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Red-shifted backbone N–H photocaging agents†

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Light is a uniquely powerful tool for spatiotemporal control of molecular structure, necessitating the development of new photocaging approaches. This communication describes the design, synthesis, and reactivity of two new photoreactive boronic acid reagents for backbone N–H modification and subsequent photocleavage.

Photocleavable protecting groups (photocages) provide exquisite tools for unmasking desired functional groups, conformational structure, and molecular function. Light is a non-invasive external stimulus that allows precise spatial and temporal control over molecular uncaging, in ways that are difficult to achieve with chemical reagents or other stimuli.[1,2] The creativity with which chemists deploy photocaged structures in diverse applications creates a continual need for new reagents and new photocleavage paradigms.[1–6] Many photocage designs rely on UV light,[7–10] which has DNA- and protein-related consequences in living systems.[11,12] Red-shifting cleavage wavelengths into the visible and near-IR range is an important and active area of research that allows improved tissue penetration and spatial uncaging precision.[13–16] While substantial progress has been made in the development of red-shifted photocleavage of C(sp^3)–X and acyl–X bonds, the cleavage of C(sp^2)–X bonds is largely unexplored.

The discovery of copper-mediated, histidine-directed backbone arylation/alkenylation with boronic acid reagents provides access to *N*-alkenyl or *N*-aryl polypeptide structures that are generally inaccessible by biosynthetic approaches or traditional chemical peptide synthesis.[17,18] This backbone modification directly disrupts the hydrogen-bonding that defines secondary structure and creates an interesting opportunity for backbone photocaging.

We described[19] a vinylogous nitroveratryl structure (**1a**, Fig. 1a), which effectively promotes photocleavage of C(sp^2)–N bonds. The putative mechanism of this process (Fig. 1b) relies on hydrogen-atom abstraction followed by selective nucleophilic attack of water on the resulting extended conjugated system. We wanted to explore the mechanistic potential and generality of this pathway by testing the extent to which modulating the chromophore structure could alter photocleavage properties, and especially to improve photocleavage performance at red-shifted wavelengths. In this context, it is worth noting that while red-shifted photocleavage of typical C(sp^3)–X structures has been most successful with other cleavage mechanisms, it has yet to be adapted to C(sp^2) cases.[13,15,20] We now report the synthesis and reactivity investigation of two new boronic acid reagents for backbone photocaging (**1b–c**, Fig. 1).

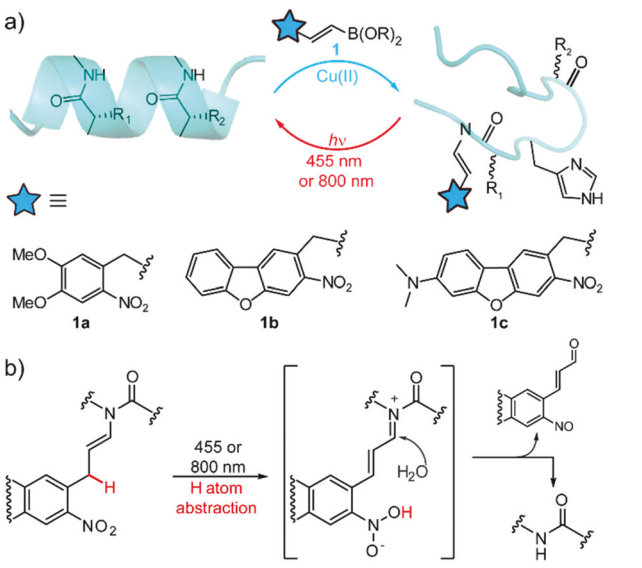

Fig. 1 (a) Schematic depiction of histidine-directed backbone caging/uncaging. (b) Proposed mechanism of C(sp^2)–N photocleavage.

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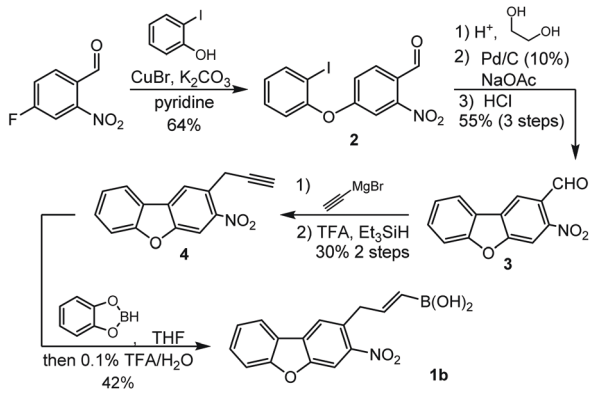


Scheme 1 Synthesis of boronic acid **1b**.

