Tetrahedron Letters 59 (2018) 1087–1090

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

# Toward bioluminescence in the near-infrared region: Tuning the emission wavelength of firefly luciferin analogues by allyl substitution

Nobuo Kitada [a], Tsuyoshi Saitoh [b,c,*], Yuma Ikeda [d], Satoshi Iwano [e], Rika Obata [f], Haruki Niwa [a], Takashi Hirano [a], Atsushi Miyawaki [e], Koji Suzuki [d], Shigeru Nishiyama [c], Shojiro A. Maki [a,*]

[a] Department of Engineering Science, Graduate School of Informatics and Engineering, University of Electro-Communications, Chofu, Tokyo 182-8585, Japan
[b] International Institute for Integrative Sleep Medicine, University of Tsukuba, Tennodai 1-1-1, Tsukuba-shi, Ibaraki 305-8577, Japan
[c] Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan
[d] Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan
[e] Laboratory for Cell Function Dynamics Brain Science Institute, RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan
[f] Research and Education Center for Natural Sciences, Keio University, Hiyoshi 4-1-1, Kohoku-ku, Yokohama 223-8521, Japan

ARTICLE INFO

*Article history:*
Received 13 December 2017
Revised 24 January 2018
Accepted 26 January 2018
Available online 9 February 2018

*Keywords:*
Luciferin derivatives
Bioluminescence
Near infrared
Firefly

ABSTRACT

The synthesis and bioluminescence of allyl-substituted luciferin derivatives as substrates for firefly luciferase are reported. The allylation of luciferins induced bathochromic shift (15–40 nm) of the bioluminescence emission. Upon combination with other chemical modifications for bioluminescence wavelength tuning, novel red emitting luciferin analogues were obtained with emission maxima at 685 and 690 nm.


Luminescent (e.g. fluorescent and bioluminescent) systems that emit light in the red or near-infrared (NIR) region have recently been reported as powerful chemical probes for the observation of biological phenomena and other applications.[1–4] In particular, the demand for NIR-luminescent systems for non-invasive and operationally simple diagnosis techniques, such as *in vivo* imaging, has grown drastically.[5] The optical window (650–950 nm) is expected to allow sensitive detection during *in vivo* imaging by avoiding unexpected absorption of visible and IR light by living specimen. While various NIR-fluorescent systems have been developed, including cyanines[6,7] or boron-dipyrromethenes (BODIPYs),[8] only few NIR-bioluminescent systems have been reported.[9–16] In the luciferin bioluminescence reaction, photons are emitted from the excited state of oxyluciferin (**2**), which is a metabolic product of the firefly luciferin (**1a**) after adenylation by firefly luciferase, followed by a reaction with molecular oxygen (Fig. 1).[17] The excited state of **2** emits only at ca. 560 nm in the presence of natural luciferase. During the course of our studies on the development of luciferin analogues,[10,11,16] we discovered that the replacement of benzothiazole with other aromatic rings causes a dramatic shift of the emission wavelength. We have described a method to tune the emission wavelength of luciferins (Fig. 1), in which the extension of the π-conjugation between the thiazoline moiety and the aromatic ring affords a large bathochromic shift (ca. 100 nm), while substitution of the hydroxide moiety with dimethylamine provides a smaller bathochromic shift (ca. 30 nm).[10] This approach has been applied to other luciferin derivatives, such as naphthyl luciferin[18] (NapLuc, **3a**; $n = 0$) and dimethylanilyl luciferin (DmaLuc, $n = 0$) to obtain the red-emitting vinyl analogues NapVLuc (**4a**), DmaVLuc (**5a**), and DmaDVLuc (**6a**) (Fig. 2). Especially **6a** exhibits promising bioluminescence properties in the NIR region ($\lambda_{em} = 680$ nm) that could potentially be exploited for the *in vivo* imaging of deep tissue.[19,20] However, the further extension of the π-conjugation of luciferin analogues renders the compound instable, resulting in a dramatic suppression of the luminescence activity. Another type of NIR luciferins has been developed based on the modification of D-aminoluciferin. Cyclic alkylaminoluciferins (CycLucs)[14] and *N*-cycloalkylaminoluciferins such as cybLuc[15] exhibit red (600–650 nm) bioluminescence and these luciferins can be used for *in vivo* bioimaging of deep tissue such as brain tissue.[15,21] Modifications of D-luciferin (**1a**) by halogenation,[22–24] alkylation,[25] and alkynylation[26] at the C-4′, -5′, and -7′ positions

