Synthesis of Carbon-11-, Fluorine-18-, and Iodine-125-Labeled $GABA_A$-Gated Chloride Ion Channel Blockers: Substituted 5-*tert*-Butyl-2-phenyl-1,3-dithianes and -dithiane Oxides

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Received December 28, 1994®

A series of substituted 5-*tert*-butyl-2-phenyl-1,3-dithianes and 5-*tert*-butyl-2-phenyl-1,1,3,3-tetraoxo-1,3-dithianes was synthesized as ligands for the $GABA_A$ receptor complex-associated neuronal chloride ion channels. The *in vitro* binding affinities of these compounds for the GABA-gated chloride ion channel were determined by their ability to compete with [^{3}H]TBOB for binding to rat brain slices. Of the eight compounds tested, *trans*-5-*tert*-butyl-2-(4-cyanophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane, **9b**, *trans*-5-*tert*-butyl-2-(4-fluorophenyl)-1,1,3,3-tetraoxo-1,3-dithiane, **10**, and *trans*-5-*tert*-butyl-2-(4-iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane, **11**, showed moderately high binding affinities (K_i = 41, 180, and 105 nM, respectively). Four radioligand candidates from this series, 5-*tert*-butyl-2-(4-cyanophenyl)-2-[^{11}C]methyl-1,3-dithiane, [^{11}C]**6**, 5-*tert*-butyl-2-(4-[^{18}F]fluorophenyl)-1,3-dithiane, [^{18}F]**7**, 5-*tert*-butyl-2-(4-[^{18}F]-fluorophenyl)-1,1,3,3-tetraoxo-1,3-dithiane, [^{18}F]**10**, and 5-*tert*-butyl-2-(4-[^{125}I]iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane, [^{125}I]**11**, have been successfully prepared for evaluation as *in vivo* imaging agents useful for positron emission tomography and single photon emission computed tomography. Preliminary *in vivo* studies indicate significant uptake into mouse brain for [^{18}F]**7**, [^{18}F]**10**, and [^{125}I]**11**.

Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system, opening neuronal chloride ion channels upon binding to the $GABA_A$ receptor complex. The subsequent influx of chloride ions results in a hyperpolarization of the neuron and consequently inhibition of firing.[1,2] Deficiencies in GABA-mediated neuromodulation have been implicated in a number of neurological abnormalities including epilepsy and anxiety disorders.[1,2] Many of the pharmaceuticals used to manage these disease states, including benzodiazepines, barbiturates, and steroids, have been shown to act through modulation of the effects of GABA by binding to ancillary sites on the $GABA_A$ receptor complex.[1-3]

Much of the investigation thus far of the $GABA_A$ receptor complex, including all of the radiotracers used in positron emission tomography (PET) and single photon emission computed tomography (SPECT),[4] has been associated with the benzodiazepine receptor site. It has been established recently, however, that a number of subtypes of the $GABA_A$ receptor complex exist and that their response to the various benzodiazepine ligands is nonhomogeneous.[3,5-7] Evidence suggests that not all subtypes of the $GABA_A$ complex even contain a benzodiazepine binding site.[8] In addition, the various ancillary binding sites on the $GABA_A$ complex are allosterically linked to one another and to the GABA-gated chloride channel itself. It would be useful to have a direct functional marker of the GABA-gated ion channel which might tell simply whether the ion channels are open or closed. With such markers, it might be possible to study the interplay of the various ancillary sites and the changes in integrity and function of the $GABA_A$ receptor complex in disease states.

Possible candidates for this type of functional marker are the picrotoxin-like chloride channel blockers which include the polychlorocycloalkanes, *tert*-butylbicyclophosphorothionates and *tert*-butylbicycloorthobenzoates.[5,9-11] These compounds have been identified as noncompetitive inhibitors of GABAergic neurotransmission.[12] Unlike the benzodiazepines and barbiturates which bind to sites on the extracellular domain of the $GABA_A$ protein complex, the picrotoxins are thought to act through binding to sites within the ion channel itself. Analogs of these "channel blockers" could provide probes for studying the GABA-gated chloride channel. In particular, PET or SPECT imaging agents based on these compounds could provide *in vivo* functional markers for the $GABA_A$ receptor complex.

In previous attempts to prepare radiolabeled channel binding ligands for the $GABA_A$ receptor-coupled Cl^- channel, the fluorine-18- and iodine-123-labeled derivatives of *tert*-butyl bicycloorthobenzoate (TBOB, Figure 1, **1a**) were successfully synthesized.[13,14] Unfortunately, neither of these radiotracers showed any potential as an *in vivo* imaging agent. In particular, the fluorine-18-labeled derivative, **1b**, exhibited poor stability toward hydrolysis, and the iodine-123-labeled compound, **1c**, was shown to bind extensively to plasma proteins. As a result neither compound showed significant brain uptake of radioactivity *in vivo*.

Recently several substituted 5-*tert*-butyl-2-phenyl-1,3-dithianes and the corresponding sulfone and sulfoxide derivatives have been identified as noncompetitive inhibitors of the $GABA_A$ receptor complex (Figure 1), exhibiting low nanomolar affinities for GABA receptor

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® Abstract published in *Advance ACS Abstracts*, June 1, 1995.

0022-2623/95/1838-2663$09.00/0

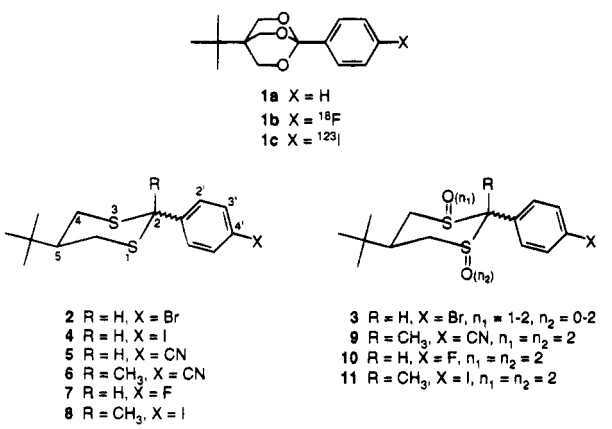


Figure 1. Structures of *tert*-butyl bicycloorthobenzoate (TBOB), **1a**, and the fluorine-18- and iodine-123-labeled analogs **1b–c**; structure and numbering of 2- and 4′-substituted 5-*tert*-butyl-2-phenyl-1,3-dithianes, **2**; and -1,1,3,3-tetraoxo-1,3-dithianes, **3**.

complexes in bovine and mouse brain.[12,15] Like TBOB, these phenyldithianes bind to the picrotoxin site but are much more resistant to hydrolysis than the bicycloorthobenzoates. Oxidations of the sulfur atoms in this class of compounds not only increase binding affinities in most cases but also provide a means of reducing lipophilicity and thus the plasma protein binding potential of these ligands.

Casida and co-workers have reported the structure–activity relationships for the 5-*tert*-butyl-2-phenyl-1,3-dithianes with respect to substitutions at both the 4′- and 2-positions (Figure 1).[16] These studies found the 4′-bromo, 4′-iodo, and 4′-cyano derivatives, **2**, **4**, and **5** respectively, to be extremely potent compounds (with LD_{50} values in the range of 4–20 μg/g in houseflies). In addition, potencies were either unchanged or substantially improved by the addition of a 2-methyl substituent. The 2-(4-cyanophenyl)-2-methyl-1,3-dithiane analog, **6**, exhibited a greater than 30-fold increase in toxicity *vs* **5**. Unfortunately, no $GABA_A$ receptor complex binding affinities were reported for these compounds.

