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# Photoaffinity Radioligand for NADH:Ubiquinone Oxidoreductase: [*S*-$C^3H_2$](Trifluoromethyl)diazirinyl-pyridaben

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## SUMMARY

Pyridaben is a new and very potent insecticide and miticide that acts by inhibiting the activity of NADH:ubiquinone oxidoreductase (the most complex of all the respiratory enzymes). The binding site, presumed to be the same as that of rotenone and fenazaquin, resides at an unknown location within the 43-polypeptide-subunit Complex I. To define the structure of the pyridaben-inhibition site(s), we prepared [*S*-$C^3H_2$](trifluoromethyl)diazirinyl-pyridaben as a photoaffinity probe. Tritium was incorporated by reducing 4-(trifluoromethyl)diazirinylphenylacetyl fluoride to the benzyl alcohol with freshly prepared $LiB^3H_4$ at 97% tritium enrichment. The tritium-labeled alcohol was converted to the benzyl bromide derivative and coupled to 2-*tert*-butyl-4-chloro-5-mercapto-3(*2H*)-pyridazinone to obtain the photoaffinity probe (56 Ci/mmol) with an $IC_{50}$ of 3.0 nM for NADH:ubiquinone oxidoreductase activity of bovine heart electron transport particles. [*S*-$C^3H_2$](Trifluoromethyl)diazirinyl-pyridaben is an improved photoaffinity radioligand combining outstanding potency for inhibiting NADH:ubiquinone oxidoreductase activity, high specific activity close to the theoretical value, and a preferred photolabile substituent (known to combine high reactivity and generation of a carbene species at wavelengths not damaging to proteins).

Key words: lithium borotritide, NADH:ubiquinone oxidoreductase, photoaffinity, pyridaben, radioligand, tritium labeling.

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## INTRODUCTION

NADH:ubiquinone oxidoreductase (or Complex I; EC 1.6.99.3), the first enzyme in the electron transfer chain, removes electrons from NADH and passes them via a series of enzyme-bound redox centers to the electron acceptor ubiquinone (*1*). Defects in Complex I leading to reduced activity are implicated in several neurological diseases and deterioration of respiratory enzyme activities with normal aging (*2-5*). With 95% of the molecular oxygen metabolized in the mitochondria by the electron transfer chain, inhibition of NADH:ubiquinone oxidoreductase activity is concomitant with superoxide radical formation leading to oxidative stress (*6-11*). Rotenone, one of the most important botanical insecticides, is a potent inhibitor of NADH:ubiquinone oxidoreductase activity (*12,13*). Pyridaben and fenazaquin, like rotenone, are commercial insecticides/acaricides (see structures in the Figure, below) that also inhibit this oxidoreductase and are therefore candidate probes for studying Complex I (*12,14*).

