Photoactivatable 2-(4'-Azidotetrafluorophenyl)-5-*tert*-butyl-1,3-dithiane-Bis-sulfone and Related Compounds as Candidate Irreversible Probes for the GABA-Gated Chloride Channels

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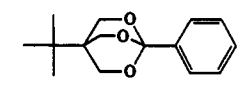
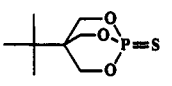
Syntheses of 2-substituted photoactivatable derivatives of 5-*tert*-butyl-1,3-dithiane and their oxidized bis-sulfone derivatives are described with the aim of developing original photoaffinity probes for the GABA-gated chloride channel. Diazocyclohexadienone as well as fluorinated arylazido derivatives were chosen as photosensitive moieties. The dithiane molecules were synthesized by condensation of 2-*tert*-butylpropane-1,3-dithiol with the appropriate substituted benzaldehydes. The diazocyclohexadienonyl derivatives were synthesized either by diazotization of the corresponding dithiane arylamine precursors (compounds 1, 2, 17, and 18) or by diazotization of the bis-sulfone dithiane arylamine (compounds 19 and 20). The reversible binding properties of the photosensitive probes were established on bovine cortex P_2 membranes by displacement of [^{3}H]-*tert*-butylbicycloorthobenzoate. While the bromo-substituted diazocyclohexadienoyl dithiane derivatives (compounds 1 and 2) exhibited a K_i of about 2-4 μM 2-(4'-azidotetrafluorophenyl)-5-*tert*-butyl-1,3-dithiane-bis-sulfone (compound 3) gave a K_i of 0.2 μM. On irradiation, probe 3 produced a 25% irreversible loss of TBOB binding sites in brain membranes. Moreover, this loss was fully protectable by TBOB, demonstrating the specificity of the photochemical inactivation by compound 3 for the convulsant site of the $GABA_A$ receptor.

The γ-aminobutyric acid$_A$ ($GABA_A$) receptor is a member of the ligand-gated ion-channel-receptor superfamily.[1] This receptor is a heterooligomeric complex on which several binding sites have been defined, including GABA, benzodiazepines, barbiturates, convulsants, and anesthetic steroids.[2] Picrotoxin and related convulsants (*tert*-butylbicycloorthobenzoate, TBOB, and *tert*-butylbicyclophosphorothionate, TBPS) block GABAergic neurotransmission by binding to a chloride-channel-associated site and allosterically preventing the GABA-mediated increase in Cl^- permeability.[3] The convulsant binding site can potentially be characterized using irreversible site-directed radiolabeled probes for which two series derived from the TBOB molecule have been described. The isothiocyanate-TBOB derivatives were used as affinity probes[4] while the azido-TBOB derivatives and trioxabicyclooctanes (TBOs) containing the diazocyclohexadienone group were proposed as photoaffinity probes.[5]

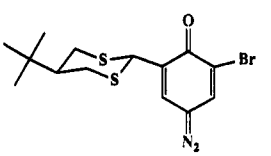
The search for efficient probes with the ability to label the entire proteic environment of a binding site including nonfunctionalized side chains of amino acid residues prompted us to develop photoaffinity probes to characterize the convulsant site of the $GABA_A$ receptor complex. Dithiane-derived molecules have been described recently to interact similarly to TBOB at the convulsant site and in some cases with equal or higher potency.[6] There are two advantages of this series over the TBOB-derived probes: the increased chemical stability under acidic conditions and the possibility of introducing radioactivity at the 2-position using carbanion chemistry. Perfluorated azidophenyl derivatives represent a new development in photoaffinity labeling technology.[7,8] These probes have been described as giving reactive singlet nitrenes, leading in particular to insertion products with aliphatic and aromatic hydrocarbons.[9] The present study describes the syntheses and the biochemical and photochemical properties not only of 2-diazocyclohexadienone but also of 2-azidoperfluoroaryl derivatives of 5-*tert*-butyldithiane and the corresponding disulfones.



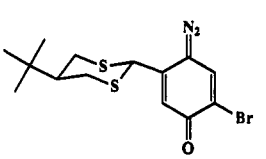
TBOB



TBPS



1



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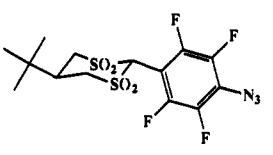

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Chemistry

The syntheses (Scheme I) of the dithiane derivatives were achieved by condensing the appropriate aromatic aldehyde with the readily available 2-*tert*-butylpropane-1,3-dithiol[7] in the presence of BF_3-Et_2O (Table I). The reaction times were adapted to have predominant formation of the thermodynamic trans isomer. The necessary aldehydes were either commercially available or obtained

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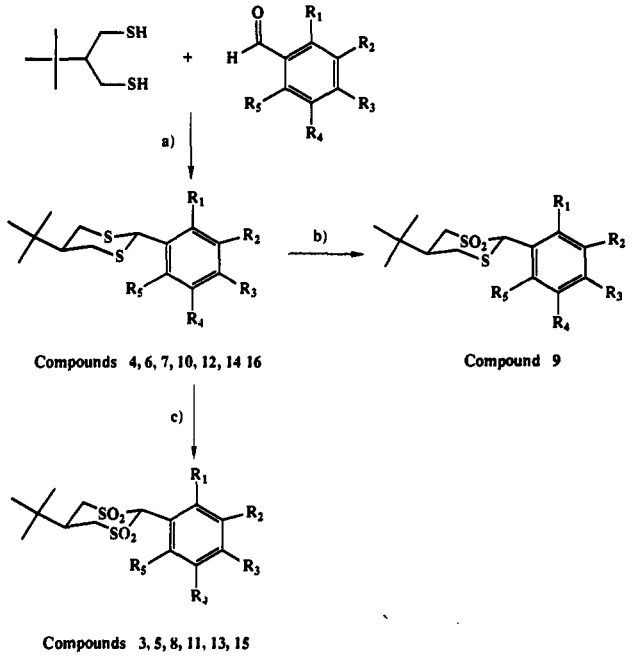
Scheme I. Syntheses of the Analogs of 2-Phenyl-5-*tert*-butyl-1,3-dithiane and 2-Phenyl-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane[a]



[a] Reaction conditions: (a) BF_3–Et_2O, CH_2Cl_2, room temperature; (b) (1) MCPBA, CH_2Cl_2, –78–0 °C, (2) $KMnO_4$, $MgSO_4$, acetone, 0 °C; (c) oxone, MeOH/H_2O or acetone/H_2O, reflux.

by bromination of the corresponding nitrophenol derivatives[11] (precursors for compounds 10 and 14, Table I). 4-Azidotetrafluorobenzaldehyde was prepared by aromatic substitution of pentafluorobenzaldehyde with NaN_3.[7] Dithianes 4, 7, 12, and 16 were subsequently oxidized to the corresponding disulfones 5, 8, 13, and 3 by using Oxone[12] as the oxidant. The brominated disulfone derivatives 11 and 15 were more easily synthesized by bromination of disulfones 8 and 13. Finally, the monosulfone derivative 9 was obtained by successive oxidations with MCPBA and $KMnO_4$[13] of dithiane 7. Table I summarizes the synthetic results in these series. Syntheses of the diazocyclohexadienonyl probes 1, 2, 17, 18, 19, and 20 were achieved in a two-step procedure from their corresponding nitrophenol derivatives as shown in Scheme II. Clearly, according to the satisfactory yields, the dithiane series is much less acid sensitive than the corresponding ortho esters, i.e., the diazotization reaction can be performed using isoamyl nitrite in the presence of acetic acid. The intermediate 4-aminophenol derivatives, which were obtained by different reduction procedures for the aromatic nitro group, were not characterized and were directly diazotized. $NaBH_4$ with Pd/C[14] was preferred over catalytic hydrogenation for the reduction of the brominated nitrophenol derivatives 10 and 14, while the reduction of disulfone nitrophenol 8 required the use of ammonium formate as the reducing agent.[15]

The UV characteristics and stabilities in pH 7.4 buffer of three newly synthesized photoactivatable probes (compounds 1, 2, and 3) are shown in Table II (these probes were selected for their moderate to high affinity in receptor binding studies). The diazo species show, as expected, a stronger absorption at higher wavelength ($\lambda_{max} \geq 360$ nm) and also a complete disappearance of the higher absorption peak upon irradiation, while the photodecomposition of the azido compound 3 is more complex. The three compounds are of comparable stability, which is sufficient to be used in photoaffinity labeling experiments.

