JOC Article

Model Compounds of Caged Capsaicin: Design, Synthesis, and Photoreactivity

Alan R. Katritzky,* Yong-Jiang Xu, and Anatoliy V. Vakulenko

Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, Florida 32611-7200

Allan L. Wilcox and Keith R. Bley*

NeurogesX, Inc., 981F Industrial Road, San Carlos, California 94070

katritzky@chem.ufl.edu

Received May 9, 2003

Molecules were prepared with substituted nitrobenzyl groups covalently bonded to *N*-(4-hydroxy-3-methoxybenzyl)acetamide (**2**) by ether or carbonate linkages. These compounds decomposed under irradiation at 363 nm. Those with carbonate linkages decomposed at slower rates than those with ether linkages. Molecules with dimethoxy-substituted benzyl groups decomposed more slowly than monomethoxy-substituted benzyl groups due to the electronic characteristics of the benzylic carbon.

Introduction

It is well-known that the absorption of light by molecules can cause a plethora of physical processes and chemical reactions, including cleavage of chemical bonds. Absorption of light and subsequent bond cleavage has been used to protect functional groups in organic synthesis[1] and to mask the biological activity of compounds for controlled introduction into biological systems. Biologically "masked" molecules are referred to as caged compounds and are used to study processes such as biological energy regulation, neuronal responses, enzyme structure, and function.

Caged compounds are useful in biologically studies because the timing, location, and rate of cleavage can be precisely controlled. Caged compounds mask features of a structure important for biological recognition and release biologically active molecules on photochemical cleavage. Photolysis of caged compounds is an excellent technique to examine fast kinetics and/or spatial heterogeneity of biochemical responses in cells, tissues, or protein crystals.[2] The technique has allowed study of neurotransmitters,[3] nucleotides,[4] nucleosides,[5] deoxyglucose,[6] glycine,[7] peptides[8] in biological systems, and calcium uptake.[9]

Capsaicin **1**[10] and congeneric structures[11] (Figure 1) have attracted much attention because of their use as analgesics,[12] diagonistic aids,[13] and vasodilators.[14] Recently, 4,5-dimethoxy-2-nitrobenzylcapsaicin has been used as pharmacological tool for the photochemical gating of heterologously expressed ion chanels.[15] Capsaicin is a natural product isolated from hot peppers and is structurally related to vanillin, a natural product from the vanilla plant. Capsaicin and related compounds are classified as vanilloids and possess substructures related to guaiacol (*o*-methoxyphenol), which is found in common plants, such as rue and broom. Nelson first determined the chemical structure of capsaicin,[16] and the first total synthesis was reported in 1930.[17]

Capsaicin is a small molecule with low melting point and moderate hydrophobicity. These properties facilitate penetration of skin stratum corneum by capsaicin from topical applications. Capsaicin has been used for many years as topical treatment for chronic pain conditions, including postherpetic neuralgia, painful diabetic neuropathy, and osteoarthritis.[10c,12] However, capsaicin is classified as an irritant, causing localized burning sensation, erythema, or stinging, and aerosolized capsaicin can induce coughing or sneezing.[18] Therefore, capsaicin must be handled with care.

* Corresponding author.

(1) Bochet, C. G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 125.
(2) Adams, S. R.; Tsien, R. Y. *Annu. Rev. Physiol.* **1993**, *55*, 755.
(3) Hess, G. P.; Grewer, C. In *Methods in Enzymology*; Marriott, G., Ed.; Academic Press: New York, 1998; Vol. 291, pp 443−473.
(4) Raimbault, C.; Perraut, C.; Marcillat, O.; Buchet, R.; Vial, C. *Eur. J. Biochem.* **1997**, *250*, 773.
(5) (a) Meldrum, R. A.; Chittock, R. S.; Wharton, C. W. In *Methods in Enzymology*; Marriott, G., Ed.; Academic Press: New York, 1998; Vol. 291, pp 483−495. (b) Cohen, B. E.; Stoddard, B. L.; Koshland, D. E. *Biochemistry* **1997**, *36*, 9035.
(6) Watanabe, S.; Hirokawa, R.; Iwamura, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3375.
(7) Grewer, C.; Jäger, J.; Carpenter, B. K.; Hess, G. P. *Biochemistry* **2000**, *39*, 2063.
(8) Odaka, M.; Furuta, T.; Kobayashi, Y.; Iwamura, M. *Photochem. Photobiol.* **1996**, *63*, 800.
(9) James, I. F.; Walpole, C. S. J.; Hixon, J.; Wood, J. N.; Wrigglesworth, R. *Mol. Pharmacol.* **1988**, *33*, 643.
(10) (a) Buck, S. H.; Burks, T. F. *Pharmacol. Rev.* **1986**, *38*, 179. (b) Holzer, P. *Pharmacol. Rev.* **1991**, *43*, 143. (c) Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51*, 159.
(11) Bennett, D. J.; Kirby, G. W. *J. Chem. Soc. C* **1968**, 442.
(12) USAN Council. *Clin. Pharmacol. Ther.* **1986**, *40*, 243.
(13) Molnár, J.; György, L.; Unyi, G.; Kenyeres, J. *Acta Physiol. Acad. Sci. Hung.* **1969**, *35*, 369.
(14) *Martindale. The Extra Pharmacopoeia*, 28th ed.; Reynolds, J. E. F., Ed.; The Pharmaceutical Press: London, 1982; p 1629.
(15) Zemelman, B. V.; Nesnas, N.; Lee, G. A.; Miesenböck, G. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1352.
(16) Nelson, E. K. *J. Am. Chem. Soc.* **1919**, *41*, 1115.
(17) Späth, E.; Darling, S. F. *Ber. Dtsch. Chem. Ges.* **1930**, *63B*, 737.

