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Development of new quinoline-based photo-labile groups for photo-regulation of bioactive molecules

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ABSTRACT

A series of quinoline-based photo-removable protecting (caging) groups were synthesized for the development of new chemical tools to photo-regulate bioactive molecules in living cells and tissues with improved properties. Compared with the recently developed 8-bromo-7-hydroxyquinolinyl (BHQ) chromophore, change of the bromine substituent to a pyridine group led to a new photo-labile group (3′-PyHQ) with an increased water solubility, a lower self-fluorescence, and a higher photolysis efficiency. It was proposed that the replacement of a halogen group by a pyridine-like heterocycle may provide a general strategy to improve the existing photo-caging groups.

Photo-labile protecting groups offer an ideal method for regulating cellular functions with a high spatiotemporal resolution and therefore, have become increasingly important in the biological research. 'Caging' is a specific term which describes the use of a photolytic chromophore to encapsulate bioactive molecules in an inactive form whose activity can be restored by photo-irradiation. A variety of molecular structures, such as 2-nitrobenzyl,[2] 1-(4,5-dimethoxy-2-nitrophenyl)-ethyl,[3,4] 4,5-dimethoxy-2-nitrobenzyl,[5] 6-bromo-7-hydroxy-coumarin-4-yl-methyl,[6] [7-(diethyl-amino)-coumarin-4-yl]-methyl,[7] and 7-dinitro-indolinyl[8] have been developed as photo-caging groups. They have been successfully used to photo-regulate metal ions (such as calcium),[9] neurotransmitters,[5,10] carboxylic acids,[11,12] proteins,[13–16] nucleotides,[17–19] peptides,[20] RNAs,[6,21] and DNAs.[22–31] Nevertheless, the existing photo-caging groups are still limited by various unfavorable properties including poor water solubility, high self-fluorescence, and/or lower photolysis sensitivity.

To solve the problems, Dore and co-workers recently developed the BHQ (8-bromo-7-hydroxyquinoline) group based on the quinoline structure. Up to now BHQ has been successfully utilized for the photo-releasing of acetate, phosphates and diol groups that are commonly found in bioactive molecules such as neurotransmitters.[1,11,12] Compared with the previous photo-caging groups, BHQ demonstrates many favorable photo-chemical and photo-physical properties such as a larger two-photon cross-section, an improved water solubility, and a higher uncaging quantum yield. These properties are highly important for the study of living cells and tissue cultures.[10]

In the present study we seek to generate an improved photo-labile protecting group with a better water solubility, a lower self-fluorescence, and a higher photolysis sensitivity as compared to BHQ. To this end we synthesize a series of BHQ derivatives (**1**–**7**, Fig. 1) to cage acetate as a model bioactive compound. Note that during the course of our study Dore and co-workers also examined the possibility to improve BHQ by the substitution changes.[12] They reported that swapping the bromine substituent for a nitro, cyano, or chloro or exchanging the hydroxy for dimethylamino or sulfhydryl significantly alters the photo-chemical and photo-physical properties of the quinoline chromophore. Compared with Dore's work, our strategy to modify BHQ is different as shown below.

Our first strategy was to change the position of the electron-donating hydroxyl group from the 7-position to the 6-position. The rationale for this strategy is the previously proposed solvent-

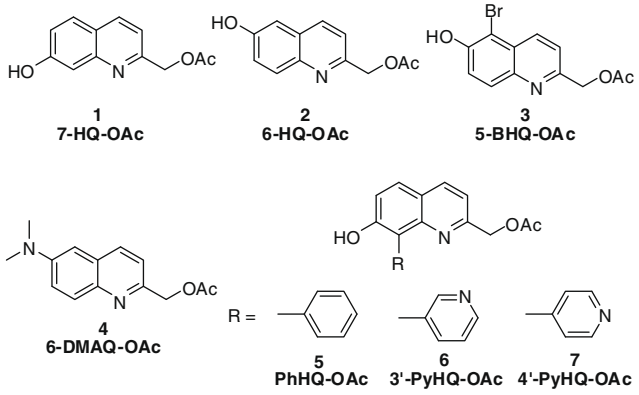

Figure 1. Quinoline-based photo-removable protecting groups.