Results

Reversible Binding of the Synthesized Molecules. Table III summarizes the binding potencies of the dithiane-, disulfone-, and TBO-derived molecules which have been synthesized and determined by displacement of [^{3}H]TBOB in the absence of light using bovine brain P_2 membranes. Clearly, among the different chemicals which have been synthesized, the bicyclic ortho esters are more potent than the dithianes which in turn are more potent than the corresponding disulfones in displacing [^{3}H]TBOB. Notably, the 4-azidotetrafluorophenyl disulfone derivative 3 is the most potent photoaffinity probe described in this article (K_i = 0.18 μM). Unfortunately, the corresponding dithiane derivative, which might have resulted in a higher affinity derivative, is unstable in buffer. As already observed in the bicyclic ortho ester series, the diazocyclohexadienonyl derivatives do not show high affinity for the TBOB binding site. However, the brominated diazocyclohexadienonyl derivatives 1 and 2 show acceptable potencies (K_i = 2.2 and 3.6 μM, respectively).

Irreversible Binding at the TBOB Binding Site. Among the synthesized photoactivatable probes, the most potent compounds in displacing [^{3}H]TBOB, molecules 1, 2, and 3, were tested for their ability to irreversibly inactivate the TBOB binding site upon irradiation. Figures 1, 2, and 3 show the results obtained during these photoinactivation experiments, including the necessary controls. The appropriate irradiation conditions must be established, including the time course, the energy, and the wavelength of irradiation. This was particularly important for the azido derivative since this chemical absorbs below 300 nm and therefore requires wavelengths of photoactivation which interfere with the absorption spectra of protein. We used a 261-nm irradiation wavelength for the azido derivative 3, which corresponds to the λ_{max} of this chemical. We adapted the energy of irradiation at that wavelength (not shown) so that the TBOB binding properties would be minimally altered. An optimal time of 15 min was determined (not shown) which corresponded to a compromise between receptor stability and receptor inactivation in the absence and in the presence of probe 3, respectively. Concerning the experiments with the diazo derivative 1, the irradiation conditions were more easily established; we used again a wavelength close to the λ_{max} of the chemical (363 nm), and the chosen irradiation time and energy corresponded to the time course of the disappearance of this chemical in a separate photodecomposition experiment (not shown).

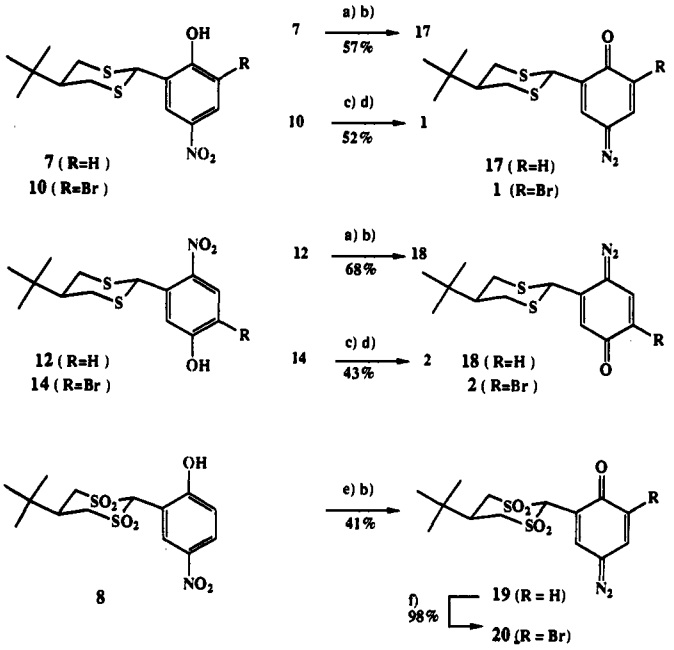
Figures 1, 2, and 3 show the photoaffinity labeling experiments of the TBOB binding site on the $GABA_A$ receptor using probes 1, 2, and 3. Each set of experiments determined the remaining [^{3}H]TBOB binding under different conditions, as indicated in the figure legends. The extent of photoinactivation is determined by comparison of the residual [^{3}H]TBOB binding sites with and without irradiation. Selectivity for the convulsant site is determined using labeled TBOB to protect against ligand-induced photoinactivation. All of the binding sites could not be recovered following incubation of the membranes with probe 1 (20 μM) and particularly probe 2 (30 μM) in the dark (Figures 1B and 2B). Irradiation resulted in 10% and 14% additionnal irreversible inhibition for probes 1

Table I. Analytical Data (yield, melting point, and analysis) for 2-(Substituted phenyl)-5-*tert*-butyl-1,3-dithianes and Their S-Oxidation Products (mono- and disulfone derivatives)

					dithiane				disulfone				monosulfone			
R_1	R_2	R_3	R_4	R_5	compd	yield, %	mp, °C	anal.	compd	yield, %	mp, °C	anal.	compd	yield, %	mp, °C	anal.
OH	H	H	H	H	4	74	149	C, H	5	83	>300	C, H				
OH	H	H	Br	H	6	75	168	C, H								
OH	H	H	NO_2	H	7	83	182	C, H, N	8	98	>300	C, H, N	9	71	278	C, H, N
OH	Br	H	NO_2	H	10	45	163	C, H, N	11	87[a]	>300					
NO_2	H	H	OH	H	12	72	208	C, H, N	13	94	>300	C, H, N				
NO_2	H	Br	OH	H	14	36	223		15	98[a]	>300	C, H				
F	F	N_3	F	F	16	50			3	58						

[a] Bromination after oxidation (Br_2, AcOEt, 0 °C, $HNEt_2$).

Scheme II. Syntheses of the Diazocyclohexadienonyl Probes in a Two-Step Procedure from Their Corresponding Nitrophenol Derivatives[a]



[a] Reaction conditions: (a) H_2 2 atm, Pd/C, AcOEt; (b) isoamyl nitrite, CH_3COOH, room temperature; (c) $NaBH_4$, Pd/C, THF/NH_4Cl; (d) isoamyl nitrite, AcOEt/CH_3COOH, 4/6, 0 °C; (e) $HOOCNH_4$, Pd/C, MeOH; (f) Br_2, $HNEt_2$, 5 °C.