10.1021/jo034616t CCC: $25.00
Published on Web 10/23/2003

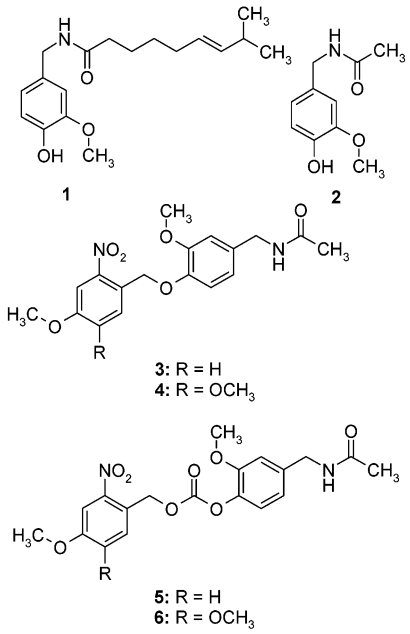


FIGURE 1.

Studies of the effect of variation in molecular structure on the activity of capsaicinoids demonstrate that blocking the phenolic OH or a change in the size of the side chain leads to loss of vanilloid receptor 1 (VR1, or TRPV1) agonist activity.[10c] Activation of the TRPV1 receptor can initiate calcium-induced neurotoxicity in sensory neurons. A rational way to decrease the irritating properties of capsaicin and increase the ease and safety of its use is to photoactivate caged capsaicin near its site of action, causing a rapid and full activation of TRPV1 receptors. In theory, this should result in less irritation since excessive depolarization and intracellular calcium accumulation will cause sensory neurons to become nonfunctional.[19] The target molecules of the present work contain substituents on the phenolic group of *N*-(4-hydroxy-3-methoxybenzyl)acetamide (**2**).

Targets based on **2** were designed to model properties of capsaicin. The study of structures based on **2** provides benefits of economics and safety. The parent **2** is easily prepared from vanillin or vanillylamine and lacks the noxious and irritating properties of capsaicin. Effects of changes in structure on reactivity in derivatives of **2** should parallel those in capsaicin and synthetic methods developed for preparation of derivatives of **2** should be directly applicable to capsaicin. We now report our study of the effects of changes in structure on hydroxyl-caged derivatives of *N*-(4-hydroxy-3-methoxybenzyl)acetamide **2**. Specifically, we describe the syntheses of the photolabile caged molecules **3**−**6** (Figure 1), using various nitrobenzyl carbonates and nitrobenzyl ethers as caging groups, and some initial investigations of their photolysis.

Results and Discussion

Design of Target Structures. Targets were designed by consideration of the photolytic reactivity of related structures, for example, 2-nitrobenzyl-caged tyrosine,[20] serotonin,[21] and phenylephrine.[22] Caged tyrosine is cleaved upon exposure to 350 nm light for 12 h. 4,5-Dimethoxy-2-nitrobenzyl-caged serotonin releases the neurotransmitter upon excitation by 308 or 337 nm laser pulses. Phenylephrine derivatives are photolyzed by flashes from a xenon arc lamp with a 300−350 nm filter or a frequency doubled ruby laser at 347 nm in buffer solution and in smooth muscle preparations. The latter wavelength of light is suitable for biological samples because photochemical damage to proteins and nucleotides is minimized.[23] The 4,5-dimethoxy groups in a caged nitrobenzyl phenylephrine derivative increases the maximum UV absorption wavelength from 272 to 330 nm and suggests electron-rich nitrobenzyl groups can be cleaved from caged compounds at longer wavelengths, an advantage when working in biological systems. Dimethoxy substitution of caged nitrobenzyl phenylephrine also increases the rate of photolysis relative to the unsubstituted nitrobenzyl phenylephrine analogue.[22a] This observation supports that electron-releasing benzyl substituents promote photolytic cleavage of 2-nitrobenzyl phenolic ethers.

