119190-26-0; **29**, 119169-91-4; **30**, 119169-92-5; **31**, 119169-81-2; **32**, 119169-75-4; **33**, 119169-76-5; **34**, 119169-74-3; **35**, 124688-65-9; **36**, 124688-66-0; **37**, 124688-67-1; **38**, 124688-68-2; **39**, 124688-69-3; **40**, 124688-70-6; **41**, 50-22-6; **42**, 3597-46-4; **43**, 124688-71-7; **44**, 124688-72-8; **45**, 124688-73-9; **48**, 124688-74-0; **49**, 124688-75-1; **50**, 119190-45-3; **51**, 119170-37-5; **52**, 119170-38-6; **53**, 119170-39-7; **54**, 119190-37-3; **55**, 119169-94-7; **56**, 119169-79-8; **57**, 119169-78-7; **58**, 119170-24-0; **59**, 119169-65-2; **60**, 124688-76-2; **61**, 124688-77-3; **62**, 119170-25-1; **63**, 119170-08-0; **64**, 119170-09-1; **65**, 119190-42-0; **66**, 124688-78-4; **67**, 124688-79-5; **68**, 124688-80-8; **69**, 124688-81-9; **70**, 119170-28-4; **71**, 119170-29-5; **72**, 119170-30-8; **73**, 124688-82-0; **74**, 124688-83-1; **75**, 124688-84-2; **76**, 119169-93-6; **77**, 124688-85-3; **78**, 124688-86-4; **79**, 124688-87-5; **80**, 124688-88-6; **81**, 124688-89-7; **82**, 124688-90-0; **83**, 124688-91-1; **84**, 124688-92-2; **90**, 124716-19-4; **91**, 124716-20-7; **92**, 124716-21-8; **93**, 124716-22-9; **94**, 124688-93-3; **95**, 124688-94-4; **96**, 124688-95-5; **97**, 124688-96-6; **98**, 124688-97-7; **99**, 124688-98-8; **100**, 124688-99-9; **101**, 124689-00-5; **102**, 124689-01-6; *i*-Pr_2NH, 108-18-9; *N*-phenyltriflimide, 37595-74-7; 1,3-dithienium fluoroborate, 39915-66-7; tris(methylthio)methane, 5418-86-0; ethylene glycol, 107-21-1; steroid 5α-reductase, 72412-84-1.

Supplementary Material Available: Selected physical and analytical data (C, H, N, partial NMR, and MS) for compounds described in this paper (19 pages). Ordering information is given on any current masthead page.

Novel [(Diazomethyl)carbonyl]-1,2,3,4-tetrahydronaphthalene Derivatives as Potential Photoaffinity Ligands for the 5-HT_{1A} Receptor

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The photolabile (diazomethyl)carbonyl function was introduced into the 8-position of 2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene in three ways, resulting in the ether 8-[[(diazomethyl)carbonyl]methoxy]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**2**), the ester 8-(diazoacetoxy)-2-(N,N-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**3**), and the ketone 8-[(diazomethyl)carbonyl]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**4**). Specific binding of these compounds at the 5-hydroxytryptamine$_{1A}$ sites in rat brain membranes labeled with 1 nM [^{3}H]-8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT) showed IC_{50} values of ca. 75, 125, and 25 nM, respectively, for the three compounds. Photolysis of methanolic solutions of **2**–**4** in the absence of receptor proteins lead in each case to an abundance of Wolff-rearranged products. In the case of ether **2**, subsequent β-elimination to 8-OH-DPAT removed this compound from serious consideration as a photoaffinity ligand. Ester **3** and ketone **4** were photolysed in vitro. Whereas ester **3** was ineffective in decreasing the specific binding of [^{3}H]-8-OH-DPAT, ketone **4** decreased 40% of the specific binding of [^{3}H]-8-OH-DPAT in the presence (but not the absence) of ultraviolet light. Thus this ketone emerges from these studies as a good candidate for a photoaffinity label for the 5-hydroxytryptamine$_{1A}$ receptor.

Among the many proteins that respond to 5-hydroxytryptamine (5-HT, serotonin), the 5-HT_{1A} receptor is distinguished by both ligand-binding and functional characteristics. The selectivity and nanomolar affinity of the agonist [^{3}H]-8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-OH-DPAT, **1**) for this receptor has made this the ligand of choice to label the receptor.[1] Unlabeled 8-OH-DPAT has been used to assign a number of in vivo physiological functions to the 5-HT_{1A} receptor, including some aspects of the "serotonin behavioral syndrome", anxiety, depression, feeding behavior, cardiovascular effects, and thermoregulation.[2] The cellular responses to 5-HT_{1A} agonists, opening of a Ca^{2+}-insensitive K^+ channel[3] and regulation of adenylyl cyclase,[4] are mediated via coupling to signal-transducing G-proteins, although exactly which G-protein(s) remains to be determined. It is also problematical whether agonist-occupied 5-HT_{1A} receptors in vivo can both stimulate[5,6] and inhibit the forskolin-stimulated[4,7] adenylyl cyclase as is observed in vitro. Receptor signal transduction mechanisms have in the last decade been addressed most successfully in experiments using the purified receptors, G-proteins, and effectors. More recently, chimeric receptors have been constructed and used to delineate ligand binding from signal-transducing domains.[8]

The direct characterization of the receptor protein(s) represents an approach to receptor taxonomy and function that is complementary to classical pharmacological methods using high-affinity reversible ligands. Technical advances in molecular biology and protein chemistry have made available both the DNA clone for the 5-HT_{1A} receptor and its expression in cell cultures.[9] Thus, the 5-HT_{1A} receptor can be expected to join the growing number of transmembrane proteins whose topology and function may be extensively mapped. A reliable, selective photoaffinity probe will be one of the tools required for these investigations, and we are developing novel reagent ligands for this purpose.

