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Synthesis of a Tritium-Labeled, Fipronil-Based, Highly Potent, Photoaffinity Probe for the GABA Receptor

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3-{4-[1-(2,6-Dichloro-4-trifluoromethylphenyl)pyrazolo]}-3-(trifluoromethyl)diazirine is a fipronil-based (i.e. fiprole), high-affinity probe for the GABA receptor. For synthesis of the tritium-labeled version of this trifluoromethyldiazirinylfiprole ([^{3}H]TDF) the required intermediate, 3-{4-[1-(2,6-dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodopyrazolo]}-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 diiodofiprole with tritium, Pd/C, and triethylamine in ethyl acetate and afforded [^{3}H]TDF with a specific activity of 15 Ci/mmol and 99% radiopurity.

Fipronil (**1**)[1] is a major insecticide[2] acting as a noncompetitive blocker of the γ-aminobutyric acid (GABA) receptor/chloride channel.[3] It is the most important example of the phenylpyrazole or fiprole insecticides. Understanding the noncompetitive blocker site of the GABA-gated chloride channel would be greatly facilitated by a fiprole radioligand[4] and particularly by a photoaffinity probe[5] 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.[6]

We envisioned that the radiolabeled portion of our photoaffinity probe could be introduced as the final synthetic step by selective tritium reduction of an iodoarene.[7] Our synthesis began from commercially available 3,5-dichloro-4-fluorobenzotrifluoride and 4-iodopyrazole (**3**), which was prepared in one step from pyrazole with iodine and ceric ammonium nitrate (CAN) as depicted in Scheme 1.[8] Nucleophilic aromatic substitution with potassium carbonate in DMF at 100 °C smoothly produced the desired phenylpyrazole **4**. We found that this route was more reliable than attempting to iodinate, such as with *N*-iodosuccinimide or iodine monochloride, after the phenyl and pyrazole rings had been brought together. Iodine−magnesium exchange[9] of **4** with isopropylmagnesium chloride in THF gave the corresponding Grignard reagent, which was quenched with freshly prepared *N*-(trifluoroacetyl)piperidine. The α-amino alkoxide[10] generated upon addition of the Grignard reagent

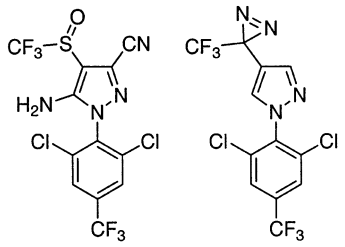

FIGURE 1. Fipronil and a candidate photoaffinity probe.

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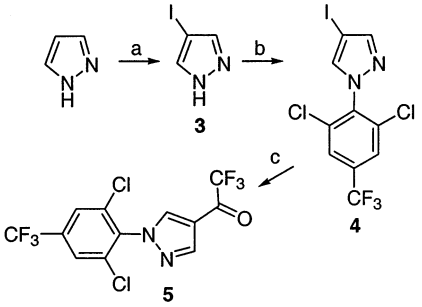
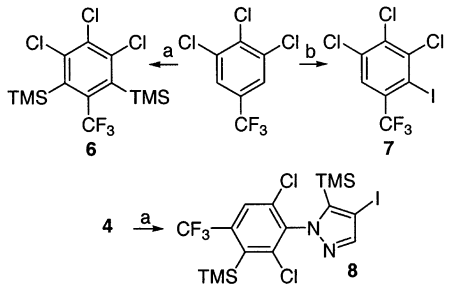
SCHEME 1[a]



[a] Reagents and conditions: (a) I_2, CAN, CH_3CN, rt, 97%; (b) 3,5-dichloro-4-fluorobenzotrifluoride, K_2CO_3, DMF, 100 °C, 99%; (c) (i) *i*-PrMgCl, THF, 0 °C, (ii) *N*-(trifluoroacetyl)piperidine, −78 °C to room temperature, 79%.

SCHEME 2[a]

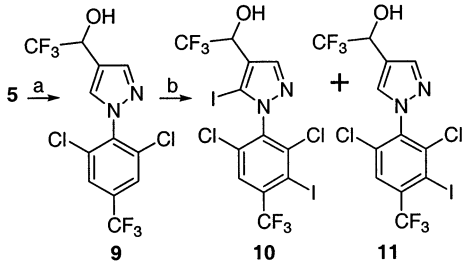


[a] Reagents and conditions: (a) (i) LDA, THF, −78 °C, (ii) TMSCl, −78 °C to room temperature, 89% (**6**) and 91% (**8**); (b) (i) LDA, THF, −78 °C, (ii) I_2, THF, −78 °C to room temperature, 94%.

to the amide provided 4-trifluoroacetylpyrazole **5** after aqueous workup.

