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Development and Photo-Responsive Peptide Bond Cleavage Reaction of Two-Photon Near-Infrared Excitation Responsive Peptide

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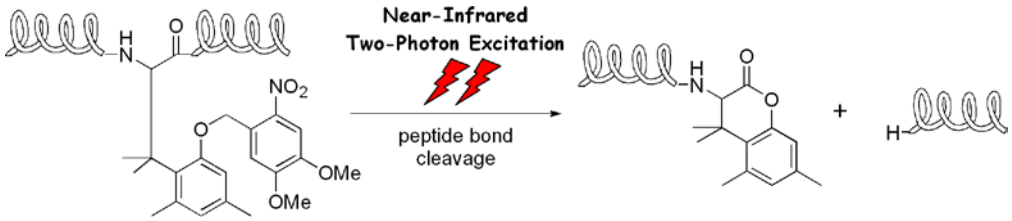
Akira Shigenaga,* Jun Yamamoto, Yoshitake Sumikawa, Toshiaki Furuta, Akira Otaka*



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TETRAHEDRON LETTERS

Development and Photo-Responsive Peptide Bond Cleavage Reaction of Two-Photon Near-Infrared Excitation Responsive Peptide

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Abstract—Two-photon near-infrared excitation-responsive amino acid was developed. It was incorporated into a peptide, and focused near-infrared pulsed laser irradiation-induced peptide bond cleavage reaction at the C-terminal position of the photo-responsive amino acid was observed.

Development of a method to control the function of peptide or protein in a spatiotemporal manner is indispensable in the field of chemical biology and drug delivery. Ultraviolet (UV) irradiation-induced bond cleavage reaction or conformational change of peptide/protein backbone has been successfully applied in order to control function.[1] However, UV irradiation sometimes causes serious damage to living organisms, and one-photon UV photolysis occurs at a whole optical path without three-dimensional spatial precision. Near-infrared (NIR) two-photon photolysis can overcome these problems because NIR, which has a wavelength longer than that of UV, is less damaging to cells compared with UV, and simultaneous absorption of two photons occurs only at the point of focus of a femtosecond pulsed laser with three-dimensional precision.[2] To our knowledge, however, peptide bond cleavage reaction triggered by NIR two-photon photolysis has yet to be reported. Previously, we reported a stimulus-responsive processing (peptide bond cleavage) device and its application for controlling peptidyl function in living cells.[3] In this paper, we describe the development and photo-reactivity of an NIR two-photon excitation (2PE)-responsive processing device which induces a peptide bond cleavage reaction after exposure to a focused NIR pulsed laser.

In the UV one-photon excitation (1PE)-responsive system developed by our group,[3b,c] UV-induced removal of protective group (PG) on the phenolic hydroxyl group of a trimethyl lock moiety[4] triggers a processing reaction as shown in Scheme 1 (PG=*o*-nitrobenzyl). Therefore, we attempted to introduce a 2PE-responsive protective group, which can be cleaved by NIR two-photon absorption, at the PG position. NIR 2PE-responsive protective groups such as Bhc group,[5,6] quinoline derivatives,[7] nitroindoline derivatives,[8] and *o*-nitrobenzyl derivatives[9,10] have been extensively studied to date. A 4,5-dimethoxy-2-nitrobenzyl group was originally developed by Patchornik *et al.* as a UV 1PE-responsive protective group,[11] and it has recently been applied as an NIR 2PE-responsive caged compound to control the function of living cells.[10] Because of its stability and ease of preparation, we attempted to introduce a 4,5-dimethoxy-2-nitrobenzyl group at the PG position.

Fmoc-protected 2PE-responsive amino acid **4** possessing a 4,5-dimethoxy-2-nitrobenzyl group at the PG position was synthesized as shown in Scheme 2. Phenol **1**[3b] was alkylated with 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene[12] in the presence of K_2CO_3 to afford 4,5-dimethoxy-2-nitrobenzyl ether **2**. After removal of the TBS group of **2** under acidic conditions, the generated hydroxyl group was oxidized with PDC to give aldehyde **3**. After oxidation of aldehyde **3** with sodium chlorite, the Boc group on the obtained carboxylic acid derivative was replaced with Fmoc group by acid treatment, followed by reaction with FmocOSu to generate Fmoc-protected 2PE-responsive amino acid **4**. The total yield of amino acid derivative **4** amounted to 72% over 6 steps beginning from phenol **1**. Finally, amino acid **4** was incorporated in model peptide **5** using Fmoc solid phase peptide synthesis (Fmoc SPPS) to examine its photo-reactivity.

