Synthesis and Chemiluminescent Activity of 8,9-Dihydrobenzo[*g*]pyridazino[4,5-*b*]indole-7,10(11*H*)-diones

Masateru Kurumi, Kenji Sasaki*, Hiroko Takata and Taiji Nakayama

Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan

Received October 16, 2000

Substituted and unsubstituted naphthylamines were transformed into the corresponding triazole derivatives, which were converted to dimethyl 1*H*-benz[*g*]indole-2,3-dicarboxylates by photocyclization. The reaction of the diesters with hydrazine hydrate gave the corresponding 8,9-dihydrobenzo[*g*]-pyridazino[4,5-*b*]indole-7,10(11*H*)-diones (**5**). One of compounds **5** was found to have chemiluminescent activity similar to luminol.

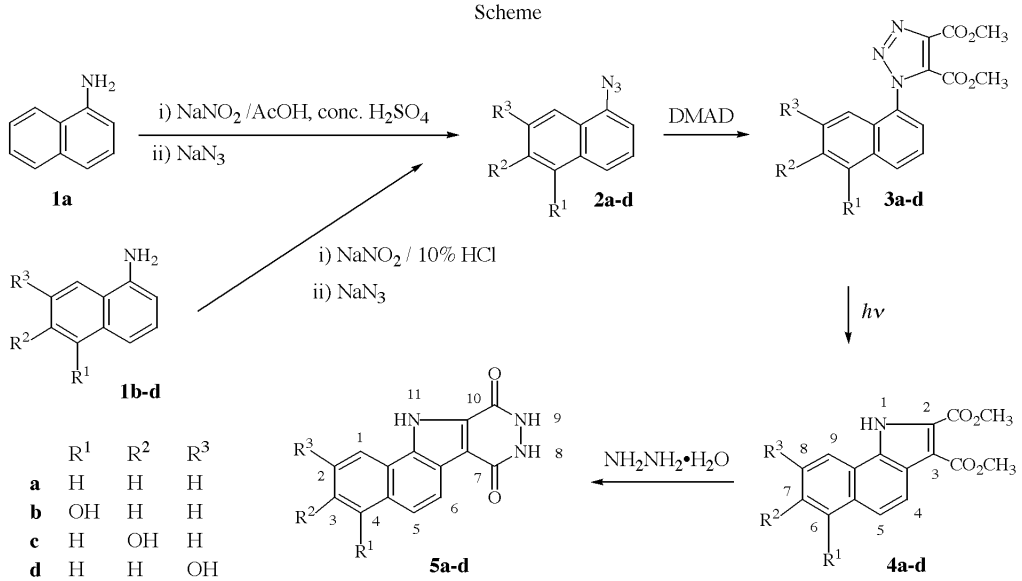
J. Heterocyclic Chem., **38**, 629 (2001).

The chemiluminescent assay is an attractive analytical method because of its high sensitivity, rapid reaction and wide dynamic range [1]. Luminol is the typical chemiluminescent compound and its analytical usefulness has extensively been studied [2]. It is well known that polyheterocyclic compounds, containing the pyridazinedione moiety, have chemiluminescent activity as luminol analogue, and many studies on these derivatives have been performed [3]. We have previously reported the synthesis of pyridazino[4,5-*b*]indole-1,4(2*H*,3*H*)-diones some of which were found to have chemiluminescent properties, similar to that of luminol [4]. This result prompted us to continue studying pyridazinoindole-1,4-dione derivatives and their chemiluminescent properties. We describe here the synthesis of 8,9-dihydrobenzo[*g*]pyridazino[4,5-*b*]-indole-7,10(11*H*)-diones **5** and evaluation of their chemiluminescent activity.

