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BODNs as biocompatible brominating reagents: visible-light photocatalytic tyrosine modification under physiologically favorable conditions

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Rakuto Yoshida,[a] Yuichiro Hori,[b] Daisuke Uraguchi*[a,c] and Keisuke Asano*[a]

The photochemical reactivity of 1-bromo-2-oxo-1,2-dihydronaphthalene-1-carboxylates (BODNs) was demonstrated. They are inert in the dark under physiological conditions but become active as brominating reagents for tyrosine modification under visible light irradiation in the presence of a photocatalyst. The BODNs can be applied to protein labeling with bromo groups as sensitive mass tags.

Photocatalytic modification of amino acid side chains offers methodologies for labeling of peptides and proteins in a spatiotemporally controlled manner, which proves useful for probing cellular events, including biomolecular communications.[1,2] Labeling tools have been developed to selectively install marking tags (X) on molecules of interest within complex biomolecular systems, with the labeled compounds subsequently identified through mass spectrometry (Figure 1a).[2] Most labeling techniques target the modification of relatively reactive and abundant amino acid residues, including lysine and cysteine, and numerous marking tags installed on proteins of interest impart high sensitivity in mass analyses. However, labeling amino acid residues with little surface exposure[3] such as tyrosine results in low sensitivity because of the small number of installed tags. Thus, using a labeling tag with characteristic signals is desirable for these targets. In this context, bromination emerges as a promising labeling method for installing sensitive mass tags as bromo groups result in diagnostic isotopic patterns owing to the unusual abundance of bromine isotopes (^{79}Br: 50.69%, ^{81}Br: 49.31%, Figure 1a), which are detectable amidst numerous other signals. The introduction of bromo groups has been reported to be effective in the sequence determination of artificial macromolecules.[4] However, photocatalytic labeling of biomolecules through bromination is underdeveloped. The sole precedent of photochemical bromination of peptides and proteins involves highly cytotoxic ultraviolet (UV) laser irradiation (Figure 1b),[5] and there has been no catalytic reaction, wherein photocatalysts serve as molecular antennae and provide a high spatial resolution.[1] Hence, the lack of biocompatible methods for photocatalytic reactions represents a bottleneck in the development of bromination labeling techniques. A solution to this problem is to develop brominating reagents whose reactivity is activatable by a photocatalyst and light irradiation under biocompatible conditions. In this study, we discovered the photochemical reactivity of 1-bromo-2-oxo-1,2-dihydronaphthalene-1-carboxylates (BODNs), which are stable in the dark at 37 °C but

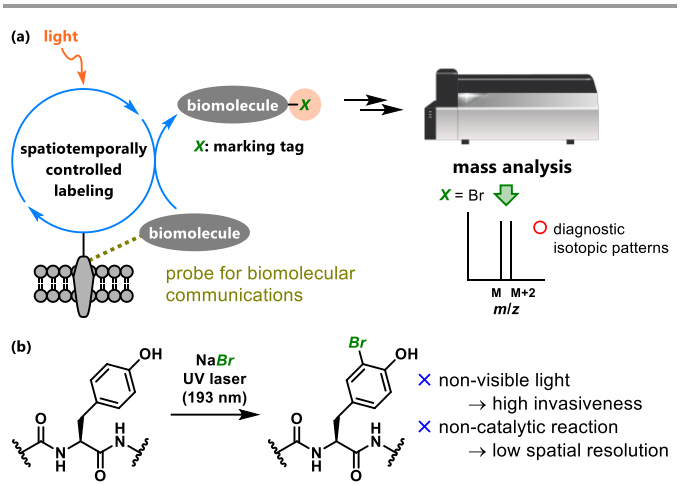


Fig. 1 Advantages and challenges in photocatalytic bromination labeling. (a) Spatiotemporally controlled labeling of biomolecules and bromination as installation of sensitive mass tags. (b) Precedent photochemical bromination of peptides and proteins.