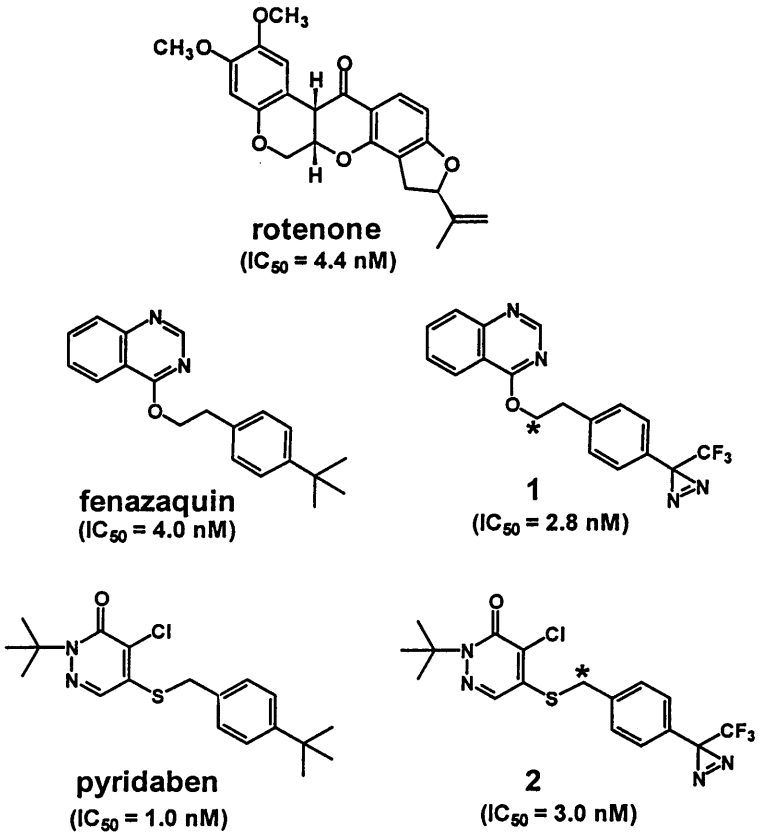



**Figure.** Inhibitors of NADH:ubiquinone oxidoreductase activity. Asterisks indicate the position of tritium incorporation in the photoaffinity radioligand. $IC_{50}$ is the concentration for 50% inhibition.

Pyridaben, optimized for miticidal and insecticidal activity (*15-17*), is generally the most potent compound of this type (*12*) (Figure). Photoaffinity labeling has increasingly become a tool of choice for defining receptor-ligand interactions and protein purification and mapping of active sites (*18,19*). In the past, we prepared a tritium-labeled photoaffinity probe (**1**) (4.7 Ci/mmol) based on fenazaquin and observed specific photoaffinity labeling of a 22-24 kDa protein from the stalk region of ATP synthase, either subunit d or the oligomycin-sensitivity conferral protein (*14,20*). From the study we concluded that fenazaquin (and by implication rotenone and possibly pyridaben) has at least two specific binding sites in electron transport particles (ETP), the anticipated high-affinity site in NADH:ubiquinone oxidoreductase and apparently a low-affinity site of unknown function in the stalk region of ATP synthase (*14*).

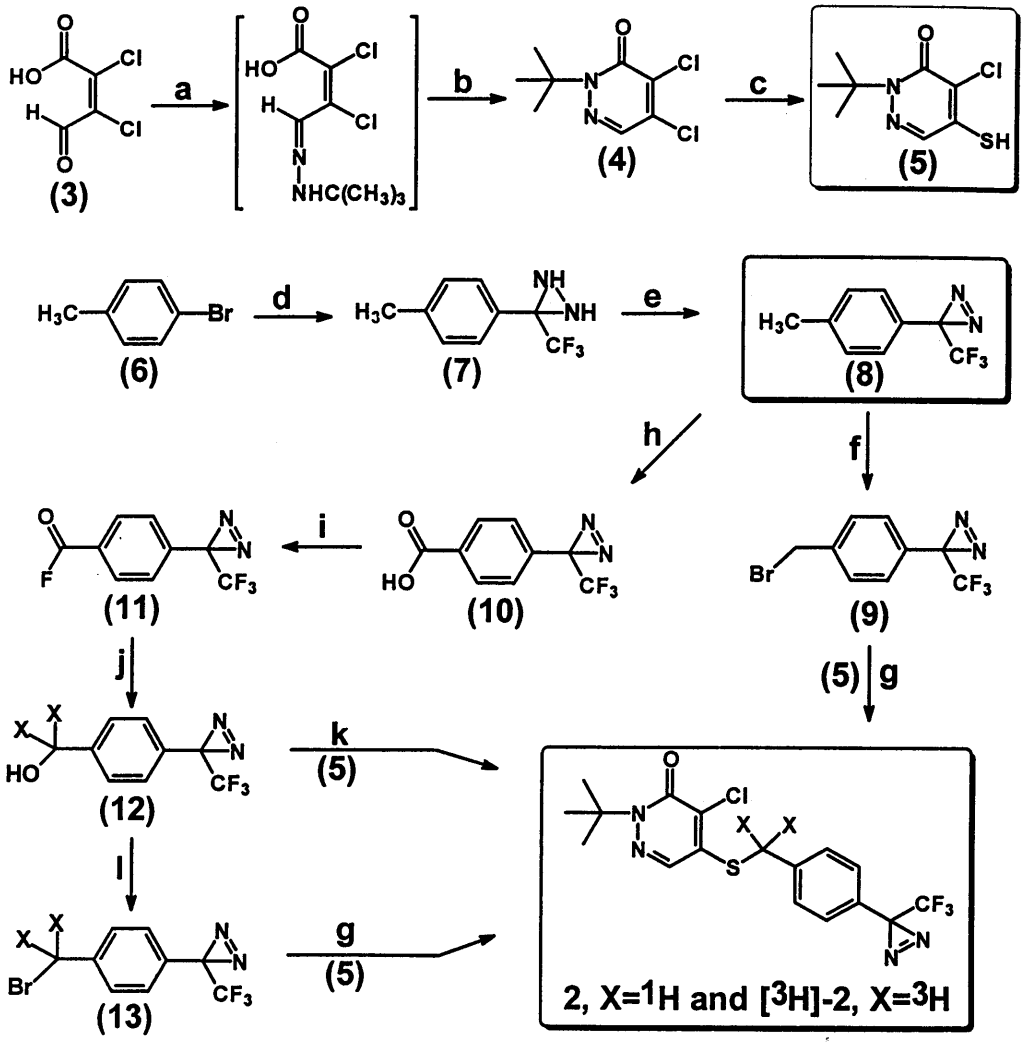
We report here the synthesis of a tritium-labeled photoaffinity analog (**2**) of pyridaben with a specific activity of 56 Ci/mmol, close to the theoretical value for incorporation of two tritium atoms.