Several attempts were made at directed ortho lithiation[11] 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[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 metalations is based on the fact that LDA is stable in the presence of TMSCl[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

SCHEME 3[a]



[a] Reagents and conditions: (a) $NaBH_4$, EtOH, rt, 97%; (b) (i) LDA, THF, −78 °C, (ii) I_2, 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 metalation of the pyrazole ring in addition to the phenyl group (Scheme 2). Although not detailed here, we determined that with limiting base and electrophile, metalation of the pyrazole ring in **4** precedes metalation 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 $NaBH_4$ 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). ^{1}H NMR indicated the major product (**10**) was iodinated on both the phenyl and pyrazole rings. The second product (**11**) was iodinated only on the phenyl ring and there were also traces of an iodinated pyrazole derivative and starting material that were not isolated from the crude mixture. We found it most efficient to use diiodo product **10** and recycle the rest to be iodinated again.

This iodo-containing alcohol was oxidized back to the corresponding trifluoromethyl ketone (**12**) most efficiently with Dess−Martin periodinane (Scheme 4).[14] With our trifluoromethyl ketone containing two iodine atoms in hand we followed standard protocol for the conversion to the diazirine.[15] The ketone was converted to its oxime (**13**) with hydroxylamine hydrochloride in pyridine and ethanol. This oxime was next reacted with tosyl chloride and triethylamine with catalytic DMAP[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.

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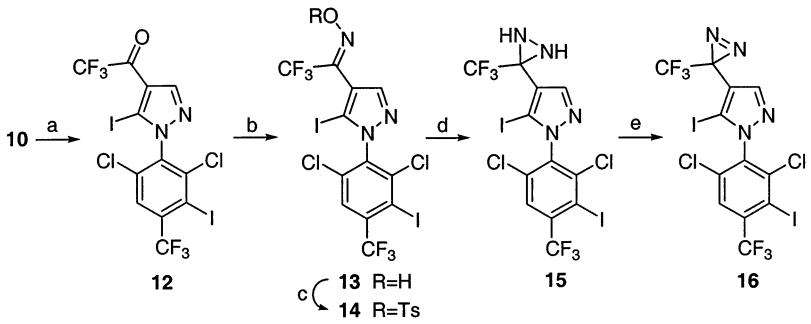
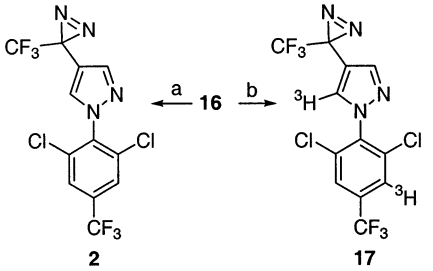
(16) DMAP-catalyzed tosylation was far superior to noncatalyzed reaction in pyridine.

SCHEME 4[a]



[a] Reagents and conditions: (a) Dess−Martin periodinane, dichloromethane, rt, 98%; (b) NH_2OH−HCl, pyridine, ethanol, 50 °C, 96%; (c) TsCl, DMAP, TEA, dichloromethane, rt, 100%; (d) NH_3, Et_2O, −78 °C to room temperature, 92%; (e) I_2, TEA, MeOH, rt, 94%.

SCHEME 5[a]



[a] Reagents and conditions: (a) H_2, Pd/C, TEA, EtOAc, rt, 45%; (b) T_2, Pd/C, TEA, EtOAc, rt.

Oxidation of the diaziridine to diazirine **16** was achieved with iodine and triethylamine in methanol.[17]

Finally reduction of diiodoarene **16** with H_2, 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 [^{3}H]TDF.

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 normal-phase TLC followed by scintillation counting of the labeled region of silica from the chromatoplate. Radiolabeled photoaffinity probe **17** was stable for several months when stored as a dilute solution in the dark at −20 °C.