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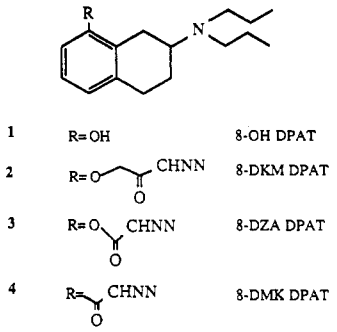
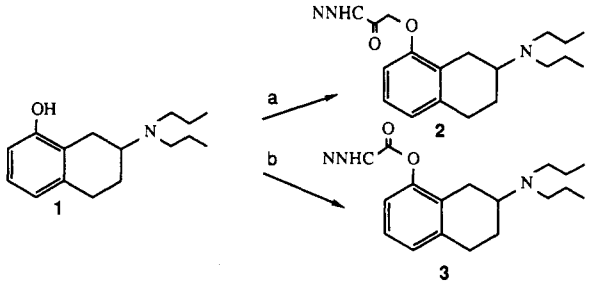
Chart I



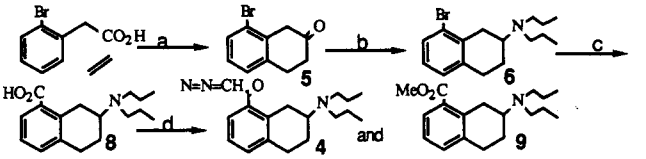
In the design of our compounds, we sought to maximize both receptor affinity/selectivity properties and the chemical reactivity of the photolabile function. Because of its excellent qualities as a 5-HT_{1A} ligand, 1 was selected as the structural basis for our modified compounds. In addition, the phenol function offered an immediate synthetic entry into two of the proposed compounds, and synthetic precursors to 1 appeared to be suitable models for analogous precursors to the third compound (vide infra). Although two other research groups are investigating the photoaffinity labeling of the 5-HT_{1A} receptor using aryl azide reagents,[10,11] several lines of reasoning lead us to select the (diazomethyl)carbonyl rather than the aryl azide function as the photolabile group. Although (diazomethyl)carbonyl derivatives may suffer the drawbacks of instability at low pH, propensity for Wolff rearrangement to a less reactive, more chemoselective ketene, (occasional) extreme photosensitivity, and the requirement for photolysis at wavelengths that are lower—and thus potentially more damaging to proteins—than those necessary to photolyze aryl azides, there are compensating advantages to this functional group. It is smaller than the aryl azide, and thus less likely to perturb receptor topography upon interaction or insertion at the ligand binding site. The increased reactivity of the carbene (generated from (diazomethyl)carbonyls) over the nitrene (generated from aryl azides) makes the former particularly useful in affinity labeling applications: Direct insertion of (unrearranged) carbene is not only up to 3 orders of magnitude faster but is also less chemoselective and therefore more readily inserts into lipophilic as well as hydrophilic binding regions.[12,13] (Diazomethyl)carbonyl compounds have been used successfully to label a variety of functional proteins, although the majority of the work on membrane-bound neurotransmitter receptors has been done with compounds that carry the aryl azide function.[12-14] It was therefore of great interest to see if our results based on the use of the carbene-generating ligands would complement those obtained from groups[10,11] using the nitrene-generating aryl azide compounds to photoaffinity label the 5-HT_{1A} receptor. We now report the synthesis of three (diazomethyl)carbonyl compounds (Chart I) as potential photoaffinity labels for the 5-HT_{1A} receptor and the 5-HT_{1A} binding affinities of these ligands under reversible and photolytically induced irreversible conditions.

Scheme I[a]



[a] (a) $NNCHC(O)CH_2Br/K_2CO_3$/MeOH–THF/room temperature for 72 h. (b) (i) *n*-BuLi/THF; (ii) *p*-TsNNCHCOCl/CH_2Cl_2; (iii) NEt_3.

Scheme II[a]



[a] (a) PCl_5, $AlCl_3$, CH_2Cl_2, −78 °C to room temperature; (b) dipropylamine, refluxing benzene, then H_2, PtO_2; (c) *n*-BuLi, THF, CO_2; (d) (i) $ClCOCOCl/CH_2Cl_2$, 0 °C, (ii) CH_2N_2, Et_2O.

Results

Chemistry. Since ease of tritium labeling was a synthetic goal, we took advantage of the existence of commercially available [^{3}H]-8-OH-DPAT and used routes to obtain the ether 8-[[(diazomethyl)carbonyl]methoxy]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-DKM-DPAT, **2**) and the ester 8-(diazoacetoxy)-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-DZA-DPAT, **3**), each in a single step from **1**. As shown in Scheme I, direct alkylation of **1** with bromodiazoacetone[15] in the presence of 3 equiv of K_2CO_3 gave **2**. Generation of the phenoxide anion of **1** with 2.5 equiv of *n*-butyllithium and addition of this anion to glyoxal chloride *p*-tolylsulfonyl)hydrazone,[16] followed by two successive treatments of the reaction mixture with 4 equiv each of triethylamine, gave **3**.