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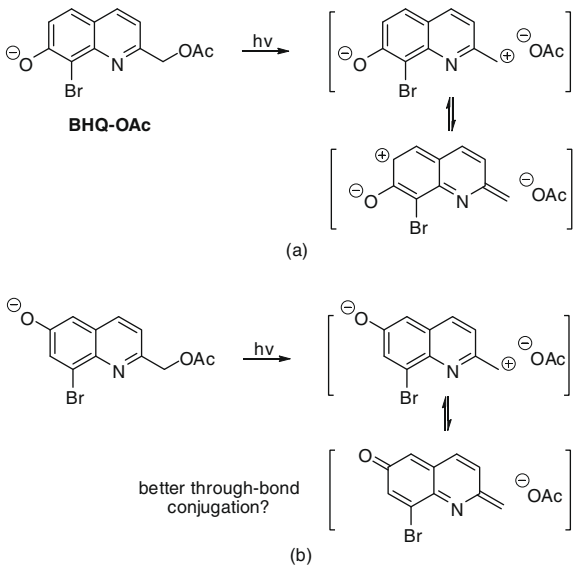


Figure 2. Proposed mechanism for the BHQ photolysis reaction.

assisted photoheterolysis (S_N1) reaction mechanism for the photoreleasing of the BHQ group.[32] As shown in Figure 2a, irradiation of BHQ-OAc should lead to a singlet excited state which can cleavage its carbon–oxygen bond to generate a zwitterion-like intermediate. If this mechanism is correct, placement of an electron-donating hydroxyl group at the 6-position should increase the stability of the zwitterion-like intermediate (Fig. 2b) and thereby, improve the properties of the photo-caging group.

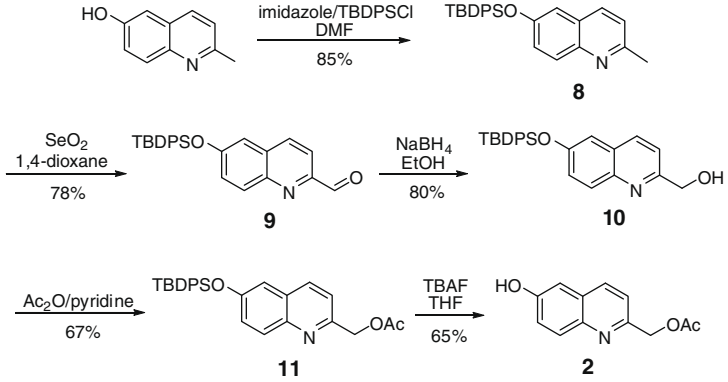
On the basis of the above proposal, we synthesized compounds **1**–**4**. To provide an example for the synthesis, 6-HQ-OAc (**2**) was prepared from 2-methylquinolin-6-ol. As shown in Scheme 1, protection of the 6-OH of 2-methylquinolin-6-ol by *t*-butyldiphenylsilyl chloride gave **8** in 85% yield. Oxidation of the methyl group of **8** by using SeO_2 in 1,4-dioxane provided **9** in 78% yield. Reduction of **9** by $NaBH_4$ in EtOH afford **10** in 80% yield. Esterification of **10** with acetic anhydride gave **11** in 67% yield. Finally, the removal of the silyl protecting group with TBAF produced **2** in 65% yield. The compounds **1**, **3**, and **4** were also synthesized by using similar synthetic procedures.

Our second approach to improve the BHQ group was to incorporate an aromatic heterocycle into the chromophore. The advantage of this approach is twofold: (1) the presence of an aromatic heterocycle such as pyridine may improve water solubility; (2) the aromatic heterocycle may improve photon cross-section and quantum yield because it enlarges the conjugation plane. Thus compound **6** (3′-PyHQ-OAc) was synthesized from 2-methylquinolin-7-ol (Scheme 2). First, bromination of 2-methylquinolin-7-ol with Br_2 in acetic acid gave **12** in 78% yield. Protection of the 7-OH group by the MOMCl provided **13** in 93% yield. Subsequently compound **14** was obtained by Suzuki cross-coupling between **13** and pyridine-3-ylboronic acid (yield = 74%). Removal of the MOM protecting group with 35% HCl in CH_3OH provided **15** in 89% yield. Re-protection of the 7-OH group by *t*-butyldiphenylsilyl chloride gave **16** in 79% yield. Oxidation of the 2-methyl group by the SeO_2 afforded **17** in 84% yield, which was reduced by $NaBH_4$ to produce **18** in 89% yield. Esterification of **18** with acetic anhydride provided **19** in 72% yield. Finally, the removal of the silyl protecting group with TBAF in THF gave **6** in 64% yield. Note that we had to use both the MOM and TDBPS protecting groups in the synthesis, because TDBPS-protected compound could not undergo Suzuki coupling possibly due to the steric hinderance, whereas the MOM-group cannot survive the SeO_2 oxidation. The compounds **5** and **7** were synthesized by using similar synthetic procedures.

