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Development of the 8-aza-3-bromo-7-hydroxycoumarin-4-ylmethyl group as a new entry of photolabile protecting groups

Hikaru Takano [a], Tetsuo Narumi [a,*,†], Nami Ohashi [a], Akinobu Suzuki [b], Toshiaki Furuta [b], Wataru Nomura [a], Hirokazu Tamamura [a,*]

[a] Institution of Biomaterial and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kandasurugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Department of Biomolecular Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

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ABSTRACT

A significant substitution effect of the position of the bromo group on the photosensitivity of the 8-azacoumarin chromophore leads to the development of a highly photosensitive 8-aza-3-bromo-7-hydroxycoumarin-4-ylmethyl (aza-3-Bhc) group that shows excellent photolytic efficiency and hydrophilicity with long-wavelength absorption maxima. The newly identified aza-3-Bhc group can be applied to caged glutamates for ester-type and carbamate-type protections of carboxyl and amino functionalities.

1. Introduction

Photosensitive biologically active compounds (referred to as caged compounds) have attracted considerable attention due to their practical potentials as phototriggers of biological functions.[1] Progress in the field has been driven by the development of new photolabile protecting group types,[2] such as nitrobenzyl,[3] benzoin,[4] phenacyl,[5] and coumarin.[6] Although a number of chromophores have been applied to photolabile protecting groups, 8-azacoumarin derivatives have not been applied to the caged compounds until our identification of several advantages as an attractive chromophore for caging chemistry, such as excellent water solubility, high molar absorptivity, and efficient photorelease at low pH as described in our previous report.[7] This report describes the unexpected substitution effects on the photosensitivity of 8-azacoumarin chromophore, leading to the development of a novel 8-aza-3-bromo-7-hydroxycoumarin-4-ylmethyl caging group (aza-3-Bhc **1**) that shows excellent photolytic efficiency and hydrophilicity with long-wavelength absorption maxima and high molar absorptivity, whose features are superior to those of the 6-bromo derivatives **2** (Fig. 1).

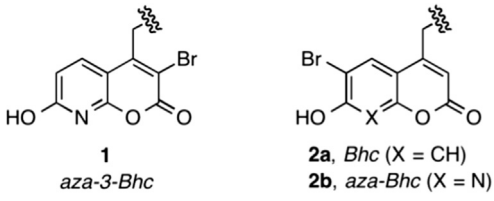

Fig. 1. Structures of azacoumarin- and coumarin-based Bhc groups.

2. Results and discussion

Our study on the development of the aza-3-Bhc group emerged from the bromination of azacoumarin derivative **3** that provided the 6-brominated compound **4a** in a 67% yield as a major product,[7] accompanied with a 16% yield of the 3-brominated compound **4b** as a minor product (Scheme 1a, Supplementary data). Bromination of the coumarin chromophore provides several advantages for photolysis reactions including lowering the pK_a of the adjacent hydroxyl group accelerating the formation of the strongly absorbing anion and the promotion of intersystem crossing to the triplet, which is considered to be the photochemically reactive state.[6d]

* Corresponding authors. Tel./fax: +81 53 478 1198 (T.N.); tel.: +81 3 5280 8036; fax: +81 3 5280 8039 (H.T.); e-mail addresses: ttnarum@ipc.shizuoka.ac.jp (T. Narumi), tamamura.mr@tmd.ac.jp (H. Tamamura).
† Present address. Department of Applied Chemistry and Biochemical Engineering, Faculty of Engineering, Shizuoka University, Hamamatsu, Shizuoka 432-8561, Japan.

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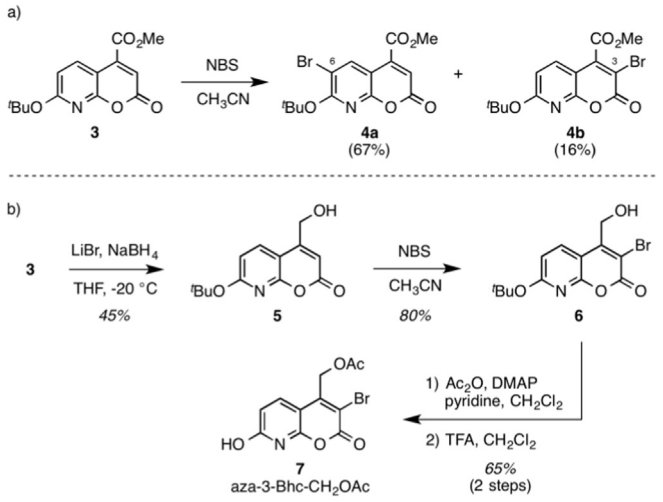


Scheme 1. Synthesis of brominated 8-azacoumarin esters **4** and aza-3-Bhc-CH_2OAc **7**.

