for 60 h. Analysis of the reaction mixture gave 9.7 g (yield 49%) of telomers (M = 152) containing 96% lactone **3**.

2-Ethylidene-6-hepten-5-olide (3): IR (Nujol) ν (C=O) 1710 s cm^{-1}; bp 76 °C/10^{-2} Torr; ^1H NMR ($CDCl_3$) δ 1.73 (dd, 3 H, Me, $^5J \approx 1$ Hz and ^3J = 7.2 Hz), 1.95-2.07 (m, 2 H, CH_2CH), 2.30-2.60 (m, 2 H, $=CCH_2CH_2$), 4.72 (m, 1 H, CH_2CH), 5.17 (dd, 1 H, CH = CH_2, cis, $^3J_{cis}$ = 10.6 and ^2J = 1.1 Hz), 5.29 (dd, 1 H, CH = CH_2, trans, $^3J_{trans}$ = 17.2 and ^2J = 1.1 Hz), 5.83 (m, 1 H, CH = CH_2), 7.14 (q of t, 1 H, CH_3CH, ^3J = 7.2, $^4J \approx 2$ Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.05 (Me), 21.16 ($=CCH_2-$), 26.86 ($-CH_2CHO$), 77.89 ($-CHO$), 115.53 ($=CH_2$), 125.64 ($C=CHMe$), 135.51 ($CHCH_2$), 139.46 ($CHMe$), 164.80 (C=O); MS, m/e 152 (37), 137 (12), 124 (27), 96 (100), 81 (28), 68 (75), 67 (48), 55 (29), 54 (40), 53 (37), 41 (34), 39 (60). Anal. Calcd for $C_9H_{12}O_2$ (M = 152.19): C, 71.03; H, 7.95. Found: C, 70.84; H, 7.98.

2-Ethyl-2,4-heptadien-4-olide (5): ^1H NMR ($CDCl_3$) δ Z isomer (E isomer in square brackets) 1.08 [≈ 1.08] (t, $CH_3CH_2C=$, ^3J = 7.5 Hz), 1.19 [1.18] (t, $CH_3CH_2CH=$, ^3J = 7.5 Hz), 2.39 [2.32] (m, CH_2), 5.16 [5.64] (t, $-CH_2CH=C$, ^3J = 7.8 Hz), 6.97 [7.27] (t, $C=CHC=$, ^4J = 1.4 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 11.7 ($CH_3CH_2C=$, E and Z isomers) 13.5 ($CH_3CH_2CH=$, E + Z), 14.35 (CH_2CH, E), 18.40 ($CH_2C=$, E + Z), 19.45 ($-CH_2CH$, Z), 115.04 ($-CH_2CH=C$, E), 115.78 ($-CH_2CH=C$, Z), 131.86 ($OC=C$, Z), 134.88 ($=CCH=C$, E), 136.16 ($C=CHC=$, Z), 147.97 ($=CC_2H_5$, E + Z), 170.4 (C = O, E + Z); MS for Z, m/e 152 (23), 137 (12), 110 (66), 109 (11), 82 (85), 81 (36), 77 (17), 69 (19), 67 (17), 55 (100); MS for E, m/e 152 (10), 137 (9), 110 (67), 82 (84), 81 (34), 77 (16), 69 (17), 67 (15), 55 (100).

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Registry No. 1a, 79110-94-4; **1b**, 105813-68-1; **2a**, 79079-77-9; **2b**, 113379-49-0; **3**, 67693-94-1; (*Z*)-**5**, 74888-98-5; (*E*)-**5**, 74888-97-4; **8** (R = Ph), 105784-57-4; **8** (R = Cy), 105813-67-0; **9**, 111287-12-8; **10**, 97954-27-3; **11**, 113379-50-3; **12**, 21797-13-7; *cis*-**17**, 113379-43-4; *trans*-**17**, 113472-62-1; **18**, 113379-45-6; CO_2, 124-38-9; $[(\overline{C_{10}H_8N})\overline{Pd\{Ph_2PCH=C(O)OEt\}}]$, 79110-93-3; $[(o\text{-}\overline{C_6H_4CH_2NMe_2})\overline{Pd\{Ph_2PCH_2C(O)=O\}}]$, 105784-57-4; $PdCl_2(PPh_3)_2$, 13965-03-2; $[Pd(dppm)(CH_3CN)_2][BF_4]_2$, 113379-47-8; $[Pd(dppm)(CH_3CN)_2][CF_3SO_3]_2$, 113379-48-9; 1,3-butadiene, 106-99-0.

Biologically Useful Chelators That Release Ca^{2+} upon Illumination†

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Abstract: A series of Ca^{2+}-selective chelators incorporating a photosensitive *o*-nitrobenzhydryl ether, alcohol, or ester (λ_{max} 350-360 nm, $\epsilon \approx 5500$ M^{-1} cm^{-1}) were synthesized. The key step of the syntheses required the novel and mild trimethylsilyl triflate catalyzed Friedel-Crafts alkylation of a *N*,*N*-dialkylaniline by a nitrobenzaldehyde or its acetal. Before photolysis, the chelators show dissociation constants for Ca^{2+} of about 10^{-7} M, roughly matching the typical free [Ca^{2+}] inside unstimulated cells. Considerable adjustment of the affinities is possible by subtle variations in the stereochemistry of the linkage between the two halves of the binding site. Irradiation around 365 nm smoothly converts the chelators into *o*-nitrosobenzophenones whose Ca^{2+} affinity is 10-30-fold weaker than the unphotolyzed compounds. The photolyses have quantum efficiencies of 0.01-0.04 and release Ca^{2+} with rate constants of 5-3000 s^{-1} after a flash, with free benzhydrols remarkably faster than their ethers. Therefore, these chelators can be used to generate controlled fast jumps in intracellular free [Ca^{2+}] to mimic and analyze a host of important cellular responses, especially in nerve and muscle.

Brief, localized fluctuations of intracellular free Ca^{2+} concentrations are believed to control neurosynaptic transmission, hormone secretion, muscle contraction, and a myriad of other physiological functions.[1,2] The ability to generate similarly fast and localized rises in free [Ca^{2+}] would be a powerful tool both in studying the physiology of intact cells and the biochemistry of their many Ca^{2+}-sensitive proteins. Recently, flash photolysis has been used in biological systems to generate sudden jumps in the concentration of adenosine triphosphate, cyclic nucleotides, cholinergic agonists, and protons.[3-9] The resulting concentration jumps cause cellular or biochemical responses whose kinetics can give valuable insights into the molecular sensing mechanisms. Photochemistry in principle can generate concentration changes that are much faster and spatially more controllable than possible with rapid mixing techniques; photolysis is also applicable to the inside of intact cells, where turbulent mixing would not be possible or desirable. Perhaps the main current limitation is the need to design and synthesize compounds that "cage" active natural trigger substances. The "caged" substance should itself be biologically inert yet readily and rapidly release the active agent once illuminated.

† BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid, or ethylene glycol bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEEDTA, *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid; MOPS, 3-morpholinopropanesulfonic acid; OTf, trifluoromethanesulfonate, CF_3SO_2-; TMS, trimethylsilyl, Me_3Si-; Tris, tris(hydroxymethyl)aminomethane.

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(1) Campbell, A. K. *Intracellular Calcium*; Wiley: New York, 1983.
(2) Carafoli, E. *Annu. Rev. Biochem.* **1987**, *56*, 395-433.
(3) Gurney, A. M.; Lester, H. A. *Physiol. Rev.* **1987**, *67*, 583-617.
(4) Lester, H. A.; Nerbonne, J. M. *Annu. Rev. Biophys. Bioeng.* **1982**, *11*, 151-175.
(5) Kaplan, J. H.; Forbush, B.; Hoffman, J. F. *Biochemistry* **1978**, *17*, 1929-1935.
(6) McCray, J. A.; Herbette, L.; Kihara, T.; Trentham, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7237-7241.
(7) Nerbonne, J. M.; Richard, S.; Nargeot, J.; Lester, H. A. *Nature (London)* **1984**, *310*, 74-76.
(8) Walker, J. W.; Somlyo, A. V.; Goldman, Y. E.; Somlyo, A. P.; Trentham, D. R. *Nature (London)* **1987**, *327*, 249-252.
(9) Walker, J. W.; McCray, J. A.; Hess, G. P. *Biochemistry* **1986**, *25*, 1799-1805.

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Table I[a]

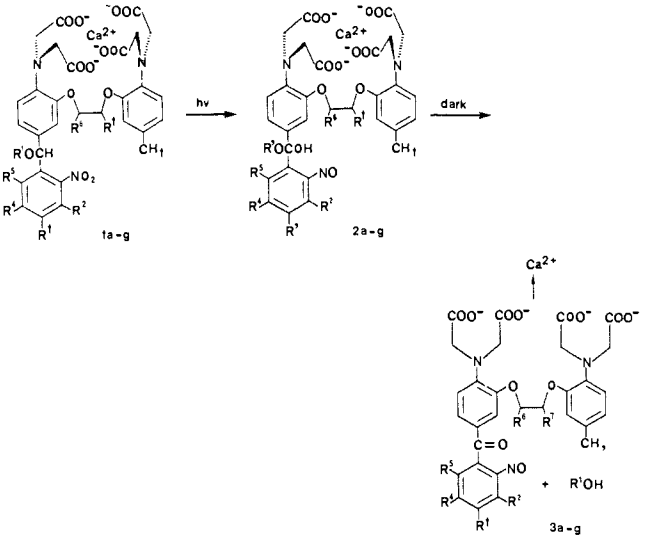
chelator	R^1	R^2	R^3	R^4	R^5	R^6	R^7	$K_d^{Ca^{2+}}$, μM[b] before	$K_d^{Ca^{2+}}$, μM[b] after	$K_d^{Mg^{2+}}$, mM[c] before	$K_d^{Mg^{2+}}$, mM[c] after	ϕ:[d] Ca^{2+} saturation low	ϕ:[d] Ca^{2+} saturation high	τ:[e] Ca^{2+} saturation low	τ:[e] Ca^{2+} saturation medium	τ:[e] Ca^{2+} saturation high
1a, nitr-1	CH_3	H	H	H	H	H	H	0.16	6	*f*	*f*	*f*	*f*	*f*	*f*	*f*
1b, nitr-2	CH_3	H	OCH_2O		H	H	H	0.16	6-10	*f*	*f*	0.05	0.01	240 ± 26 ms	203 ± 5 ms	200 ± 26 ms
1c, nitr-3	CH_3	OCH_3	H	H	NO_2	H	H	*f*	*f*	*f*	*f*	0.04	0.015	*f*	*f*	*f*
1d, nitr-4	CH_3	H	OCH_2O		H	*cis*-$(CH_2)_3$		0.040	0.6-1	*f*	*f*	*g*	*g*	*f*	*f*	*f*
1e, nitr-5	H	H	OCH_2O		H	H	H	0.145	6.3	8.5	8	0.035	0.012	409 ± 60 μs	315 ± 28 μs	272 ± 38 μs
1f, nitr-6	$COCH_3$	H	OCH_2O		H	H	H	*f*	*f*	*f*	*f*	*g*	*g*	*f*	(318 ± 34 μs)[45]	*f*
1g, nitr-7	H	H	OCH_2O		H	*cis*-$(CH_2)_3$		0.054	3	5.4	5	0.042	0.011	249 ± 42 μs	1.81 ± 0.16 ms[h]	281 ± 43 μs

[a] All measurements were made at 0.1-0.15 M ionic strength, pH 7.0-7.4, and 20-23 °C (see the Experimental Section and Figures 1 and 2 for details). [b] Dissociation constant for Ca^{2+}, i.e., $K_d^{Ca^{2+}} = [Ca^{2+}][\text{free chelator}]/[Ca^{2+}\text{-complex}]$. Before and after photolysis. [c] Dissociation constant for Mg^{2+}, i.e., $K_d^{Mg^{2+}} = [Mg^{2+}][\text{free chelator}]/[Mg^{2+}\text{-complex}]$. Before and after photolysis. [d] Quantum efficiency of photolysis at zero or saturating $[Ca^{2+}]$. [e] Time constant or reciprocal rate constant for main component of absorbance increase, attributed to formation of benzophenones **3a-g**. [f] Not determined. [g] Not precisely determined but similar to values for **1e**, nitr-5. [h] An additional fast component was present whose rate constant could not be determined reproducibly.

