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Substitution Effect on the One- and Two-photon Sensitivity of DMAQ "Caging" Groups

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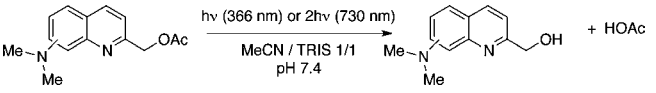
ABSTRACT



The systematic SAR study of a "caging" group showed a strong influence of the position of the donor dimethylamino group on the efficiency of photolysis of the DMAQ (2-hydroxymethylene-(*N,N*-dimethylamino)quinoline) caged acetate under one-photon near-UV or two-photon near-IR excitation. Photorelease of L-glutamate by the most efficient 8-DMAQ derivative strongly and efficiently activated glutamate receptors, generating large, fast rising responses similar to those elicited by glutamate photoreleased from the widely used MNI-caged glutamate.

Cell physiology and experimental neuroscience rely increasingly on the use of photoactivable "caging" groups that permit the release of biologically active ligands with high spatial and temporal accuracy.[1] None of the currently available cages satisfy fully the stringent photophysical and physicochemical conditions for two-photon excitation applied in cell physiology or neuroscience: most show slow fragmentation kinetics, poor one-photon- (OP) and particularly two-photon (TP) sensitivity, poor solubility and show hydrolytic instability in physiological solution. The main difficulty in the rational design of such compounds is the lack of reliable models and general rules that could predict structure-related photophysical and fragmentation proprieties. While absorption spectra can be accurately predicted by *ab initio* computation methods, the fate of the excited state remains largely unpredictable, depending on the chemical structure and also on the photolysis conditions. The rational design of caged substrates stands essentially on empirical trial and error methods.[2]

As part of a larger program on the development of caged compounds,[3] we were interested in examining substituent effects on the aminoquinoline (AQ) scaffold introduced earlier by the Dore group (Figure 1).[4]

The parent 2-hydroxymethylene-8-bromo-7-hydroxyquinoline, (BHQ **1**, Figure 1) shows fast fragmentation kinetic in physiological solutes, high uncaging sensitivity, solubility and low fluorescence.[4f,5] It was shown earlier, that both photophysical properties and the fragmentation of the quinoline scaffold are markedly influenced by the substitution pattern.[4e,f,j] In addition dialkylaminoquinoline derivatives having the NR_2 substituent in the 6 or 7 position and bearing dicarboxylic substituents (of interest for water solubilization) were recently shown to exhibit acceptable two-photon absorption in the near IR region (with typical

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(1) Selected reviews on "caged" compounds: (a) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921. (b) Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619–628. (c) Ellis-Davies, G. C. R. *Chem. Rev.* **2008**, *108*, 1603–613. (d) Warther, D.; Gug, S.; Specht, A.; Bolze, F.; Nicoud, J.-F.; Mourot, A.; Goeldner, M. *Bioorg. Med. Chem.* **2010**, *18*, 7753–7758. (e) Brieke, C.; Rohrbach, F.; Gottschalk, A.; Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 8446–8476. (f) *Photochem. Photobiol. Sci.* **2012**, *11* (Thematic issue).

(2) Bandara, H. M. D.; Walsh, T. P.; Burdette, S. C. *Chem.—Eur. J.* **2011**, *17*, 3932–3941.

(3) Petit, M.; Bort, G.; Doan, B.-T.; Sicard, C.; Ogden, D.; Scherman, D.; Ferroud, C.; Dalko, P. I. *Angew. Chem., Int. Ed.* **2011**, *50*, 9708–9711.

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TPA cross-section σ_2 ranging from 10 to 20 GM at the maximum) for a small molecule.[4j] Interestingly the position of the NMe_2 affects the location of the TPA maximum but not its magnitude. The 7 position leads to a blue shift of both one-photon and two-photon absorption spectra.[4j] 2-Hydroxymethylene-7-dimethylaminoquinoline (7-DMAQ, **2**) derivatives are red-shifted analogues of the BHQ platform **1**.[4e] The efficiency of the one photon photolysis (εQ_u) of the 7-DMAQ acetate was less than for the parent BHQ-OAc (211 vs 754 $M^{-1}\,cm^{-1}$ at 366 nm, measured in KMOPS, pH 7.2)[4f] although high enough for the use in physiological experiments. We report here that the structural analogue 8-DMAQ (**5**) is considerably more efficient than the 7-DMAQ (**2**) under OPA or TPA photolysis conditions. This study also represents the first systematic investigation of the position of the substituents on the fragmentation of a caging group.

The reference compound 7-DMAQ-OAc (**10**) and the 6-DMAQ-OAc (**14**)[6] were prepared using modified Dore's protocol, as highlighted in Scheme 1.[4f] The 7-DMAQ acetate **10** was prepared from 3-(*N*,*N*-dimethylamino)aniline **6**, that was transformed regioselectively to 7-(*N*,*N*-dimethylamino)-quinaldine **8** under Döbner-Miller conditions (43%). The transformation of **8** to the corresponding hydroxymethylene derivative **9** was performed by selenium dioxide oxidation in dioxane at 80 °C[4a,f] (34%) followed by $NaBH_4$ reduction (99%) in ethanol to isolate the desired alcohol **9** that was converted to the acetate **10** under standard conditions (86%).

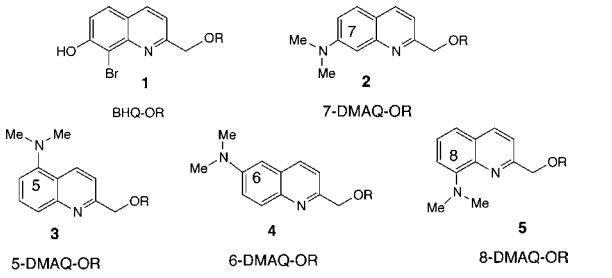
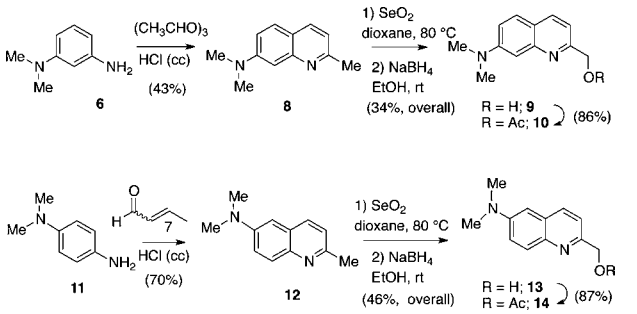
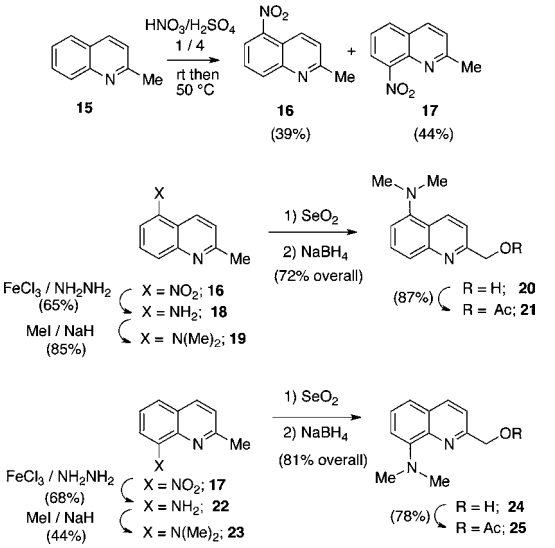


Figure 1. Quinolin-derived photoremovable "caging" groups.

Scheme 1. Synthesis of the 7-DMAQ and the 6-DMAQ Acetates **10** and **14**



Scheme 2. Synthesis of 5-DMAQ and 8-DMAQ Acetates **21** and **25**



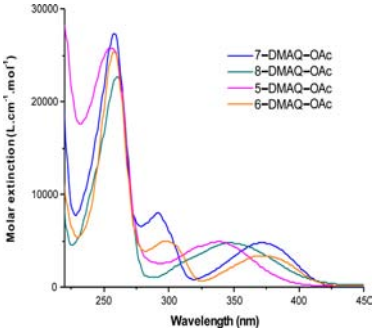


Figure 2. Absorption spectra of (*N*,*N*-dimethylamino)-2-acetoxymethyl quinolines **10**, **14**, **21** and **25** in acetonitrile/TRIS buffer (20 mM) 1/1 at 293 K.

The preparation of the 2-acetoxymethyl-6-(*N*,*N*-dimethylamino)quinoline (6-DMAQ-OAc, **14**) followed

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(5) Eckardt, T.; Hagen, V.; Schade, B.; Schmidt, R.; Schweitzer, C.; Bendig, J. *J. Org. Chem.* **2002**, *67*, 703–710.

Table 1. Photophysical Properties of Chromophores **10**, **14**, **21** and **25**

	λ_{max}[a] (nm)	ε_{max}[a,b] ($M^{-1}cm^{-1}$)	$\varepsilon_{(366\ nm)}$[a,b] ($M^{-1}cm^{-1}$)	Q_u[b,c] (%)	εQ_u[b] ($M^{-1}cm^{-1}$)	$\delta_{u,730\ nm}$ (GM)	λ_{TPA}^{max} (nm)	σ_2^{max} (GM)	$\sigma_{2,(730\ nm)}$ (GM)	$\sigma_2 Q_u$ (GM)
5-DMAQ-OAc (**21**)	340	4900	2770	0	0	0	≤700	≤1.5	0.8	0
6-DMAQ-OAc (**14**)	369	3380	3330	0	0	0	740	3.0	2.0	0
7-DMAQ-OAc (**10**)	371	4790	4670	3.0	140	0.05	760	2.8	2.2	0.07
8-DMAQ-OAc (**25**)	347	4800	4090	17.3	708	0.67	700	10	3	0.52

[a] Measured in acetonitrile/TRIS buffer (20 mM) 1/1 at 293 K. [b] Measured at 366 nm. [c] Samples were prepared in 0.1 mM concentration in acetonitrile/TRIS buffer 1/1 solvent mixture (pH 7.4). For full experimental protocol, see Supporting Information.

a similar strategy (Scheme 1). The condensation of 4-(*N*,*N* dimethylamino)aniline **11** and crotonaldehyde **7** in HCl (cc) afforded the 6-(*N*,*N*-dimethylamino)quinaldine **12**, in considerably higher yield (70%) than by using paraformaldehyde. Quinaldine **12** was transformed to the acetoxymethylene derivative **14** by SeO_2 oxidation (58%), reduction by $NaBH_4$ (**13**, 80%) and acetylation (87%).

The 5- and 8-(*N*,*N*-dimethylamino) acetates, (5-DMAQ-OAc, **21** and 8-DMAQ-OAc, **25**) were prepared from quinaldine **15** as starting material (Scheme 2). Nitration of **15** in a 1:4 mixture of HNO_3 (cc) and H_2SO_4 (cc) afforded a mixture of a roughly equivalent amounts of C(5), and C(8) mononitrated compounds **16** and **17**, that were separated on silica gel (39% and 44%). The structure of the position isomer **23** and thus the structures of **16** and **17** were unambiguously attributed by chemical correlation (for full experimental details see Supporting Information (SI)). Isomers **16** and **17** were transformed to the corresponding dimethylamino quinaldines respectively by using Bechamp reduction followed by methylation under standard conditions (NaH, MeI). The desired acetates **21** and **25** were obtained after benzylic oxidation using SeO_2 followed by reduction and acetylation (Scheme 2).

DMAQ acetates **10**, **14**, **21** and **25** were practically insoluble in 10 mM TRIS buffer; thus, all analyses were performed in a 1/1 mixture of acetonitrile/TRIS buffer (20 mM, pH 7.4). The absorption spectra of compounds **10**, **14**, **21** and **25** were recorded in a mixture of acetonitrile/TRIS (1/1) solution (pH 7.4) at 293 K in the 200−600 nm wavelength range and the calculated molar extinction spectra are plotted in Figure 2. Compounds **10**, **14**, **21** and **25** have qualitatively similar absorption spectra. The first absorption maxima of compounds **10**, **14**, **21** and **25** are located between 340 and 371 nm having molar extinction at the absorption maxima ε_{max} between 3380 and 4900 $M^{-1}cm^{-1}$ (Figure 2, Table 1).

As compared to 6- and 7-DMAQ acetates **14** and **10**, which absorb at 369 and 371 nm, respectively, 5- and 8-DMAQ acetates **21** and **25** are significantly blue-shifted (by more than 20 and 30 nm, respectively). Interestingly, when 6- and 7-DMAQ bear carboxylic ester/acid substituents in position 2 and 4, the absorption is further red-shifted in addition to increased aqueous solubility conveyed by the free acid derivative.[4j]

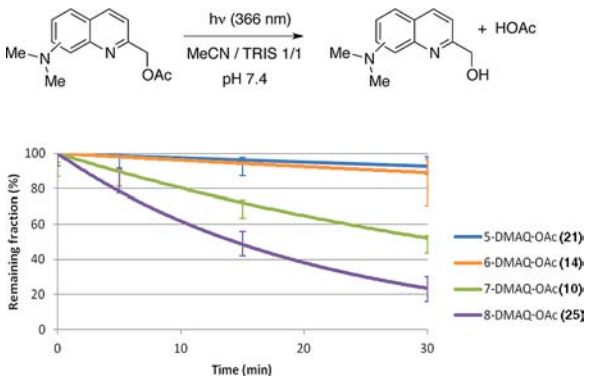

Figure 3. Photolysis of 5-DMAQ-OAc (**21**), 6-DMAQ-OAc (**14**), 7-DMAQ-OAc (**10**), and 8-DMAQ-OAc (**25**) at 366 nm with the calculated time course of the photolyses of DMAQ acetate samples determined by HPLC.

DMAQ acetate samples **10**, **14**, **21** and **25** were photolyzed by UV (366 nm: OPA) and near IR (730 nm: TPA) irradiation. The time course of UV photolysis was monitored by HPLC and the consumption of the starting materials against the time is plotted in Figure 3. No quantitative analysis of photoreleased acetic acid was made. The photolysis at 366 nm of 5- and 6-DMAQ acetates **21** and **14** are too slow to record photofragmentation within 2 h of irradiation and these compounds are photochemically stable at this wavelength. In contrast, 8-DMAQ-OAc (**25**) was photolyzed 6-fold more efficiently at 366 nm than the reference compound 7-DMAQ-OAc (**10**) (Table 1). Notably, compound **25** showed a slow dark hydrolysis rate under these condition with $t_{1/2}$ = 192 h (pH 7.4). The two-photon photolysis of DMAQ acetates **10**, **14**, **21** and **25** was then performed by using 100 fs pulsed 730 nm laser light.

The two-photon uncaging cross section (δ_u) values measured directly from the fractional conversion are collected in Table 1. The irradiation did not afford detectable

(6) 6-DMAQ acetate was prepared earlier by Liu et al. The photolysis of this compound was not reported due to the low aqueous solubility of **14**. See in: Li, Y.-M.; Shi, J.; Cai, R.; Chen, X.-Y.; Guo, Q.-X.; Liu, L. *Tetrahedron Lett*. **2010**, *51*, 1609–1612.

(7) Holding potential −60 mV, Purkinje neuron in a cerebellar slice from 20 day old rat. Experiments were in presence of 2 μM TTX, 10 μM SR 95531, 50 μM D-AP5 and 1 μM Agatoxin IVA: Palma-Cerda, F.; Auger, C.; Crawford, D. J.; Hodgson, A. C. C.; Reynolds, S. J.; Cowell, J. K.; Swift, K. A. D.; Cais, O.; Vyklicky, L.; Corrie, J. E. T.; Ogden, D. *Neuropharm*. **2012**, *63*, 624–634.

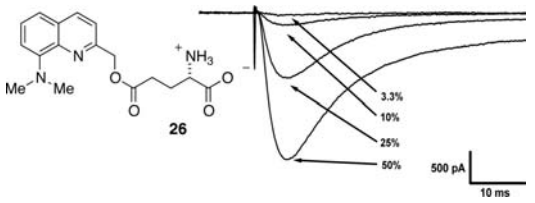

Figure 4. Voltage clamp recording of responses of cerebellar Purkinje neuron evoked by photolysis of 1 mM **26**. Time of the flashlamp pulse (duration 1 ms) is shown by the fast downward pulse due to the trigger, fraction of the maximum intensity of 300−380 nm light indicated for each trace.[7]

photolysis products from 5- and 6-DMAQ acetates **21** and **14** while compound **25** was photolyzed more efficiently than the reference **10** with δ_u values reaching 0.67 GM (10^{-50} cm^{4}s/photon). In order to get better insight into the origin of this marked difference, we performed complementary two-photon induced fluorescence experiments (TPEF) in solution (see SI). The TPEF measurements yield the TPEF action cross sections $\sigma_2\Phi$ (where Φ is the fluorescence quantum yields) from which σ_2 values can be derived. The maximum TPA cross-section (σ_2^{max}) obtained for compounds **10**, **14**, **21** and **25** are collected in Table 1 along with the TPA cross section determined at 730 nm. We observed that whereas compounds **10** and **14** show similar (and modest) maximum TP absorption cross-section in the NIR region, compound **25** shows a 3 times larger σ_2^{max}, indicating that the substitution on position 8 by a strong electron-donating substituent leads not only to larger Q_u but also to larger TPA responses in the NIR region. This most probably originates from an electronic charge shift occurring upon excitation that leads to larger TPA response and favors subsequent photorelease (see SI). Interestingly, we note that δ_u values derived from direct TP uncaging experiments are in fairly good agreement with those obtained from independent measurement of Q_u and σ_2 ($\delta_u = \sigma_2 Q_u$). This agreement suggests that uncaging proceeds via similar mechanisms upon OP or TP excitation.

Notably, under the same conditions both the reference 7-DMAQ acetate, and MNI-glutamate (one of the most popular caged compound) have a two-photon photolysis cross-section of 0.05 GM (10^{-50} cm^{4}s/photon). Thus **25** although having similar one-photon efficiency to MNI-glutamate, has one order higher two-photon sensitivity.

As 8-DMAQ-OAc (**25**) showed improved photolysis characteristics compared to the reference 7-DMAQ-OAc (**10**), the corresponding glutamate **26**, was prepared by using standard protocol (for full experimental details see SI). Compound **26** was more sensitive to hydrolysis at pH 7.4 than the acetate (**25**) thus **26** was isolated as the corresponding acetate salt. In contrast, the caged glutamate was hydrolytically stable in a pH 4.5 solution at rt for one week. To test the photorelease of glutamate from 8-DMAQ-Glu (**26**) and the effects of **26** itself upon neuronal preparations, experiments were done with 1 ms flashlamp photolysis in cerebellar slices prepared from 20 day postnatal rats (Figure 4). Whole cell patch clamp recordings were made from Purkinje neurons. L-Glutamate was released by wide-field photolysis over a 0.2 mm diameter field with 1 ms pulses from a xenon flashlamp at 300−380 nm. Photorelease of glutamate from **26** pre-equilibrated in the bath at 1 mM concentration generated fast rising responses with amplitude and rise-time similar to those elicited by glutamate release from MNI-caged glutamate in the same conditions. Experimental records are illustrated in Figure 4.[8]

In summary, the first systematic structure-TPA-photolysis study in which the influence of the position of the dimethylamino donor group on the photolysis was examined has shown, that the 8-DMAQ "caged" compounds are considerably more sensitive than the parent 7-DMAQ derivatives by irradiation in a 1/1 mixture of acetonitrile/TRIS buffer at 366 nm (OPA, continuous) and 730 nm (TPA, fs pulses) light. Compound **26** showed fast and efficient activation of glutamate receptors in Purkinje neurons by photoreleased L-glutamate (Figure 4). However, interference from hydrolytically released glutamate precludes use of the 8-DMAQ ester, **26**, in longer-term physiological experiments. Nonetheless, the data indicate that the chromophore is more efficient than the previously reported amino-quinoline cages. The systematic study of other type of substituents, mechanistic investigations for the rationalization of the observed phenomena as well as the preparation of hydrolytically more stable derivatives is underway.

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Supporting Information Available. Full experimental procedures and analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(8) No *in vivo* two-photon uncaging experiment was performed with the caged glutamate **26** in this series due to its low dark stability at pH 7.4.