Preliminary studies revealed that the 8-aza-7-hydroxycoumarin chromophore as well as the 6-bromo-7-hydroxycoumarin has a pK_a value, which is lower than 7.4, and mostly assumes the deprotonated (ionic) form under physiological conditions without additional electron-withdrawing groups. Therefore, the absorption spectrum of **9** has a single peak as the spectrum of **8** (Fig. 2). These features imply the possibility that the regioisomeric 3-brominated 8-azacoumarin chromophore would also work as a hydrophilic caging group. Furthermore, several papers[8] that identified strong substitution effects on quinolone chromophores prompted us to invoke that the substitution pattern could positively affect photophysical and photochemical properties of 8-azacoumarin chromophore.

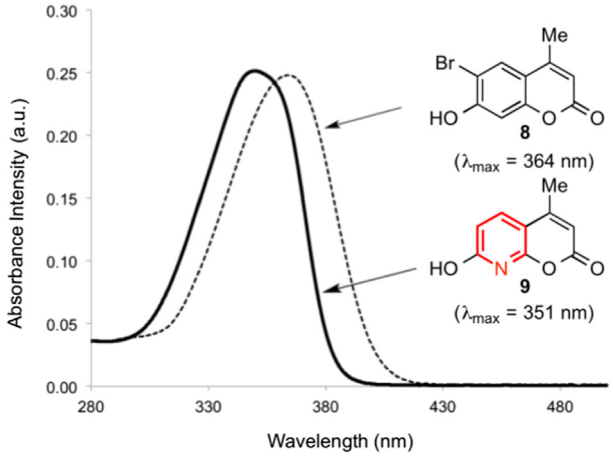
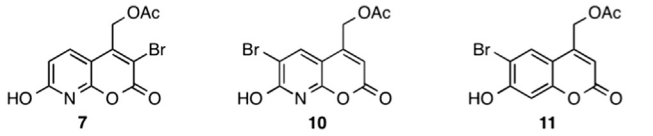


Fig. 2. Absorption spectra of Bhc derivative **8** (dash line) and 8-aza-hc **9** (solid line).

The 3-brominated acetate aza-3-Bhc-CH_2OAc **7** was synthesized from **3** in four steps (Scheme 1b). Briefly, reduction of **3** with $LiBH_4$ gave the corresponding alcohol **5** in moderate yield. In contrast with the bromination of **3** that gave a mixture of regioisomers, the bromination of alcohol **5** proceeded regioselectively to provide the 3-brominated alcohol **6** in 80% yield, which was subjected to acetylation followed by TFA treatment to give the desired aza-3-Bhc-CH_2OAc **7**.

Initially, we investigated the photophysical and hydrophilic behaviors of aza-3-Bhc-CH_2OAc **7** (Table 1). Compared with the original Bhc-CH_2OAc **11**, the absorption maximum of **7** shifted to longer wavelength from 370 nm for **11** to 378 nm for **7**, whereas that of **10** shifted to shorter wavelength (λ_{max}=362 nm). The molar absorptivity at the maximum wavelength (ε_{max}) of **7** is 27,086 M^{-1} cm^{-1}, which is also higher than those of **10** (ε_{max}=21,107 M^{-1} cm^{-1}) and **11** (ε_{max}=16,584 M^{-1} cm^{-1}). These results indicated that the 3-brominated 8-azacoumarin would be a superior chromophore to the 6-brominated coumarins. As expected, aza-3-Bhc-CH_2OAc **7** has a pK_a value below the physiological pH and also shows high aqueous solubility, which is important for the photorelease of high concentrations of the caged compounds under physiological conditions. HPLC monitoring of the hydrolysis stability revealed that while aza-3-Bhc-CH_2OAc **7** was more sensitive to hydrolysis in PBS (pH 7.4) than aza-Bhc-CH_2OAc **10**, the hydrolysis stability of **7** in KMOPS buffer (10 mM MOPS; 3-morpholinepropane-1-sulfonic acid, and 100 mM KCl, pH 7.1) is comparable to that of **10** (Fig. 3).

