

学位論文要旨

Design and Synthesis of Photolabile Protecting Group with Two Photon Absorption and Reactivity: Application to Caged Compounds

(2光子吸収と反応性に優れた光解離性保護基の設計と合成：ケージド化合物への応用)

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Calcium ions (Ca^{2+}) significantly contribute to various biological processes such as cell messaging (as second messengers), neurotransmitter secretion, muscle contraction, nerve impulses, and non-muscle motility. If the release of neurotransmitters is controlled spatiotemporally, it is expected to investigate the mechanisms of life phenomena related to human memory and learning. One of the research methods for elucidating mechanism of such physiologically active substances is using caged compounds. A caged compound in which a physiologically active substance is protected by a photocleavable protecting group and made inactive can regenerate the physiologically active substance by light irradiation, and its role in vivo can be studied. By providing the photocleavable protective group with two-photon absorption (2PA) ability in the near infrared region (680-1050 nm), it becomes possible to release the physiologically active substance at the deep part. Because 2P absorption can use the lower energy than the 1P photolysis, the risk of cell damage becomes low. In 2012, Goldner and his worker have succeeded in developing biphenyl skeleton as two photon responsive caged glutamate, which has two-photon uncaging efficiency up to 11 GM. In comparison with these compounds, the addition of another benzene ring has been shown to give higher 2PA cross-section values, as reported by Turro and co-workers in 2004 and Kamath and co-workers in 2014. So, in this study, ethylene glycol tetraacetic acid (EGTA)-substituted *p*-nitroterphenyl (PNTTP) derivatives with two different electron-donating groups (dimethylamino (NMe_2) and methoxy (OMe)) were synthesized and their photoreactions were investigated.

Terphenyl EGTA ethyl esters (**3a,b**) and terphenyl EGTA (**4a,b**) were synthesized successfully using Suzuki-Miyaura coupling between the bromide⁴⁾ and biphenyl-substituted boronic acid (Figure 1). At room temperature, these compounds were photoactivated by 1P excitation in C_6D_6 and CD_3OD and 2P excitation in DMSO and HEPES buffer pH 7.4.

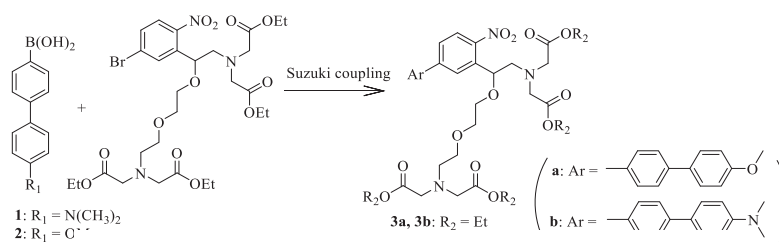


Figure 1. Synthesis of Terphenyl-EGTA ethyl ester (**3a, 3b**)
and Terphenyl-EGTA (**4a, 4b**)

At 360 nm, the uncaging reaction of terphenyl-EGTA **4a,b** in CD_3OD were detected. The fragmentation product was determined by $^1\text{H-NMR}$ analysis (Figure 2). From this result, it was confirmed that the terphenyl skeleton functions as a caged compound. In addition, four compounds **3a,b** and **4a,b** were irradiated with near-infrared light using a titanium-sapphire laser, and the photoreaction was monitored by HPLC analysis. As a result, the NMe_2 group, which is a stronger electron-donating group, has led to a higher 2P cross-section ($\sigma_2 = 75 \text{ GM}$ at 780 nm) compared to the OMe group ($\sigma_2 = 9 \text{ GM}$ at 720 nm) in a pH = 7.4 HEPES buffer solution. The quantum yield ($\Phi = 0.04$) for the decomposition of the NMe_2 -substituted caged compound was much lower than that for the OMe -substituted caged compound ($\Phi = 0.40$) (Figure 3).

