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Two-Photon "Caging" Groups: Effect of Position Isomery on the Photorelease Properties of Aminoquinoline-Derived Photolabile Protecting Groups

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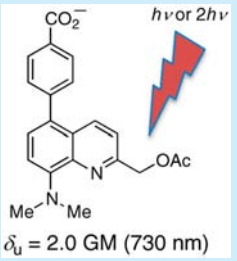
Supporting Information

ABSTRACT: High two-photon photolysis cross sections and water solubility of probes are important to avoid toxicity in biomedical applications of photolysis. Systematic variation of the position of a carboxyl electron-withdrawing group (EWG) on photolysis of 8-dimethylaminoquinoline protecting groups identified the C5-substituted isomer as a privileged dipole. The 5-benzoyl-8-DMAQ substitution yields a caging group with an enhanced two-photon uncaging cross section (δ_u = 2.0 GM) and good water solubility ($c \leq 50$ mM, pH 7.4).



The light-controlled release of ligands is increasingly applied in studies of organized biological systems. These probes have the potential to trigger perturbations in cells and tissues at the molecular level by site-selective interactions with high temporal resolution and at controlled ligand concentrations.[1] They can be activated by near-UV light, or by pulsed NIR light under two-photon (TP) irradiation conditions. TP photolysis has the advantage of maintaining the localization of excitation deep within turbid biological tissue because scattered NIR photons do not produce out of focus excitation permitting better spatiotemporal resolution than near-UV irradiation deep within tissues. However, TP absorption is inherently much less efficient at nontoxic laser intensities, requiring much larger TP cross sections than in current probes such as nitrobenzyl or nitroindolinyl cages, or in ruthenium coordination based probes.[1] The aim here was to develop small hydrophilic caging groups with large TP cross sections. The quinoline platform has the potential for applications under one- and two-photon excitation conditions; these probes have high photolysis efficiencies, long wavelength absorption, and low competing fluorescence.[2] Moreover, the photolysis of bromohydroxyquinoline (BHQ) and dimethylaminoquinoline (DMAQ) derivatives (Figure 1) is fast enough not to extend the volume of TP excitation beyond the illuminated region by diffusion of intermediates[3] and fast enough to activate synaptic glutamate receptors.[4]

In contrast to two-photon excited fluorophores, the rational design of TP uncaging chromophores is less well understood and appears more complex. Here and in forthcoming papers we have developed an empirical approach based on matching the polarizations of the chromophore and photofragmenting elements. This has allowed *in fine* high TP uncaging cross sections to be achieved. We,[5] and others[6] have demonstrated that the photophysical properties and the fragmentation of the DMAQ scaffold are strongly influenced by structural variations. In a recent systematic study we demonstrated a marked dependency of the photolysis efficiency on the position of the dimethylamino donor group under single-photon absorption and TP absorption (TPA) conditions.[5a] We showed that the 8-DMAQ caged acetate **2** (R = Ac, Figure 1) was considerably more efficient in single and TP photolysis than analogues having the dimethylamino substituent at the C5, C6, or C7 positions, respectively, suggesting an optimal position for photolysis

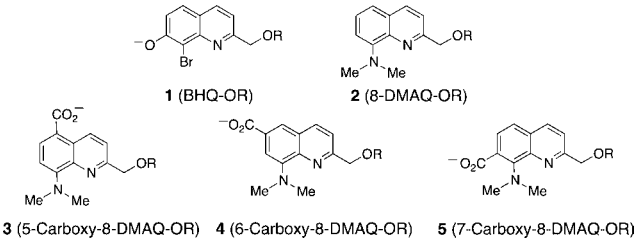


Figure 1. Quinolin-derived photosensitive probes.

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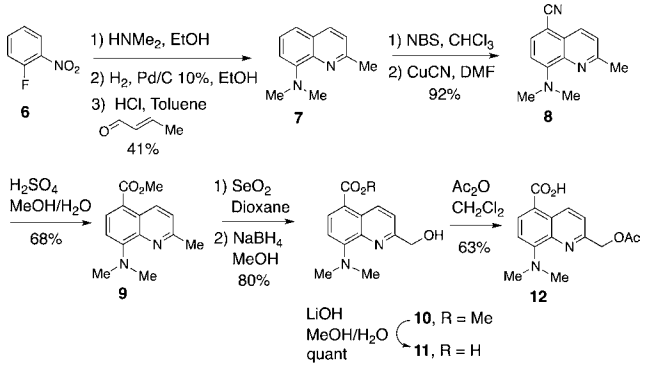
efficiency. Here, the effect of an electron-withdrawing group (EWG) on the photolysis of 8-DMAQ is examined.