Table 1
Photophysical and hydrophilic properties of aza-3-Bhc-CH_2OAc **7**, aza-Bhc-CH_2OAc **10**, and Bhc-CH_2OAc **11**



Compd	λ_{max}[a] (nm)	ε_{max}[b] (M^{-1} cm^{-1})	C_s[c] (μM)	pK_a[d]
Aza-3-Bhc-CH_2OAc (**7**)	378	27,086	3260	5.08
Aza-Bhc-CH_2OAc (**10**)	362	21,107	10,832	4.22
Bhc-CH_2OAc (**11**)	370	16,584	602	5.88

[a] Long-wavelength absorption maxima in PBS (0.1% DMSO).
[b] Molar absorptivity at the absorption maxima.
[c] Concentration at saturation in PBS (0.1% DMSO).
[d] Determined using citric/phosphate buffer in the pH range 2.6–7.0.

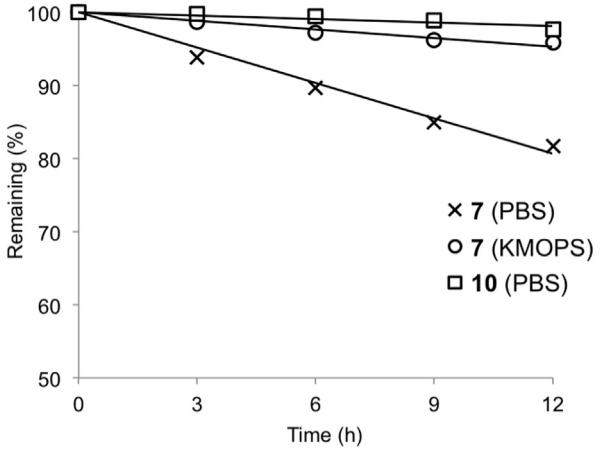
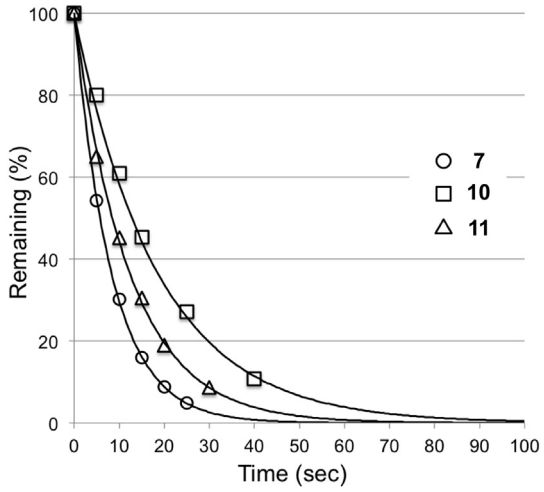


Fig. 3. Hydrolysis stability of compounds **7** and **10** at room temperature in dark. Time course of compounds **7** and **10** in PBS or KMOPS was analyzed by reversed-phase HPLC.

Having recognized promising photophysical and hydrophilic behaviors of the 3-brominated 8-azacoumarin chromophore, we evaluated the photochemical properties of aza-3-Bhc-CH_2OAc **7** under the photolysis in 5 μM KMOPS buffer solution at pH 7.2 at 350 nm. The time course of photolysis reaction of **7** was monitored by HPLC in terms of the consumption of the starting materials (Table 2), and indicates that the photolytic reaction at 350 nm of **7** follows a single-exponential decay as in the result of the original Bhc compound **11**. The time to reach 90% conversion (t_{90}) for photolysis of **7** is 19 s, which is shorter than those of the 6-

Table 2
Time course for photolysis reactions of aza-3-Bhc-CH_2OAc **7**, aza-Bhc-CH_2OAc **10**, and Bhc-CH_2OAc **11** and selected photochemical properties

