Supporting Information

for

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

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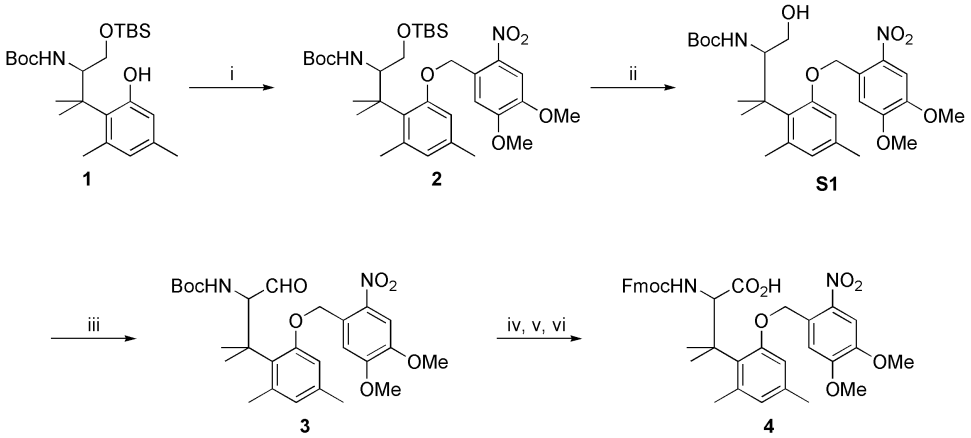
General Methods: All reactions were carried out under a positive pressure of argon. For column chromatography, silica gel (KANTO KAGAKU N-60) was employed. Exact mass spectra were recorded on Waters MICROMASS® LCT PREMIER™ or Bruker Esquire200T. ^{1}H or ^{13}C NMR spectra were recorded using a JEOL GSX400 spectrometer at 400 MHz frequency or a JEOL JNM-AL300 spectrometer at 75 MHz respectively. Chemical shifts are calibrated to the solvent signal. For HPLC separations, a Cosmosil $5C_{18}$-AR-II analytical column (Nacalai Tesque, 4.6×250 mm, flow rate 1 mL/min) or a 5C18-AR-II preparative column (Nacalai Tesque, 20×250 mm, flow rate 10 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% aqueous solution of TFA (*v/v*, solvent A) and 0.1% TFA in acetonitrile (*v/v*, solvent B) was used for HPLC elution. Photolysis by UV irradiation was performed using Moritex MUV-202U with the filtered output (>365 nm) of a 3000 mW/cm^2 Hg-Xe lamp. Femtosecond near-IR pluses from a mode-locked Ti-sapphire laser (Tsunami pumped by Millenium V; Spectra-Physics) were used for an NIR two-photon excitation experiment.

Preparation of NIR 2PE Responsive Amino Acid 4.



Reagents and conditions. (i) 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, 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).

{1-(*tert*-Butyldimethylsilanyloxymethyl)-2-[2-(4,5-dimethoxy-2-nitrobenzyloxy)-4,6-dimethyl-phenyl]-2-methylpropyl}carbamic acid *tert*-butyl ester (2).

To a stirred solution of phenol[S1] **1** (841 mg, 1.92 mmol) in DMF (17.0 mL) were added K_2CO_3 (637 mg, 4.61 mmol) and 4,5-dimethoxy-2-nitrobenzyl bromide[S2] (636 mg, 2.31 mmol), and the resulting suspension was stirred overnight. After addition of aqueous NH_4Cl solution, the reaction mixture was stirred for 30 min followed by addition of H_2O and extraction with diethyl ether. The organic phase was washed with H_2O, saturated aqueous solution of NH_4Cl and brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2,