In conclusion, a more efficient synthetic route to intermediate **5** was described and the directed ortho-metalation chemistry for this system has been developed. This methodology allowed for the synthesis of the novel, tritium-labeled, fipronil-based, highly potent, photoaffinity probe **17** via several diiodo intermediates. Target compound **17** ([^{3}H]TDF) contains both the radiolabel (tritium) and the photoreactive substituent (diazirine) necessary for a useful photoaffinity probe. The synthesis of [^{3}H]TDF should now allow for the photoaffinity labeling of the GABA receptor and its insecticide binding site.

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

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; ^{1}H NMR δ ($CDCl_3$) 7.84 (s, 1H), 7.75 (s, 2H), 7.63 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 146.8, 138.6, 135.5, 135.4, 133.3 (q, 35 Hz), 125.9, 122.2 (q, 270 Hz), 58.4. Anal. Calcd for $C_{10}H_4Cl_2F_3IN_2$: C, 29.51; H, 0.99; N, 6.89. Found: C, 29.36; H, 0.99; N, 6.78.

1-(2,6-Dichloro-4-trifluoromethylphenyl)-4-trifluoroacetylpyrazole (5).[6] 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 brine and then dried with sodium sulfate. The concentrated oil was purified by column chromotography (5% ethyl acetate in hexane) to give ketone **5** (2.43 g, 79%) as a white solid: mp 59−61 °C; ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H), 8.31 (s, 1H), 7.79 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 174.5 (q, J = 38 Hz), 143.0, 137.9, 136.9, 135.2, 134.2 (q, J = 35 Hz), 126.2, 122.0 (q, J = 270 Hz), 117.9, 116.3 (q, J = 290 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 brine, dried, and concentrated to give the crude solid in almost quantitative yield. Recrystallization from methanol provided **6** (296 mg, 89%): mp 100−101 °C; ^{1}H NMR δ ($CDCl_3$) 0.48 (q, J = 1 Hz, 18H); ^{13}C NMR ($CDCl_3$) δ 141.4, 141.2 (q, J = 32 Hz), 140.7 (q, J = 5 Hz), 135.5, 123.9 (q, J = 270 Hz),

(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 $C_{13}H_{18}Cl_3F_3Si_2$: C, 39.65; H, 4.61. Found: C, 39.26; H, 4.75.

2-Iodo-3,4,5-trichlorobenzotrifluoride (7). The procedure for the synthesis of **6** was followed to scale with the noted differences: 3,4,5-trichlorobenzotrifluoride (847 mg, 3.4 mmol), 10 mmol of LDA, iodine (2.58 g, 10 mmol), and washing with 5% aqueous sodium bisulfite to give **7** on recrystallization (1.19 g, 94%): mp 30−31 °C; ^{1}H NMR δ ($CDCl_3$) 7.69 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 141.9, 135.0, 134.7 (q, J = 32 Hz), 134.5, 126.9 (q, J = 7 Hz), 121.5 (q, J = 270 Hz), 95.5. Anal. Calcd for $C_7HCl_3F_3I$: C, 22.40; H, 0.27. Found: C, 22.53; H, 0.25.

1-(2,6-Dichloro-4-trifluoromethyl-3-trimethylsilylphenyl)-4-iodo-5-trimethylsilylpyrazole (8). The procedure for the synthesis of **6** was followed to scale with the noted differences: **4** (200 mg, 0.49 mmol), 1.62 mmol of LDA, and TMSCl (187 mg, 1.72 mmol) to give **8** (246 mg, 91%) after preparative TLC (3% ethyl acetate in hexane): mp 107−109 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (s, 1H), 7.82 (s, 1H), 0.49 (q, J = 1.5 Hz, 9H), 0.15 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 147.6, 144.8, 144.1, 140.9, 139.3, 137.9 (q, J = 32 Hz), 136.0, 126.2 (q, J = 7 Hz), 122.7 (q, J = 270 Hz), 68.7, 2.1, 1.1. Anal. Calcd for $C_{16}H_{20}Cl_2F_3IN_2Si_2$: C, 34.86; H 3.66; N, 5.08. Found: C, 34.80; H, 3.82; N, 5.01.