* Corresponding authors at: International Institute for Integrative Sleep Medicine, University of Tsukuba, Tennodai 1-1-1, Tsukuba-shi, Ibaraki 305-8577, Japan (T. Saitoh).
*E-mail addresses:* tsuyoshi-saito.gf@u.tsukuba.ac.jp (T. Saitoh), s-maki@uec.ac.jp (S.A. Maki).

https://doi.org/10.1016/j.tetlet.2018.01.078
0040-4039/

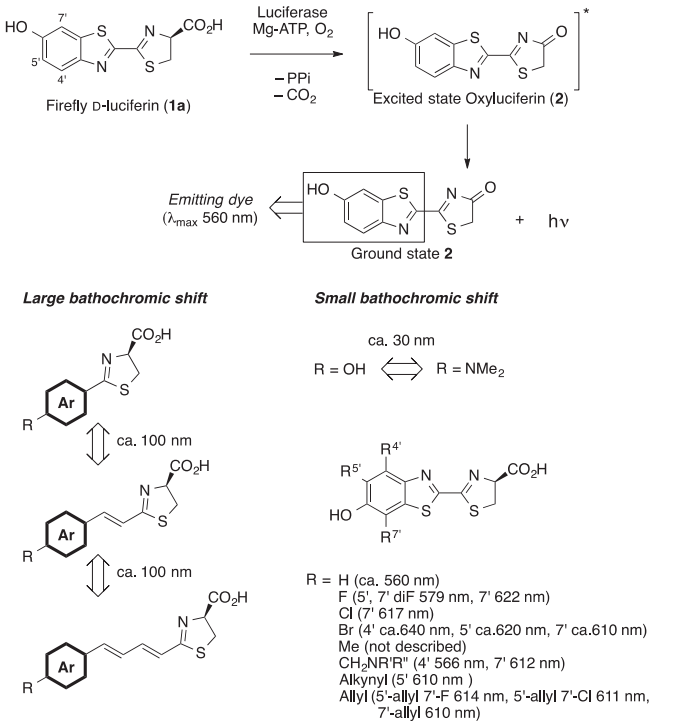



**Fig. 1.** Bioluminescence reaction mechanism and structure–wavelength relationship for luciferins.

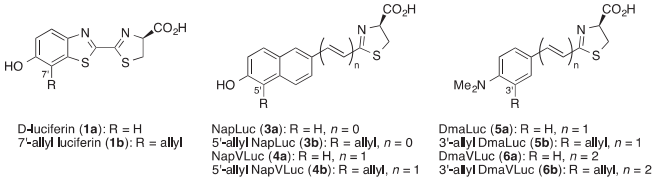
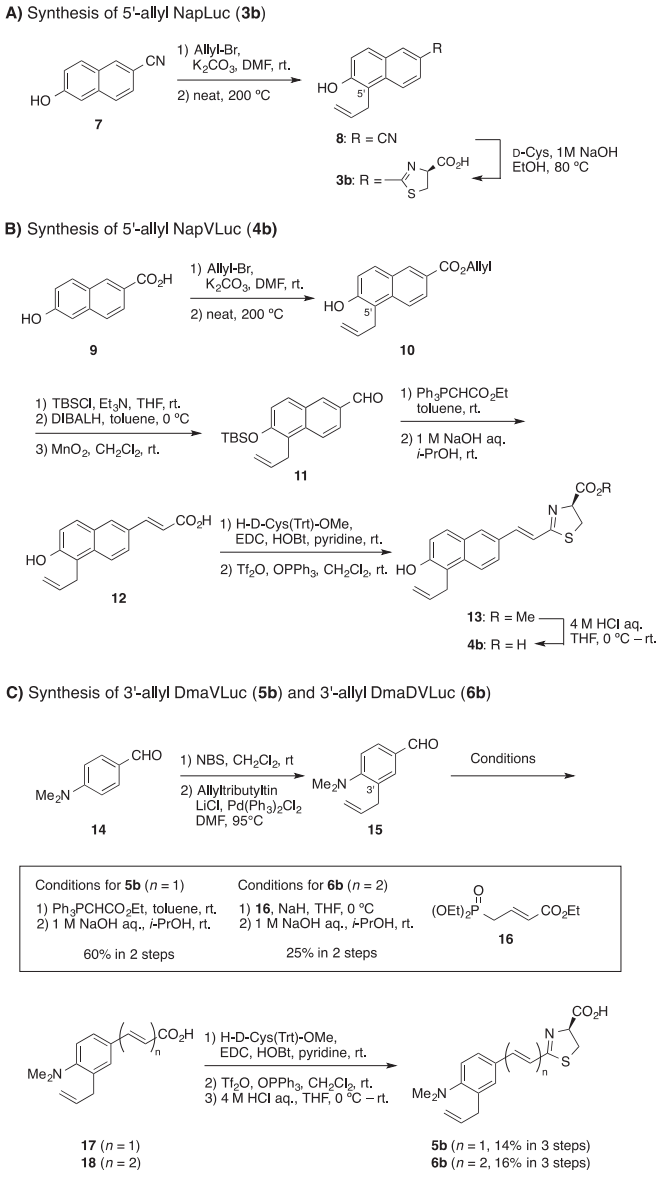