The synthetic route to **5** from naphthylamine derivatives **1** is shown in Scheme. 1-Azidonaphthalene **2a** was prepared in 31% yield by the method of Forster *et al*. [5] and was used as a starting material in the next step without further purification. Similar reactions of **1b-d** gave the corresponding **2b-d** in low yields. Spagnolo *et al*. have obtained **2b,d** from **1b,d** by diazotization followed by transformation into the azides [6]. Consequently we tried this method for the preparation of **2b-d** which were obtained in moderate yield. Compounds **2b-d** were used in the next step without further purification. The reactions of compounds **2a-d** with dimethyl acetylenedicarboxylate (DMAD) at room temperature for 10 days in the dark afforded the corresponding triazoles **3a-d**. These triazoles **3a-d** were transformed into the corresponding dimethyl 1*H*-benz[*g*]indole-2,3-carboxylates **4a-d** *via* photocyclization using a 500 watt mercury high pressure lamp by the method of Nagawa *et al*. [7]. ^{1}H-Nmr data for **4a** has been reported by Mitchell *et al*. [8] and our data for this compound agrees with theirs.

Finally, compounds **4a-d** were converted to the corresponding benzopyridazinoindoles **5a-d** in 92-97% yields by reaction with hydrazine hydrate. These reactions proceeded by using a large excess of hydrazine hydrate and heating at reflux. The spectra and analytical data for these compounds are consistent with their structures.

Scheme



	R^1	R^2	R^3
a	H	H	H
b	OH	H	H
c	H	OH	H
d	H	H	OH

Evaluation of the chemiluminescent activity of **5a-d** was performed in the presence of Triton X-100, hydrogen peroxide and *Arthromyces ramosus* peroxidase (ARP) in phosphate buffer (for pH 4.0, 6.0 and 8.0) or carbonate buffer (for pH 10.0) solutions. Compound **5a** exhibited chemiluminescence at pH 10.0 with an intensity 1/30th of that of luminol. Compounds **5b-d** showed no chemiluminescence at any pH value. Details on the chemiluminescent activity of **5a** will be reported in the future.

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. The FAB-mass spectra were obtained on a VG 70 mass spectrometer and glycerol or *m*-nitrobenzyl alcohol was used as a matrix. The ir spectra were recorded on a Japan Spectroscopic IRA-102 diffraction grating infrared spectrophotometer and frequencies are expressed in cm^{-1}. The ^{1}H-nmr spectra were recorded on a Varian VXR-200 instrument operating at 200 MHz with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ) and J values in Hz, and the signals are designated as follows: s, singlet; d, doublet; dd, doublet doublet; ddd, doublet doublet doublet; t, triplet; dt, doublet triplet; q, quartet; m, multiplet. Photocyclization was performed using an Eikohsha High-Pressure Mercury Arcs EHB-W1 (500 watt). The chemiluminescence intensity was measured with a Shimadzu Multiconvertible Spectrophotometer Double-40.

Azidonaphthalene (**2a**).

The title compound was obtained by the method of Forster *et al*. [5]. The column chromatography on silica gel eluting with *n*-hexane-ethyl acetate (19:1, v/v) was employed for purification. The resulting material was used in the next step without further purification, 31% yield, Ir (neat) cm^{-1}: 2110 (N_3).

1-Azido-5-naphthol (**2b**).

The title compound was obtained by the method of Spagnolo *et al*. [6]. The column chromatography on silica gel eluting with *n*-hexane-ethyl acetate (9:1, v/v) was employed for purification. The resulting material was used in the next step without further purification, 60% yield, Ir (chloroform) cm^{-1}: 3200 (OH), 2120 (N_3).

1-Azido-6-naphthol (**2c**).

The title compound was obtained essentially by the method of Spagnolo *et al*. [6]. 1-Amino-6-naphthol (**1c**, 4.65 g, 29.3 mmol) in 10% hydrochloric acid (156 ml), sodium nitrite (2.17 g, 31.4 mmol) in water (15 ml) and sodium azide (2.33 g, 35.8 mmol) in water (20 ml) were employed. Extraction of the reaction mixture with ethyl acetate gave almost pure **2c** (3.00 g, 16.2 mmol), which was used in the next step without further purification, 55% yield, Ir (chloroform) cm^{-1}: 3300 (OH), 2150 (N_3).