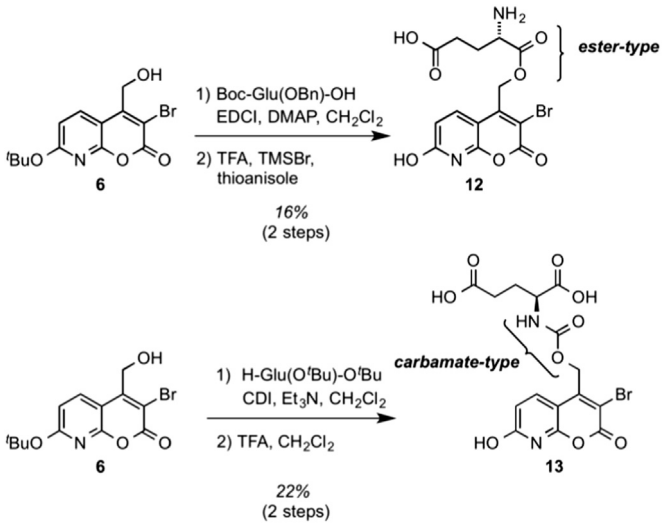


Compd	t_{90} (s)	ε_{350}[a] (M^{-1} cm^{-1})	Φ_{chem}[b]	$\varepsilon_{350}\cdot\Phi_{chem}$[c]
Aza-3-Bhc-CH_2OAc (**7**)	19	20,175	0.17	2667
Aza-Bhc-CH_2OAc (**10**)	42	20,583	0.059	1211
Bhc-CH_2OAc (**11**)	28	13,774	0.13	1806

[a] Molar absorptivity at 350 nm.
[b] Quantum yields for the disappearance of starting materials upon irradiation at 350 nm.
[c] Product of the photolysis quantum yield and molar absorptivity.

brominated coumarins **10** and **11** (t_{90}=42 s for **10** and 28 s for **11**, respectively). The photolysis quantum yields of disappearance of starting materials were calculated from the single decay curves using the equation $\Phi=1/(I\times10^3\varepsilon t_{90})$ as reported by Tsien.[9] Notably, the quantum yield of disappearance of **7** (Φ_{chem}=0.17) is approximately three times higher than that of **10** (Φ_{chem}=0.059) and significantly higher than that of **11** (Φ_{chem}=0.13). In addition, the photolytic efficiency,[10] the product of the photolysis quantum yield (Φ_{chem}) and molar absorptivity (ε) of **7** ($\varepsilon_{350}\cdot\Phi_{chem}$=2667), is approximately 1.5–2.2-fold higher than that of **10** ($\varepsilon_{350}\cdot\Phi_{chem}$=1211) and **11** ($\varepsilon_{350}\cdot\Phi_{chem}$=1806). These results indicate that the bromo substitution on position 3 of the 8-azacoumarin chromophore leads not only to improve the photophysical behavior but also to increase the photosensitivity. Although the reason for the significant enhancement of photochemical reactivity of **7** is not fully understood at this stage, these observations suggest that the aza-3-Bhc group has a powerful set of photophysical, photochemical, and hydrophilic properties for caging chemistry.

Next, we examined the synthetic methods for the introduction of the aza-3-Bhc group to biologically relevant compounds (Scheme 2). The aza-3-Bhc group is not limited to the caging group for ester-types. In addition to the α-Glu ester **12**, the aza-3-Bhc group can be applied to the α-Glu carbamate **13** for the protection of an amino functionality, which can be removed efficiently with light of wavelength of 365 nm to produce the corresponding alcohol.[11] Compared to the corresponding Bhc-protected compounds **14** and **15**,[6d] both aza-3-Bhc-protected compounds **12** and **13** showed improved photosensitivity (Table 3). The time to reach 90% conversion (t_{90}) for photolysis of **12** is 32 s, which corresponds to be approximately 20% faster than that of the corresponding Bhc-protected α-Glu ester **14** (t_{90} of **14**=38 s). A similar superiority of the aza-3-Bhc group was observed with the carbamate substrates **13** and **15**. In accordance with the greater quantum yields of disappearance and comparable molar absorptivities, the photolytic efficiency of both **12** and **13** is higher than those of **14** and **15** (relative



Scheme 2. Synthesis of aza-3-Bhc-caged glutamates.

value of $\varepsilon\cdot\Phi$ of **12**/**14**=1.19 and of **13**/**15**=1.27). These results indicate that the newly identified aza-3-Bhc chromophore can serve as a variant of Bhc chromophore in biologically relevant compounds. Further studies for the application of the 8-aza-3-Bhc group to the protection of other functionalities, such as alcohols, phosphoric acids, and thiols are in progress.

