in potassium phosphate buffer (0.1M, pH = 7.4) and chloroform.

Fluorescence measurements (emission/excitation) were carried out on a Jobin Yvon Fluorolog fluorescence spectrofluorometer. Fluorescent Spectra were taken by adding probe (1-5 μL of 0.5 mM stock solution in DMSO) to either 1000μL potassium phosphate buffer (0.1M, pH 7.4) or chloroform to a quartz cuvette (4-clear sides) and excited at the excitation maxima as stated in Table S1.

Fluorescence quantum yields are the average of three independent measurements that were determined by excitation at either 390nm if using Coumarin 102 (ϕ = 0.58 in ethanol) or 354 nm if using Coumarin 120 (ϕ = 0.75 in ethanol) as fluorescence standards,[2] depending on the proximity to the excitation maxima of the probe. The full procedure has been described in a document provided by the instrument manufacturer Jobin Yvon (http://www.jobinyvon.com/usadivisions/Fluorescence/applications/quantumyieldstrad.pdf). The calculation was based on comparison of the gradients of fluorescence emission over UV absorbance for the probe and the standard. Solvent refractive index correction was not made between ethanol and PH=7.4 buffer.

Photostability of FFN206

In order to determine the photostability of FFN206 under the fluorometric assay conditions, fluorescence emission of a solution of FFN206 (0.5 μM in PBS) was repeatedly measured on a BioTek H1MF plate reader using the same excitation and emission wavelengths as above (λ_{ex} = 368 nm, λ_{em} = 464 nm). Measurements were taken every 15 s for 10 min. No significant decrease in fluorescence intensity was observed over this time period.

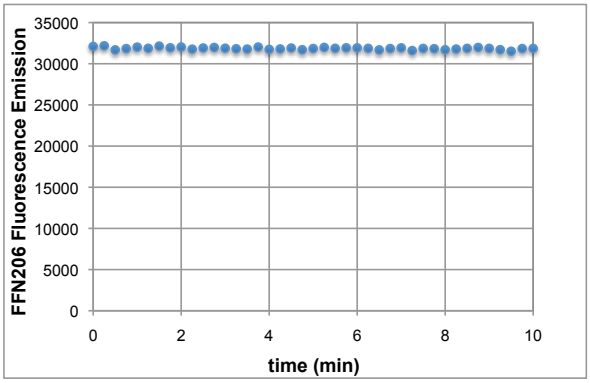


Figure S1 Photostability of FFN206. No significant attenuation of FFN206 signal was observed after repeated measurements using a plate reader.

Part III: Biological studies

(1) Results for initial fluorometric FFN206 uptake assay

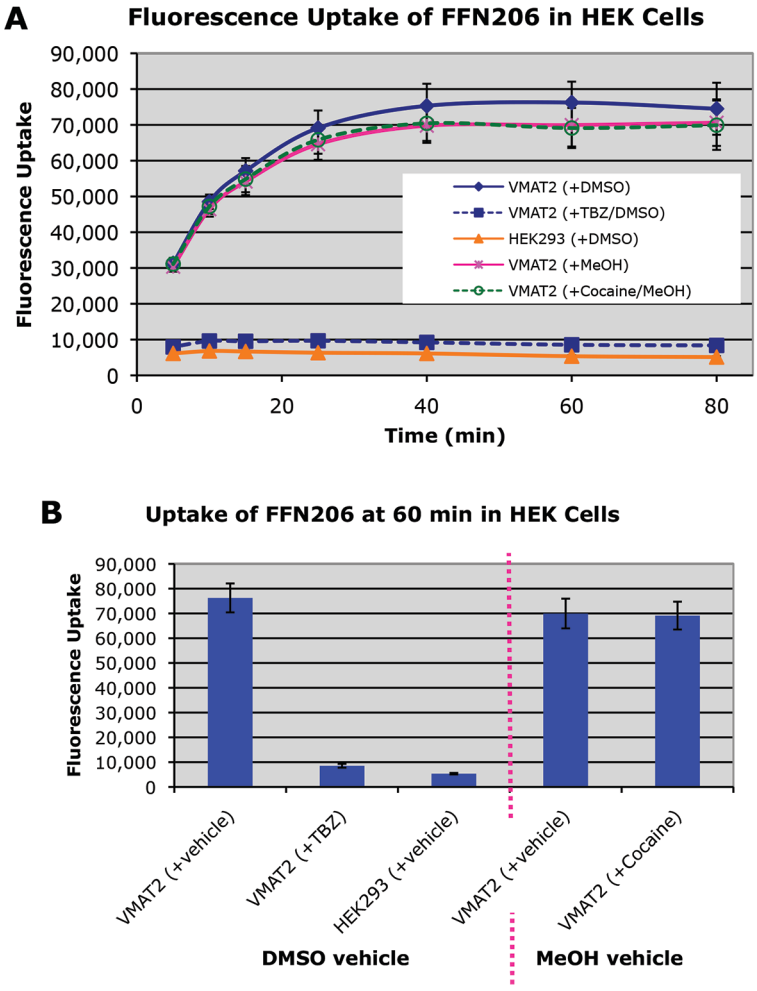


Figure S2 Time dependence of FFN206 uptake in 96-well microplate format. (A) VMAT2-HEK and control HEK cells were pretreated with vehicle controls, TBZ or cocaine (2μM), followed by treatment with FFN206 (1μM) for the designated times. Accumulation of FFN206 was measured by fluorescence immediately after washing wells at the designated times. (B) Representation of FFN206 fluorescence in cells from the same experiment after 60 minutes. Reported values represent means ± SEM derived from 3 independent experiments.