The presence of strong donor−acceptor couples that promote internal charge transfer (ICT), as well as extended conjugation, is a key parameter of increased TPA.[7] The synthesis of the donor−acceptor heteroaryl platform was motivated by the earlier observation that these structures promote strong ICT and the compounds show distinct TPA and photochemical properties, as either fluorescent dyes[8a] or photoremovable protecting groups.[8b] The next step of our study was thus the examination of the influence of an EWG group such as carboxylate on the photolysis of the most efficient phototrigger 8-DMAQ **2**. The choice of carboxylate was dictated by the observation that a stronger EWG such as nitrile or nitro groups invariably inhibited the photofragmentation of the probe.[9]

Preparations of carboxy-substituted 8-DMAQ-analogues **3**−**5** are depicted in Schemes 1−3. The synthesis of the C5-carboxy

Scheme 1. Synthesis of the 5-Carboxy-8-DMAQ Acetate, 12

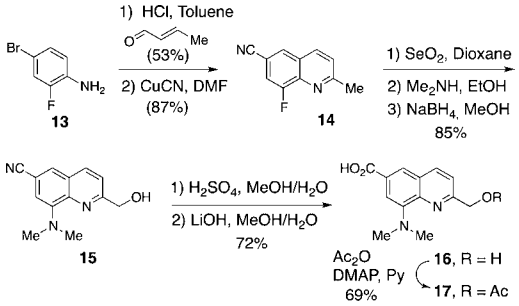


isomer **12** started from *o*-fluoro nitrobenzene **6**. The dimethylamino group was introduced by S_NAr reaction (98%), (Scheme 1), and the nitroaryl was then converted to the corresponding aniline by Pd-catalyzed reduction. The formed aniline was transformed to quinaldine, **7**, in the presence of crotonaldehyde following modified Döbner−Miller conditions (41% over two steps).[5a] The C5-nitrile derivative, **8**, was prepared in a short sequence that included selective monobromination of the quinaldine (NBS, $CHCl_3$) and transformation of the bromo-derivative to the nitrile **8** using CuCN (1.7 equiv) in DMF at 170 °C (92% two steps overall) (Scheme 1). The desired **12** was obtained after methanolysis of the nitrile (68%) (Scheme 1), oxidation/reduction of the carbaldehyde, saponification (LiOH, MeOH/H_2O), and acetylation (63% over two steps).

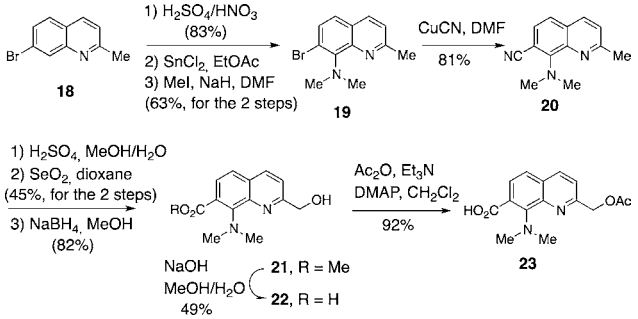
A similar strategy was used for the preparation of the C6-carboxy isomer, **17**. Compound **17** was prepared from 4-bromo-2-fluoro nitrobenzene, **13**, that was transformed to the 6-nitrile derivative, **14**, in 87% yield (Scheme 2). The dimethylamino group was introduced by S_NAr substitution of the fluorine, and then the carbinol **15** was obtained by $NaBH_4$ reduction of the aldehyde. Finally, the C6-carboxy isomer **17** was obtained from **15** by methanolysis followed by saponification of the formed methyl ester and acetylation (69%) (Scheme 2).

The C7-carboxy isomer, **23**, was prepared from the advanced 7-bromoquinoline intermediate **18**[5a] by regioselective mononitration followed by $SnCl_2$ reduction and dimethylation of the amino-bromoquinaldine intermediate (Scheme 3). The desired product, **23**, was obtained after a couple of functional group adjustments that involved the transformation of the nitrile **20** to

Scheme 2. Synthesis of the 6-Carboxy-8-DMAQ Acetate, 17



Scheme 3. Synthesis of the 7-Carboxy-8-DMAQ Acetate, 23



the methyl ester (45%), formation of the lateral C2 chain by oxidation/reduction, saponification, and acetylation (92%).

