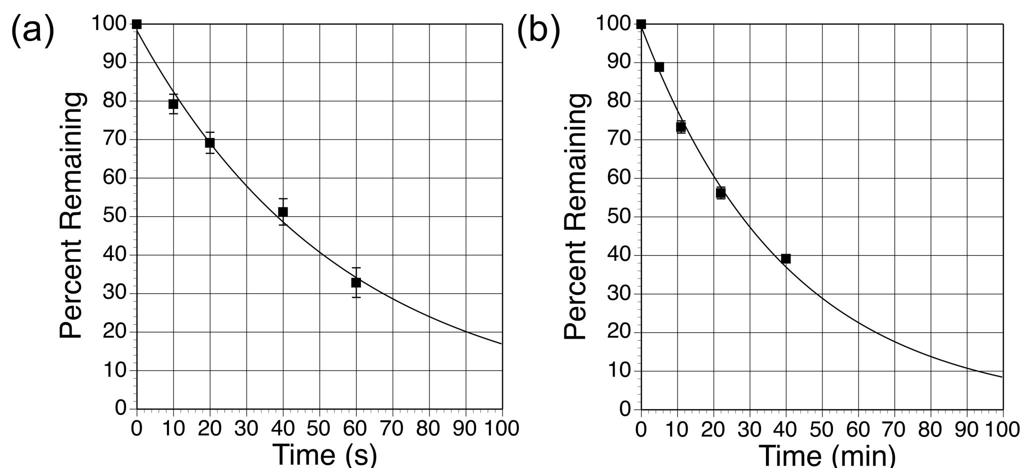


Figure S1. Time course for photolysis of BHQ-OPh at (a) 365 nm (1PE) and (b) 740 nm (2 PE) in KMOPS buffer (pH 7.2) to accompany Figure 2a and Table 1 in the main text. The percent remaining was determined by HPLC and is the average of at least 3 runs. Lines are least-squares fits of a single exponential decay. From these curves, Q_u and δ_u were calculated. Error bars represent the standard deviation of the measurement.

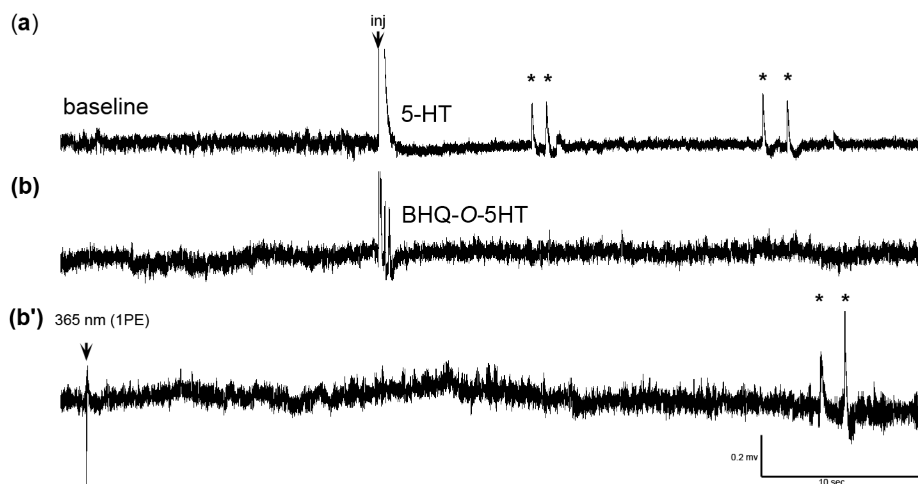


Figure S2. In vivo extracellular field recordings of 5-HT induced changes in neural activity in the optic tectum of 7-day old zebrafish larva to accompany Figure 6 in the main text. Baseline recordings from the optic tectum of 7-day old larvae show low amplitude neural activity. **(a)** Micro-injection of 1 nL of a 250-μM buffered 5-HT solution into the optic tectum elicited periodic high amplitude spiking (asterisks) within 10 s of the injection. Response shown was observed in 27 out of 29 experiments. **(b)** Micro-injection of 1 nL of a 500-μM buffered BHQ-*O*-5HT solution did not alter baseline activity. Response shown was observed in 12 out of 12 experiments. **(b′)** Photolysis of BHQ-*O*-5HT by exposure of the embryo to four 1-ms pulses of 365-nm light elicited periodic high amplitude spiking (asterisks) within 20-30 s. Response shown was observed in 12 out of 12 experiments.