Table S1 Comparison of IC_{50} and K_i values for selected VMAT2 inhibitors

Drugs	This work			Literature	
	IC_{50}±S.E. (μM)	**Hill slope**	**K_i±S.E. (μM)**	**IC_{50}±S.E. (μM)**	**K_i±S.E. (μM)**
Reserpine	0.019± 0.001	2.76	0.0100± 0.0005	0.012± 0.004[a]	0.012[b]
Haloperidol	0.071± 0.003	0.96	0.038± 0.002	0.10±0.01[a]	
TBZ	0.32±0.01	1.63	0.171± 0.007	0.3±0.04[c]	
Lobeline	1.01±0.07	0.99	0.55±0.04	0.88± 0.001[d]	
DTBZ	0.017± 0.001	1.32	0.0093± 0.0007		0.006[e]
Fluoxetine	1.07±0.05	1.07	0.58±0.03	3.8±0.5[a]	
Ketanserin	0.105± 0.005	0.97	0.056± 0.003	0.32±0.03[f]	
S(+)-Methamphetamine	4.53±1.42	0.73	2.43±0.77	9.10±1.02[f]	

[a] Yasumoto, S.; Tamura, K.; Karasawa, J.; Hasegawa, R.; Ikeda, K.; Yamamoto, T.; Yamamoto, H. *Neurosci Lett* **2009**, *454*, 229-232. [b] Chaudhry, F. A.; Boulland, J.-L.; Jenstad, M.; Bredahl, M. K. L.; Edwards, R. H. In *Pharmacology of Neurotransmitter Release. Handbook of Experimental Pharmacology*; Sudhof, T. C., Stark, K., Eds.; Springer-Verlag Berlin Heidelberg: 2008; Vol. 184. [c] Peter, D.; Vu, T.; Edwards, R. H. *J Biol Chem* **1996**, *271*, 2979-2986. [d](1) Teng, L.; Crooks, P. A.; Sonsalla, P. K.; Dwoskin, L. P. *J Pharmacol Exp Ther* **1997**, *280*, 1432-1444; (2) Teng, L.; Crooks, P. A.; Dwoskin, L. P. *J Neurochem* **1998**, *71*, 258-265. [e] Zheng, G.; Dwoskin, L. P.; Crooks, P. A. *AAPS J* **2006**, *8*, E682-692.[f] Partilla, J. S.; Dempsey, A. G.; Nagpal, A. S.; Blough, B. E.; Baumann, M. H.; Rothman, R. B. *J Pharmacol Exp Ther* **2006**, *319*, 237-246.

(2) K_m of FFN206 at VMAT2

Procedure for Kinetic study of FFN206 uptake in VMAT2-HEK cells. The apparent K_m of FFN206 at VMAT2 was determined using the following protocol. Cells were plated as described in METHODS, and on the day of experiment, the growth medium of confluent VMAT2-HEK cells was aspirated and the cells were incubated with TBZ (2 μM, 100 μL/well) or DMSO vehicle for 1 hour in experimental media. A cocktail (100 μL/well) containing FFN206 (2 μM) and TBZ (2 μM) was added and the cells were incubated for the required time. Uptake was terminated with one PBS wash (200 μL/well)

and the cells were treated with fresh PBS buffer (120 µL). The fluorescence uptake in each well was immediately recorded in a plate reader MicroMax 384 connected to a Jobin Yvon Fluorolog fluorometer with excitation and emission wavelengths set at 369 nm and 464 nm respectively. The specific uptake was defined as the overall fluorescence uptake (with DMSO vehicle) subtracted by that in the presence of TBZ. The specific uptake was nearly linear against the incubation time of the probe when the concentration of FFN206 is 5 µM or less and the incubation time is within 12 min. The initial uptake rates for each concentration can thus be calculated and fit to the Michaelis-Menten equation using KaleidaGraph software to give apparent K_m value for FFN206 uptake. Each point is an average of 5 independent measurements performed at least in duplicate of 2 plates.

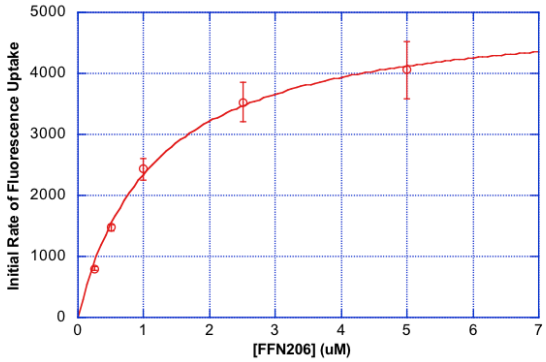


Figure S3 Plot of the initial rate of fluorescence uptake versus concentration of FFN206. The initial rates were calculated from specific uptake (total uptake subtracted by uptake in the presence of 2 μM TBZ). The apparent K_m value of FFN206 was determined to be 1.16 ± 0.10 μM. Error bars are standard errors (S.E.). The data shown in the curve were average of 5 independent experiments. Each experiment was run with two plates and duplicate wells per plate. The apparent K_m is listed as [FFN206] ± S.E.