[a] *Institute for Catalysis, Hokkaido University, Sapporo, Hokkaido 001-0021, Japan.*
[b] *Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka, Fukuoka 819-0395, Japan.*
[c] *List Sustainable Digital Transformation Catalyst Collaboration Research Platform, Institute for Chemical Reaction Design and Discovery (ICReDD List-PF), Hokkaido University, Sapporo, Hokkaido 001-0021, Japan.*
E-mail: uraguchi@cat.hokudai.ac.jp; asano@cat.hokudai.ac.jp
† Electronic Supplementary Information (ESI) available: Experimental procedures, analytical and spectroscopic data for synthetic compounds, and copies of NMR. See DOI: 10.1039/x0xx00000x

become activated as brominating reagents through visible light photocatalysis. Notably, fluorescent dyes commonly used in bioimaging probes exhibit activity as photocatalysts, providing methodologies for the bromination labeling of tyrosine-containing peptides and proteins. Because tyrosine residues are known to play important roles in protein-protein interactions (PPI) despite their poor exposure on general protein surfaces,[6] the development of labeling techniques with sensitive chemical handles for mass analyses would contribute to the elucidation of significant biomolecular communications.

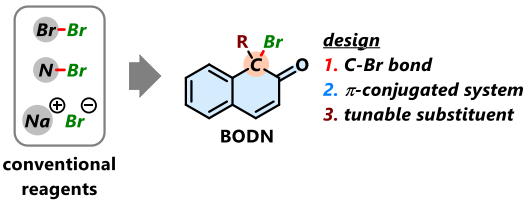
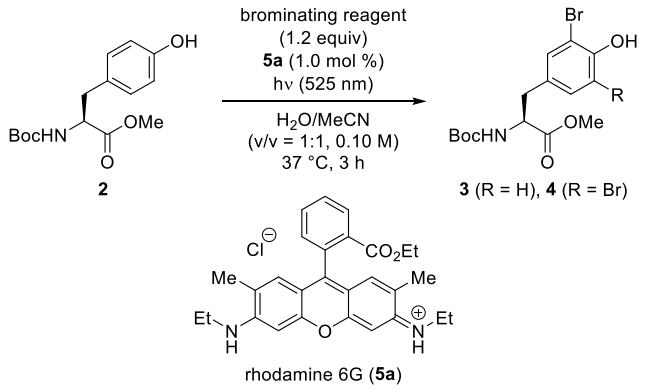


Fig. 2 Design of biocompatible reagents for photocatalytic bromination.

In this study, we aimed to develop a methodology for tyrosine labeling through photocatalytic bromination. Various reagents were assayed in a model reaction: bromination of tyrosine

Table 1 Investigations of brominating reagents[a]



Entry	Brominating reagent	Yields without light [**3**, **4**] (%)		Yields with light [**3**, **4**] (%)	
		3	**4**	**3**	**4**
1	Br_2	43	28	33	27
2	NBS	73	<1	63	<1
3	NaBr	<1	<1	<1	<1
4	**1a**	<1	<1	<1	<1
5	**1b**	<1	<1	<1	<1
6	**1c**	<1	<1	<1	<1
7	**1d**	11	<1	43	7
8	**1e**	<1	<1	76	<1
9	**1f**	<1	<1	59	<1
10	**1g**	<1	<1	71	6
11	**1h**	<1	<1	<1	<1

[a] Reactions were run using **2** (0.10 mmol), the brominating reagent (0.12 mmol), and **5a** (0.0010 mmol) in H_2O/MeCN (v/v =1:1, 1.0 mL). ASAHI SPECTRA CL-1503/CL-H1-525-7-1-B (525 nm) was used as a light source.