Electrophysiology in the Optic Tectum of 7 dpf Larval Zebrafish (Figure S2)
Animals. Larval zebrafish (*Danio rerio*) of the WIK strain were obtained from animals maintained in the University of Georgia Zebrafish Facility following standard procedures (Westerfield, 2007). Embryos and larvae were staged using standard staging criteria (Kimmel, et al., 1995; Westerfield, 2007). All experiments conformed to the guidelines on the ethical use of animals. All experimental procedures were conducted according to National Institutes of Health guidelines under protocols approved by the University of Georgia Institutional Animal Care and Use Committee and were designed to minimize animal suffering.

Solutions. The 5-HT used for this study was purchased from Sigma-Aldrich (St. Louis, MO). The concentration of 5-HT used was 250 μM for each experiment. All neurotransmitters were dissolved in phosphate buffered saline (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) containing 0.5% dimethyl sulfoxide. Solid BHQ-*O*-5HT was dissolved in phosphate buffered saline containing 0.5% dimethyl sulfoxide, making a 500 μM solution.

Electrophysiology. Larval zebrafish, 7 days post-fertilization (7 dpf) of age, were immobilized by exposure to 0.5 mM pancuronium bromide in normal Ringer's solution (116 mM NaCl, 2.9 mM KCl, 1.8 mM $CaCl_2$, 5.0 mM HEPES, pH 7.2.) and mounted in 1.2% agarose made with normal Ringer's in a 35-mm petri dish, to which 2850 μL Ringer's solution was added. A sharp glass pipet microelectrode (15–20 MΩ impedance), loaded with normal Ringer's solution, was inserted into the optic tectum and the chloride-coated silver reference wire was placed was placed touching a dorsal region of the tail. For drug delivery, a second sharp glass pipet was inserted into the optic tectum in close proximity to the primary electrode. After a 2-minute baseline was recorded, 1 nL of the test compounds were microinjected into the optic tectum and the neurological responses recorded. To control for light flashes used in the uncaging experiments, a fiber optic cable was used to flash the larval set-up (400 V, 2,000 mF × 4 at 2-s intervals) one min after microjection. To test the ability of caged 5-HT to alter the neurological activity of the larvae, BHQ-*O*-5HT was microinjected into the optic tectum of a larval zebrafish. It was observed that injection of the caged-compound did not disrupt or alter the native electrophysiological state of the larvae. Once it had been established that microinjection of the caged-compound would not disrupt the native neural activity, the BHQ-*O*-5HT was photolysed (400 V, 2,000 mF × 4 at 2-s intervals) using a fiber optic cable to flash the larval set-up. For all experiments, the set-up procedure was carried out in dark room conditions. Electrical activity was recorded using an Axoclamp 900a amplifier (Axon Instruments, Union City, Ca, USA). The amplified voltage was passed through a Hum Bug Noise Eliminator (AutoMate Scientific, Berkley, CA, USA), band-pass filtered from 1 Hz–0.1 kHz, and digitized at 10 kHz using a Digidata 1440 interface and stored on a PC using pClamp software (version 10.3, Axon).

References
Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F. (1995). Stages of embryonic development of the zebrafish. Dev. Dyn. *203*, 253-310.

Westerfield, M. ed. (2007). The Zebrafish Book: A Guide for the Laboratory Use of Zebrafish (*Danio rerio*), 5th Edition (Eugene, OR: University of Oregon Press).