## RESULTS AND DISCUSSION

**Choice of (trifluoromethyl)diazirinyl-pyridaben as the photoaffinity probe.** The (trifluoromethyldiazirinyl)phenyl moiety is a preferred photoreactive group because of favorable UV absorbance at 340 nm, chemical stability towards harsh conditions during synthesis, and generation of a carbene species at wavelengths not damaging to proteins (*20* and references cited therein). Like fenazaquin, pyridaben has a 4-*tert*-butylphenyl substituent but is generally a more potent inhibitor of NADH:ubiquinone oxidoreductase (Figure). In our studies with fenazaquin we replaced the *tert*-butyl substituent with trifluoromethyldiazirinyl without affecting either the potency of the compound for inhibiting NADH:ubiquinone oxidoreductase or the general level of insecticidal activity (*20*). This suggested that the same replacement might be suitable in the pyridaben series, a proposal supported by retention of the miticidal activity when 4-*tert*-butyl is replaced by 4-trimethylsilyl, 4-*n*-butyl and 4-phenyl (*15*).

**Thiopyridazinone 5 (Scheme).** The thiopyridazinone was prepared by converting mucochloric acid (**3**) to the dichloro compound (**4**) (*21,22*) and introducing the thiol group at temperatures lower than 100 °C using $Na_2S$ instead of NaHS as reported earlier (*16,17*).

**(Trifluoromethyl)diazirinyl-pyridaben (2) (Scheme).** The trifluoromethyldiazirinyl moiety is conveniently introduced in 42% overall yield starting from 4-bromotoluene (**6**) (*23*). The conversion of 3-(4-methylphenyl)-3-trifluoromethyldiaziridine (**7**) to the diazirine (**8**) was carried out with iodine instead of $Ag_2O$ as the oxidizing reagent; whereas $Ag_2O$ is expensive, must be made fresh from $AgNO_3$ and NaOH, and used in large excess, this oxidation can be carried out with iodine (*24*) or *tert*-butyl hypochlorite (*25*) since compound (**7**) did not have an acid-sensitive group. 3-(4-Bromomethylphenyl)-3-trifluoromethyl-3*H*-diazirine (**9**) was obtained




**Scheme.** Preparation of 2-*tert*-butyl-4-chloro-5-mercapto-3(2*H*)-pyridazinone (**5**) and the tritium-labeled photoaffinity probe ([$^3$H]-**2**).

