Supplementary Material (ESI) for *Chemical Communications*

Supplementary Information

A bromocoumarin-based linker for synthesis of photocleavable peptidoconjugates with high photosensitivity

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Synthesis of Bac linker building block 1

The starting precursor **2** was prepared in two steps from commercially available materials as previously reported.[S1]

Synthesis of 3

To a suspension of **2** (500 mg, 1.84 mmol) in anhydrous DMF (15 mL) were added potassium carbonate (0.51 g, 3.69 mmol) and 2-(*tert*-butoxycarbonylamino)ethyl bromide (2.06 g, 9.19 mmol). After stirring at room temperature for 48 h, the solvent was removed under vacuum. The resulting residue was dissolved in $CHCl_3$ (50mL) and washed with water (50 mL × 2) and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH = 10/1) to give compound **3** as white powder (380 mg, 50 %).

^{1}H-NMR (*d*-DMSO, 500 MHz): δ 7.91 (s, 1H), 7.22 (s, 1H), 6.96 (br, 1H), 6.34 (s, 1H), 5.64 (br, 1H), 4.72 (d, 2H), 4.16 (t, 2H), 3.35 (m, 2H), 1.38 (s, 9H).

MALDI-TOF-MS (CHCA): calcd for $[M+Na]^+$ = 436.04; obsd 435.75

Synthesis of 4

To a suspension of **3** (300 mg, 0.72 mmol) in CH_2Cl_2 (18mL) on an ice bath was added trifluoroacetic acid (2ml). After stirring for 15 min, the solution was allowed to warm to room temperature and stirred for a further 2 h. The solvent was removed under vacuum and the resulting residue was re-dissolved in anhydrous DMF (20 mL). To the solution were added *N*,*N*-diisopropylethylamine (DIEA, 502 μL, 2.95 mmol) and 9-fluorenylmethyl succinimidyl carbonate (Fmoc-OSu, 292 mg, 0.87 mmol). The reaction mixture was stirred at room temperature for 0.5 h. After evaporation, the residue was dissolved in EtOAc (50 mL) and washed with 0.1 N aqueous HCl (50 mL × 2). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography on silica gel ($CHCl_3$ to $CHCl_3$/MeOH = 15/1) to give compound **4** as white powder (360 mg, 93 %).

^{1}H-NMR (*d*-DMSO, 500 MHz): δ 7.91 (s, 1H), 7.88 (d, 2H), 7.69 (d, 2H), 7.51 (br, 1H), 7.40 (t, 2H), 7.31 (t, 3H), 7.24 (s, 1H), 6.34 (s, 1H), 5.65 (t, 1H), 4.72 (d, 2H), 4.33 (d, 2H), 4.23-4.18 (m, 3H), 3.42 (m, 2H).

MALDI-TOF-MS (CHCA): calcd for $[M+Na]^+$ = 558.05; obsd 558.09

Synthesis of 1

To a suspension of **4** (300 mg, 0.56 mmol) in CH_2Cl_2 (20mL) were added DIEA (878 μl, 5.16 mmol) and 4-nitrophenyl chloroformate (1.15 g, 5.70 mmol). After stirring for 15 min, the solution was allowed to warm to room temperature and stirred for a further 2 h. The reaction solution was washed with 0.01 M aqueous HCl (50 mL × 2), and the organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude residue was purified by column chromatography on silica gel ($CHCl_3$) to give compound **1** as white powder (356 mg, 91%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.32 (d, 2H), 7.75 (d, 2H), 7.71 (s, 1H), 7.59 (d, 2H), 7.43 (d, 2H), 7.38 (t, 2H), 7.30 (t, 2H), 6.89 (s, 1H), 6.50 (s, 1H), 5.42 (s, 2H), 5.24 (br, 1H), 4.45 (d, 2H), 4.22 (t, 1H), 4.17 (t, 2H), 3.72 (m, 2H).

MALDI-TOF-MS (CHCA): calcd for $[M+Na]^+$ = 723.06; obsd 723.50.

Peptide Synthesis

The peptides were synthesized manually on a Rink Amide resin by standard Fmoc-based solid-phase peptide synthesis protocol. Fmoc-Arg(Pbf)-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Tyr(*t*Bu)-OH, and **1** were used as building blocks. In the synthesis of **5**, (+)-biotin was also used. Fmoc deprotection was performed with 20% piperidine in DMF and coupling reactions were performed with a mixture of Fmoc-amino acid, diisopropylcarbodiimide (DIC), and HOBt in DMF. Incorporation of the Bac linker was carried out by double coupling using **1** (2 eq) and DIEA (2 eq) in DMF. Acetylation was performed with acetic anhydride and DIEA in DMF. In the case of the branched peptidoconjugate **6**, the Mtt group of protected FLAG resin **7** was selectively removed by treatment with CH_2Cl_2 containing 2% TFA and 5% triisopropylsilane. All coupling and Fmoc deprotection steps were monitored by the Kaiser test.[S2] Following chain assembly, global deprotection and cleavage from the resin was performed with TFA containing 2.5% ethanedithiol and 2.5% H_2O. The crude peptide products were precipitated by Et_2O 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): linear form **5** calcd for $[M+H]^+$ = 1867.67, obsd 1868.03;

branched form **9** calcd for $[M+H]^+$ = 2530.49, obsd 2530.23.

