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COMMUNICATION

Singlet oxygen-responsive photorelease of tyramine

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Abstract

A system capable of photoreleasing tyramine has been developed. The photodonor system consists of an isoindole cage of tyramine and a bis-cyclometalated Ir(III) complex singlet oxygen ($^1O_2$) photosensitizer. Photoirradiation at a wavelength of 365 nm produces tyramine, as evidenced by mass spectrometry and ^{1}H NMR spectroscopy. The photorelease proceeds through two steps involving the formation of a Diels–Alder-type [2 + 4] cycloadduct of $^1O_2$, followed by the slow and spontaneous decomposition of the adduct into tyramine and 2-benzoylbenzophenon.

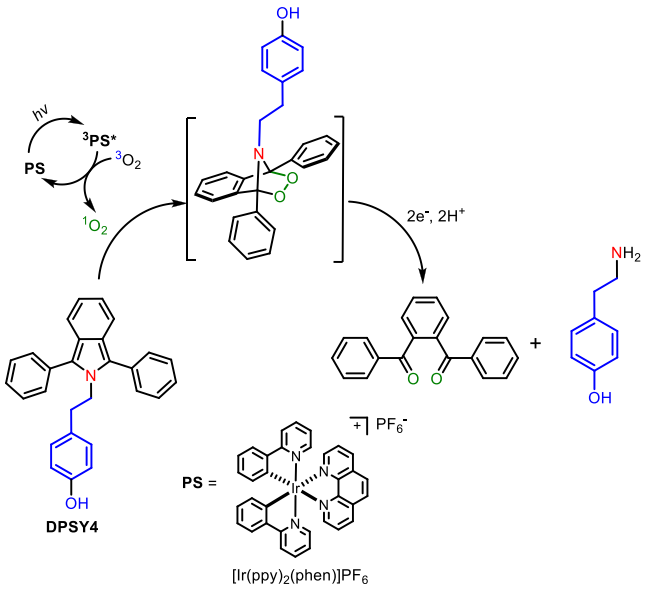
KEYWORDS
iridium complex, photocage, photodelivery, singlet oxygen, tyramine

Tyramine, 2-(4-hydroxyphenyl)ethylamine, belongs to the family of biogenic trace amines in the human body. Although the trace level of tyramine remains lower than those of classical biogenic amino neurotransmitters, such as γ-aminobutyric acid, local concentrations at neural synapses are considerably high.[1] Growing evidence indicates that tyramine is intimately involved in a variety of neurophysiological processes. For examples, Borowsky and coworkers found that neurotransmission is mediated by tyramine binding of G protein-coupled receptors.[2] The disruption in the homeostasis of tyramine elicits fatal neurodegenerative conditions. Psychiatric disorders, such as depression, hypertension, and schizophrenia, are linked to the failure of optimal level of tyramine.[3] The other pathological conditions, including migraine and Parkinsonism, are also proposed to be related with the dysregulation of tyramine.[4,5] However, despite the pathophysiological importance, the molecular mechanism underlying the biological actions of tyramine has yet been fully established. The immaturity is due to the underdevelopment of molecular tools of tyramine. In particular, stimuli-responsive molecular donors of tyramine remain sparse in the literature, although they are anticipated to be employed for therapeutic purposes.

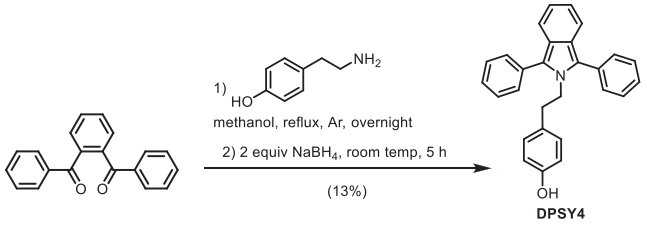
One viable approach to attaining high-precision release is to employ photons. The photoinduced release can be executed noninvasively with very high spatiotemporal precisions. In addition, the progress of photoinduced release can be controlled by a photon dose-dependent manner. Thus, it is anticipated that photodonors of tyramine will serve as powerful tools to study the biological actions of tyramine. Despite the prospects, there is only one report of a photodonor of tyramine. Etchenique and co-workers found that a Ru(II) diimine complex could liberate a tyramine monodentate ligand under photoillumination at a wavelength of 450 nm.[6]