1-Azide-7-naphthol (**2d**).

The title compound was obtained by the method of Spagnolo *et al*. [6]. The extraction of the reaction mixture with ethyl acetate gave the brown material (6.80 g) including **2d** and some impurities, which was used in the next step without further purification, Ir (chloroform) cm^{-1}: 3250(OH), 2110 (N_3).

Triazole Derivatives **3.**

General Procedure.

This procedure is similar to the method of Nagawa *et al*. [7]. That is, DMAD was added to a solution of **2** in dioxane and the resulting mixture was stirred at room temperature for 10 days in the dark. After evaporation of the solvent, the residue was column chromatographed on silica gel or directly recrystallized to isolate the desired **3**.

Dimethyl 1-Naphthyl-1*H*-1,2,3-triazole-4,5-dicarboxylate (**3a**).

The title compound was obtained by the method of Nagawa *et al*. [7] except for the solvent. In our case dioxane was employed. Colorless needles (from diethyl ether), 74% yield, mp 127-128 °C (lit. [7] mp 122-123 °C, lit. [8] mp 128-129 °C).

Dimethyl 1-(5-Hydroxy-1-naphthyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (**3b**).

A mixture of **2b** (4.73 g, 25.6 mmol) and DMAD (14.5 g, 102 mmol) in dioxane (50 ml) was employed. Column chromatography eluting with *n*-hexane-ethyl acetate (11:9, v/v) gave **3b** (6.70 g, 20.5 mmol) which was recrystallized from chloroform-*n*-hexane as colorless granules, 80% yield, mp 161-162 °C. FAB-ms m/z: 328 (MH^+). Ir (potassium bromide) cm^{-1}: 3240 (OH), 1740 (CO). ^{1}H-nmr (deuteriochloroform): δ 3.70 (3H, s, 5-ester CH_3), 4.05 (3H, s, 4-ester CH_3), 5.99 (1H, br s, deuterium oxide exchangeable, OH), 6.79 (1H, dt, J_d = 8.5, J_t =1.0, 8'-H), 6.90 (1H, dd, J = 7.5, J = 1.0, 6'-H), 7.33 (1H, dd, J = 8.5, J = 7.5, 7'-H), 7.52-7.66 (2H, m, 2'- and 3'-H), 8.46 (1H, ddd, J = 9.8, J = 4.9, J = 1.0, 4'-H).

Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84. Found: C, 58.85; H, 4.11; N, 12.70.

Dimethyl 1-(6-Hydroxy-1-naphthyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (**3c**).

A mixture of **2c** (3.18 g, 17.2 mmol) and DMAD (9.80 g, 68.8 mmol) in dioxane (34 ml) was employed. Column chromatography eluting with *n*-hexane-ethyl acetate (6:4, v/v) gave **3c** (4.44 g, 13.6 mmol) which was recrystallized from benzene as pale brown plates, 79% yield, mp 171-173 °C. FAB-ms m/z: 328 (MH^+). Ir (potassium bromide) cm^{-1}: 3310 (OH), 1735 (CO). ^{1}H-nmr (deuteriochloroform): δ 3.72 (3H, s, 5-ester CH_3), 4.05 (3H, s, 4-ester CH_3), 5.50 (1H, br s, deuterium oxide exchangeable, OH), 7.13 (1H, dd, J = 9.2, J = 2.2, 7'-H), 7.19 (1H, br d, J = 9.2, 8'-H), 7.27 (1H, d, J = 2.2, 5'-H), 7.39 (1H, dd, J = 7.3, J = 1.3, 2'-H), 7.53 (1H, dd, J = 8.3, J = 7.3, 3'-H), 7.90 (1H, br d, J = 8.3, 4'-H).

Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84. Found: C, 58.76; H, 4.14; N, 12.67.