(3) K_m determination with Digitonin

To determine if the rate determining step of FFN206 uptake is dependent on the plasma membrane, an experiment using digitonin to permeabilize the membrane was adapted from Schuldiner, et al.[3] Measurements were taken using a BioTek H1MF plate

reader in bottom read mode, and a similar protocol to that described above. First, K_m was measured on this platform under non-permeabilizing conditions to serve as an independent control. Cells stably transfected with VMAT2 (VMAT2-HEK) were seeded at a density of 3.0 x 10^4 cells/well in white clear-bottom 96-well plates and allowed to proliferate in growth medium for ~2 days at 37 ºC to reach confluence. On the day of experiment, the growth medium of confluent VMAT2-HEK cells was aspirated and the cells were incubated with DTBZ (2 μM, 100 μL/well) or DMSO vehicle for 1 hour in experimental medium. Experimental medium (100 μL/well) containing either FFN206 or a cocktail of FFN206 and DTBZ (2 μM) was added and the cells were incubated for required duration of time. The uptake was terminated with two rapid PBS washes (2x200 μL/well) and the cells were maintained in fresh PBS buffer (120 μL). The fluorescence uptake in each well was immediately recorded in a plate reader BioTek Synergy H1MF Hybrid Reader (3x3 and 5x5 area scan, bottom read mode) with excitation and emission wavelengths set at 369 nm and 464 nm respectively. The specific uptake was defined as the overall fluorescence uptake (with DMSO vehicle) subtracted by that in the presence of DTBZ. The specific uptake rates were linear for concentrations of FFN206 up to 6 μM with a corresponding incubation time of 12 min. The initial uptake rates for each concentration can thus be fit to the Michaelis-Menten equation using GraphPad Prism 5 software to give apparent K_m value for FFN206 uptake. Each independent experiment consisted of duplicate plates.

For experiments using digitonin, cells were treated as above with an additional pretreatment of digitonin (10 μM, 10 min) in clear DMEM (without phenol red) + 25 mM HEPES (Life Sciences) containing 1% BSA, with DTBZ (2μM) or DMSO vehicle

immediately before initiating uptake. After digitonin pretreatment, the permeabilization medium was removed, cells were washed (100 μL PBS), and FFN206 solutions in the same experimental medium (without digitonin) supplemented with 5 mM Na_2ATP were applied for the time required. Treatment and wash protocols were otherwise the same as described above. The specific uptake rates were linear for concentrations of FFN206 up to 6 μM for a corresponding incubation time of 9 min.

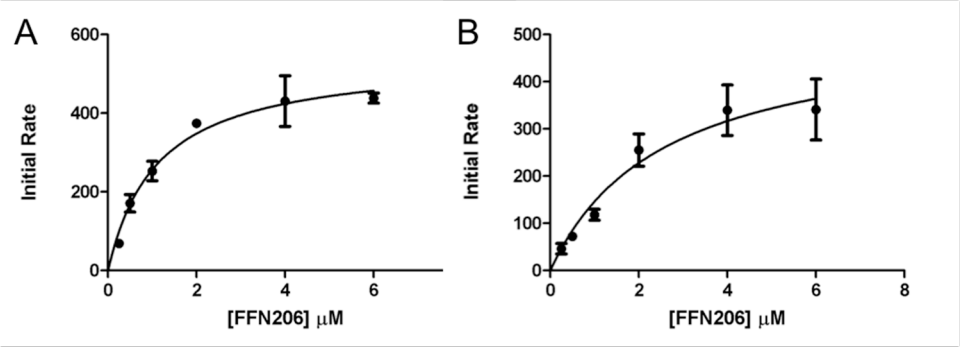


Figure S4 Apparent K_m values of FFN206 at VMAT2 in the absence and presence of digitonin were determined using a BioTek H1MF plate reader. (A) The apparent K_m was determined to be 1.12 ± 0.24, (mean ± SEM), in agreement with the value determined using a Micromax384 plate reader (1.16 ± 0.10, mean ± SEM). (B) Treatment of the cells with 10 μM digitonin (10 min) does not decrease the apparent K_m as measured by this method (2.26 ± 0.92, (mean ± SEM)), suggesting that diffusion across the plasma membrane is not the rate determining step of uptake.

(4) Measurement of Affinity to VMAT2 by Radioactive Uptake Assay

The inhibitory affinity of FFN206 for transport by VMAT2 in a membrane preparation was subsequently assessed in a radioactivity uptake assay. The uptake assay was performed as described previously.[4] Briefly, VMAT2-HEK cells were sonicated, the nuclei pelleted, and ~80 μg supernatant protein was added to a pre-warmed reaction solution of 0.32 M sucrose, 10 mM HEPES, pH 7.4, 2.5 mM $MgSO_4$, 4 mM KCl, 2 mM ATP and 20 nM [^{3}H]-serotonin (2 min, 30° C). The reaction was terminated by dilution in cold reaction solution followed by filtration and scintillation counting of the filter to

determine the amount of serotonin accumulated. We found that FFN206 indeed competes for [^{3}H]-serotonin uptake in this assay, providing apparent half maximal inhibitory concentrations (IC_{50}) of 1.15 μM respectively (Figure S5), with the potency of FFN206 being close to that of endogenous dopamine itself (IC_{50}=0.92 ± 0.05 μM).[5]