(a) $(CH_3)_3CNH\text{-}NH_2.HCl$, $Na_2CO_3$, $H_2O$; (b) $CH_3COOH$, 79% steps (a) and (b); (c) $Na_2S$, $H_2O$, HCl, 55%; (d) 65%, ref. (*23*); (e) $I_2$, $Et_3N$, $CHCl_3$, 65%; (f) $(C_6H_5CO)_2O_2$, NBS, $CCl_4$, 90%; (g) (**5**), $Et_3N$, $Et_2O$, 65%, X = $^1$H, 53%, X = $^3$H; (h) $KMnO_4$, $C_5H_5N$, 38%; (i) cyanuric fluoride, $C_5H_5N$, $CH_2Cl_2$, 95%; (j) $LiBX_4$, THF, MeOH, 84%, X = $^1$H, 71%, X = $^3$H; (k) $Ph_3P$, DEAD, (**5**), THF, 27%, X = $^1$H; (l) $CBr_4$, $Ph_3P$, $CH_2Cl_2$, 91%, X = $^1$H, 17%, X = $^3$H.

using *N*-bromosuccinimide (NBS) and benzoyl peroxide in $CCl_4$ at reflux (*26*) then coupled at room temperature to thiopyridazinone (**5**) in the presence of triethylamine to obtain unlabelled **2**.

**[S-$C^3H_2$](Trifluoromethyl)diazirinyl-pyridaben ([$^3$H]-2) (Scheme).** 3-(4-Methylphenyl)-3-trifluoromethyl-3*H*-diazirine (**8**) was first oxidized to 4-(1-azi-2,2,2-trifluoroethyl)benzoic acid (**10**) by powdered solid $KMnO_4$ in pyridine at 50 °C according to Nassal (*23*), and then converted to the acyl fluoride (which is more stable than the acyl chloride to neutral nucleophiles such as water and methanol). Like acyl chlorides (*27,28*), two atoms of tritium can be introduced by a simple tritide reduction of acyl fluorides to alcohols. Others have used $LiB^3H_4$ to reduce mixed anhydrides to tritiated alcohols (*29*). The acyl fluoride analog (**11**) was prepared according to Kokotos and Noula (*30*). However, the acyl fluorides they prepared were not isolated but were kept in the reaction solvent ($CH_2Cl_2$) and reduced to alcohols by addition of $NaBH_4$ followed by methanol (*30*). In our studies, we found that either $NaBH_4$ or $LiBH_4$ can be used; $LiBH_4$ is a much faster reducing agent and can be prepared in a radioactive form at higher specific activity compared to $NaBH_4$. The sequence of adding the reducing agent, or the use of THF or $CH_2Cl_2$ as the solvent, does not affect the conversion of acyl fluorides to alcohols. Though not described here, these results were confirmed by using $NaB^2H_4$. To introduce tritium into the ligand, $LiB^3H_4$ was prepared (*31*), then a solution of the acyl fluoride was immediately added followed by methanol at 0 °C. The tritiated alcohol (**12**) was converted to the benzyl bromide (**13**) and coupled to the thiopyridazinone using triethylamine. Direct coupling of the unlabeled alcohol to the thiopyridazinone using the thio-Mitsunobu reaction gave very unsatisfactory yields (*32*).

**NADH:ubiquinone oxidoreductase inhibition.** Pyridaben and **2** were assayed with bovine heart mitochondrial electron transport particles (*20*) as the source best understood from the standpoint of structure and function including the amino acid sequences for its subunits (*1,33*). (Trifluoromethyl)diazirinyl-pyridaben **2** has comparable potency to pyridaben and selected other NADH:ubiquinone oxidoreductase inhibitors including rotenone (*20*) (Figure).