In a subsequent study that explored the effect of *S*-oxidation state on binding affinity, the 2-(4-bromophenyl)-1,3-dithiane, **2**, exhibited an affinity of ~300 nM for the GABA receptor complex ion channel in mouse brain.[15] The binding affinity of **2** for GABA-gated chloride channel in mouse brain was improved by 1 order of magnitude (IC_{50} = 34 nM) by oxidation to the monosulfoxide, and the fully oxidized disulfone, **3** ($n_1 = n_2 = 2$), exhibited an increase in affinity of another order of magnitude (IC_{50} = 3 nM). This increase in affinity with increasing oxidation state appears to be a general property of the 2-phenyldithiane class of channel blockers and, as mentioned previously, provides for easy manipulation of lipophilicity.

We have, therefore, undertaken the synthesis and pharmacological evaluation of a series of substituted 5-*tert*-butyl-2-phenyl-1,3-dithianes, **5–8**, and 5-*tert*-butyl-2-phenyl-1,1,3,3-tetraoxo-1,3-dithianes, **9–11**, as ligands of the $GABA_A$ receptor complex. We have also prepared selected radiolabeled analogs of this series of ligands for evaluation as imaging agents for the neuronal $GABA_A$ receptor-gated chloride channels. 5-*tert*-Butyl-2-(4-cyanophenyl)-2-[^{11}C]methyl-1,3-dithiane, [^{11}C]**6**, 5-*tert*-butyl-2-(4-[^{18}F]fluorophenyl)-1,3-dithiane, [^{18}F]**7**, and the corresponding 1,1,3,3-tetraoxide derivative, [^{18}F]**10**, were synthesized for evaluation as PET imaging agents, and 5-*tert*-butyl-2-(4-[^{125}I]iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane, [^{125}I]**11**, was prepared as a model compound for future efforts at preparing an ^{123}I-labeled analog useful for SPECT imaging. The *in vitro* binding affinities of these compounds were determined by radioligand competition assays using [^{3}H]TBOB.[17]

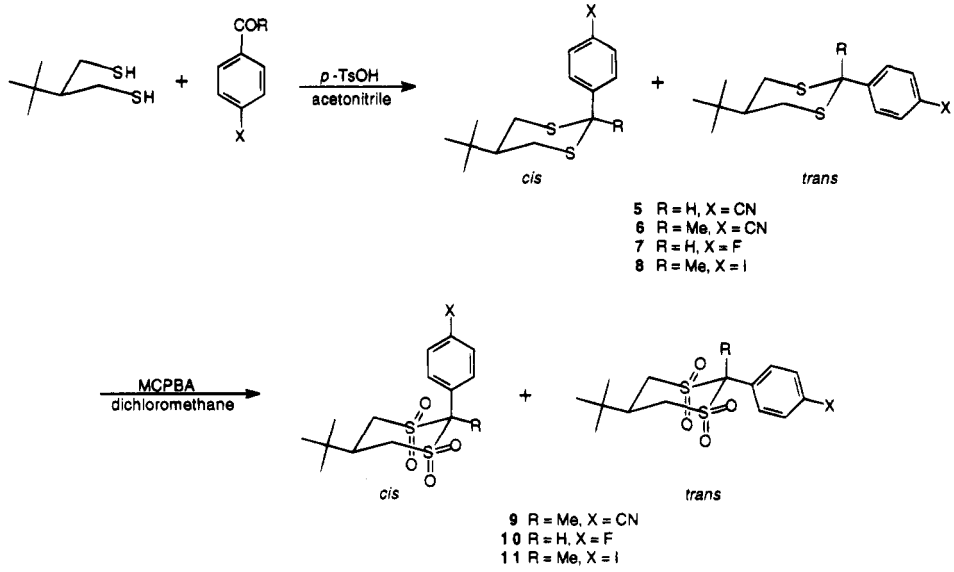
Results

Synthesis of Unlabeled Dithianes and Dithiane Oxides. The series of dithianes and dithiane oxides which were synthesized in unlabeled form for *in vitro* binding assays are shown in Scheme 1. Condensation of the appropriate 4-substituted benzaldehyde or acetophenone with 2-*tert*-butyl-1,3-propanedithiol[18] in the presence of *p*-toluenesulfonic acid by the method of Elliot *et al.*[16] provided 80–99% yields of the dithianes **5–8**. All syntheses gave a mixture of *cis* and *trans* products with respect to the relative configuration of the *tert*-butyl and phenyl moieties. Subsequent oxidation of the *cis/trans* mixtures of **6–8** with excess *m*-chloroperoxybenzoic acid (MCPBA) provided, after workup and chromatographic purification, the corresponding 1,1,3,3-tetraoxo-1,3-dithianes, **9–11**, respectively. Again both *cis* and *trans* configurational isomers were formed, but in all cases the *trans/cis* ratios for the tetraoxodithianes were significantly higher than those for the corresponding dithianes. Thus, **10** and **11** gave predominantly the *trans* isomer (90% and 75%, respectively). Due to the notoriously low yield of this oxidation in our hands, it is difficult to determine whether this *trans* selectivity is due to selective oxidation of the *trans* dithiane or acid-catalyzed epimerization of the *cis* isomers. However, this observation is consistent with results reported previously by Wacher *et al.*[15]

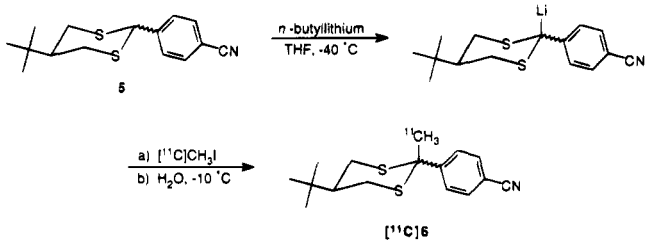
Synthesis of the Carbon-11-Labeled Dithiane [^{11}C]6. The synthesis of [^{11}C]**6**, as outlined in Scheme 2, was accomplished *via* a regioselective methylation of **5**, taking advantage of standard dithiane metalation chemistry.[19,20] Thus, the precursor **5** was treated with butyllithium at low temperature to give the 2-lithiodithiane derivative. This was then alkylated using [^{11}C]methyl iodide to provide, after workup and chromatographic purification, [^{11}C]**6** in 6% radiochemical yield (decay corrected, $t_{1/2}$ = 20 min) and >99% radiochemical purity. Total synthesis time was 35 min. Radiochemical yields and reaction times were not optimized.

Synthesis of the Fluorine-18-Labeled Dithiane [^{18}F]7 and Tetraoxodithiane [^{18}F]10. The starting material for this synthesis was 4-[^{18}F]fluorobenzaldehyde which, as depicted in Scheme 3, was synthesized using standard procedures from 4-(trimethylammonio)-benzaldehyde trifluoromethanesulfonate.[21] The labeled aldehyde was then condensed with 2-*tert*-butyl-1,3-propanedithiol at elevated temperature to reduce reaction times. This provided a 1:2 *cis/trans* mixture of [^{18}F]**7** in 68% radiochemical yield (decay corrected, $t_{1/2}$ = 110 min) and >99% radiochemical purity and with a specific activity of 270 Ci/mmol at end of synthesis (EOS). Subsequent oxidation of [^{18}F]**7** with excess MCPBA provided, after chromatographic purification, [^{18}F]**10** in 21% radiochemical yield (decay corrected) and

Scheme 1. Synthesis of Unlabeled Dithianes **5**–**8** and Dithiane Oxides **9**–**11**



Scheme 2. Synthesis of *cis/trans*-5-*tert*-butyl-2-(4-cyanophenyl)-2-[^{11}C]methyl-1,3-dithiane, [^{11}C]**6**