Since Ca(II) has no useful redox or covalent chemistry in aqueous solution, it has to be "caged" by complexation with a chelator whose affinity for Ca^{2+} starts high but falls sharply upon photolysis. Such a chelator must fulfill several stringent requirements to be biologically useful: (1) Because the concentration of free calcium is about 10^{-7} M in nearly all resting cells, the chelator before photolysis must bind Ca^{2+} with a similar or lower dissociation constant in order to store a significant amount of Ca^{2+} at equilibrium. Such strong binding must exist at pH 7 and ≥0.1 M ionic strength. Obviously, the luxury of working in organic solvents to improve solubility and increase binding affinities is not available here. (2) Because the typical intracellular concentration of free Mg^{2+} is near 10^{-3} M, the dissociation constant for Mg^{2+} should exceed 10^{-3} M so that the chelator neither perturbs cell $[Mg^{2+}]$ significantly nor is blocked by it. Therefore, the chelator needs a selectivity of $\geq 10^4$ for Ca^{2+} over Mg^{2+}. (3) After photochemical conversion, the chelator should have a Ca^{2+} dissociation constant $\geq 10^{-6}$ M to be able to raise intracellular free $[Ca^{2+}]$ to such levels, which are typical for physiologically stimulated cells. (4) The photochemistry and Ca^{2+} release should be mostly complete in $\leq 10^{-3}$ s, because Ca^{2+} activates many processes in as little as a few milliseconds. (5) The required wavelength and intensity of light should not acutely perturb or damage living cells. Wavelengths below 300 nm probably would be unacceptable in most applications due to macromolecular damage and tissue opacity. Also the photochemistry should not generate toxic byproducts.[3,4]

Some work has been reported on photochemical modulation of chelator affinities for cations,[10-19] but no structure has yet been produced that comes near satisfying the above criteria. The favored photochemical mechanism has been cis-trans isomerization to alter the conformation of the cation binding site. Such a mechanism has the problem that often the desired isomerization is itself hindered by coordination of the cation, which stabilizes the isomer that binds it.[16-19] We report here the design, synthesis, and characterization of the first series of chelators (**1a-g**) that can satisfy all the above requirements. The design began with the requirements for high Ca^{2+} affinity and Ca^{2+}/Mg^{2+} selectivity.

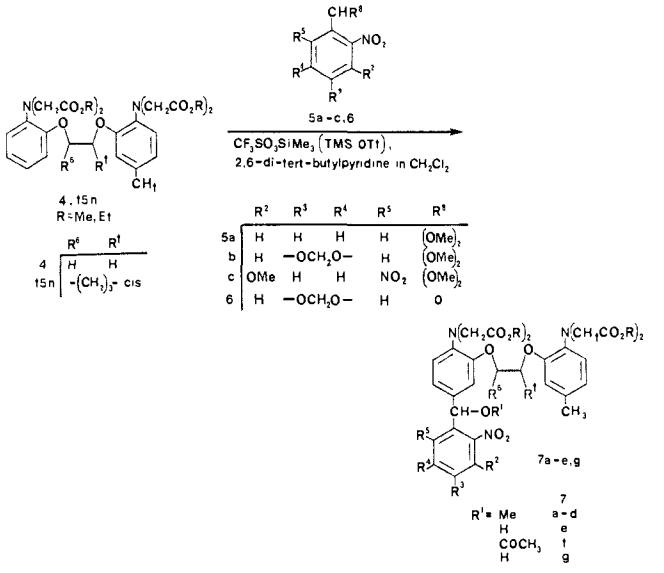
Scheme I



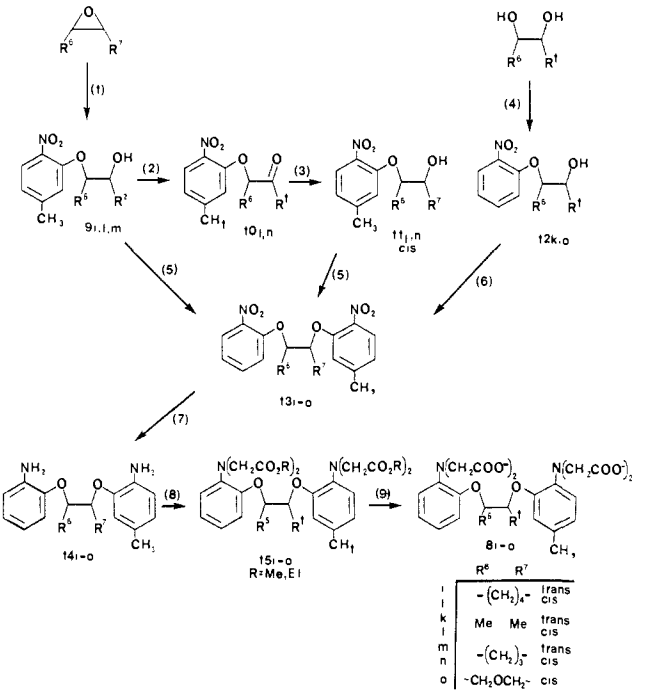
The only synthetic chelators known to have such properties[20] near pH 7 are a few aminopolycarboxylates such as EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid, and its aromatic homologue BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid.[21] We considered BAPTA the best starting point for modification because of its fast kinetics of Ca^{2+} binding and release, its insensitivity to pH variations above pH 7, and the ease of adjusting its Ca^{2+} affinity by substitutions on its benzene rings. Electron-donating or -withdrawing substituents respectively raise or lower the affinities for H^+, Ca^{2+}, and Mg^{2+} as expected from analogous linear free energy relationships.[21] Photochemical generation of an electron-withdrawing substituent would produce the desired drop in Ca^{2+} affinity without steric disruption of the binding site. A suitable photochemical reaction was envisaged to be the known rearrangement[22-26] of *o*-nitrobenzhydrols to nitrosobenzophenones (Scheme I, Table I). From the point of view of the chelator, an insulating benzyl group would be converted into an electronegative ketone substituent.[27] This basic plan proved to work well, though further molecular optimizations were

(10) Irie, M. *J. Am. Chem. Soc.* **1983**, *105*, 2078-2079.
(11) Shinkai, S.; Manabe, O. *Top. Curr. Chem.* **1984**, *121* (Host Guest Complex Chem. 3), 67-104.
(12) Blank, M.; Soo, L. M.; Wassermann, N. H.; Erlanger, B. F. *Science (Washington, D.C.)* **1981**, *214*, 70-72.
(13) Irie, M.; Kato, M. *J. Am. Chem. Soc.* **1985**, *107*, 1024-1028.
(14) Yamashita, I.; Fujii, M.; Kaneda, T.; Misumi, S.; Otsubo, T. *Tetrahedron Lett.* **1980**, *21*, 541-544.
(15) Desvergne, J. P.; Bitit, N.; Bouas-Laurent, H. *J. Chem. Res. Synop.* **1984**, 214-215.
(16) Shinkai, S.; Nakaji, T.; Ogawa, T.; Shigematsu, K.; Manabe, O. *J. Am. Chem. Soc.* **1981**, *103*, 111-115.
(17) Shinkai, S.; Shigematsu, K.; Kusano, Y.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* **1981**, 3279-3283.
(18) Shinkai, S.; Ogawa, T.; Kusano, Y.; Manabe, O.; Kikukawa, K.; Goto, T.; Matsuda, T. *J. Am. Chem. Soc.* **1982**, *104*, 1960-1967.
(19) Shinkai, S.; Shigematsu, K.; Sato, M.; Manabe, O. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2735-2739.
(20) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum: New York, 1974; Vol. 1.
(21) Tsien, R. Y. *Biochemistry* **1980**, *19*, 2396-2404.
(22) Morrison, H. A. In *The Chemistry of the Nitro and Nitroso Groups*; Feuer, H., Ed.; Interscience: New York, 1969; pp 165-213.
(23) Chow, Y. L. In *The Chemistry of Amino, Nitroso and Nitro Compounds and Their Derivatives, Supplement F Part 1*; Patai, S.; Ed.; Wiley: New York, 1982; pp 181-290.
(24) Pillai, V. N. R. *Synthesis* **1980**, 1-26.
(25) Binkley, R. W.; Flechtner, T. W. In *Synthetic Organic Photochemistry*; Horspool, W. M., Ed.; Plenum: New York, 1984; pp 375-423.
(26) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333-6335.
(27) Tsien, R. Y.; Zucker, R. S. *Biophys. J.* **1986**, *50*, 843-853.

Scheme II



Scheme III[a]



still required to increase the initial affinity for Ca^{2+} and the speed of Ca^{2+} release.

Results

Synthesis. We tried several ways to convert the readily available[28] ester **4** into a benzhydrol derivative (Scheme II). The standard direct route of aryllithium or arylmagnesium addition to a nitrobenzaldehyde[29] was not readily compatible with the eventual presence of four carboxylates, despite elaborate efforts to protect those carboxylates[30] or delay their introduction until after an organometallic step. Conventional proton or Lewis acids failed to give useful products[31] from electrophilic attack of nitrobenzaldehydes on **4**, probably because acidity sufficient to activate the aldehyde deactivated the amine ester. Under forcing conditions, tars and triphenylmethane derivatives were the only products. Friedel–Crafts acylations of **4** with nitrobenzoyl chlorides[32] or substituted amides[33] likewise failed to give nitrobenzophenones. It is well known that aromatic amines are often unsuited to those electrophilic substitutions that require strongly acidic conditions.[34] Success eventually came with the use of trimethylsilyl trifluoromethanesulfonate (TMS OTf), a Lewis acid unusual in its compatibility with amine bases.[35-37] TMS OTf plus the hindered base 2,6-di-*tert*-butylpyridine promoted electrophilic attack of aromatic acetals (**5a–c**) on amine **4** under very mild conditions, a few hours at 0 °C in CH_2Cl_2 (Scheme II). Even the highly hindered acetal **5c** or the free aldehyde **6** underwent smooth reaction at room temperature. The dimethyl acetals **5a–c** produced benzhydrol methyl ethers **7a–d**, whereas the free aldehyde **6** yielded trimethylsilyl ethers that could readily be cleaved to the free benzhydrols (**7e,g**) with fluoride anion. Alkaline hydrolysis of these chelator esters released the free chelator tetraanions **1a–g**, whose properties as light-sensitive Ca^{2+} chelators are shown in Table I. To prepare the acetyl ester **1f** for comparison with the methyl ether **1b** and free benzhydrol **1e**, the free acid form of **1e** was acetylated with acetic anhydride and then hydrolyzed at pH 7 to destroy cyclic or mixed anhydrides.