Unlike the previously prepared DMAQ derivatives[5,6] the carboxy substituted reagents **12**, **17**, and **23** were found to be highly water-soluble to concentrations ≤50 mM at pH 7.4 in TRIS buffer.[10] However, for comparison with previous data all analyses were performed in a 1/1 mixture of acetonitrile/TRIS buffer (20 mM, pH 7.4, 293 K) for consistency. (For the calculated molar extinction spectra, see Supporting Information.) While all compounds had qualitatively similar absorption spectra, C6 and C7 isomers showed lower ε^{max} compared to the C5 isomer. Carboxylates **12**, **17**, and **23** were slightly red-shifted compared to the reference 8-DMAQ acetate, **2** (λ_{max} = 347 nm) showing λ_{max} 349−368 nm (Table 1), a fact that is consistent with the earlier observations of 6- and 7-DMAQ bearing carboxylic ester/acid substituents in position C2 and C4.[5a] Interestingly 7-carboxy-8-DMAQ acetate showed more complex spectra compared to the other two indicating either different type of associations or a cooperative effect between the *ortho* substituents. Molar extinctions at the absorption maxima ε^{max} varied between 1.9 and 3.6 mM^{-1} cm^{-1} (Table 1), similar to other DMAQ derivatives.[3,5,6]

DMAQ acetate samples **12**, **17**, and **23** were photolyzed by 366 nm light (for further details see SI). Photolysis products were compared to the free carbinols **11**, **16**, and **22**, respectively. No quantitative analysis of the photoreleased acetic acid was made. Before irradiation it was ascertained that all acetate samples **12**, **17**, and **23** are hydrolytically stable and the background hydrolysis in the dark is less than 1% per hour at rt and at pH 7.4 (see SI). All carboxy-DMAQ-OAc (**12**, **17**, and **23**) undergo photolysis at 366 nm, and the fragmentation followed the C5 > C6 > C7 order (Table 1). While the C6 isomer **17** was slightly less efficient than the reference compound **2** under UV irradiation, compound **12** was photolyzed 1.5-fold more efficiently than the reference 8-DMAQ acetate, **2** (Table 1). This order is analogous with the earlier observed trend showing

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Table 1. Photophysical Properties of Chromophores 12, 17, 23, 27, and 36

	λ_{abs}^{max} (nm)	ε^{max} (mM^{-1} cm^{-1})	ε^{366} (mM^{-1} cm^{-1})	λ_{em}^{max} (nm)	Stokes shift (cm^{-1})[a]	Φ_f[b]	Q_u[e] (%)	$\varepsilon^{366}Q_u$ ($M^{-1}cm^{-1}$ ×100)
12	368	3.6	3.5	533	8500	0.01[c,d]	32	1120
17	349	2.4	2.0	533	10 100	0.02[c] 0.03[d]	27	540
23	368	1.9	1.8	477	6200	<0.01[c,d]	3	54
27	340	1.2	1.0	517	9100	<0.01[c,d]	21	210
36	343	2.0	1.6	520	9600	<0.01[c,d]	14	224

[a]Stokes shift = $1/\lambda_{abs} - 1/\lambda_{em}$. [b]$\Phi$ = fluorescence quantum yield. [c]Standard: quinine in H_2SO_4 (0.5 M) (Φ = 0.546). [d]Standard: fluorescein in NaOH (0.1 M) (Φ = 0.90). [e]Standard: 7-DMAQ-OAc in TRIS (20 mM)/acetonitrile 1/1.

increased quantum yields for *para- vs meta-* or *ortho*-substituted donor–acceptor (push–pull) chromophores.[11] In summary, the photolysis experiments have identified for 8-DMAQ the privileged position of the EWG substituent for optimal uncaging efficiency.