Next, the photo-responsive processing reaction of peptide **5** was examined (Scheme 3). Prior to the two-photon NIR absorption experiment, we performed a one-photon UV absorption experiment to characterize processing products and to estimate photophysical properties of peptide **5**. Diastereomerically purified peptide **5**[13] in phosphate buffer (pH 7.6, 20 mM) with 50% *v*/*v* acetonitrile was irradiated by UV (>365 nm) for 3 min, and the reaction mixture was incubated at 37 °C. Reaction progress was monitored by HPLC, and the peptides were characterized by electrospray ionization mass spectrometry (ESI-MS). After 4 h of incubation, peptide **5** was completely converted to corresponding processing products **6** and **7** as shown in Figure 1. Next, the time course for photolysis of peptide **5** by irradiation with 365 nm UV light (1.41×10^{16} photon s^{-1}) was monitored. As shown in Figure 2a, the uncaging (photo-induced removal of 4,5-dimethoxy-2-nitrobenzyl group) reaction of peptide **5** shows first-order dependence on the concentration of peptide **5**. Quantum yield of peptide **5** for UV 1PE was then estimated based on the decay curve and the extinction coefficient of **5**; results are summarized in Table 1. Compared with reference compound **8**,[6i] which also possesses a 4,5-dimethoxy-2-nitrobenzyl group, the extinction coefficient of peptide **5** at 365 nm (ε_{365}) is similar to that of **8**, whereas quantum yield of disappearance of **5** (Φ_{365}) is higher than that of **8**. The reason for this high quantum yield is not clear at present; however, it might be due to the influence of a peptide moiety and not by the solvent effect.[14] Actually, the photophysical parameters (ε_{365} and Φ_{365}) of **8** in 50% *v*/*v* acetonitrile in phosphate buffer (pH 7.6, 20 mM) were estimated as 5,063 M^{-1} cm^{-1} and 0.003, respectively. These values are almost identical to that in K-MOPS buffer; therefore, no obvious solvent effect on the photophysical parameters was observed under these conditions.

These results encouraged us to examine an NIR two-photon absorption experiment. Two-photon excitation reaction of peptide **5** using a focused NIR pulsed laser was examined as shown in Scheme 3b. Solution of peptide **5** in 50% *v*/*v* acetonitrile/phosphate buffer (pH 7.6, 20 mM) was irradiated with a focused NIR pulsed laser (740 nm, 3.48×10^{12} photon s^{-1}), and the time course concentration of peptide **5** was monitored by HPLC. Based on the decay curve of peptide **5** depicted in Figure 2b, two-photon uncaging action cross-section at 740 nm (δ_{u740}) was estimated as 0.23 GM (Goeppert-Meyer, 1 GM=10^{-50} cm^4 s $photon^{-1}$) (Table 1). This δ_{u740} value is higher than that reported for 4,5-dimethoxy-2-nitrobenzyl derivatives including reference compound **8**,[6i,15] presumably due to the high quantum yield of peptide **5** as mentioned above. According to the literature,[6i] a δ_u value exceeding 0.1 GM is preferred for biological application of 2PE-responsive caged compounds. Therefore, the NIR 2PE-responsive processing system reported here can be potentially applicable for biological studies.

In conclusion, we developed a two-photon NIR excitation-responsive peptide. The peptide bond at the C-terminal position of the photo-responsive amino acid was successfully cleaved by irradiation of a focused NIR pulsed laser at 740 nm to yield the processing products, and the δ_{u740} value was sufficient for application in biological studies. To our knowledge, this is the first example of a peptide bond cleavage reaction triggered by NIR two-photon excitation.[17] Applications of this unprecedented NIR 2PE-responsive processing device for spatiotemporal control of peptide/protein function in living cells are in progress.

