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Convergent and short-step syntheses of *dl*-Cypridina luciferin and its analogues based on Pd-mediated cross couplings

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Abstract

dl-Cypridina luciferin and its analogues were synthesized from 2-aminopyrazine by an eight-step method that included two regio-selective Pd-mediated cross couplings, and their chemi- and bioluminescent activities were compared. Analogues having a 3-benzofuranyl or a 3-benzothienyl group in the place of a 3-indolyl group showed luciferase affinities similar to Cypridina luciferase but with a lower luminescent efficiency, suggesting that the NH group is unimportant for molecular recognition whereas the indolyl group is crucial for efficient luminescence.
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Cypridina luciferin (**1a**) is an imidazopyrazinone and involved in the bioluminescence of the crustacean Cypridina (*Vargula*) *hilgendorfii* and deep-sea fishes. Cypridina luciferin shows a typical luciferin–luciferase reaction to emit a blue light of 465 nm from an oxyluciferin–luciferase complex.[1] Bioluminescent imidazopyrazinones were synthesized from properly substituted 2-aminopyrazines, which are synthesized by the pyrazine ring construction of a coupling pair of a glyoxal, an α-aminoamidine or an α-aminonitrile and an α-ketooxime (Fig. 1). The first total synthesis of Cypridina luciferin was achieved by Kishi et al. in 1966[2] by using a former coupling pair and the synthesis of ethioluciferamine was reported in 1971 by White and Karpetsky[3] by a $TiCl_4$-mediated coupling of an α-aminonitrile and an α-ketooxime. Several analogues were prepared based on Kishi's method and the structure–activity relationship and mechanistic aspects of bio- and chemiluminescence were investigated extensively by Goto.[4]

In the course of our studies on bio- and chemiluminescent imidazopyrazinones, we have developed a new synthetic approach to imidazopyrazinones by a Pd-mediated cross coupling, and achieved the

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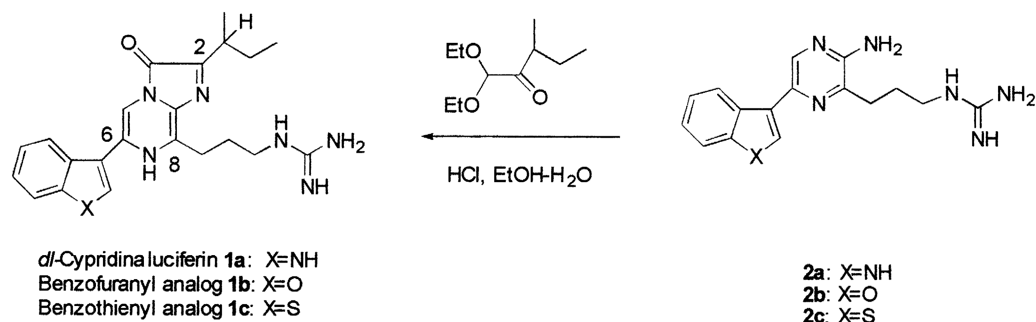

Fig. 1.

synthesis of coelenterazine and its analogues.[5] We have extensively applied the new method to Cypridina luciferin as well as its analogues having different substituents at the 6-position. Here we would like to report novel short-step syntheses of Cypridina luciferin and its analogues having 3-benzofuranyl or 3-benzothienyl chromophores together with their bioluminescent activities.

The synthesis was started with 2-amino-3,5-dibromopyrazine prepared from commercially available 2-aminopyrazine by bromination with Br_2.[6] Regio-selective introduction of a C3 unit was achieved with *N*-Boc-propargylamine under Sonogashira coupling[7] conditions to yield an alkynyl compound (**5**) which was hydrogenated with PtO_2 to form bromopyrazine **6** (Fig. 2). A coupling reaction of bromopyrazine **6** with an indole was achieved by Suzuki coupling[8a,b] with *N*-tosylindolyl-3-boronic acid [**7**, X=NTs, Y=$B(OH)_2$][9] to afford indolylaminopyrazine **8a** in 80% yield after hydrolysis with a 1:1 mixture of 5 M NaOH aq. and 1,4-dioxane. Deprotection reaction with TFA afforded ethioluciferamine hydrochloride in 87% yield. Total yield of ethioluciferamine in six steps was 30% from commercially available 2-aminopyrazine.