Table II. UV Characteristics (λ_{max} and ϵ), Stability in pH 7.4 Buffer (half-life time), and Photodecomposition (half-life time) for the Three Photoactivatable Probes Tested (compounds 1, 2, and 3)[a]

	compound		
	1	2	3
λ_{max}, nm	362	365	261
ϵ, L mol^{-1} cm^{-1}	26 500	26 000	11 000
stability			
T, °C	37	37	25
$t_{1/2}$, h	5.5	3.3	8.8
photodecomposition			
E, μV	50	50	120
λ, nm	363	363	261
$t_{1/2}$, min	4.2	7.0	7.0

[a] All these experiments were done in phosphate buffer (200 mM NaCl, 10 mM $NaH_2PO_4 \cdot H_2O$) at pH 7.4.

and 2, respectively (Figures 1B* and 2B*). However, this inactivation was not diminished in the presence of high concentrations of unlabeled TBOB (Figures 1C, 1C*, 2C, and 2C*), indicating that the irreversible inhibition was nonselective. In contrast, probe 3 was completely reversible without irradiation (Figure 3B) and upon irradiation photoinactivated 25% of the [^{3}H]TBOB binding sites (Figure 3B*). This photoinactivation was completely protectable by 4 μM TBOB (Figure 3C*). An additional control experiment using the prephotolyzed probe 3 did not result in irreversible inhibition (data not shown), indicating that the probe-3-induced inactivation was not due to nondissociating photoproducts.

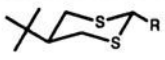
Discussion

Among the different neurotransmitter receptors which have been characterized through cloning and sequencing techniques, the $GABA_A$ receptor offers probably the most complex picture in terms of receptor heterogeneity.[1,16] Receptor heterogeneity produced by the combination of different subunits generates the coexistence of receptor populations in the central nervous system which presumably possess structurally diverse ligand binding sites. This represents for the chemist a new challenge in the design and synthesis of ligands which would be selective for a defined receptor subtype, i.e., by using a recombinant receptor expressed in a heterologous cell line.[17] The identification of the different residues constituting the binding site, through irreversible labeling experiments,[18] offers new possibilities for the study of ligand–receptor interactions, particularly for heterogeneous receptor populations.

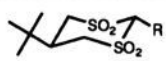
Eight candidate photoaffinity probes containing the dithiane central spacer unit were synthesized for the convulsant site of the $GABA_A$ receptor. The dithianes offer over the corresponding bicyclic ortho ester series the advantage of being more easily synthesized, this being mainly due to a better chemical stability particularly under acidic conditions. Table III shows the structure of all the ligands newly synthesized as well as their binding potency in displacing [^{3}H]TBOB from its binding site. Clearly, the dithiane series is less potent than the corresponding bicyclic ortho esters. Concerning the disulfone derivatives, although these compounds show in general a lower affinity for this site when compared to the dithianes, the results are sometimes surprising, i.e., the 2-(4′-bromophenyl)-dithiane–bis-sulfone derivative (Table III) has been described as being extremely potent in displacing TBPS (K_i = 3 nM[6a]). Also of interest is the good potency of the photoactivatable bis-sulfone probe 3 (K_i = 0.18 μM). The perfluorated azidophenyl derivatives represent a new development in the photoaffinity labeling technolog.[7,8] These probes have been described as giving reactive singlet nitrenes, leading in particular to insertion products with aliphatic and aromatic hydrocarbons.[9]

Three candidate photoaffinity ligands were tested as irreversible probes for the [^{3}H]TBOB binding site of the $GABA_A$ receptor from bovine brain. These molecules are derived from the 5-*tert*-butyldithiane series and incorporate 2-(bromodiazocyclohexadienonyl) moietes (molcules 1 and 2) while compound 3 is derived from the 5-*tert*-

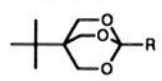
Table III. Affinities of Ligands for the Convulsant Site of the $GABA_A$ Receptor[a]



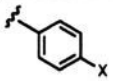
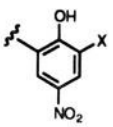
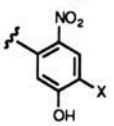
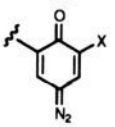
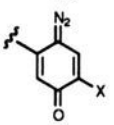
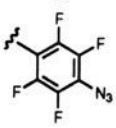
A



B



C

	structure A				structure B				structure C	
R	compd	X = H	compd	X = Br	compd	X = H	compd	X = Br	X = H	X = Br
		0.25[b]		0.28[b]		0.51[b]		0.003[b]	0.018[c]	0.009[d]
	**4**	1.8 ± 0.3			**5**	23.9 ± 0.1			0.059 ± 0.002	
	**7**	0.56 ± 0.03	**10**	0.22 ± 0.02	**8**	18 ± 1	**11**	0.53 ± 0.01		
	**12**	8 ± 4	**14**	4.5 ± 0.2	**13**	52 ± 6	**15**	>30		
	**17**	14 ± 2	**1**	2.2 ± 1.0	**19**	250*	**20**	>30*		
	**18**	30 ± 6	**2**	3.6 ± 0.1					5.9[c]	0.6[c]
	**16**	unstable			**3**	0.18 ± 0.02				

[a] The inhibition constants (K_is) were determined in bovine cortical P_2 membranes using [^{3}H]TBOB as described in the Experimental Section unless otherwise noted. Values in micromolar are means ± SEM from two to three independent experiments calculated by $K_i = IC_{50}/(1 + L/K_D)$, where IC_{50} is the concentration producing 50% inhibition of specific binding, L is the radioligand concentration, and K_D is the radioligand dissociation constant. The asterisk denotes values taken from a single experiment. [b] Reference 6a (IC_{50}/[^{35}S]TBPS). [c] Reference 5b (IC_{50}/[^{3}H]TBOB. [d] Reference 21 (IC_{50}/[^{35}S]TBPS).

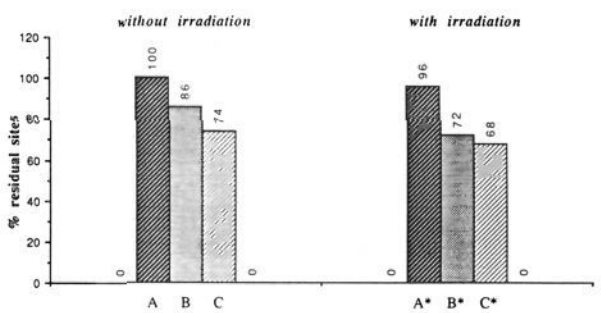


Figure 1. Photoaffinity labeling experiment using probe **1**. Membranes and drugs, at the indicated concentrations, were incubated for 1 h at 25 °C and subsequently irradiated (with irradiation) or not irradiated (without irradiation) for 15 min at 15 °C at 363 nm with a light intensity of 50 μV. Thereafter, the filter dissociation procedure and the [^{3}H]TBOB binding assay proceeded as described in the Experimental Section: (A) membranes, (B) membranes + **1** (20 μM or 9 Ki), and (C) membranes + **1** (20 μM or 9 Ki) + TBOB (4 μM or 200 Ki).

