

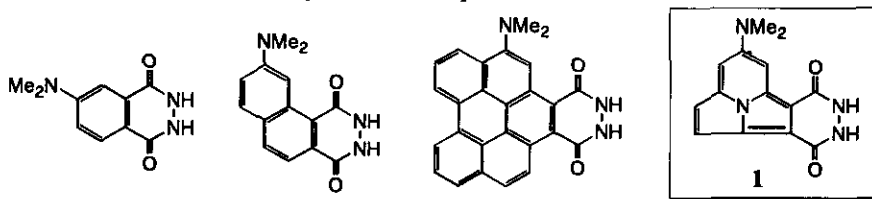
SYNTHESIS OF A LUMINESCENT COMPOUND: 8-DIMETHYL-AMINOPYRIDAZINO[4,5-*a*][2.2.3]CYCLAZINE-1,4(2*H*,3*H*)-DIONES

Yoshinori Tominaga,^{*a} Kaori Komiya,^a Seigo Kataoka,^a Yasuhiro Shigemitsu^b, Takashi Hirota,^c and Kenji Sasaki^c

^aFaculty of Pharmaceutical Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8131, Japan; ^bChemistry & Design Division, Technology Center of Nagasaki, 2-1303-8 Ikeda, Omura, Nagasaki 856-0026, Japan; ^cFaculty of Pharmaceutical Sciences, Okayama University, 1-1-1, Tsushima, Okayama 700-8530, Japan,

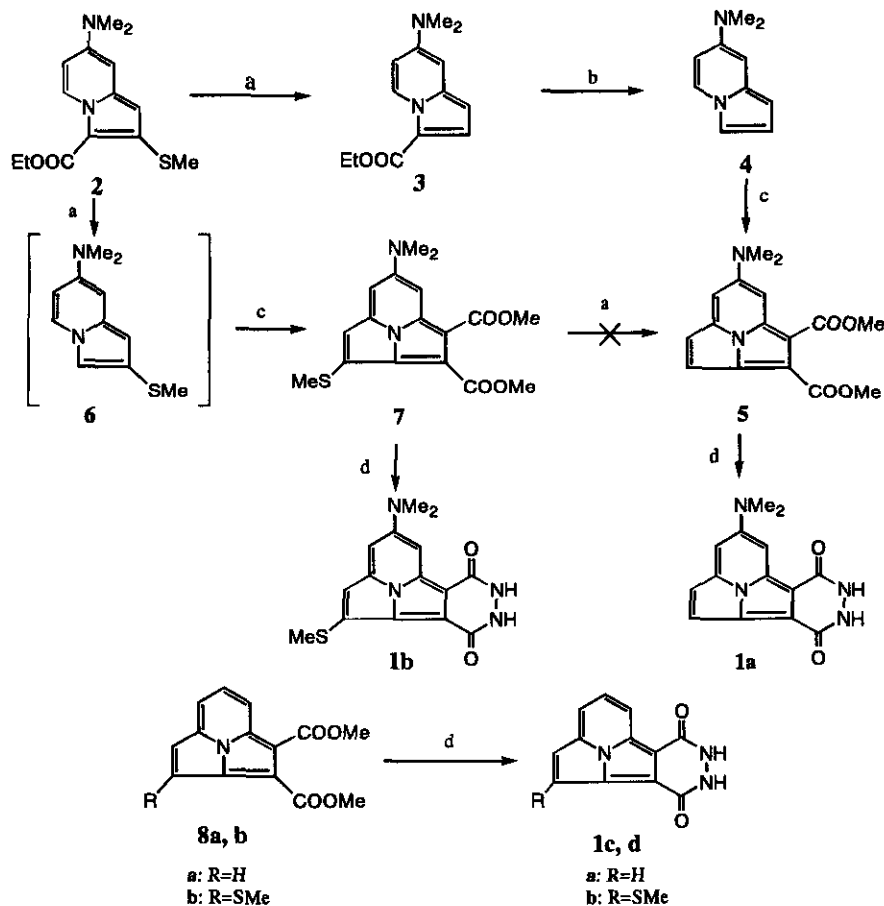
Abstract-----Some pyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-diones (**1a-d**) as a luminescent compound were synthesized *via* several steps from indolizine derivatives. The key intermediates, dimethyl 6-dimethylamino[2.2.3]cyclazine-1,2-dicarboxylates (**5**, **7**) were synthesized by the [8 + 2] cycloaddition reaction of 7-dimethylaminoindolizines (**4**, **6**) with dimethyl acetylenedicarboxylate in the presence of Pd-C in refluxing toluene.