## EXPERIMENTAL PROCEDURES

**Materials and Methods.** Silica gel TLC was performed for analysis with precoated plastic sheets (4x8 cm, 0.25 mm gel layer) and fluorescent indicator (Polygram R SILG/UV254, Macherey-Nagel, Germany) and for preparative purposes with precoated silica gel GF plates (20x20 cm, Analtech). NMR spectra were recorded with the Bruker AM-300 spectrometer. Chemical shifts (δ) are reported for $^1$H at 300 MHz and for $^{13}$C at 75 MHz relative to internal tetramethylsilane. Mass spectra were acquired by GC/MS with the Hewlett-Packard 5971A or 5985B instrument in the electron impact (EI) mode (70 eV, 200 °C). Fast atom bombardment (FAB)-MS (both low and high resolution, LR and HR) was carried out with the Fisons ZAB2-EQ spectrometer. UV absorbances were measured on a Hewlett-Packard 8452A diode array spectrophotometer. Melting points (uncorrected) were recorded on a Fisher-Johns melting point apparatus. All reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) except

cyanuric fluoride, which was purchased from Lancaster (Windham, NH). Solvents were of reagent or HPLC grade. THF was distilled from sodium benzophenone under nitrogen in a recirculating still, with a deep blue color maintained in the distillation pot. Liquid scintillation counting was carried out on a Packard 1500 liquid scintillation system, using Optifluor cocktail. HPLC was performed on a normal phase silica gel column (5"x 0.25" Si 60, 5 μm) using Waters model 510 pumps. UV detection was at 304 nm on a Hewlett Packard 1040A diode array spectrophotometer. The specific activity of the photoaffinity probe was determined by 1) comparison of the UV absorbance with that of a standard analytical sample and liquid scintillation counting of the isolated HPLC peak and 2) using $^{1}H$ and $^{3}H$ NMR (IBM AF spectrometer at 300 and 320 MHz respectively, with $C_6{}^2H_6$ and 5-mm probe). The candidate probe **2** was >99% pure based on NMR and HPLC. Compounds with photosensitive substituents were used in subdued light.

### Preparation of 2-*tert*-Butyl-4-chloro-5-mercapto-3(2*H*)-pyridazinone (5) (Scheme)

**2-*tert*-Butyl-4,5-dichloropyridazinone (4):** to a mixture of mucochloric acid (**3**) (3.38 g, 20 mmol) in water (30 mL) at 0 °C was added anhydrous $Na_2CO_3$ (1.06 g, 10 mmol) to give a colorless solution. To this was added *tert*-butylhydrazine.HCl (2.5 g, 20 mmol). A precipitate appeared in a few min and the mixture was further stirred at 0 °C for 2 h. The hydrazone product was filtered and washed with cold water and dried in open air to give 4.32 g. The crude hydrazone was then dissolved in glacial acetic acid (40 mL) which was refluxed for 20 min. After cooling to room temperature, the solution was concentrated and purified by silica gel chromatography using $CHCl_3$ as eluent to give 3.5 g of a white solid in 79% yield, m.p. 63-65 °C, $R_f$ = 0.6 in 20% EtOAc:hexane. $^{1}H$ NMR ($CDCl_3$) δ: 7.7(s, 1H, 6-H); 1.65(br s, 9H, *t*-butyl). $^{13}C$ NMR ($CDCl_3$) δ: 156.8, 135.7, 134.7, 133.4, 66.9, 27.6.

**2-*tert*-Butyl-4-chloro-5-mercapto-3(2*H*)-pyridazinone (5):** compound (**4**) (1.1 g, 5.0 mmol) in water (15 mL) was stirred with $Na_2S$ (1.17 g, 15 mmol) at 70 to 100 °C until all of the solid was dissolved (about 4 h). The solution was then cooled to room temperature and concentrated HCl (12 N) was carefully added to give a yellowish precipitate, which was filtered and washed with cold water. Crystallization from hexane gave 0.60 g of a white powder (55% yield), m.p. 100-102 °C. $^{1}H$ NMR ($CDCl_3$) δ: 7.6(s, 1H, 6-H), 4.0(s, 1H, SH); 1.63(br s, 9H, *t*-butyl). $^{13}C$ NMR ($CDCl_3$) δ: 155.8, 138.0, 132.5; 130.3, 66.0, 27.6.

