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Synthesis of Fluorescent Substrate Analogues for Mechanistic Study of Luciferase

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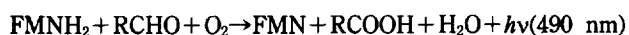
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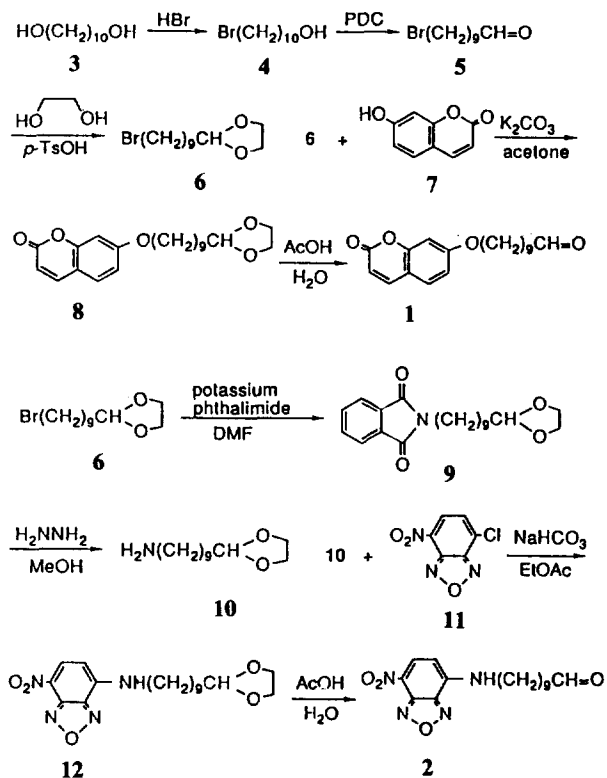
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Bacterial luciferase catalyzes the oxidation of a mixture of FMNH₂ and a long chain aliphatic aldehyde by oxygen to produce FMN, corresponding fatty acids, water, and bioluminescence.

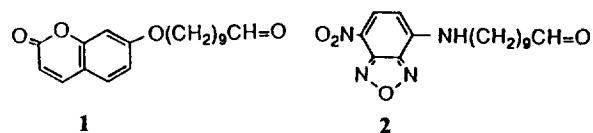


Despite many years of intensive efforts, the mechanism of the bioluminescence by bacterial luciferase has not been fully understood on the molecular level. According to mechan-



Scheme 1.

ism¹⁻³ proposed by Hastings, an excited FMN 4a-hydroxide is assumed to be the primary excited species emitting bioluminescence. However, there are some experimental evidences⁴⁻⁶ that are inconsistent with his hypothesis and the possibility of certain other species being the primary excited state cannot be ruled out. In this report, we describe the synthesis of two fluorescent substrate analogues (1 and 2) to be used as probes for mechanistic study of bacterial luciferase.



$\lambda_{\text{max, emit}} = \text{ca. } 450 \text{ nm}^7$

$\lambda_{\text{max, emit}} = \text{ca. } 550 \text{ nm}^8$

If the oxidation of 2 gives emission with additional components (550 nm), while that of 1 does with no additional component, it could be interpreted as a supportive of the hypothesis that excited FMN 4a-hydroxide is the primary excited species. If the oxidation of both 1 and 2 give emission with additional component (450 and 550 nm respectively), it indicates that certain species other than FMN 4a-hydroxide is a primary excited species. Some reports^{9,10} describe the usefulness of fluorescent substrate analogues similar to those in this study. However, fluorescence maxima of these analogues are either inappropriate (higher than 490 nm) or more limited (lower than 450 nm) for a mechanistic test.

The substrate analogues 1 and 2 were synthesized by the following synthetic routes.

Bromide 4¹¹ was obtained by bromination of 3 followed by separation through liquid-liquid extraction.¹² The product

crystallized from pentane is melted at ambient temperature. Mild oxidation of **4** by pyridinium dichromate¹³ followed by ketalization under a Dean-Stark trap yielded the bromide **6**. When methyl ethyl ketone instead of acetone was used as a solvent in the condensation of **6** and 7-hydroxycoumarin (**7**), the reaction time was reduced from 5 days to one day, but the yield was a little lower. The amine **10** was obtained from **6** by Gabriel synthesis.¹⁴ Since NBD-chloride (**11**) was unstable in organic solvents containing acid neutralizing base, the condensation of **10** and **11** was carried out in ethyl acetate over basic aqueous solvent. In the detection of **1**, **2**, **8**, and **12** on TLC plates, their strong fluorescence under UV lamp was helpful.

Experimental

Infrared spectra were recorded on a Perkin-Elmer 1330 Infrared spectrophotometer. ¹H NMR spectra were measured on a Bruker ARX-300 NMR spectrometer in CDCl₃ using tetramethylsilane as an internal standard. Coupling constants are expressed in Hz.

10-Bromodecanol (4)¹¹. Colorless oil; yield 65%; ¹H NMR δ 3.63 (t, 2H, H-1), 3.41 (t, 2H, H-10), 1.85 (tt, 2H, H-9), 1.56 (tt, 2H, H-2), 1.5-1.2 (m, 12H, H-3 to 8), $J_{1,2}=6.7$, $J_{9,10}=6.9$; IR (neat) 3320, 1050, 640, 560 cm⁻¹.