QSAR analysis provides mechanistic details of the photochemical cleavage of benzylic compounds useful in the design of target structures.[24] For example, photochromic bleaching rates (k) of 2-(2-nitrobenzyl)pyridines[25] are sharply increased by electron releasing substituents as shown by eq 1.

$$\log k = (-2.62 \pm 0.40)\sigma - (1.69 \pm 0.17) \quad \text{(eq 1)}$$

$$N = 9,\ r^2 = 0.97,\ Q^2 = 0.95,\ s = 0.20$$

The coefficient of σ in eq 1 describes a strong effect of the electronic nature of the benzylic carbon on the photolysis rates of 2-(2-nitrobenzyl)pyridines. Because the photolysis of 2-nitrobenzyl compounds proceeds through an aci-nitro intermediate,[26] the effect of substituents on the nitro group might be important in the rate-determining step of the reaction. However, when 2-(2-nitrobenzyl)pyridines in this dataset are parametrized using σ for the effect of substituents on the nitro group, the correlation is low ($r^2 = 0.65$) with photochromic bleaching rates ($\log k$). Therefore, eq 1 suggests the effect of substituents on the benzylic carbon is more important than that of substituents on the nitro group in determining rate of photolysis and is consistent with a positive correlation of photolytic cleavage rate with more electron-rich benzylic carbons.

Bochet recently showed the effects of substituents on wavelength sensitivity and photoreactivity of 2-nitroben-

(18) Rains, C.; Bryson, H. M. *Drugs. Aging* **1995**, *7*, 317.
(19) Dray, A. *Biochem. Pharmacol.* **1992**, *44*, 611.
(20) Sreecumar, R.; Ikebe, M.; Fay, F. S.; Walker, J. W. In *Methods in Enzymology*; Marriott, G., Ed.; Academic Press: New York, 1998; Vol. 291, pp 78−94.
(21) Breitinger, H.-G. A.; Wieboldt, R.; Ramesh, D.; Carpenter, B. K.; Hess, G. P. *Biochemistry* **2000**, *39*, 5500.
(22) (a) Walker, J. W.; Martin, H.; Schmitt, F. R.; Barsotti, R. J. *Biochemistry* **1993**, *32*, 1338. (b) Boyle, W. A.; Muralidharan, S.; Maher, G. M.; Nerbonne, J. M. *J. Photochem. Photobiol. B* **1997**, *41*, 233.
(23) McCray, J. A.; Trentham, D. R. *Annu. Rev. Biophys. Biophys. Chem.* **1989**, *18*, 239; *Chem. Abstr.* **1989**, *111*, 53299w.
(24) QSAR equations in this paper are found in *CQSAR 4.5 Database;* Biobyte Corp.: Claremmont, CA.
(25) Weinstein, J.; Bluhm, A. L.; Sousa, J. A. *J. Org. Chem.* **1966**, *31*, 1983.
(26) Peng, L.; Goeldner, M. *J. Org. Chem.* **1996**, *61*, 185.

zyl carbamates.[27] Similar to photolysis of 2-(2-nitrobenzyl)pyridines, electron-releasing benzyl substituents increase the rate of photolysis (k) of 2-nitrobenzyl carbamates (eq 2) under 420 nm light

$$\log k = (-0.22 \pm 0.08)\sigma^{+} - (1.59 \pm 0.07) \qquad \text{(eq 2)}$$

$$N = 5,\ r^2 = 0.96,\ Q^2 = 0.91,\ s = 0.02$$

Equation 2 supports the concept that substituents with electron-releasing properties to the benzylic carbon increase rates of photolysis of 2-nitrobenzyl carbamates. Equation 2 further demonstrates the electronic nature of the benzylic group is most important for the rate-determining step because when the 2-nitrobenzyl carbamates are parametrized for the nitro group, the correlation with rates of photolysis is poor ($r^2 = 0.70$).