[a] (1) 5-Methyl-2-nitrophenol, K^+ 5-methyl-2-nitrophenolate, DMF. (2) Pyridinium chlorochromate, CH_2Cl_2. (3) Lithium trisiamylborohydride (LS-Selectride), THF, −78 °C. (4, 5) 2-fluoronitrobenzene, NaH, DME. (6) 3-fluoro-4-nitrotoluene, NaH, DMF. (7) H_2, catalytic Pd/C, EtOH–EtOAc, 1 atm, 20 °C. (8) $BrCH_2CO_2R$, 1,8-bis(dimethylamino)naphthalene (Proton Sponge, Aldrich), NaI, CH_3CN. (9) Aqueous KOH + dioxane–MeOH.

Attempts to raise the Ca^{2+} affinities without affecting the Mg^{2+} affinities focused on incorporating the central ethylenedioxy linkage of the parent structure **4** into a cyclopentane or cyclohexane ring. Whereas the parent had been assembled by phenoxide attack on 1,2-dibromoethane,[28] analogous S_N2 substitution on cyclic tosylates proved unproductive as suspected. However, 5-methyl-2-nitrophenoxide attack on cycloalkene oxides yielded *trans*-2-(5-methyl-2-nitrophenoxy)cyclohexanol or -pentanol (Scheme III). Trans chelators could then be prepared by reaction of the alkoxide with 2-fluoronitrobenzene to give the diether, followed by reduction of the nitro groups, N-alkylation with an alkyl bromoacetate, and saponification. Cis isomers could not be made by tosylation of the above trans-substituted cycloalkanols followed by phenoxide displacement of the tosylate. Instead, the alcohols were inverted by oxidation[38] to the ketone and then stereoselective reduction with a bulky hydride reagent, lithium trisiamylborohydride.[39] In some cases (**12k,o**), the diol with correct configuration was readily available, and successive reaction of the two alkoxides with 2-fluoronitrobenzene and 3-fluoro-4-nitrotoluene yielded the diethers.

Absorbance Spectra, Ca^{2+} Binding, and Photolysis. Before photolysis, the absorbance spectra of **1a–g** are essentially summations of the spectra of the chelator moieties and the alkoxy-substituted nitrobenzyl groups. Only the latter contribute significant absorbance at wavelengths > 320 nm, so that in this region the total absorbance is practically unaffected by Ca^{2+} binding. Below 300 nm, the UV spectrum contains enough contribution from the aminophenyl chromophores of the chelator moieties to permit measurement of Ca^{2+} binding constants from spectra at varied free Ca^{2+} concentrations (Figure 1A). These Ca^{2+} dissociation constants (Table I) are near 10^{-7} M and show that the nitrobenzyl substituent has only a minor effect on the Ca^{2+} af-

(28) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440–3450.
(29) Hey, D. H.; Mulley, R. D. *J. Chem. Soc.* **1952**, 2276–2287.
(30) Parham, W. E.; Bradsher, C. K. *Acc. Chem. Res.* **1982**, *15*, 300–305.
(31) Albrecht, K. *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 3292–3299.
(32) Boetius, M.; Romisch, H. *Ber. Dtsch. Chem. Ges.* **1935**, *68*, 1924–1933.
(33) Shah, R. C.; Deshpande, R. K.; Chaubal, J. S. *J. Chem. Soc.* **1932**, 642–652.
(34) March, J. *Advanced Organic Chemistry*, 3rd. ed.; Wiley: New York, 1985; p 485.
(35) Fleming, I. *Chem. Soc. Rev.* **1981**, *10*, 83–111.
(36) Noyori, R.; Murata, S.; Suzuki, M. *Tetrahedron* **1981**, *37*, 3899–3910.
(37) Emde, H.; Domsch, D.; Feger, H.; Frick, U.; Gotz, A.; Hergott, H. H.; Hoffman, K.; Kober, W.; Krageloh, K.; Oesterle, T. S.; Steppan, W.; West, W.; Simchen, G. *Synthesis* **1982**, 1–22.
(38) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650.
(39) Krishnamurthy, S.; Brown, H. C. *J. Am. Chem. Soc.* **1976**, *98*, 3383–3384.

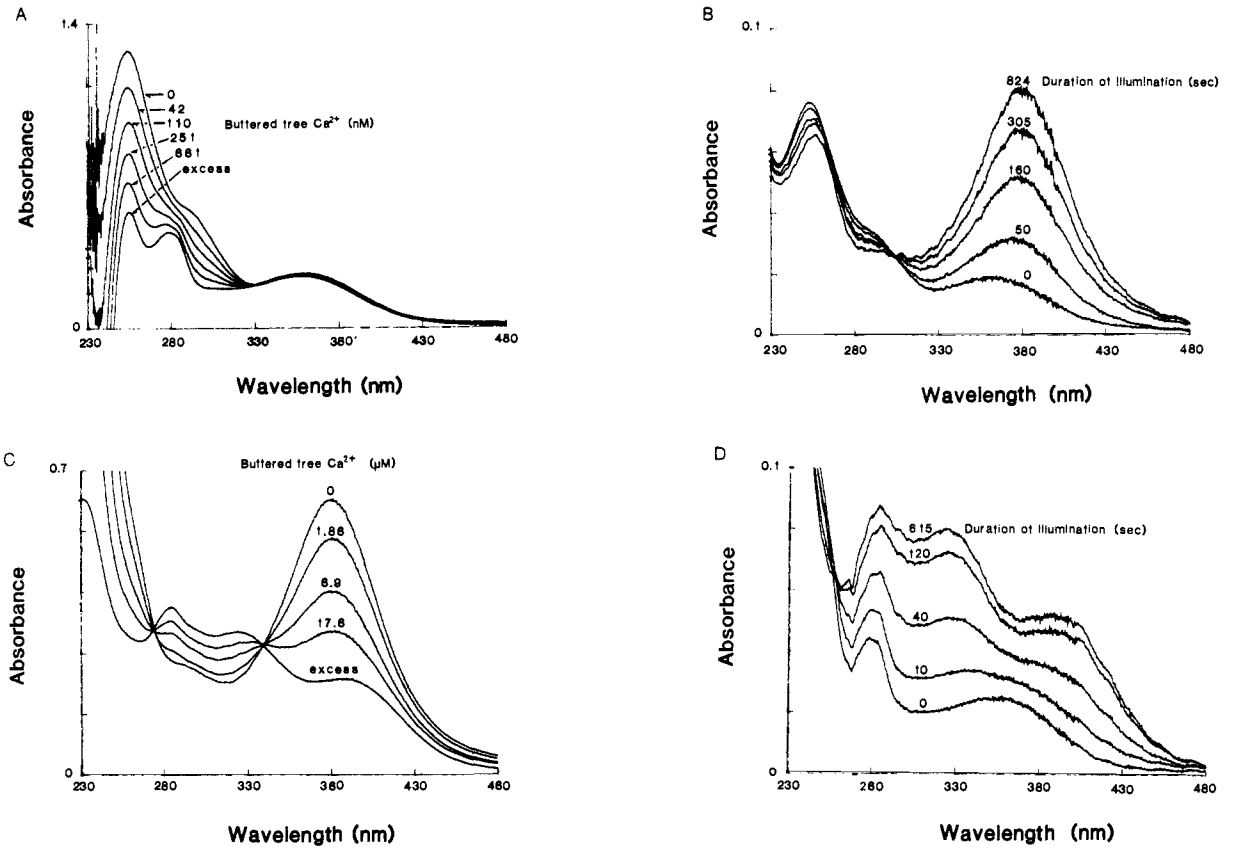


Figure 1. (A) Absorbance spectra of unphotolyzed nitr-5 (**1e**) as a function of free $[Ca^{2+}]$. The titration was done at 22 °C with 10 mL of 100 mM KCl, 10 mM Tris·HCl, 10 mM K_2H·HEEDTA, and 50 μM nitr-5 as starting materials, adjusting the pH to 8.48, recording the spectrum, and then discarding 1.0 mL of this solution and replacing with 1.0 mL of 100 mM KCl, 10 mM Tris·HCl, 10 mM KCaHEEDTA, 50 μM nitr-5, readjusting the pH to 8.48, and recording the spectrum, which was then in 9 mM K_2H·HEEDTA and 1 mM KCaHEEDTA. Subsequent iterations to reach n mM KCaHEEDTA, $(10 - n)$ mM HEEDTA, n = 2–10, were done by discarding $10.0/(11 - n)$ mL and replacing with equal volumes of the 10 mM KCaHEEDTA, 50 μM nitr-5 stock. After n = 10 had been reached to give a free Ca^{2+} between 10^{-5} and 10^{-4} M, addition of 1 mM $CaCl_2$ had no further effect on the spectrum. For clarity only six spectra are included in the figure, n = 0, 2, 4, 6, 8, and 10. Each spectrum is labeled with the calculated free $[Ca^{2+}]$ imposed by the HEEDTA buffer, assuming a log effective stability constant[20] of 6.78 at pH 8.48. (B) Absorbance spectra of nitr-5 (**1e**) undergoing photolysis in the absence of Ca^{2+}. Nitr-5 was dissolved at 10 μM in 100 mM KCl, 10 mM Tris, 5 mM MOPS, 4 mM K_2H_2EGTA and pH titrated to 7.2 with HCl. Spectra were obtained after 0, 10, 25, 50, 90, 160, 305, 512, and 824 s of 365-nm illumination at 2.84×10^{-8} einsteins cm^{-2} s^{-1} from the Spectroline lamp. For clarity only the 0-, 50-, 160-, 305-, and 824-s spectra have been reproduced here. The 512- and 824-s spectra were identical, confirming completion of photolysis after those times. Solutions were at 22 ± 2 °C and continually stirred by magnetic follower during illumination. (C) Absorbance spectra of nitrosobenzophenone **3e** (photolyzed nitr-5) as a function of free $[Ca^{2+}]$. The nitrosobenzophenone was produced by irradiating a 50 μM solution of **1e** in 100 mM KCl, 10 mM K·MOPS, 10 mM K_2H·HEEDTA, pH 7.07, with the Spectroline lamp at 365 nm to completion. The titration was then performed as in A but the pH was maintained at 7.07, at which the calculated log effective stability constant[20] for HEEDTA was 5.35. (D) Absorbance spectra during photolysis of the Ca^{2+} complex of nitr-5 (**1e**). The method was as described in B except that the EGTA was replaced by 2 mM $CaCl_2$, and spectra were measured after 0-, 3-, 10-, 20-, 40-, 70-, 120-, 200-, 303-, and 615-s illumination. The last two spectra were identical, confirming completion. For clarity only about every other spectrum is shown here. Photoisomerization approaches completion more rapidly here in high $[Ca^{2+}]$ than in zero $[Ca^{2+}]$ (B).

finities of the unsubstituted parent chelators. Photolysis of **1a–g** at 365 nm in the absence of Ca^{2+} produced a major new absorbance peaking at 380 nm, consistent[40] with the increased conjugation in the expected 4-amino-2′-nitrosobenzophenones **3a–g** (Figure 1B). Binding of Ca^{2+} now markedly reduced the 380-nm peak (Figure 1C), in close analogy to related chelators in which Ca^{2+} effectively decouples the amino groups from the rest of the chromophore.[21,28,41] The affinities for Ca^{2+} (Table I) were now 30–40-fold weaker than before photolysis, a large change easily explained by the negative mesomeric effect of the para carbonyl. The same end product was obtained whether Ca^{2+} was added before or after photolysis (Figure 1, parts D and A, respectively), and all photolyses showed good isosbestic points. The only noticeable difference in photolysis between the free chelators and their Ca^{2+} complexes was the higher quantum efficiency of the latter, typically 0.04 compared to 0.01 without Ca^{2+}.