1-(2,6-Dichloro-4-trifluoromethylphenyl)-4-[1-hydroxy-(2,2,2-trifluoroethyl)]pyrazole (9). Purified ketone **5** (1.92 g, 5.1 mmol)[19] in ethanol (30 mL) at 5 °C was treated portionwise with sodium borohydride (0.22 g, 5.8 mmol). After being stirred for 3 h at room temperature the reaction was quenched with ice and water (100 mL). An ethyl acetate (100 mL) extract was washed with water and brine, concentrated, and triturated with dichloromethane to give the compound as a white solid upon filtration. The mother liquor was concentrated and triturated with hexane to give a second crop. These white solids were dried under vacuum to give **9** (1.86 g, 97%): mp 145−146 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (s, 1H), 7.75 (s, 2H), 7.71 (s, 1H), 5.18 (p, J = 6 Hz, 1H), 2.86 (d, J = 6 Hz, 1H); ^{13}C NMR (acetone-d_6) δ 141.2, 140.6, 136.3, 133.6 (q, J = 34 Hz), 132.5, 127.0, 126.0 (q, J = 280 Hz), 123.5 (q, J = 270 Hz), 119.5, 66.3 (q, J = 33 Hz). Anal. Calcd for $C_{12}H_6Cl_2F_6N_2O$: C, 38.02; H, 1.60; N, 7.39. Found: C, 37.93; H, 1.62; N, 7.44.

1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-4-[1-hydroxy-(2,2,2-trifluoroethyl)]-5-iodopyrazole (10). The procedure for the synthesis of **6** was followed to scale with the noted differences: **9** (600 mg, 1.58 mmol), 9.5 mmol of LDA, iodine (2.58 g, 10 mmol), and washing with 5% sodium bisulfite to afford a crude brown tar. Flash chromatography with ethyl acetate/hexane (1/9 slowly increased to 1/4) provided **10** (552 mg, 55%) as a solid: ^{1}H NMR (acetone-d_6) δ 8.13 (s, 1H), 7.98 (s, 1H), 6.06 (d, J = 6.2 Hz 1H), 5.10 (p, J = 6.7 Hz 1H); ^{13}C NMR (acetone-d_6) δ 144.6, 142.9, 139.4, 138.9 (q, J = 32 Hz), 136.9, 128.6 (q, J = 7 Hz), 126.1 (q, J = 280 Hz), 124.8, 122.7 (q, J = 275 Hz), 97.4, 89.3, 67.9 (q, J = 32 Hz). Anal. Calcd for $C_{12}H_4Cl_2F_6I_2N_2O$: C, 22.84; H, 0.64; N, 4.44. Found: C, 22.71; H, 0.82; N, 4.07.

1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-4-[1-hydroxy-(2,2,2-trifluoroethyl)]pyrazole (11). Continued flash chromatography of the reaction that afforded **10** with ethyl acetate/hexane (1/3) gave **11** (199 mg, 25%) as a solid: ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1H), 7.80 (s, 1H), 7.67 (s, 1H), 5.13 (q, J = 6.3 Hz, 1H), 3.53 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 142.9, 140.5, 138.3, 137.9 (q, J = 32 Hz), 134.9, 130.7, 127.3 (q, J = 7 Hz), 124.2 (q, J = 280 Hz), 121.4 (q, J = 280 Hz), 117.6, 96.2, 66.2 (q, J = 34 Hz). Anal. Calcd for $C_{12}H_5Cl_2F_6IN_2O$: C, 28.54; H, 1.00; N, 5.55. Found: C, 28.75; H, 1.07; N, 5.28.

1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodo-4-(trifluoroacetyl)pyrazole (12). To a solution of **10** (560 mg, 0.89 mmol) in dichloromethane (8 mL) was added Dess−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 extrated 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 NMR and used as such for further synthesis. ^{1}H NMR ($CDCl_3$) δ 8.33 (q, J = 2 Hz, 1H), 7.86 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 174.0 (q, J = 38 Hz), 144.7, 143.4, 139.0 (q, J = 34 Hz), 137.2, 135.5, 127.3 (q, J = 7 Hz), 126.0, 121.1 (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 $C_{13}H_6Cl_2F_6I_2N_2O_2$: C, 23.63; H, 0.92; N, 4.24. Found: C, 23.83; H, 0.89; N, 4.14.

1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodo-4-(trifluoroacetyl)pyrazole Oxime (13). Trifluoromethyl 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-d_6) δ 12.18 (br s, 1H), 8.16 (s, 1H), 7.98 (s, 1H); ^{13}C NMR (acetone-d_6) δ 144.4, 143.8, 139.9 (q, J = 32 Hz), 139.3, 138.9 (q, J = 32 Hz), 136.7, 128.6 (q, J = 5 Hz), 122.7 (q, J = 280 Hz), 120.4, 119.2 (q, J = 280 Hz), 97.5, 89.9. Anal. Calcd for $C_{12}H_3Cl_2F_6I_2N_3O$: C, 22.38; H, 0.47; N, 6.53. Found: C, 22.48; H, 0.32; N, 6.29.