Our design of alkenylboronic acid **1b** was inspired by a reported nitrodibenzofuran structure used as a cysteine thiol photocage with appreciably improved photocleavage kinetics and wavelength dependence,[6] and we developed a synthesis starting from 4-fluoro-2-nitrobenzaldehyde (Scheme 1). The synthetic plan focused on construction of the dibenzofuran moiety *via* a C–H activation/cyclization and late-stage introduction of the propargyl group, followed by hydroboration. This approach was necessary to address limited stability of both the 2-propargyl nitroaromatic moiety and boronate esters. An Ullman coupling allowed access to the diaryl ether **2**.

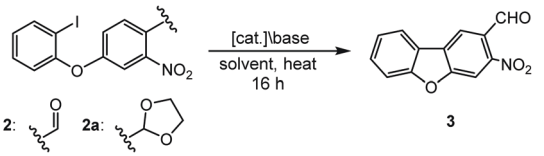
Precedent for the key cyclization exists with both homogeneous (entry 1, Table 1)[21] and heterogenous (entries 2–6)[22] palladium catalysts. In our hands, homogeneous conditions on the acetalized starting material with $Pd(OAc)_2$ gave sluggish reactivity, reaching 20% conversion in 2 days (entry 1). Heterogeneous conditions were initially plagued by irreproducibility where yields reported in *N*,*N*-dimethylacetamide (DMA) ranged from 14–40% depending on the batch of DMA (entry 2).

Intramolecular C–H arylation in DMF followed by acetal hydrolysis gave a remarkably clean reaction, and pure aldehyde **3** could be isolated by simple recrystallization (entry 6) in a reliable yield of 66%. It was also possible to produce the product aldehyde **3** directly without carboxaldehyde protection, albeit in more modest yield (27%, entry 5). Alkynylation with ethynylmagnesium bromide, reduction with triethylsilane under acidic conditions, and uncatalyzed hydroboration with catecholborane followed by acidic workup furnished the desired boronic acid **1b**.

The dimethylamino-substituted analogue **1c** was also targeted to increase light absorption at longer wavelengths and access a two-photon photocleavage mechanism under IR irradiation.[21] The above route was amenable to accessing **1c** with some changes to reactions and conditions (Scheme 2). The synthesis commenced with iodination of 3-(dimethylamino)phenol.[21] With this substrate, diaryl ether formation was best accomplished by KO^{t}Bu-promoted nucleophilic aromatic substitution in the absence of copper.[21] Analogous to our previous route, ring closure, Grignard addition, and triethylsilane reduction afforded the alkyne **8**. Surprisingly, formation of a requisite alkenylboronate compound **1c** was best performed with pinacol borane in the presence of zirconocene hydrochloride catalyst,[23] conditions ineffective for hydroboration of the parent alkyne **4**.

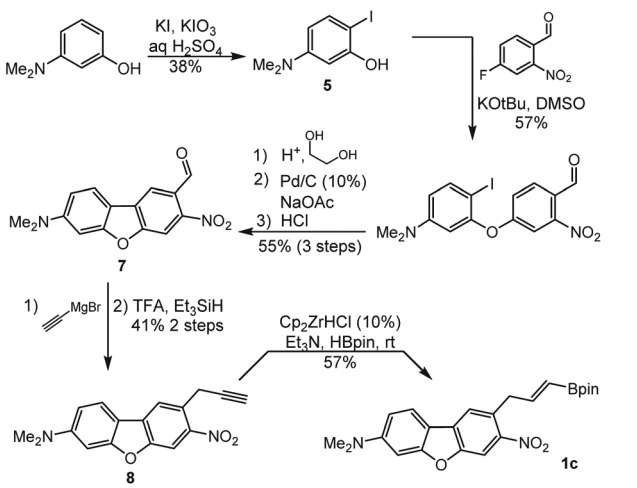
We first examined the reactivity of photosensitive reagent **1b** for backbone modification and subsequent photorelease of peptide **pep1**, a collagen-type sequence that exhibits triple-helix folding behavior known to be disrupted by backbone N–H alteration (seq: Ac-$(POG)_3$POGHOG$(POG)_3$-NH_2,[19,24] and peptide **pep2**, a hormone releasing peptide (LHRH) that contains a pyroglutamate–histidine motif (seq: **pE**–HWSYGLRPG-NH_2), which previous efforts have established as an especially reactive sequence for histidine-directed backbone modification. In aqueous buffer at pH 7.0, **pep1** reacted under histidine-directed Chan–Lam coupling conditions, producing a peptide with an alkenyl modification at Gly9 in 70% conversion as assessed by HPLC and MALDI-MS. Peptide **pep1b** was purified by preparative HPLC to obtain analytically pure

