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## Supporting Information

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## Supporting Information

for

### A Caged Phosphopeptide-Based Approach for Photochemical Activation of Kinases in Living Cells

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#### **Synthesis of Bhc-caged phosphotyrosine building block 1**

The starting precursor **2** was prepared in three steps from commercially available materials as previously reported.<sup>[S1]</sup>

*Synthesis of 3:* To a solution of **2** (580 mg, 1.84 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was added triethylamine (0.56 mL, 4.1 mmol) and 2-cyanoethyl diisopropylchlorophosphoramidite (0.46 mL, 2.1 mmol). The mixture was stirred at room temperature for 30 min, diluted with saturated aqueous NaHCO<sub>3</sub> (15 mL), and extracted with CHCl<sub>3</sub> (2 x 15 mL). The combined organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated. The resulting oil residue was re-dissolved in Et<sub>2</sub>O and evaporated to give compound **3** as yellow solid (905 mg, 95 %). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.75 (s, 1H), 7.14 (s, 1H), 6.45 (s, 1H), 5.30 (s, 2H), 4.80 (m, 2H), 3.91 (m, 1H), 3.83 (m, 1H), 3.67 (m, 2H), 3.51 (s, 3H), 2.67 (t, 2H), 1.22 (d, 12H). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 300 MHz, H<sub>3</sub>PO<sub>4</sub> as external standard): δ 150.7.

*Synthesis of 1:* To a solution of *N*-α-Fmoc-L-tyrosine (500 mg, 1.24 mmol) in anhydrous THF (5 mL) was added *N*-methylmorpholine (136 μL, 1.24 mmol) and *tert*-butyl-

dimethylchlorosilane (187 mg, 1.24 mmol). After stirring at room temperature for 15 min to yield the protected Fmoc-tyrosine **4, 3** (838 mg, 1.63 mmol) and 1*H*-tetrazole (130 mg, 1.86 mmol) were added to the above solution. The mixture was stirred for 3 h, affording the intermediate phosphite. Subsequently, *tert*-butyl hydroperoxide (5.0-6.0 M solution in decane, 500  $\mu$ L) was added to the reaction solution. The mixture was stirred for a further 2 h. After evaporation, the crude residue was dissolved in EtOAc (15 mL) and washed with 5 % aqueous NaHSO<sub>4</sub> (15 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated. The crude residue was purified by column chromatography on silica gel (eluent; 0.2 % AcOH / 2.5 % MeOH / CHCl<sub>3</sub>). The collected fraction was washed with saturated aqueous NaHCO<sub>3</sub> (15 mL). The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered, and evaporated to give compound **1** as yellow solid (904 mg, 88 %). <sup>1</sup>H NMR ([D]DMSO, 500 MHz):  $\delta$  7.96 (s, 1H), 7.84 (d, 2H), 7.61 (d, 2H), 7.37 (t, 2H), 7.31 (t, 2H), 7.28 (d, 2H), 7.25 (s, 1H), 7.18 (d, 2H), 6.33 (s, 1H), 5.44 (m, 2H), 5.41 (s, 2H), 4.36 (d, 2H), 4.20-4.15 (m, 4H), 3.41 (s, 3H), 3.07-2.85 (m, 2H), 2.97 (t, 2H). <sup>31</sup>P NMR (CDCl<sub>3</sub>, 300 MHz, H<sub>3</sub>PO<sub>4</sub> as an external standard):  $\delta$  -5.96.

MALDI-TOF-MS (Dithranol): calcd for [M+Na]<sup>+</sup> = 855.09; obsd 855.11.

### Peptide synthesis

The peptides were synthesized manually on a Rink Amide resin by a standard Fmoc-based solid-phase peptide synthesis protocol. Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Met-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Val-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Tyr(PO(OBzl)OH)-OH, and **1** were used as building blocks. Fmoc deprotection was performed with 20 % piperidine in DMF, and coupling reactions were performed in DMF with a mixture of Fmoc-amino acid and i) diisopropylcarbodiimide (DIC) and HOBt or ii) HBTU, HOBt, and DIEA. For the incorporation of **1**, the amino acid was pre-activated using the coupling reagents (ii) and then mixed with the resin. Following chain assembly, global deprotection and cleavage from the resin was performed with TFA containing 2.5 % ethanedithiol, 2.5 % H<sub>2</sub>O, and 1 % triisopropylsilane. The crude peptide products were precipitated by Et<sub>2</sub>O and purified by reversed-phase HPLC using a semi-preparative YMC-Pack ODS-A column with a linear gradient of 0.1 % aqueous TFA and acetonitrile containing 0.1 % TFA. The peptides were identified by MALDI-TOF-MS (CHCA): **5** calcd for

$[M+\text{Na}]^+ = 1359.08$ , obsd 1359.10; **9** calcd for  $[M+\text{Na}]^+ = 1375.21$ , obsd 1375.23.