10-Bromodecanal (5). Colorless oil; Yield 89%; bp. 105-108 °C (13 mm); ¹H NMR δ 9.77 (t, 1H, H-1), 3.41 (t, 2H, H-10), 2.43 (dt, 2H, H-2), 1.85 (tt, 2H, H-9), 1.7-1.2 (m, 12H, H-3 to 8), $J_{1,2}=1.8$, $J_{2,3}=7.4$, $J_{9,10}=6.9$; IR (neat) 1720, 640, 560 cm⁻¹.

Ethylene Ketal of 10-Bromodecanal (6). Colorless oil; Yield 76%; bp. 112-116 °C (13 mm.); ¹H NMR δ 4.84 (t, 1H, H-1), 3.99-3.82 (sym. m, 4H, ketal), 3.40 (t, 2H, H-10), 1.85 (tt, 2H, H-9), 1.64 (dt, 2H, H-2), 1.5-1.2 (m, 12H, H-3 to 8), $J_{1,2}=4.9$, $J_{9,10}=6.9$; IR (neat) 1140, 1120, 1030, 940, 640, 560 cm⁻¹.

Ethylene Ketal of 10-(Coumarin-8-yloxy)decanal (8)

Pale yellow solid; Yield 65%; mp. 99-100 °C; ¹H NMR δ 7.62 (d, 1H, H-4'), 7.35 (d, 1H, H-5'), 6.83 (dd, 1H, H-6'), 6.80 (d, 1H, H-8'), 6.23 (d, 1H, H-3'), 4.84 (t, 1H, H-1), 3.99 (t, 2H, H-10), 3.99-3.82 (sym. m, 4H, ketal), 1.80 (tt, 2H, H-9), 1.64 (dt, 2H, H-2), 1.5-1.2 (m, 12H, H-3 to 8), $J_{1,2}=4.8$, $J_{9,10}=6.2$, $J_{3',4'}=9.5$, $J_{5',6'}=8.4$, $J_{6',8'}=2.4$; IR (KBr) 1730, 1710, 1610, 1230, 1125, 1020 cm⁻¹.

10-(Coumarin-8-yloxy)decanal (1). Pale yellow solid; Yield 70%; mp. 105-107 °C; ¹H NMR δ 9.77 (t, 1H, H-1), 7.62 (d, 1H, H-4'), 7.36 (d, 1H, H-5'), 6.83 (dd, 1H, H-6'), 6.80 (d, 1H, H-8'), 6.24 (d, 1H, H-3'), 4.01 (t, 2H, H-10), 2.42 (dt, 2H, H-2), 1.81 (tt, 2H, H-9), 1.8-1.2 (m, 12H, H-3 to 8), $J_{1,2}=1.8$, $J_{2,3}=7.3$, $J_{9,10}=6.5$, $J_{3',4'}=9.4$, $J_{5',8',6'}=8.4$, $J_{6',8'}=2.4$; IR (KBr) 1740, 1710, 1610, 1230, 840 cm⁻¹.

Ethylene Ketal of 10-Phthalimidodecanal (9)

White solid; Yield 62%; mp. 85-86 °C; ¹H NMR δ 7.84 & 7.70 (sym. m, 4H, *o*- and *m*-H's respectively), 4.84 (t, 1H, H-1), 4.0-3.8 (sym. m, 4H, ketal), 3.67 (t, 2H, H-10), 1.8-1.2 (m, 16H, H-2 to 9), $J_{1,2}=4.8$, $J_{9,10}=7.3$; IR (KBr) 1690, 1120, 1050,

960 cm⁻¹.

Ethylene Ketal of 10-Aminodecanal (10). Colorless oil; Yield 54%; bp. 92-94 °C (4.5 mm.); ¹H NMR δ 4.84 (t, 1H, H-1), 4.02-3.79 (sym. m, 4H, ketal), 2.69 (t, 2H, H-10), 1.96 (s, NH₂), 1.64 (m, 2H, H-2), 1.5-1.2 (m, 14H, H-3 to 9), $J_{1,2}=4.8$, $J_{9,10}=7.0$; IR (neat) 3330, 3220, 1140, 1120, 1030, 940 cm⁻¹.

Ethylene Ketal of 10-[7-(4-Nitrobenzo-2-oxa-diazolyl)]decanal (12). Orange solid; Yield 52%; mp. 102-103 °C; ¹H NMR δ 8.49 (d, 1H, H-6'), 6.2 (s, 1H, NH), 6.17 (d, 1H, H-5'), 4.84 (t, 1H, H-1), 3.99-3.82 (sym. m, 4H, ketal), 3.48 (dt, 2H, H-10), 1.81 (tt, 2H, H-9), 1.64 (dt, 2H, H-2), 1.5-1.2 (m, 12H, H-3 to 8), $J_{1,2}=4.8$, $J_{9,10}=ca. 6.7$, $J_{NH,10}=ca. 6.7$, $J_{5',6'}=8.7$; IR (KBr) 3300, 1570, 1490, 1320, 1120, 1020 cm⁻¹.

10-[7-(4-Nitrobenzo-2-oxa-diazolyl)]decanal (2). Orange solid; Yield 71%; mp. 92-94 °C; ¹H NMR δ 9.77 (t, 1H, H-1), 8.49 (d, 1H, H-6'), 6.22 (s, 1H, NH), 6.17 (d, 1H, H-5'), 3.49 (dt, 2H, H-10), 2.43 (dt, 2H, H-2), 1.81 (tt, 2H, H-9), 1.7-1.2 (m, 12H, H-3 to 8), $J_{1,2}=1.4$, $J_{2,3}=7.2$, $J_{9,10}=ca. 6.6$, $J_{NH,10}=ca. 6.6$, $J_{5',6'}=8.7$; IR (KBr) 3340, 1710, 1580, 1490, 1310 cm⁻¹.

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