Acknowledgments

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13. Because racemic Fmoc amino acid **4** was used for Fmoc SPPS, diastereomeric mixture of peptide **5** was generated. These diastereomers were separated by HPLC and the peptide eluted earlier was used for subsequent photolysis experiment.
14. A quantum yield of disappearance of Bz-Gly-ODMNB (DMNB: 4,5-dimethoxy-2-nitrobenzyl) in 3:2 acetonitrile/water was reported as 0.08. Singh, A. K.; Khade, P. K. *Tetrahedron* **2005**, *61*, 10007-10012.
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17. A referee pointed out that our approach brings together two previously known things; a stimulus-responsive peptide bond cleavage device[3] and a 2PE-responsive protective group.[10,11] However, we think that combination of these two is novel, and the resulting 2PE-responsive peptide bond cleavage system with potential applicability for biological studies is unprecedented.

Legends

Scheme 1. Photo-responsive processing system (PG: photo-removable protective group).

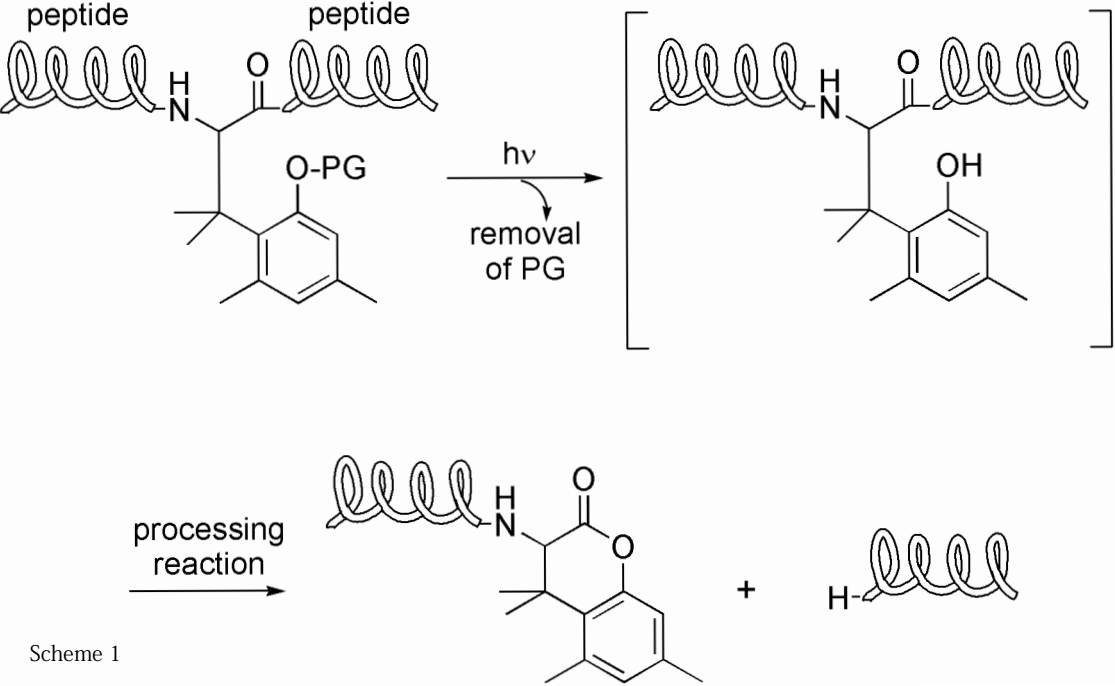
Scheme 2. Reagents and conditions. (i) 1-bromomethyl-4,5-dimethoxy-2-nitrobenzene,[12] K_2CO_3, DMF, 97%. (ii) AcOH, H_2O, THF, 96%. (iii) PDC, CH_2Cl_2, 82%. (iv) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, acetone, *tert*-BuOH, H_2O. (v) HCl, 1,4-dioxane. (vi) FmocOSu, Na_2CO_3, acetonitrile, H_2O, 94% (3 steps). (vii) Fmoc SPPS.

Scheme 3. Reagents and conditions. (a) UV irradiation (>365 nm), 50% *v*/*v* acetonitrile in phosphate buffer (pH 7.6, 20 mM). (b) NIR pulsed laser irradiation (740 nm), 50% *v*/*v* acetonitrile in phosphate buffer (pH 7.6, 20 mM).

Figure 1. HPLC profiles (a) before and (b) after 3 min of UV irradiation followed by 4 h of incubation at 37 °C. Peptides were detected by UV absorbance at 220 nm. Asterisked peak is 4,5-dimethoxy-2-nitrosobenzaldehyde derivative generated by photolysis of 4,5-dimethoxy-2-nitrobenzyl group.