Dimethyl 1-(7-Hydroxy-1-naphthyl)-1*H*-1,2,3-triazole-4,5-dicarboxylate (**3d**).

A mixture of the residue (6.70 g) obtained in the preparation for **2d** and DMAD (21.0 g, 150 mmol) in dioxane (73 ml) was employed. After evaporation of the reaction mixture, the crystalline residue was directly recrystallized from ethyl acetate-*n*-hexane to afford **3d** (12.0 g, 37.0 mmol) as colorless granules, 83% overall yield from **1d**, mp 216-218 °C. FAB-ms m/z: 328 (MH^+). Ir (potassium bromide) cm^{-1}: 3220 (OH), 1738 (CO). ^{1}H-nmr (deuteriochloroform): δ 3.69 (3H, s, 5-ester CH_3), 4.01 (3H, s, 4-ester CH_3), 6.56 (1H, d, J = 2.4, 8'-H), 6.59 (1H, br s,

deuterium oxide exchangeable, OH), 7.23 (1H, dd, J = 8.9, J = 2.4, 6'-H), 7.42 (1H, dd, J = 8.0, J = 7.4, 3'-H), 7.52 (1H, dd, J = 7.4, J = 1.4, 2'- or 4'-H), 7.87 (1H, d, J = 8.9, 5'-H), 7.99 (1H, br d, J = 8.0, 2'- or 4'-H).

Anal. Calcd. for $C_{16}H_{13}N_3O_5$: C, 58.72; H, 4.00; N, 12.84. Found: C, 58.56; H, 4.04; N, 12.53.

Dimethyl 1*H*-Benz[*g*]indole-2,3-dicarboxylates **4.**

General Procedure.

This procedure is essentially similar to the method of Mitchell *et al*. [8]. That is, solution of **3** in methanol was irradiated with a 500 watt high-pressure mercury lamp under nitrogen atmosphere. After evaporation of the solvent the residue was directly recrystallized or subjected to column chromatography on silica gel for purification.

Dimethyl 1*H*-Benz[*g*]indole-2,3-dicarboxylate (**4a**).

The title compound was obtained by the method of Mitchell *et al*. [8] except for the solvent. In our case methanol was employed. Colorless needles (from benzene-*n*-hexane), 83% yield, m.p. 191-191.5 °C (lit. [8] mp 191-192 °C).

Dimethyl 6-Hydroxy-1*H*-benz[*g*]indole-2,3-dicarboxylate (**4b**).

A solution of **3b** (300 mg, 0.917 mmol) in methanol (600 ml) was irradiated for 3.5 hours. Column chromatography eluting with chloroform gave unreacted **3b** (146 mg, 0.446 mmol). The eluate using the same eluting system gave **4b** (73 mg, 0.244 mmol) which was recrystallized from chloroform-*n*-hexane as pale brown granules, 52% yield, mp 244-245 °C. FAB-ms m/z: 300 (MH^+), 268 (MH^+ - 32). Ir (potassium bromide) cm^{-1}: 3430, 3320 (NH and OH), 1695, 1685 (CO). ^{1}H-nmr (deuteriomethanol): δ 3.96, 3.98 (each 3H, each s, CH_3 x 2), 6.90 (1H, dd, J = 8.0, J = 1.0, 7-H), 7.39 (1H, t, J = 8.0, 8-H), 7.86, 7.99 (each 1H, AB q, J = 9.0, 4- and 5-H), 7.94 (1H, dd, J = 8.0, J = 1.0, 9-H),

Anal. Calcd. for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.68. Found: C, 64.08; H, 4.43; N, 4.59.

Dimethyl 7-Hydroxy-1*H*-benz[*g*]indole-2,3-dicarboxylate (**4c**).