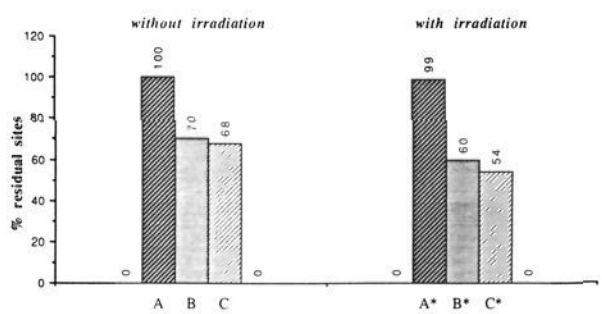


Figure 2. Photoaffinity labeling experiment using probe **2**. Conditions were the same as stated in Figure 1: (A) membranes, (B) membranes + **2** (30 μM or 8 Ki), and (C) membranes + **2** (30 μM or 8 Ki) + TBOB (4 μM or 200 Ki).

butyldithiane–bis-sulfone series and incorporates a 2-(4′-azidotetrafluorophenyl) group. The diazo species **1** and **2** do not induce specific photoirreversible receptor inactivation. Although compound **2** gave a substantial loss of [^{3}H]TBOB binding capacity (about 40% using 30 μM **2**), a comparable inactivation was also observed in the absence of light, and more important, neither inactivation could be protected by an excess of unlabled TBOB or other ligands (not shown) acting at this site. A nonprotectable affinity labeling is a possible explanation for these experimental results; however, this possibility was not pursued. On the other hand, the fluorophenyl)azido probe

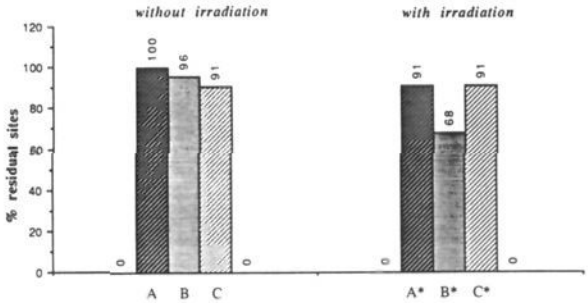


Figure 3. Photoaffinity labeling experiment using probe **3**. Conditions were the same as stated in Figure 1 except that the sample was irradiated for 20 min at 15 °C at 261 nm with a light intensity of 120 μV: (A) membranes, (B) membranes + **3** (6 μM or 33 Ki), and (C) membranes + **3** (6 μM or 33 Ki) + TBOB (4 μM or 200 Ki).

3 gave promising results. The photolytic conditions used resulted in less than 10% loss of receptor binding capacity, and probe **3** could be entirely dissociated in the absence of light under identical conditions. However, in the presence of light, probe **3** photoinactivated 25% of the [^{3}H]TBOB binding sites, and this inactivation was totally protectable by unlabeled TBOB (4 μM). Taken together, these results demonstrate that the (fluorophenyl)azido probe **3** selectively photoirreversibly inhibits [^{3}H]TBOB binding, indicating covalent modification of the convulsant site of the $GABA_A$ receptor.

A direct comparison with the previously described photoaffinity probes can be made at that point. The diazocyclohexadienonyl probes in either the TBO-derived series[5] or the dithiane series (present study) resulted in ligands having fairly low affinity for the TBOB binding site, K_is ranging from 0.6 μM for BrN_2 dienone[5] to 30 μM for compound **18**. On the other hand, although these probes have interesting photochemical properties (appropriate irradiation wavelengths and formation of highly reactive species), they have not given a high level of selective photoinactivation. In contrast, the arylazido probes, which have been described, possess higher affinity: K_is range from 180 nM for compound **3** to 82 nM[19] for the 4-azido-TBO derivative.[5] Concerning the irreversible properties, compound **3** gave the promising results, leading to appreciable receptor photoinactivation in a reproducible fashion and specific for the TBOB binding site. We did not attempt to increase the percent of irreversible labeling through successive addition–irradiation steps. In addition, a major synthetic advantage of probe **3** over the TBO–azido molecule is due to its stability under acidic conditions. This property opens the possibility of introducing the azido moiety through diazotation–azidation procedures on an amine precursor and thus allows the use of titriation procedures for the synthesis of a radiolabeled probe.

Experimental Section

Materials and Methods. Chemistry. Melting points were obtained on Reichert microscope or on a Mettler FP62 capillary melting-point apparatus and are uncorrected. ^{1}H and ^{13}C NMR experiments were recorded on Bruker Model WP SY200 (200-MHz and 50-MHz) spectrometers and ^{19}F NMR on a Bruker Model AM400 (376.5-MHz) spectrometer with proton decoupling. Chemical shifts are reported in δ (ppm), and appropriate solvent resonance spectra were consistently used as internal references ($CDCl_3$, 7.27 in proton and 77.0 in carbon NMR, and acetone-d_6, 2.05 in proton and 29.8 in carbon NMR). Infrared (IR) spectra were recorded as KBr disks or in solution ($CHCl_3$) on a Perkin-Elmer 1600 series Fourier transform IR spectrophotometer with the bands reported as cm^{-1}. Mass spectra (MS) were obtained using an electron-impact system (LKB 9000S, direct introduction) or on a fast atom bombardment system (ZAB HF system, VG analytical Manchester FAB system). UV spectra were recorded on a Kontron Uvikon 860 spectrophotometer. The compounds were analyzed for C, H, and N by the service de Microanalyse du CNRS de l'Université Louis Pasteur (Strasbourg), and the results are within 0.4% of the theoretical value. Thin-layer chromatography (TLC) was performed with Merck 5715 (F254, 0.25-mm silica gel) glass TLC plates. Spots were visualized under UV light or under visible light by spraying the TLC plate with a ninhydrin spray solution. Column chromatography utilized silica gel Merck 9385 (particle size 40–63 μm). The solvents used were dried and purified by standard methods.[20]

The analytical data (reaction yields, melting points, and analyses) of the precursors of the photoactivatable probes are found in Table I.

Dithianes 4, 6, 7, 10, 12, 14, and 16 (Table I). 2-*tert*-Butylpropane-1,3-dithiol was prepared from diethyl *tert*-butylmalonate.[7] The necessary aldehydes were either commercially available (dithianes **4**, **6**, **7**, and **12**) or synthesized (dithianes **10** and **14**) by bromination of the corresponding 4-nitrophenol derivatives using standard procedures,[8] while 4-azido-2,3,5,6-tetrafluorobenzaldehyde was synthesized as described.[9]

2-*tert*-Butylpropane-1,3-dithiol (1 equiv) was added to a solution of aldehyde (1.1 equiv) in dry CH_2Cl_2 containing boron trifluoride etherate (0.2 equiv). The solution was stirred at room temperature for several hours (2–12 h, reaction followed by TLC). After evaporation of the solvent, the reaction mixture was purified by silica gel column chromatography. The predominant trans isomers **4**, **6**, **7**, **10**, **12**, **14**, and **16** were eluted with either ethyl acetate/hexane or CH_2Cl_2/hexane mixtures. The crystalline derivatives were recrystallized from CH_2Cl_2/hexane.

2-(2′-Hydroxyphenyl)-5-*tert*-butyl-1,3-dithiane (4): IR (KBr) 3366, 2952, 2912, 1595, 1456, 1279, 1226, 1183, 756; ^{1}H NMR δ 7.30–7.26 (m, 1H, H, aromatic), 7.21 (dd, 1 H, H aromatic), 6.92–6.88 (m, 2H, H aromatic), 6.36 (s, 1H, OH), 5.36 (s, 1H, H_2), 3.00 and 2.85 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 13.8 Hz, $^3J_{ax-eq}$ = 2.7 Hz, $^3J_{ax-ax}$ = 10.8 Hz), 1.78 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.8 Hz, $^3J_{ax-ax}$ = 10.8 Hz), 0.98 (s, 9H, *t*-Bu).