Evaluation of Photochemical Properties[S1]

The photocleavable peptidoconjugate solutions (**5**, 9 μM; **6**, 8 μM) in 1 mL of K-MOPS buffer (10 mM MOPS, 100 mM KCl, pH 7.2) 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 (≈ 10 $mJ \cdot s^{-1}$). Aliquots of 10 μL were taken at defined time points and analyzed by RP-HPLC on an analytical COSMOSIL $5C_{18}$-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 (Φ) was determined by the following equation (2):

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

where I is the light intensity ($einstein \cdot cm^{-2} \cdot s^{-1}$), ε_{350} is the molar absorption coefficient ($M^{-1} \cdot cm^{-1}$) of the Bac group at 350 nm, and $t_{90\%}$ is the irradiation time in seconds for 90% consumption of the starting material calculated from the 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.

References

[S1] T. Furuta, S. S.-H. Wang, J. L. Dantzker, T. M. Dore, W. J. Bybee, E. M. Callaway, W. Denk and R. Y. Tsien, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 1193-1200.

[S2] E. Kaiser, R. L. Colescott, C. D. Bossinger and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595-598.

Supplementary Figures

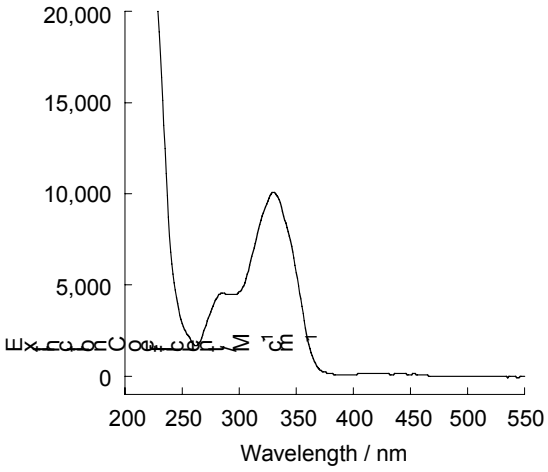

Fig. S1 UV/vis spectrum of **5** in K-MOPS buffer. λ_{max} = 330 nm.

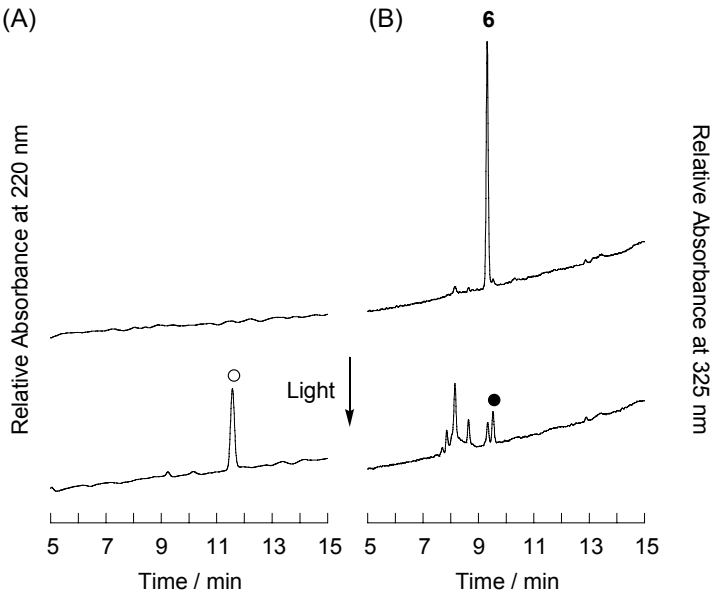

Fig. S2 HPLC traces of **6** before (top) and after (bottom) UV photolysis (350 nm, 2 min). Conditions: 8 μM in K-MOPS buffer. HPLC was performed on an ODS column with a linear gradient of acetonitrile containing 0.1% TFA (solvent A) and 0.1% aqueous TFA (solvent B). (A) Detection at 220 nm. Gradient: A/B = 4:90 to 10:60 for 15 min. Peak ○ denotes Ac-FLAG (Ac-DYKDDDDK-NH_2). (B) Detection at 325 nm. Gradient: A/B = 10:90 to 40:60 for 15 min. Peak ● denotes Ac-R_7-Bac_{OH}.