hexane/AcOEt=20/1) and 1.17 g of ether **2** (1.86 mmol, 97%) was obtained as an yellow amorphousness: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=-0.04 (3H, s), -0.03 (3H, s), 0.85 (9H, s), 1.36 (9H, s), 1.57 (3H, s), 1.58 (3H, s), 2.17 (3H, s), 2.55 (3H, s), 3.53 (1H, dd, *J*=10.4 and 4.2 Hz), 3.58 (1H, dd, *J*=10.4 and 4.2 Hz), 3.94 (3H, s), 3.97 (3H, s), 4.70 (1H, dt, *J*=10.1 and 4.2 Hz), 4.84 (1H, d, *J*=10.1 Hz), 5.51 (1H, d, *J*=16.0 Hz), 5.57 (1H, d, *J*=16.0 Hz), 6.48 (1H, s), 6.58 (1H, s), 7.40 (1H, s), 7.78 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=-5.6 (CH_3), -5.5 (CH_3), 18.1 (C), 20.6 (CH_3), 25.8 (CH_3×3), 25.9 (CH_3), 27.6 (CH_3), 28.3 (CH_3×3), 29.0 (CH_3), 45.0 (C), 56.3 (CH_3), 56.3 (CH), 56.6 (CH_3), 63.7 (CH_2), 69.5 (CH_2), 78.4 (C), 108.0 (CH), 110.4 (CH), 114.1 (CH), 128.4 (CH), 130.4 (C), 131.6 (C), 136.4 (C), 138.4 (C), 138.8 (C), 147.8 (C), 154.2 (C), 155.9 (C), 158.5 (C); HRMS (ESI-TOF) calc. for $C_{33}H_{52}N_2NaO_8Si$ ([*M*+Na]$^+$): 655.3391, found: 655.3362.

{2-[2-(4,5-Dimethoxy-2-nitrobenzyloxy)-4,6-dimethylphenyl]-1-hydroxymethyl-2-methyl-propyl}carbamic acid *tert*-butyl ester (S1).

Glacial acetic acid (15.0 mL) and water (5.00 mL) were added to a solution of silyl ether **2** (1.17 g, 1.86 mmol) in THF (5.00 mL). The reaction mixture was stirred overnight. After extraction with AcOEt, the obtained organic phase was washed with water (×3) and brine, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane/AcOEt=2/1) and 926 mg of alcohol **S1** (1.79 mmol, 96%) was obtained as an yellow amorphousness: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.35 (9H, s), 1.54 (3H, s), 1.55 (3H, s), 2.17 (3H, s), 2.53 (3H, s), 3.53 (1H, dd, *J*=10.5 and 7.8 Hz), 3.66 (1H, d, *J*=10.5 Hz), 3.94 (3H, s), 3.97 (3H, s), 4.70 (1H, dd, *J*=9.3 and 7.8 Hz), 4.80 (1H, d, *J*=9.3 Hz), 5.52 (1H, d, *J*=15.6 Hz), 5.56 (1H, d, *J*=15.6 Hz), 6.50 (1H, s), 6.59 (1H, s), 7.33 (1H, s), 7.79 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=20.7 (CH_3), 26.0 (CH_3), 27.6 (CH_3), 28.3 (CH_3×3), 28.7 (CH_3), 44.2 (C), 56.4 (CH_3), 56.7 (CH_3), 58.9 (CH), 64.0 (CH_2), 69.5 (CH_2), 79.3 (C), 108.2 (CH), 110.4 (CH), 114.1 (CH), 128.7 (CH), 130.0 (C), 130.8 (C), 136.8 (C), 138.1 (C), 139.0 (C), 148.0 (C), 154.3 (C), 157.0 (C), 158.5 (C); HRMS (ESI-TOF) calc. for $C_{27}H_{39}N_2O_8$ ([*M*+H]$^+$): 519.2706, found: 519.2697.

{2-[2-(4,5-Dimethoxy-2-nitrobenzyloxy)-4,6-dimethylphenyl]-1-formyl-2-methylpropyl}-carbamic acid *tert*-butyl ester (3).