2-(2′-Hydroxy-5′-bromophenyl)-5-*tert*-butyl-1,3-dithiane (6): IR (KBr) 3405, 2963, 1493, 1411, 1284, 1103, 816; ^{1}H NMR δ 7.4 (d, 1H, $H_{6'}$, ^4J = 2.4 Hz), 7.31 (dd, 1H, $H_{4'}$, ^4J = 2.4 Hz, ^3J = 8.6 Hz), 6.78 (d, 1H, $H_{3'}$, ^3J = 8.6 Hz), 6.43 (s, large, 1H, OH), 5.28 (s, 1H, H_2), 2.99 and 2.83 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 13.9 Hz, $^3J_{ax-eq}$ ≈ 2.6 Hz, $^3J_{ax-ax}$ = 11.0 Hz), 1.76 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.6 Hz, $^3J_{ax-ax}$ = 10.9 Hz), 0.97 (s, 9H, *t*-Bu).

2-(2′-Hydroxy-5′-nitrophenyl)-5-*tert*-butyl-1,3-dithiane (7): IR (KBr) 3487, 2957, 1590, 1524, 1490, 1342, 1282; ^{1}H NMR δ 8.24 (d, 1H, $H_{6'}$, ^4J = 2.7 Hz), 8.14 (dd, 1H, $H_{4'}$, ^4J = 2.8 Hz, ^3J = 9.0 Hz), 7.40 (s, large, 1 H, OH), 6.98 (d, 1H, $H_{3'}$, ^3J = 9.0 Hz), 5.39 (s, 1H, H_2), 3.02 and 2.87 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2JH = 14.0 Hz, $^3J_{ax-eq}$ = 2.6 Hz, $^3J_{ax-ax}$ = 11.0 Hz), 1.78 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.6 Hz, $^3J_{ax-ax}$ = 11.0 Hz), 0.99 (s, 9H, *t*-Bu); ^{13}C NMR δ 160.5 ($C_{2'}$), 141.1 ($C_{5'}$), 126.0 and 125.8 ($C_{4'}$ and $C_{6'}$), 123.5 ($C_{1'}$), 117.8 ($C_{3'}$), 46.5 (C_2), 45.8 (C_5), 34.0 (CCH_3), 32.9 (C_4, C_6), 27.2 (CH_3).

2-(2′-Hydroxy-3′-bromo-5′-nitrophenyl)-5-*tert*-butyl-1,3-dithiane (10): IR (KBr) 3405, 2968, 2910, 2880, 1606, 1578, 1520, 1462, 1426, 1337; ^{1}H NMR δ 8.40–8.38 (m, 2H, H aromatic), 6.86 (s, 1H, OH), 5.55 (s, 1H, H_2), 3.10 and 2.95 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 14.0 Hz, $^3J_{ax-eq}$ = 2.8 Hz, $^3J_{ax-ax}$ = 10.8 Hz), 1.78 (AA′BB′*X*, 1H, H_5, $^3J_{cis}$ ≈ 2.9 Hz, $^3J_{trans}$ = 10.8 Hz), 0.9 (s, 9H, *t*-Bu); ^{13}C NMR δ 154.6 ($C_{2'}$), 140.9 ($C_{5'}$), 127.4 ($C_{4'}$), 125.3 ($C_{1'}$), 124.2 ($C_{6'}$), 110.2 ($C_{3'}$), 45.4 and 44.0 (C_2 and C_5), 33.4 (CCH_3), 32.7 (C_4 and C_6), 26.7 (CH_3).

2-(2′-Nitro-5′-hydroxyphenyl)-5-*tert*-butyl-1,3-dithiane (12): IR (KBr) 3218, 2960, 1593, 1518, 1435, 1366; ^{1}H NMR δ 7.97 (d, 1H, $H_{3'}$, ^3J = 8.9 Hz), 7.31 (d, 1H, $H_{6'}$, ^4J = 2.8 Hz), 6.86 (dd, 1H, $H_{4'}$, ^4J = 2.8 Hz, ^3J = 9.0 Hz), 6.01 (s, 1H, H_2), 3.00 and 2.91 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 14.0 Hz, $^3J_{ax-eq}$ = 3.7 Hz, $^3J_{ax-ax}$ = 10.3 Hz), 1.76 (AA′BB′*X*, 1H, $^3J_{ax-eq}$ = 3.3 Hz, $^3J_{ax-ax}$ = 10.3 Hz), 0.98 (s, 9H, *t*-Bu); ^{13}C NMR δ 161.6

($C_{5'}$), 139.7 ($C_{2'}$), 135.9 ($C_{1'}$), 127.8 ($C_{3'}$), 116.8 and 115.5 ($C_{4'}$ and $C_{6'}$), 46.2 and 46.1 (C_2 and C_5), 33.7 (CCH_3), 33.6 (C_4 and C_6), 27.1 (CH_3).

2-(2′-Nitro-4′-bromo-5′-hydroxyphenyl)-5-*tert*-butyl-1,3-dithiane (14): IR (KBr) 3220, 2952, 1578, 1520, 1395, 1323, 1265, 1202; ^{1}H NMR δ 8.22 (s, 1H, $H_{3'}$), 7.54 (s, 1H, $H_{6'}$), 5.96 (s, 1H, H_2), 3.05 and 2.90 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 14.0 Hz, $^3J_{ax-eq}$ = 5.5 Hz, $^3J_{ax-ax}$ = 10.5 Hz), 1.78 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 5.5 Hz, $^3J_{ax-ax}$ = 10.4 Hz), 0.97 (s, 9H, *t*-Bu); ^{13}C NMR δ 156.4 ($C_{5'}$), 140.5 ($C_{2'}$), 135.2 ($C_{1'}$), 129.2 ($C_{3'}$), 116.9 ($C_{6'}$), 108.9 ($C_{4'}$), 45.7 and 45.1 (C_2 and C_5), 33.4 (CCH_3), 33.2 (C_4 and C_6), 26.7 (CH_3).

2-(4′-Azidotetrafluorophenyl)-5-*tert*-butyl-1,3-dithiane (16): IR ($CHCl_3$) 2966, 2124 (N_3), 1479, 1410, 1369, 1276, 1228, 1094, 907, 733; UV (phosphate pH 7.4 buffer) λ_{max} = 263 nm, ϵ = 13 000, (methanol) λ_{max} = 255 nm, ϵ = 20 000; ^{1}H NMR δ 5.52 (t, 1H, H_2, $^4J_{H-F}$ = 2.1 Hz), 3.01 and 2.88 (*AA′BB′*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 14.0 Hz, $^3J_{ax-eq}$ = 2.8 Hz, $^3J_{ax-ax}$ = 10.8 Hz), 1.84 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.8 Hz, $^3J_{ax-ax}$ = 10.8 Hz), 0.99 (s, 9H, *t*-Bu); ^{13}C NMR δ 46.1 (C_2), 42.8 (C_5), 34.2 (C_4 and C_6), 34.1 (CCH_3), 27.2 (CH_3); ^{19}F NMR (proton decoupldng) δ −139.95 (m, 2F, $F_{2'}$ and $F_{6'}$), −151.55 (dd, 2F, F_3 and F_5, $^3J_{F-F}$ = 21.0 Hz, $^5J_{F-F}$ = 9.5 Hz); MS-FAB$^+$ (2-nitrobenzylic alcohol, *m/z*) 731 (MH^+ + M), 703 (MH^+ + (M − N_2)), 675 (MH^+ − N_2 + (M − N_2)), *366* (MH^+), 365 (M^+), 337 (M − N_2), 175 (M − diazocyclohexadienone).