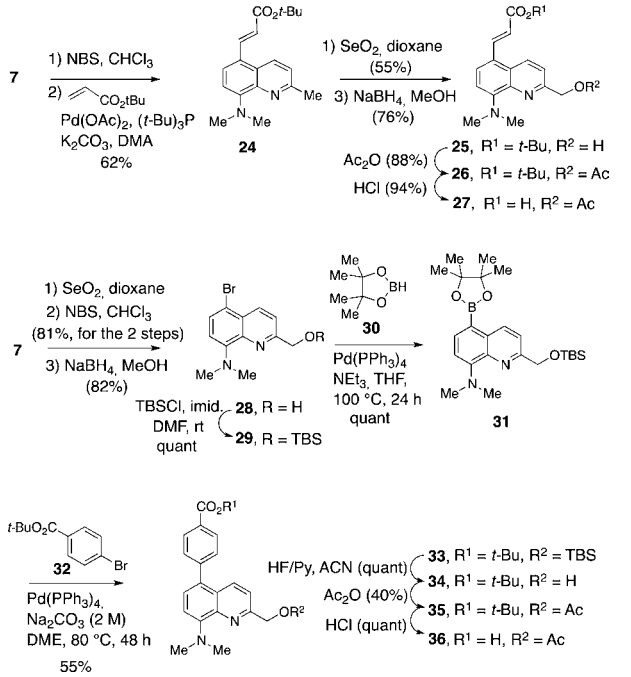
The two-photon uncaging cross section at 730 nm ($\delta_u = \sigma_2 Q_u$) of the best performing **12** was measured directly from the fractional conversion of the acetate to the free carbinol. A 45 μL volume was irradiated in a 3 mm path length quartz cuvette by the beam of a Ti-Sapphire mode-locked laser (MaiTai BB; SpectraPhysics) at 730 nm wavelength with 100 fs pulses at 80 MHz. The expanded beam was focused with a 50 mm lens so that the whole of the excitation volume was contained in the cuvette. Samples were irradiated for 2–4 h at 100 mW average power. The loss of the cage was measured by HPLC, and the photolysis cross sections calculated from the rate of reduction of the fractional cage concentration at the laser beam parameters are given above. The 5-carboxy-8-DMAQ acetate **12** had a δ_u = 0.11 GM (10^{-50} cm^4 s/photon) photolysis cross section, roughly six times less than that of the reference DMAQ acetate, **2** (δ_u = 0.67 GM).

The extension of π-conjugation has been shown to be, in some cases, a viable strategy for increasing TP sensitivity;[12] therefore, it was interesting to examine the effect of the insertion of an extra olefin, and also an aryl group in the dipole at position C5. The synthesis of the acrylate derivative **27** started from quinaldine **7**. Regioselective halogenation followed by Heck coupling afforded the α,β-unsaturated ester **24** in 62% yield (Scheme 4). The oxidation/reduction of the quinaldine followed by acylation and deprotection afforded the desired **27**. Likewise, the transformation of **7** to the carbinol **28** having the required halogen linchpin at C5 was realized by selective monobromination of the corresponding aldehyde followed by reduction (Scheme 4). The TBS-protected carbinol, **29**, was transformed to the boronate ester **31** by Pd-mediated coupling. We found that the electronically mismatched Suzuki–Miyaura reaction could be realized at 80 °C resulting in the fully protected probe, **33**, in a slow reaction (48 h). The replacement of the TBS by acetate and the deprotection of the carboxyl group afforded the desired **36**.

The UV absorption of **27** and **36** showed qualitatively similar spectra to **12** with blue-shifted λ_{max} at 340 and 343 nm with ε^{max} = 1.2 and 2.0 (mM^{-1} cm^{-1}, Table 1), respectively. Also, the UV photolysis of **27** and **36** by 366 nm irradiation showed similar patterns to that of the parent **12** but was less efficient (Q_u = 21 and 14%; εQ_u = 210 and 224 M^{-1} cm^{-1}, respectively). The TP photolysis at 730 nm fs pulsed laser light showed, however, a marked difference with a modest TP uncaging cross section for **27** of δ_u = 0.25 GM, while a large increase of the two-photon uncaging cross section was exhibited for **36** with δ_u = 2.0 GM.

The low TP uncaging cross section of **12** and **27** compared to the parent 8-DMAQ acetate **2** is surprising, as these probes may

Scheme 4. Synthesis of the 5-Acryl- 27 and 5-Benzoyl-8-DMAQ Acetates 36



be characterized by more efficient ICT. The diminished TP sensitivity may eventually be attributed to the laser parameters used, as the duration, shape, and intensity of the input pulse have a marked influence on the control of population of the quantum system: an eventual overexcitation may populate also higher lying energy levels leading thus to an altered relaxation path.

In summary, the synthesis of **36**, having a larger two-photon uncaging cross section (δ_u = 2.0 GM) than 8-DMAQ, validates the dipolar polarization based optimization strategy used here and opens the way for the rational design of more efficient TP optimized probes.

■ ASSOCIATED CONTENT

Supporting Information

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

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Notes

The authors declare no competing financial interest.

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