### Synthesis of the photoaffinity probe (Scheme)

**3-(4-Methylphenyl)-3-trifluoromethyl-3*H*-diaziridine (7):** this compound was prepared according to Nassal (*23*) from 4-bromotoluene (**6**) in 65% overall yield.

**3-(4-Methylphenyl)-3-trifluoromethyl-3*H*-diazirine (8):** to a solution of diaziridine **7** (3.12 g, 15.8 mmol) in $CHCl_3$ (20 mL) and triethylamine (3.31 mL, 23.7 mmol), iodine was added at room temperature in small portions until the deep purple color persisted (about 2.10 g of iodine). The reaction was further stirred for 15 min and then concentrated *in vacuo*. The dark brown residue was partitioned between water and ether and washed with 0.1 N HCl solution, then with a saturated solution of $Na_2S_2O_3$ to reduce the unreacted iodine. The organic phase was dried ($MgSO_4$), filtered and concentrated. Purification by silica gel flash chromatography using $CHCl_3$ as eluent gave 2.0 g (65% yield) of a colorless oil, $R_f$ = 0.8 in 20% EtOAc:hexane. $^{1}H$ NMR ($CDCl_3$) δ: 7.19(d, J = 8.15 Hz, 2H, aromatic); 7.08(d, J = 8.15 Hz, 2H, aromatic); 2.35(s, 3H, $CH_3$). $^{13}C$ NMR ($CDCl_3$) δ: 139.7, 129.4, 126.6, 126.3, 124.1, 120.5, 76.8($C(N_2)$), 19.1(q, J = 40 Hz, $CF_3$), 20.6 (br s, $CH_3$).

**3-(4-Bromomethylphenyl)-3-trifluoromethyl-3*H*-diazirine (9):** a solution of (**8**) (0.20 g, 1.0 mmol), benzoyl peroxide (15 mg, 0.062 mmol) and NBS (0.47 g, 2.64 mmol) in dry $CCl_4$ (10 mL) was stirred at 70 °C for 3 h. The solution was then cooled to room temperature and filtered. The filtrate was concentrated *in vacuo* and purified by flash chromatography using $CHCl_3$ as eluent to give 0.25 g of a colorless oil in 90% yield, $R_f$ = 0.76 in 20% EtOAc:hexane. $^1H$ NMR ($CDCl_3$) δ: 7.4(d, J = 8.11 Hz, 2H, aromatic); 7.15(d, J = 8.11 Hz, 2H, aromatic); 4.5(s, 2H, $CH_2Br$). $^{13}C$ NMR ($CDCl_3$) δ: 139.5, 129.4, 127.1, 126.9, 126.8, 123.8, 76.81, 32.0($CH_2Br$), 28.6(m, $CF_3$). MS-EI: $M^+$($^{79}Br$)(20%), $M^+$+2($^{81}Br$)(20%), $M^+$-$N_2$(80%), $M^+$-2-$N_2$(80%), 171($M^+$-$N_2$-Br, 100%), 151($M^+$-$N_2$-Br-F, 80%).