2-(2′-Hydroxy-5′-nitrophenyl)-5-*tert*-butyl-1,1-dioxo-1,3-dithiane (9). Monosulfone **9** was synthesized according to a described synthetic route.[6,11] MCPBA (70 mg, 0.29 mmol) was added slowly with stirring to a cooled (−78 °C) solution of **7** (80 mg, 0.26 mmol) in dry CH_2Cl_2 (10 mL). The temperature was rasied to −10 °C over a period of 3 h, and the solvent was removed and the residue taken up in ethyl acetate (100 mL). The MCPBA was removed by washing with a solution of Na_2CO_3, 1 N (3 × 20 mL). The organic layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated to yield the crude product (white solid, 65 mg, 77% as a mixture of dithiane derivatives having SO equatorial and SO axial). This crude mixture was added to $MgSO_4$ (60 mg) in acetone (5 mL). The reaction mixture was stirred vigorously, and $KMnO_4$ (21 mg, 0.13 mmol) was added in small portions. The reaction mixture was stirred for 4 h at 0 °C. Acetone was evaporated and the residue taken up in ethyl acetate (50 mL). The organic layer was washed successively with a saturated solution of $Na_2S_2O_5$, water, and a saturated solution of NaCl and finally dried over $MgSO_4$. The solvent was removed, and the crude product was purified by silica gel column chromatography (ethyl acetate/hexane, 1/1−1/0, v/v) to yield **9** (white crystals, 60 mg): IR (KBr), 3288, 2963, 1593, 1560, 1523, 1339, 1299, 1115; ^{1}H NMR (acetone-d_6) 8.57 (d, 1H, $H_{6'}$; ^4J = 2.8 Hz), 8.18 (dd, 1H, $H_{4'}$, ^3J = 9.1 Hz, ^4J = 2.9 Hz), 7.16 (d, 1H, $H_{3'}$, ^3J = 9.1 Hz), 5.99 (s, 1H, H_2), 3.47−3.22 (m, 3H, H_4 axial and H_6), 3.00 (ddd, 1H, H_4 equatorial in α of S, ^2J = 14.7 Hz, $^3J_{ax-eq}$ = 2.0 Hz, $^4J_{ax-ax}$ = 1.4 Hz), 2.5 (dddd, 1H, H_5, $^3J_{ax-ax}$ = 6.6 and 7.4 Hz, $^3J_{ax-eq}$ = 3.2 and 2.0 Hz), 1.04 (s, 9H, *t*-Bu).

Oxone Oxidation:[10] Compounds 5, 8, 13, and 3 (Table I). Oxone (10 equiv of potassium peroxymonosulfate) solubilized in water was added to a solution of dithiane (1 equiv) in an equal volume of methanol (for **5**, **8**, and **13**) or acetone (for **3**). This mixture was stirred first at room temperature for 12 h and then at reflux for 8 h, after which time the solvent was removed. The residue was partitioned between water and ethyl acetate. The organic layer was separated, washed with a saturated solution of NaCl, and dried over $MgSO_4$, and the solvent was removed. Purification by silica gel column chromatography gave the tetraoxide dithiane derivatives as white powders.

2-(2′-Hydroxy-5′-nitrophenyl)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (8): IR (KBr) 3411, 2930, 1594, 1533, 1502, 1351, 1310, 1141; ^{1}H NMR (acetone-d_6) δ 8.92 (d, 1H, $H_{6'}$, ^4J = 2.9 Hz), 8.29 (dd, 1H, $H_{4'}$, ^4J = 2.9 Hz, ^3J = 9.1 Hz), 7.22 (d, 1H, $H_{3'}$, ^3J = 9.1 Hz), 6.60 (s, 1H, H_2), 3.83 and 3.63 (*AA′BB′*X, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 14.4 Hz, $^3J_{ax-eq}$ = 2.3 Hz, $^3J_{ax-ax}$ = 12.0 Hz), 2.52 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.3 Hz, $^3J_{ax-ax}$ = 12.1 Hz), 1.13 (s, 9H, *t*-Bu).

2-(2′-Nitro-5′-hydroxyphenyl)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (13): IR (KBr) 3369, 2962, 1596, 1522, 1320, 1148, 1130; ^{1}H NMR (acetone-d_6) δ 8.17 (d, 1H, $H_{3'}$, ^3J = 9.1 Hz), 7.78 (d, 1H, $H_{6'}$, ^4J = 2.7 Hz), 7.20 (dd, 1H, $H_{4'}$, ^4J = 2.8 Hz, ^3J = 9.1 Hz), 7.07 (s, 1H, H_2), 3.93 and 3.67 (*AA′BB′*X, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 14.4 Hz, $^3J_{ax-eq}$ = 2.1 Hz, $^3J_{ax-ax}$ = 12.2 Hz), 2.48 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.2 Hz, $^3J_{ax-ax}$ = 12.2 Hz), 1.12 (s, 9H, *t*-Bu).

2-(4′-Azidotetrafluorophenyl)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (3): UV (phosphate pH 7.4 buffer) λ_{max} = 261 nm, ϵ = 11 000, (methanol) λ_{max} = 262 nm, ϵ = 23 000; IR (KBr) 3422, 2928, 2125 (N_3), 1654, 1507, 1485, 1352, 1322, 1142; ^{1}H NMR (acetone-d_6) δ 6.46 (t, 1H, H_2, small *J*), 3.83 and 3.71 (*AA′BB′*H, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 14.3 Hz, $^3J_{ax-eq}$ = 3.0 Hz, $^3J_{ax-ax}$ = 11.5 Hz), 2.55 (AA′BB′*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.8 Hz, $^3J_{ax-ax}$ = 11.3 Hz), 1 .12 (s, 9H, *t*-Bu); ^{13}C NMR (acetone-d_6) δ 75.3 (C_2), 55.4 (C_4 and C_6), 40.9 (C_5), 34.2 (CCH_3), 27.3 (CH_3); ^{19}F NMR (proton decoupling) δ −127.54 (ddd, 1F, $F_{2'}$ or $F_{6'}$, $^3J_{F-F}$ = 20.0 Hz, $^5J_{F-F}$ = 9.3 Hz, $^4J_{F-F}$ = 8.5 Hz), −139.85 (ddd, 1F, $F_{2'}$ or $F_{6'}$, $^3J_{F-F}$ = 20.4 Hz), $^5J_{F-F}$ = 8.7 Hz, $^4J_{F-F}$ = 8.0 Hz, m −150.76 (dd, 1F, $F_{3'}$ or $F_{5'}$, $^3J_{F-F}$ = 19.9 Hz, $^5J_{F-F}$ = 8.7 Hz), −151.63 (dd, 1F, $F_{3'}$ or $F_{5'}$, $^3J_{F-F}$ = 20.7 Hz, $^5J_{F-F}$ = 9.3 Hz); MS-FAB$^+$ (nitrobenzylic alcohol, *m/z*) 583 (MH^+ + nitrobenzylic alcohol), 537 (MH^+ + benzylic alcohol), 430 (MH^+); exact mass FAB$^+$ (polyethyleneglycol 400) for (M + H)$^+$ calculated 430.0518, found 430.0506.