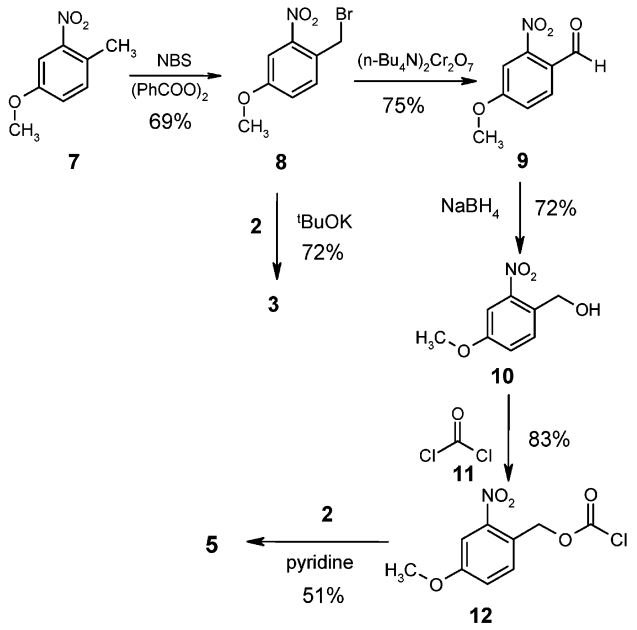
Concern that the parent **2** might need optimization as a leaving group from a photolytically activated intermediate led us to design molecules with the carbonate linkage in order to modulate the rate of release of **2**. A 2-nitrobenzyl group linked by a carbonate to the anticancer drug 5-fluorodeoxyuridine (5-F-dU) is activated by UV light. The structure is nontoxic to cells and upon exposure to light (>350 nm) releases 5-F-dU. Photolysis of 2-nitrobenzyl group linked by a carbonate to 5-F-dU in vitro results in inhibition of cell growth.[28]

We sought to target nitrobenzyl-protected analogues with electron-donating substituents to provide the most rapid release of **2** upon photolysis. Four target molecules (**3**−**6**) were selected to examine the effect of the electronic nature of the benzyl group and two different types of linkage groups bonded to **2**, an ether (**3** and **4**), or a carbonate (**5** and **6**) linkage (Figure 1). Two different substitution patterns on the nitrobenzyl group were selected to give a range of electronic properties of the benzylic carbon atom. The 4-monomethoxy-substituted nitrobenzyl has a more electron-rich benzylic carbon atom (negative coefficient $\sigma = -0.27$) than that of the 3,4-dimethoxy-substituted nitrobenzyl compound ($\sigma = -0.15$) because the methoxy substituent in the meta position is electron withdrawing ($\sigma = +0.12$) with respect to the benzyl carbon atom. On the basis of QSAR discussed above, it was expected that monomethoxy-substituted nitrobenzyl molecules **3** and **5** would decompose more rapidly than their dimethoxy analogues under conditions of photolysis.

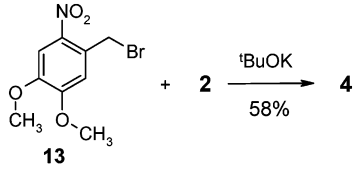
Syntheses. Syntheses of Target Molecules 3 and 5. Target molecule **3** was synthesized by the coupling reaction of compound **2** with 1-(bromomethyl)-4-methoxy-2-nitrobenzene (**8**) in the presence of an equal amount of potassium *tert*-butoxide to enhance the reactivity of the phenolic group in **2** (Scheme 1). Compound **8** was obtained by the bromination of commercially available 4-methyl-3-nitroanisole (**7**).[29]

The synthesis of molecule **5** was carried out using coupling reaction of compound **2** with 1-[(chlorocarbonyl)-oxy]methyl-4-methoxy-2-nitrobenzene (**12**) in the presence of anhydrous pyridine instead of potassium *tert*-butoxide as a base. The key starting material **12** was prepared in 83% yield by reaction of phosgene (**11**) with 4-methoxy-2-nitrobenzyl alcohol (**10**), which was obtained from **8** by (i) oxidization of **8** by tetrabutylammonium dichromate to get 4-methoxy-2-nitrobenzaldehyde (**9**)[29] (75%) and (ii) reduction of **9** using $NaBH_4$ to give **10** (72%) (Scheme 1). The structures of **3**, **5**, and all the precursors were fully characterized by ^{1}H and ^{13}C NMR and microanalysis (except **12**, which is used in subsequent reactions without purification).

SCHEME 1



SCHEME 2



Compound **2** was synthesized from 4-hydroxy-3-methoxybenzylamine hydrochloride and acetic anhydride in 58% yield.[30] Attempted chloroformylation of **2** to 1-[(acetylamino)methyl]-4-[(chlorocarbonyl)oxy]-3-methoxybenzene failed.

Synthesis of Target Molecules 4 and 6. Target molecule **4** was prepared in 58% yield by the similar coupling reaction as that described for the synthesis of **3** using commercially available 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene (**13**) instead of compound **8** (Scheme 2).

Following a procedure similar to that described for the preparation of **5**, target molecule **6** was obtained in 42% yield starting from 1-[(chlorocarbonyl)oxy]methyl-4,5-dimethoxy-2-nitrobenzene (**15**),[31] which was prepared in 84% yield by the reaction of phosgene (**11**) with 4,5-dimethoxy-2-nitrobenzyl alcohol (**14**) (Scheme 3). The structures of **4**, **6**, and **15** were also fully characterized by ^{1}H and ^{13}C NMR and microanalysis.