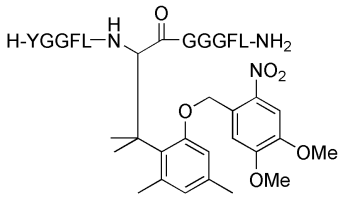
To a stirred solution of alcohol **S1** (926 mg, 1.79 mmol) in dichloromethane (13.0 mL) was added PCC (1.54 g, 7.14 mmol), and the resulting suspension was stirred for 6 h. After addition of the Cerite 535, the reaction mixture was filtered through the Cerite 535. The obtained organic layer was washed with saturated aqueous solution of NH_4Cl, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (SiO_2, hexane/AcOEt=8/1 then 4/1) and 756 mg of aldehyde **3** (1.46 mmol, 82%) was obtained as an yellow amorphousness: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.38 (9H, s), 1.54 (3H, s), 1.63 (3H, s), 2.20 (3H, s), 2.54 (3H, s), 3.93 (3H, s),

3.98 (3H, s), 5.13 (1H, d, *J*=8.8 Hz), 5.35 (1H, d, *J*=8.8 Hz), 5.54 (1H, d, *J*=15.6 Hz), 5.60 (1H, d, *J*=15.6 Hz), 6.54 (1H, s), 6.64 (1H, s), 7.27 (1H, s), 7.79 (1H, s), 9.51 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=20.9 (CH_3), 26.0 (CH_3), 27.9 (CH_3), 28.4 (CH_3×3), 28.6 (CH_3), 44.3 (C), 56.5 (CH_3), 56.8 (CH_3), 66.0 (CH), 69.5 (CH_2), 79.7 (C), 108.3 (CH), 110.7 (CH), 114.1 (CH), 128.7 (C), 128.9 (CH), 129.5 (C), 137.7 (C), 138.4 (C), 139.3 (C), 148.2 (C), 154.3 (C), 156.0 (C), 158.3 (C), 201.3 (CH); HRMS (EST-TOF) calc. for $C_{27}H_{37}N_2O_8$ ([*M*+H]$^+$): 517.2550, found: 517.2545.

3-[2-(4,5-Dimethoxy-2-nitrobenzyloxy)-4,6-dimethylphenyl]-2-(9*H*-fluoren-9-ylmethoxy-carbonylamino)-3-methylbutyric acid (4).

Sodium dihydrogen phosphate (66.0 mg, 0.549 mmol), 2-methyl-2-butene (262 μL, 2.47 mmol) and $NaClO_2$ (217 mg, 1.92 mmol) were added to a solution of aldehyde **3** (189 mg, 0.366 mmol) in acetone/*tert*-BuOH/H_2O (17/12/3 *v*/*v*/*v*, 12.8 mL), and the resulting mixture was stirred for 4 h. To the reaction mixture was added saturated aqueous solution of NH_4Cl and the obtained mixture was extracted with AcOEt. The organic phase was dried over Na_2SO_4 and concentrated in vacuo. Hydrogen chloride in AcOEt (4 M, 2.80 mL) was added to the crude product, and the resulting mixture was stirred for 1.5 h. After concentration in vacuo, the obtained residue was dissolved in acetonitrile/10% *w*/*v* aqueous solution of Na_2CO_3 (3/1 *v*/*v*, 8.00 mL). To the resulting solution was added FmocOSu (136 mg, 0.403 mmol), and the reaction mixture was stirred for 6 h. After being acidified by 5% *w*/*v* aqueous solution of $KHSO_4$, the reaction mixture was extracted with diethyl ether. The organic phase was washed with brine and concentrated in vacuo. The obtained crude product was purified by column chromatography (SiO_2, chloroform/MeOH=1/0 then 100/1) and 226 mg of Fmoc derivative **4** (0.345 mmol, 94%) was obtained as an yellow amorphousness: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.63 (3H, s), 1.66 (3H, s), 2.14 (3H, s), 2.50 (3H, s), 3.83 (3H, s), 3.93 (3H, s), 4.06-4.14 (1H, m), 4.18 (1H, dd, *J*=10.5 and 6.8 Hz), 4.33 (1H, dd, *J*=10.5 and 6.8 Hz), 5.49 (1H, d, *J*=9.5 Hz), 5.51-5.65 (1H, m), 5.54 (1H, d, *J*=15.6 Hz), 5.61 (1H, d, *J*=15.6 Hz), 6.48 (1H, s), 6.56 (1H, s), 7.21-7.40 (5H, m), 7.43 (1H, d, *J*=7.3 Hz), 7.48 (1H, d, *J*=7.3 Hz), 7.73 (2H, d, *J*=7.3 Hz), 7.76 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=20.9 (CH_3), 25.9 (CH_3), 27.9 (CH_3), 28.6 (CH_3), 44.6 (C), 47.2 (CH), 56.4 (CH_3), 56.7 (CH_3), 59.9 (CH), 67.1 (CH_2), 69.7 (CH_2), 108.2 (CH), 110.2 (CH), 114.0 (CH), 120.1 (CH×2), 125.1 (CH), 125.2 (CH), 127.1 (CH), 127.1 (CH), 127.8 (CH), 127.9 (CH), 128.7 (C), 128.9 (CH), 130.1 (C), 137.4 (C), 138.0 (C), 139.0 (C), 141.3 (C×2), 143.9 (C×2), 148.0 (C), 154.3 (C), 156.1 (C), 158.7 (C), 176.5 (C); HRMS (ESI-TOF) calc. for $C_{37}H_{38}N_2NaO_9$ ([*M*+Na]$^+$): 677.2475, found: 677.2498.