Figure 2. Time course for photolysis of peptide **5** under (a) UV at 365 nm (light intensity: 1.41×10^{16} photon s^{-1}) (b) NIR pulsed laser at 740 nm (light intensity: 3.48×10^{12} photon s^{-1}). Solid lines are least-squares fits of simple decaying exponentials.

Table 1. Photophysical properties of photo-responsive compounds.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peptide
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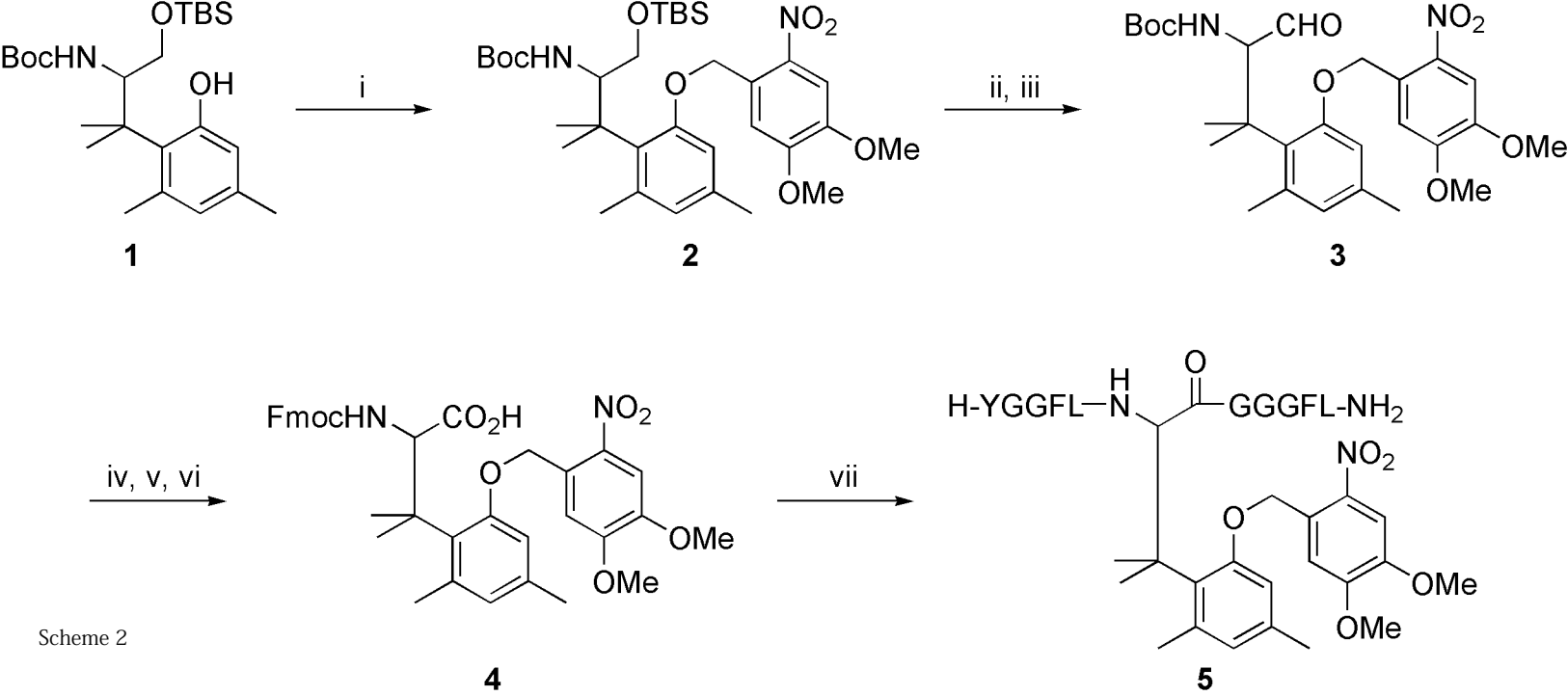
N
O
O-PG
hν
removal
of PG
OH
processing
reaction
+
H-