Table 1 Optimization of cyclization conditions[a]



Entry	SM	Catalyst	Base	Solvent	Yield (%)
1[b]	**2a**	$Pd(OAc)_2$	Cs_2CO_3	DMA	20[c]
2	**2**	Pd/C	NaOAc	DMA	14–40[d]
3	**2**	Pd/C	NaOAc	NMP	<5[c]
4	**2**	Pd/C	NaOAc + Me_2NH	DMA	<5[c]
5	**2**	Pd/C	NaOAc	DMF	27
6	**2a**	Pd/C	NaOAc	DMF	66[e]

[a] Condns: 4-(2-Iodophenoxy)-2-nitrobenzaldehyde (**2**) (120 mg), catalyst (5 mol%), base (3 equiv.) in 12 mL of solvent, 140 °C. [b] 2 d at 80 °C. [c] Yield determined by NMR. [d] Yield dependent on solvent source. [e] Yield after acetal hydrolysis.



Scheme 2 Synthesis of boronate pinacol ester **1c**.

material (Fig. 2b, cyan spectrum). A solution of **pep1b** in buffer with isoamylamine (reactive side product scavenging reagent) was then irradiated with blue LED light (nominal 450 nm), which, most pleasingly, cleaved the photosensitive modification and released peptide **pep1** (Fig. 2b, red spectrum). Similarly, **pep2** was modified with **1b** and **1c** to full conversion in aqueous buffer at pH 6.0 to produce **pep2b** and **pep2c**, respectively (Fig. 2c and S5†). Purified peptides (cyan spectrum) were then irradiated with blue light for 4 h in isoamylamine buffer, cleaving the modifications to release **pep2** in both cases (red spectrum).

Similar results were obtained with peptide **pep3** (leuprolide, pE-HWSY-^{D}Leu-LRP-NHEt). When treated with boronic acid **1b**, clean conversion of leuprolide to modified **pep3b** was observed by HPLC in as little as 1.5 h (Fig. 3b, cyan spectrum), and subsequent irradiation with a blue LED light (nominal 455 nm) smoothly released the parent peptide **pep3**. Photorelease kinetics were measured with the blue LED source, following established chemical actinometry procedures to determine LED intensity (Fig. 3b and c).[25] The NDBF-caged substrate (NDBF = 3-nitrodibenzofuran), **pep3b**, exhibited significantly faster photocleavage ($t_{90\%}$ = 37 s, $\Phi\cdot\sigma = 4.7 \times 10^6$ cm^2 mol^{-1}). Both the 1st-generation nitrophenyl cage **pep3a** ($t_{90\%}$ = 257 s, $\Phi\cdot\sigma = 6.7 \times 10^5$ cm^2 mol^{-1}) and the dimethylamino-substituted analogue **pep3c** ($t_{90\%}$ = 907 s, $\Phi\cdot\sigma = 1.9 \times 10^5$ cm^2 mol^{-1}) exhibit appreciably less efficient kinetics, consistent with previous reports.[21] The improved photocleavage with reagent **1b** enables appreciable uncaging within seconds of blue light irradiation (Fig. 3c). These results indicate that **1c** disfavors a 1-photon uncaging mechanism, mirroring previous findings.[21]

Two photon excitation provides myriad benefits, including spatial localization, tissue penetration, and decreased off-target activity. The boronate ester **1c** was designed to optimize two-photon uncaging, based on a previous report of a related structure for photouncaging.[21] Photocleavage of the **pep3b/c** conjugates under near-IR irradiation indicated that both reagents **1b** and **1c** are capable of 2-photon uncaging. Pure