Preparation of NIR 2PE Responsive Peptide 5.



Peptide **5** was synthesized in a manner similar to that reported previously.[S1] Analytical HPLC condition: linear gradient of solvent B in solvent A, 20 to 80% over 30 min. Retention time=18.5 or 19.9 min, respectively for each diastereomer. Preparative HPLC condition: linear gradient of solvent B in solvent A, 42 to 52% over 30 min. MS (ESI-IT, reconstructed) calc. for $C_{71}H_{94}N_{13}O_{17}$ ([*M*+H]$^+$): 1400.7, found 1401.0 and 1401.0. Diastereomerically purified peptide **5** eluted earlier was used for subsequent photolysis experiments.

Ultraviolet One-photon Excitation Experiment.

Diastereomerically purified peptide **5** (0.10 mg, 0.070 μmol) in acetonitrile (316 μL) was added to phosphate buffer (20 mM, pH 7.6, 316 μL), and the resulting mixture was irradiated by UV (>365 nm) for 3 min. The reaction mixture was then incubated at 37 ℃ and the reaction was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10 to 80% over 30 min. **5**: retention time=23.4 min. **6**: retention time=21.8 min, MS (ESI-IT) calc. for $C_{41}H_{53}N_6O_8$ ([*M*+H]$^+$): 757.4, fouind 757.5. **7**: retention time=11.5 min, MS (ESI-IT) calc. for $C_{21}H_{33}N_6O_5$ ([*M*+H]$^+$): 449.3, found 449.3.

Estimation of Quantum Efficiency for One-Photon Excitation.

Typical procedure: Light intensity was determined as 1.41×10^{16} photon s^{-1} using a potassium ferrioxalate actinometer. As the reaction solvent, 50% *v*/*v* acetonitrile in phosphate buffer (pH 7.6, 20 mM) was used. Quantum yield of disappearance of peptide **5** was estimated according to a previous report.[S3] To calculate a remaining percentage of **5**, *m*-cresol was used as an internal standard. Product of the photolysis quantum yield and the molar absorptivity at 365 nm was calculated as 470 M^{-1} cm^{-1} based on the time course for photolysis of peptide **5** (Figure S1a). Because molar absorptivity of peptide **5** was determined as 5,889 M^{-1} cm^{-1} (Figure S1b), quantum yield for disappearance of peptide **5** was estimated as 0.080.

