Sensitized two-photon photochemical deprotection

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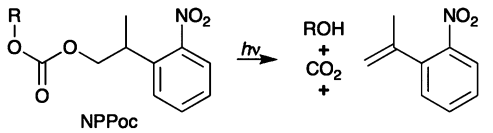
The photoremovable protecting group NPPoc has little sensitivity to two-photon excitation, limiting its use in applications requiring high spatial control of its photochemistry. In the presence of a triplet sensitizer with a large two-photon absorption cross-section, however, the two-photon uncaging action cross-section is improved to levels useful in a variety of applications.

Two-photon photochemistry provides some advantages over conventional one-photon processes, particularly in the biological sciences. The use of two IR photons in lieu of a near-UV photon obviates the risk of damage to UV-sensitive biomolecules and provides high spatial control at the focal point of an IR laser, where high photon flux meets the requirements for two-photon absorption (TPA). This principle is used in two-photon fluorescence microscopy[1] (where TPA creates an excited state that fluoresces) and two-photon uncaging (where the excited state undergoes a chemical reaction).[1,2] The latter process relies on a photoremovable protecting group that is responsive to two-photon excitation, an area of ongoing scientific interest and challenge.

A number of protecting groups that are removable by TPA have been reported, such as the Bhc and BHQ groups.[3] A key characteristic of any photoremovable protecting group is its two-photon uncaging action cross-section, or δ_u, which is measured in units of Göppert-Mayer (GM), or 10^{-50} cm^4 s photon^{-1}. A protecting group should have $\delta_u > 0.5$ GM to be useful in two-photon photodeprotection. The δ_u's of Bhc and BHQ are around 0.8 GM at 740 nm,[3] whereas some newer groups (such as BNSF) have a δ_u of 5–10 GM.[4]

DNA microarray fabrication relies on photochemical deprotection using a modified photolithographic method, light-directed parallel chemical synthesis.[5] Methods for semiconductor photolithography have advanced at a rapid pace, particularly concerning the feature sizes that can be practically created. Current capabilities enable features as small as 65 nm to be written, reflecting Moore's law (the doubling every two years of the number of transistors on an integrated circuit).[6] The same progress has not been seen with DNA microarrays. Feature size in DNA photolithography began at 500 μm and has reached only as low as 4 μm.[7]

In the fabrication of DNA microarrays, a parameter as important as feature size is the cycle yield (reflecting both a photodeprotection step and a phosphoramidite coupling step) in the addition of successive nucleotide subunits. It controls not only the quality of the ultimate DNA probes but also the length of the sequences that can be prepared. Superior performance in DNA synthesis is exhibited by photoremovable groups of the NPPoc family (eqn (1)), which give near-quantitative yields.[8]



(1)

One characteristic of essentially all photoremovable groups is that they asymptotically approach completion (expected for any first-order process), which as shown in our earlier kinetic modeling[9] affects the spatial definition of photochemical deprotection in microarray fabrication. One exception to this behavior has been reported, however: when the deprotection of a NPPoc group at 420 nm on a microarray is performed with sensitization by thioxanthone, the reaction proceeds essentially linearly to completion.[10] While unexpected and uncommon, Steiner *et al.* have performed experiments and provided theory that explains this behavior.[11] Thioxanthone's significantly higher 365 nm absorption (ε 4918 cm^{-1} M^{-1} compared to 225 cm^{-1} M^{-1} for NPPoc) followed by energy transfer to the NPPoc group also provides a 2–3-fold enhancement in deprotection rate on the microarray.

Our initial efforts to improve upon the performance of photoremovable groups for light-directed synthesis relied on sequential one-photon processes to accomplish the equivalent of two-photon deprotection.[9] Aiming to develop a more robust technology that offers the attractive cycle yields and kinetics of sensitized NPPoc deprotection and adds the high spatial definition of TPA, we found examples in stereolithography where thioxanthone has been used as a two-photon photo-initiator of polymerization.[12] We postulated that TPA of thioxanthone (TPA cross-section, δ_a = 5 GM)[13] with high peak power laser pulses would create excited states in a localized area that could then transfer their energy only to nearby NPPoc groups for photochemical deprotection.

Such a sensitization strategy enables a modular approach for maximizing the spatial definition *and* yield in photodeprotection. That is, a protecting group with a high photochemical deprotection yield for a particular functional group can be paired with a sensitizer with a high two-photon cross-section, so long as the energy transfer is favorable. This approach is far more attractive than seeking novel photoremovable protecting groups that provide *both* quantitative deprotection yields and high TPA. Neither property is understood well enough that it can be predicted *a priori*, so empirical approaches based on synthesis and examination of many novel groups would be required. Other work has used similar "antenna" strategies for the generation of singlet oxygen[14]

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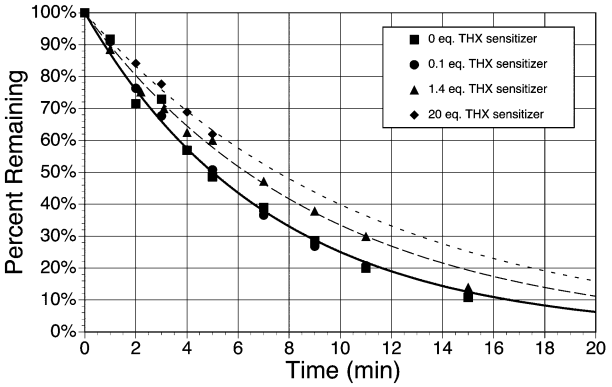


Fig. 1 Time course of sensitized deprotection of 3′-NPPoc-T at 365 nm in the presence and absence of the triplet sensitizer isopropyl thioxanthone (THX). The lines are a least-squares fit of a single exponential decay.

