Supporting Information

New GABA/Glutamate Receptor Target for [^{3}H]Isoxazoline Insecticide

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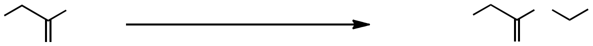
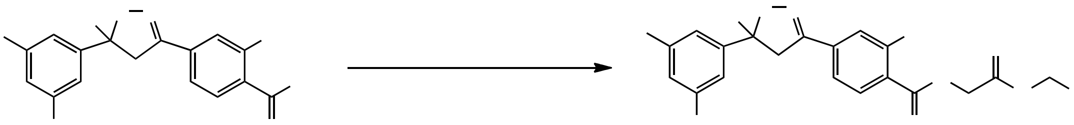
General Methods. All reactions were performed in flame-dried glassware fitted with rubber septa under a nitrogen atmosphere unless otherwise specified. Liquid reagents and solvents were transferred via syringe under nitrogen. Tetrahydrofuran (THF), diethyl ether and toluene were dried over alumina under a nitrogen atmosphere in a GlassContour solvent system. Dichloromethane (CH_2Cl_2) was distilled over calcium hydride. All other solvents and reagents were used as received unless otherwise noted. Reaction temperatures above 23 °C were controlled by an IKA® temperature modulator. Reactions were monitored by thin layer chromatography using SiliCycle silica gel 60 F254 precoated plates (0.25 mm) which were visualized using UV light, *p*-anisaldehyde stain, $KMnO_4$ or CAM stain. Sorbent silica gel (particle size 40-63 μm) was used for flash chromatography. ^{1}H and ^{13}C NMR were recorded on Bruker AVB-400, AV-500, or AV-600 MHz spectrometers with ^{13}C operating frequencies of 100, 125, and 150 MHz, respectively, in $CDCl_3$ or C_6D_6) at 23 °C. Chemical shifts (δ) are reported in ppm relative to the residual solvent signal ($CDCl_3$ $\delta = 7.27$ for ^{1}H NMR and $\delta = 77.2$ for ^{13}C NMR). Data for ^{1}H NMR are reported as follows: chemical shift (multiplicity, coupling constant, number of hydrogens). Multiplicity is abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Mass spectral data were obtained from the Mass Spectral Facility at the University of California, Berkeley. HPLC analysis of [^{3}H]A1443 was performed using a Prevail C18 column (5 μm, 4.6 x 150 mm) with mobile phase A (0.05% trifluoroacetic acid in water) and mobile phase B (acetonitrile) at a flow rate of 1.0 mL/min and a gradient of t=0 min 50% B, 10 min 90% B, 12 min 60% B. Under these conditions, the retention time of [^{3}H]A1443 was 7.362 min.

Starting Materials. Commercially available starting materials were used as received. Boc-Gly-OH, *N,N'*-dicyclohexylcarbodiimide, 4-dimethylaminopyridine and thionyl chloride were purchased from Aldrich. Trifluoroethylamine was purchased from Matrix Scientific. Carboxylic acid **2** was prepared following the patented procedure.[1]