3. Conclusion

We have reported the development of the aza-3-Bhc group as a new entry of photolabile protecting groups for caging chemistry through the strong influence of the position of a bromo substituent on the photosensitivity of the 8-azacoumarin chromophore. The 3-brominated 8-azacoumarin **7** is considerably more efficient than the 6-brominated regioisomer **10**. Aza-3-Bhc-CH_2OAc **7** shows excellent photolytic efficiency with a bathochromic shift of the absorption maximum of 8-azacoumarin from 362 to 378 nm. Moreover, we have disclosed the potentials of the aza-3-Bhc group as protecting groups of carboxyl and amino functionalities for caged glutamates. A key to the development of the aza-3-Bhc group is the acidic azacoumarin chromophore, whose feature can provide a new opportunity to improve the photosensitivity by chemical modifications that are distinct from those in previous approaches. Efforts to elucidate the reason for the markedly enhanced photosensitivity by the 3-bromo substituent and studies on the two-photon sensitivity of the 8-azacoumarin chromophore are currently in progress.

4. Experimental section

4.1. General methods

All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of nitrogen, using commercially supplied solvents and reagents unless otherwise noted. CH_2Cl_2 was distilled from CaH_2 and stored over molecular sieves. Thin-layer chromatography (TLC) was performed on Merck $60F_{254}$ precoated silica gel plates and were visualized by fluorescence quenching under UV light and by staining with phosphomolybdic acid, *p*-anisaldehyde, or ninhydrin, respectively. Flash column chromatography was carried out using silica gel 60 N (Kanto Chemical Co., Inc.).

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Table 3
Selected photophysical and photochemical properties of aza-3-Bhc-caged glutamates **12** and **13**, and Bhc-caged glutamates **14** and **15**

Compd	λ_{max}[a] (nm)	ε_{max}[b] (M^{-1} cm^{-1})	ε_{365}[c] (M^{-1} cm^{-1})	t_{90}[d] (s)	Φ_{chem}[e]	$\varepsilon_{365}\cdot\Phi_{chem}$[f]	rel. $\varepsilon\cdot\Phi$[g]
Aza-3-Bhc-Glu-ester (**12**)	378	13,648	12,153	32	0.17	2063	1.19
Aza-3-Bhc-Glu-carbamate (**13**)	376	17,068	15,623	10	0.43	6717	1.27
Bhc-Glu-ester (**14**)	370	16,912	16,466	38	0.11	1740	—
Bhc-Glu-carbamate (**15**)	370	17,822	17,636	13	0.30	5280	—

[a] Long-wavelength absorption maxima in PBS (0.1% DMSO).
[b] Molar absorptivity at the absorption maxima.
[c] Molar absorptivity at 365 nm.
[d] Time to reach 90% conversion.
[e] Quantum yields for the disappearance of starting materials upon irradiation at 365 nm.
[f] Photolytic efficiency: product of the photolysis quantum yield and molar absorptivity.
[g] Relative value of photolytic efficiency [aza-3-Bhc/Bhc]. For full experimental protocol, see Supplementary data.

4.2. Characterization data

^{1}H NMR (400 or 500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using a Bruker Avance II spectrometer with a CryoProbe. Chemical shifts are reported in δ (ppm) relative to Me_4Si (in $CDCl_3$) as internal standard. Infrared (IR) spectra were recorded on a JASCO FT/IR 4100, and are reported as wavenumber (cm^{-1}). Low- and high-resolution mass spectra were recorded on a Bruker Daltonics micrOTOF (ESI-MS) spectrometers in the positive and negative detection modes.

4.3. HPLC condition

For analytical HPLC, a Cosmosil C18-ARII column (4.6×250 mm, Nacalai Tesque, Inc., Kyoto, Japan) was employed with a linear gradient of MeCN containing 0.1% (v/v) TFA at a flow rate of 1 cm^3 min^{-1} an Agilent HP 1100 system with DAD detection (Agilent Technologies JAPAN Ltd., Tokyo, Japan) and JASCO PU-2086 plus (JASCO corporation, Ltd., Tokyo, Japan), and eluting products were detected by UV at 340 nm.