In this research, we developed a photodonor of tyramine (DPSY4). DPSY4 was based on 1,3-diphenyl-2*H*-isoindole (Scheme 1). Tyramine was incorporated into the isoindole core through the nitrogen atom. DPSY4 is highly reactive toward the Diels–Alder-type [2 + 4] cycloaddition reaction with singlet dioxygen ($^1O_2$, hereafter). The $^1O_2$-adduct, an endoperoxide, is spontaneously cleaved into tyramine and 2-benzoylbenzophenone (BBP, hereafter). We employed a $^1O_2$ photosensitizer to trigger the two-step release of tyramine. We previously validated the $^1O_2$-mediated photouncaging of hydrogen sulfide[7] and β-phenethylamines.[8] This strategy is valuable as the photoexcitation wavelength can be conveniently controlled by choosing a $^1O_2$ photosensitizer.[9] Herein, we report the synthesis and reactivities of DPSY4. The photorelease reaction was characterized with mass spectrometry and ^{1}H NMR and UV–vis absorption spectroscopy.

DPSY4 was prepared through consecutive reductive amination reactions between BBP and tyramine in the presence of $NaBH_4$ in a 13% yield (Scheme 2). DPSY4 exhibited insufficient stabilities under light and an aerobic condition. This limited stability prohibited long-



SCHEME 1 Photorelease of tyramine mediated by photosensitized singlet oxygen ($^1O_2$)



SCHEME 2 Synthesis of DPSY4

term storage and the large-scale synthesis. All the spectroscopic studies were, thus, performed with freshly prepared samples.

Tyramine release from DPSY4 was examined in aerated CH_3CN containing $[Ir(ppy)_2(phen)]PF_6$ (ppy = 2-phenylpyridinato; phen = 1,10-phenanthroline), a $^1O_2$ photosensitizer, under continuous photoirradiation at a wavelength of 365 nm (4 W). $[Ir(ppy)_2(phen)]PF_6$ was chosen as it produces the triplet-excited state with a unitary quantum yield. The triplet state energy of $[Ir(ppy)_2(phen)]PF_6$ is 2.13 eV which is greater than that (0.97 eV) of $^1O_2$.[10] This energetic disposition facilitates spontaneous triplet–triplet energy transfer to the ground-state molecular oxygen to form $^1O_2$. Actually, the quantum yield for $^1O_2$ photosensitization (Φ_Δ) of $[Ir(ppy)_2(phen)]PF_6$ was determined to be as high as 0.52.[10]

Figure 1 compares the liquid chromatograms of tyramine, BBP, $[Ir(ppy)_2(phen)]^+$, DPSY4, and a mixture of DPSY4 and $[Ir(ppy)_2(phen)]^+$. Peaks corresponding to the authentic tyramine and BBP appeared at retention time at 0.6 and 3.7 min, respectively. DPSY4 and $[Ir(ppy)_2(phen)]^+$ eluted at retention time 10.1 and 1.6 min. Photoirradiation (365 nm, 4 W) of the mixture of 1.0 mM DPSY4 and 100 μM $[Ir(ppy)_2(phen)]PF_6$ led to the disappearance of the DPSY4 peak with the concomitant emergence of the peaks marked with * (retention time = 0.6–1.0 min) and ** (retention time = 3.7 min). The former peak contained multiple bands; mass spectra (positive mode, ESI) contained the *m/z* values of 195.12, 210.23, and 407.11, which could be assigned as $[H(N\text{-oxide of tyramine})(CH_3CN)]^+$, $[H(tyramine)(THF)]^+$, and $[KNa(tyramine)_2(H_2O)_2Cl]^+$, respectively. The close matches between the theoretical isotope distributions and the experimental values support the assignments (Figure 1b and Figure S2). The latter peak revealed the *m/z* value of 287.11. This mass value should be assigned as $[H(BBP)]^+$, as its theoretical isotope distribution coincides perfectly with the experimental result (Figure 1c). These observations collectively indicate the photoinduced cleavage of DPSY4 into tyramine and BBP and are consistent with our proposed mechanism (Scheme 1). DPSY4 retained sufficient stability unless photons and O_2 were provided (Figure S3). The result indicates that the tyramine release proceeded by $^1O_2$.