A solution of **3c** (100 mg, 0.306 mmol) in methanol (500 ml) was irradiated for 10 minutes. Column chromatography eluting with chloroform-ethyl acetate (17:3, v/v) gave **4c** (52.3 mg, 0.175 mmol) which was recrystallized from ethyl acetate-*n*-hexane as pale yellow granules, 57% yield, mp 246-247 °C. FAB-ms m/z: 300 (MH^+), 268 (MH^+ - 32). Ir (potassium bromide) cm^{-1}: 3395, 3325 (NH and OH), 1702, 1680 (CO). ^{1}H-nmr (deuteriomethanol): δ 3.96, 3.97 (each 3H, each s, CH_3 x 2), 7.14 (1H, dd, J = 8.8, J = 2.4, 8-H), 7.21 (1H, d, J = 2.4, 6-H), 7.42 (1H, d, J = 9.0, 5-H), 7.83 (1H, d, J = 9.0, 4-H), 8.34 (1H, d, J = 8.8, 9-H).

Anal. Calcd. for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.68. Found: C, 64.06; H, 4.51; N, 4.41.

Dimethyl 8-Hydroxy-1*H*-benz[*g*]indole-2,3-dicarboxylate (**4d**).

A solution of **3d** (300 mg, 0.917 mmol) in methanol (500 ml) was irradiated for 45 minutes. Column chromatography eluting with chloroform-ethyl acetate (17:3, v/v) gave **4d** (142 mg, 0.475 mmol) which was recrystallized from chloroform-*n*-hexane as pale yellow granules, 52% yield, mp 247-248 °C. FAB-ms m/z: 300 (MH^+), 268 (MH^+ - 32). Ir (potassium bromide) cm^{-1}: 3445, 3280 (NH and OH), 1718, 1690 (CO). ^{1}H-nmr (deuteriomethanol): δ 3.95, 3.98 (each 3H, each s, CH_3 x 2), 7.07 (1H, dd, J = 8.8, J = 2.4, 7-H), 7.49 (1H, d, J = 8.9, 5-H), 7.72 (1H, d, J = 8.9, 4-H), 7.76 (1H, d, J = 2.4, 9-H), 7.76 (1H, d, J = 8.8, 6-H).

Anal. Calcd. for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.68. Found: C, 64.59; H, 4.50; N, 4.66.

8,9-Dihydrobenzo[*g*]pyridazino[4,5-*b*]indole-7,10(11*H*)-diones (**5**).

General Procedure.

A mixture of **4** and hydrazine hydrate in ethanol (or methanol) was refluxed for an appropriate period. After evaporation of the solvent and excess of hydrazine hydrate, the resulting crystalline residue was dissolved in dimethyl sulfoxide and diluted hydrochloric acid was added to the resulting solution. Then the precipitated crystalline solid was collected *in vacuo*, washed with water and recrystallized from an appropriate solvent to afford **5**.

8,9-Dihydrobenzo[*g*]pyridazino[4,5-*b*]indole-7,10(11*H*)-dione (**5a**).

A solution of **4a** (1.60 g, 5.65 mmol) and hydrazine hydrate (56.0 g, 1.12 mol) in ethanol (280 ml) was refluxed for 2.5 hours. Recrystallization from methanol gave **5a** (1.33 g, 5.30 mmol) as white granules, 94% yield, mp >300 °C. FAB-ms m/z: 252 (MH^+). Ir (potassium bromide) cm^{-1}: 3517, 3222, 3092, 2972 (NH, broad), 1618 (CO). ^{1}H-nmr (DMSO-d_6): δ 7.63-7.72 (2H, m, J = 8, J = 1.6, 2- and 3-H), 7.75 (1H, d, J = 8.7, 5-H), 8.06 (1H, br d, J = 8, 4-H), 8.14 (1H, d, J = 8.7, 6-H), 8.80 (1H, d, J = 8, 1-H), 11.60 (2H, br s, deuterium oxide exchangeable, NH x 2), 13.45 (1H, br s, deuterium oxide exchangeable, NH).

Anal. Calcd. for $C_{14}H_9N_3O_2$: C, 66.93; H, 3.61; N; 16.73. Found: C, 66.84; H, 3.75; N, 16.44.