For 8-[(diazomethyl)carbonyl]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-DMK-DPAT, **4**) a Burkhalter–Campbell condensation[17] was used to prepare 1,2,3,4-tetrahydronaphthalen-2-one **5** (Scheme II). Reductive amination of **5** with *N*,*N*-di-*n*-propylamine required an initial acid-catalyzed dehydration to the intermediate iminium salt. The crude iminum salt was then hydrogenated over Adams catalyst to the 2-(*N*,*N*-dipropylamino)-1,2,3,4-tetrahydronaphthalene derivative **6**. The conversion of **6** to amino acid **8** was accomplished by halogen–metal exchange, followed by carbonylation of the aryllithium with CO_2 gas. Acid **8** was converted via the dicyclohexyamine salt to the acid chloride and allowed to react with excess diazomethane to yield **4**, accompanied by ester **9**. The ester and the diazomethyl ketone were separated by flash chromatography.

Photolytic Behavior in Methanol in the Absence of Membranes. The fate of the (diazomethyl)carbonyl

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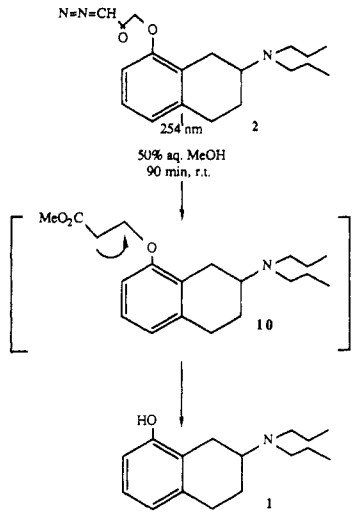
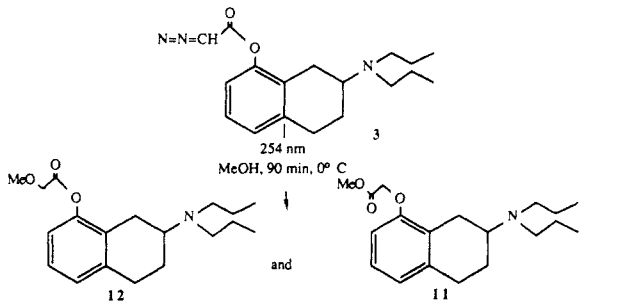
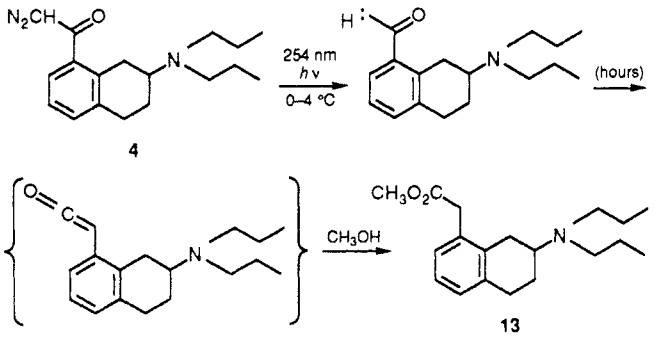
Scheme III



Scheme IV



Scheme V



groups of compounds **2–4** upon photolysis in the presence of methanol as a model nucleophile was examined. The ether **2** gave exclusively 8-OH-DPAT, probably arising as a β-elimination product of the ester **10** that results from methanolysis of the intermediate ketene derived from Wolff rearrangement[15] (Scheme III). Under similar conditions compound **3** gave the ketene-derived ester **11** (40%) and the direct insertion product **12** (60%) (Scheme IV). Ketone **4** gave exclusively Wolff-rearrangement ester **13** as the major isolated product (Scheme V).

Binding to [^{3}H]-8-OH-DPAT Sites in Rat Brain. A. Under Reversible Conditions. Compounds **2–4** as well as the photostable analogue of **4**, ester **9**, were evaluated in competition binding using [^{3}H]-8-OH DPAT to label binding sites on membranes from the 5-HT_{1A} receptor-rich regions of rat hippocampus and/or cortex. The data shown in Figure 1 demonstrate competition by compounds **2** and **3** for a single population of [^{3}H]-8-OH-DPAT sites in both brain regions. Receptor affinity, however, is in the 70–150 nM range for both compounds. In contrast, ketone **4** has an IC_{50} value of 20 nM in rat hippocampus membranes,

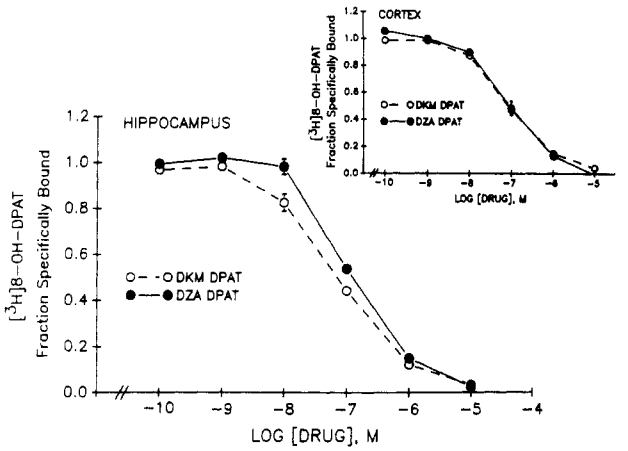


Figure 1. Competition of 8-DKM-DPAT (O) and 8-DZA-DPAT (●) for sites labeled by 1 nM [^{3}H]-8-OH-DPAT in rat hippocampus and cortex (inset) membranes. The IC_{50} values for 8-DKM-DPAT in hippocampus and cortex respectively are 78.1 ± 12.9 and 72.0 ± 17.2 nM; for 8-DZA-DPAT, 155 ± 42 and 107 ± 24 nM. All procedures were conducted under partially subdued laboratory lighting. Details of the binding assay are found in ref 21. Each point represents the mean ± SE of three separate experiments performed in triplicate. Nonspecific binding is defined by 10 μM 5-HT.