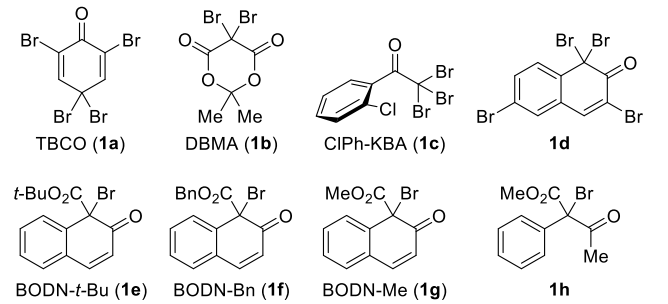


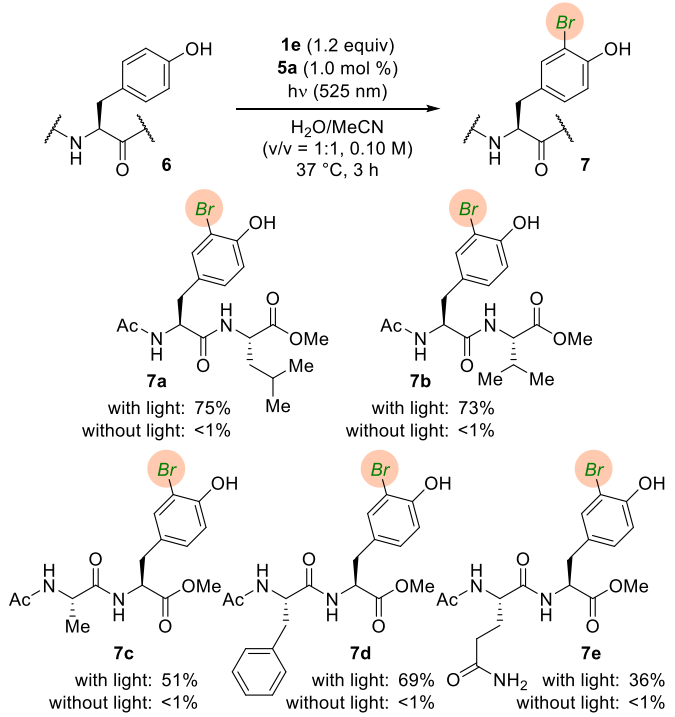
Fig. 3 Synthesized and precedent C–Br bond-based reagents.

derivative **2** using rhodamine 6G (**5a**) as a photocatalyst and light at a wavelength of 525 nm at 37 °C in aqueous media (Table 1). Bromine (Br_2) and *N*-bromosuccinimide (NBS) underwent thermal reactions in the dark at physiological temperatures because of their high reactivities (Table 1, entries 1 and 2). In contrast, sodium bromide (NaBr), which was used in the preceding experiment (Figure 1c),[5] was too inert to be activated in the photocatalytic reaction under visible light (Table 1, entry 3). To ensure appropriate stability to prevent background reactions, we then explored brominating reagents bearing a C–Br bond (Figure 2, design 1), which is more stable than the Br–Br and N–Br bonds contained in conventional reagents. Known brominating reagents **1a**–**1d** bearing C–Br bonds (Figure 3)[8] were initially investigated. TBCO (**1a**),[8a] DBMA (**1b**),[8b] and ClPh-KBA (**1c**)[8c] were sufficiently stable to inhibit dark reactions; however, their photocatalytic reactions failed (Table 1, entries 4–6). To enhance the photochemical reactivity, we investigated 1,1,3,6-tetrabromonaphthalen-2(1*H*)-one (**1d**)[8d] bearing an expanded π-conjugated system (Figure 2, design 2). **1d** provided brominated tyrosines **3** and **4** under light irradiation, although moderate background bromination also took place in the dark (Table 1, entry 7). The promising results with **1d** encouraged us to investigate BODNs bearing an analogous π-conjugated structure with a substituent at the α-position, which can be easily altered and affect the reactivity (Figure 2, design 3), while BODNs have not been previously used as brominating reagents. BODNs **1e**–**1g** were readily synthesized through two steps from 2-hydroxy-1-naphthoic acid.[7] **1e** selectively produced monobrominated tyrosine **3** under light irradiation, whereas no bromination occurred in the dark (Table 1, entry 8). These results indicate that **1e** possesses stability preventing background reactions, and has reactivity controllable by visible light irradiation under biocompatible conditions. BODNs **1f** and **1g** with different ester substituents at the α-position also proved useful, with variation in yields indicating tunable reactivity by changing the substituent at the C1-position (Table 1, entries 9 and 10). The cyclic π-conjugated structure of BODNs proved to be crucial for the photochemical reactivity, as an acyclic analog **1h** was not reactive regardless of the light irradiation (Table 1, entry 11). These investigations identified BODNs as brominating reagents applicable for biocompatible photocatalytic labeling.