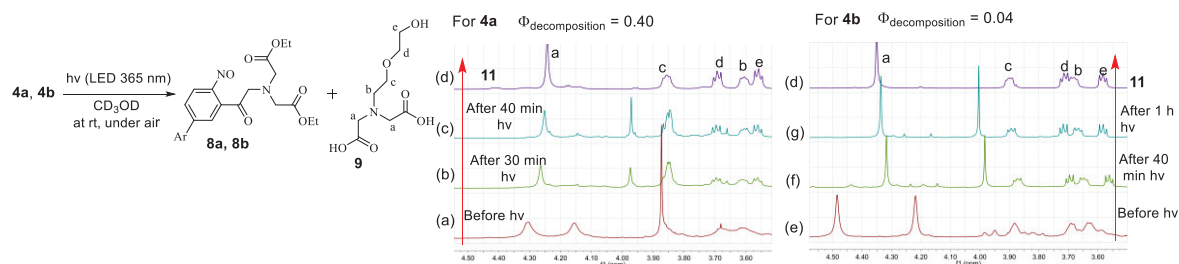


Figure 2. NMR of compound **4a**, **4b** (CD_3OD , 400MHz) before and after irradiation at 360 nm.

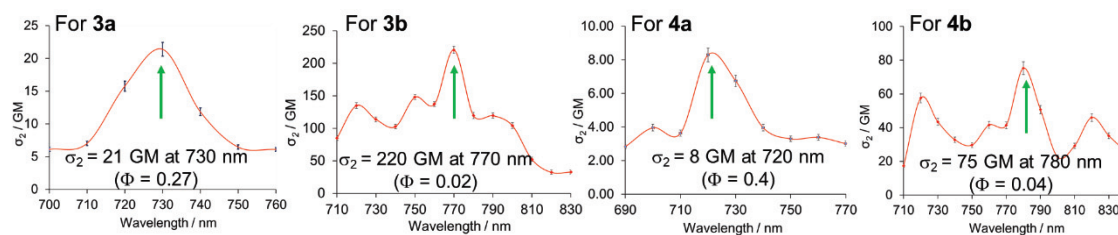


Figure 3. 2PA cross-section spectra of compounds **3a,b** and **4a,b**.

In the previous study, terphenyl chromophores **TP** and biphenyl skeleton (**BP**) application for the 2P-responsive caged compounds was investigated. However, because the dihedral angle of biphenyl unit was smaller than that of **TP**, leading to the σ_2 of **TP** was relatively lower than that of the **BP**,

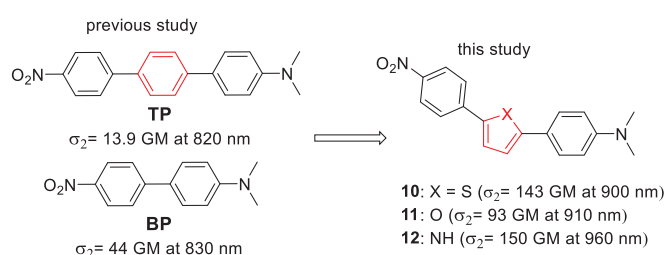


Figure 4. The 2PA of **TP**, **BP** and **10-12**.

although the number of π electron increases. The planarity would increase the transition dipole moment between the acceptor and donor moieties, and π -conjugation. Therefore, a new chromophore having more planar structure would increase the 2P response in NIR region, reducing the damage of cells in physiological experiments. In this study, the terphenyl derivatives **10-12**, in which the central aromatic ring is replaced by heteroaromatic rings such as thiophene, furan, and pyrrole, were designed and synthesized. The donor ($p\text{-NMe}_2\text{C}_6\text{H}_4$ group) and acceptor ($p\text{-NO}_2\text{C}_6\text{H}_4$ group) substituents are connected with the π -linker. The effect of the central heterocyclic rings system would play a vital role in the photophysical properties and two-photon absorption character. As the results, two-photon of terphenyl derivatives, in which the heterocycle linkers such as thiophene, furan, and pyrrole replaced the central aromatic ring showed much improved 2PA cross-section value (Figure 4).

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- (2) Thuy Thi Thu Pham, Satish Jakkampudi, Ko Furukawa, Fung-Yu Cheng, Tzu-Chau Lin, Yoki Nakamura, Norimitsu Morioka, Manabu Abe. *Journal of Photochemistry & Photobiology, A: Chemistry* **2021**, 409, 113154.
- (3) Thuy Thi Thu Pham, Youhei Chitose, Tran Thi Thanh Tam, Tzu-Chau Lin, Manabu Abe. *Chem.Lett* **2021**, submitted for publication.