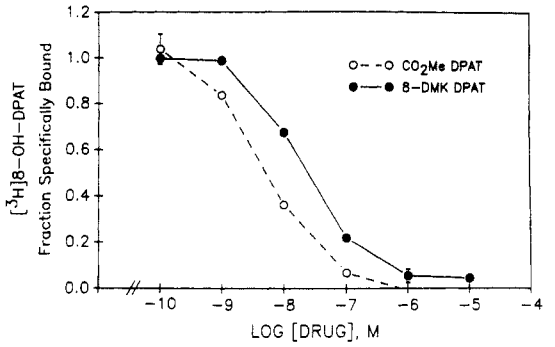


Figure 2. Competition of 8-DMK-DPAT (●) and 8-CO_2Me-DPAT (O) for sites labeled by 1 nM [^{3}H-8-OH-DPAT in rat cortex membranes. The IC_{50} value for 8-DMK-DPAT is 24 ± 2.2 nM; for 8-CO_2Me, 5.4 ± 0.4 nM. The procedure is identical with that described in the legend to Figure 1.

as shown in Figure 2. The ester 8-(carbomethoxy)-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**9**) has an IC_{50} value of 5 nM (Figure 2).

B. Photolytically Induced Irreversible Binding. Since ether **2** demonstrated both low affinity and little useful photolytic behavior, no further investigation of this compound was undertaken. For ester **3** and ketone **4**, incubation of each compound at saturating concentrations (10 μM) with membranes at 4 °C (as described in the Experimental Section) was followed by a 2-min exposure to ultraviolet light from a Hanovia source at a distance of 20 cm. The membranes were then freed from unbound compound by centrifugation and washing and subjected to [^{3}H]-8-OH-DPAT binding. Figure 3 (for ester **3**) and 4 (for ketone **4**) show specific binding under four sets of conditions: with and without the photolabile compound, and in the presence and absence of ultraviolet light. A low degree of photolytic inactivation was observed for the ester **3**, which is consistent with the poor affinity (ca. 100 nM) of ester **3** for the 5-HT_{1A} site in hippocampus membranes. Two successive treatments of the membranes with ester **3** in the presence of ultraviolet light did not appreciably improve the photoinactivation. In contrast, ketone **4**, with only a single incubation/photolysis treatment, decreased

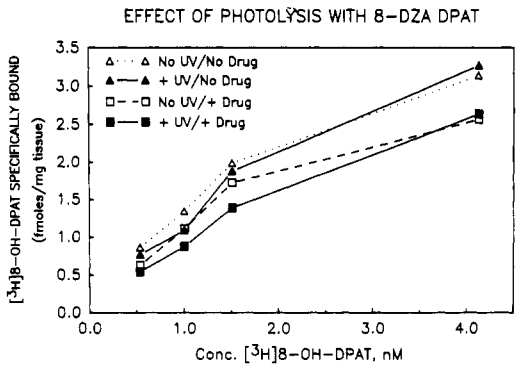


Figure 3. Effect on 5-HT_{1A} binding sites of exposing membranes to 8-DZA-DPAT under photolyzing conditions. Rat cerebral cortical membranes (prepared as described in the Experimental Section) were divided into four fractions and exposed to the following conditions: ultraviolet light (UV) in the presence of 8-DZA-DPAT, UV in the absence of 8-DZA-DPAT, 8-DZA-DPAT with no exposure to UV, or no exposure to either UV or 8-DZA-DPAT (control). Each tissue fraction was then washed and measured. Shown is a representative experiment with each point equal to the mean of triplicate determinations. This experiment was repeated two additional times. In two of the experiments, analysis of variance, followed by Newman-Keul's post hoc comparison, showed a statistically significant ($p < 0.05$) decrease in the binding of [^{3}H]-8-OH-DPAT to the membrane fraction exposed to both 8-DZA-DPAT and ultraviolet light when compared to the other three treatments. In one of the experiments no significant difference was found between the fraction exposed to both 8-DZA-DPAT and ultraviolet light and the fraction exposed to UV light in the absence of drug, while a significant difference did exist between the other two fractions and that exposed to both 8-DZA-DPAT and UV light. The average decrease in binding caused by the combination of 8-DZA-DPAT and UV light over the four [^{3}H]-8-OH-DPAT concentrations for the three experiments was 19.6% (range = 6–30%).

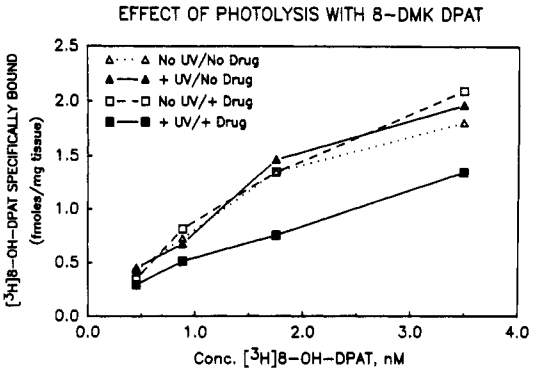


Figure 4. Effect on 5-HT_{1A} binding sites of exposing membranes to 8-DMK-DPAT under photolyzing conditions. Rat cerbral cortical membranes (prepared as described in the Experimental Section) were divided into four fractions and exposed to the following conditions: ultraviolet light (UV) in the presence of 8-DMK-DPAT, UV in the absence of 8-DMK-DPAT, 8-DMK-DPAT with no exposure to UV, or no exposure to either UV or 8-DMK-DPAT (control). Each tissue fraction was then washed, and the specific binding of [^{3}H]-8-OH-DPAT at four separate concentrations was measured. Shown are the four individual experiments with each point representing the mean of triplicate determinations. In each experiment, analysis of variance, followed by Newman-Keul's post hoc comparison, showed a statistically significant ($p < 0.05$) decrease in the binding of [^{3}H]-8-OH-DPAT to the membrane fraction exposed to both 8-DMK and UV light when compared to the other three treatments. The average decrease in binding caused by the combination of 8-DMK-DPAT and UV light over the four [^{3}H]-8-OH-DPAT concentrations for the four experiments was 38.2% (range = 28–56%).

the specific [^{3}H]-8-OH-DPAT binding by 40% (Figure 4).