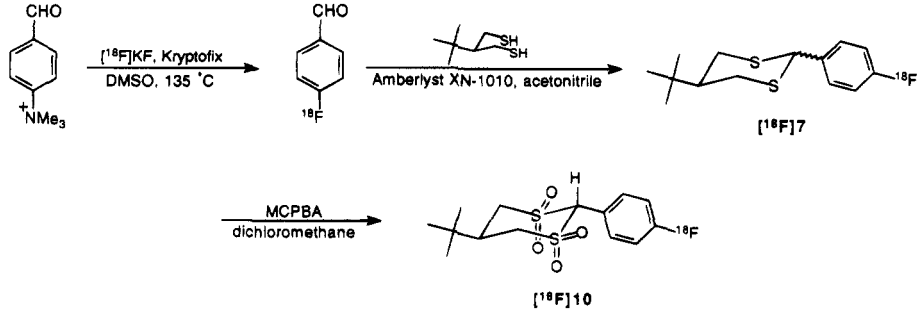
with a radiochemical purity of >99%. The specific activity of [^{18}F]**10** was 45 Ci/mmol (EOS), and the total synthesis time from solubilized $^{18}F^-$ was 168 min. Radiochemical yields and reaction times were not optimized. Only the *trans* isomer of [^{18}F]**10** was observed, which was not surprising based on the 1:9 *cis/trans* ratio obtained for the synthesis of unlabeled **10**.

Synthesis of 5-*tert*-Butyl-2-(4-[^{125}I]iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane ([^{125}I]11). Synthesis of the iodine-125-labeled analog of dithiane **8** through direct solid-state iodide exchange failed due to preferential oxidation of the dithiane. Conversely, the radiolabeled compound [^{125}I]**11** was easily prepared from its unlabeled analog **11** (Scheme 4) using a solid-state exchange reaction with [^{125}I]NaI in the presence of ammonium sulfate at 145 °C. Purification *via* ion-exchange chromatography (Amberlite IRA400, OH form) gave [^{125}I]**11** in 64% radiochemical yield and >99% radiochemical purity and with a specific activity of >150 Ci/mmol. Reaction time including purification was ~3 h.

***In Vitro* Radioligand Competition Analysis.** Compounds **5**–**11** were evaluated for their ability to compete with [^{3}H]TBOB binding in rat brain slices using an autoradiographic binding assay.[17] 5-*tert*-Butyl-2-(4-ethynylphenyl)-1,3-dithiane, **12**, was also tested in this assay as a standard compound of known affinity.[15,22,23] The K_i values for the compounds tested, along with partition coefficients calculated *de novo* for each compound by the hydrophobic fragmental constant method of Rekker and Mannhold,[24] are shown in Table 1. All compounds competed with [^{3}H]TBOB for binding to the GABA-gated chloride ion channel of rat brain. As expected based on previous SAR studies,[15] dithianes **6**–**8** showed 1 order of magnitude lower affinity relative to the corresponding tetraoxide analogs **9**–**11**. Compounds **5**–**9** were initially tested as the *cis/trans* mixture, whereas **10**, **11**, and **12** were the pure *trans* isomers. Tetraoxodithiane **9** exhibited the highest affinity of all the compounds tested, including standard compound **12**. Separation of the *cis* and *trans* isomers of **9** was accomplished using centrifugally accelerated, radial chromatography, and the pure isomers were assayed for binding affinity. The *trans* isomer, **9b**, showed nearly a 5-fold higher affinity than the *cis* isomer, **9a**, for the GABA-gated chloride channel.

***In Vivo* Biodistribution Studies in Mouse Brain.** All three radiotracers showed initial uptake into mouse brain followed by a gradual washout over the first 30

Scheme 3. Synthesis of *cis/trans*-5-*tert*-Butyl-2-(4-[^{18}F]fluorophenyl)-1,3-dithiane, [^{18}F]**7** and *trans*-5-*tert*-Butyl-2-(4-[^{18}F]fluorophenyl)-1,1,3,3-tetraoxo-1,3-dithiane, [^{18}F]**10**



Scheme 4. Synthesis of *trans*-5-*tert*-Butyl-2-(4-[^{125}I]iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane, [^{125}I]**11**

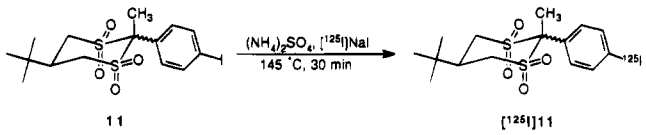
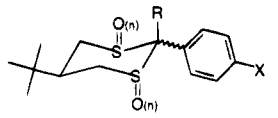


Table 1. *In Vitro* Affinities of 1,3-Dithianes **7**−**10** and 1,1,3,3-Tetraoxo-1,3-dithianes **11**−**13** for [^{3}H]TBOB Binding Sites in Rat Brain Slices



compound	*t*-Bu/Ph isomer	R	X	*n*	K_i (nM) mean ± SEM (n = 3)	log P^a
5	*cis/trans*	H	CN	0	240 ± 52	4.375
6	*cis/trans*	CH_3	CN	0	3100 ± 575	4.895
7	*cis/trans*	H	F	0	1100 ± 150	4.974
8	*cis/trans*	CH_3	I	0	1900 ± 260	6.496
9	*cis/trans*	CH_3	CN	2	87 ± 13	0.351
9a	*cis*	CH_3	CN	2	200 ± 27	
9b	*trans*	CH_3	CN	2	41 ± 8	
10	*trans*	H	F	2	180 ± 34	0.430
11	*trans*	CH_3	I	2	105 ± 15	1.952
12[b]	*trans*	H	C≡CH	0	85 ± 28	4.955
[^{3}H]TBOB					6.0 ± 0.1, 29.1 ± 3.8[c]	1.546

[a] The octanol/water partition coefficient for each compound was calculated *de novo* using the hydrophobic fragmental constant approach of Rekker and Mannhold, ref 24. [b] 5-*tert*-Butyl-2-(4-ethynylphenyl)-1,3-dithiane was the kind gift of J. E. Casida, University of California, Berkeley. [c] K_D values reported for [^{3}H]-TBOB from refs 12 and 17, respectively.

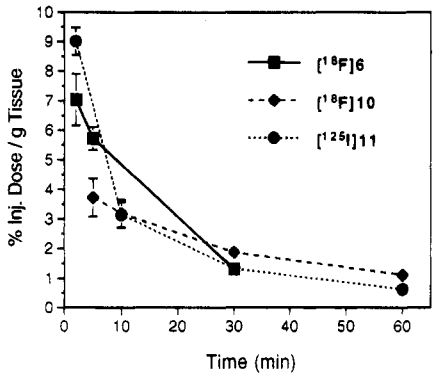


Figure 2. Time course in mice of [^{18}F]**6**, [^{18}F]**10**, and [^{125}I]**11** radioactivity for whole brain. Data are expressed as % injected dose/g of tissue at the specified times points post injection and are the average of four animals. Error bars denote standard deviation.

min (Figure 2). For both [^{18}F]**6** and [^{125}I]**11**, regional distributions of radioactivity were uniform at all time points examined (data not shown). However, as shown in Table 2, [^{18}F]**10** exhibited significantly higher amounts of radioactivity retained at 30 min in regions corresponding to high [^{3}H]TBOB binding *in vitro* (cortex and cerebellum) *vs* pons-medulla ($p < 0.05$), and a trend toward higher concentrations in these two regions *vs* striatum ($p < 0.1$).