The generation of tyramine was further validated by ^1H NMR (300 MHz) spectroscopy. Figure 2 shows the evolution of ^1H NMR spectra of aerated CD_2Cl_2 containing 3.0 mM DPSY4 and 100 μM $[Ir(ppy)_2(phen)]PF_6$ recorded during the course of continuous photoirradiation (365 nm, 4 W). Two triplet peaks in the ethylene unit of DPSY4 appeared at 4.56 (H_a) and 2.49 (H_b) ppm. Photoirradiation provoked the appearance of a triplet peak at 2.41 ppm and a quartet peak at 3.45 ppm. These peaks are assigned as α-(H_b') and β-protons (H_a') with respect to the free amine of tyramine, respectively. The broad multiplet peak due to —NH_2 emerged at 3.20 ppm. These spectral signatures are consistent with the generation of tyramine, although DPSY4 was not fully consumed under the photoirradiation condition.

As the final phase of our study, the kinetics of the photoinduced uncaging reaction was monitored using UV–vis absorption spectroscopy. Aerated CH_3CN of 10 μM DPSY4 and 0.3 μM $[Ir(ppy)_2(phen)]PF_6$ was photoirradiated at a wavelength of 365 nm (4 W), during which UV–vis absorption spectra were taken. The fresh DPSY4 exhibited a broad, structured absorption band in the region of 290–430 nm (Figure 3a). This vibronically resolved band is due to the π–π* transition localized within the isoindole core. Photoirradiation led to a hypochromic shift (i.e., a decrease in the absorbance) of this band, which indicates the disruption of the isoindole unit. The shift accompanied a hyperchromic shift (i.e., an increase in the absorbance) of a peak at a wavelength of 251 nm. This 251 nm peak is reminiscent of BBP (see the dotted gray curve in Figure 3a); therefore, the chromic shifts are consistent with the mechanism that involves the conversion of DPSY4 into BBP.

Note that the UV–vis spectral changes are devoid of isosbestic points. The absence of an isosbestic point suggests the presence of a chromic intermediate species, because tyramine is spectroscopically silent in the observation region. The chromic intermediate is likely an endoperoxide of DPSY4. Although the endoperoxide species could not be