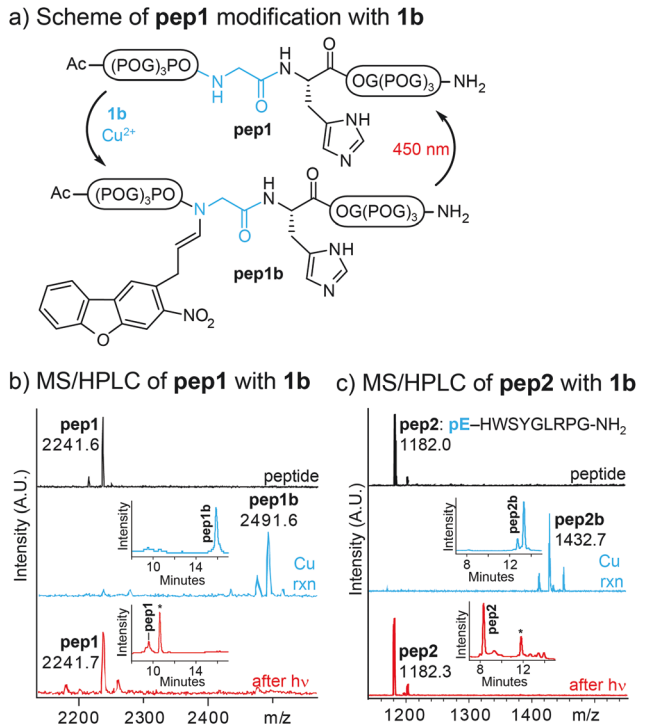


Fig. 2 Chemical photocaging/uncaging of collagen mimetic peptide (**pep1**) and hormone releasing peptide (**pep2**) with **1b**. (a) Schematic depiction. (b) MALDI–TOF MS of **pep1** [M + H] before (black) and after (cyan) copper-mediated N–H photocaging. (red) After irradiation with a blue LED (nominal 450 nm). (c) MALDI–TOF MS of **pep2** [M + H] before (black) and after (cyan) Cu-mediated N–H photocaging. Irradiation with a blue LED (nominal 450 nm) (red) causes photocleavage. (insets in b/c) HPLC analysis before (cyan) and after (red) irradiation. * = non-peptidyl impurity. Condns: **pep1** (100 μM), **1b** (2 mM), $Cu(NO_3)_2$ (1 mM), NMM buffer + 10% v/v DMSO (pH 7.0), 37 °C; **pep2** (100 μM), boronate **1b** (2 mM), $Cu(NO_3)_2$ (1 mM), NMM buffer + 40% v/v DMSO (pH 6.0), 37 °C.