Discussion

The 5-*tert*-butyl-2-phenyl-1,3-dithiane class of GABA-gated chloride channel blockers is an attractive target

Table 2. Regional Distribution Data for [^{18}F]**10** in Mouse Brain at 30 min Postinjection

brain region	[^{18}F]**10** *in vivo* (% inj dose/g of tissue)[a]	[^{3}H]TBOB *in vitro* (pmol/mg of protein)[b]
cerebral cortex	2.01 ± 0.26	1.57 ± 0.03
cerebellum	1.93 ± 0.21	1.34 ± 0.02
striatum	1.69 ± 0.23[c]	0.63 ± 0.01
pons-medulla	1.73 ± 0.16[d]	0.50 ± 0.01

[a] Data are the average of four animals ± standard deviation. Comparisons between brain regions were performed using paired Student's *t*-tests. [b] Data are specific binding *in vitro* for [^{3}H]TBOB from ref 29. [c] $p < 0.1$ *vs* cortex and cerebellum. [d] $p < 0.05$ *vs* cortex and cerebellum.

for developing radiolabeled *in vivo* imaging agents. They are easily synthesized from the condensation of commercially available aldehydes and ketones with 2-*tert*-butyl-1,3-propanedithiol. Although the 2-*tert*-butyl-1,3-propanedithiol used in these reactions was not commercially available, the synthesis of this compound is very straightforward and can be performed on large scale.[18] The dithiol can then, if handled properly, be stored at −4 °C for several months. This coupling reaction allows for the simple and rapid production of a wide range of 4′-substituted 2-phenyl-1,3-dithianes in excellent yield, because the aldehydes and ketones can be purchased with a variety of *para* substituents.

Wacher *et al.*[15] have reported that 5-*tert*-butyl-2-phenyl-1,3-dithianes substituted at the 4′-position with either a bromine or ethynyl moiety could be easily converted to the corresponding mono-, di-, tri-, and tetraoxo derivatives in good yields using MCPBA. The distribution of oxidation products formed could be manipulated by controlling the peroxide stoichiometry. There are a number of possible oxidation products for each dithiane, including both stereoisomers and configurational isomers (with respect to *cis/trans* configuration as well as equatorial *vs* axial *S*-oxidation), each with unique chemical characteristics, and, potentially, unique biodistribution and kinetic properties. As several of these oxidation products can be synthesized with minimal effort in a single step from a common starting material and can be separated using HPLC (data not shown), numerous additional 5-*tert*-butyl-2-phenyl-1,3-dithianes and dithiane oxides can eventually be evaluated as *in vivo* radioligands for the GABA-gated chloride channel site.

We limited our initial efforts to the synthesis of the parent dithianes **5**−**8** and their tetraoxo derivatives **9**−**11** in order to simplify the chemistry, radiolabeling, and chromatography. The tetraoxodithianes **9** and **11** were obtained in modest yields (44% and 25%, respectively) with the remainder of the material composed of several unidentified oxidation products. As expected based on the excellent yields obtained previously with the 2-(4-fluorophenyl)-1,3-dithiane,[25] the yield of **10** was somewhat better at 76%.

All of the unlabeled target compounds **5**−**11** were assayed for their ability to compete with [^{3}H]TBOB for binding sites in rat brain slices. From the K_i values obtained (Table 1), it is obvious that none of the dithianes **5**−**8** had high affinity for the $GABA_A$ receptor complex. The 1,1,3,3-tetraoxodithianes **9**−**11**, on the other hand, exhibited high binding affinities, in the same range as standard compound **12**. For a given 4′-substituent, the *in vitro* affinities of the tetraoxodithiane was significantly higher than the unoxidized dithiane,

consistent with the literature.[15] The binding affinity of the *trans* isomer **9b** was nearly 5-fold higher than the *cis* **9a**. This is consistent with the observations of Elliot *et al.*[16] of higher affinities for the *trans*-5-*tert*-butyl-2-methyl-2-phenyl-1,3-dithianes. Unfortunately, this *trans* binding preference is not consistently observed for all dithianes and dithiane oxides. The corresponding *cis* isomers of compounds **10** and **11** could not be isolated from the reaction mixture, and we were unable to obtain *in vitro* binding affinities for those isomers.

The apparent affinity of **12** in our assay system (85 nM) is lower than that reported previously (IC_{50} = 4 nM in mouse and 21 nM in bovine).[15,22] This discrepancy could possibly be explained on the basis of differences in assay conditions as the K_D value of TBOB (29.1 nM) in our autoradiographic assay system also differs significantly from that reported in bovine brain homogenates (6.0 nM) by Hawkinson and Casida.[12] Alternatively, species variability could account for the discrepancy in affinities for TBOB and compound **12** (see above).

On the basis of these *in vitro* results, compounds **9**–**11** were selected as suitable candidates for evaluation as *in vivo* imaging agents. Of the two radiolabeling approaches utilized, substitution at the 4′-position or methylation of the 2-position, carbon-11 labeling by methylation at the 2-position of the dithiane is perhaps the most promising. The alkylation of both dithianes and many of the dithiane oxides is a well-established procedure.[20,26–28] This chemistry is easily adaptable to radiochemical syntheses with short-lived radionuclides, as these alkylation reactions can be performed rapidly and selectively as the last synthetic step. As the 4′-substituent has little or no effect on alkylation at the 2-position, this labeling procedure allows for broad structural diversity. Finally, as the GABA-gated chloride channel seems to have a high tolerance for small alkyl substituents at the benzylic position of these 2-phenyl-1,3-dithianes, presumably any high-affinity dithiane analog might be ^{11}C-methylated at this position. Although the reaction conditions and radiochemical yields were not optimized, the feasibility of this synthetic approach to carbon-11-labeling ligands for the picrotoxin binding site was exemplified by the successful synthesis of **[^{11}C]6**.

The fluorine-18-labeling procedure to form **[^{18}F]7** and **[^{18}F]10** was somewhat limited in scope as the radionuclide must be introduced near the beginning of the synthesis and synthesis times were rather long. Nevertheless, sufficient amounts of radiolabeled products could be prepared for subsequent *in vivo* studies (see below). This synthetic approach has the advantage of providing a radiolabeled intermediate, the 5-*tert*-butyl-2-(4-[^{18}F]fluorophenyl)-1,3-dithiane, which can be further elaborated to multiple dithiane oxides.[25] Synthesis and biological testing if these analogs is currently underway.

The iodine-125-labeling reaction of **11** gave excellent chemical and radiochemical results but is, likewise, rather limited in scope. This reaction is run under highly oxidizing conditions so that only the di-, tri-, or tetraoxodithianes can be labeled. Of these, the trioxodithianes have been shown to have much lower affinities than the corresponding mono-, di-, or tetraoxides.[15] This leaves only **[^{125}I]11** and the corresponding disulfoxides as possible candidates for radiolabeling as SPECT radiotracers.