Scheme 1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OMe
3
iv, v, vi
FmocHN
CO2H
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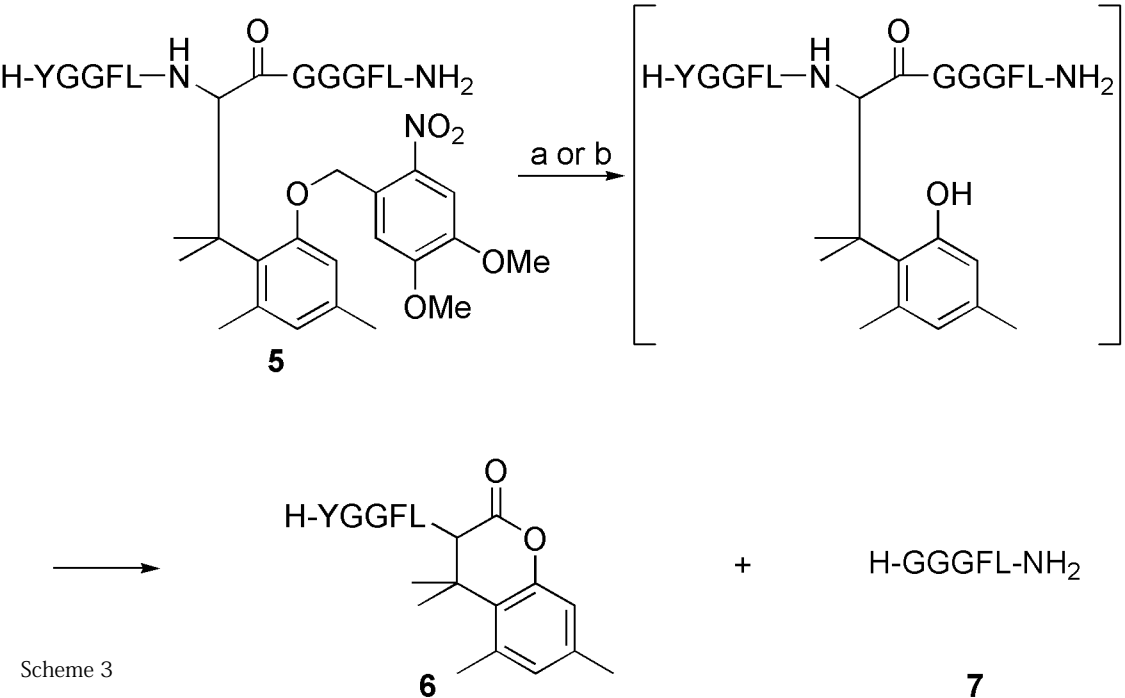
5

Scheme 2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N
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OMe
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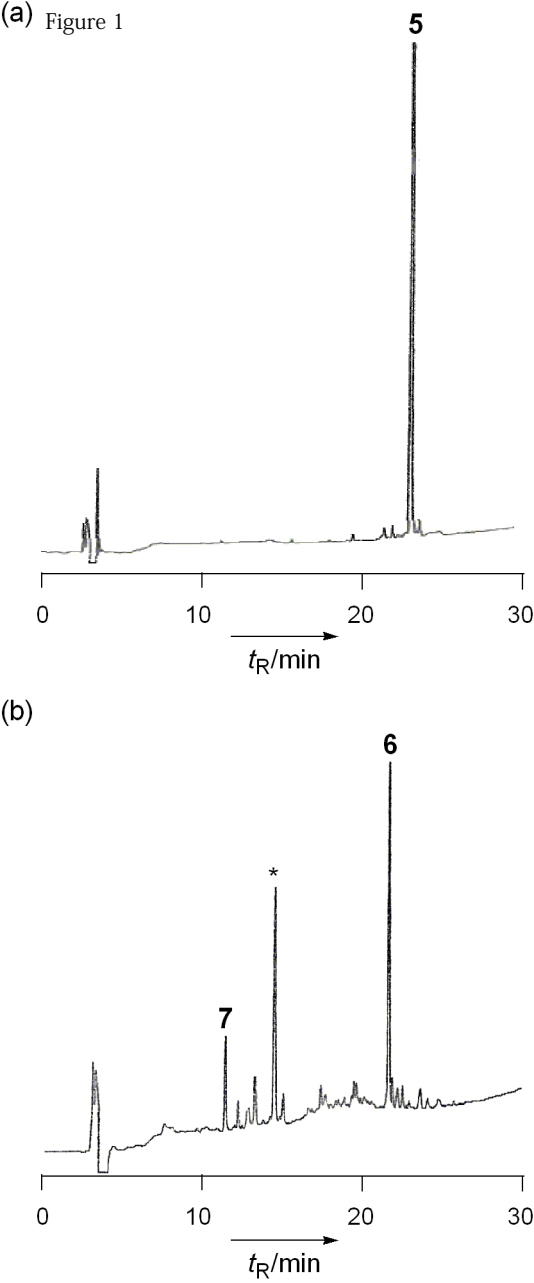
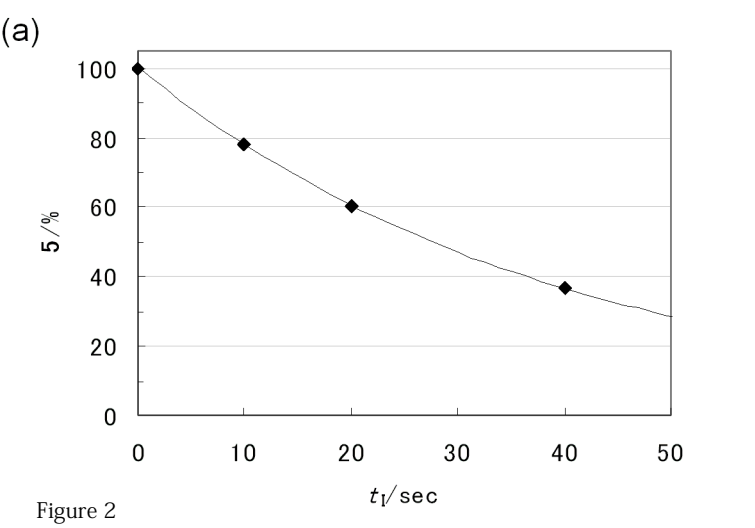
O
6
+
H-GGGFL-NH2
7