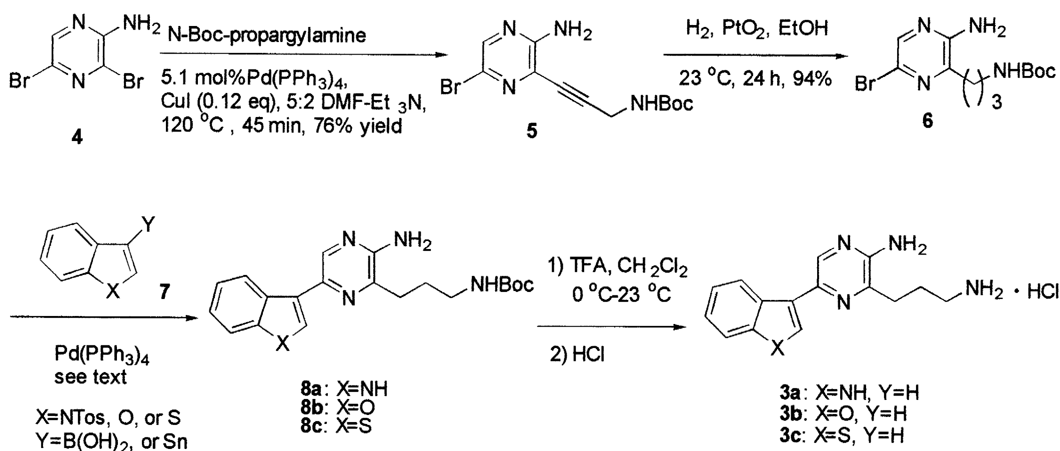

Fig. 2.

Ethioluciferamine was converted to ethioluciferin by using a modified method[10] in 96% yield. Cyclization of ethioluciferin with a keto-acetal afforded *dl*-Cypridina luciferin[11,12] in 24% yield.

Cypridina luciferin analogues (**1b** and **1c**) with an oxygen or a sulfur atom instead of the NH group were synthesized by a similar manner. Coupling reactions were achieved smoothly with tributylstannanes under Stille coupling conditions.[5a] Compounds **8** (X=O and X=S) were obtained in a yield of 47 and 72%, respectively. A three-step transformation of **8b** and **8c** was carried out under the same conditions

to afford *dl*-Cypridina luciferin analogues (**1b**: X=O and **1c**: X=S) at moderate yields of 16 and 29%, respectively.[11,12] The luminescence properties of *dl*-Cypridina luciferin and the analogues with luciferase (bioluminescence) or without luciferase (chemiluminescence) are summarized in Table 1. The analogues were bioluminescent but with lower efficiencies. However, the kinetic parameters, as well as the enhancement of luminescence by luciferase (BL/CL), were similar each other, suggesting that the NH group of the indolyl group in luciferin is not essential for binding luciferase[13] but important for high luminescent efficiency. The reason for low chemiluminescent efficiency of the analogues is not completely clear. Detailed study of the structure–activity relationship is in progress in our laboratory.

Table 1
Luminescence properties of synthesized *dl*-Cypridina luciferin (**1a**) and analogues (**1b** and **1c**) and natural *S*-Cypridina luciferin at 23°C

X	O (**1b**)	S (**1c**)	NH (**1a**)	NH (natural)[6]
Relative Light Yield of BL[1]	4	7	64	100
$Km/\mu M$[2]	5.0	0.99	0.27	0.46
$\lambda max/nm$[3]	443	445	455	455
Relative Light Yield of CL[4]	0.6	0.8	12	11
BL/CL[5]	7	9	5	9

1) Bioluminescence (BL) was performed at 1.7×10^{-6} M in 10 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid buffer containing 0.1 M NaCl, pH 7.5 with *Cypridina* luciferase (0.2-0.3 μg/mL). 2) Determined by Lineweaver-Burk plot. 3) Uncorrected bioluminescence spectrum. 4) Chemiluminescence (CL) was measured at 1.7×10^{-6} M in diethylene glycol monomethyl ether with 3% 0.1 M acetate buffer, pH 5.6. 5) Ratio of relative light yields of BL and CL. 6) *dl*-Cypridina luciferin was shown to exhibit 100% and 60% luminescence abilities of natural one in chemiluminescence and bioluminescence, respectively (lit. 11).

Bioluminescence[14] is a highly sensitive and non-destructive detection method applicable to monitor various transient biological events such as a calcium wave during fertilization.[15] Recently, genes of bioluminescent proteins including apoaequorin, firefly luciferase, *Renilla* luciferase, and Cypridina luciferase have been cloned and used as a reporter gene studying critical biological events. Cypridina luciferase was shown to be useful for monitoring gene expression in measuring a secreted enzyme.[16] Our synthetic method may be applicable in future for the preparation of various types of Cypridina luciferin in sufficiently large quantities for use in the area of life sciences.