The first crucial consideration in developing a new radiotracer for brain imaging is whether the agent will cross the blood–brain barrier and be retained in brain tissues. Prior attempts to prepare *in vivo* radioligands for the GABA$_A$-gated chloride ion channel, based on the bicycloorthobenzoate structure, failed due to a distinct lack of brain uptake of the radiolabeled compounds.[13,14] Unlike the labeled TBOB analogs, the radiolabeled 5-*tert*-butyl-2-phenyl-1,3-dithianes and 1,1,3,3-tetraoxodithianes exhibit substantial uptake of radioactivity into brain tissues. Compounds **[^{18}F]7**, **[^{18}F]10**, and **[^{125}I]-11** show initial brain uptakes (5 min postinjection) of 7%, 4%, and 3%, respectively, of the injected dose per gram of tissue. Thus, brain permeability will not be a limitation in the development of a dithiane-based radiotracer for the GABA$_A$-gated chloride ion channel. Regional brain distributions for **[^{18}F]7** and **[^{125}I]11** did not demonstrate statistically significant selectivity at any of the time points studied. Compound **[^{18}F]10** demonstrated statistically significant distribution differences in cortex and cerebellum *vs* pons-medulla and a trend toward a higher radioactivity in cortex and cerebellum *vs* striatum. These data are consistent with the *in vitro* binding distribution of [^{3}H]TBOB (Table 2).[29] This is encouraging in light of the fact that all of the regional distribution data for this ionophore are based on *in vitro* assay systems from which all endogenous GABA and effector ligands have been carefully removed. There are no data as yet, other than LD_{50} values, on how these 5-*tert*-butyl-2-phenyl-1,3-dithiane-based ligands behave in the intact biological system. In addition, unlike many other neurotransmitter receptors (such as dopamine receptors) where regional distributions can differ by 1 order of magnitude or more, the GABA$_A$ receptor complex is ubiquitous to all brain regions. Even in optimized *in vitro* assay systems the regional distribution differences are on the order of only 2 or 3 to 1. Another possible consideration is that the GABA$_A$ receptor complex is a multisubunit ionophore for which numerous isoforms, composed of different combinations of subunits, have been identified.[3,5] It is conceivable that the dithiane channel blockers may exhibit a differential preference among these various GABA$_A$ receptor complex isoforms, although there is currently no evidence for GABA$_A$ receptor complexes with altered or diminished affinities for the existing classes of picrotoxin site ligands. Either or both of these factors could account for the lack of distinct regional uptake or binding for some of the 5-*tert*-butyl-2-phenyl-1,3-dithianes *in vivo* in these initial studies. Thus, our preliminary *in vivo* data are encouraging, and extensive biological evaluation, including pharmacological manipulation of the receptor complex, will likely be necessary to determine the usefulness of these compounds as *in vivo* imaging agents for the GABA$_A$-gated chloride ionophore.

Conclusion

In this work, we have demonstrated that the 5-*tert*-butyl-2-phenyl-1,3-dithianes and corresponding *S*-oxidation products, a new class of high-affinity ligands for the GABA$_A$ receptor complex chloride ion channel, can be radiolabeled with positron- or γ-emitting radionuclides. As there are many combinations of phenyl ring

substituents, 2-alkyl substituents, and sulfur oxidation states that will result in high-affinity ligands, we feel there is an excellent potential that a suitable *in vivo* radiopharmaceutical for PET or SPECT imaging of this channel in human brain will eventually be developed. Efforts to prepare and evaluate ligands of this chemical class will continue.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Brüker (360 MHz) NMR instrument in $CDCl_3$, and chemical shifts are reported in δ values (parts per million) relative to an internal reference of tetramethylsilane (TMS, δ 0.00) unless otherwise noted. Abbreviations used in NMR analyses are as follows: d = doublet, dd = doublet of doublets, m = multiplet, s = singlet, t = triplet, tt = triplet of triplets. Chemical ionization (CI) and electron ionization (EI) mass spectra were obtained on a V.G. Analytical 70−250S spectrometer, and high-resolution spectra are within 0.0015 m/z. The ionization gas for CIMS and high-resolution CIMS was ammonia. Elemental analyses were performed by the University of Michigan CHN/AA Laboratory (Ann Arbor, MI) and were within 0.4% of the calculated values, unless otherwise noted. All starting materials for chemical syntheses were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). *m*-Chloroperoxybenzoic acid was purified before use by repeated extraction of a dichloromethane solution with aqueous $NaHCO_3$ to remove *m*-chlorobenzoic acid. Sep-Pak cartridges used for radiochemical syntheses (25 × 10 mm N-alumina and 10 × 10 mm C18-silica) were purchased from the Waters Chromatography Division of Millipore Corp. (Milford, MA).

Chromatographic purification of unlabeled compounds was performed *via* centrifugally accelerated, radial, thin layer chromatography (chromatotron) using 4 mm thick silica gel rotors and gradient elution (dichloromethane/methanol or hexane/dichloromethane) at a flow rate of 9 mL/min.

[^{11}C]Carbon dioxide, produced by proton irradiation of a nitrogen target at 20 μA for 2 min, was converted to [^{11}C]methyl iodide by lithium aluminum hydride reduction followed by treatment with hydroiodic acid. All syntheses with carbon-11 were done in small glass vessels placed in a remote apparatus which was manipulated from outside the hot cell. Radiochemical yields were calculated based on 240 mCi of [^{11}C]CO_2 produced at end of bombardment (EOB) and are decay corrected.

[^{18}F]Fluoride was produced by proton irradiation of a 10% [^{18}O]water target at 15 μA for 30 min. This aqueous fluoride was converted to [^{18}F]KF by addition of K_2CO_3 and solubilized for reaction by addition of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (Kryptofix 222), removal of the water by evaporation followed by acetonitrile azeotrope, and redissolving the residue in dry dimethyl sulfoxide (DMSO). Radiochemical yields were calculated based on the radioactivity (mCi) of [^{18}F]KF solubilized and are corrected for decay over the duration of the synthesis. Specific activities are decay corrected to end of synthesis (EOS).

[^{125}I]Sodium iodide was purchased from Nordion International, Inc. (Kanata, Ontario, Canada) as a solution in 0.1 N NaOH. Radiochemical yield for the iodine-125-labeled compound was calculated based on the radioactivity (mCi) of [^{125}I]NaI used for the synthesis.

The [^{3}H]*tert*-butylbicycloorthobenzoate ([^{3}H]TBOB, specific activity = 25.0 Ci/mmol) used in radioligand competition assays was purchased from Amersham Inc. (Arlington Heights, IL). All remaining reagents used in these assays were purchased from Sigma Chemicals (St. Louis, MO) and were of the highest possible purity.

General Synthesis for Dithianes 5−8. Using the method of Wacher *et al.*,[15] a solution of the appropriately substituted aldehyde or ketone in acetonitrile and a catalytic amount of *p*-toluenesulfonic acid was stirred under a nitrogen atmosphere. After a few minutes to allow dissolution of the catalyst, 1 molar equiv of 2-*tert*-butyl-1,3-propanedithiol[18] was added and the reaction mixture was allowed to stir overnight at room temperature under nitrogen. The solvent was removed *via* rotary evaporation, and the residue was redissolved in dichloromethane, washed with a saturated aqueous $NaHCO_3$ solution, and dried over anhydrous $MgSO_4$. Filtration and solvent removal provided, in all cases, a *cis/trans* mixture of the desired product as a white amorphous solid. Isomer ratios were determined by both ^{1}H NMR and HPLC analysis. Separation of the isomers was performed *via* centrifugally accelerated, radial, thin layer chromatography (chromatotron) using silica gel rotors and hexane/dichloromethane gradient elution.