Discussion

The photolabile (diazomethyl)carbonyl function has been introduced onto the 2-amino-1,2,3,4-tetrahydronaphthalene skeleton in three different ways, giving rise to ether **2**, ester **3**, and ketone **4**. In each case tritium incorporation is provided for in the reductive amination of the respective ketone intermediates; for **2** and **3** the starting material **1** is commercially available (via this reaction) in tritiated form.

The observed photochemical behavior of these compounds has provided a best candidate for tritium labeling. Since, upon photolysis in methanol, ether **2** undergoes extensive Wolff rearrangement followed by β-elimination to give 8-OH-DPAT, this compound was excluded as a potential photoaffinity ligand for the 5-HT_{1A} receptor. Similarly, ester **3**, pharmacologically handicapped by low affinity and chemically handicapped by nearly 50% Wolff rearrangement, displayed no useful photoinactivation properties in vitro. Ketone **4**, in contrast, provided rapid photoinactivation of 40% of the specific [^{3}H]-8-OH-DPAT binding sites in a single photolysis. This inactivation occurs despite the fact that only the ester **13** (from Wolff rearrangement) was isolated from a solution photolysis in the absence of protein. A caveat in evaluating the results from the methanolic photolysis experiments is that, while these experiments may be a useful adjunct to in vitro photolyses, the reaction conditions cannot reproduce fully the reaction conditions in the presence of receptor proteins. Methanol is a relatively weak nucleophile, and the reaction with the photoligands is, in methanol, completely intermolecular whereas in the binding experiments it is intramolecular. In order to isolate and identify the reaction products, high (ca. 10 mM) ligand concentrations were used in methanol, as contrasted with 10 μM concentrations in membranes. The times required to *complete* the reactions in methanol were far in excess of the times used to photolabel the receptor; during the latter one cannot assume that starting photoligand has been completely consumed. Thus the methanolic photolyses may give an overestimate of Wolff-rearranged product. This is particularly true for ketone **4**, which required several hours at 4 °C in methanol for complete disappearance, gave only rearrangement product **13**, and yet in membranes provided rapid and specific inactivation of the [^{3}H]-8-OH-DPAT binding. Thus ketone **4** emerges as a promising candidate for tritium incorporation and development as a photoaffinity label for the 5-HT_{1A} receptor.

The two other laboratories investigating the photoaffinity labeling of the 5-HT_{1A} receptor have appended a photolabile aryl azide group onto their respective choices of high-affinity ligands. Ransom et al. have photolyzed [^{3}H]-1-[(*p*-azidophenyl)ethyl]-4-[(trifluoromethyl)-phenyl]piperazine ([^{3}H]*p*-N_3PAPP) in bovine cortical membranes and obtained binding to a 55-kDa protein that shows characteristics of a 5-HT_{1A} binding protein, but have not been able to measure reduction in [^{3}H]-8-OH-DPAT binding commensurate with the presumed covalent attachment of their ligand. Emerit et al. have used 8-methoxy-2-[*N*-*n*-propyl-*N*-[3-[(*p*-azidophenyl)amino]-propyl]amino]-1,2,3,4-tetrahydronaphthalene to obtain 55–60% irreversible inhibition of [^{3}H]-8-OH-DPAT binding, but two or three successive photolytic treatments of the membranes with concentrations of 150 times the K_d value are required in order to achieve this blockade. Ketone **4** offers advantages over both of these compounds in that significant photoinduced reduction of specific [^{3}H]-8-OH-DPAT binding can be demonstrated after a single photolysis. Photolytic insertion of [^{3}H]-**4** will enable

us to compare the molecular weight of the labeled protein with those reported by Emerit et al. and Shih et al.

Since the consequences of modifications of the 1,2,3,4-tetrahydronaphthalene nucleus have not been explored extensively, it is of interest to determine the structure–activity relationships in functional receptor preparations and the binding discrimination for these compounds. These experiments are currently underway. Some preliminary conclusions can be drawn, however, regarding both affinities and photochemical behavior of these novel 1,2,3,4-tetrahydronaphthalenes. Experimental and computational lines of evidence support the idea that the volume of the C-8 substituent (with a maximum around 24 Å^3) rather than the electronic properties contributes to 5-HT$_{1A}$ receptor affinity.[18] Functionalization of the 8-phenolic group as either an ether (**2**) or ester (**3**) decreases the affinity of the substituted 1,2,3,4-tetrahydronaphthalene to a similar extent (12–20-fold) at 5-HT$_{1A}$ binding sites. Elimination of the oxygen at C-8 altogether provides a smaller group at this position and restores much of the receptor affinity for the modified ligand. The proposed inverse relationship of size to affinity is bolstered by the observation that the IC$_{50}$ of the ester 8-carbomethoxy-DPAT (**9**) for specific [^{3}H]-8-OH-DPAT sites is 5 nM, which is comparable to the affinity of the receptor for 8-OH-DPAT.

In summary, a probe has been developed that shows good potential as a photoaffinity label for the 5-HT$_{1A}$ receptor. The results of the ^{3}H-labeling and functional receptor studies will be reported elsewhere.