**Fig. 2.** Structure of luciferins **1**–**6**.

have also been reported to produce intriguing bioluminescence features. Recently, we have reported the synthesis of C-7′ ally luciferin (**1b**) and its interesting bio-orthogonal luminescence properties.[27] The C-7′ allylation of **1a** induced a bathochromic shift (40 nm) of the emission wavelength, implying a weaker enzymatic recognition of the benzothiazole ring and the formation of an excited state with lower energy. However, the detailed effects of such an allyl substitution on the bioluminescence emission of other known luciferase substrates remain to be elucidated. Based on these observations, we herein report the application of said allylation method for the further tuning of the bioluminescence wavelength of known luciferins.

The effect of allyl substitution on the firefly bioluminescence of luciferin derivatives (**3b**, **4b**, **5b**, and **6b**) was investigated (Fig. 2). The synthesis of **3b** started with the *O*-allylation of 6-cyano-2-naphthol (**7**) to afford an allyl ether, followed by a Claisen rearrangement, which selectively proceeded at the C-7′ position to afford **8** in high yield. A cyclocondensation with D-cysteine ultimately converted **8** into 5′-allyl NapLuc (**3b**) (Scheme 1A). To access 5′-allyl NapVLuc (**4b**), a twofold *O*-allylation of commercially available 6-hydroxy-2-naphthoic acid (**9**) was carried out, followed by a Claisen rearrangement to afford 5′-allyl naphthalene **10**. After protection of the hydroxyl group with TBS, the allyl ester was reduced with DIBALH and subsequently oxidized into aldehyde **11** (Scheme 1B). A Wittig reaction then furnished a vinyl




**Scheme 1.** Synthesis allyl-substituted luciferins (**3b**, **4b**, **5b**, **6b**).

ester, which yielded carboxylic acid **12** upon hydrolysis with NaOH aq. Subsequently, **12** was condensed with H-D-Cys(Trt)-OMe, followed by the formation of a thiazoline ring with $Tf_2O$ and $OPPh_3$[28] to furnish **13**. The desired luciferin **4b** was ultimately isolated following the hydrolysis of the methyl ester moiety. Similarly, the synthesis of 3′-allyl DmaLuc (**5b**) and DmaVLuc (**6b**) started with the bromination of commercially available 4-dimethylaminobenzaldehyde (**14**), followed by a Pd-catalyzed Stille coupling with allyltributyltin to afford allyl arene **15** (Scheme 1C). A Wittig or Horner–Wadsworth–Emmons reaction of the aldehyde with phosphonic acid diester **16** afforded the corresponding vinyl and dienyl esters, which yielded the carboxylic acids **17** and **18** upon hydrolysis with NaOH aq. The desired luciferins **5b** and **6b** were isolated in the same manner as **4b**.

The bioluminescence (BL) spectra of the synthetic luciferins with recombinant firefly luciferase (Ppy; *Photinus pyralis*) are shown in Fig. 3. The allylated NapLuc **3b** displays a bathochromic shift (15 nm) of the emission maximum compared to that of **3a** (Table 1). On the other hand, allylated NapVLuc **4b** exhibits a larger bathochromic shift (35 nm) of its peak maximum relative to that of