(27) Bochet, C. G. *Tetrahedron Lett.* **2000**, *41*, 6341.
(28) Wei, Y.; Yan, Y.; Pei, D.; Gong, B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2419.
(29) Mohan, R.; Katzenellenbogen, J. A. *J. Org. Chem.* **1984**, *49*, 1238.
(30) Nelson, E. K. *J. Am. Chem. Soc.* **1919**, *41*, 2121.

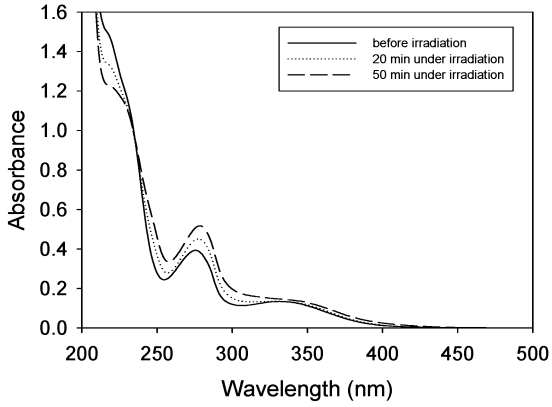
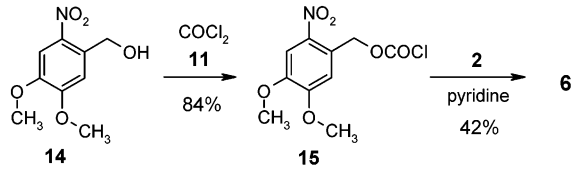


FIGURE 2. UV−vis spectra of compound **3** (starting concentration, 4.0 mg/mL).

SCHEME 3



Photolyses. Photolyses were carried out in the same concentration (4.0 mg/mL) under 363 nm UV light for each molecule. ^{1}H NMR spectra and UV−vis absorption data were used to monitor the photolysis characteristics of compounds **3**−**6**.

The ^{1}H NMR spectra showed that all compounds decomposed under irradiation. For **3**, about 57% of the ether linkage was cleaved after 20 min. In the same time interval, 20% of **4** was transformed and less than 7% of **5** decomposed (from the ^{1}H NMR spectrum). Similar NMR spectra were recorded for **6** before and after 20 min irradiation; the only difference was two very small new peaks at 3.89 and 3.92 ppm.

After 80 min (4 × 20 min) of irradiation, the singlet at 5.62 ppm assigned to the $ArCH_2O-$ group disappeared from the ^{1}H NMR spectrum of **3**, indicating that **3** had decomposed completely. Similarly, **4** cleaved completely after 140 min of irradiation. The ^{1}H NMR spectrum of **5** showed some starting material after 380 min of irradiation. Judging by the ^{1}H NMR spectrum, about 80% **6** still remained after 480 min of irradiation.

The color of the solutions also changed: after 20 min of irradiation, the solution of **3** changed to medium yellow, **4** changed to bright yellow, that of **5** was light yellow, while the solution of **6** was nearly colorless. As irradiation continued, the color of all the solutions became progressively deeper. At the end of the irradiation, all of the solutions were deep brown, including **6**.

UV−vis spectra were studied before and after photolysis. The spectra of **3** are shown in Figure 2. A decreased absorbance in the 200−235 nm region and increased absorbance in the 235−325 nm region is observed with irradiation. Similar spectra were recorded with **5**. A difference between the spectra of **3** and **5** is that the change of the absorbance in **5** is significantly slower than

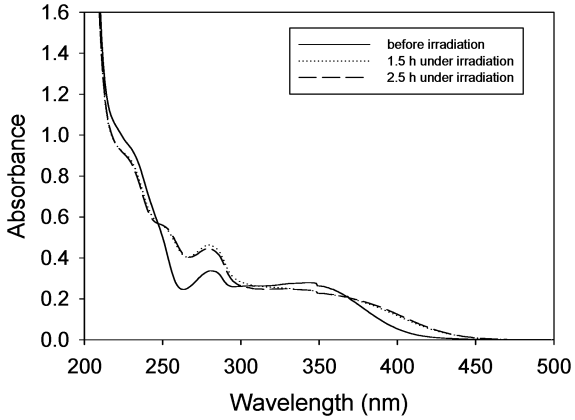


FIGURE 3. UV−vis spectra of compound **4** (starting concentration, 4.0 mg/mL).

that for **3**. The spectral changes for **4** and **6** are similar to those for **3** and **5**. Spectra of **4** and **6** show a decreased absorbance in the 200−247 nm and 308−367 nm regions and an increased absorbance in the 247−308 nm and above 367 nm region (Figure 3).