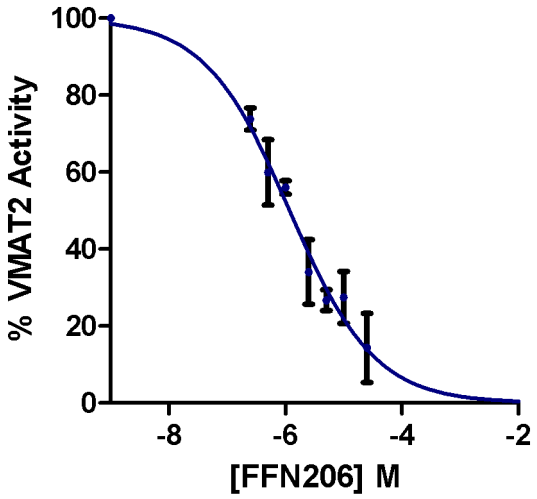


Figure S5 FFN206 inhibits the uptake of [^{3}H]-serotonin in VMAT2-expressing membrane preparations. For FFN206: IC_{50} = 1.15 ± 0.24 μM. Error bars are standard error (S.E.) derived from 3 independent experiments. Each independent experiment was run in triplicate. All IC_{50} values are listed as [inhibitor] ± S.E.

(5) Determination of High Throughput Screening (HTS) parameters

Data for the HTS assay were recorded on BioTek Synergy H1MF Hybrid Plate Reader using poly-D-lysine coated 96-well micro–assay plate. VMAT2-HEK cells were seeded at a density of 3.0 x 10^4 cells/well (by addition of 200 μL of a pre-diluted cell suspension) in white clear-bottom 96-well plates (allowed to attach the plate in the biosafety cabinet for ~20 min) and allowed to proliferate in growth medium for 50-54 h at 37 ºC to reach full confluence. On the day of experiment, the growth medium was aspirated and the cells were incubated for 30 min in experimental media with DTBZ (2

μM, 100 μL/well) or DMSO vehicle. A solution of FFN206 (2 μM) or a cocktail of FFN206 (2 μM) and DTBZ (2 μM) in the experimental medium (100 μL/well) were added and the cells were incubated for 1 h. The final layout of the plate consisted of 48 wells of positive control (2 μM DTBZ) and 48 wells of uninhibited negative control (DMSO vehicle). The uptake was terminated with PBS wash (2x200 μL/well) and the cells were maintained in fresh PBS buffer (120 μL). The fluorescence uptake in each well was immediately recorded in bottom-read mode (using three different methods – single point, area scan (matrix 3x3) and area scan (matrix 5x5)) in the plate reader with excitation and emission wavelengths set at 369 nm and 464 nm respectively. Z' value, coefficient of variation (CV) and signal to basal ratio (S/B) were calculated for each plate run in duplicate per experiment. Presented are the arithmetic means of these values calculated from 5 independent experiments consisting of duplicate plates.

$$z' = 1 - \frac{3\times \sum stdev}{mean(-) - mean(+)}$$

$$CV = \frac{stdv}{mean}\times 100$$

$$S/B = \frac{(-)mean}{(+)mean}$$

Figure S6 Equations used to calculate high-throughput Screening parameters

Table S2 High throughput screening parameters as measured by a BioTek H1MF plate reader recoring single points from each well (fastest for HT) compared to area scans of various dimensions. Values are reported as means ± SD.

	Single Point	Area Scan (3x3)	Area Scan (5x5)
Z'	0.68±0.07	0.79±0.03	0.82±0.03
CV (-)	7.0±2.1	4.6±0.7	4.0±0.5
CV (+)	11.0±1.0	7.2±0.8	7.2±1.0
S/B	5.4±0.9	6.2±0.9	6.4±1.0

Part IV: References

[1] Vadola, P.A. and Sames, D. (2012) Catalytic coupling of arene C-H bonds and alkynes for the synthesis of coumarins: substrate scope and application to the development of neuroimaging agents, *J. Org. Chem. 77*, 7804-7814.

[2] Reynolds, G. A., Drexhage, K. H. (1975) New coumarin dyes with rigidized structure for flashlamp-pumped dye lasers, *Opt. Commun. 13*, 222-225.