Experimental Section

Chemistry. All reactions were conducted under nitrogen or argon. Tetrahydrofuran (THF) was distilled from benzophenone ketyl immediately prior to use. Other reaction solvents and reagents were purified as described below or were the highest grade commercially available. Thin-layer chromatography (TLC) analyses of reactions were run on Polygram SilG/UV 254 plates and visualized under ultraviolet light, with (2,4-dinitrophenyl)-hydrazine, Ehrlich's reagent, and/or dichlorotolidine. Unless otherwise indicated, preparative chromatography was done on silica gel under flash conditions.[19] Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian FT 80-A instrument, in $CDCl_3$, unless otherwise indicated. Infrared spectra were recorded on a Beckman IR8 instrument. Ultraviolet spectra were recorded on a Beckman Model 34 instrument. Low-resolution mass spectra were obtained on a 5980-A Hewlett Packard mass spectrometer and high-resolution mass spectra on an MS-30 Kratos spectrometer, by Dr. Chalres Iden of the Department of Chemistry at State University of New York at Stony Brook. Elemental analyses were obtained from the Microanalytical Service at the Rockefeller University.

8-[[(Diazomethyl)carbonyl]methoxy]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (2). A solution of bromodiazoacetone[15] (235.6 mg, 1.58 mmol), K_2CO_3 (515 mg, 3.74 mmol), and 1[20] (296 mg, 1.2 mmol base) in a 5 mL of 4:1 THF/MeOH was allowed to stir at room temperature for 72 h in the dark. The reaction mixture was poured into water, the pH adjusted to 9, and the basic fraction extracted into ether. The organic phase was dried, and the solvent was removed in vacuo to give a dark oily residue. Chromatography (5:95 MeOH–$CHCl_3$ plus 5 drops of NH_4OH/100 mL) gave 160 mg of **2** as a yellow oil (50% of converted starting material) and 54 mg of recovered 1 that could be recycled. Anal. ($C_{19}H_{27}N_3O_2$) Calcd: C, 69.30; H, 8.21; N, 12.77. Found: C, 69.57; H, 8.45; N, 12.80. NMR: δ 6.49–7.17 (3 H, ArH), 5.78 (s, 1 H, CH=N=N), 4.54 (2 H, s, OCH_2CO), 2.70–3.32 (5 H, m, NCH over NCH_2), 2.40–2.70 (4 H, m, $ArCH_2$), 1.10–2.35 (6 H, m, CH_2CH_2), 0.90 (6 H, t, CH_3). IR (neat oil): 2120, 1670 cm^{-1}. UV (EtOH): λ_{max} 206 nm (ϵ = 16 500), 262 (ϵ = 10 200). MS: m^+/z 330 (MH^+), 329 (M), 300 (M – C_2H_5). HRMS: 329.2111.

8-(Diazoacetoxy)-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (3). To a 0 °C solution of **1** (241 mg, 0.98 mmol) in 8 mL of THF was added a hexane solution of *n*-butyllithium (1.53 mL of a 1.6 M solution, 2.45 mmol). The anion solution was stirred at 0 °C for 10 min and then at room temperature for 20 min, before being transferred under nitrogen to a 0 °C solution of glyoxal chloride (*p*-tolylsulfonyl)hydrazone (524 mg, 2.8 mmol) in 8 mL of methylene chloride. The reaction mixture was allowed to come to room temperature and stirred for 2.5 h before the first 4 equiv of triethylamine was added. The reaction mixture was then stirred 1 additional h, a second 4 equiv of triethylamine was added, and stirring was continued for 30 min before the reaction was quenched by the addition of excess cold water. The crude reaction mixture was partly concentrated in vacuo and then taken up in ethyl acetate. The crude basic fraction was extracted and purified by chromatography (2:98 MeOH/$CHCl_3$ plus 10 drops of NH_4OH/100 mL) to give 98.7 mg of **3** (57% of converted **1**) and 107.3 mg of **1**. Anal. $C_{18}H_{25}N_3O_2$ (C, H, N). NMR: δ 6.84–7.19 (3 H, ArH), 4.97 (1 H, s, CH=N=N), 2.68–3.12 (5 H, m, NCH over NCH_2), 2.19–2.59 (4 H, m, $ArCH_2$), 1.09–2.16, (6 H, m, CH_2CH_2), 0.88 (6 H, t, J = 7 Hz, CH_3). IR (neat): 2120, 1710 cm^{-1}. UV (EtOH): λ_{max} 249 nm (ϵ = 19 600), 210 (ϵ = 16 600). MS: m^+/z: 315 (M), 285 (M – C_2H_5). HRMS: 315.1963.

8-Bromo-1,2,3,4-tetrahydronaphthalen-2-one (5). To a 0 °C solution of (2-bromophenyl)acetic acid (3.1 g, 14.4 mmol) in 10 mL of methylene chloride was added, in small portions, solid PCl_5 (3.75 g, 18 mmol), and the reaction mixture was allowed to stir for 15 min at 0 °C and then 30 min at room temperature. The acid chloride was then transferred under nitrogen over the course of 1 h to a −78 °C suspension of $AlCl_3$ (3.83 g, 28.7 mmol) in 20 mL of methylene chloride. The reaction mixture was stirred 1 h at −78 °C and maintained at this temperature while ethylene was bubbled vigorously through the solution for 30 min. The cooling bath was removed, and the solution was stirred overnight before being poured into excess ice water. The methylene chloride phase was washed successively with 2 N HCl, $NaHCO_3$, and NaCl, then dried, and concentrated in vacuo to a residual oil. Chromatography (0.5:99.5 ethyl acetate/hexane followed by 1:99 ethyl acetate/hexane) gave 786 mg (24%) of **5** as a white solid. NMR: δ 7.13–7.62 (3 H, ArH), 3.74 (2 H, s, $ArCH_2CO$), 3.18 (2 H, t, J = 6.2, 7.0 Hz, $ArCH_2$), 2.65 (2 H, t, J = 7.1, 6.4 Hz, $COCH_2CH_2$). IR ($CHCl_3$): 1740 cm^{-1}. This material was used immediately in the subsequent reaction.