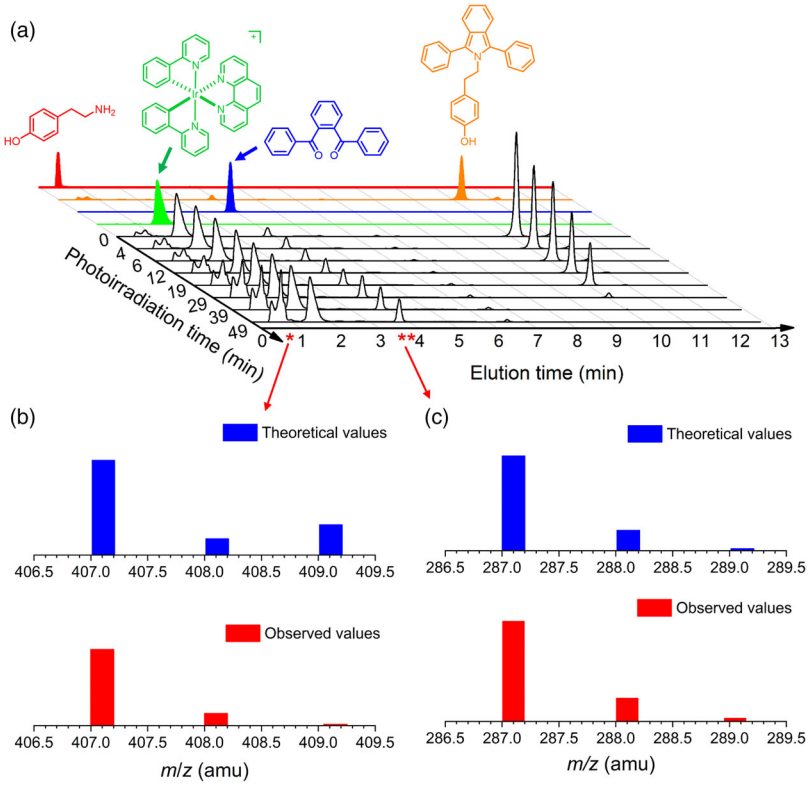


FIGURE 1 Photoinduced release of tyramine from DPSY4. (a) Liquid chromatograms of aerated CH_3CN containing 1.0 mM DPSY4 and 100 μM $[Ir(ppy)_2(phen)]PF_6$ recorded during continuous photoirradiation at a wavelength of 365 nm (4 W). The colored chromatograms correspond to the authentic samples, including tyramine (red), $[Ir(ppy)_2(phen)]PF_6$ (green), BBP (blue), and DPSY4 (orange). (b,c) Mass spectra for the peaks marked with * (elution time = 0.6 min) and ** (elution time = 3.7 min) which correspond to the photoreleased tyramine (b) and BBP (c), respectively. (b) Top, theoretical values for $[KNa(tyramine)_2(H_2O)_2Cl]^+$ (m/z = 407.11); bottom, observed values. (c) Top, theoretical values for $[H(BBP)]^+$ (m/z = 287.11); bottom, observed values

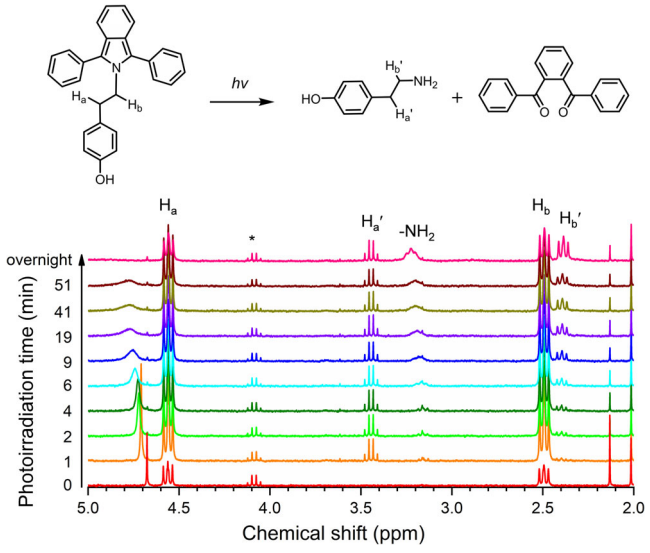


FIGURE 2 ^1H NMR (300 MHz) spectra of aerated CD_2Cl_2 containing 3.0 mM DPSY4 and 100 μM $[Ir(ppy)_2(phen)]PF_6$ recorded during continuous photoirradiation at a wavelength of 365 nm (4 W). The peak marked with * is the residual solvent (EtOAc)

detected by our mass spectrometry and ^1H NMR spectroscopy due to its high instability, its intermediacy is essential for the formation of BBP and tyramine.

In order to follow the stepwise uncaging mediated by the endoperoxide, we recorded the temporal changes of the 251 nm absorbance. This wavelength was chosen as it exhibited a stepwise decay-and-rise profile (Figure 3b). Nonlinear least-squares fit of the biphasic profile to an exponential decay-and-rise model returned a decay time constant (τ_1) and a rise time constant (τ_2) of 27 s and 1471 s, respectively. The shorter value of τ_1 than τ_2 indicates the fast formation of the endoperoxide of DPSY1, followed by the slow decomposition into BBP and tyramine. The tyramine release slower than the endoperoxide formation is undesirable because tyramine may be over-oxidized by $^1O_2$ during continuous photoirradiation in the presence of $[Ir(ppy)_2(phen)]PF_6$. The photochemical quantum yield for the tyramine release was determined based on the ferrioxalate actinometry, to be 5.3% (365 nm, photon flux = 2.3×10^{-8} einstein s^{-1}). This value is smaller than those (6% and 9%) reported for photodonors of β-phenethylamines,[8] which is likely due to the oxidative degradation of the released tyramine by $^1O_2$. One viable approach to accelerating the tyramine release would involve substituent control that weakens the O—O bond of the endoperoxide. It would be instrumental to introduce electron-withdrawing substituents at the peripheral phenyl rings of DPSY4.