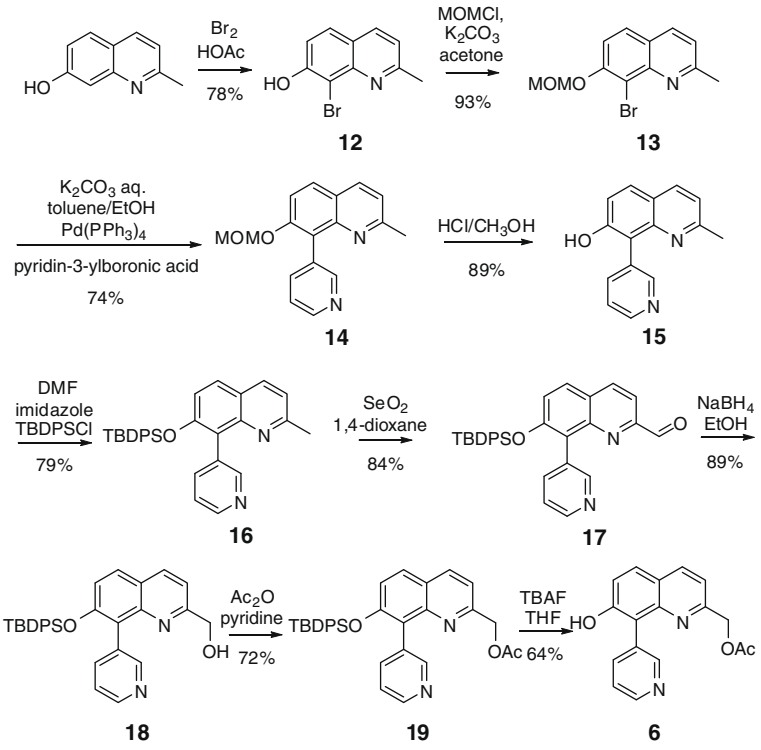
The UV–vis spectra reveal that compounds **3** and **4** have characteristic absorbance peaks similar to BHQ-OAc, although the λ_{max} value for compounds **1**, **2**, and **5**–**7** are slightly lower (Fig. 3). On the other hand, significant differences are observed for the fluorescence spectra of these compounds (Fig. 4). Compared to BHQ-OAc, compounds **2**, **6**, and **7** exhibit a much lower fluorescence emission. In particular, compound **2** shows almost no self-fluorescence. A lower self-fluorescence of **2**, **6**, and **7** should facilitate the application of these photo-caging groups when they are used together with other fluorescent indicators.[1] Note that the dimethylamino-substituted compound (**4**) exhibits the strongest emission bands at 498 nm. This observation is consistent with the previous studies on similar compounds by Dore and co-workers.[12]

It has been demonstrated that successful use of 'caged' compounds in cellular studies requires satisfactory solubility in aqueous solutions at moderately high ionic strength.[1] Thus the water solubility of compounds **1**–**7** was determined by measuring the fluorescence or absorption intensity as a function of their concentrations, respectively.[33–35] As shown in Figure 5, the solubility of compound **6** in water (0.5% DMSO) is 250 μM, which is nearly 10-fold higher than the parent compound, BHQ-OAc. Besides, compounds **1**–**3** have better water solubility to various degrees. The results supported our proposal that the solubility of a photo-caging group can be improved by the incorporation of heterocycles. Note that the solubility of compound **4** is fairly low (less than 5 μM) and it has a high self-fluorescence. Therefore, we concluded that **4** could not be suitable for further applications.

Furthermore, we studied the photolysis efficiency of compounds **1**–**7**. Upon photolysis with 365 nm photons under simulated physiological conditions in KMOPS (100 mM KCl, 10 mM MOPS, pH 7.2), BHQ-OAc and compounds **1–7** were all converted



Scheme 1. The synthesis of 6-HQ-OAc (**2**).