Next, the photocatalytic control of bromination was demonstrated by monitoring the reaction with **1e**, **5a**, and 525

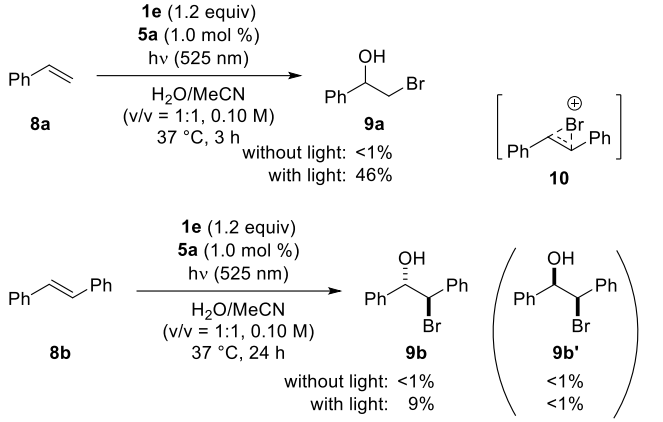
nm light (see Schemes S1 and S2 in the ESI). While bromination proceeded under light irradiation in the presence of **5a** (Scheme S1a), the absence of **5a** or light resulted in no reaction (Schemes S1b and S1c). Both the catalyst and light were essential for bromination. Furthermore, the reaction conducted at 0.010 M was much slower than that at 0.10 M (Schemes S1a and S1d), indicating sensitivity to the concentration.[2] In addition, the reaction was arbitrarily initiated by light irradiation in the presence of **5a** (Scheme S2). These results indicate that the reaction is dependent on the proximity to the catalyst as a molecular antenna and light irradiation as a switch in the reaction, thereby leading to the spatial and temporal control of bromination.

The photocatalytic bromination could also be applied to tyrosine-containing peptides (Scheme 1). Peptides bearing leucine (**7a**), valine (**7b**), alanine (**7c**), phenylalanine (**7d**), and glutamine (**7e**) were efficiently brominated under the light irradiation, while no bromination occurred in the dark. The photocatalytic reactions selectively provided the desired product **7** without any detected side products, and **7** exhibited diagnostic signals in the mass spectra analyses (see Scheme S3 in the ESI). In addition, this catalytic reaction was specifically efficient for tyrosine modification, and the bromination of other aromatic amino acids, including phenylalanine, tryptophan, and histidine, was sluggish (see Scheme S4 and S5 in the ESI).



Scheme 1 Bromination of tyrosine-containing peptides.

Mechanistic investigations were also conducted. As indicated by the ultraviolet-visible (UV-Vis) absorption of **1e** shown in Scheme S6 in the ESI, **1e** absorbed visible light with wavelengths up to approximately 430 nm. It was further observed that irradiation with light at a wavelength of 430 nm caused the decomposition of **1e** while it was intact in the dark (see Scheme S7 in the ESI), indicating that **1e** was excited by the light. In addition, irradiation with 430 nm light enabled the bromination of **2** with **1e** in the absence of any catalyst (see Scheme S8a in the ESI), whereas no bromination occurred, and most of **2** was recovered under no light irradiation (see Scheme S8b in the ESI). These results indicate that the excited BODN **1e*** served as a brominating reagent. Notably, the developed catalytic reaction enabled the use of less invasive light with a longer wavelength compared to that used in non-catalytic reactions (525 nm vs. 430 nm).



Scheme 2 Reactions of styrene and *trans*-stilbene.