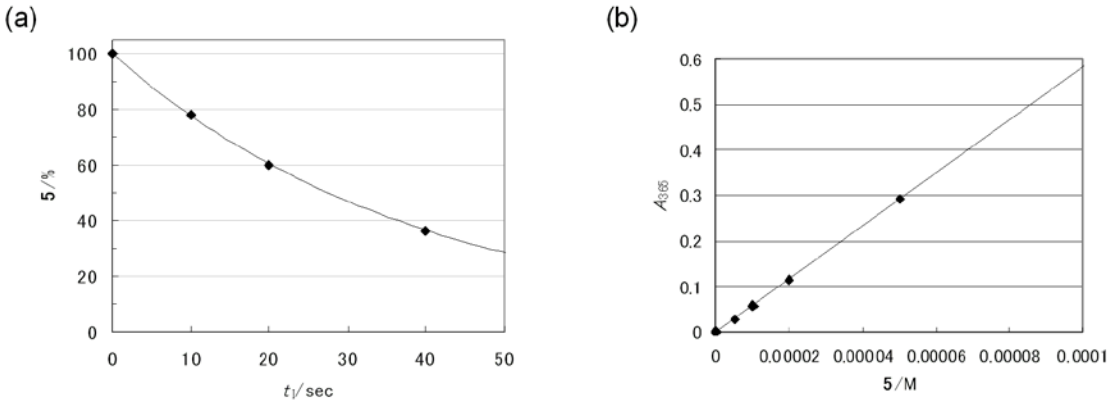


Figure S1. (a) Time course for photolysis of peptide **5** under UV at 365 nm (light intensity: 1.41×10^{16} photon s^{-1}). (b) Plot of concentration of peptide **5** versus absorbance at 365 nm.

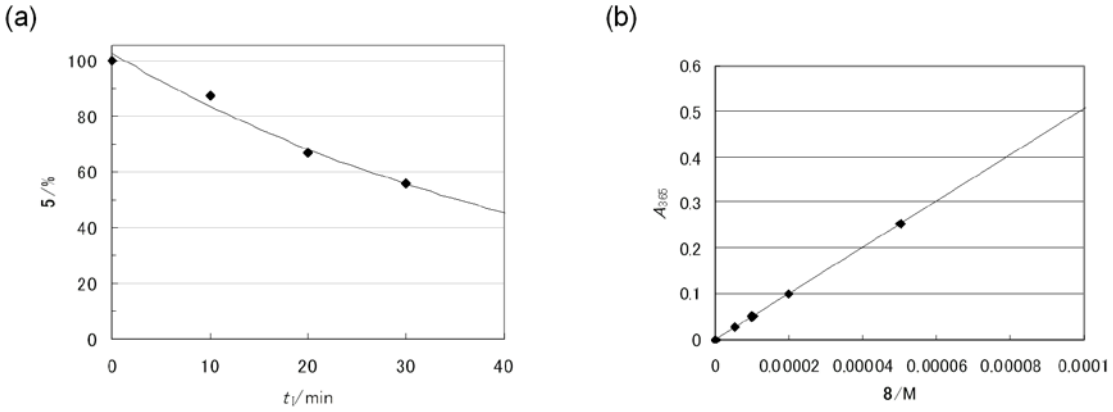


Figure S2. (a) Time course for photolysis of reference compound **8** under UV at 365 nm (light intensity: 1.19×10^{16} photon s^{-1}). (b) Plot of concentration of acetate **8** versus absorbance at 365 nm.

Near-Infrared Two-Photon Excitation Experiment.

NIR two-photon excitation experiment was performed, and δ_u value was calculated as similar to that reported in previous report.[S4] Irradiation intensity was estimated as 3.48×10^{12} photon s^{-1} when referenced to a fluorescein, for which fluorescence quantum yield Φ_F (0.9) and two-photon absorption cross-section δ_F (30 GM at 740 nm) have been characterized.[S5] A 10 μM solution of diastereomerically purified peptide **5** in phosphate buffer (20 mM, pH 7.6) with 50% *v*/*v* acetonitrile was subjected to photolysis.

References

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