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References

1. Shimomura, O.; Johnson, F. H.; Masugi, T. *Science* **1969**, *164*, 1299–1300.
2. Kishi, Y.; Goto, T.; Inoue, S.; Sugiura, S.; Kishimoto, H. *Tetrahedron Lett.* **1966**, 3445–3450.
3. Karpetsky, T. P.; White, E. H. *J. Am. Chem. Soc.* **1971**, *93*, 2333–2334. Idem, *Tetrahedron* **1973**, *29*, 3761–3773.
4. Goto, T. *Pure Appl. Chem.* **1968**, *17*, 421–441.
5. (a) Nakamura, H.; Takeuchi, D.; Murai, A. *Synlett* **1995**, 1227–1228. (b) Nakamura, H.; Aizawa, M.; Murai, A. *Synlett* **1996**, 1015–1017. (c) Nakamura, H.; Wu, C.; Murai, A.; Inouye, S.; Shimomura, O. *Tetrahedron Lett.* **1997**, *38*, 6405–6406.
6. Sato, N. *J. Heterocycl. Chem.* **1982**, 673–674.
7. Sonogashira, K. *Comprehensive Organic Synthesis*; Trost, B. M.; Fleming, I., Eds.; Pergamon Press: Oxford, 1991; Vol. 3, Chapter 2.3, pp. 521–529.
8. (a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483. (b) It could not be achieved by Stille coupling with 1-*t*-butyldimethylsilyl-3-tributylstannylindole but might be possible with 1-tosyl-3-trimethylstannylindole: Cianttini, P. G.; Morera, E.; Ortar, G. *Tetrahedron Lett.* **1994**, *35*, 2405–2408.
9. Zheng, Q.; Yang, Y.; Martin, A. R. *Heterocycles* **1994**, *37*, 1761–1772.
10. Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497–2502.
11. The reaction conditions were not optimized yet at small scale reactions, and the condensation with a ketoaldehyde was reported as good (80%) at a several hundred mg scale. Sugiura, S.; Inoue, S.; Goto, T. *Yakugaku Zasshi* **1970**, *90*, 707–710.
12. All compounds gave satisfactory spectral data. Compound **1a**: pale orange crystals (HBr salts); mp 168–172; HR-FDMS *m/z* 405.2279 (M^+); calcd for $C_{22}H_{27}N_7O$: 405.2277; ^1H NMR (400 MHz, CD_3OD) 0.95 (3H, t, *J*=7 Hz), 1.45 (3H, d, *J*=7 Hz), 1.80–1.99 (2H, m), 2.33 (2H, quintet, *J*=7 Hz), 3.17 (1H, sextet, *J*=7 Hz), 3.41 (2H, q, *J*=7 Hz), 3.44 (2H, q, *J*=7 Hz), 7.18–7.21 (2H, m), 7.50 (1H, d, *J*=7 Hz), 8.01 (1H, s), 8.07 (1H, d, *J*=7 Hz), 8.44 (1H, s); UV (1:1 EtOH:H_2O) λ_{max} 434 nm (ε 8800). Compound **1b**: pale yellow crystals (HCl salts); FABMS *m/z* 407 $(M+H)^+$; ^1H NMR (400 MHz, CD_3OD) 0.95 (3H, t, *J*=7 Hz), 1.47 (3H, d, *J*=7 Hz), 1.80–1.99 (2H, m), 2.33 (2H, quintet, *J*=7 Hz), 3.18 (1H, sextet, *J*=7 Hz), 3.40 (2H, q, *J*=7 Hz), 3.43 (2H, q, *J*=7 Hz), 7.39–7.45 (2H, m), 7.62 (1H, d, *J*=7 Hz), 8.15 (1H, s), 8.49 (1H, d, *J*=7 Hz), 8.60 (1H, s); UV (1:1 EtOH:H_2O) λ_{max} 424 nm (ε 6800). Compound **1c**: pale yellow crystals (HCl salts); HR-FABMS *m/z* 422.1921 (M^+); calcd for $C_{22}H_{26}N_6OS$: 422.1889; ^1H NMR (400 MHz, CD_3OD) 0.96 (3H, t, *J*=7 Hz), 1.48 (3H, d, *J*=7 Hz), 1.80–2.00 (2H, m), 2.32 (2H, quintet, *J*=7 Hz), 3.20 (1H, sextet, *J*=7 Hz), 3.44 (2H, q, *J*=7 Hz), 3.45 (2H, q, *J*=7 Hz), 7.41–7.52 (2H, m), 8.00 (1H, d, *J*=7 Hz), 8.19 (1H, s), 8.30 (1H, d, *J*=7 Hz), 8.61 (1H, s); UV (1:1 EtOH:H_2O) λ_{max} 430 nm (ε 10400).
13. For a recent review, see: Wilson, T.; Hastings, J. W. *Annu. Rev. Cell Dev. Biol.* **1998**, *14*, 197–230.
14. Similar findings in an indolactam–protein kinase C interaction were reported by: Kozikowski, A. P.; Ma, D.; Du, L.; Lewin, N. E.; Blumberg, P. M. *J. Am. Chem. Soc.* **1995**, *117*, 6666–6672.
15. Kubota, H. Y.; Yoshimoto, Y.; Yoneda, M.; Hiramoto, Y. *Dev. Biol.* **1987** *19*, 129–136.
16. (a) Inouye, S.; Ohmiya, Y.; Toya, Y.; Tsuji F. I. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9584–9587. (b) Thompson, E. M.; Adenot, P.; Tsuji, F. I.; Renard, J. P. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 1317–1321. (c) Miesenböck, G.; Rothman, J. E. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 3402–3407.