5-*tert*-Butyl-2-(4-cyanophenyl)-1,3-dithiane (5). The title compound was synthesized as described starting with 1.00 g (7.63 mmol) of 4-cyanobenzaldehyde to provide a 2.6:1 mixture of *cis*- and *trans*-**5** in 80% yield. This compound has been synthesized previously,[16] but a full characterization has not been reported. Therefore, an analytical sample of each isomer was separated *via* chromatotron and characterized. ***Cis isomer*:** mp 104−107 °C; ^{1}H NMR ($CDCl_3$) δ 7.93 (2H, AA′BB′, J = 8.4 and 1.9 Hz, 2ArH), 7.67 (2H, AA′BB′, J = 8.4, 1.9, and 1.6 Hz, 2ArH), 4.86 (1H, s, H-2), 2.73 (2H, dd, J = 14.0 and 3.2 Hz, 4_{eq} and 6_{eq}), 2.58 (2H, dd, J = 14.0 and 10.8 Hz, 4_{ax} and 6_{ax}), 1.86 (1H, tt, J = 10.8 and 3.2 Hz, H-5), 0.88 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 277 (M^+, 88), 57(100); HR EIMS 277.0966 (calcd 277.0959). Anal. ($C_{15}H_{19}NS_2$) C, H, N. ***Trans isomer*:** mp 151−153 °C; ^{1}H NMR ($CDCl_3$) δ 7.63 (2H, AA′BB′, J = 8.5, 2.0, and 1.5 Hz, 2ArH), 7.58 (2H, AA′BB′, J = 8.4, 2.0, and 1.5 Hz, 2ArH), 5.15 (1H, s, H-2), 3.00 (2H, dd, J = 14.2 and 2.3 Hz, 4_{eq} and 6_{eq}), 2.84 (2H, dd, J = 14.1 and 11.2 Hz, 4_{ax} and 6_{ax}), 1.76 (2H, tt, J = 11.2 and 2.4 Hz, H-5), 0.97 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 277 (M^+, 93), 57 (100); HR EIMS 277.0952 (calcd 277.0959). Anal. ($C_{15}H_{19}NS_2$) C, H, N.

5-*tert*-Butyl-2-(4-cyanophenyl)-2-methyl-1,3-dithiane (6). The title compound was synthesized as described starting with 380 mg (2.62 mmol) of 4-acetylbenzonitrile to provide a 1:1.25 mixture of *cis*- and *trans*-**6** in 99% yield. This compound has been synthesized previously,[16] but a full characterization has not been reported. Therefore, an analytical sample of each isomer was separated *via* chromatotron and characterized: ***Cis isomer*:** mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 8.13 (2H, AA′BB′, J = 8.8, 2.0, and 1.9 Hz, ArH), 7.68 (2H, AA′BB′, J = 8.8, 2.0, and 1.9 Hz, ArH), 2.69 (2H, dd, J = 14.4 and 2.5 Hz, 4_{eq} and 6_{eq}), 2.34 (2H, dd, J = 14.3 and 11.6 Hz, 4_{ax} and 6_{ax}), 1.74 (1H, tt, J = 11.6 and 2.5 Hz, H-5), 1.67 (3H, s, CH_3), 0.82 (9H, s, $C(CH_3)_3$); EIMS m/z 291 (M^+); HR EIMS 291.1108 (calcd 291.1115). Anal. ($C_{16}H_{21}NS_2$) C, H, N. ***Trans isomer*:** mp 163−165 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (2H, AA′BB′, J = 8.7, 2.0, and 1.9 Hz, ArH), 7.66 (2H, AA′BB′, J = 8.7, 2.0, and 1.9 Hz, ArH), 3.03 (2H, dd, J = 14.4 and 11.4 Hz, 4_{ax} and 6_{ax}), 2.84 (2H, dd, J = 14.4 and 2.9 Hz, 4_{eq} and 6_{eq}), 2.22 (3H, s, CH_3), 1.77 (1H, tt, J = 11.4 and 2.9 Hz, H-5), 1.01 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 291 (M^+, 99), 130 (100); HR EIMS 291.1118 (calcd 291.1115). Anal. ($C_{16}H_{21}NS_2$) C, H, N.

5-*tert*-Butyl-2-(4-fluorophenyl)-1,3-dithiane (7). The title compound was synthesized as described starting with 200 mg (1.61 mmol) of 4-fluorobenzaldehyde providing a 1:2 mixture of *cis*- and *trans*-**7** in quantitative yield. A small sample was separated *via* chromatotron to provide pure analytical samples of both isomers. ***Cis isomer*:** mp 62−64 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (2H, AA′BB′, J = 9.3, 3.1, and 2.2 Hz, ArH), 7.04 (2H, AA′BB′, J = 9.3, 3.1, and 2.2 Hz, ArH), 4.89 (1H, s, H-2), 2.73 (2H, dd, J = 13.8 and 3.6 Hz, 4_{eq} and 6_{eq}), 2.65 (2H, dd, J = 13.8 and 10.4 Hz, 4_{ax} and 6_{ax}), 1.85 (1H, tt, J = 10.4 and 3.6 Hz, H-5), 0.88 (9H, s, $C(CH_3)_3$); EIMS m/z 270 (M^+); HR EIMS 270.0901 (calcd 270.0912). Anal. ($C_{14}H_{19}FS_2$) C, H. ***Trans isomer*:** mp 125−126 °C (sublimed); ^{1}H NMR ($CDCl_3$) δ 7.45 (2H, AA′BB′, J = 9.2, 3.1, and 2.2 Hz, ArH), 7.02 (2H, AA′BB′, J = 9.2, 3.1, and 2.2 Hz, ArH), 5.12 (1H, s, H-2), 2.97 (2H, dd, J = 14.1 and 2.4 Hz, 4_{eq} and 6_{eq}), 2.83 (2H, dd, J = 14.1 and 11.2 Hz, 4_{ax} and 6_{ax}), 1.75 (1H, tt, J = 11.2 and 2.5 Hz, H-5), 0.96 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 270 (M^+, 42), 162 (56), 57 (100); HR EIMS 270.0918 (calcd 270.0912). Anal. ($C_{14}H_{19}FS_2$) C, H.

5-*tert*-Butyl-2-(4-iodophenyl)-2-methyl-1,3-dithiane (8). Title compound was synthesized as described, starting with 500 mg (2.03 mmol) of 4′-iodoacetophenone, to provide a 1:1.2 mixture of *cis*- and *trans*-**8** in 97% yield. A small sample was separated *via* chromatotron to provide pure analytical samples of both isomers. ***Cis isomer***: mp 161–163 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (2H, AA′BB′, J = 7.8, 2.2, and 1.9 Hz, 2ArH), 7.68 (2H, AA′BB′, J = 7.8, 2.2, and 1.9 Hz, 2ArH), 2.66 (2H, dd, J = 14.3 and 2.4 Hz, 4_{eq} and 6_{eq}), 2.39 (2H, dd, J = 14.2 and 11.6 Hz, 4_{ax} and 6_{ax}), 1.72 (1H, tt, J = 11.5 and 2.5 Hz, H-5), 1.66 (3H, s, CH_3), 0.82 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 392 (M^+, 44), 262 (100); HR EIMS 392.0120 (calcd 392.0129). Anal. ($C_{15}H_{21}IS_2$) C, H. ***Trans isomer***: mp 129–131 °C; ^{1}H NMR ($CDCl_3$) δ 7.69 (2H, AA′BB′, J = 8.8, 2.5, and 2.0 Hz, 2ArH), 7.55 (2H, AA′BB′, J = 8.8, 2.5, and 2.0 Hz, 2ArH), 3.02 (2H, dd, J = 14.4 and 11.5 Hz, 4_{ax} and 6_{ax}), 2.82 (2H, dd, J = 14.5 and 2.8 Hz, 4_{eq} and 6_{eq}), 2.20 (3H, s, CH_3), 1.76 (1H, tt, J = 11.5 and 2.8 Hz, H-5), 0.98 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 392 (M^+, 41), 262 (100); HR EIMS 392.0129 (calcd 392.0129). Anal. ($C_{15}H_{21}IS_2$) C, H.