Table II[a]

chelator	R^6	R^7	$K_d^{Ca^{2+}}$,[b] μM	$K_d^{Mg^{2+}}$,[c] mM
8h, benz4	H	H	0.079	8.1
8i, trans-6	*trans*-$(CH_2)_4$		0.220	10
8j, cis-6	*cis*-$(CH_2)_4$		0.400	5.9
8k, trans-4	*trans*-CH_3	CH_3	0.102	8.9
8l, cis-4	*cis*-CH_3	CH_3	1	7.8
8m, trans-5	*trans*-$(CH_2)_3$		6	>100
8n, cis-5	*cis*-$(CH_2)_3$		0.020	4.2
8o, cisO-5	*cis*-CH_2OCH_2		0.050	7.2

[a] Measurements are in 0.1–0.15 M ionic strength, pH 7.0–7.6, and at 20–22 °C (see the Experimental Section for details). [b] Dissociation constant for Ca^{2+}. [c] Dissociation constant for Mg^{2+}.

Our search for ways to increase the Ca^{2+} affinities and Ca^{2+}/Mg^{2+} selectivities of the chelators focused on modifying the OCH_2CH_2O linkage between the two halves of the parent chelator **8h**. As shown in Table II, incorporation of either a *cis*- or *trans*-cyclohexane (**8j** or **8i**, respectively) ring weakened the Ca^{2+} affinity by 3–4-fold. Cyclopentanes showed much more discrimination between cis and trans isomers, the cis isomer (**8n**) giving

(40) For comparison, bis(4-dimethylamino)benzophenone absorbs at 372 nm in a polar solvent, acetic acid: Adam, F. C. *J. Mol. Spectrosc.* **1960**, *4*, 359–371. A small further bathochromic shift would be expected from an even more polar solvent, water, as well as from the slightly greater electron-withdrawing power of a nitrosodimethoxybenzoyl substituent compared to a (dimethylamino)benzoyl group.

(41) Tsien, R. Y. *Annu. Rev. Biophys. Bioeng.* **1983**, *12*, 91–116.

a 4-fold improvement over the parent whereas the trans isomer (**8m**) was nearly 2 orders of magnitude worse than the parent. When the *cis*-cyclopentane was changed to a *cis*-3,4-tetrahydrofuran linkage (**8o**), most of the improvement in affinity was lost. Oddly, when two methyl substituents were added to the linkage instead of a ring annelation, the ranking of cis (**8l**) vs trans (**8k**) isomers was reversed, the latter now being the stronger chelator. The overall superiority of the *cis*-cyclopentanes was the motivation for inclusion of that linkage in **1d** and **1g**.

Flash Photolysis Kinetics. When **1b** was photolyzed by a single brief light flash, the absorbance monitored at 365 nm hardly changed during the flash itself, but then rose toward a new higher steady state in a dark reaction with a single exponential rate constant of 4–5 s^{-1} at room temperature.[27] This rate constant could not reflect Ca^{2+} loss because it remained the same in the total absence of Ca^{2+} or when [Ca^{2+}] was so high that the photolysis product was unable to shed Ca^{2+} (Table I). Because the amplitude of the absorbance change was large and strongly affected by [Ca^{2+}], as described above, the absorbance change could not be ascribed to the initial formation of the nitroso hemiketal, but must instead have reflected the collapse of the hemiketal to the benzophenone (Scheme I). To study the final release of Ca^{2+}, a buffer such as *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetate (HEEDTA) was used to set free [Ca^{2+}] above the K_d for the unphotolyzed chelator but below that for the photolyzed, so that the light could cause a maximal change in degree of occupancy. The uptake of Ca^{2+} by the buffer after a flash proved to have the same 5 s^{-1} rate as benzophenone formation. Therefore, once the benzophenone had been slowly formed, release of Ca^{2+} was fast. It is not surprising that the final step of Ca^{2+} dissociation is fast, because analogous chelators are known from temperature-jump studies[42] to exchange Ca^{2+} with dissociation rate constants of 10^2 to $>10^3$ s^{-1} (see also ref 43). Benzophenone formation was found to be subject to weak buffer catalysis. For example, addition of 200 mM imidazole while maintaining the pH at 7.1 increased the rate constant to 15 s^{-1}. Very little information is available on the kinetics of hemiketal breakdown in general;[44] flash photolysis of nitrobenzyl derivatives may provide a useful way of generating significant nonequilibrium concentrations of these species.

A logical way to increase the speed of benzophenone formation would be to replace the OMe of **1b** by a better leaving group, for example OAc as in **1f**. Surprisingly, the OH leaving group in **1e** was equally good. Both **1e** and **1f**[45] showed absorbance transients with rate constants near 3000 s^{-1} (Figure 2a), several hundred fold faster than **1b**. Again, this rate constant was nearly independent of [Ca^{2+}] from <1 nM to >1 mM. At intermediate [Ca^{2+}] appropriate for measuring actual Ca^{2+} release, the same rate was still obtained for the rate of Ca^{2+} uptake by the HEEDTA buffer, provided the latter was in sufficient concentration, >10 mM. At lower concentrations of HEEDTA, the uptake rate became proportional to the buffer concentration. If Ca^{2+} has to dissociate completely from the photolyzed chelator before binding to the HEEDTA, the dissociation rate from the former must exceed 3000 s^{-1}, and the association rate to the latter must be around 3×10^5 M^{-1} s^{-1}.

Only in the case of **1g** was the release of Ca^{2+} slow enough to distinguish from benzophenone formation. At 400 nM [Ca^{2+}], intermediate between the pre- and postphotolysis dissociation constants for Ca^{2+}, the absorbance after a flash (Figure 2B) was the sum of two exponentials. The faster one corresponded to the usual >3000-s^{-1} formation of benzophenone. The slower one, 550 s^{-1}, was presumed to be the additional absorbance change upon loss of Ca^{2+}, since it was not observable at <1 nM or >1 mM [Ca^{2+}] (Table I). It is not surprising that Ca^{2+} release is considerably slower from **3g** than from **3e**, since **3g** has significantly higher Ca^{2+} affinity and a more rigid conformation of its binding site.

The flash photolyses of **1e–g** also showed a small slow component in addition to the major fast component(s) described above.

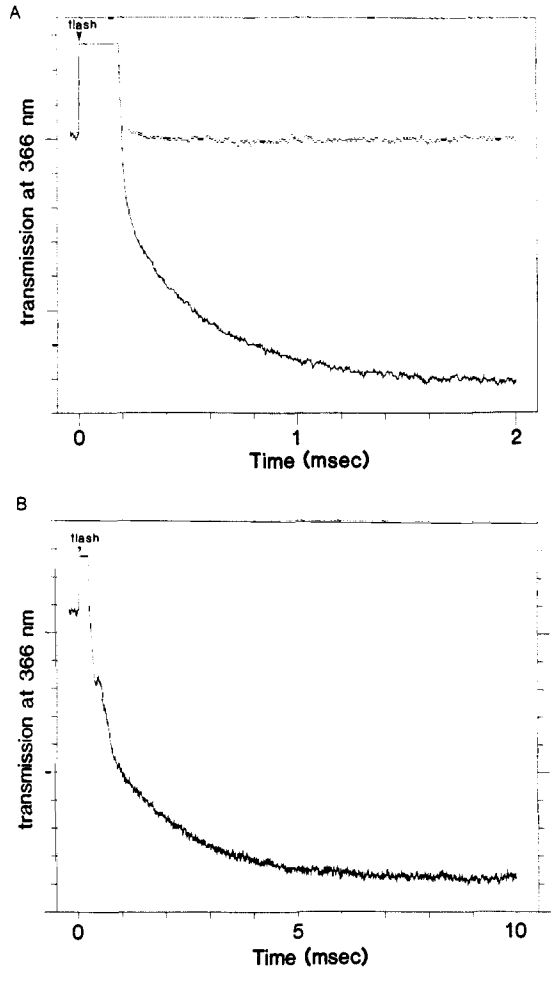
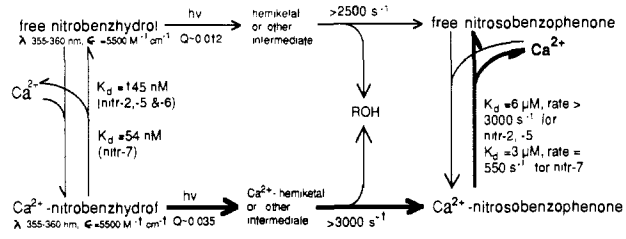


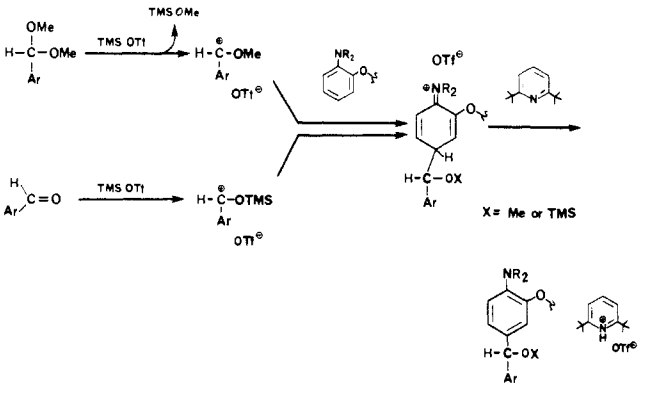
Figure 2. Relaxation kinetics of chelators upon flash photolysis at nonsaturating free [Ca^{2+}]. (A) Compound **1e** (nitr-5) was dissolved at 50 μM in 150 mM KCl, 20 mM MOPS, 2.4 mM KCaHEEDTA, 7.6 mM K_2H·HEEDTA, pH 7.20, free [Ca^{2+}] = 1.0 μM. Transmitted light intensity was monitored with a mercury source, interference filter (366 nm, 20-nm bandwidth), photomultiplier detector, and Biomation transient recorder. Each small division of the ordinate corresponds to 0.003 39 absorbance unit. The upward spike starting at time zero is the artifact from the unfiltered xenon flashlamp. The solid trace is from nitr-5. For comparison the dotted trace is from a photochemically inert sample, bromthymol blue in 150 mM KCl and 12 mM HCl, with the dye concentration was adjusted to match the transmitted light intensity from the nitr-5 sample. This control record shows that the recovery from the flash artifact is clearly separable from the subsequent gradual increase in absorbance of the nitr-5. (B) Analogous flash photolysis of **1g** (nitr-7) at 50 μM in 150 mM KCl, 20 mM MOPS, 1.1 mM KCaHEEDTA, 8.9 mM K_2H·HEEDTA, pH 7.20, free [Ca^{2+}] 0.4 μM. The ordinate scale is identical with that in A.