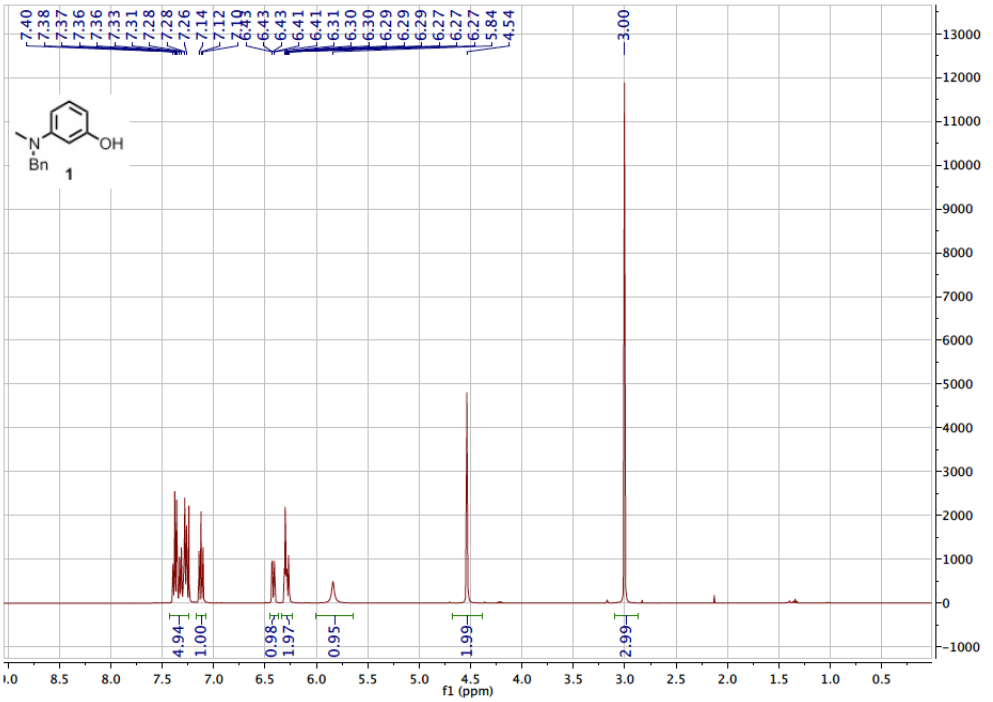
[3] Yelin, R., and Schuldiner, S. (1995) The pharmacological profile of the vesicular monoamine transporter resembles that of multidrug transporters, *FEBS Lett. 377*, 201-207.

[4] Finn, J. P., and Edwards, R. H. (1997) Individual residues contribute to multiple differences in ligand recognition between vesicular monoamine transporters 1 and 2, *J. Biol. Chem. 272*, 16301-16307.

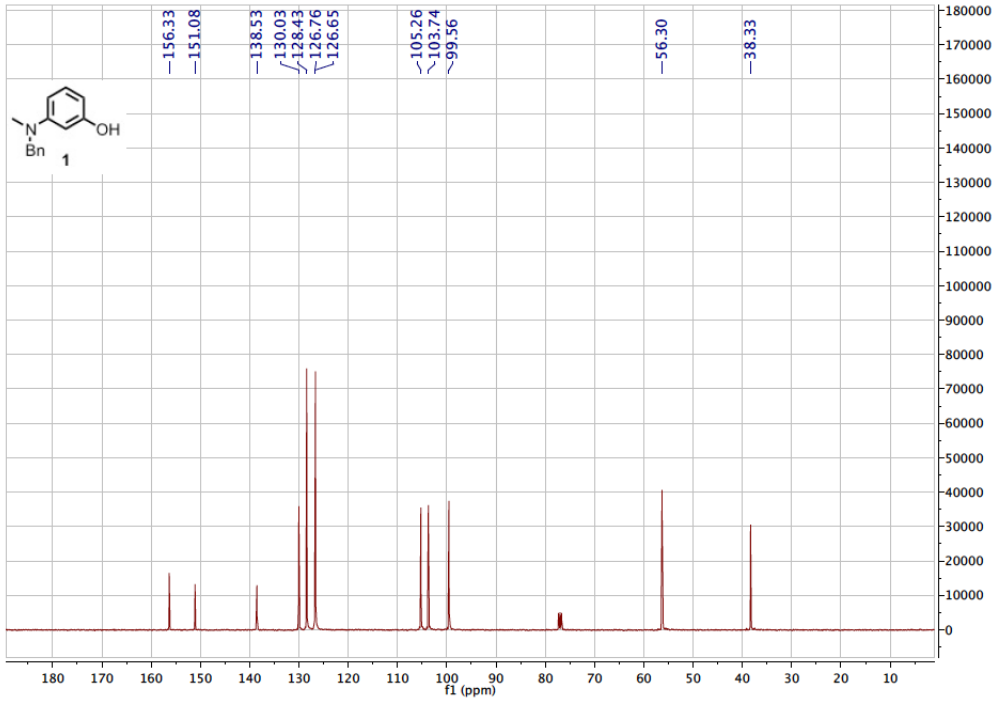
[5] Partilla, J. S., Dempsey, A. G., Nagpal, A. S., Blough, B. E., Baumann, M. H., and Rothman, R. B. (2006) Interaction of Amphetamines and Related Compounds at the Vesicular Monoamine Transporter, *J. Pharmacol. Exp. Ther. 319*, 237-246.