4.4. Experimental procedures of 8-azacoumarin derivatives 5, 6, 7, 12, and 13

*4.4.1. 7-(tert-Butoxy)-4-(hydroxymethyl)-2H-pyrano[2,3-b]pyridin-2-one (**5**).* A suspension of LiBr (1.09 g, 12.6 mmol) and $NaBH_4$ (410.2 mg, 10.8 mmol) in THF (36.0 mL) was stirred under nitrogen at 50 °C for 2 h, producing the solution of $LiBH_4$ (ca. 0.3 M). To the solution was added compound **3** (1.03 g, 3.72 mmol) in THF (36.0 mL), and the mixture was stirred at −20 °C for 2 h. The reaction mixture was quenched by 1 M HCl aq, and the organic layer was removed under reduced pressure. The residue was extracted with EtOAc, washed with brine, and dried over Na_2SO_4. Concentration under reduced pressure followed by flash column chromatography over silica gel with *n*-hexane/EtOAc (1:1) gave the title compound **5** (411.8 mg, 45% yield) as white powder. Mp: 236–244 °C (dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64 (s, 9H), 4.85 (m, 2H), 6.45 (m, 1H), 6.60 (m, 1H), 7.74 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.3, 60.9, 82.5, 104.9, 109.1, 110.5, 134.6, 153.9, 157.4, 161.5, 164.6; IR (ATR) ν 3395 (OH), 1704 (CO); HRMS (ESI), *m*/*z* calcd for $C_9H_8NO_4$ $[M-tert\text{-}Bu+2H]^+$ 194.0453, found 194.0450.

*4.4.2. 3-Bromo-7-(tert-butoxy)-4-(hydroxymethyl)-2H-pyrano[2,3-b]pyridin-2-one (**6**).* To a solution of compound **5** (121.4 mg, 0.487 mmol) in CH_3CN (1.37 mL) was added NBS (428.6 mg, 125 mmol), and the mixture was stirred at room temperature for 4 h. After being concentrated under reduced pressure, the residue was dissolved in EtOAc, washed with H_2O, and dried over Na_2SO_4. Concentration under reduced pressure followed by flash column chromatography over silica gel with *n*-hexane/EtOAc (1:1) gave the title compound **6** (127.1 mg, 80% yield) as a dark green solid. Mp: 254–257 °C (dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64 (s, 9H), 5.02 (s, 2H), 6.67 (m, 1H), 8.13 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.7, 61.9, 82.9, 106.1, 109.3, 111.3, 136.8, 150.3, 156.23, 157.7, 164.7; IR (ATR) ν 3465 (OH) 2977 (CH), 2929 (CH), 1707 (CO), HRMS (ESI), *m*/*z* calcd for $C_{13}H_{14}BrNNaO_4$ $[M+Na]^+$ 350.0004, found 350.0000.

*4.4.3. (3-Bromo-7-hydroxy-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl) methylacetate (**7**).* To a solution of compound **6** (127.1 mg, 0.389 mmol) and DMAP (6.20 mg, 0.0507 mmol) in CH_2Cl_2 (5.6 mL) were added sequentially pyridine (313.1 μL, 3.89 mmol) and acetic anhydride (183.7 μL, 1.94 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ aq, and dried over Na_2SO_4. Concentration under reduced pressure gave the corresponding acetate (138.1 mg, 96% yield). To a solution of the corresponding acetate (138.1 mg, 0.374 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (1 mL), and the mixture was stirred at room temperature for 30 min. Concentration under reduced pressure followed by flash column chromatography over silica gel with $CHCl_3$/MeOH (5:1) to give the title compound **7** (80.1 mg, 68% yield) as a white solid. Mp: 273–276 °C (dec); ^{1}H NMR (500 MHz, CD_3OD) δ 1.99 (s, 3H), 5.35 (s, 2H), 6.53 (d, *J*=9.0 Hz, 1H), 7.97 (d, *J*=9.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.5, 61.1, 102.0, 106.1, 109.4, 136.2, 150.9, 158.4, 160.4, 166.8, 170.0; IR (ATR) ν 2923 (OH) 1736 (CO), HRMS (ESI), *m*/*z* calcd for $C_{11}H_8BrNNaO_5$ $[M+Na]^+$ 335.9484, found 335.9485.