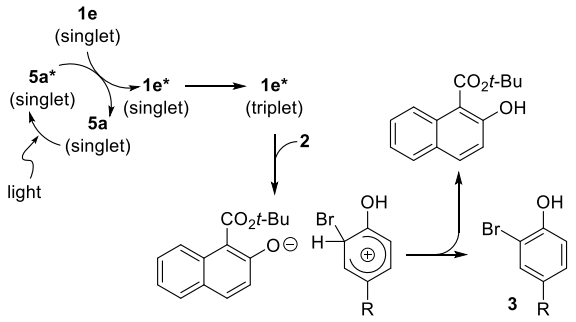


Fig. 4 Proposed mechanism.

Fluorescence-quenching experiments were conducted using **1e**, **2**, and **5a** (see Scheme S9 in the ESI). The Stern–Volmer plots indicate that the excited catalyst was quenched by **1e** but not by **2**. To gain insight into whether the bromination proceeded through the formation of cationic or radial species following the excitation of **1e**, the reactions of alkenes were investigated. The photocatalytic reaction of styrene (**8a**) provided bromohydrin **9a**, suggesting a mechanism involving cationic species. Moreover, *trans*-stilbene (**8b**) afforded *trans*-bromohydrin **9b**, whereas *cis*-bromohydrin **9b'** was not yielded (Scheme 2). The stereospecificity suggests that the reaction proceeded through the formation of bromiranium ion intermediate **10** rather than a mechanism involving radical species, and activated BODN **1e*** served as a cationic brominating reagent (Figure 4). Furthermore, the energy transfer from **5a*** to **1e** was assigned to a Dexter mechanism rather than a Förster mechanism because the absorption band of **1e** and the emission band of **5a**

did not overlap (see Scheme S10 in the ESI). Dexter mechanisms enable shorter-range energy transfer than Förster mechanisms, providing a higher spatial resolution for photocatalytic reactions.[9]

Moreover, **5a**, which contains no heavy atoms, functioned as a photocatalyst. In the present reaction system, the triplet state of excited BODN **1e***, which has the required lifetime for the reaction, was probably the active species. Generally, molecules containing heavy atoms are necessary as catalysts to generate triplet active species, in which the triplet states of the excited catalysts transfer their energy to the reagents and generate triplet active species. Here, however, the bromine atom of **1e** likely provided heavy-atom effects, and the singlet states of the light-irradiated catalysts **5a*** excited **1e** to the singlet state of **1e***, which underwent intersystem crossing mediated by its bromine atom to generate the triplet state of **1e*** (Figure 4). The singlet energy transfer from **5a*** to **1e** was supported by the fact that the efficiency of the reaction under air, including O_2, which is known to be a triplet quencher,[10] was comparable to that under an argon atmosphere (see Scheme S11 in the ESI). Thus, various photocatalysts were useful (see Scheme S12 in the ESI), and photocatalytic reactions of **1e** were possible not only with rose bengal (**5d**) and eosin Y (**5e**) but also with **5a**, 5-TAMRA (**5b**), and fluorescein (**5c**), which contain no heavy atoms and have been used as dyes of fluorescent probes for bioimaging.[11,12] This means that selective labeling techniques established based on the fluorescent probes would be useful for installing the catalysts for the present bromination in cellular systems, for example, specific installation of fluorescein derivatives on cell surfaces.[12a] The uncommon feature of fluorescent probes also being useful as catalyst probes facilitates the application of BODNs to chemical biology research and offers unique probing technology. The reaction mechanism, which is compatible with the presence of O_2 and does not generate toxic reactive oxygen species, is also advantageous for the development of chemical biology tools.

In summary, we discovered the photochemical reactivity of BODNs, which did not induce background reactions at 37 °C in aqueous media but were activated as brominating reagents under the visible light irradiation in the presence of the photocatalysts for tyrosine modification. The photocatalytic reactions enabled the use of less invasive light with a longer wavelength compared to non-catalytic reactions and spatiotemporal control of bromination. Notably, fluorescent dyes are useful as photocatalysts in the present reaction system, and they will readily open up the application of BODNs as chemical biology tools enabling the installation of attractive labeling tags on tyrosine-containing peptides and proteins. Studies on bromination labeling using BODNs in biomolecular systems are currently underway and will be reported in due course.

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Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article has been included as part of the ESI.†

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