1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodo-4-(trifluoroacetyl)pyrazole *O*-(*p*-Tolylsulfonyl)oxime (14). To oxime **13** (339 mg, 0.53 mmol) in dichloromethane (4 mL) was added triethylamine (0.22 mL, 1.6 mmol) at room temperature. Tosyl chloride (121 mg, 0.63 mmol) addition was followed by a catalytic amount of DMAP (6.5 mg, 53 μmol). After 2 h ethyl acetate and water were added, the layers separated, and the organic phase washed with sodium bicarbonate, water, and brine. After drying with sodium sulfate, filtration and evaporation provided tosylate **14** (421 mg, 100%) as a solid. ^{1}H NMR ($CDCl_3$) δ 7.93 (d, J = 8.2 Hz, 2H), 7.84 (s, 2H), 7.40 (d, 8.2 Hz, 2H), 2.49 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 146.6 (q, J = 36 Hz), 146.3, 143.7, 142.6, 138.7 (q, J = 32 Hz), 137.5, 135.8, 131.3, 129.8, 129.4, 127.2 (q, J = 5 Hz), 121.3 (q, J = 280 Hz), 119.3 (q, J = 280 Hz), 115.1, 96.0, 89.4, 21.7. Anal. Calcd for $C_{19}H_9Cl_2F_6I_2N_2O_3S$: C, 28.59; H, 1.14; N, 5.27. Found: C, 28.89; H, 0.91; N, 5.03.

3-{4-[1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodopyrazolo]}-3-(trifluoromethyl)diaziridine (15). Ether (2 mL) was added to tosylate **14** (421 mg, 0.53 mmol) in a thick-walled glass tube and the mixture was cooled to −78 °C. A stream of ammonia gas was bubbled in until about 1 mL had condensed at which time the tube was sealed. The reaction was allowed to warm to room temperature and stirred overnight and then cooled again before opening the tube. Removal of the ammonium tosylate via filtration and evaporation of the solvent under reduced pressure provided diaziridine **15** (311 mg, 92%) after flash chromatography (10% ethyl acetate in hexane) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.14 (s, 1H), 8.04 (s, 1H), 3.73 (d, J = 8.7 Hz, 1H), 3.51 (d, J = 8.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 145.0, 139.8 (q, J = 32 Hz), 139.4, 137.3 (q, J = 7 Hz), 128.7 (q, J = 5 Hz), 127.4, 125.2 (q, J = 280 Hz), 123.1(q, J = 270 Hz), 122.1, 97.9, 91.1, 53.5 (q, J = 36 Hz). Anal. Calcd for $C_{12}H_4Cl_2F_6I_2N_4$: C, 22.42; H, 0.63; N, 8.71. Found: C, 22.67; H, 0.77; N, 8.53.

3-{4-[1-(2,6-Dichloro-3-iodo-4-trifluoromethylphenyl)-5-iodopyrazolo]}-3-(trifluoromethyl)diazirine (16). To diaziridine **15** (67.3 mg, 0.10 mmol) in methanol (1 mL) was added triethylamine (42 μL, 0.30 mmol) followed by iodine (31 mg, 0.12 mmol). This dark solution was stirred at room

(19) An equivalent amount of crude ketone can also be used with excess $NaBH_4$ to reduce the remaining *N*-(trifluoroacetyl)piperidine 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.

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 ($CDCl_3$) δ 8.04 (s, 1H), 7.82 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 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 $(C_{12}H_2Cl_2F_6I_2N_4 + H)^+$ 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.[6] In addition, **16** (4.0 mg, 6 μmol) was dissolved in ethyl acetate (200 μL) and to this was added triethylamine (20 μL) 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 ($CDCl_3$) δ 7.76 (s, 2H), 7.68 (s, 1H), 7.55 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 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)-5-tritiopyrazolo]}-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 [^{3}H]TDF (**17**). [^{3}H]TDF was purified by reverse-phase HPLC on a YMC ODSA C18 column (20 × 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 μCi/mL) in ethyl acetate in the dark at −20 °C. After several months of storage under these conditions the radiopurity had remained >95%.

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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.

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