2-(2′-Hydroxy-3′-bromo-5′-nitrophenyl)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (11). To an ice cold solution of **8** (150 mg, 0.4 mmol) solubilized in ethyl acetate (10 mL) was added bromine (0.48 mmol) and diethylamine (0.48 mmol). The reaction mixture was stirred at 0 °C for 2 h. The mixture was evaporated was the residue partitioned between water and ethyl acetate. The organic layer was washed with a diluted solution of $Na_2S_2O_5$, dried over $MgSO_4$, and evaporated. Recrystallization in ethyl acetate gave **11** (159 mg, yellow crystals): ^{1}H NMR (acetone-d_6) δ 8.79 (d, 1H, H aromatic, ^4J = 2.9 Hz), 8.40 (d, 1H, H aromatic, ^4J = 3 Hz), 7.08 (s, 1H, H_2), 3.57 and 3.45 (*AA′BB′*X, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 13.5 Hz, $^3J_{ax-eq}$ = 2.4 Hz, $^3J_{ax-ax}$ = 11.8 Hz), 2.42 (AA′BB′*X*, 1H H_5, $^3J_{ax-eq}$ = 2.4 Hz, $^3J_{ax-ax}$ = 12.0 Hz), 0.95 (s, 9H, *t*-Bu).

2-(2′-Nitro-4′-bromo-5′-hydroxyphenyl)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (15). Reaction of **13** (150 mg, 0.4 mmol) with bromine to afford **15** (180 mg, yellow powder, 98%) used the procedure described for the synthesis of **11**. **15**: IR (KBr) 3355, 2960, 1654, 1525, 1326, 1141; ^{1}H NMR (acetone-d_6) δ 8.45 (s, 1H, $H_{3'}$), 7.85 (s, 1H, $H_{6'}$), 7.00 (s, 1H, H_2), 3.93 and 3.70 (*AA′BB′*X, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 14.5 Hz, $^3J_{ax-eq}$ = 2.0 Hz, $^3J_{ax-ax}$ = 12.3 Hz), 2.46 (AA′BB′*X*, 1H, H_6, $^3J_{ax-eq}$ = 2.0 Hz, $^3J_{ax-ax}$ = 12.3 Hz), 1.11 (s, 9H, *t*-Bu).

Reduction and Diazotization Procedures. 2-(5′-Diazo-3′,6′-cyclohexadien-2′-one)-5-*tert*-butyl-1,3-dithiane (17). A solution of **7** (22 mg, 0.07 mmol) in methanol (5 mL) containing 5 mg of 10% Pd/C was hydrogenated for 30 min at 2 atm. The reaction mixture was filtered through a pad of Celite, and the Celite was washed with a further 10 mL of ethyl acetate. Evaporation of the solvents from the filtrate afforded a colorless powder (19 mg of amine, 96%). Diazotization of the aminophenol was done in the absence of light. To a stirred solution of amine (19 mg, 0.067 mmol) in glacial acetic acid (1 mL) at room temperature was added isoamyl nitrite (6 μL, 0.07 mmol). Ice cold ethyl acetate (50 mL) was added, and the organic layer was washed with a saturated solution of $NaHCO_3$ until the pH of the aqueous layer became neutral. The organic layer was dried over $MgSO_4$, and the solvent was evaporated at 10−15 °C. The crude product was purified by silica gel column chromatography. Elution with ethyl acetate/hexane (1/1−2/1, v/v) afforded **17** (11.7 mg, yellow powder, 59%): UV (phosphate pH 7.4 buffer) λ_{max} = 354 nm, ϵ = 26 500, (methoxy ethanol) λ_{max} = 361, nm ϵ = 24 500; ^{1}H NMR (acetone-d_6) δ 7.90 (d, 1H, $H_{6'}$, ^4J = 2.9 Hz), 7.70 (dd, 1H, $H_{4'}$, ^3J = 9.8 Hz, ^4J = 2.9 Hz), 6.26 (d, 1H, $H_{3'}$, ^3J = 9.8 Hz), 5.61 (s, 1H, H_2), 2.91 (d, 4H, H_4 and H_6, ^3J = 6.8 Hz), 1.65 (qt, 1H, H_5, ^3J = 6 .9 Hz), 0.96 (s, 9H, *t*-Bu); MS-FAB$^+$ (2-nitrobenzylic alcohol, *m/z*) 295 (MH^+), 267 (MH^+ − N_2), 175 (M^+ − diazocyclohexadienone).

2-(2′-Diazo-3′,6′-cyclohexadien-5′-one)-5-*tert*-butyl-1,3-dithiane (18). Reduction of 4-nitrophenol **12** (20 mg, 0.064 mmol) and diazotization of the corresponding aminophenol used the procedures described for the synthesis of **17** and afforded **18** (yellow powder, 10.4 mg, 55%): UV (phosphate pH 7.4 buffer) λ_{max} = 356 nm, ϵ = 27 000; ^{1}H NMR (acetone-d_6) δ 7.69 (d, 1H,

$H_{3'}$, ^3J = 9.6 Hz), 6.35 (d, 1H, $H_{6'}$, ^4J = 1.8 Hz), 6.19 (dd, 1H, $H_{4'}$, ^3J = 9.6 Hz, ^4J = 1.8 Hz), 5.41 (s, 1H, H_2), 3.02 (d, 4H, H_4 and H_6, ^3J = 6.9 Hz), 1.66 (qt, 1H, H_5, ^3J = 7.0 Hz), 0.96 (s, 9H, *t*-Bu); MS-FAB$^+$ (2-nitrobenzylic alcohol, *m/z*) 295 (MH^+), 267 ($MH^+ - N_2$), 175 (M^+ – diazocyclohexadienone).

2-(3'-Bromo-5'-diazo-3',6'-cyclohexadien-2'-one)-5-*tert*-butyl-1,3-dithiane (1). To a solution of 10 (65 mg, 0.16 mmol) in THF (3 mL) containing 15 mg of 10% Pd/C was added $NaBH_4$ (60 mg, 1.6 mmol). The mixture (green color) was stirred at room temperature for 2 h, and a saturated solution of NH_4Cl (2 mL) was added dropwise (at this moment, reduction was proceeding). The reaction mixture was filtered through a pad of Celite which was washed with a further 50 mL of ethyl acetate. The organic layer was separated, washed first with water and then with a saturated solution of NaCl, dried over $MgSO_4$, and evaporated. Purification by silica gel column chromatography (ethyl acetate/hexane, 2/8, v/v) afforded the aminophenol (47 mg, white powder, 81%). The aminophenol was diazotazed in ethyl acetate/glacial acetic acid (4/6, 1 mL) with isoamyl nitrite (110 μL, 1.3 mmol in 990 μL of ethyl acetate) by dropwise addition over a period of 30 min. Workup as described for 17 and purification by silica gel column chromatography (ethyl acetate/hexane, 2/8, v/v) afforded 1 (31 mg, brown solid, 64%): UV (phosphate pH 7.4 buffer) λ_{max} = 362 nm, ϵ = 26 500; ^{1}H NMR ($CDCl_3$) δ 7.81 (d, 1H, H aromatic ^4J = 2.9 Hz), 7.78 (d, 1H, H aromatic, ^4J = 2.9 Hz), 5.66 (s, 1H, H_2), 2.98–2.84 (m, 4H, H_4 and H_6), 1.76–1.66 (m, 1H, H_5), 0.96 (s, 9H, *t*-Bu); MS-FAB$^+$ (2-nitrobenzylic alc ohol, *m/z*) 375 [$(MBr^{81})H^+$], 373 [$(MBr^{79})H^+$], 347 [$(MBr^{81})H^+ - N_2$], 346 [$(MBr^{81}) - N_2$], 345 [$(MBr^{79})H^+ - N_2$], 344 [$(MBr^{79}) - N_2$), 175 (M^+ – diazocyclohexadienone), exact mass FAB$^+$ (polyethyleneglycol 400) for ($C_{14}Br^{79}H_{17}N_2OS_2$ + H) calculated 373.0044, found 373.0061.