The 2-nitrophenylethyl-caged peptide **8** was synthesized as described above using the *N*- $\alpha$ -Fmoc-phospho(1-nitrophenylethyl-2-cyanoethyl)-tyrosine building block previously reported.<sup>[S2]</sup>

### Evaluation of Photochemical Properties<sup>[S3]</sup>

*One-photon UV excitation:* The caged peptide solutions (8 or 100  $\mu\text{M}$ , 1 mL) in K-MOPS buffer (10 mM MOPS, 100 mM KCl, pH 7.2) containing 0.1 % DMSO were placed in a Pyrex test tube of 12 mm diameter and irradiated in a RPR-200 preparative reactor (Rayonet, The Southern New England Ultraviolet Co.) equipped with two RPR 350 nm lamps ( $\sim 10 \text{ mJ}\cdot\text{s}^{-1}$ ). Aliquots of 10  $\mu\text{L}$  were taken at defined time points and analyzed by RP-HPLC on an analytical COSMOSIL 5C<sub>18</sub>-AR-II column with a linear gradient of 0.1 % aqueous TFA and acetonitrile containing 0.1 % TFA using absorbance detection at 220 or 325 nm. The apparent first-order rate constant for the photolytic consumption of the starting material ( $k$ ) was obtained by fitting a plot of the remaining % of the starting material ( $P$ ) as a function of irradiation time ( $t$  in seconds) to the first-order equation (1):  $P = 100 \cdot \exp(-k \cdot t)$ . The quantum yield of photolysis ( $F$ ) was determined by the following equation (2):

$$F = 1/(1000 \cdot I \cdot e_{350} \cdot t_{90\%})$$

where  $I$  is the light intensity ( $\text{einsteins}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ ),  $e_{350}$  is the molar absorption coefficient ( $\text{M}^{-1}\cdot\text{cm}^{-1}$ ) of Bhc caging group at 350 nm, and  $t_{90\%}$  is the irradiation time in seconds for 90 % consumption of the starting material calculated from equation (1). The UV intensity  $I$  was measured by potassium ferrioxalate actinometry. Experiments were carried out in triplicate and the average values were used for calculations.

*Two-photon IR excitation:* The caged peptide solutions (8  $\mu\text{M}$ , 15  $\mu\text{L}$ ) in K-MOPS buffer containing 0.1 % DMSO were placed in a sub-micro fluorometer cell (16.10F-Q-10/Z15, 10 mm path length, 1 x 1 mm window; Starna). Femtosecond near IR pulses from a mode-locked Ti/sapphire laser (Tsunami pumped by Millenia V; Spectra-Physics) were focused on the center of the sample chamber with a 25 mm focus length lens (06LXP003/076; Melles Griot). Aliquots of 10  $\mu\text{L}$  were taken at defined time points and analyzed by RP-HPLC as described above. The two-photon uncaging action cross-section ( $d_u$ ) was estimated by referencing to fluorescein, of which fluores-

cence quantum yield  $F_F$  (0.9) and two-photon absorption cross-section  $d_{uF}$  (30 GM at 740 nm) were reported previously<sup>8</sup>. The  $d_u$  was calculated from equation (3):

$$d_u = (N_p \cdot K \cdot F_F \cdot d_{uF} \cdot C_F) / (\langle F(t) \rangle \cdot C_S)$$

where  $N_p$  is the number of product molecules formed per unit time (molecules·s<sup>-1</sup>);  $K$  is the collection efficiency of our experimental setup used to measure the fluorescence of fluorescein emitted at a right angle to the beam and passed through a 520 ± 10 nm bandpass filter (03F1V109; Melles Griot);  $C_F$  is the concentration of fluorescein (mol·L<sup>-1</sup>);  $\langle F(t) \rangle$  is the time averaged fluorescence (photon·s<sup>-1</sup>) collected by the detector (silicon photodiode radiometer, SED033 on an IL-1700; International Light); and  $C_S$  is the initial concentration of the caged peptide (mol·L<sup>-1</sup>). Experiments were carried out in triplicate and the average values were used for calculations.

### Live-cell imaging

NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) containing 10 % fetal bovine serum at 37 °C under 5 % CO<sub>2</sub>. Plasmid transfection (the plasmid encoding GFP-PHAkt was kindly provided by Prof. Yukiko Gotoh) was carried out using Lipofectamine and Plus Reagents (Invitrogen) in Opti-MEM I Reduced-Serum Medium (Gibco) according to the manufacturer's protocol. After 24 h of expression, cells were microinjected with peptide using an InjectMan NI 2 micro-manipulator (Eppendorf). Cells were observed with a Leica TCS NT confocal laser microscope (Leica Microsystems). The fluorescence images were acquired using the 488 nm line of an argon laser for excitation and a 530 nm band-pass filter for emission, or the 568 nm line of a krypton laser for excitation and a 590 nm long-pass filter for emission. The specimens were viewed using a 100x oil immersion objective.

Caged peptide (100 μM) and fluoro-ruby (dextran-tetramethylrhodamine, 10 kDa; Molecular Probes) in PBS(-) were microinjected into NIH3T3 cells. Fluoro-ruby (50 μg/mL) was used as a red marker. After microinjection, cells were re-incubated for 30 min and the morphology of the microinjected cells did not change dramatically over that period of time. Irradiation was carried out using a UV hand lamp (SLUV-4; AS ONE) at 365 nm for 2 min and images were acquired 15 min after irradiation. For PI3K inhibition experiments, cells were pre-incubated in DMEM containing LY294002 (100 μM) for 1 h and then used for subsequent experiments.

## References

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