Appendix I: NMR Spectra

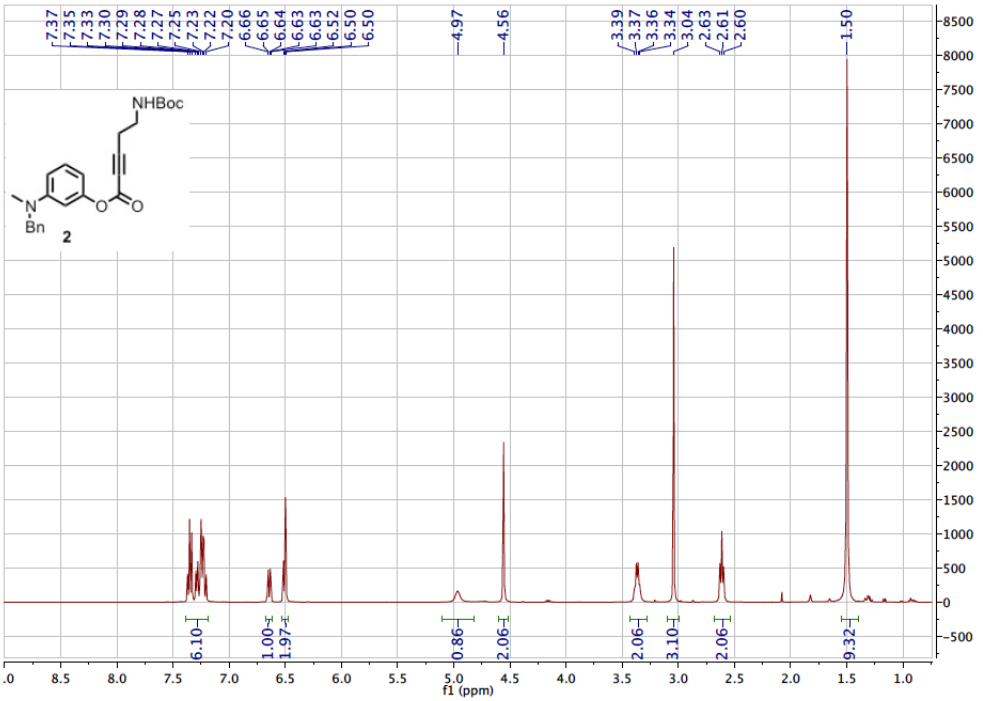
Compound 1 ^{1}H NMR:



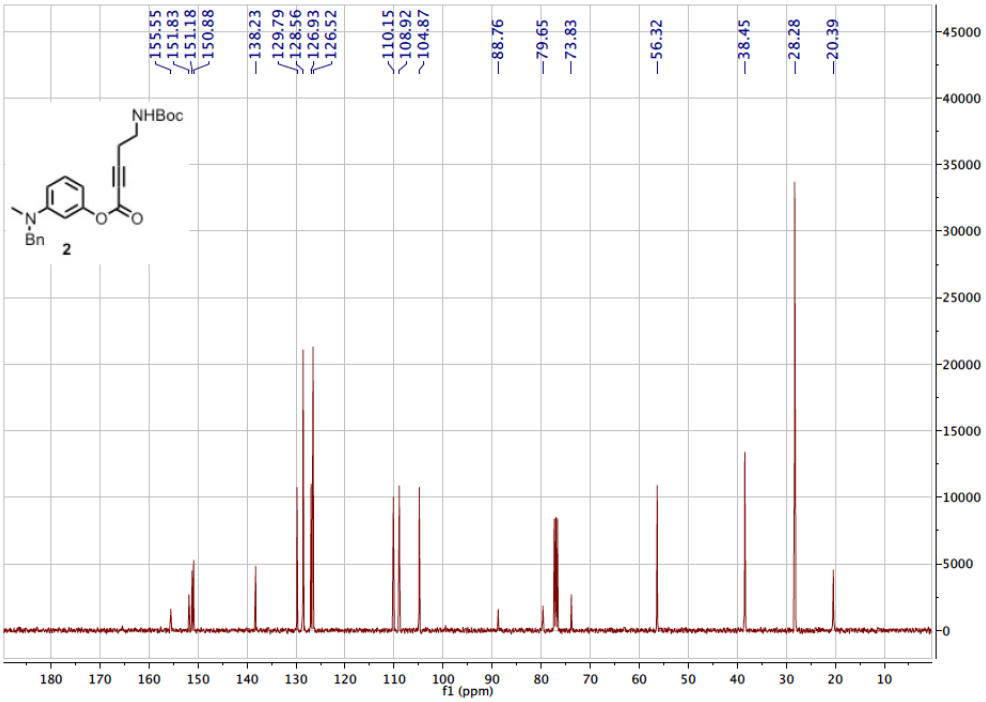
Compound 1 ^{13}C NMR:



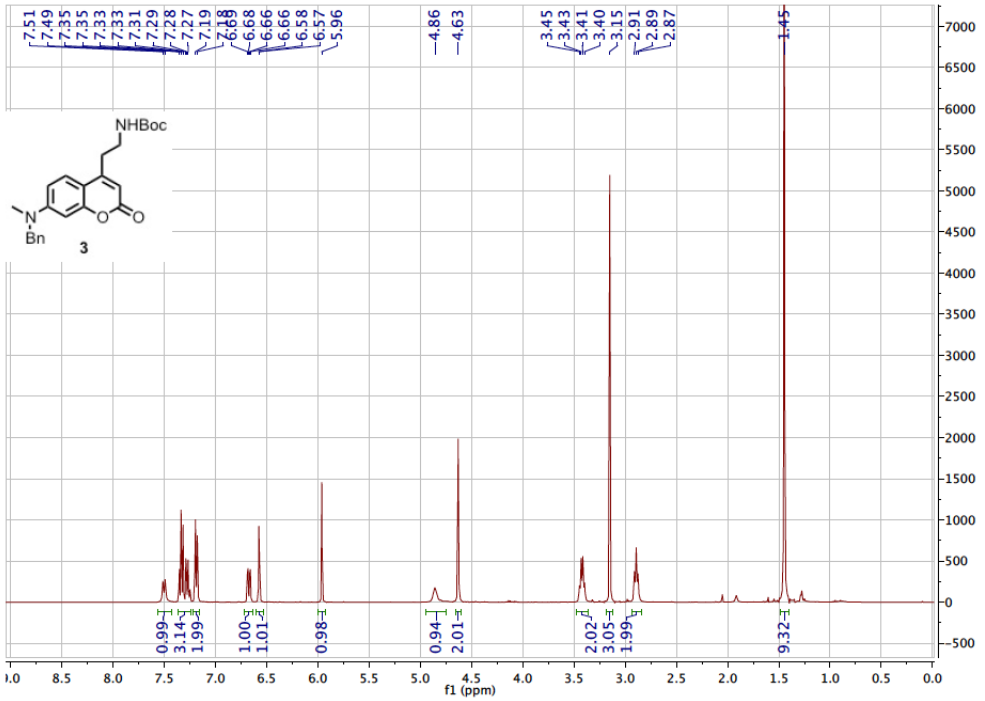
Compound 2 ^{1}H NMR:



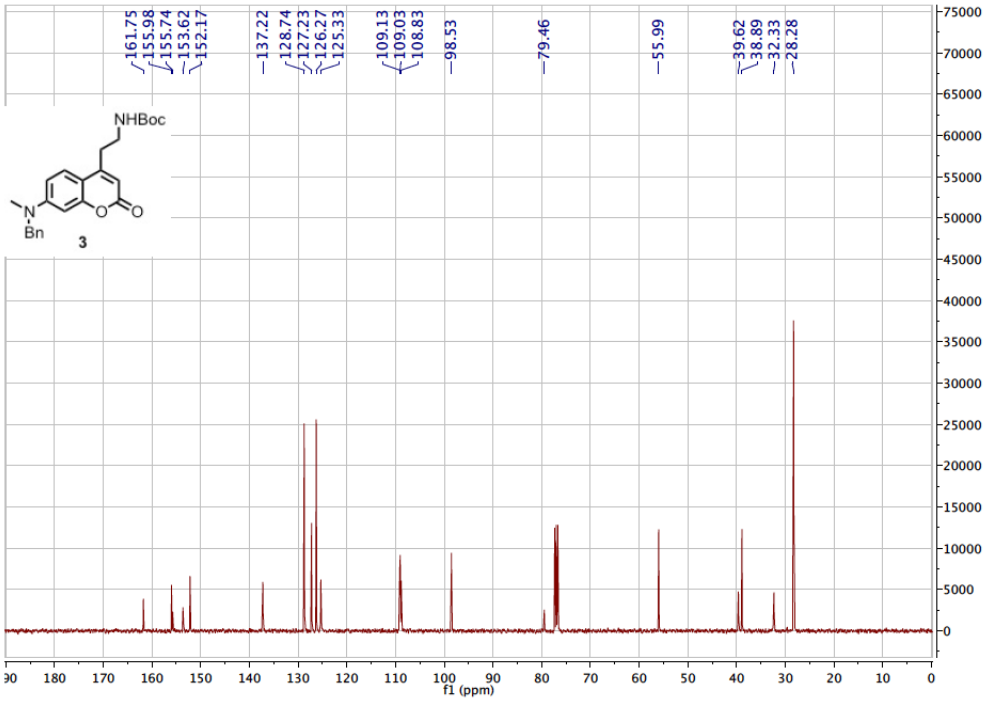
Compound 2 ^{13}C NMR:



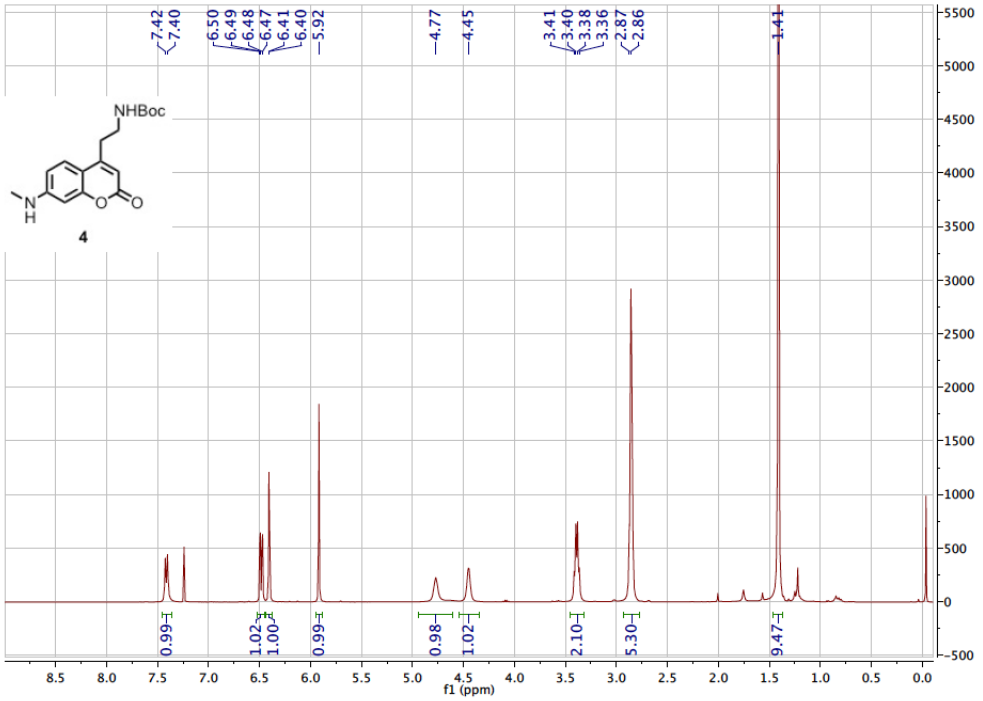
Compound 3 ^{1}H NMR:



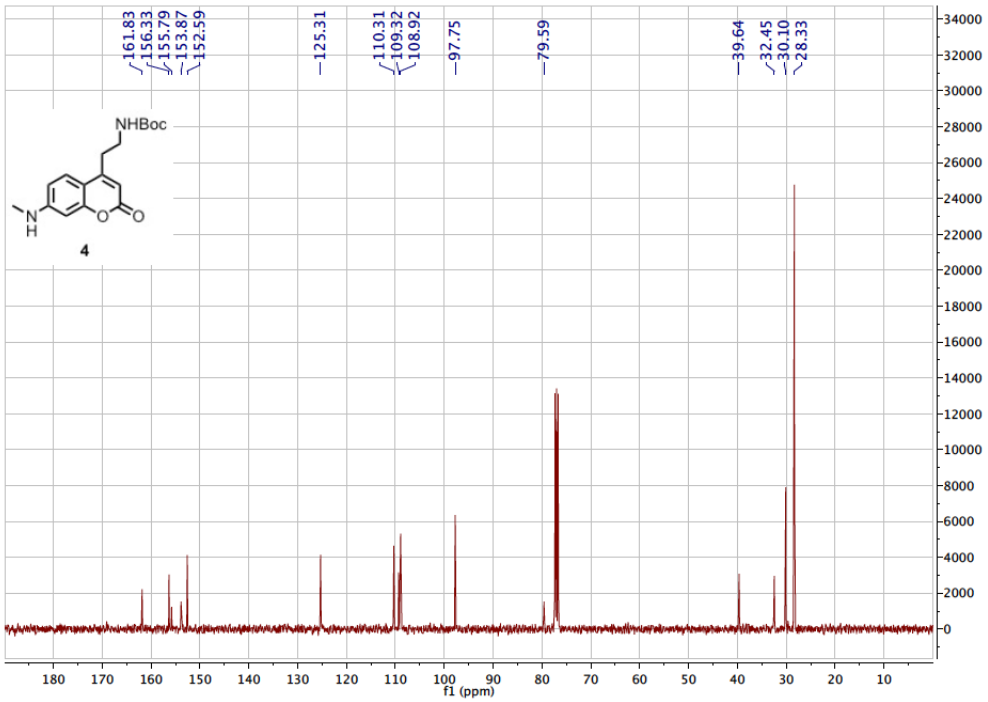
Compound 3 ^{13}C NMR:



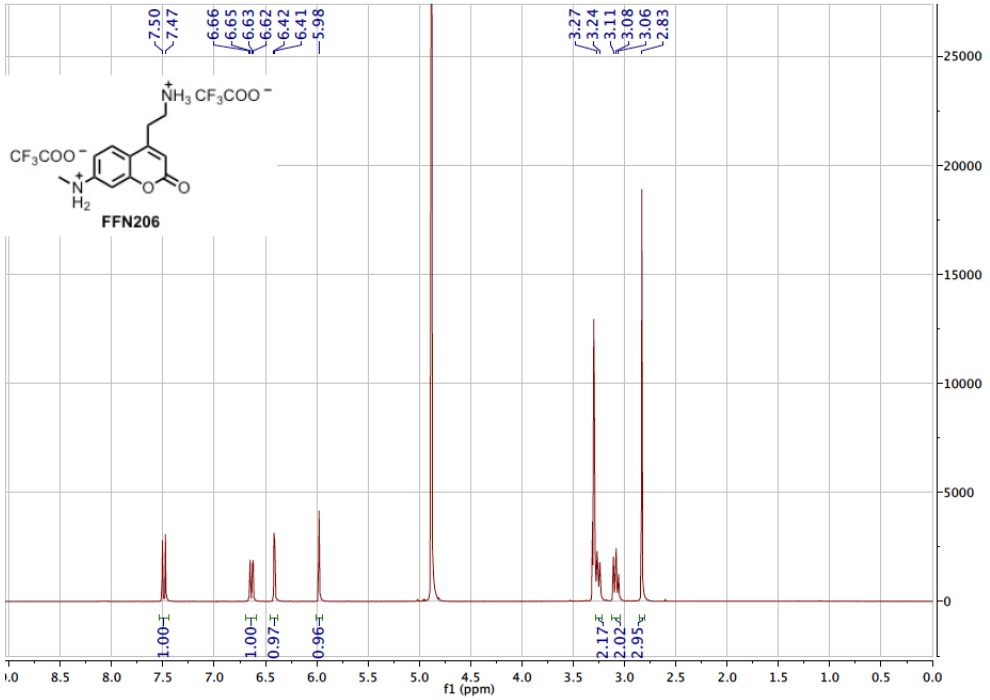
Compound 4 ^{1}H NMR:



Compound 4 ^{13}C NMR:



FFN206 ^{1}H NMR:



FFN206 ^{13}C NMR: