Fipronil (1) is a major insecticide acting as a non-competitive blocker of the γ-aminobutyric acid (GABA) receptor/chloride channel. It is the most important example of the phenylpyrazole or fiprole insecticides. Understanding the noncompetitive blocker site of the GABA receptor/chloride channel would be greatly facilitated by a fiprole radioligand and particularly by a photoaffinity probe active on both insect and mammalian systems. We recently introduced a candidate fipronil-based photoaffinity probe 2, which shows very high potency at Drosophila and human β3 GABA receptors.

We envisioned that the radiolabeled portion of our photoaffinity probe could be introduced as the final synthetic step by selective tritium reduction of an iododiprole, high-affinity probe for the GABA receptor. For synthesis of the tritium-labeled version of this trifluoromethyldiazirinylfiprole ([³H]TDF) the required intermediate, 3-(4-[1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazolo]-3-trifluoromethyl)diazirine, was prepared in 10 steps from pyrazole and 3,5-dichloro-4-fluorobenzotrifluoride. One of the key transformations was lithiation and subsequent iodination of the 4-(2,2,2-trifluoro-1-hydroxyethyl)pyrazole intermediate. The last step involved reduction of the diiododiprole with tritium, Pd/C, and triethylamine in ethyl acetate and afforded [³H]TDF with a specific activity of 15 Ci/mmol and 99% radiopurity.

FIGURE 1. Fipronil and a candidate photoaffinity probe.


SCHEME 1\textsuperscript{a}

\[ \text{Reagents and conditions: (a) I}_2, \text{CAN}, \text{CH}_3\text{CN}, \text{rt, 97}\%; (b) 3,5-dichloro-4-fluorobenzotrifluoride, K_2CO_3, DMF, 100 °C, 95\%; (c) (i) i-PrMgCl, THF, 0 °C, (ii) N-(trifluoroacetyl)pyridine, −78 °C to room temperature, 79\%. \]

\[ \text{SCHEME 2}\textsuperscript{a} \]

\[ \text{Reagents and conditions: (a) (i) LDA, THF, −78 °C, (ii) TMSCl, −78 °C to room temperature, 89\% (6) and 91\% (8); (b) (i) LDA, THF, −78 °C, (ii) I}_2, \text{THF, −78 °C to room temperature, 94\%.} \]

\[ \text{to the amide provided 4-trifluoroacetylpyrazole 5 after aqueous workup.} \]

Several attempts were made at directed ortho lithiation\textsuperscript{13} of 5 as well as subsequent synthetic intermediates up to and including 2. In each case, however, there was considerable decomposition under the standard conditions for lithiation/iodination\textsuperscript{12} and the desired products could not be isolated. We therefore took a closer look at our system as it pertains to ortho lithiation and metalation chemistry. Reaction of commercially available 3,4,5-trichlorobenzotrifluoride with excess LDA at −78 °C followed by quenching with excess TMSCl gave bis-TMS derivative 6 in excellent yield (Scheme 2). Following the same ortho lithiation procedure and then quenching with iodine provided mono-iodo derivative 7 in nearly quantitative yield. Presumably, only the monoanion is formed on reaction of LDA and so the difference in one versus two substitutions in these metations is based on the fact that LDA is stable in the presence of TMSCl\textsuperscript{13} and the second metalation can occur in situ. Similarly, with methyl iodide or allyl bromide as the electrophile two methyl groups or one allyl group was added (not shown) as would be predicted on the basis of the stability of LDA with each of these electrophiles. Since this

\[ \text{Reagents and conditions: (a) NaBH}_4, \text{EtOH, rt, 97\%; (b) (i) LDA, THF, −78 °C, (ii) I}_2, \text{THF, −78 °C to room temperature, 55\% (10) and 25\% (11).} \]

procedure worked well and predictively on this type of phenyl ring we moved forward and attempted ortho lithiations on phenylpyrazole 4. Following the same procedure as in the synthesis of 6, we obtained bis-TMS derivative 8 almost exclusively, showing the ease of metation of the pyrazole ring in addition to the phenyl group (Scheme 2). Although not detailed here, we determined that with limiting base and electrophile, metation of the pyrazole ring in 4 precedes metation of the phenyl ring.

Results with the model systems encouraged us to continue experimenting with ortho lithiation chemistry. Reduction of 4-trifluoromethyl ketone 5 with NaBH\textsubscript{4} in ethanol (Scheme 3) smoothly afforded alcohol 9 and fortunately this was completely stable to the lithiation conditions. Reaction of 9 with excess LDA provided lithiated intermediates as indicated by the formation of the dark red color. Quenching with iodine followed this ortho lithiation; however, this reaction was not ideal as it produced a mixture of substitution products (Scheme 3). \textsuperscript{1}H NMR indicated the major product (Scheme 3). Following the same protocol for the conversion of 12 to the diazirine.\textsuperscript{15} The ketone was converted to its oxime \textsuperscript{14} with our\textsubscript{14} DMAP-catalyzed tosylation was far superior to noncatalyzed tosylation (Scheme 4). With our trifluoromethyl ketone containing two iodine atoms in hand we followed standard protocol for the conversion to the diazirine.\textsuperscript{15} The ketone was converted to its oxime \textsuperscript{13} with hydroxylamine hydrochloride in pyridine and ethanol. This oxime was next reacted with tosyl chloride and triethylamine with catalytic DMAP\textsuperscript{16} in dichloromethane to afford the corresponding oxime O-tosylate 14. Conversion of 14 to diaziridine 15 was brought about with ammonia in ether under pressure in a sealed tube.


\textsuperscript{16}DMAP-catalyzed tosylation was far superior to noncatalyzed reaction in pyridine.
Synthesis of a Photoaffinity Probe for the GABA Receptor

**SCHEME 4**

<table>
<thead>
<tr>
<th>a</th>
<th>Reagents and conditions: (a) Dess–Martin periodinane, dichloromethane, rt, 98%; (b) NH₂OH–HCl, pyridine, ethanol, 50 °C, 96%; (c) TsCl, DMAP, TEA, dichloromethane, rt, 100%; (d) NH₃, Et₂O, −78 °C to room temperature, 92%; (e) I₂, TEA, MeOH, rt, 94%.</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>Oxidation of the diaziridine to diazirine 16 was achieved with iodine and triethylamine in methanol.¹⁷</td>
</tr>
<tr>
<td>c</td>
<td>Finally reduction of diiodoarene 16 with H₂, 10% Pd/C, and triethylamine in ethyl acetate gave 2 in 45% isolated yield (Scheme 5). The identical reaction with tritium gas, however, afforded an intermediate that contained one tritium atom but also possessed an iodine atom. Further reduction with tritium gas provided the desired radiolabeled photoaffinity probe 17 or [³H]TDF.</td>
</tr>
</tbody>
</table>
| d | Comparison of radiolabeled probe 17 with the cold standard 2 showed them to be identical by reverse-phase HPLC and normal-phase TLC. The specific activity of the radiolabeled photoaffinity probe was determined to be 15 Ci/mmol. Radioflow chromatogram analysis provided a radiopurity of 99.3% and this was corroborated by scintillation counting of the crude solid isolated from a chromatoplate. The labeled region of silica from the chromatoplate. ¹⁷

**SCHEME 5**

<table>
<thead>
<tr>
<th>a</th>
<th>Reagents and conditions: (a) H₂, Pd/C, TEA, EtOAc, rt, 45%; (b) T₂, Pd/C, TEA, EtOAc, rt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>Oxidation of the diaziridine to diazirine 16 was achieved with iodine and triethylamine in methanol.¹⁷</td>
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</tr>
</tbody>
</table>

**Experimental Section**

1-(2,6-Dichloro-4-trifluoromethylphenyl)-4-iodopyrazole (4). Potassium carbonate (1.39 g, 10 mmol) was added to a solution of 4-iodopyrazole (1.61 g, 8.32 mmol) in DMF (20 mL). 3,5-Dichloro-4-fluorobenzotrifluoride (1.92 g, 8.24 mmol) was introduced and the mixture stirred vigorously at 100 °C for 4 h. As the reaction was cooling, water was added dropwise until a white precipitate began to form. Additional water was added to bring the total volume to 80 mL, once the reaction had cooled to room temperature. The white precipitate was collected by suction filtration, washed with water, and recrystallized from aqueous methanol to give phenylpyrazole 4 (3.33 g, 99%): mp 118–120 °C; ¹H NMR (CDCl₃) 7.84 (s, 1H), 7.75 (s, 2H), 7.63 (s, 1H); ¹³C NMR (CDCl₃) δ 146.8, 138.6, 136.5, 135.4, 133.3 (q, 35 Hz), 125.9, 122.2 (q, 270 Hz), 58.4. Anal. Calcd for C₁₀H₇Cl₂F₃N₂: C, 62.90; H, 3.05; N, 7.36. Found: C, 62.85; H, 2.98; N, 7.41.

1-(2,6-Dichloro-4-trifluoromethylphenyl)-4-trifluoroacetophenone (5). A solution of isopropylmagnesium chloride in THF (4.9 mL, 9.8 mmol) was added briskly to compound 4 (3.33 g, 8.81 mmol) in THF (30 mL) at 0 °C. The resulting yellow solution was stirred for 1 h and cooled to −78 °C, and N-(trifluoroacetyl)piperidine (1.78 g, 1.48 mL, 9.8 mmol) added quickly.¹⁸ The solution was stirred for 2 h as it warmed to room temperature and quenched with saturated ammonium chloride, then ethyl acetate (100 mL) was added. The organic layer was washed with water and then dried with sodium sulfate. The concentrated oil was purified by column chromatography (5% ethyl acetate in hexane) to give ketone 5 (2.43 g, 79%) as a white solid: mp 59–61 °C; ¹H NMR (CDCl₃) δ 8.37 (s, 1H), 8.31 (s, 1H), 8.24 (s, 1H), 8.12 (s, 1H), 7.73 (s, 2H), 7.63 (s, 1H); ¹³C NMR (CDCl₃) δ 174.5, 146.8, 138.6, 136.5, 135.2, 134.2 (q, 35 Hz), 126.0, 122.0 (q, 270 Hz), 117.9, 116.3 (q, 270 Hz).

2,6-Bis(trimethylsilyl)-3,4,5-trichlorobenzotrifluoride (6). A solution of 3,4,5-trichlorobenzotrifluoride (211 mg, 0.85 mmol) in anhydrous THF (4 mL) was cooled to −78 °C. LDA prepared from butyllithium and diisopropylamine in THF or a commercially available solution in heptane/THF/ethylbenzene (1.18 mL, 2.0 M, 2.37 mmol) was added dropwise followed by stirring for 1 h. The reaction was allowed to come to room temperature after addition of TMSCl (0.236 mL, 1.86 mmol) and stirred for 2 h. The reaction was quenched with saturated aqueous ammonium chloride (10 mL) and then ethyl acetate (10 mL) was added. The organic layer was washed with water and then dried with sodium sulfate. The crude solid was obtained in almost quantitative yield. Recrystallization from methanol provided 6 (296 mg, 89%): mp 100–101 °C; ¹H NMR (CDCl₃) 0.48 (q, J = 1 Hz, 18H); ¹³C NMR (CDCl₃) δ 141.4, 141.2 (q, J = 32 Hz), 140.7 (q, J = 5 Hz), 135.5, 123.9 (q, J = 290 Hz).

![Image](96x457 to 253x555)

(17) Silver(I) oxide gave comparable yield and purity but it was essential that it be prepared fresh each time.

(18) The yield was lower with slow addition of isopropylmagnesium chloride and N-trifluoroacetyl)piperidine compared with the optimized conditions.

2.4. Anal. Calcd for C_{13}H_{18}Cl_{3}F_{3}Si_{2}: C, 39.65; H, 4.61. Found: C, 39.38; H, 4.63.

2.4. Anal. Calcd for C_{13}H_{18}Cl_{3}F_{3}Si_{2}: C, 39.65; H, 4.61. Found: C, 39.38; H, 4.63.

(19) An equivalent amount of crude ketone can also be used with excess NaH to reduce the remaining N-(trifluoroacetylpiperidine) to piperidine and 2,2,2-trifluoroethanol. For an example of trifluoroacetamide reduction see: Bukownik, R. R.; Wilcox, C. S. J. Org. Chem. 1988, 53, 463-471.

Martin periodinane (1.15 g, 2.7 mmol) and this mixture was stirred overnight at room temperature. The reaction mixture was diluted with ether (20 mL) and washed with sodium thiosulfate in saturated aqueous sodium bicarbonate, then sodium bicarbonate, and water. The combined aqueous phases were extracted with ether (20 mL) and the organic phases were dried with sodium sulfate, filtered, and evaporated to give ketone 12 (560 mg, 98%) as an oil, which was pure according to TLC and used as such for further synthesis. ^1 H NMR (CDCl₃) δ 8.33 (q, J = 2 Hz, 1H), 7.86 (s, 1H); ^13 C NMR (CDCl₃) δ 174.0 (q, J = 32 Hz), 144.7, 143.4, 139.0 (q, J = 34 Hz), 137.2, 135.5, 127.3 (q, J = 7 Hz), 126.0, 123.5 (q, J = 280 Hz), 116.1 (q, J = 290 Hz), 96.1, 93.9. An analytical sample was crystallized from methanol as its methyl hemiacetal. Anal. Calcd for C_{13}H_{11}Cl_{3}F_{3}Si_{2}: Found: C, 73.83; H, 0.32; N, 4.14.

1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodo-(trifluoroacetyl)pyrazole Oxime (13). Trifluoroethyl ketone derivative 12 (550 mg, 0.87 mmol) was dissolved in pyridine (4 mL) and ethanol (1.5 mL). Hydroxylamine hydrochloride (67 mg, 0.98 mmol) was added and the reaction temperature increased to 50 °C, then the mixture was stirred for 14 h. Ethyl acetate (30 mL) and water (10 mL) were added to the cooled mixture and the organic layer was washed twice with 0.1 N hydrochloric acid and once with brine. Drying, filtration, and evaporation provided oxime 13 (539 mg, 96%) as a solid. ^1 H NMR (acetone-d₆) δ 12.18 (br s, 1H), 8.16 (s, 1H), 7.98 (s, 1H); ^13 C NMR (acetone-d₆) δ 144.4, 143.8, 139.9 (q, J = 32 Hz), 139.3, 138.9 (q, J = 32 Hz), 136.7, 128.0 (q, J = 280 Hz). Anal. Calcd for C_{19}H_{11}Cl_{2}F_{6}I_{2}N_{2}O₃S: Found: C, 23.68; H, 0.47; N, 6.53. Found: C, 22.48; H, 0.32; N, 6.29.
temperature for 2 h and then ethyl acetate was added followed by washing with aqueous sodium bisulfate, sodium carbonate, and brine. The organic layer was dried with sodium sulfate, filtered, and concentrated to give 16 (63 mg, 94%) as a solid after flash chromatography with 3% ethyl acetate in hexane. 

$^1$H NMR (CDCl$_3$) $\delta$ 8.04 (s, 1H), 7.82 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 144.8, 144.5, 138.6 (q, $J$ = 32 Hz), 135.9, 127.2, (q, $J$ = 4.5 Hz), 125.9, 121.3 (q, $J$ = 280 Hz), 118.2 (q, $J$ = 260 Hz), 96.0, 86.3, 22.9 (q, $J$ = 40 Hz). FAB-MS 641 (MH)$^+$; HRMS calcd for (C$_{12}$H$_2$Cl$_2$F$_6$I$_2$N$_4$ + H)$^+$ 640.7735, found 640.7729.

3-[[4-[1-(2,6-Dichloro-4-trifluoromethylphenyl)pyrazolo]-3-(trifluoromethyl)diazirine (2). The compound was synthesized from 5 as previously described. In addition, 16 (4.0 mg, 6 $\mu$mol) was dissolved in ethyl acetate (200 $\mu$L) and to this was added triethylamine (20 $\mu$L) and 10% Pd/C (4.0 mg). The septum-sealed flask was briefly purged with hydrogen gas and then a balloon filled with hydrogen gas (1.03 atm) was attached. After 4 h the system was opened and flash chromatography (3% ethyl acetate in hexane) of the reaction mixture gave 3 (1.1 mg, 45%) as an oil. $^1$H NMR (CDCl$_3$) $\delta$ 7.76 (s, 2H), 7.68 (s, 1H), 7.55 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 139.8, 138.5, 135.4, 133.7 (q, $J$ = 34 Hz), 131.0, 126.0, 122.1 (q, $J$ = 270 Hz), 121.8 (q, $J$ = 270 Hz), 113.5, 24.2 (q, $J$ = 45 Hz).

3-[[4-[1-(2,6-Dichloro-3-tritio-4-trifluoromethylphenyl)pyrazolo]-3-(trifluoromethyl)diazirine (17). Following the above procedure for 2 replacing hydrogen with tritium gas and properly modifying the reactor to handle the radioactive atmosphere provided [3H]TDF (17). [3H]TDF was purified by reverse-phase HPLC on a YMC ODSA C18 column (20 x 100 mm) with 30% water (with 0.05% TFA) and 70% acetonitrile. The purified probe (7.8 mCi) had a specific activity of 15 Ci/mmol and radiopurity of >99% determined by TLC cochromatography and radioautography. 17 was stored as a solution (680 $\mu$Ci/mL) in ethyl acetate in the dark at –20 °C. After several months of storage under these conditions the radiopurity had remained >95%.

**Acknowledgment.** We thank current or former laboratory colleagues Nanjing Zhang, Pierluigi Caboni, and Nilantha Sirisoma for helpful suggestions. Robert Fazio and Robert Andresini at ViTrax (Placentia, CA) carried out the radiosynthesis and radioligand purification. The project described was supported by Grant R01 ES008419 from the National Institute of Environmental Health Sciences (NIEHS), NIH, and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH.

**Supporting Information Available:** General experimental procedures, synthesis of 3 and N-(trifluoroacetyl)piperidine, reverse-phase HPLC chromatogram of 2, $^1$H NMR and reverse-phase HPLC chromatogram of 16, and radioflow chromatogram of 17. This material is available free of charge via the Internet at http://pubs.acs.org.