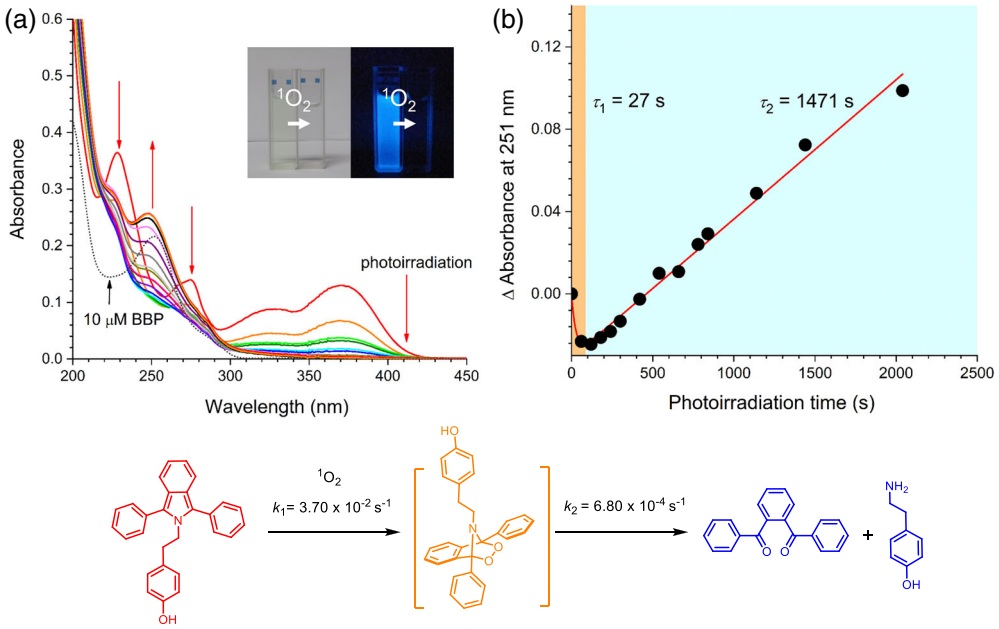


FIGURE 3 (a) UV–vis absorption spectra of aerated CH_3CN containing 10 μM DPSY4 and 0.3 μM $[Ir(ppy)_2(phen)]PF_6$ recorded during continuous photoirradiation at a wavelength of 365 nm (4 W; 0–34 min). The inset photos show absorption and fluorescence emission of the mixture solution before and after the 34 min photoirradiation. (b) Temporal changes of the absorption difference at a wavelength of 251 nm. The red curve is the fit to an exponential decay-and-rise model. τ_1 and τ_2 are time constants of the fit, which correspond to the formation of the endoperoxide intermediate (orange structure in the bottom scheme) and the dissociation of the endoperoxide into BBP and tyramine (blue structures in the bottom scheme). k_1 and k_2 are calculated through the relationships, $k_1 = 1/\tau_1$ and $k_2 = 1/\tau_2$

To summarize, we have synthesized and characterized a photodonor of tyramine. The photodonor contained 1,3-diphenylisoindole incorporating tyramine through its nitrogen atom. A $^1O_2$ photosensitizer was combined with the isoindole derivative of tyramine. Photoinduced release of tyramine occurred through a two-step reaction, which involved the formation of a $^1O_2$-endoperoxide of the tyramine donor, followed by slow decomposition of the endoperoxide into tyramine and BBP, as supported by UV–vis absorption spectroscopy. Finally, the tyramine production was validated unambiguously by mass spectrometry and ^1H NMR spectroscopy. One disadvantage of using the tyramine donor is the poor stability against air and light. We anticipate that judicious substituent control over the isoindole core will improve the stability of the donor and the rate for tyramine production.

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