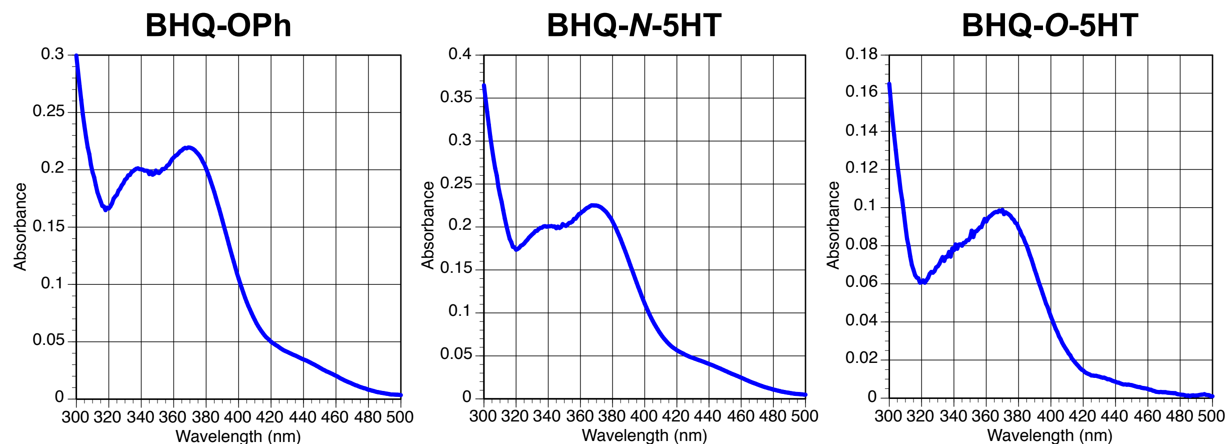
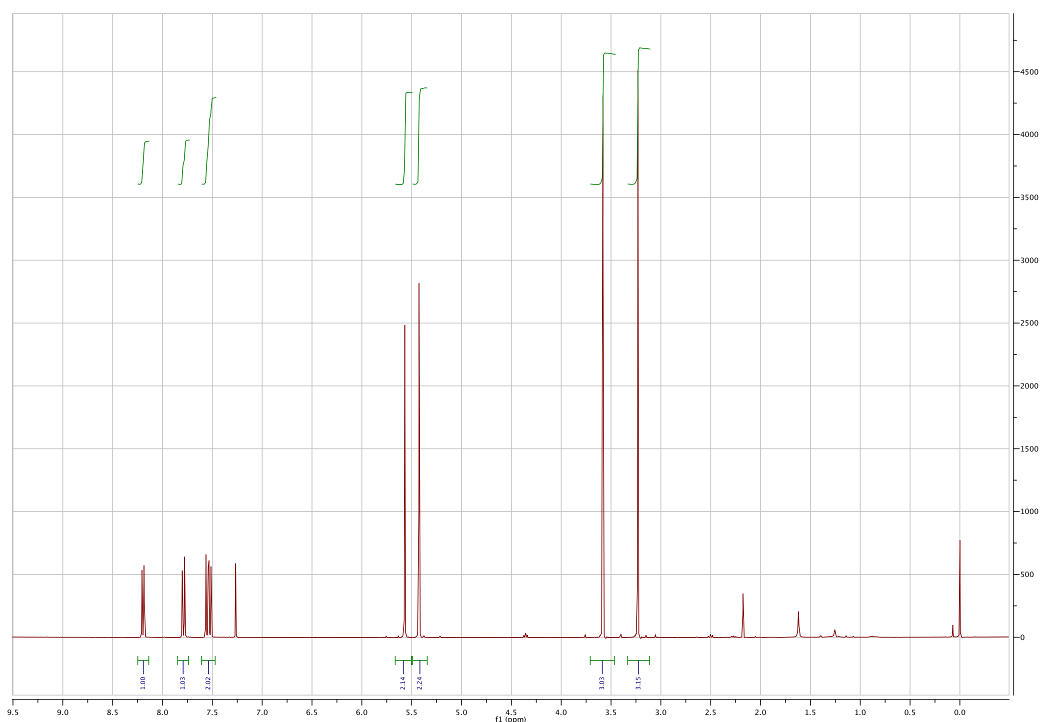
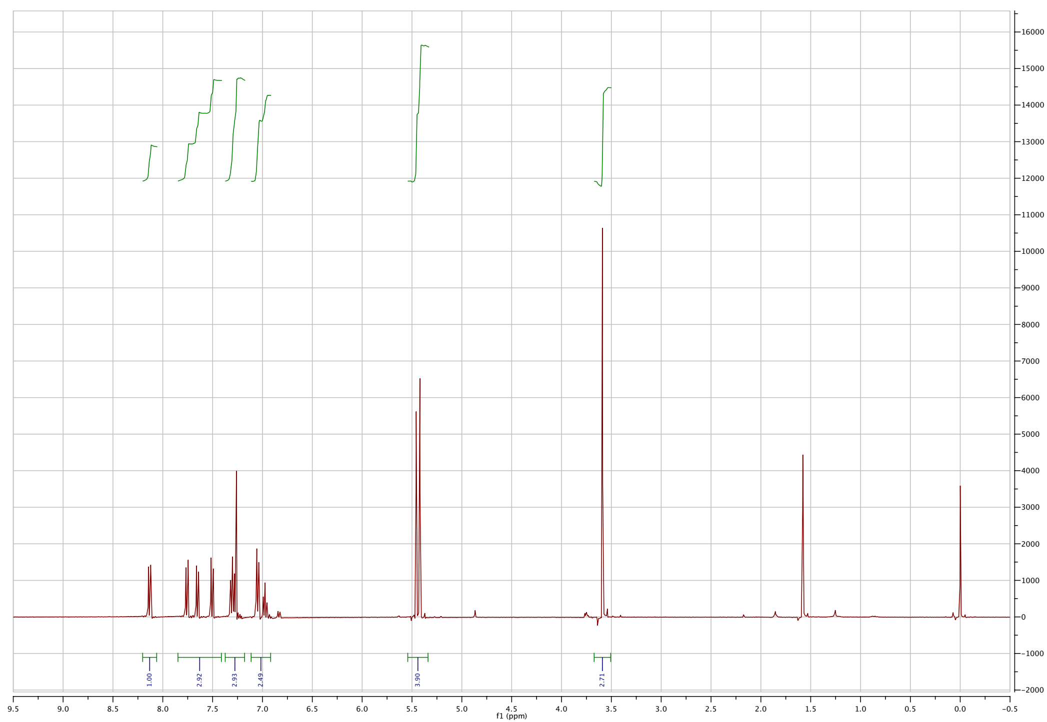