**4-Chloro-2-*tert*-butyl-5-[[[4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl]methyl]-thio]-3(*2H*)-pyridazinone [2]:** a solution of (**9**) (279 mg, 1.0 mmol), (**5**) (345 mg, 1.6 mmol) and triethylamine (0.3 mL, 2.0 mmol) in anhydrous ether (10 mL) was stirred at room temperature for 4 h. Water (5.0 mL) was added followed by extraction with EtOAc, drying ($MgSO_4$), filtering and concentrating to give a solid residue. Purification by silica gel flash chromatography using $CHCl_3$ as eluent gave a cream colored solid in 65% yield. $R_f$ = 0.45 in 100% $CHCl_3$, or 0.6 in 20% EtOAc:hexane, m.p. 115-116 °C. $^1H$ NMR ($CDCl_3$) δ: 7.56(s, 1H, 6-H, pyridazinone); 7.45(d, J = 8.10 Hz, 2H, aromatic); 7.19(d, J = 8.10 Hz, 2H, aromatic); 4.27(s, 2H, $SCH_2$); 1.62(br s, 9H, *t*-butyl). $^1H$ NMR ($C_6D_6$) δ: 6.97(s, 1H); 6.75(d, J = 8.4 Hz, 2H); 6.71(d, J = 8.4 Hz, 2H); 3.25(s, 2H, $SCH_2$); 1.55(br s, 9H, *t*-butyl). $^{13}C$ NMR ($CDCl_3$) δ: 155.5, 140.1, 136.8, 134.2, 131.6, 130.2, 129.8, 129.1, 127.1, 123.8, 76.1, 66.3, 35.3, 29.2(m), 27.6. MS-FAB-LR: $MH^+$:417(52%), 361(30%), 154(100%). MS-FAB-HR: $C_{17}H_{16}{}^{35}ClF_3N_4OSH^+$: calculated 417.0764, found 417.0764. $\lambda_{226}$, ε = 26,000; $\lambda_{298}$, ε = 11,000 in absolute ethanol.

**4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoic acid (10):** this intermediate was prepared in 38% yield by $KMnO_4$ oxidation of (**8**) in pyridine as described by Nassal (*23*).

**4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzoyl fluoride (11):** to a solution of (**10**) (98 mg, 0.43 mmol) in dry $CH_2Cl_2$ (2.0 mL) was added pyridine (34 μL, 0.43 mmol) and cyanuric fluoride (77 μL, 0.86 mmol) at -20 °C. The reaction mixture was stirred at -10 to -20 °C for 2 h before cold water was added. The mixture was brought up to room temperature and extracted with $CH_2Cl_2$, dried ($MgSO_4$), filtered and concentrated *in* vacuo to give a slightly yellow oil in 95% yield. $R_f$ = 0.43 in 20% EtOAc:hexane. $^1H$ NMR ($CD_2Cl_2$) δ: 8.1(d, J = 8.30 Hz, 2H, aromatic); 7.3(d, J = 8.30 Hz, 2H, aromatic).

**4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl alcohol (12):** the acyl fluoride (**11**) (90 mg, 0.39 mmol) in dry THF (2.0 mL) was added to a solution of $LiBH_4$ (14 mg, 0.39 mmol) in methanol (1.0 mL) at 0 °C. The reaction produced gas instantly. It was then warmed to room temperature and stirred for 1 h. Aqueous $H_2SO_4$ (0.1 N, 1.0 mL) was added and most of the solvent was removed *in vacuo*. To the residue, water (2.0 mL) was added followed by extraction with EtOAc, drying ($MgSO_4$), filtration and concentration to give 70 mg of a colorless oil (84% yield), $R_f$ = 0.27 in 20% EtOAc:hexane. $^1H$ NMR ($CDCl_3$) δ: 7.4(d, J = 7.88 Hz, 2H, aromatic); 7.2(d, J = 7.88 Hz, 2H, aromatic); 4.7(s, 2H, $CH_2OH$). Identical to the literature (*23*).

**4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl bromide (13):** a solution of the above alcohol (83 mg, 0.38 mmol) and $CBr_4$ (189 mg, 0.57 mmol) in dry $CH_2Cl_2$ (2.0 mL) was added at 0 °C to a solution of $Ph_3P$ (150 mg, 0.57 mmol) in $CH_2Cl_2$ (2.0 mL). The resulting solution was warmed to room temperature and stirred for an additional 2 h. It was then concentrated and purified by silica gel chromatography using $CHCl_3$ as eluent to give 98 mg (91% yield) of a colorless oil. $^1H$ NMR was identical to (**9**) which was prepared from (**8**) as described above.