8-Bromo-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (6). A solution of **5** (402 mg, 1.79 mmol), *N*,*N*-dipropylamine (1404 mg, 14 mmol), and *p*-toluenesulfonic acid monohydrate (37 mg, 0.2 mmol) in 25 mL of freshly distilled benzene was refluxed with a Dean-Stark water separator for 72 h and then concentrated in vacuo. The residual oil was dissolved in 100 mL of absolute ethanol and shaken over 100 mg of PtO_2 under 55 psi H_2 in a Parr hydrogenation apparatus for 24 h. the catalyst was filtered off, the solvent evaporated in vacuo, and the basic fraction isolated to give crude **6** as a purple oil. Chromatography on neutral alumina (1:99 ethyl acetate/hexane) gave 185 mg (53%) of **6** as a colorless oil. NMR: δ 6.92–7.13 (3 H, ArH), 2.59–2.89 (5 H, m, NCH over NCH_2), 2.25–2.50 (4 H, m, $ArCH_2$), 1.1–2.0 (4 H, q, J = 7.6 Hz, CH_2CH_3), 0.89 (6 H, t, J = 6.9, 7.2 Hz, CH_3). IR (neat): 2980 cm^{-1} (C–H stretch). Anal. $C_{16}H_{24}BrN$ (C, H, N).

8-Carboxy-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8). To a −78 °C solution of **6** (185 mg, 0.71 mmol) in 20 mL of THF was added, dropwise, a hexane solution of *n*-butyllithium (2.6 mL of a 1.6 M solution, 4.2 mmol). The bright red-orange solution was allowed to stir at −78 °C for 1 h and then warmed to −10 °C. Carbon dioxide gas was bubbled through, immediately decolorizing the solution, and the bubbling was continued for 1 h; the reaction mixture was stirred 1 additional h after the CO_2 inlet was removed and then neutralized with 1 equiv of trifluoroacetic acid (81 mg, 55 μL, 0.71 mmol). The volatiles were removed in vacuo, the residue dissolved in water,

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and the pH adjusted to 6.0 by careful dropwise addition of trifluoroacetic acid. This solution was applied to a Dowex 50 W × 4 column (H^+ form), washed with 150 mL of water, and eluted in 1 N NH_4OH, to give 111 mg (57%) of acid **8**. NMR: δ 7.0–7.8 (3 H, m, ArH), 3.6–4.0 (5 H, m, NCH over NCH_2), 2.5–3.5 (4 H, m, $ArCH_2$), 1.25–2.5 (6 H, m, $ArCH_2CH_2$ over CH_2CH_3), 1.0 (6 H, t, CH_3). IR (neat): 3100–3500 (br), 3000, 1720, 1700 cm^{-1}. Anal. $C_{17}H_{25}NO_2$ (C, H, N).

8-[(Diazomethyl)carbonyl]-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (4). To a 0 °C solution of acid **8** (74.4 mg, 0.27 mmol) in 2 mL of methylene chloride was added dicyclohexylamine (59 μL, 0.29 mmol). The cooling bath was removed and the salt allowed to stir at room temperature for 2 h and then concentrated in vacuo. Ethanol was added and the solution was again concentrated in vacuo; this procedure was repeated once more to remove any water azeotropically before dissolving the residue in methylene chloride. The methylene chloride solution was cooled to 0 °C, and a solution of oxalyl chloride (351 μL, 4.2 mmol) in 1 mL of methylene chloride was added dropwise. The reaction mixture was stirred for 15 min at 0 °C and then 5 min at room temperature before removal of all volatiles in vacuo. Ice-cold chloroform was added and the solution was concentrated in vacuo; addition and removal of cold chloroform was repeated, and then the residue was dissolved in cold THF and filtered, with two rinses of cold THF, through a pad of $MgSO_4$/Celite. The yellow filtrate was added to a 0 °C ethereal solution of diazomethane (ca. 3.3 mmol, generated from 1 g of Diazald) and the solution allowed to come to room temperature overnight. Concentration in vacuo gave 97 mg of an oily residue that was chromatographically separated (1:99 MeOH/$CHCl_3$ plus 5 drops of NH_4OH/100 mL) into 20.7 mg (26%) of ester **9** and 26.7 mg (33%) of diazo ketone **4**.

4. NMR: δ 7.15 (3 H, ArH), 5.52 (1 H, s, CH=N=N), 2.7–3.0 (5 H, m, NCH over NCH_2), 2.3–2.6 (4 H, m, $ArCH_2$), 1–2.3 (6 H, m over q, $ArCH_2CH_2$ over CH_2CH_3), 0.87 (6 H, t, J = 6.3, 6.8 Hz, CH_3). IR (neat): 2210, 1790, 1630 cm^{-1}.

UV: (EtOH) λ_{max} 288 (ε = 9900), 259 (ε = 9200), 109 (ε = 12000). HRMS: $C_{18}H_{25}H_3O$.

9. NMR: δ 7.11–7.63 (3 H, ArH), 3.87 (3 H, s, CO_2CH_3), 3.0–3.5 (5 H, m, NCH over NCH_2), 2.7–3.1 (4 H, m, $ArCH_2$), 1.5–2.6 (6 H, m over q, $ArCH_2CH_2$ over CH_2CH_3), 0.87 (6 H, t, J = 6.9, 7.1 Hz, CH_3). IR: 1725 cm^{-1}. Anal. ($C_{18}H_{27}NO_2$) Calcd: C, 74.70; H, 9.30; N, 4.80. Found: C, 73.97; H, 9.28; N, 4.82.