Figure S3. Representative UV-vis spectra of BHQ-OPh (68 μM), BHQ-*N*-5HT (110 μM), and BHQ-*O*-5HT (49 μM) in KMOPS buffer (pH 7.2) to accompany Table 1.

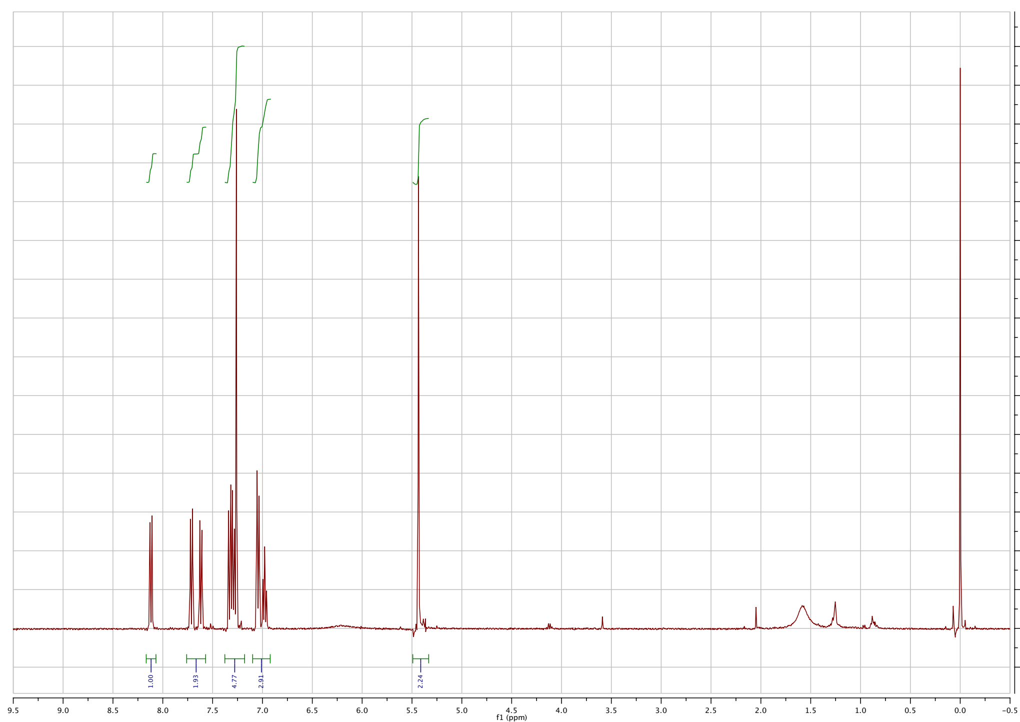
^{1}H NMR Spectra (see synthetic preparations in the Experimental Procedures section)
MOM-BHQ-OMs (chloroform-d)