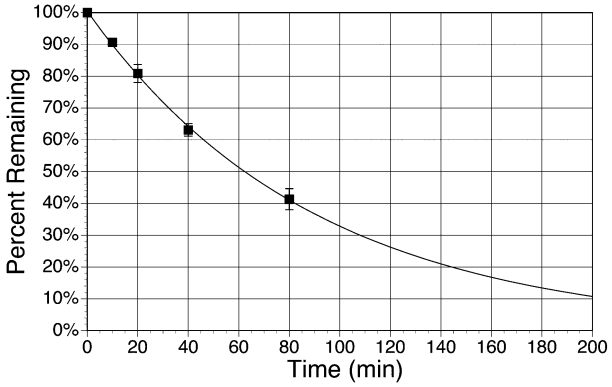
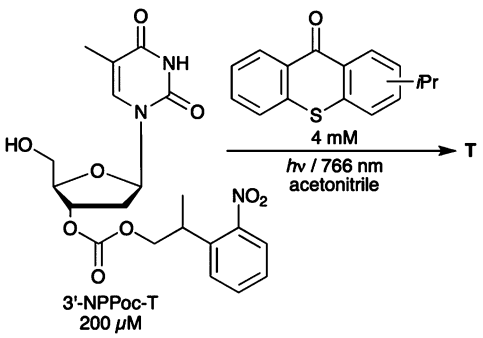


Fig. 2 Time course of sensitized deprotection of 3′-NPPoc-T at 766 nm. The initial concentration was 100 μM, and the sample size was 20 μL. The line is a least-squares fit of a single exponential decay. Error bars represent the standard deviation of the measurement.

and for the release of biological effectors (but only in an intramolecular sense).[15]

To establish a method to measure the rate of a sensitized two-photon deprotection, sensitized one-photon deprotection of 3′-NPPoc-T (eqn (2)) was first studied. 3′-NPPoc-T was irradiated in non-degassed acetonitrile using a Spectroline SB-100P Hg lamp (365 nm). The sensitizer was isopropyl thioxanthone (THX), which is obtained commercially as an (immaterial) mixture of positional isomers. The concentrations of starting material and the product thymine (T) were determined by HPLC using DMT-T as an internal standard and were normalized to the % conversion. Representative data are shown in Fig. 1. Interestingly, increasing concentrations of sensitizer slowed deprotection. The fastest rate was observed with 10 mol% of thioxanthone, and the slowest was observed with 20 equiv. (*vide infra*). Steiner made similar observations in his solution experiments and attributed them to oxygen quenching of the THX triplet.[16]

Sensitized deprotection of 3′-NPPoc-T using TPA was studied at 766 nm† using a fs-pulsed, mode-locked, Ti:sapphire laser as described in past work.[3] The average laser power was 390 mW, the pulsewidth (dt) was 137 fs, and the repetition rate was 76 MHz. Study of the dependence of deprotection efficiency on thioxanthone concentration showed that higher concentrations were best. The fastest deprotection was observed at the highest concentration examined, a 20× excess of sensitizer (eqn (2)). The normalized conversion determined by HPLC (average of 3 runs) is shown in Fig. 2.



(2)

Analysis of these data enabled the two-photon uncaging cross-section (δ_u) for the combination of thioxanthone and 3′-NPPoc-T to be determined using previously described methods;[3] it is 0.86 GM. The distinction between one-photon and two-photon sensitized deprotection is apparent, where at the 20× concentration of thioxanthone, the latter is greatest and the former is poorest.

The reported results establish a new method for two-photon sensitized photochemical removal of a protecting group. They suggest that a broad range of photoremovable groups may be adapted to TPA *via* sensitization, and that this principle should apply to many sensitizer/photoremovable group pairs, provided they have downhill energy transfer. The method provides the ability to modify the protecting group and sensitizer at will to fit the requirements of particular molecules to be protected/deprotected. The specific combination examined here provides a two-photon uncaging cross-section δ_u that is comparable to many existing two-photon photoremovable groups used in caging studies. This result is superior to one reported method for two-photon uncaging based on the NPPoc group.[17] The high concentration of THX could be addressed by using a NPPoc group that carries its own thioxanthone sensitizer,[16] essentially a variant on the intramolecular "antenna" strategy. It also seems likely that improvements can be made on thioxanthone as a two-photon sensitizer, since two-photon photoinitiators with $\delta_a > 100$ GM are known, such as 7-(benzo[*d*]thiazol-2-yl)-9,9-didecyl-*N*,*N*-diphenyl-9H-fluoren-2-amine (DPABz).[13] This method may find its greatest application in the de-caging of biological effectors, but could also be used in direct-write methods in molecular photolithography.[18]

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Notes and references

† This wavelength was chosen because it is twice the λ_{max} of the thioxanthone sensitizer (383 nm) and probably close to the optimum wavelength for TPA.

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