General Synthesis of 1,1,3,3-Tetraoxo-1,3-dithianes 9–11.[15] To a stirred solution of the dithiane in dichloromethane at 0 °C was added 5 molar equiv of MCPBA as a solution in dichloromethane. This reaction mixture was allowed to warm to room temperature and stir overnight. The organic solution was then extracted with a saturated aqueous $NaHCO_3$ solution to remove *m*-chlorobenzoic acid, dried over anhydrous $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. The resulting white solid was purified by chromatotron using silica gel rotors and a dichloromethane/methanol gradient elution to provide moderate yields of the 1,1,3,3-tetraoxo-1,3-dithianes.

5-*tert*-Butyl-2-(4-cyanophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane (9). The title compound was synthesized as described, starting with 150 mg (0.516 mmol) of **6**, to provide a 1:2 mixture of *cis* and *trans* **9** in 44% yield. An analytical sample of this isomer mixture was separated by chromatotron. ***Cis isomer***: mp >260 °C; ^{1}H NMR (acetone-d_6, δ 2.04) δ 8.13 (2H, AA′BB′, J = 9.0, 2.2, and 2.17 Hz, 2ArH), 7.88 (2H, AA′BB′, J = 9.0, 2.2, and 2.17 Hz, 2ArH), 3.69 (2H, dd, J = 15.0 and 3.3 Hz, 4_{eq} and 6_{eq}), 3.59 (2H, dd, J = 15.0 and 11.8 Hz, 4_{ax} and 6_{ax}), 2.51 (1H, tt, J = 11.8 and 3.3 Hz, H-5), 2.20 (3H, s, CH_3), 1.09 (9H, s, $C(CH_3)_3$); CIMS m/z 373 $(M + NH_4)^+$; HR 373.1254 (calcd 373.1256). Anal. ($C_{16}H_{21}NO_4S_2$) C, 54.07; H, 5.96; N, 3.94. Found: C, 52.73; H, 5.74; N, 3.81. ***Trans isomer***: mp >260 °C; ^{1}H NMR (acetone-d_6, δ 2.04) δ 8.31 (2H, AA′BB′, J = 8.8 Hz, 2ArH), 7.92 (2H, AA′BB′, J = 8.8 Hz, 2ArH), 3.83 (2H, dd, J = 14.9 and 12.7 Hz, 4_{ax} and 6_{ax}), 3.55 (2H, dd, J = 15.0 and 1.9 Hz, 4_{eq} and 6_{eq}), 2.56 (1H, tt, J = 12.7 and 2.0 Hz, H-5), 2.43 (3H, s, CH_3), 1.14 (9H, s, $C(CH_3)_3$); CIMS m/z 373 $(M + NH_4)^+$; HR 373.1269 (calcd 373.1256). Anal. ($C_{16}H_{21}NO_4S_2$) C, H, N.

5-*tert*-Butyl-2-(4-fluorophenyl)-1,1,3,3-tetraoxo-1,3-dithiane (10). The title compound was synthesized as described, starting with 214 mg (0.793 mmol) of **7**, to provide predominantly *trans*-**10** in 76% yield, with <10% of the *cis* isomer as detected by ^{1}H NMR. The *trans*-**10** was purified by chromatotron and characterized. We were unable to purify a sufficient quantity of the *cis* isomer for characterization. ***Trans isomer***: mp >260 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (2H, m, 2ArH), 7.19 (2H, m, 2ArH), 5.22 (1H, s, H-2), 3.58 (2H, dd, J = 2.0 and 14.8 Hz, H-4_{eq} and H-6_{eq}), 3.09 (2H, dd, J = 12.8 and 14.3 Hz, H-4_{ax} and H-6_{ax}), 2.62 (1H, tt, J = 2.0 and 12.5 Hz, H-5), 1.08 (9H, s, $(CH_3)_3$); EIMS m/z (relative intensity) 334 (M^+, 25), 122 (100); HR EIMS 334.0699 (calcd 334.0709). Anal. ($C_{14}H_{19}FO_4S_2$) C, H.

5-*tert*-Butyl-2-(4-iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane (11). The title compound was synthesized as described, starting with 834 mg (2.13 mmol) of **8**, and provided a meager 25% yield of predominantly *trans*-**11**, with ~25% of the *cis* isomer as detected by ^{1}H NMR. The *trans*-**11** was purified by chromatotron and characterized. We were unable to purify a sufficient quantity of the *cis* isomer for characterization. ***Trans isomer***: mp 247–250 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (2H, AA′BB′, J = 9.3, 2.7, 2.4, and 2.1 Hz, ArH), 7.53 (2H, AA′BB′, J = 9.3, 2.7, 2.4, and 2.1 Hz, ArH), 3.41 (2H, dd, J = 14.8 and 2.8 Hz, 4_{eq} and 6_{eq}), 3.20 (2H, dd, J = 14.7 and 12.4 Hz, 4_{ax} and 6_{ax}), 2.56 (1H, tt, J = 12.3 and 2.9 Hz, H-5), 2.20 (3H, s, CH_3), 1.01 (9H, s, $C(CH_3)_3$); EIMS m/z (relative intensity) 456 (M^+, 5), 392 (60), 262 (100); HR EIMS 455.9929 (calcd 455.9926). Anal. ($C_{15}H_{21}IO_4S_2$) C, H.

5-*tert*-Butyl-2-(4-cyanophenyl)-2-[^{11}C]methyl-1,3-dithiane ([^{11}C]6). A solution of **5** (1.5 mg, 5.42 μmol) was dissolved in dry tetrahydrofuran (250 μL), placed under nitrogen in a 1.0 mL V-vial, and cooled to −40 °C. To this was added *n*-butyllithium (6.0 μmol, 3.0 μL of a 2.0 M solution in cyclohexane), and the resulting yellow solution was allowed to stand at −40 °C for 10 min to ensure complete metalation. [^{11}C]Methyl iodide carried by a nitrogen stream was then bubbled through this reaction mixture with simultaneous disappearance of the yellow color. The reaction vial was sealed and allowed to warm to −10 °C over about 5 min. Deionized H_2O (1 mL) was added to quench the reaction. This mixture was loaded on a C18 Sep-Pak and washed with H_2O (3 × 2 mL), and the Sep-Pak was blown dry with a nitrogen stream. The product was then eluted with dichloromethane and passed directly through a short column (80 × 5 mm) of anhydrous Na_2SO_4 followed by an N-Alumina Sep-Pak column (dichloromethane, 6 mL total elution volume). Compound [^{11}C]**6** was thus obtained in a 6% radiochemical yield in >99% radiochemical purity and was shown to coelute with **6** on HPLC analysis (t_R = 9.0 (*trans*) and 10.0 min (*cis*) on a C8-silica column (10 × 250 mm, using 75:25 acetonitrile/H_2O, 1.0 mL/min). Total synthesis time from end of bombardment (EOB) was 35 min. Due to difficulties in the chromatographic separation of [^{11}C]**6** and **5**, specific activity was not determined for this compound.

5-*tert*-Butyl-2-(4-[^{18}F]fluorophenyl)-1,3-dithiane ([^{18}F]7). The starting material for this synthesis, 4-[^{18}F]fluorobenzaldehyde, was prepared from 4-(*N,N,N*-trimethylammonio)-benzaldehyde trifluoromethanesulfonate and [^{18}F]KF using standard procedures.[21] To a mixture of labeled aldehyde (4 mCi) and Amberlyst XN-1010 ion-exchange resin in acetonitrile (250 μL) in a 2.5 mL V-vial was added 2-*tert*-butyl-1,3-propanedithiol. The reaction vial was sealed and heated at 135 °C for 30 min. The crude reaction mixture was purified by loading it onto a C18 Sep-Pak, washing with deionized H_2O (1 × 10 mL), and eluting with dichloromethane (6 mL). This organic solution was sequentially dried by passing it through a short column of anhydrous Na_2SO_4 (12 × 24 mm) followed by passing it through a N-Alumina Sep-Pak to provide a 68% radiochemical yield (decay corrected) of [^{18}F]**7** as a 1:2 *cis/trans* mixture in >99% radiochemical purity and a specific activity of 270 Ci/mmol (EOS). Compound [^{18}F]**7** was determined to coelute with **7** upon HPLC analysis (t_R = 12.1 (*trans*) and 13.3 min (*cis*) on a C8-silica column (10 × 250 mm) using 70:30 acetonitrile/H_2O, 1.0 mL/min). Total synthesis time including purification was 53 min.