MOM-BHQ-OPh (chloroform-d)



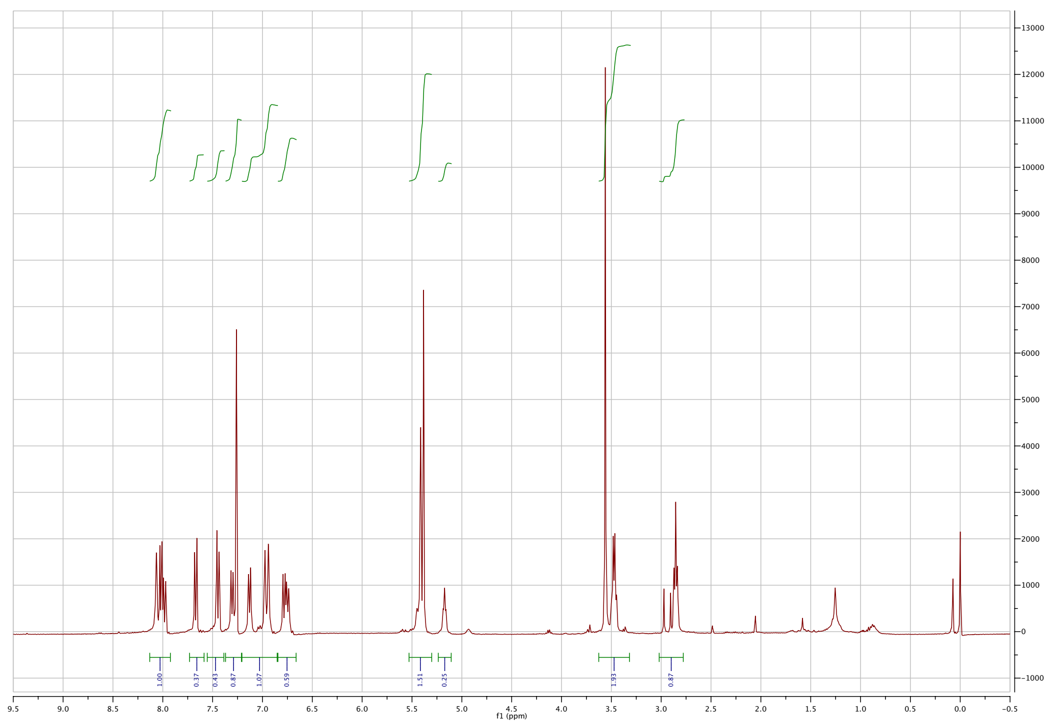
BHQ-OPh (chloroform-d)



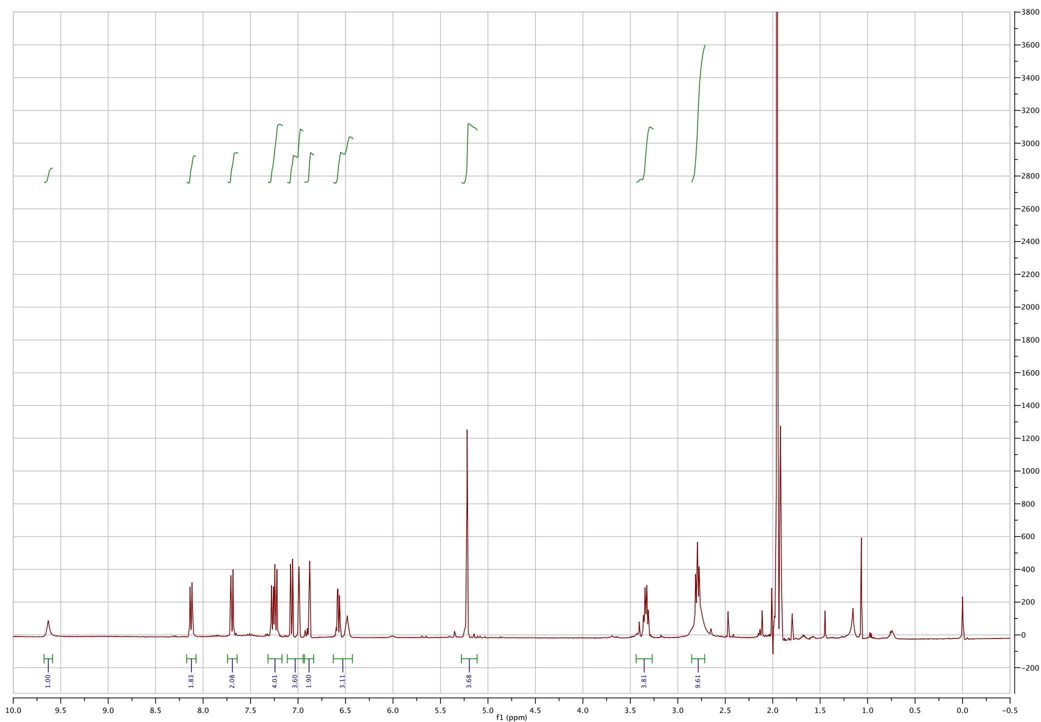
MOM-BHQ-Carbonylimidazole (chloroform-d)