4-Hydroxy-8,9-dihydrobenzo[*g*]pyridazino[4,5-*b*]indole-7,10(11*H*)-dione (**5b**).

A solution of **4b** (300 mg, 1.00 mmol) and hydrazine hydrate (24.0 g, 480 mmol) in methanol (54 ml) was refluxed for 2.5 hours. Recrystallization from ethanol gave **5b** (260 mg, 0.970 mmol) as pale yellow prisms, 97% yield, mp >300 °C. FAB-ms m/z: 268 (MH^+). Ir (potassium bromide) cm^{-1}: 3210, 3055 (NH and OH, broad), 1655 (CO, broad). ^{1}H-nmr (DMSO-d_6): δ 6.99 (1H, d, J = 8.0, 3-H), 7.44 (1H, t, J = 8.0, 2-H), 7.99, 8.05 (each 1H, AB q, J = 8.0, 5- and 6-H), 8.22 (1H, d, J = 8.0, 1-H), 10.23 (1H, br s, deuterium oxide exchangeable, OH), 11.55 (2H, br s, deuterium oxide exchangeable, NH x 2), 13.27 (1H, br s, deuterium oxide exchangeable, NH).

Anal. Calcd. for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N; 15.72. Found: C, 63.06; H, 3.58; N, 15.63.

3-Hydroxy-8,9-dihydrobenzo[*g*]pyridazino[4,5-*b*]indole-7,10(11*H*)-dione (**5c**).

A solution of **4c** (1.25 g, 4.18 mmol) and hydrazine hydrate (33.0 g, 660 mmol) in ethanol (22 ml) was refluxed for 5 hours. Recrystallization from ethanol-*n*-hexane gave **5c** (1.03 g, 3.86 mmol) as white granules, 92% yield, mp >300 °C. FAB-ms m/z: 268 (MH^+). Ir (potassium bromide) cm^{-1}: 3200, 3060 (NH and OH, broad), 1635 (CO). ^{1}H-nmr (DMSO-d_6) δ: 7.16 (1H, dd, J = 9, J = 2, 2-H), 7.28 (1H, d, J = 2, 4-H), 7.51 (1H, d, J = 9, 5-H), 8.02 (1H, d, J = 9, 6-H), 8.62 (1H, d, J = 9, 1-H), 9.84 (1H, br s, deuterium oxide exchangeable, OH), 11.51 (2H, br s, deuterium oxide exchangeable, NH x 2), 13.24 (1H, br s, deuterium oxide exchangeable, NH).

Anal. Calcd. for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N; 15.72. Found: C, 62.73; H, 3.51; N, 15.80.

2-Hydroxy-8,9-dihydrobenzo[*g*]pyridazino[4,5-*b*]indole-7,10(11*H*)-dione (**5d**).

A solution of **4d** (300 mg, 1.00 mmol) and hydrazine hydrate (24.0 g, 480 mmol) in methanol (54 ml) was refluxed for 2 hours. Recrystallization from ethanol-*n*-hexane gave **5d** (251 mg, 0.937 mmol) as colorless granules, 94% yield, mp >300 °C. FAB-ms m/z: 268 (MH^+). Ir (potassium bromide) cm^{-1}: 3230, 3020, 2880 (NH and OH, broad), 1625 (CO). ^{1}H-nmr (DMSO-d_6): δ 7.15 (1H, dd, J = 8.7, J = 2.3, 3-H), 7.59 (1H, d, J = 8.6, 5-H), 7.87 (1H, d, J = 8.7, 4-H), 7.90 (1H, d, J = 8.6, 6-H), 8.03 (1H, br s, 1-H), 9.87 (1H, br s, deuterium oxide exchangeable, OH), 11.52 (2H, br s, deuterium oxide exchangeable, NH x 2), 13.27 (1H, br s, deuterium oxide exchangeable, NH).