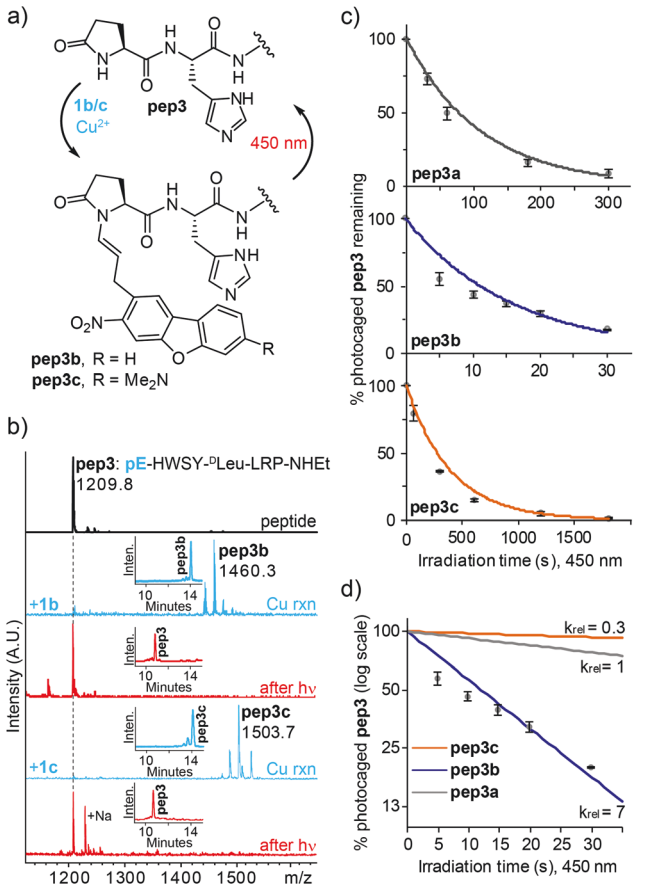


Fig. 3 Chemical photocaging/uncaging kinetics of **pep3b/c**. (a) Schematic depiction. (b) MALDI-TOF MS of **pep3** [M + H] before (black) and after (cyan) Cu-mediated N–H photocaging. After irradiation of **pep3b** and **pep3c** with a blue LED (nominal 455 nm) (red). (insets) HPLC analysis before (cyan) and after (red) irradiation. (c) Uncaging kinetics of photocaged **pep3** by HPLC at various irradiation times (450 nm); n = 3, error bars = std dev. (d) Logarithmic depiction of relative rate constants of **pep3** uncaging. Condns: **pep3** (100–200 μM), boronate (1–4 mM), $Cu(NO_3)_2$ (1–3 mM), NMM buffer + 0–20% v/v DMSO (pH 7.0), 37 °C; individual peptide conditions can be found in the ESI.†

samples of caged peptides **pep3b** and **pep3c** in buffer with DTT additive (15 mM) were irradiated with a Ti:sapphire laser at 800 nm. The progress of the uncaging was followed by single ion monitoring (SIM) LC/MS (Fig. 4). Following 20 minutes of irradiation, **pep3b** shows modest conversion to **pep3** (Fig. 4a, teal/green), while **pep3c** exhibited nearly complete conversion to **pep3** (Fig. 4b, teal/green). We observe 2-photon photorelease rates similar to that of a related system for cleavage of C–S bonds, indicating that photocleavage cross section of **pep3c** is similar to the 0.13 GM measured previously.[26]

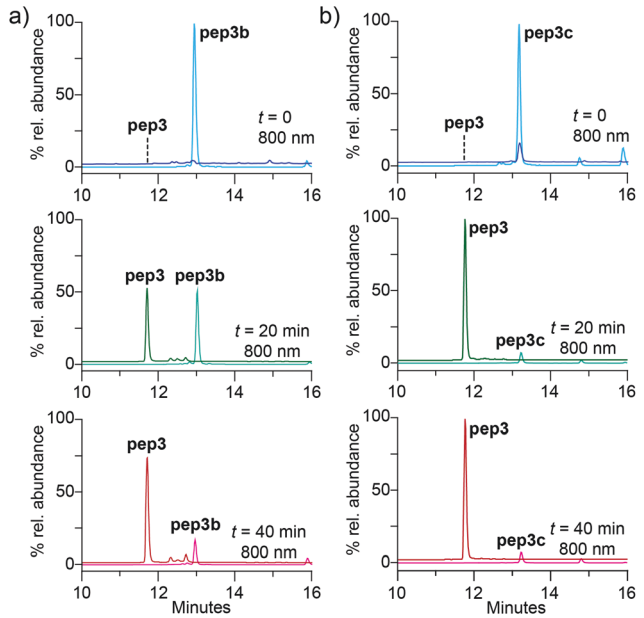


Fig. 4 Two-photon uncaging of **pep3** at 800 nm. (a/b) LC–MS chromatographs (SIM) of **pep3** (darker colors) and **pep3b/c** (lighter colors). Measurements were taken before (blue/cyan), after 20 min (green/teal), and after 40 min (red/pink) of irradiation with 800 nm light. Caging condns: **pep3** (100–200 μM), boronate (2–4 mM), $Cu(NO_3)_2$ (1–3 mM), NMM buffer + 10–20% v/v DMSO (pH 7.0), 37 °C. For uncaging, DTT (15 mM) was added.

We developed new photocaging reagents that cleave at $C(sp^2)$–N bonds, enabling photocaged backbone N–H bonds. The nitrodibenzofuran core allows more efficient uncaging under both blue (1-photon) and red (2-photon) illumination. Photocleavage under 1-photon conditions exhibits drastic differences in behavior depending on substitution pattern. Both photocages demonstrate 2-photon cleavage capabilities, with **1c** providing more efficient 2-photon response, despite negligible 1-photon cleavage. As such, the 2-photon-selective uncaging properties of **1c** may prove useful for orthogonal, sequential uncaging. The absorption, photocleavage, and two-photon-absorption profiles of these reagents mirror those of analogous systems for cleavage at sp^3 carbon atoms and indicate that structure–function relationships observed in traditional photocleavage frameworks may be readily applied to vinylogous analogues for $C(sp^2)$–N photocleavage.

Conflicts of interest

There are no conflicts to declare.

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