Scheme 2. The synthesis of 3′-PyHQ-OAc (**6**).

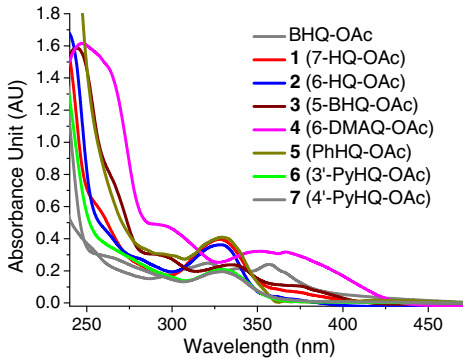


Figure 3. UV–vis spectra of BHQ-OAc and compounds **1**–**7** (100 μM in KMOPS).

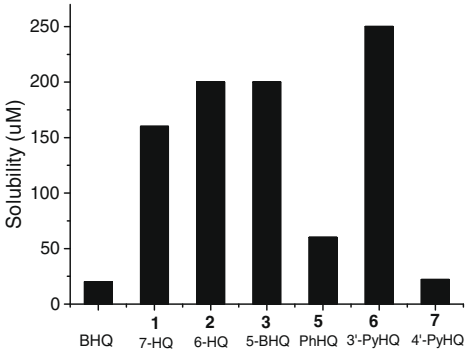


Figure 5. The water solubility of BHQ-OAc and compounds **1**–**3**, **5**–**7** (aqueous solutions with 0.5% DMSO).

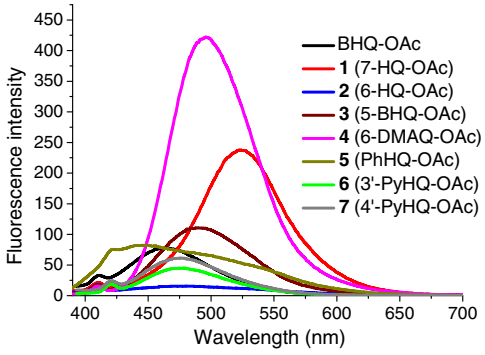


Figure 4. Fluorescence spectra of BHQ-OAc and compounds **1**–**7** (50 μM in KMOPS, λ_{ex} = 365 nm).

to the corresponding hydroxy derivative and acetate. The speed of each photo-chemical reaction was monitored by HPLC measurements. The results (Fig. 6) indicated that compounds **5–7** photolyze nearly 1.5 times faster than BHQ-OAc, which is possibly due to their large uncaging cross-section. On the other hand, compounds **1** and **2** photolyze slightly more slowly than the parent compound BHQ-OAc. Finally, it is surprising to observe that compound **3** does not photolyze efficiently. We considered that the high photolysis sensitivity may contribute to a lower phototoxicity in the study of biomolecules.

Summarizing the above results,[36–42] we can conclude that compounds **2** (6-HQ-OAc) and **6** (3′-PyHQ-OAc) are better photo-caging groups than BHQ-OAc. Both **2** and **6** exhibit much lower self-fluorescence. They both show dramatically better water solubility. Furthermore, **6** photolyzes about 1.5 times faster than BHQ-OAc, although **2** photolyzes slightly more slowly than BHQ-OAc. Given the fact that BHQ represents one of the best photo-caging groups available at the present stage, we believe that our newly developed photo-caging groups (i.e., 6-HQ and 3′-PyHQ) may find important applications for photo-regulation of various bioactive molecules under physiological conditions. Furthermore, we have demonstrated in the present study that the replacement of a halogen

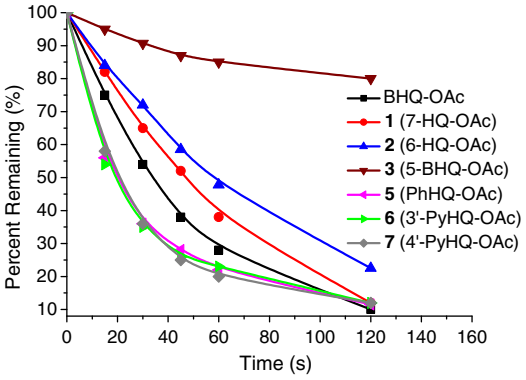


Figure 6. Time courses for the photolysis of BHQ-OAc and compounds **1**–**7** at 365 nm.

group by a pyridine-like heterocycle may provide a general strategy to improve the existing photo-caging groups.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.071.