**Preparation of 2 via the thio-Mitsunobu reaction:** to a solution of $Ph_3P$ (42 mg, 0.16 mmol) in dry THF (0.5 mL) was added diethyl azodicarboxylate (DEAD) (29 μL, 0.16 mmol) and the resulting yellow solution was stirred at room temperature for 30 min. To this were added alcohol (**12**) (23 mg, 0.11 mmol) and thiopyridazinone (**5**) (29 mg, 0.13 mmol) in dry THF (1.0 mL). The reaction was stirred at room temperature overnight. Then, it was concentrated and purified by silica gel chromatography using $CHCl_3$ as eluent to give 12 mg of pure product in 27% yield. $^1H$ NMR identical to **2**.

**Synthesis of 4-chloro-2-*tert*-butyl-5-[[[4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl]-$^3$H-methyl]thio]-3(2*H*)-pyridazinone [$^3$H]-2:** $LiB^3H_4$ was prepared according to the literature (*31*) on a 0.6 mmol scale. The THF solution was cooled to 0 °C (ice bath) and a solution of the acyl fluoride (**11**) (0.11 mmol) in dry THF (1.1 mL) was added. To this mixture methanol was added (350 μL) and the solution was stirred at room temperature for 1.5 h. Then aqueous $H_2SO_4$ (0.1 N, 0.5 mL) was injected to destroy excess $LiB^3H_4$ and most of the solvents were removed under reduced pressure. The residue was again diluted with water (1.0 mL) and extracted with EtOAc, dried ($MgSO_4$), filtered and concentrated under a stream of nitrogen to give 4.36 Ci (71% radiochemical yield) of the crude tritiated alcohol. To this product, $CBr_4$ (55 mg, 0.16 mmol) was added and the mixture was dissolved in dry $CH_2Cl_2$ (2.0 mL) and added at 0 °C to a solution of $Ph_3P$ (43 mg, 0.16 mmol) in dry $CH_2Cl_2$ (0.5 mL). The resulting mixture was stirred at room temperature for 2 h before the solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ (1.0 mL) and counted to give 3.2 Ci. The benzyl bromide produced was purified by silica gel (10 g) flash chromatography using $CHCl_3$ as eluent to give 720 mCi (17% radiochemical yield) of product which comigrated on a TLC plate with (**9**). The halide in $CHCl_3$ was concentrated under a stream of nitrogen and then dissolved in anhydrous ether (2.0 mL). To this solution, triethylamine (0.10 mL) and (**5**) (6.0 mg, 0.027 mmol) were added and the mixture was stirred at room temperature overnight. Most of the solvent was removed under a stream of nitrogen and, to the residue, water (1.5 mL) and EtOAc (5.0 mL) were added. The organic phase was pipetted out and the aqueous phase was extracted with EtOAc (3x3.0 mL), dried ($MgSO_4$), filtered and concentrated under a stream of nitrogen. The residue was dissolved in methanol (1.0 mL) and counted to give 532 mCi. This was purified by HPLC using the silica gel normal phase column and 10% THF:hexane ($R_f$ = 6.1 min at a flow of 1.0 mL/min) to afford 380 mCi (53% radiochemical yield) of the product of more than 99% radiochemical purity (based on $^3$H NMR and HPLC). $^1H$ NMR ($C_6D_6$) δ: 6.96(s, 1H, pyridazinone); 6.75(d, J = 8.4 Hz, 2H, aromatic); 6.71(d, J = 8.4 Hz, 2H, aromatic); 1.56(s, 9H, *t*-butyl). $^3H$ NMR ($^1$H-decoupled) δ: 3.19(s, $2^3$H); ($^1$H-coupled) d: 3.19(s, $2^3$H).

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