5-*tert*-Butyl-2-(4-[^{18}F]fluorophenyl)-1,1,3,3-tetraoxo-1,3-dithiane ([^{18}F]10). Labeled dithiane [^{18}F]**7** in 3 mL of dichloromethane was treated at room temperature with a large excess of MCPBA. After 30 min the solvent was removed using a stream of nitrogen, and the reaction was quenched by addition of saturated aqueous $Na_2S_2O_5$ (5 mL) followed by saturated aqueous Na_2CO_3 (10 mL). This aqueous mixture was loaded onto a C18 Sep-Pak, washed with aqueous Na_2CO_3 (3 × 8 mL) and H_2O (1 × 10 mL), and eluted with dichloromethane (6 mL). The organic solution was then dried over anhydrous Na_2SO_4, concentrated to ~500 μL, and purified by normal-phase HPLC (silica gel, 5 μm, dichloromethane/methanol, 99.5:0.5) to provide [^{18}F]**10** in 21% radiochemical yield (decay corrected) with >99% radiochemical purity and a specific activity of 45 Ci/mmol (EOS). Compound [^{18}F]**10** was shown to coelute with **10** upon HPLC analysis (t_R = 4.5 min (*trans* only) on a C8-silica column (10 × 250 mm) using 70:30 acetonitrile/H_2O, 1.0 mL/min). Total synthesis time, including HPLC purification, from [^{18}F]**7** was 115 min.

5-*tert*-Butyl-2-(4-[^{125}I]iodophenyl)-2-methyl-1,1,3,3-tetraoxo-1,3-dithiane ([^{125}I]11). A solution of **11** (20 μg, *cis/trans* mixture) in ethanol (20 μL), an aqueous $(NH_4)_2SO_4$ solution (5.0 mg in 15 μL of deionized H_2O), and three layers

of glass beads (German BSG, 3 mm) were placed successively in a 3 mL multidose vial. To this was added 3.32 mCi of [^{125}I]-NaI *via* syringe. The syringe was rinsed with acetone (2 × 50 μL), which was added to the reaction vial, and the sides of the reaction vial were washed with ethanol (2 × 50 μL). The reaction vial was crimp-capped and fitted with a disposable plastipak syringe as a distillate condenser. The condenser was connected successively to a charcoal (14 mesh) trap and a sodium thiosulfate trap. The reaction mixture was then evaporated to dryness at 135 °C in an oil bath. Air (20 mL) was then slowly introduced over 1 min *via* syringe in order to dry the reaction mixture completely. The dry reaction mixture was maintained at 145 °C for an additional 30 min and then cooled to room temperature. The resulting solid mixture was redissolved in ethanol (2 mL) and then transferred to an anion-exchange column (Amberlite IRA 400, OH form, strongly basic) to remove residual free [^{125}I]iodide. Elution with ethanol (2 mL) afforded 2.14 mCi (64%) of **[^{125}I]11** in >99% radiochemical purity, as determined by TLC analysis (silica, chloroform/ethanol, 97:3) using radioisotope detection. The specific activity of this *cis/trans* mixture was determined to be >150 Ci/mmol. Total synthesis time including purification was 3 h.

Radioligand Competition Studies. Male Sprague–Dawley rats (Harlan Labs, Indianapolis, IN; weight 175–199 g) were decapitated, and their brains were rapidly removed and frozen in Lipshaw embedding matrix surrounded by powdered dry ice. Twenty-micrometer-thick sections were cut horizontally on a Lipshaw cryostat, thaw-mounted onto 2× subbed gelatin-coated slides, and stored at −20 °C until the time of assay. All assays were performed 24 h after decapitation. Sections were run in triplicate. Slide-mounted tissue sections were warmed to room temperature and prewashed for 3 × 10 min in buffer (50 mM Tris-HCl + 1 mM EDTA, pH 7.4) at 4 °C and dried under a stream of cool air. Binding of [^{3}H]TBOB (20 nM) was carried out in buffer (50 mM Tris-HCl + 120 mM NaCl, pH 7.4) for 90 min at room temperature. The incubation was terminated by two 30 min rinses in buffer (50 mM Tris-HCl, pH 7.4) at 4 °C followed by a brief dip in distilled water. Each slide was then dried under a stream of hot air. Nonspecific binding was assessed in the presence of 20 μM picrotoxin. For analysis of the dithianes' effect on [^{3}H]TBOB binding, tissue sections were incubated with 20 nM [^{3}H]TBOB and nine concentrations of dithiane ranging from 1 nM to 100 μM for **5–8** and **12** and from 100 pM to 10 μM for **9–11**. Autoradiograms were generated by apposing the slides to tritium-sensitive film (Hyperfilm, Amersham) in light-tight cassettes along with standards containing known amounts of radioactivity. After 3 weeks, films were developed in Kodak D-19. Ligand binding was quantitated with computer-assisted densitometry using the MCID system (Imaging Research Inc., St. Catherine's, Ontario). To quantify ligand binding density, the optical density of coexposed standards was determined and a standard curve generated by fitting standard optical density values to standard radioactivity values with a fourth-degree polynomial regression equation. Standards were commercial ^{14}C plastic standards (ARC, St. Louis, MO) calibrated against previously described ^{3}H-brain paste standards constructed to give a known amount of radioactivity per milligram of protein.[30] Use of the standards and derived standard curve allowed conversion of areal optical density to pmol/mg protein values. Areas measured were the cortical laminae V-VI. Six to ten readings from three animals were averaged, and IC_{50} values for the dithianes were calculated by log-logit analysis. Calculation of K_i values was performed using a K_D for [^{3}H]-TBOB of 29.1 ± 3.8 nM.[17]

***In Vivo* Distribution Studies in Mouse Brain.** Female CD-1 mice (20–25 g, Charles River Laboratories, Wilmington, MA) were anesthetized with diethyl ether, and 10–15 μCi of the radiolabeled compound (**[^{18}F]7**, **[^{18}F]10**, or **[^{125}I]11**, formulated as a solutions in 10% aqueous alcohol) was injected *via* the tail vein. At 5, 10, 30, and 60 min, groups of animals (n = 4) were sacrificed by decapitation, and the brain was rapidly removed and dissected into samples of striatum, cortex, hippocampus, cerebellum, pons-medulla, and the remainder of brain. Tissue samples were weighed and counted in an automatic γ-counter. The amounts of radioactivity in each brain region was calculated as % injected dose/g of tissue. Regions of high and low [^{3}H]TBOB binding *in vitro* were compared using paired Student's t tests with $p < 0.05$ being considered significant.

Acknowledgment. The authors would like to thank Dr. John E. Casida for his advice and the kind gift of 5-*tert*-butyl-2-(4-ethynylphenyl)-1,3-dithiane. This work was supported by grants from the National Institutes of Health (NS 15655, NS 19613, AG 08671, and T-32-CA09015 (to S.E.S.)) and the Department of Energy (DE-FG021-87ER60561).

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JM940866D