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36. *Compound* **1**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.94 (d, J = 8.3, 1H), 7.30 (d, J = 8.9, 1H), 7.24 (d, J = 1.6, 1H), 7.21 (d, J = 8.3, 1H), 6.77 (dd, J = 8.8, 2.1, 1H), 5.29 (s, 2H), 2.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 170.8, 159.6, 155.3, 147.9, 137.6, 128.8, 122.0, 120.0, 116.5, 108.3, 66.3, 20.9.
37. *Compound* **2**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 8.03 (dd, J = 11.7, 9.1, 2H), 7.43 (d, J = 8.5, 1H), 7.34 (dd, J = 9.1, 2.6, 1H), 7.14 (d, J = 2.6, 1H), 5.38 (s, 2H), 2.17 (s, 3H); ^{13}C NMR (100 MHz, DMSO, δ ppm) 170.3, 155.7, 152.8, 142.2, 135.2, 130.2, 128.7, 122.4, 119.9, 108.4, 66.9, 20.8.
38. *Compound* **3**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 8.38 (d, J = 8.7, 1H), 7.99 (d, J = 9.2, 1H), 7.54 (d, J = 8.7, 1H), 7.50 (d, J = 9.2, 1H), 5.38 (s, 2H), 2.19 (s, 3H); ^{13}C NMR (100 MHz, DMSO, δ ppm) 170.3, 153.7, 152.9, 142.7, 134.2, 129.7, 127.6, 121.9, 121.1, 103.3, 66.4, 20.8.
39. *Compound* **4**: ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.99–7.91 (m, 2H), 7.39–7.32 (m, 2H), 6.80 (d, J = 2.8, 1H), 5.32 (s, 2H), 3.08 (s, 6H), 2.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 170.9, 151.5, 148.9, 135.0, 129.8, 129.2, 120.3, 119.8, 104.9, 67.9, 40.8, 21.1.
40. *Compound***5**: ^{1}H NMR (400 MHz, DMSO, δ ppm) 8.21 (d, J = 8.4, 1H), 7.81 (d, J = 8.9, 1H), 7.41 (d, J = 4.2, 4H), 7.38 (d, J = 8.9, 1H), 7.31 (dq, J = 8.9, 4.2, 1H), 7.26 (d, J = 8.4, 1H), 5.15 (s, 2H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, DMSO, δ ppm) 170.5, 155.9, 155.6, 146.9, 137.0, 135.8, 132.0, 128.4, 127.4, 126.5, 122.4, 121.9, 119.2, 116.01, 66.6, 20.8.
41. *Compound***6**: ^{1}H NMR (400 MHz, DMSO, δ ppm) 10.20 (s, 1H), 8.61 (dd, J = 2.1, 0.7, 1H), 8.50 (dd, J = 4.8, 1.7, 1H), 8.28 (d, J = 8.4, 1H), 7.88 (d, J = 8.9, 1H), 7.85–7.80 (m, 1H), 7.45 (ddd, J = 7.8, 4.8, 0.7, 1H), 7.39 (d, J = 8.9, 1H), 7.31 (d, J = 8.4, 1H), 5.17 (s, 2H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, DMSO, δ ppm) 170.2, 156.1, 155.8, 152.1, 147.0, 146.4, 139.1, 137.0, 131.3, 129.0, 122.6, 121.7, 119.0, 118.5, 116.1, 66.3, 20.6.
42. *Compound* **7**: ^{1}H NMR (400 MHz, DMSO, δ ppm) 10.28 (s, 1H), 8.60 (dd, J = 4.4, 1.6, 2H), 8.28 (d, J = 8.4, 1H), 7.89 (d, J = 8.9, 1H), 7.43 (dd, J = 4.4, 1.6, 2H), 7.38 (d, J = 8.9, 1H), 7.31 (d, J = 8.4, 1H), 5.17 (s, 2H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, DMSO, δ ppm) 170.2, 156.2, 155.6, 148.5, 146.1, 143.8, 137.0, 129.4, 127.1, 121.6, 119.3, 119.0, 116.2, 66.3, 20.7.