2-(2'-Diazo-4'-bromo-3',6'-cyclohexadien-5'-one)-5-*tert*-butyl-1,3-dithiane (2). Reduction of 14 (37 mg, 0.094 mmol) to the aminophenol (37 mg, white powder, 69%) and diazotization as described for 1 afforded compound 2 (15.3 mg, brown powder, 63%): UV (phosphate pH 7.4 buffer) λ_{max} = 365 nm, ϵ = 26 000; ^{1}H NMR ($CDCl_3$) δ 7.82 (s, 1H, H aromatic), 6.75 (s, 1H, H aromatic), 5.08 (s, 1H, H_2), 3.00 and 2.82 (*AA'BB'*X, 4H, H_4, H_6 equatorial and H_4, H_6 axial, ^2J = 14.0 Hz, $^3J_{ax-eq}$ = 2.4 Hz, $^3J_{ax-ax}$ = 11.2 Hz), 1.67 (AA'BB'*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.4 Hz, $^3J_{ax-ax}$ = 11.2 Hz), 0.96 (s, 9H, *t*-Bu); MS-FAB$^+$ (2-nitrobenzylic alcohol, *m/z*) 375 [$(MBr^{81})H^+$], 373 [$(MBr^{79})H^+$], 347 [$(MBr^{81})H^+ - N_2$], 345 [$(MBr^{79})H^+ - N_2$], 175 (M^+ – diazocyclohexadienone).

2-(5'-Diazo-3',6'-cyclohexadien-2'-one)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (19). To a solution of 8 (100 mg, 0.26 mmol) containing 10 mg of 10% Pd/C in methanol (10 mL) was added NH_4CO_2H (160 mg, 2.6 mmol). The reaction was complete after 30 min at room temperature as determined by TLC (ethyl acetate/hexane, 2/1, v/v). The reaction mixture was filtered through a pad of Celite which was washed with ethyl acetate (20 mL). Evaporation of the solvent from the filtrate and purification by silica gel column chromatography (ethyl acetate/hexane, 1/1–1/0, v/v) afforded the aminophenol (86 mg, white powder, 95%). Diazotization with isoamyl nitrite as described for 17 and purification by silica gel column chromatography (ethyl acetate/hexane 1/1–1/0, v/v) afforded 19 (yellow crystals, 38 mg, 43%): UV (phosphate pH 7.4 buffer) λ_{max} = 348 nm, ϵ = 22 000; ^{1}H NMR (acetone-d_6) δ 8.25 (d, 1H, $H_{6'}$, ^4J = 3.0 Hz), 7.82 (dd, 1H, $H_{4'}$, ^4J = 3.0 Hz, ^3J = 9.8 Hz), 6.56 (s, 1H, H_2), 6.35 (d, 1H, $H_{3'}$, ^3J = 9.8 Hz), 3.76 and 3.50 (*AA' BB'*X, 4H, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 14.0 Hz, $^3J_{ax-eq}$ = 2.1 Hz, $^3J_{ax-ax}$ = 12.7 Hz), 2.38 (AA'BB'*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.1 Hz, $^3J_{ax-ax}$ = 12.7 Hz), 1.11 (s, 9H, *t*-Bu).

2-(3'-Bromo-5'-diazo-3',6'-cyclohexadien-2'-one)-5-*tert*-butyl-1,1,3,3-tetraoxo-1,3-dithiane (20). To a stirred solution of 19 (13 mg, 0.036 mmol) in ethyl acetate/methanol (8/2, 3 mL) was added bromine (0.5 mL from a solution of 100 μL of Br_2, 0.1 mmol in 10 mL of ethyl acetate) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C, and $HNEt_2$ (4.2 μL, 0.04 mmol) was added. The crude mixture was directly chromatographed (ethyl acetate/hexane, 1/1) and gave 20 (15.5 mg, yellow powder, 98%): UV (phosphate pH 7.4 buffer) λ_{max} = 355 nm, ϵ = 18 000 (solubilized in methanol/DMSO, 1/1, before dilution in buffer); ^{1}H NMR (acetone-d_6) δ 8.37 (d, 1H, H aromatic, ^4J = 2.8 Hz), 8.33 (d, 1H, H aromatic, ^4J = 2.8 Hz), 6.62 (s, 1H, H_2), 3.80 and 3.54 (*AA'BB'*X, 4H, H_4, H_6 axial and H_4, H_6 equatorial, ^2J = 14.3 Hz, $^3J_{ax-eq}$ = 2.1 Hz, $^3J_{ax-ax}$ = 12.2 Hz), 2.38 (AA'BB'*X*, 1H, H_5, $^3J_{ax-eq}$ = 2.1 Hz, $^3J_{ax-ax}$ = 12.2 Hz), 0.96 (s, 9H, *t*-Bu); ^{13}C NMR δ 135.4 and 134.0 ($C_{4'}$ and $C_{6'}$), 73.3 (C_2), 54.3 (C_4 and C_6), 41.0 (C_5), 27.4 (CH_3).

Receptor Assays. Binding Assays. [^{3}H]TBOB (from Amersham, 19.4 or 30 Ci/mmol) was used to assay the potency of TBO, dithiane, and disulfone derivatives as inhibitors of the GABA-gated chloride channel in bovine brain cortical membranes in the absence of light (ref 5b). The receptor source was EDTA, water-dialyzed brain P_2 membranes in 200 mM NaCl–10 mM sodium phosphate (pH 7.4) assay buffer. Receptor assays involved the P_2 protein (1 mg) in 1 mL of assay buffer containing [^{3}H]TBOB (5 nM) alone or with unlabeled TBPS (2 μM) to correct for nonspecific binding (30% relative to the total binding). The suspensions were incubated for 1 h at 37 °C (or 25 °C for 3) to achieve equilibrium and then subjected to rapid filtration on Whatman GF/C filters and rinsed three times with ice cold assay buffer (3 mL); the bound radioactivity was determined by liquid scintillation counting. IC_{50} values were determined as previously described.[5b]

Photolabeling Experiments. Monochromatic light was obtained from a 1000-W xenon–mercury lamp (Hanovia) connected to a grating monochromator (Jobin-Yvon). The light intensity was measured (in volts) with a thermopile (Kipp and Zohnen) and adjusted through an iris diaphragm to the desired intensity. The light beam was focused through a quartz lens on a refrigerated assay cell to form a 10-mm high and 2-mm wide spot. Aliquots of membrane preparation (1 mg/mL) in 500 mM NaCl–10 mM sodium phosphate (pH 7.4) buffer were incubated for 1 h at 25 °C with the indicated concentrations of photosensitive substances with or without the addition of TBOB (4 μM). Thereafter, 4-mL aliquots of incubation mixture were irradiated at 15 °C, with gentle stirring for the indicated time (15 min for 1 and 2 and 20 min for 3). The diazo derivatives were irradiated at 363 nm, near their maximal absorption wavelengths, for compounds 1 and 2 and the azido derivative 3 at 261 nm at the maximal absorption wavelength. Irradiated membrane suspensions (3 mL) were diluted 5,5-fold with 10 mM sodium phosphate (pH 7.4) buffer and incubated for 20 min at 37 °C. Samples in triplicate (3 × 5 mL) were then adsorbed under partial vacuum on GF/B (Whatman) glass-fiber filters and given nine successive rinses (3 mL of 10 mM phosphate pH 7.4 buffer) to remove the unbound ligands. This dissociation procedure was followed by the [^{3}H]TBOB filter binding assay, performed according to reference 5b.

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