To summarize, four photolabile caged molecules were synthesized and the behavior of each studied under UV irradiation at 363 nm. Molecules with ether linkages (**3** and **4**) decomposed faster than those with carbonate linkages (**5** and **6**). Molecules with an additional methoxy group (**3** and **5**) decomposed more slowly than the desmethoxy analogues (**4** and **6**), consistent with QSAR analysis of related molecules which predicts the monomethoxy-substituted nitrobenzyl molecules **3** and **5** are more sensitive than their dimethoxy analogues **4** and **6** to conditions of photolysis. The relative reactivity of 2-nitrobenzyl ether and carbonate-linked molecules support that compounds with more electron-rich benzylic carbons **3** and **5** decompose more rapidly than their less electron-rich analogues **4** and **6**.

Experimental Section

THF was distilled from sodium benzophenone prior to use. Melting points were determined using a Bristoline hot-stage microscope and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra (300 and 75 MHz, respectively) were recorded on a Gemini 300 NMR spectrometer in $CDCl_3$ (with TMS for ^{1}H and chloroform-*d* for ^{13}C as the internal reference). The UV−vis spectra were recorded on a Varian Cary 100 Conc spectrophotometer. Elemental analyses were performed on a Carlo Erba-1106 instrument. HRMS were measured on an AEI-30 mass spectrometer. Column chromatography was performed on silica gel unless otherwise noted. All of the reactions were carried out under N_2.

Photolysis. UV 450 W Immer. Lamp with 363 nm filter was used as monochromatic light source. A quartz cuvette with a 1-cm light-path length was used to measure absorption. The intensity of the incident light, which was measured by the standard method,[32] is 5.25×10^{-9} Ein/s.

1-(Bromomethyl)-4-methoxy-2-nitrobenzene (8). A solution of 2-nitro-4-methoxytoluene **7** (0.5 g, 3.03 mmol), *N*-bromosuccinimide (0.539 g, 3.03 mmol), and benzoyl peroxide (1.5 mg) in 10 mL of carbon tetrachloride was refluxed under strong illumination using a 400-W General Electric sunlamp. After 7 h, the solution was cooled and the solid

(31) Amit, B.; Zehavi, U.; Patchornik, A. *J. Org. Chem*. **1974**, *39*, 192.

(32) Heller, H. G.; Langan, J. R. *J. Chem. Soc., Perkin Trans. 2* **1981**, 341.

succinimide was removed by filtration. The filtrate was concentrated to yield brown oil. Purification by column chromatography (eluent: hexanes/benzene = 1/1) afforded 0.49 g (69%) of the product **8** as light yellow needles: mp 63−64 °C (lit.[29] mp 63−64 °C); ^{1}H NMR δ 3.88 (s, 3H), 4.80 (s, 2H), 7.13 (dd, J = 2.7, 8.7 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 2.7 Hz, 1H); ^{13}C NMR δ 29.1, 55.9, 110.4, 119.9, 124.7, 133.6, 148.5, 160.0.

4-Methoxy-2-nitrobenzaldehyde (9). A solution of **8** (0.56 g, 2.26 mmol) and bis(tetrabutylammonium) dichromate (1.47 g, 2.0 mmol) in 5 mL of $CHCl_3$ was heated under reflux for 8 h. TLC analysis showed the disappearance of **8**. The reaction was rapidly cooled in ice bath and filtered through a pad of silica gel (2 g) to eliminate the inorganic and tetrabutylammonium salts. The silica was then washed with diethyl ether (20 mL). Evaporation of the combined organic solvents afforded the crude organic product. Purification by flash chromatography (eluent: $CHCl_3$) afforded 0.30 g (75%) **9** as yellowish needles: mp 91−92 °C (lit.[29] mp 91−92 °C); ^{1}H NMR δ 3.98 (s, 3H), 7.24 (dd, J = 2.3, 8.7 Hz, 1H), 7.52 (d, J = 2.3 Hz, 1H), 7.98 (d, J = 8.7 Hz, 1H), 10.29 (s, 1H); ^{13}C NMR δ 56.3, 109.6, 119.1, 123.4, 131.4, 151.6, 163.7, 186.9.

(4-Methoxy-2-nitrophenyl)methanol (10). Sodium borohydride (0.06 g, 1.65 mmol) was added in small portions to an ice-cold solution of **9** (0.3 g, 1.65 mmol) in dry methanol (20 mL) with stirring. The mixture was left at 0−5 °C for 1 h. Then the solvent was evaporated, and chloroform (30 mL) was added to the residue obtained. The organic layer was washed with sodium bicarbonate (5%, 20 mL) and H_2O (30 mL) and dried over $MgSO_4$. The product obtained was recrystallized from benzene/hexanes (0.22 g, 72%) as colorless needles: mp 79−81 °C; ^{1}H NMR δ 2.63 (br s, 1H), 3.89 (s, 3H), 4.87 (s, 2H), 7.20 (dd, J = 2.6, 8.5 Hz, 1H), 7.57−7.61 (m, 2H); ^{13}C NMR δ 55.9, 62.3, 109.7, 120.4, 128.7, 131.5, 148.4, 159.4. Anal. Calcd for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.31; H, 4.93; N, 7.48.