Photolysis of Compounds 2–4 in Methanol. Irradiation of 254 nm was produced by a General Electric germicidal lamp in an enclosed box or by a suspended minerallamp, similarly enclosed. In each experiment, the methanolic solutions were stirred in an open beaker and cooled by placing either on an ice bath or in a temperature-controlled 4 °C cold room. The starting compound was maintained under identical conditions but wrapped in foil to exclude light.

Photolysis of 2 in Methanol. A solution of **2** (8.5 mg, 0.026 mmol) in 1 mL of 50% aqueous MeOH was stirred at 4 °C 4 cm from the ultraviolet light source for 1 h and monitored by TLC. Following concentration in vacuo, the residue was chromatographed on a pipet of silica gel, eluting first with 0.5% and then 3% MeOH/$CHCl_3$ plus 5 drops of NH_4OH/100 mL. A small amount of unchanged **2** was recovered, followed by 3 mg of a material as the major isolated product (Scheme IV), whose NMR spectrum and migration on TLC were identical with those of **1**.

Photolysis of 3 in Methanol. A solution of **3** (11 mg, 0.03 mmol) in 1 mL of MeOH was stirred at 4 °C 4 cm from the ultraviolet light source for 90 min. Periodic analysis by TLC showed that all **3** had disappeared by 1 h and that two new major spots, both more polar, had appeared. The mixture was concentrated in vacuo and then separated by chromatography on a pipet column of silica gel, using a stepwise gradient of 1–4% MeOH in $CHCl_3$ plus 5 drops of NH_4OH/100 mL. NMR was used to identify the products (Scheme III). The least polar spot (R_f = 0.29) corresponded to 2.8 mg (60%) of isolated 8-(methoxyacetoxy)-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**12**), while the more polar spot (R_f = 0.19) corresponded to an isolated 1.7 mg (40%) of 8-(carbomethoxymethoxy)-2-(*N*,*N*-di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (**11**). A third spot, of highest polarity, was observed on the analytical plate but was not isolated in sufficient quantities to characterize; this spot did not comigrate with **1**.

12: NMR (only diagnostic peaks are reported): δ 4.7 (2 H, s, OCH_2CO_2Me), 3.75 (3 H, s, CO_2CH_3).

11: NMR δ 4.30 (2 H, s, $MeOCH_2CO$), 3.5 (3 H, s, $COCH_3$).

Photolysis of 4 in Methanol. Ketone **4** (12 mg, 0.04 mmol) in 2 mL of 50% aqueous MeOH was subjected to the same photolytic reaction conditions described for compounds **2** and **3**. The reaction, monitored by TLC, began to show the appearance of a new, less polar product after 90 min; small amounts of a more polar product were also observed. Five hours were required for complete disappearance of starting material. The reaction mixture was concentrated on a rotarty evaporator, and the products were extracted into $CHCl_3$, dried over $MgSO_4$, and concentrated again in vacuo. The residue was purified on a small pipet of silica gel (2.5% MeOH in $CHCl_3$ plus 5 drops of NH_4OH/100 mL), and the major product, ester **13**, identified by NMR: δ 7.25–7.04 (m, ArH), 3.68 (s, CO_2CH_3), 3.63 ($ArCH_2CO_2CH_3$), 0.8–3.5 (aliphatic protons unchanged).

Binding to 5-HT_{1A} Sites Labeled by [^{3}H]-8-OH-DPAT. A. Reversible Binding. This was performed as described.[21] Rat brain membranes from the designated region were resuspended in Tris buffer for use in the binding assay at a final concentration of 10 mg of tissue (original wet weight)/mL and 1 nM [^{3}H]-8-OH-DPAT (New England Nuclear, Boston, MA, specific activity 30–40 Ci/mmol). Specific binding was defined as the difference between binding in the absence and presence of 10 μM 5-HT.

B. Photolytic Inactivation of 5-HT_{1A} Binding Sites. Rat hippocampal membranes were prepared to final tissue concentration of 10 mg/mL in 50 mM Tris·HCl, pH 7.4, and divided into four portions of 35 mL each: Group one (control, 35 mL of ethanol, no photolysis), group two (35 mL of ethanol, photolysis), group three (35 mL of an ethanolic solution of ligand, 10 μM, photolysis), group four (35 mL of an ethanolic solution of ligand, 10 μM, no photolysis). All four suspensions, prepared in argon-bubbled 4 °C buffer, were incubated in the dark for 10 min at 37 °C and then recooled to 4 °C. Suspensions one and four were maintained in the dark while two and three were exposed to ultraviolet irradiation from a Hanovia mercury vapor lamp (ACE 783534), fitted with a VICOR 7010 filtering jacket, at a distance of 15 cm for 2 min. All four suspensions were then centrifuged (48000*g*, 10 min) and resuspended in 35 mL of incubation buffer at 4 °C; this was repeated three times. Between the second and third washes the sample was incubated at 37 °C for 10 min to insure the removal of all unbound ligands. An aliquot (1 mL) was removed from each suspension and diluted with 2 mL of binding buffer (final concentration 50 mM Tris, 3 mM $CaCl_2$, 100 mM pargyline). Saturation binding of [^{3}H]-8-OH-DPAT was determined at concentrations of 0.5, 1.0, 2.0, 5.0, and 10 nM. Specific binding was defined as that which is eliminated by 10 μM 5-HT.

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