Experimental Procedures

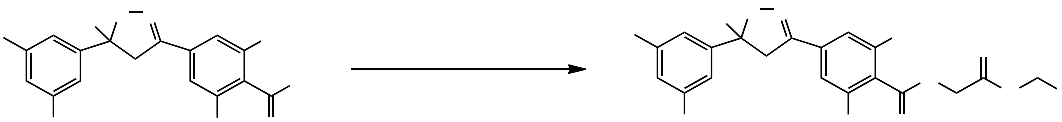


Boc-Gly-NHCH$_2$CF$_3$ (1). *N,N'*-Dicyclohexylcarbodiimide (708 mg, 3.43 mmol) was added in one portion to a 0 ºC stirring solution of *N*-Boc-Gly-OH (500 mg, 2.85 mmol) and 4-dimethylaminopyridine (34.8 mg, 285 µmol). After stirring the resulting reaction mixture at 0 ºC for 5 min, trifluoroethylamine (269 µL, 3.43 mmol) was added and the reaction mixture stirred at room temperature. After 8 h, the reaction mixture was filtered through a short pad of Celite™ and the filtrate was concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (20% EtOAc/Hex), using 30 mL silica gel, afforded dipeptide **1** (535 mg, 2.09 mmol, 73%) as a colorless solid. ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.69 (s, 1H), 5.12 (s, 1H), 3.93 (m, 2H), 3.85 (d, *J* = 5.92 Hz, 1H), 1.47 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 170.3, 156.6, 124.2 (q, *J* = 278 Hz), 80.9, 44.5, 40.7 (q, *J* = 34.91 Hz), 28.4; HRMS-ESI calcd for $C_9H_{15}N_2O_3F_3Na$ ($[M+Na]^+$): 279.0927, found 279.0924.

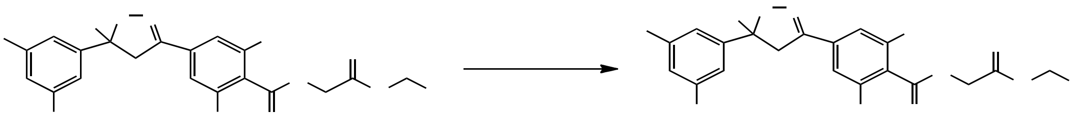


A1443. Isoxazoline A1443 was prepared following the procedure reported in a patent.[2] ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.58-7.41 (m, 6H), 6.92-6.85 (m, 1H), 6.74-6.68 (m, 1H), 4.21 (d, *J* = 5.33 Hz, 2H), 4.09 (d, *J* = 17.2 Hz, 1H), 4.01-3.91 (m, 2H), 3.71 (d, *J* = 17.2 Hz, 1H), 2.48 (s, 3H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 170.0, 169.6, 155.6, 139.0, 137.6, 137.3, 135.8, 108.09, 125.5, 124.5, 124.0 (q, *J* = 278 Hz), 130.0, 129.7, 128.1, 127.8, 128.0, 127.9, 87.5 (q, *J* = 30.6 Hz), 67.9, 43.9 (d, *J* = 47.4 Hz), 40.9 (q, *J* = 34.9 Hz), 19.9; HRMS-ESI calcd for $C_{22}H_{18}N_3O_3Cl_2F_6$ ($[M+H]^+$): 556.0624, found 556.0620.

BocHN



Iodo-A1443 (4). *N,N*-Diisopropylethylamine was added dropwise to a 0 ºC stirring solution of iodocarboxylic acid **3**[3] (20 mg, 36.8 µmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 8.47 mg, 44.2 µmol) and *N,N*-dimethylaminopyridine (450 µg, 6.68 µmol) in 368 µL CH$_2$Cl$_2$. The reaction mixture was then stirred at room temperature for 8 h, at which point it was diluted with EtOAc (50 mL) and washed with 0.1 M aq. HCl (2x40 mL), water (1x40 mL) and brine (1x40 mL). The organic layer was separated, dried with anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification of the resulting residue by flash chromatography (50% to 80% EtOAc/Hex), using 10 mL silica gel, afforded iodo-A1443 **(4)** (17.9 mg, 26.2 mmol, 71%) as an orange oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.60-7.39 (m, 4H), 6.77-6.66 (m, 1H), 6.46-6.37 (m, 1H), 4.21 (d, *J* = 5.44 Hz, 2H), 4.05 (d, *J* = 17.25 Hz, 1H), 4.02-3.94 (m, 2H), 3.67 (d, *J* = 17.2 Hz, 1H), 2.40 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.9, 168.7, 154.4, 144.0, 138.8, 137.6, 135.8, 134.8, 130.2, 130.0, 128.5, 125.4, 124.0 (q, *J* = 278 Hz), 93.1, 87.6, 43.7 (d, *J* = 16.1 Hz), 40.9 (m), 20.1; HRMS-ESI calcd for $C_{22}H_{17}N_3O_3Cl_2F_6I$ ($[M+Na]^+$): 681.9590, found 681.9584.



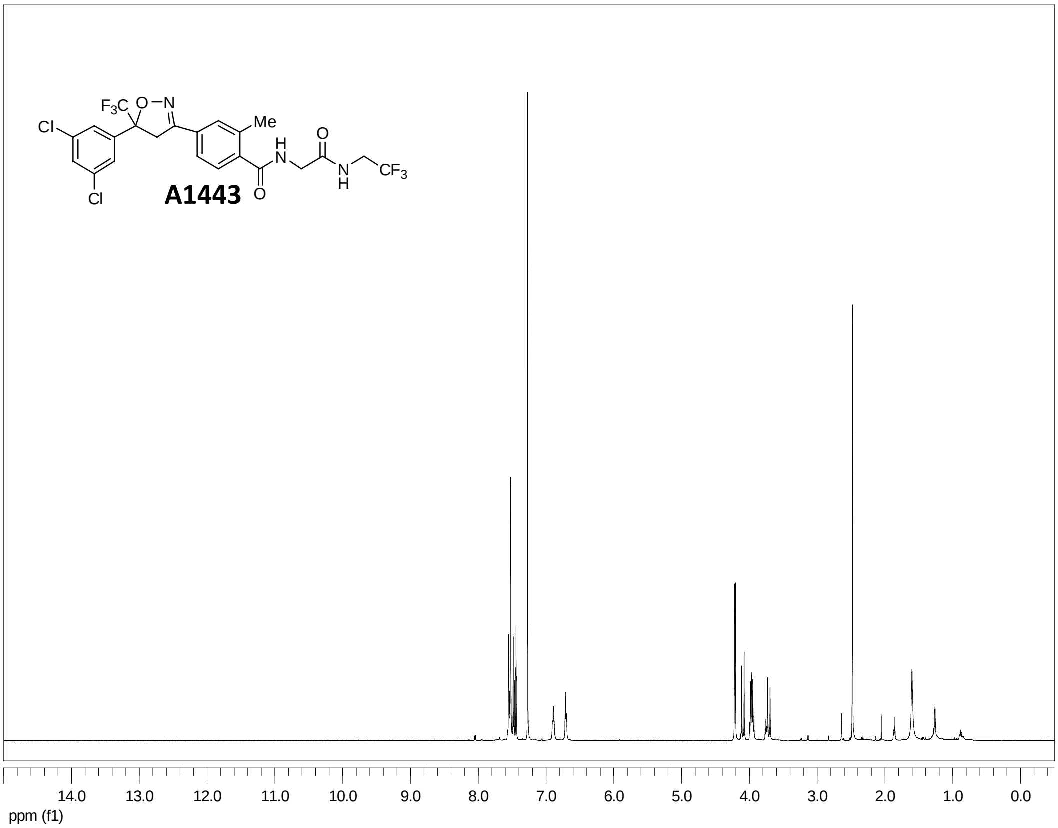
A1443 and ^{3}H-A1443 by hydrogenolysis of iodo-A1443 (4). A 4 mL vial was charged with iodo-A1443 (**4**) (1 mg, 1.47 µmol), 10% Pd/C (ca. 0.1-0.3 mg, Aldrich) and 95% EtOH (100 µL). The vial was fitted with a septum, purged with N_2 (3x) and then with H_2 (balloon, atmospheric pressure, 3x). The resulting reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered through a short pad of Celite™ and rinsed with EtOAc (3 mL). Concentration of the filtrate *in vacuo* afforded crude A1443 (1.0 mg) as a pale yellow oil. Analysis of this crude material by ^{1}H NMR and LCMS revealed near quantitative conversion to the title compound. Following replacement of H_2 by T_2, this procedure yielded ^{3}H-A1443.

References

(1) Takeshi, M.; Furukawa, Y.; Iwasa, M.; Komoda, M. EP Patent 2151437, 2010.
(2) Kousaka, H.; Fukuya, S.; Moriyama, Y.; Yaosaka, M.; Mizukoshi, T. EP Patent 2308857, 2011.
(3) Renold, P.; Zambach, W.; Maienfisch, P.; Muehlebach, M. WO. Patent 2009080250, 2009.

F_3C O N

Cl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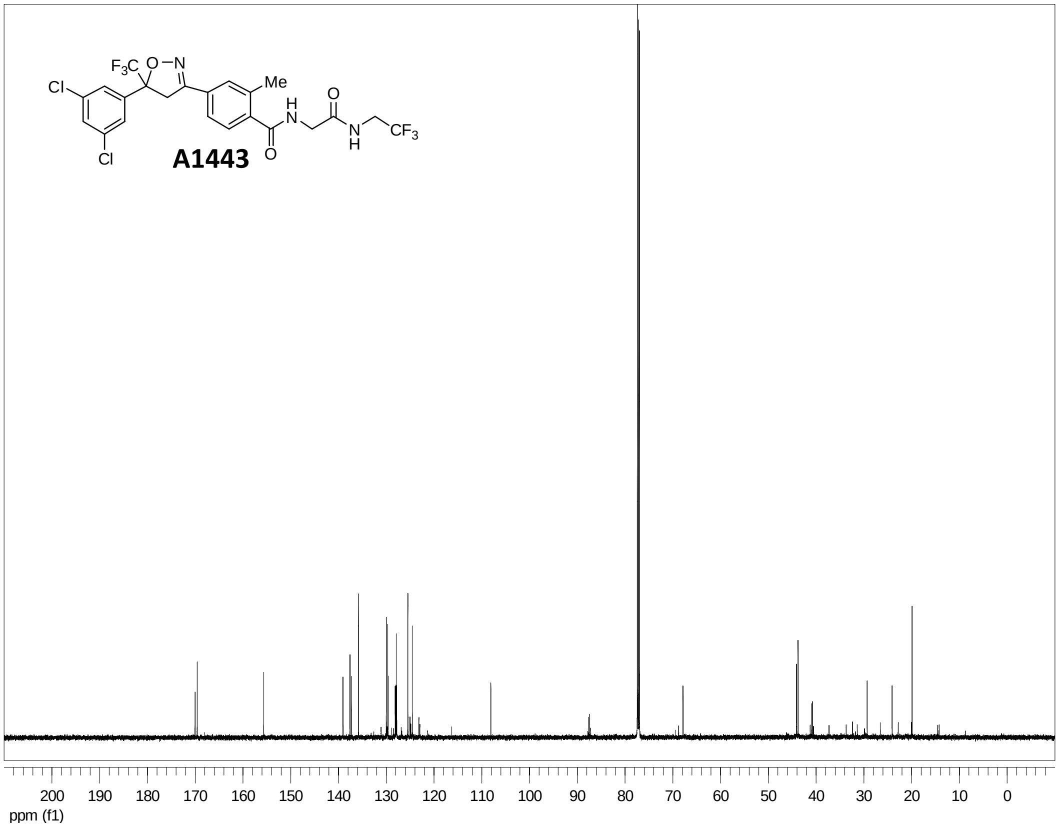
ppm (f1)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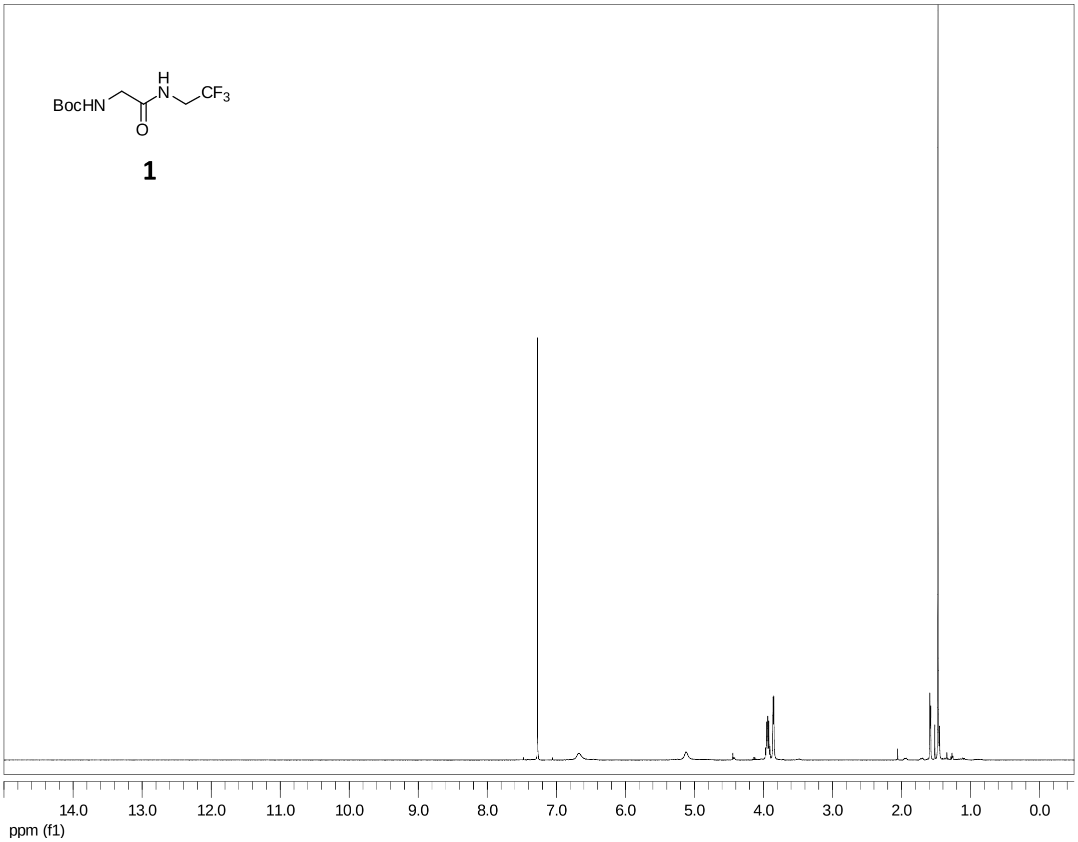
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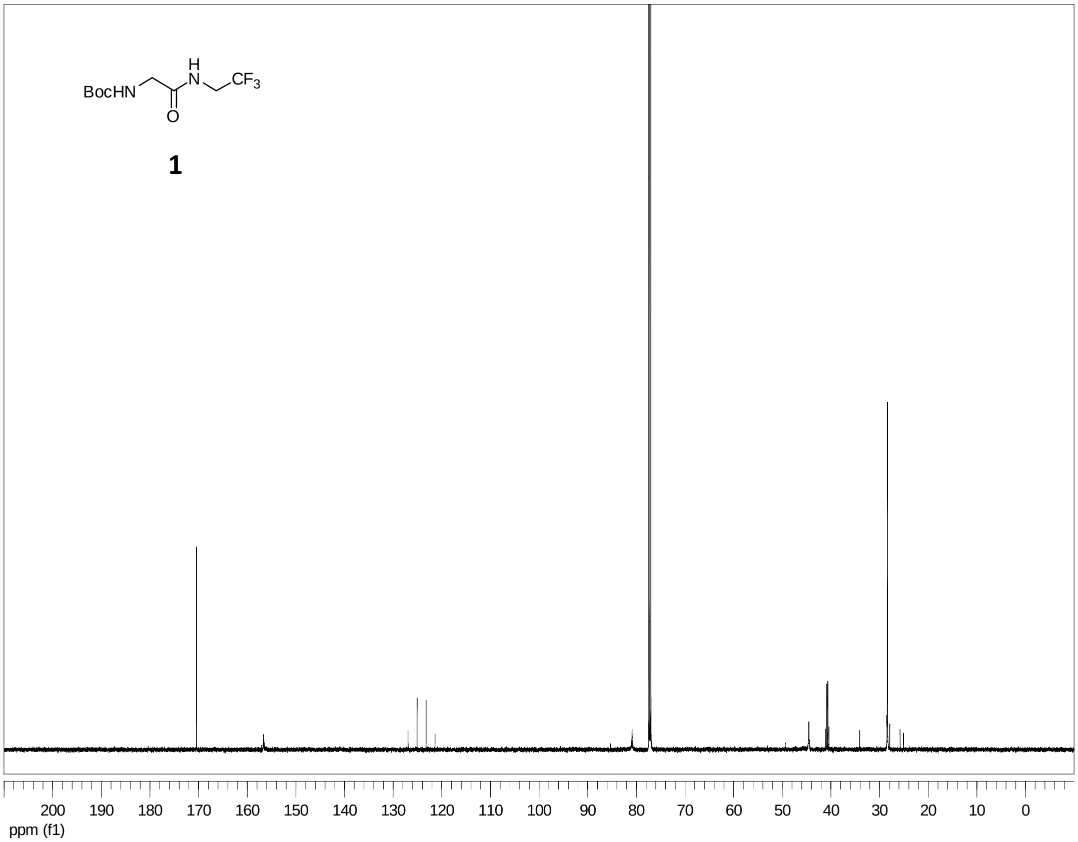
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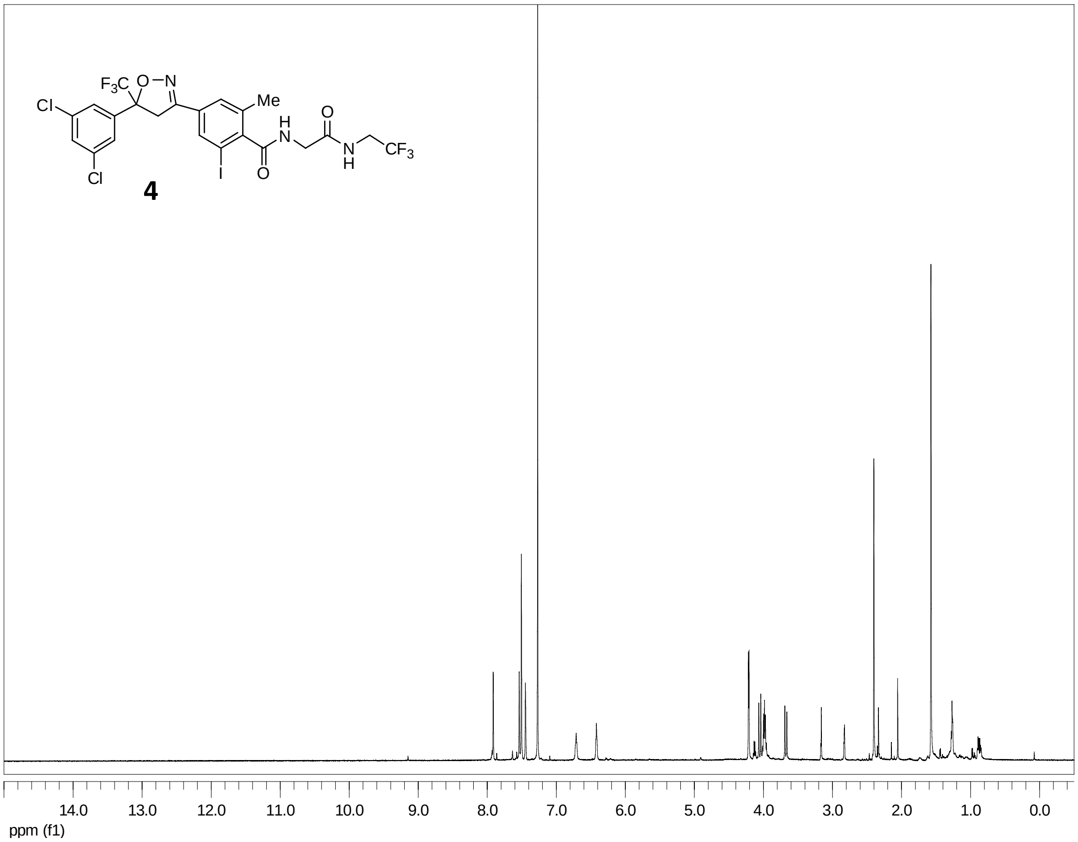
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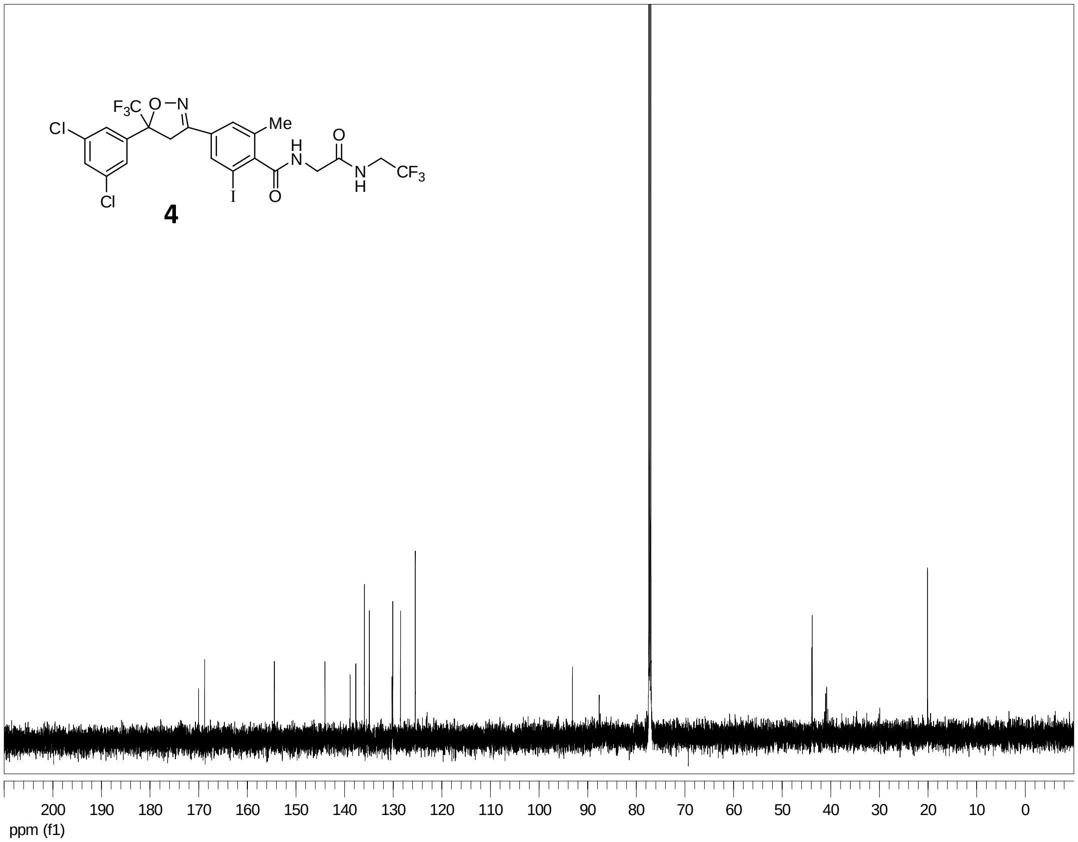
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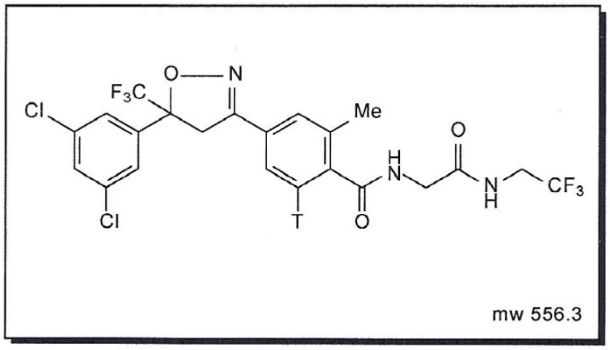
ppm (f1)

ViTrax

Technical Data Sheet

VT 100

PG3-014B, [^3H]



Lot Number: 149-033-000

Specific Activity: 14 Ci/mmol by Mass Spec.

Packaged: 8.2 mCi @ 1 mCi/mL in Ethanol

Primary Container: Type A20

Total Batch Activity: 8.2 mCi
Reserve: 0 mCi

Radiochemical Purity: >99% by HPLC on 3/22/2012

Comments: Product identity confirmed by HPLC co-elution with Authentic Standard

HPLC

Prevail C18, 5μm, 4.6 x 150mm
Mobile Phase A: 0.05% TFA/H_20
Mobile Phase B: CH_3CN
Flow Rate: 1.0 mL / min., RC Flow Det.

time program

```
 0 min 60%B
10 min 90%B
12 min 60%B
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RC Profile attached

TLC

N/A

Stability and Storage Recommendation: The exact rate of decomposition is unknown. However, it can be assumed that the product may decompose at a rate of approximately 2% per month when stored at -20° C in the original container.

Caution: This product is intended for investigational or manufacturing use only.
It is pharmaceutically unrefined and is not intended for use in humans. Responsibility for its use in humans, as a diagnostic reagent, and compliance with federal laws rests solely with the purchaser.

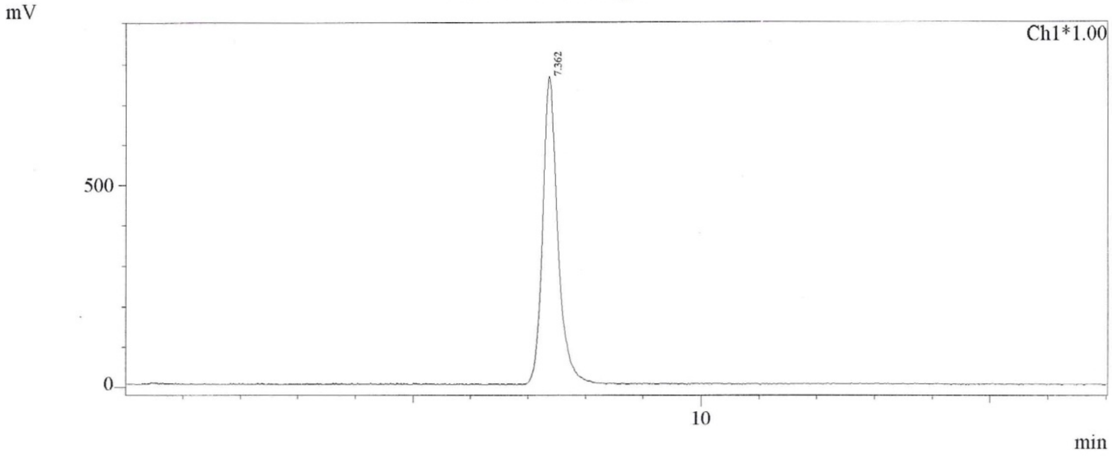
Document Issue Date: March 22, 2012

Approved By:

ViTrax

PG3-014B, [3H]
Cat# VT 100
Lot# 149-033-000
Mar 22, 2012

Radioflow Chromatogram



LC Peak Table - Ch1

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				13971272	100.00