1-[[(Chlorocarbonyl)oxy]methyl]-4-methoxy-2-nitrobenzene (12). A solution of phosgene (**11**) in toluene (4.57 mL, 20% w/w, 8.78 mmol) was added to a stirred solution of **10** (0.67 g, 3.66 mmol) in 20 mL of dry THF. Stirring was continued for 23 h at ambient temperature. The excess phosgene was removed under low vacuum and trapped with an aqueous NaOH. The remaining solvent was removed on a rotary evaporator. The yellowish oil so formed was directly used for the subsequent reaction without further purification owing to its instability: ^{1}H NMR δ 3.91 (s, 3H), 5.64 (s, 2H), 7.22 (dd, J = 2.6, 8.7 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.67 (d, J = 2.6 Hz, 1H); ^{13}C NMR δ 56.0, 69.5, 110.4, 120.1, 121.0, 131.3, 148.3, 150.4, 160.4.

1-[[(Chlorocarbonyl)oxy]methyl]-4,5-dimethoxy-2-nitrobenzene (15). Following a procedure similar to that used for **12**, the reaction of phosgene (**11**) in toluene (2.75 mL of 20% w/w, 5.28 mmol) and 4,5-dimethoxy-2-nitrobenzyl alcohol **14** (0.47 g, 2.2 mmol) went on for 36 h in 10 mL of dry THF. After workup, the residue was recrystallized from benzene to afford 0.51 g (84%) of yellowish needles: mp 125−127 °C (lit.[31] mp 125−127 °C); ^{1}H NMR δ 3.99 (s, 3H), 4.02 (s, 3H), 5.73 (s, 2H), 7.02 (s, 1H), 7.76 (s, 1H); ^{13}C NMR δ 56.4, 56.5, 69.8, 108.3, 110.4, 124.1, 139.8, 148.9, 150.3, 153.7.

***N*-(4-Hydroxy-3-methoxybenzyl)acetamide (2).** A mixture of 4-hydroxy-3-methoxybenzylamine hydrochloride (5 g, 26 mmol), acetic anhydride (9 mL, 88 mmol), and anhydrous sodium acetate (2.46 g, 30 mmol) was heated at 130 °C for 6 h. Then an excess of sodium hydroxide was added to the cooled mixture with shaking. The solution was thoroughly extracted with chloroform (3 times). The residue formed after the evaporation of the chloroform was saponified with cold alcoholic potassium hydroxide, saturated with carbon dioxide, and extracted with chloroform. After evaporation of the solvent, the product was obtained as thick brown syrup which slowly became crystalline (58%): mp 82−83 °C (lit.[30] mp 84−85 °C); ^{1}H NMR δ 2.01 (s, 3H), 3.88 (s, 3H), 4.33 (d, J = 5.5 Hz, 2H), 5.81 (br s, 2H), 6.77 (d, J = 8.0 Hz, 1H), 6.81 (s, 1H), 6.86 (d, J = 8.0 Hz, 1H); ^{13}C NMR δ 23.3, 43.7, 55.9, 110.8, 114.4, 120.8, 130.1, 145.2, 146.7, 169.8.

***N*-[3-Methoxy-4-[(4-methoxy-2-nitrobenzyl)oxy]benzyl]acetamide (3).** To a solution of **2** (0.20 g, 1 mmol) in 10 mL of THF was added *t*-BuOK (1 mL, 1M in THF). Then **8** (0.246 g, 1 mmol) was added. The resulting mixture was stirred for 24 h at rt. After removal of the solvent, the residue was purified by column chromatography (eluent: EtOAc/hexanes = 1/4). The product was recrystallized from CH_2Cl_2/Et_2O to give yellowish needles (0.26 g, 72%): mp 143−144 °C; UV (C_2H_5OH) λ max [nm] (log ϵ) 276 (34.9), 331 (11.4); ^{1}H NMR δ 2.02 (s, 3H), 3.89 (s, 3H), 3.90 (s, 3H), 4.36 (d, J = 5.8 Hz, 2H), 5.46 (s, 2H), 5.71 (br s, 1H), 6.75−6.82 (m, 2H), 6.87 (s, 1H), 7.20 (dd, J = 2.6, 8.7 Hz, 1H), 7.66 (d, J = 2.6 Hz, 1H), 7.78 (d, J = 8.8 Hz, 1H); ^{13}C NMR δ 23.3, 43.6, 55.9, 56.0, 67.8, 109.5, 111.9, 114.1, 120.1, 120.6, 125.8, 129.8, 132.0, 147.1, 147.5, 149.9, 159.2, 169.8. Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.87; H, 5.67; N, 7.67.