Anal. Calcd. for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N; 15.72. Found: C, 62.98; H, 3.49; N, 15.85.

Evaluation of Chemiluminescent Activity.

The reaction solution contains 10 m*M* phosphate buffer (for pH 4.0, 6.0 or 8.0) or 10 m*M* carbonate buffer (for pH 10.0), Triton X-100 (0.05%) and test compound (0.1 mg/ml). The concentration of hydrogen peroxide solution is 3 m*M*. *Arthromyces ramosus* peroxidase (ARP) solution was prepared as 730 Units/ml. The reaction solution (3 ml) was transferred into a sample tube and immediately placed in a water bath (37 °C) for 10 minutes. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted after additions of hydrogen peroxide solution (75 μl) and ARP solution (75 μl). The final concentration of hydrogen peroxide is 0.22 mmol/3 ml (the reaction solution) and the final concentration of ARP is 55 Units/3 ml (the reaction solution).

Acknowledgement.

A part of this work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (A) (No. 11147217) from the Ministry of Education, Science, Sports and Culture of Japan. The authors are grateful to The SC-NMR Laboratory of Okayama University for 200 MHz ^{1}H-nmr experiments.

REFERENCES AND NOTES

[1a] D. C. Young, S. D. Ryan and F. J. Dutko, *Anal. Biochem*., **215**, 24 (1993); [b] J. A. Matthews, A. Batki, C. Hynds and L. J. Kricka, *Anal. Biochem*., **151**, 205 (1985); [c] M. P. Wymann, V. Tscharner, D. A. Deraulean and M. Baggiolini, *Anal. Biochem*., **165**, 371 (1987); [d] R. Lock, A. Johansson, K. Orselius and C. Dahlgren, *Anal. Biochem*., **173**, 450 (1988); [e] H. P. Misra and P. M. Squatrito, *Arch Biochem. Biophys*, **215**, 59 (1982); [f] J. Arnhold, S. Mueller, K. Arnold and K. Sonntag, *J. Biolumin. Chemilumin*., **6**, 189 (1991); [g] G. Merenyi J. Lind and T. E. Eriksen, *J. Biolumin. Chemilumin*, **5**, 53 (1990).

[2] Bioluminescence and Chemiluminescence, ed. by A. K. Campbell, L. J. Kricka and P. E. Stanley, John Wiley & Sons, Chichester, 1994 and 1996, and references cited therein.

[3a] T. G. Burdo and W. Rudolf Seitz, *Anal. Chem*., **47**, 1639 (1975); [b] R. B. Brundrett, D. F. Roswell and E. H. White, *J. Am. Chem. Soc*., **94**, 7536 (1972); [c] C. C. Wei and E. H. White, *Tetrahedron Lett*., 3559 (1971); [d] M. Ii, H. Yoshida, Y. Aramaki, H. Masuya, T. Hada, M. Terada, M. Hatanaka and Y. Ichimori, *Biochem. Biophys. Res. Comm*., **193**, 540 (1993).

[4] M. Kurumi, K. Sasaki, H. Takata and T. Nakayama, *Heterocycles*, **53**, 2809 (2000).

[5] M. O. Forster and H. E. Fierz, *J. Chem. Soc*., **91**, 1942 (1907).

[6] P. Spagnolo, A. Tundo and P. Zanirato, *J. Org. Chem*., **43**, 2508 (1978).

[7a] Y. Nagawa, K. Honda and H. Nakanishi, *Bull. Chem. Soc. Jpn*., **60**, 2931 (1987); [b] Y. Nagawa, M. Goto, K. Honda and H. Nakanishi, *Bull. Chem. Soc. Jpn*., **62**, 3109 (1989); [c] Y. Nagawa, K. Honda and H. Nakanishi, *Heterocycles*, **51**, 1093 (1999).

[8] G. Mitchell and C. W. Rees, *J. Chem. Soc. Perkin Trans. 1*, 413 (1987).