*4.4.4. (S)-4-Amino-5-((3-bromo-7-hydroxy-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl)methoxy)-5-oxopentanoic acid (**12**).* To a solution of Boc-Glu(OBn)-OH (158.0 mg, 0.468 mmol), EDCI·HCl (541.2 mg, 2.82 mmol), and DMAP (13.8 mg, 0.113 mmol) in CH_2Cl_2 (10.2 mL) was added compound **6** (100.7 mg, 0.308 mmol), and the mixture was stirred at room temperature for 24 h. The mixture was poured into water and extracted with EtOAc, and dried over Na_2SO_4. Concentration under reduced pressure gave the crude compound (321.9 mg), which was used in the next step without further purification. A solution of the crude compound (321.9 mg, 0.498 mmol), 1 M TMS-Br/TFA (3.32 mL), and 1 M thioanisole/TFA (3.32 mL) was stirred at room temperature for 3 h. Purification by preparative HPLC (Gradient: 0 min, 0% CH_3CN in H_2O; 90 min, 40% CH_3CN in H_2O) followed by lyophilization to give a title compound **12** (23.7 mg, 16% yield) as a pale purple solid. Mp: 149–154 °C (dec); ^{1}H NMR (500 MHz, CD_3OD) δ 2.06 (m, 2H), 2.39 (m, 2H), 4.10 (m, 1H), 5.56 (m, 2H), 6.60 (d, *J*=8.5 Hz, 1H), 8.06 (d, *J*=8.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 26.6, 30.2, 53.2, 64.7, 106.3, 110.4, 112.0, 139.1, 147.5, 157.9, 158.3, 166.6, 169.9, 175.4; IR (ATR) ν 3505 (NH), 2981 (OH), 2865 (CH), 1737 (CO), 1690 (CO) 1682 (CO), HRMS (ESI), *m*/*z* calcd for $C_{14}H_{14}BrN_2O_7$ $[M+H]^+$ 400.9984, found 400.9981.

*4.4.5. (S)-2-((((3-Bromo-7-hydroxy-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl)methoxy)carbonyl)amino)-pentanedioic acid (**13**).* To a solution

of CDI (25.4 mg, 0.157 mmol) of CH_2Cl_2 was added **6** (48.5 mg, 0.148 mmol), and the mixture stirred at 0 °C for 1 h. After H-Glu(O^tBu)-O^tBu (90.4 mg, 0.306 mmol) and Et_3N (52.3 μL, 0.375 mmol) were added, the mixture was stirred at room temperature for additional 12 h. The reaction mixture was concentrated with reduced pressure. The residue was diluted with Et_2O, and washed with water and brine, dried over Na_2SO_4. Concentration under reduced pressure gave the crude compound (168.1 mg), which was used in the next step without further purification. To a solution of the crude compound (168.1 mg, 0.275 mmol) in CH_2Cl_2 (0.90 mL) was added TFA (2.7 mL), and the mixture was stirred at room temperature for 1 h. Purification by preparative HPLC (Gradient: 0 min, 5% CH_3CN in H_2O; 90 min, 40% CH_3CN in H_2O) followed by lyophilization to give a title compound **13** (17.6 mg, 22% yield) as a pale green solid. Mp: 196–198 °C (dec); ^1H NMR (500 MHz, CD_3OD) δ 1.88 (m, 1H), 2.16 (m, 1H), 2.38 (m, 2H), 4.19 (m, 1H), 5.46 (s, 2H), 6.68 (m, 1H), 8.19 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 27.8, 31.1, 54.8, 64.0, 106.3, 110.4, 110.8, 139.6, 149.4, 157.6 (2C), 158.4, 166.3, 175.2, 176.3; IR (ATR) ν 3306 (NH), 2981 (OH), 1742 (CO), 1698 (CO), HRMS (ESI), m/z calcd for $C_{15}H_{13}BrN_2NaO_9$ $[M+Na]^+$ 466.9702, found 466.9701.

4.5. Determination of saturated concentrations

Saturated concentrations of compounds were calculated from the standard curves that related peak area (340 nm) against known concentration of compounds in PBS.

4.6. Determination of the pK_a values

The pK_a values of compounds were estimated from the titration curves of absorbance against pH using 10 μM substrate solution in citric/phosphate buffer solution in the pH range 2.6–7.0 by adjusting the acidity with 10 μL of 2 M NaOH sequentially. The pH values were analyzed by a sensitive pH meter (HORIBA, F51).

4.7. Photolysis and quantum efficiency measurement

Into a Pyrex test tube of 12 mm diameter was placed 2 mL of 10 μM substrate solution in KMOP solution (pH 7.2) containing 0.1% DMSO. The solution was irradiated at 350 nm using four RPR 350 nm lamps (10 mJ s^{-1}). Aliquots of 10 μM were removed periodically and analyzed by HPLC. The light output for the quantum efficiencies measurement was performed using ferrioxalate actinometry.

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Supplementary data

This section presents the experimental details, and ^1H and ^{13}C NMR spectra. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.04.063.

References and notes

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11. See Supplementary data for details.