*MOM-BHQ-*N-*5HT(O-TIPS) (chloroform-d)*

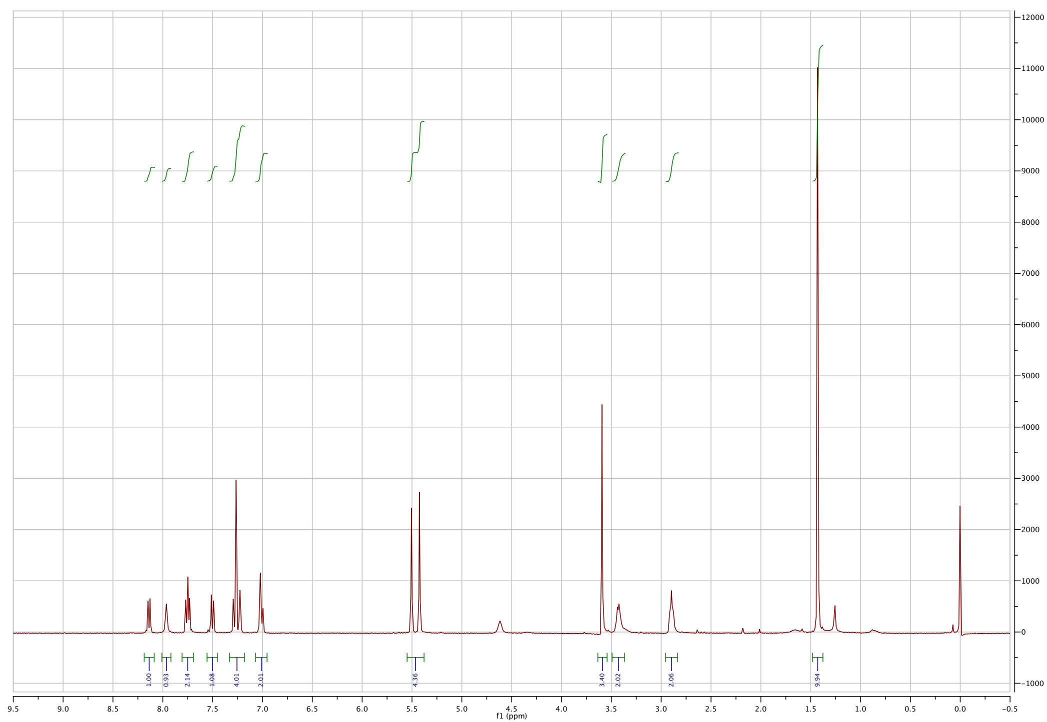
MOM-BHQ-N-*5HT (chloroform-d)*



BHQ-N-*5HT (acetone-d_6)*



*MOM-BHQ-O-5HT(*N-*Boc) (chloroform-d)*



BHQ-O-5HT (methanol-d_4)

HPLC Data (see synthetic preparations in the Experimental Procedures section)

BHQ-O-5HT

Microsorb-MV 100-5 C18 250 × 4.6 mm column

40% CH_3CN/60% H_2O (w/ 0.1 % TFA) isocratic, 1 mL/min flow rate