Scheme 3

Figure 1

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60
40
20
0
S/%
0
10
20
30
40
50
t_I/sec

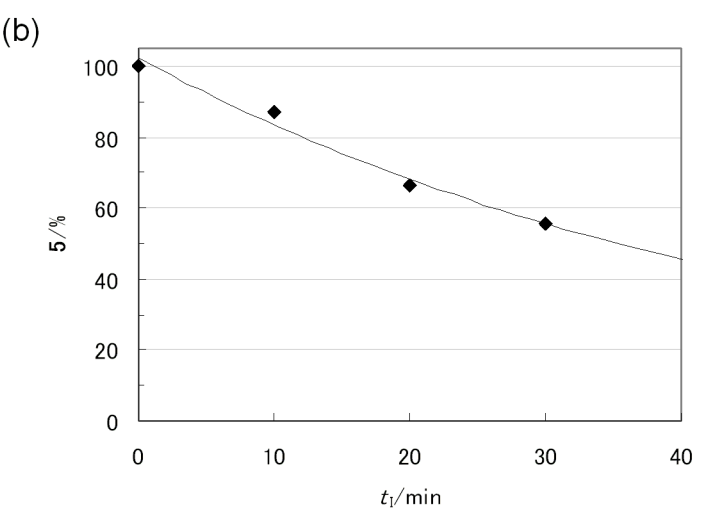
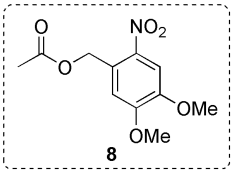

(b)
100
80
60
40
20
0
S/%
0
10
20
30
40
t_I/min

Figure 2

Table 1

	5[a]	**8**[b]
ε_{365}[c]	5,890	5,200
Φ_{365}[d]	0.080	0.005
$\Phi\varepsilon_{365}$[e]	471	26
δ_{u740}[f]	0.23	0.03



8

(a) Solvent: 50% *v*/*v* acetonitrile in phosphate buffer (pH 7.6, 20 mM). (b) Solvent: K-MOPS buffer (pH 7.2, 10 mM MOPS, 100 mM KCl). Values taken from literature.[6i] (c) Molar absorptivity at 365 nm (M^{-1} cm^{-1}). (d) Quantum yield of disappearance of starting materials upon 365 nm irradiation. (e) Product of photolysis quantum yield and molar absorptivity at 365 nm (M^{-1} cm^{-1}). (f) Two-photon uncaging action cross-section at 740 nm (GM).