Scheme IV



(42) Kao, J. P. Y.; Tsien, R. Y. *Biophys. J.*, in press.
(43) Jackson, A. P.; Timmerman, M. P.; Bagshaw, C. R.; Ashley, C. C. *FEBS Lett.* **1987**, *216*, 35–39.
(44) Ogata, Y.; Kawasaki, A. In *The Chemistry of the Carbonyl Group*; Zabicky, J., Ed.; Interscience: London, 1970; Vol. 2, pp 1–69.
(45) Because **1f** was so similar to **1e** in photolysis kinetics, we suspect that the acetyl group of **1f** had hydrolyzed spontaneously back to **1e** in the test solutions before the photolysis runs could begin. Indeed, a model for **1f**, 4′-(dimethylamino)-4,5-(methylenedioxy)-2-nitrobenzhydryl acetate, produced acetic acid with a half-life of a few minutes simply on standing in pH 7 buffer. Presumably the lability of such esters reflects stabilization of an S_N1-like intermediate by the (dialkylamino)phenyl group.

Scheme V



The slow component accounted for about 35% of the total absorbance change and had a rate of 0.17 s^{-1}, nearly independent of the Ca^{2+} level, reminiscent of but even slower than the decay transient of **1b**. Compound **1b** itself showed no such slower component.

Scheme IV summarizes the properties and dominant interconversion rates of the chelators **1b,e–g** (nitr-2, -5, -6, -7) with respect to Ca^{2+} binding and photolysis. The heavy arrows emphasize the pathways responsible for light-induced ejection of Ca^{2+}.

Discussion

Synthesis. The key step in the efficient preparation of the photosensitive benzhydrols was the reaction of dialkylanilines **4** or **7g** with aldehydes or acetals in a smooth electrophilic substitution activated by TMS OTf but not by conventional proton or Lewis acids. Almost nothing has been reported on TMS OTf as a promoter of aromatic as opposed to aliphatic electrophilic substitution or with substrates activated by amino rather than ether substituents.[46] Since previous work with TMS OTf had stressed the unreactivity of aldehydes compared with acetals or ketals,[36] we were pleasantly surprised to find that 6-nitropiperonal (**6**) required only slightly more vigorous conditions than its acetal (**5b**), 25 °C instead of 0 °C, to react with **4**. A plausible reaction mechanism is shown in Scheme V. Presumably the carbonyl oxygen can displace triflate from TMS OTf to give a [(trimethylsilyl)oxy]carbonium ion analogous to the methoxycarbonium ion formed by TMS OTf abstraction of methoxide from the dimethyl acetal. Either carbonium ion can attack **4**, but only the silyl group can be readily removed later. TMS OTf was the only Lewis acid found that activates the electrophile without deactivating the amine substrate **4**. The hindered base 2,6-di-*tert*-butylpyridine serves to mop up triflic acid that would otherwise protonate **4**. Though no attempt has been to investigate the full scope of the reaction, TMS OTf would seem to have considerable promise in promoting electrophilic aromatic substitution, especially with acid-sensitive substrates or electrophiles activatable by silyl coordination to oxygen.

Chelator Design and Properties. Table I shows the order in which the photolabile chelators were prepared. Compound **1a**, containing the simplest *o*-nitrobenzyl substituent, established the synthetic feasibility and photochemical workability of the *o*-nitrobenzhydrol design, but its biological utility would be limited by its low extinction coefficient above 300 nm. Addition of alkoxy substituents to the nitrobenzyl group as in **1b** greatly improved the long-wave UV absorbance and thereby enabled successful preliminary neurophysiological experiments.[27] However, increases in quantum efficiency, Ca^{2+} affinity, and speed of release were still desired, prompting the design and synthesis of further analogues. Compound **1c** was prepared with two *o*-nitro groups in the hope that doubling the number of potentially reactive groups might increase the quantum efficiency of oxygen transfer to the benzhydryl carbon. Such naive logic did correctly predict that a model compound, 2,6-dinitro-3-methoxybenzyl alcohol, would have a higher quantum efficiency of photoisomerization than 6-nitropiperonyl alcohol. However, **1c** proved to have quantum efficiency not significantly better than **1b**, perhaps because the steric hindrance of an aryl group replacing a benzylic hydrogen allowed only one nitro group to be correctly oriented for reaction. Since **1c** also had a lower extinction coefficient than **1b** in the long-wave UV, it was inferior overall.

Another shortcoming of **1b** was its limited affinity for Ca^{2+} before photolysis, corresponding to a dissociation constant of 160 nM at 0.1 M ionic strength. In a typical resting cell with a free Ca^{2+} concentration near 100 nM, only a fraction of the chelator molecules would carry Ca^{2+}, obviously restricting the amount that could be released. Furthermore, unphotolyzed molecules would tend to buffer the Ca^{2+} released by their photolyzed neighbors; also, the Ca^{2+}-free chelator has several-fold lower quantum efficiency than the Ca^{2+} complex. One way to increase the Ca^{2+} affinity would be to add electron-donating substituents to the aryl rings.[21] However, such substituents would also increase the Mg^{2+} and H^+ affinities and the susceptibility to autoxidation. Instead, we preferred to manipulate the stereochemistry of the binding site to favor Ca^{2+} even more strongly over potential competitors. Both the complexation constants and the UV spectra of the parent chelator BAPTA show that each Mg^{2+} or H^+ binds only to one of the two aryliminodiacetate moieties that makes up the molecule, whereas Ca^{2+} binding involves both halves simultaneously.[21] One way to favor Ca^{2+} yet further over Mg^{2+} and H^+ would be to preorganize an *s*-cis conformation of the OCH_2CH_2O bridge to make the two halves ready to fit around Ca^{2+}. Such conformational stabilization could be achieved by adding alkyl substituents to the bridge or by incorporating it into a cycloalkane ring. The most complete series of analogous chelator modifications is with EDTA, where addition of methyl groups or annelation of a five- or six-membered ring to the ethane bridge between the two amino groups can raise Ca^{2+} affinities up to 1000-fold over EDTA, as in *trans*-1,2-cyclohexanediamine-*N,N'*-tetraacetic acid.[20,47] We therefore prepared and tested analogues of **8h** with methyls or rings comprising R^6 and R^7. Somewhat to our surprise, both 1,2-cyclohexanediyl and 2,3-butanediyl bridges in either cis or trans forms (**8i–l**) slightly weakened the Ca^{2+} affinities (Table II). A *trans*-1,2-cyclopentanediyl linkage (**8m**) greatly weakened Ca^{2+} binding, perhaps by spreading the two halves of the molecule too far apart. Only *cis*-1,2-cyclopentanediyl (**8n**) significantly strengthened Ca^{2+} binding, though the 4-fold enhancement over **8h** was a much more modest effect than in the EDTA series. Nevertheless, the analogous improvement in Ca^{2+} affinity in **1g** over **1e** is quite valuable, especially because Mg^{2+} and H^+ affinities were not correspondingly increased. The main disadvantages of **1g** are a longer synthesis and some slowing of the Ca^{2+} release kinetics from 3000 to 550 s^{-1}.

The most successful series of modifications was to increase the speed of Ca^{2+} release after a flash. In **1b** the rate constant of 5 s^{-1} was limited by a slow dark reaction, the loss of methanol from hemiketal **2b**. When R^1 was changed to H as in **1e**, the single relaxation split into two components, the major one speeded 600-fold to 3000 s^{-1}, and a minor one slowed to 0.17 s^{-1}. We interpret these two components to represent two parallel pathways for breakdown of the intermediate resulting from initial photochemical removal of the hydrogen on the benzylic carbon. The slow component would proceed through the ketone hydrate **2e** analogous to the hemiketal **2b**, with final ketone formation slowed by the slightly greater basicity[48] and poorer leaving group ability of hydroxide compared with methoxide. The fast component would proceed to the ketone by proton shuffling and loss of OH from the *aci*-nitro group to the solvent, without transferral of an oxygen from nitrogen to the benzylic carbon. Analogous mechanisms have been proposed for the photolyses of *p*-nitrobenzyl

(46) The only reference that we have found to aromatic substrates is: Vorbruggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234–1255, who reported that neither anisole nor *N,N*-dimethylaniline gave a C-nucleoside with a peracylated ribose and TMS OTf, whereas 1,3,5-trimethoxybenzene did.

(47) Kroll, H.; Gordon, M. *Ann. N.Y. Acad. Sci.* **1960**, *88*, 341–352.
(48) Reeve, W.; Erikson, C. M.; Aluotto, P. F. *Can. J. Chem.* **1979**, *57*, 2747–2754.

alcohol[49] and *o*-nitrobenzaldehydes.[50] However, we have not done ^{18}O labeling, which would more conclusively decide whether the free benzhydrol can indeed photolyze to the nitrosobenzophenone without transferring an oxygen from the nitro group to the benzylic carbon.

It is noteworthy that the quantum efficiencies of photolysis are consistently higher for the Ca^{2+} complexes than for the free chelators. The metal ion can promote the photochemistry that leads to its own ejection, presumably because Ca^{2+} extrusion is driven by an irreversible dark reaction (carbonyl formation) well after the decision point between thermal deactivation and productive rearrangement has been passed. By contrast, when metal extrusion is an integral part of the primary photochemistry as in cis–trans isomerism of azo complexes, the presence of the metal is expected and found to have if anything a hindering effect.[16-19] The beneficial effect of Ca^{2+} on photolysis quantum yields of the present chelators is analogous to its enhancement of the fluorescence quantum yields of analogous indicator dyes.[21,28] The probable explanation is that Ca^{2+} binding to the iminodiacetate groups prevents them from encouraging parasitic inactivation as free dialkylamino groups are known to do.[51]

Chelator **1e** ("nitr-5") represents a reasonably satisfactory first solution to the challenge of "caging" intracellular Ca^{2+}. It has already been successfully applied in cultured rat sympathetic neurons.[52] With a single flash of a xenon lamp transmitted through a microscope illuminator, more than 50% of the 2 mM intracellular nitr-5 could be photolyzed, causing the buffered concentration of free Ca^{2+} to jump from 0.4 to 7.5 μM. This step increase in $[Ca^{2+}]$ activated a K^+-conducting channel in the neuronal membrane whose activation stoichiometry and kinetics could be quantified by varying the flash energy and $[Ca^{2+}]$ step amplitude.[52] With a suitable laser, even more complete photolysis should be achievable in a single flash. Nitr-5 has also shown that Ca^{2+} regulation of troponin rather than myosin ATP hydrolysis is rate limiting in the onset of skeletal muscle contraction.[53] Many other biological applications are in progress, thanks partly to the establishment of commercial production (e.g., Behring Diagnostics). For certain experiments, the higher starting Ca^{2+} affinity of **1g** ("nitr-7") may justify its considerably more tedious synthesis. The principle of photochemical manipulation of remote inductive substituents deserves consideration along with cis–trans photoisomerization in the design of photoactive molecules.

Experimental Section

Chemicals and solvents (HPLC grade) were used directly as obtained unless otherwise noted. CH_2Cl_2 and $CHCl_3$ were redistilled from P_2O_5, dimethoxyethane was dried over NaPb alloy, dimethylformamide was dried over 4A molecular sieve, and THF was redistilled from CaH_2.

Proton magnetic resonance spectra were recorded on a Varian EM-390 90-MHz spectrometer in $CDCl_3$ unless otherwise noted, and the chemical shifts are given in δ values from Me_4Si. UV absorbance spectra were recorded on a Cary 210 or a Perkin-Elmer Lambda Array 3840 spectrophotometer at 22 ± 2 °C. Melting points are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory in the Department of Chemistry at Berkeley.

Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F-254 or reverse-phase (RP-18 F-254 S, E. Merck) plates. For column chromatography, silica gel 60 (230–400 mesh, E. Merck) was used. Since all 2-nitrobenzaldehyde and 2-nitrobenzyl alcohol derivatives are sensitive to near ultraviolet light, manipulations were performed under an orange safety lamp.

***trans*-2-(5-Methyl-2-nitrophenoxy)cyclopentanol (9m) and Analogues 9i,l.** Cyclopentene oxide (8.73 mL, 0.1 mol), 5-methyl-2-nitrophenol (15.3 g, 0.1 mol), and potassium 5-methyl-2-nitrophenoxide (1.91 g, 0.01 mol) were dissolved in dry DMF (5 mL) and refluxed for 20 h under an Ar atmosphere. The cooled reaction mixture was diluted with aqueous NaOH solution (100 mL, 1 M) and extracted with toluene (three 50-mL portions). The combined extracts were washed with H_2O (3 × 50 mL) and dried (Na_2SO_4), toluene was removed by evaporation, and the product was distilled bulb-to-bulb at 0.15 mmHg; the fraction that distilled at 180–200 °C was collected to give **9m** (17.0 g, 72%), an orange oil which crystallized on cooling: mp 42–44 °C; ^1H NMR δ 1.9 (br m, 6 H, cyclopentyl CH_2), 2.40 (s, 3 H, CH_3), 4.40 (br m, 1 H, C*H*OH), 4.65 (m, 1 H, C*H*OAr), 6.80 (d, 1 H, J = 8 Hz, H-4), 6.93 (s, 1 H, H-6), 7.67 (d, 1 H, J = 8 Hz, H-3). Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.53; H, 6.30; N, 5.87.

Similarly, **9i** was synthesized from cyclohexene oxide. Recrystallization from hexane gave yellow crystals, yield 56%: mp 55–57 °C; ^1H NMR δ 1.50, 1.78, 2.1 (br m, 8 H, cyclohexyl), 2.40 (s, 3 H, CH_3), 3.30 (s br, 1 H, OH), 3.73 (m, 1 H, C*H*OH), 4.05 (m, 1 H, C*H*OAr), 6.78 (d, 1 H, J = 8 Hz, H-4), 6.90 (s, 1 H, H-6), 7.67 (d, 1 H, J = 8 Hz, H-3).

Reaction of *trans*-2,3-epoxybutane afforded crude **9l** as an oil (16% yield), which was used without further purification: ^1H NMR δ 1.20, 1.30 (2 d, 6 H, J = 5 Hz, butyl CH_3), 2.40 (s, 3 H, Ar CH_3), 2.72 (s, 1 H, OH), 4.00, 4.53 (2 m, 2 H, J = 3 Hz, CH), 6.85 (d, 2 H, J = 8 Hz, H-4), 6.95 (s, 1 H, H-6), 7.80 (d, 2 H, J = 8 Hz, H-3).

2-(5-Methyl-2-nitrophenoxy)cyclopentanone (10n) and -hexanone (10j). Compound **9m** (8.3 g, 35 mmol) dissolved in CH_2Cl_2 (10 mL) was added in one portion to a stirred suspension of pyridinium chlorochromate (11.3 g, 55 mmol) in CH_2Cl_2 (70 mL) and stirred at room temperature for 16 h. The reaction mixture was diluted with Et_2O (350 mL) and decanted from the dark tar, which was washed (3 × 50 mL) with Et_2O. The combined extracts were filtered through Celite and evaporated to dryness to yield an oil, which crystallized. Recrystallization from MeOH gave **10n** as yellow crystals (6.60 g, 80%): mp 65–66 °C; ^1H NMR δ 2.10 (m, 6 H, $(CH_2)_3$), 2.33 (s, 3 H, CH_3), 4.63 (t, 1 H, CH), 6.80 (d, 1 H, J = 8 Hz, H-4), 7.02 (s, 1 H, H-6), 7.70 (d, 2 H, J = 8 Hz, H-3). Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.10; H, 5.57; N, 5.94.

Oxidation of the cyclohexanol derivative **9i** required a 6-fold excess of pyridinium chlorochromate and a reaction time of 5 days to give **10j** (88% yield) as a yellow solid, recrystallized from isopropyl ether: mp 125–128 °C; ^1H NMR δ 1.6–2.6 (m's, 8 H, $(CH_2)_4$), 2.33 (s, 3 H, CH_3), 4.63 (t, 1 H, CH), 6.80 (d, 1 H, J = 8 Hz, H-4), 7.02 (s, 1 H, H-6), 7.73 (d, 2 H, J = 8 Hz, H-3).

***cis*-2-(5-Methyl-2-nitrophenoxy)cyclopentanol (11n) and -hexanol (11j).** Compound **10n** (5.86 g, 25 mmol) dissolved in dry THF (25 mL) at −10 °C was added dropwise with stirring to a solution of LS-Selectride (Aldrich) (30 mmol) in THF (30 mL) at −78 °C under a N_2 atmosphere. After 2 h, the red reaction mixture was allowed to warm up to room temperature (1 h), quenched with H_2O (4 mL) and EtOH (15 mL), made alkaline with aqueous KOH (6 mL, 10 M), and oxidized by cautious addition of 30% aqueous H_2O_2 (15 mL) with cooling. After being saturated with K_2CO_3, the aqueous layer was separated and washed with Et_2O–THF (1:1, 2 × 10 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness, and the product, **11n**, was distilled bulb-to-bulb at 180–200 °C at 0.1 mmHg to yield an orange oil (4.55 g, 77%): ^1H NMR δ 1.9 (br m, 6 H, $(CH_2)_3$), 2.40 (s, 3 H, CH_3), 3.05 (br s, 1 H, OH), 4.20, 4.66 (2 m, 2 H, CH), 6.82 (d, 1 H, J = 8 Hz, H-4), 6.90 (s, 1 H, H-6), 7.76 (d, 1 H, J = 8 Hz, H-3). Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.23; H, 6.22; N, 5.85. Thin-layer chromatography in a system that separated the cis and trans isomers, **11n** and **9m**, respectively, indicated complete conversion to the cis isomer.

Similarly, **10j** gave **11j** as a dark orange oil (54% yield) after chromatography on SiO_2 in ethyl acetate–hexane: ^1H NMR δ 1.68 (br m's, 8 H, $(CH_2)_4$), 2.40 (s, 3 H, CH_3), 2.70 (br d, 1 H, OH), 3.83 (br m, 1 H, C*H*), 4.53 (m, 1 H, C*H*OAr), 6.80 (d, 1 H, J = 8 Hz, H-4), 6.90 (s, 1 H, H-6), 7.76 (d, 1 H, J = 8 Hz, H-3).

***cis*-3-(2-Nitrophenoxy)tetrahydrofuran-4-ol (12o) and (*R*,*R*)-2-(2-Nitrophenoxy)butan-3-ol (12k).** NaH (42 mg 57% suspension in oil, 1 mmol) was added portionwise with stirring to a solution of *cis*-tetrahydrofuran-3,4-diol[54] (0.21 g, 2 mmol) and 2-fluoronitrobenzene (105 μL, 1 mmol) in dry DMF (1 mL). Thirty minutes after the final addition, the reaction mixture was diluted with H_2O (15 mL) and cooled on ice for at least 30 min. The solid precipitate of the diarylated byproduct was filtered off, the filtrate was extracted with toluene (3 × 5 mL), and the combined extracts were dried (Na_2SO_4) and evaporated to dryness to yield the product **12o** as a yellow oil that crystallized on trituration with isopropyl ether (yield 111 mg), mp 59–61 °C. TLC (5% MeOH–$CHCl_3$) showed less than 5% disubstituted byproduct. The product was used without further purification: ^1H NMR δ 3.13 (d, 1 H, OH), 3.4–4.2 (m's, 4 H, CH_2OCH_2), 4.45, 5.80 (2 m, 2 H, CH), 6.9–8.0 (m, 4 H, aromatic).

(49) Wan, P.; Yates, K. *Can. J. Chem.* **1986**, *64*, 2076–2086.
(50) George, M. V.; Scaiano, J. C. *J. Phys. Chem.* **1980**, *84*, 492–495.
(51) Drexhage, K. H. In *Dye Lasers*; Schaefer, F. P., Ed.; Springer Verlag: Berlin, 1973; pp 144–193.
(52) Gurney, A. M.; Tsien, R. Y.; Lester, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 3496–3500.
(53) Ashley, C. C.; Barsotti, R. J.; Ferenczi, M. A.; Lea, T. J.; Mulligan, I. P.; Tsien, R. Y. *J. Physiol. (London)* **1987**, *390*, 144P.
(54) Otey, F. H.; Mehltretter, C. L. *J. Org. Chem.* **1961**, *26*, 1673.

Similarly, addition of NaH (4 mmol) to a solution of (*R,R*)-(−)-2,3-butanediol (5 mmol) and 2-fluoronitrobenzene (4 mmol) in dry *N*-methylpyrrolidinone (2.5 mL), quenching with H_2O after 30 min, and extraction into ethyl acetate gave a mixture of the mono- and disubstituted products, which were separated on SiO_2 by eluting with ethyl acetate–hexane to give a yellow oil, **12k** (45%), and white solid (14%), respectively: ^1H NMR δ 1.25, 1.36 (2 d, 6 H, J = 7 Hz, CH_3), 3.15 (br s, 1 H, OH), 3.86 (br q, 1 H, C*H*OH), 4.27 (q, 1 H, C*H*OAr), 6.8–7.8 (m, 4 H, aromatic).

***cis*-1-(5-Methyl-2-nitrophenoxy)-2-(2-nitrophenoxy)cyclopentane and Analogues 13i–o.** NaH (1.05 g, 57% oil suspension, 25 mmol) was added portionwise with stirring and cooling to a solution of **11n** (4.27 g, 18 mmol) and 2-fluoronitrobenzene (2.11 mL, 20 mmol) in dry dimethoxyethane (25 mL). After having stood at room temperature for 1 h, the reaction mixture was diluted with H_2O (100 mL) and extracted with $CHCl_3$ (3 × 50 mL); the extracts were dried and evaporated to dryness to yield the crude product **13n** as an oil, which crystallized on chilling. Recrystallization from acetone–methanol gave yellow crystals (4.8 g, 74%): mp 109–111 °C; ^1H NMR δ 1.7–2.2 (m, 6 H, $(CH_2)_3$), 2.35 (s, 3 H, CH_3), 4.87 (m, 2 H, CH), 6.7–7.8 (m's, 7 H, aromatic). Anal. Calcd for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.20; H, 4.97; N, 7.79.

Similarly, **13i,j,l,m** were synthesized from the corresponding alcohols by the same method. The yield and physical and spectral properties were as follows. **13l**: yellow crystals; mp 86–90 °C (60%); ^1H NMR δ 1.3–2.2 (m's, 8 H, $(CH_2)_4$), 2.40 (s, 3 H, CH_3), 4.63 (m, 2 H, CH), 6.8–7.8 (m, 7 H, aromatic). **13j**: brown oil (SiO_2 chromatography, ethyl acetate–hexane); yield 74%; ^1H NMR δ 1.3–2.2 (m's, 8 H, $(CH_2)_4$), 2.37 (s, 3 H, CH_3), 4.70 (br d, 2 H, CH), 6.8–7.8 (m's, 7 H, aromatic). **13l**: pale yellow solid; mp 86 °C; purified by SiO_2 chromatography (64%); ^1H NMR δ 1.47 (d, 6 H, J = 6.5 Hz, CH_3), 2.42 (s, 3 H, $ArCH_3$), 4.70 (q, d, 2 H, J = 6.5, 3 Hz, CH), 6.8–8.0 (m, 7 H, aromatic). **13m**: orange oil; distilled bulb-to-bulb at 230 °C (0.25 mmHg) (63%); ^1H NMR δ 1.6–2.1 (m, 6 H, $(CH_2)_3$), 2.40 (s, 3 H, CH_3), 4.75 (m, 2 H, CH), 6.8–7.8 (m, 7 H, aromatic).

Compounds **13k** and **13o** were similarly prepared from alcohols **12k,o**, respectively, but by substituting 3-fluoro-4-nitrotoluene[55] for 2-fluoronitrobenzene. Compound **13k** was an orange oil, separated by SiO_2 chromatography (ethyl acetate–hexane), yield 45%: ^1H NMR δ 1.33 (d, 6 H, J = 6.5 Hz, CH_3), 2.40 (s, 3 H, $ArCH_3$), 4.75 (m, 2 H, CH), 6.7–7.8 (m's, 7 H, aromatic). Compound **13o** was obtained as a pale yellow precipitate on quenching the reaction mixture followed by recrystallization from ethanol, yield 63%: mp 145–148 °C; ^1H NMR δ 2.37 (s, 3 H, CH_3), 4.21 (m, 4 H, CH_2OCH_2), 5.07 (m, 2 H, CH), 6.8–7.8 (m's, 7 H, aromatic).

***cis*-1-(2-Amino-5-methylphenoxy)-2-(2-aminophenoxy)cyclopentane and Analogues 14i–o.** Compound **13n** (2.0 g, 5.58 mmol) was catalytically hydrogenated at room temperature and pressure with 200 mg of 5% Pd/C in ethyl acetate–95% aqueous EtOH (2:1). Uptake was complete within 1 h, and after a further 1 h, the reaction mixture was filtered and evaporated to dryness to yield the product **14n** as a pale brown oil, which was used in the following reaction without further purification: ^1H NMR δ 1.6–2.2 (br m, 6 H, $(CH_2)_3$), 2.18 (s, 3 H, CH_3), 3.67 (br s, 4 H, NH_2), 4.63 (m, 2 H, CH), 6.4–6.8 (m, 7 H, aromatic).

Similarly, **13i–m,o** were hydrogenated over Pd/C catalyst in ethyl acetate or ethanol, and the products **14i–m,o**, usually oils, which darkened on exposure to air, gave the expected NMR spectra and were used without further purification.

***cis*-1-[2-[Bis[(methoxycarbonyl)methyl]amino]-5-methylphenoxy]-2-[2-[bis[(methoxycarbonyl)methyl]amino]phenoxy]cyclopentane and Analogues 15i–o.** The product **14n** from the previous reaction, assumed quantitative (i.e., 5.58 mmol), 1,8-bis(dimethylamino)naphthalene (7.29 g, 34 mmol), NaI (0.45 g, 3 mmol), and methyl bromoacetate (3.22 mL, 34 mmol) were refluxed in dry CH_3CN (75 mL) under N_2 for 16 h. The cooled reaction mixture was diluted with toluene (150 mL), filtered, washed with phosphate buffer pH 2 until washings were pH 2, and washed with H_2O. The organic layer was dried ($MgSO_4$) and evaporated to dryness to yield an oil, which was chromatographed on SiO_2 with ethyl acetate–hexane as eluant to yield **15n** as a colorless oil (2.77 g, 85%), which should be stored below room temperature to prevent its ready decomposition and oxidation: ^1H NMR δ 2.0 (br m, 6 H, $(CH_2)_3$), 2.15 (s, 3 H, $ArCH_3$), 3.61 (s, 12 H, OCH_3), 4.13, 4.18 (2 s, 8 H, CH_2), 4.77 (m, 2 H, CH), 6.5–6.8 (m, 7 H, aromatic). Anal. Calcd for $C_{30}H_{38}N_2O_{10}$: C, 61.42; H, 6.53; N, 4.78. Found: C, 58.86; H, 6.35; N, 4.57.

Similarly, amines **14i–m,o** were alkylated with ethyl or methyl bromoacetate and after SiO_2 chromatography gave the expected products **15i–m,o** as oils of which only one, **15l**, crystallized (from isopropyl ether, mp 67–69 °C), yields 30–80%. ^1H NMR spectra were as expected; the shifts (ppm) of the ethane bridge protons were as follows: **15i**, 4.50; **15j**, 4.65; **15k**, 4.77; **15l**, 4.51; **15m**, 4.80; **15o**, 5.13.

Nitr-7 Tetramethyl Ester (7g). Trimethylsilyl trifluoromethanesulfonate (TMS OTf) (0.5 mL, 2.54 mmol) was added dropwise to a solution of **15n** (293 mg, 0.5 mmol), 6-nitropiperonal (**6**) (127 mg, 0.65 mmol) and 2,6-di-*tert*-butylpyridine (0.67 mL, 3 mmol) in dry $CHCl_3$ (3 mL) at room temperature under a N_2 atmosphere. After standing overnight, the reaction solution was diluted with $CHCl_3$ (10 mL), poured into saturated aqueous $NaHCO_3$ (50 mL), and separated, and the aqueous layer was extracted (2 × 15 mL) with $CHCl_3$. The combined extracts were washed with H_2O, dried ($MgSO_4$), and evaporated to dryness to yield the crude TMS ether as a yellow oil. This was dissolved in $CHCl_3$ (25 mL), and tetrabutylammonium fluoride trihydrate (475 mg, 1.5 mmol) was added in one portion. After 15 min at room temperature, the solution was evaporated to dryness, dissolved in ethyl acetate–toluene (1:9 v/v, 100 mL), washed with H_2O (3 × 20 mL), dried ($MgSO_4$), and evaporated to yield the crude product as an orange oil. Separation by SiO_2 chromatography with ethyl acetate–hexane as eluant gave **7g** as a yellow, glassy foam, yield 53%: mp 44–49 °C; ^1H NMR δ 2.0 (br m, 6 H, $(CH_2)_3$), 2.18, 2.23 (2 s, 3 H, $ArCH_3$), 3.58, 3.60, 3.62, 3.64 (4 s, 12 H, OCH_3), 3.99, 4.02, 4.15 (3 s, 8 H, NCH_2), 4.83 (br m, 2 H, cyclopentane CHO), 6.03 (s, 2 H, OCH_2O), 6.30 (d, 1 H, Ar_2CH), 6.60–6.93 (m, 6 H, aromatic), 7.11 (s, 1 H, piperonyl H-2), 7.28 (s, 1 H, OH), 7.37, 7.41 (2 s, 1 H, piperonyl H-5). Anal. Calcd for $C_{38}H_{43}N_3O_{15}$: C, 58.38; H, 5.54; N, 5.38. Found: C, 57.76; H, 5.89; N, 4.96.

Nitr-5 Tetramethyl Ester (7e). Similarly, **7e** was prepared from **4**. The trimethylsilyl ether of **7e** tetramethyl ester was isolated by SiO_2 chromatography in ethyl acetate–hexane as a pale yellow solid, yield 80%. Recrystallization from ethyl acetate–hexane gave mp 128 °C: ^1H NMR 0.12 (s, 9 H, Me_3Si), 2.22 (s, 3 H, Ar CH_3), 3.48, 3.50 (2 s, 12 H, OCH_3), 4.05 (s, 8 H, NCH_2), 4.13 (s, 4 H, CH_2CH_2), 5.98 (s, 2 H, OCH_2), 6.38 (s, 1 H, Ar_2CH), 6.48–6.83 (m, 6 H, aromatic), 7.11 (s, 1 H, piperonyl H-2), 7.25 (s, 1 H, piperonyl H-5). Anal. Calcd for $C_{38}H_{47}N_3O_{15}Si$: C, 56.08; H, 5.82; N, 5.16. Found: C, 55.82; H, 5.89; N, 5.08. The trimethylsilyl group was removed as with **7g** to yield **7e** tetramethyl ester, yield 49% (overall from **4**) of pale yellow crystals following purification by SiO_2 chromatography and recrystallization from MeOH: mp 142–143 °C; ^1H NMR δ 2.33 (s, 3 H, Ar CH_3), 2.53 (s, 12 H, OCH_3), 4.03, 4.08 (2 s, 8 H, NCH_2), 4.18 (s, 4 H, OCH_2CH_2O), 5.98 (s, 2 H, OCH_2O), 6.23 (br d, 1 H, Ar_2CH), 6.60 (m, 6 H, aromatic), 6.83 (br s, 1 H, OH), 7.08 (s, 1 H, piperonyl H-2), 7.38, 7.40 (2 s, 1 H, piperonyl H-5). Anal. Calcd for $C_{35}H_{39}N_3O_5$: C, 56.68; H, 5.30; N, 5.67. Found: C, 56.84; H, 5.36; N, 5.70.

Other Esters (7a–d) of Photolabile Chelators. Compounds **7a–d** were synthesized by a similar method except that 6-nitropiperonal (**6**) was replaced by 2-nitrobenzaldehyde dimethyl acetal (**5a**) for **7a**, 2,6-dinitro-3-methoxybenzaldehyde dimethyl acetal (**5b**) for **7c**, and 6-nitropiperonal dimethyl acetal (**4c**) for **7a** and **7d**. Acetals were made by reacting the aldehydes with methoxytrimethylsilane and TMS OTf.[36] The desilylation with tetrabutylammonium fluoride was omitted, and the products were purified by chromatography on SiO_2, yields 30–75%. **7a** (nitr-1 tetramethyl ester): ^1H NMR δ 2.20 (s, 3 H, Ar CH_3), 3.38 (s, 3 H, Ar_2OCH_3), 3.50 (2 s, 12 H, ester CH_3), 4.0–4.4 (m, 12 H, OCH_2, NCH_2), 5.83 (s, 1 H, Ar_2CH), 6.5–7.8 (m, 10 H, aromatic). **7b** (nitr-2 tetraethyl ester) was recrystallized from isopropyl ether: mp 89–90 °C; ^1H NMR (200 MHz) δ 1.11 (t, 3 H, CH_2CH_3), 2.25 (s, 3 H, Ar CH_3), 3.31 (s, 3 H, OCH_3), 4.00 (q, 8 H, OCH_2CH_3), 4.09, 4.11 (2 s, 8 H, NCH_2), 4.24 (s, 4 H, OCH_2CH_2O), 5.93 (s, 1 H, Ar_2CH), 6.09 (2 s, 2 H, OCH_2O), 6.65–6.87 (m, 6 H, aromatic), 7.14 (s, 1 H, piperonyl H-2), 7.44 (s, 1 H, piperonyl H-5). Anal. Calcd for $C_{40}H_{49}N_3O_{15}$: C, 59.18; H, 6.08; N, 5.18. Found: C, 58.83; H, 6.02; N, 5.33. **7c** (nitr-3 tetramethyl ester): ^1H NMR δ 2.17 (s, 3 H, Ar CH_3), 3.22 (s, 3 H, Ar_2OCH_3), 3.41, 3.43 (2 s, 12 H, ester CH_3), 3.85 (3 H, s, $ArOCH_3$), 3.95–4.20 (m, 12 H, OCH_2,NCH_2), 5.87 (2, 1 H, Ar_2CH), 6.4–6.8 (m, 6 H, aromatic), 6.95 (d, 1 H, J = 9 Hz, H-4), 7.85 (d, 1 H J = 9 Hz, H-5). **7d** (nitr-4 tetraethyl ester) was an orange oil: ^1H NMR δ 1.17 (t, 12 H, CH_2CH_3), 1.97 (br m, 6 H, $(CH_2)_3$), 2.13, 2.18 (2 s, 3 H, Ar CH_3), 3.27 (s, 3 H, OCH_3), 4.13 (q, 8 H, OCH_2CH_3), 4.18 (s, 8 H, NCH_2), 4.78 (br m, 2 H, cyclopentane CHO), 5.85 (s, 1 H, Ar_2CH), 6.03 (s, 2 H, OCH_2O), 6.58–6.93 (br m, 6 H, aromatic), 7.08 (s, 1 H, piperonyl H-2), 7.38, 7.40 (2 s, 1 H, piperonyl H-5). Anal. Calcd for $C_{43}H_{53}N_3O_{15}$: C, 60.62; H, 6.27; N, 4.93. Found: C, 60.31; H, 6.17; N, 4.95.

Saponification of Chelator Esters. Esters **7a–e,g** and **15i–o** were dissolved in dioxane–methanol, 1:1, and saponified by addition of an excess of 1 M aqueous KOH, warming as necessary to aid dissolution. Reaction was complete within a few hours or after standing overnight. Usually, chelators **1a–e,g** and **8i–o** were stored frozen in these diluted saponifi-

(55) Schiemann, G. *Chem. Ber.* **1929**, *62*, 1794–1805.

cation mixtures. The free acids could be isolated by acidification and collection of the precipitate.

Nitr-6 Free Acid (1f). Compound **1f** was prepared from the solid free acid of **1e** by acetylation in an excess of acetic anhydride with 3 equiv of pyridine. Reaction was complete in a few hours. Addition of Et_2O to the reaction mixture precipitated the crude product as a sticky gum, which hardened to a pale yellow solid on chilling to 0 °C. The product was washed with dry Et_2O and desiccated. These conditions with parent compound **4a** yield the dianhydride and/or a variety of mixed anhydrides as shown by NMR and IR spectra. The crude **1f** anhydride was homogeneous on reverse-phase TLC (R_f 0.2) in 30% MeOH–70% H_2O saturated with KH_2PO_4, under which conditions **1e** free acid has R_f 0.8. The tetraanion of **1f** was prepared by dissolving the anhydride in saturated aqueous $NaHCO_3$ solution with gentle warming. Anal. Calcd for $C_{33}H_{33}N_3O_{16}$: C, 54.51; H, 4.57; N, 5.78. Found: C, 54.69; H, 4.34; N, 5.57.

Calcium and Magnesium Affinities. Ca^{2+}-binding constants for chelators **1a–g** before and after photolysis and of **8h–o** were determined by monitoring UV spectra during titration of EGTA or HEEDTA buffers to varying free Ca^{2+} levels.[21,28] Either the ratio of, for example, [CaEGTA] to unbound [EGTA] was adjusted to a constant pH, or the pH was varied while [CaEGTA] = [EGTA]. These two approaches gave equivalent answers for pH >7 whenever directly tested, thanks to the pH insensitivity of BAPTA-like ligands.[21] The apparent dissociation constants of Ca/EGTA and Ca/HEEDTA were calculated as described elsewhere.[20,27,28]

Free [Mg^{2+}] was likewise controlled by Mg^{2+}/EGTA buffers, assuming an apparent dissociation constant for the Mg^{2+}·EGTA complex (including its monoprotonated form) of 6 mM at pH 7.60 in 0.1 M ionic strength.[20,27]

Quantum Efficiencies of Photolysis. The photolysis quantum efficiencies for chelators **1a–g** were determined by irradiating a buffered solution of the substrate with a known intensity of long-wave UV light either from a UVGL-58 hand mercury lamp (Ultraviolet Products, San Gabriel, CA) or from a B-100 mercury lamp (Spectronics Corp., Westbury, NY). The output of these lamps peaked at the 365-nm Hg line, but the UVGL-58 included a substantial contribution from a bell-shaped spectral distribution whose wavelengths were half-maximal at 338 and 375 nm. With these relatively low intensity lamps, minutes to tens of minutes were required for near-complete photolysis, so the overall rate of conversion was limited by the rate of photon capture and the quantum efficiency rather than the rates of any of the subsequent dark reaction steps. The specimen compartment of the Perkin-Elmer 3840 spectrometer was modified so that the thermostated sample in a 1-cm cuvet could be irradiated in place with the actinic light entering at right angles to the measurement beam. An electronic shutter (A.W. Vincent Inc., Rochester, NY) controlled the duration of each irradiation period. Between each irradiation, the absorbance spectrum was measured without having to move the cuvet. The diode array spectrophotometer required only a few seconds of dim UV to acquire a spectrum and blocked the measurement beam at all other times, so it caused negligible photolysis. Because the conversion to a relatively photostable end product appeared clean with good isosbestic points, the progress of the reaction was simply measurable by comparison of the absorbance spectrum with those of the unphotolyzed and fully photolyzed end points. Optical densities at the irradiating wavelength of 365 nm were kept below 0.2 so that inner-filtering of the irradiation could be neglected, and the progress curves were simple decaying exponentials. Quantum efficiencies were calculated[56] as $(I\epsilon t_{90\%})^{-1}$, where I is the irradiation intensity in einsteins cm^{-2} s^{-1}, ϵ is the decadic extinction coefficient in cm^2 (mol substrate)$^{-1}$ (10^3 times the conventional extinction coefficient in M^{-1} cm^{-1}), and $t_{90\%}$ is the irradiation time in seconds for 90% conversion to product. The total UV intensity I was measured each experimental day by actinometry with 6 mM potassium ferrioxalate[57] in the same setup. These intensities were $(1–5) \times 10^{-8}$ einsteins cm^{-2} s^{-1}. The quantum efficiencies reported here are somewhat lower than given in a preliminary report[27] on **1b** ("nitr-2"). Some of the discrepancy is due to upward revision of ϵ due to better purification. Also, in the earlier work the irradiation was gated by manually moving the sample from Hg lamp to spectrophotometer, a crude procedure not very accurate for exposures of only a few seconds as used in the ferrioxalate actinometry.

Kinetics of Photolysis. Kinetics of the conversion of **1b,e–g** to the nitrobenzophenone products or Ca^{2+} release were measured by standard methods of flash photolysis. For convenience of kinetic recording, the experiments were done in a Dialog apparatus[58] (Garching, FRG) normally used for temperature jumps. The xenon flash lamp was a Model 6100SP7 (Photochemical Research Associates, London, Ontario, Canada) modified to give a light pulse with a full width at half maximum of 85 μs. Neither the flash lamp nor the kinetic apparatus was optimized for light energy, so the response amplitude achieved in these experiments (Figure 2) is far from the maximum achievable during a biological experiment. Control flashes delivered to photoinactive solutions (Figure 2A, dotted trace) showed that the flash duration and detector overload recovery did not limit the time resolution. Briefly, a steady, low-intensity monitoring beam was used to record the transmittance of a chelator sample at 300, 365, or 450 nm (Hg lamp) or 375–385 nm (tungsten source plus interference filter) before and immediately after a flash from the unfiltered xenon lamp coming from right angles. The time course of the increase in absorbance was taken to signal the formation of the nitrosobenzophenone, which has a higher absorbance than the starting chelator both in high or low [Ca^{2+}]. The observed time constants were similar at all four wavelengths tested, with no sign of transient intermediates absorbing at long wavelengths. The transmitted light signal was digitized by a Biomation transient recorder and analyzed for multiple exponential decay times by a program, DISCRETE,[59,60] running on a VAX11/780. Measurements of the intrinsic absorbance of the chelator were made at very low free Ca^{2+} (2 mM EGTA in the sample), high Ca^{2+} (2 mM $CaCl_2$), and intermediate levels, 1 μM for nitr-2, -5, and -6 (**1b,e,f**), 0.4 μM for nitr-7 (**1g**), maintained by 10 mM CaHEEDTA/HEEDTA buffer. The solutions also contained 50 μM chelator, 20 mM MOPS buffer (pH 7.2), and 150 mM KCl. Independent experiments with a fast-scanning diode array spectrophotometer showed that by 1 s after the flash the entire UV difference spectrum was similar in shape to its final value, showing that the kinetics in Figure 2 correspond to formation of end product rather than a transient intermediate.

Measurements of Ca^{2+} release from nitr-5 (**1e**) were made with bromthymol blue monitored at 625 nm to detect the protons released by the binding of the Ca^{2+} to HEEDTA. The samples contained 50–100 μM nitr-5, 16 μM bromthymol blue, 150 mM KCl, 0.5 to 10 mM CaHEEDTA/HEEDTA buffer, preset to 1 μM free [Ca^{2+}] and pH 7.3–7.5. To minimize pH buffering, the samples were bubbled for several minutes with helium just prior to flash photolysis. All runs were at 23 °C.

(56) Livingston, R. In *Techniques of Chemistry. Vol. 3: Photochromism*; Brown, G. H., Ed.; Wiley: New York, 1971; pp 13–44.

(57) Hatchard, C. G.; Parker, C. A. *Proc. R. Soc. London, A* **1956**, *235*, 518–536.

(58) Rigler, R.; Rubl, C.-R.; Jovin, T. M. *Rev. Sci. Instrum.* **1974**, *45*, 580–588.

(59) Provencher, S. W. *Biophys. J.* **1976**, *16*, 27–41.

(60) Provencher, S. W. *J. Chem. Phys.* **1976**, *64*, 2772–2777.