***N*-[4-[(4,5-Dimethoxy-2-nitrobenzyl)oxy]-3-methoxybenzyl]acetamide (4).** Following a procedure similar to that used for **3**, compound **2** (0.20 g, 1 mmol) and 4,5-dimethoxy-2-nitrobenzyl bromide **13** (0.276 g, 1 mmol) were used. The product was recrystallized from CH_2Cl_2/Et_2O to give white needles (0.22 g, 56%): mp 187−188 °C; UV (C_2H_5OH) λ max [nm] (log ϵ) 280 (32.5), 345 (27.0); ^{1}H NMR 2.03 (s, 3H), 3.91 (s, 3H), 3.97 (s, 6H), 4.38 (d, J = 5.6 Hz, 2H), 5.55 (s, 2H), 5.68 (br s, 1H), 6.78−6.89 (m, 3H), 7.49 (s, 1H), 7.76 (s, 1H); ^{13}C NMR δ 23.3, 43.6, 56.0, 56.4 (2), 68.5, 107.9, 109.5, 111.9, 114.6, 120.3, 129.8, 132.3, 138.9, 147.1, 147.8, 149.9, 154.0, 169.8. Anal. Calcd for $C_{19}H_{22}N_2O_7$: C, 58.46; H, 5.68; N, 7.18. Found: C, 58.83; H, 5.70; N, 7.12.

4-[(Acetylamino)methyl]-2-methoxyphenyl-4-methoxy-2-nitrobenzyl Carbonate (5). To a stirred solution of **2** (0.58 g, 2.99 mmol) in 2.8 mL of anhydrous pyridine cooled to 0 °C was added dropwise a solution of **12** (0.81 g, 3.3 mmol) in THF (5 mL). The mixture was then stirred at rt for 41 h. The solvent was evaporated to give the crude product, which was recrystallized from chloroform/hexanes to afford yellowish microcrystals (0.62 g, 51%): mp 108−110 °C; UV (C_2H_5OH) λ max [nm] (log ϵ) 272 (36.8), 327 (12.4); ^{1}H NMR (DMSO) δ 1.89 (s, 3H), 3.77 (s, 3H), 3.89 (s, 3H), 4.25 (d, J = 5.8 Hz, 2H), 5.50 (s, 2H), 6.84 (d, J = 8.2 Hz, 1H), 7.04 (s, 1H), 7.14 (d, J = 8.1 Hz, 1H), 7.43 (dd, J = 2.6, 8.7 Hz, 1H), 7.64−7.67 (m, 2H), 8.37 (t, J = 5.8 Hz, 1H); ^{13}C NMR (DMSO) δ 22.7, 42.1, 56.0, 56.3, 66.4, 110.2, 112.2, 119.3, 120.1, 122.0, 122.1, 131.7, 138.4, 139.3, 148.7, 150.6, 152.6, 159.8, 169.4. Anal. Calcd for $C_{19}H_{20}N_2O_8$: C, 56.43; H, 4.99; N, 6.93. Found: C, 56.55; H, 4.88; N, 6.78.

4-[(Acetylamino)methyl]-2-methoxyphenyl-4,5-dimethoxy-2-nitrobenzyl Carbonate (6). Following a procedure similar to that used for **5**, the reaction of **2** (0.32 g, 1.64 mmol) and **15** (0.5 g, 1.8 mmol) was carried out. After workup, the precipitate was filtered off and washed with cool THF to give yellowish microcrystals (0.30 g, 42%): UV (C_2H_5OH) λ max [nm] (log ϵ) 279 (35.2), 344 (38.9); ^{1}H NMR (DMSO) δ 1.88 (s, 3H), 3.77 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 4.25 (d, J = 5.8 Hz, 2H), 5.56 (s, 2H), 6.85 (d, J = 8.1 Hz, 1H), 7.05 (s, 1H), 7.16 (d, J = 8.1 Hz, 1H), 7.21 (s, 1H), 7.74 (s, 1H), 8.37 (t, J = 5.7 Hz, 1H); ^{13}C NMR (DMSO) δ 22.6, 41.9, 55.8, 56.2, 56.3, 66.8, 108.3, 111.5, 112.1, 119.2, 122.0, 125.0, 138.2, 139.2, 139.8, 148.3, 150.5, 152.3, 153.2, 169.2. Anal. Calcd for $C_{20}H_{22}N_2O_9$: C, 55.30; H, 5.10; N, 6.45. Found: C, 55.50; H, 5.25; N, 6.39.

Acknowledgment. We are indebted to Dr. K. Schanze (University of Florida) for advice and help with the photoreactions. A.W. thanks Corwin Hansch for access to the database and useful discussions on QSAR analysis.

JO034616T