Polycyclic pyridazines have been extensively studied because they are of great importance in biological and medicinal chemistry.¹ We have focused our attention on the synthesis and chemiluminescent property of polycyclic fused pyridazine-1,4-dione derivatives.² Many polycyclic hydrazides have been synthesized in efforts to increase the efficiency of light production.³ We now report here the synthesis of an efficient cyclic hydrazide based on the [2.2.3]cyclazine ring system. [2.2.3]Cyclazines, which are peripheral conjugate aromatic compounds with delocalized 10 π -electrons, are interesting hetero aromatic compounds from both theoretical and synthetic standpoints.⁴



Our synthetic approach, which was achieved in a 4-step procedure, is outlined in Scheme 1. First, the desulfurization of **25** with Raney Ni in refluxing ethanol afforded ethyl 7-dimethylaminoindolizine-3-carboxylate (**3**)⁶ in 72% yield. Decarboxylation of **3** with PPA at 150°C for 1 h was smoothly carried out to give the expected 7-dimethylaminoindolizine (**4**) in 92% yield.⁷ The [8 + 2] cycloaddition reaction of **4** with dimethyl acetylenedicarboxylate (DMAD) in the presence of 5% Pd-C in refluxing toluene gave the expected product, dimethyl 6-dimethylamino[2.2.3]cyclazine-1,2-dicarboxylate (**5**)⁸ as orange needles, mp 125-126°C, in 32% yield. This compound (**5**) was not obtained by the desulfurization of

dimethyl 6-dimethylamino-3-methylthio[2.2.3]cylazine-1,2-dicarboxylate (**7**) with Raney Ni in refluxing methanol. Compound (**7**)⁹ was synthesized by the [8 + 2] cycloaddition reaction of 7-dimethylamino-2-methylthioindolizine (**6**) with DMAD in a similar manner to that described for the preparation of **5**, **10**, **11**



a: Raney Ni, reflux for 5 h in ethanol; b: PPA at 150°C for 1.5 h, 10% NaOH; c: DMAD(dimethyl acetylenedicarboxylate) reflux for 5 h in toluene; d: excess of 80% $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$

Scheme 1

The expected 6-dimethylaminopyridazino[4,5-*a*][2.2.3]cylazine-1,4(2*H*,3*H*)-diones (**1a**, **b**)^{12, 13} were obtained by the reaction of **5** and **7** with a large excess of 80% hydrazine hydrate in 90% and 77% yields, respectively, and could be purified by recrystallization from DMSO to give orange red crystals.¹⁴ Similarly, pyridazino[4,5-*a*][2.2.3]cylazine-1,4(2*H*,3*H*)-diones (**1c**, **d**)^{15, 16} were also readily prepared from the corresponding dimethyl [2.2.3]cylazine-1,2-dicarboxylate (**8a**)^{17a, b} and 3-methylthio derivative (**8b**)^{17a} in 82% and 77% yields, respectively.

The chemiluminescence (CL) experiments reported here were performed in the presence of Triton X-100, hydrogen peroxide, and horseradish peroxidase (HRP) in a phosphate buffer solution at pH 8.0.¹⁸ The CL intensity in these pyridazinedione series is shown in Table 1. Compounds (**1c**, **d**) showed nearly the same or somewhat stronger light intensity than luminol.

Table 1. Chemiluminescence Intensity of pyridazino[4,5-a][2.2.3]cyclazine-1,4(2H,3H)-diones

Compound	CL(CPS) ^a pH 8	Compound	CL(CPS) ^a pH 8
1a	1.00x10 ¹	1c	6.98x10 ⁵
1b	2.17x10 ²	1d	4.86x10 ⁵
		Luminol	6.77x10 ⁴

a) Counts per 1.0 sec. (Their values were subtracted from each background.)

A reaction solution contains 10 mmol/L phosphate buffer pH 8.0, 0.5 ml/L Triton X-100, 2.5x10⁻⁷ mol/L test compound, and 2500 U/L HRP (Each test compound was prepared to obtain concentration of 1.5x10⁻⁵ mol/L in DMSO). The solution (3 ml. of vol) was transferred to a Borosilicate glass tube (12x75 mm) and immediately placed in a water bath (37 °C) for 10 min. At the end of the incubation period, the sample tube to be counted was incorporated into a luminometer. Photons were counted for 1.0 sec. after addition of 0.3 mL of 1.1x10⁻⁹ mol/L H₂O₂ (2.5x10⁻⁹ mol/L as final concentration).

In conclusion, 8-dimethylaminopyridazino[4,5-a][2.2.3]cyclazine-1,4(2H,3H)-diones as luminescent compounds were readily obtained from dimethyl 6-dimethylamino[2.2.3]cyclazine-1,2-dicarboxylates which are prepared by the [8 + 2] cycloaddition reaction of dimethylaminoindolizines with DMAD.

REFERENCES AND NOTES

1. W. J. Coates, "Pyridazines and their Benzo Derivatives" in "Comprehensive Heterocyclic Chemistry II", Vol. 6, ed. by A. R. Katritzky, C. W. Rees, and E. F. V. Scriven, Pergamon Press, Oxford, 1993, pp. 1-91.
2. a) Y. Tominaga, N. Yoshioka, S. Kataoka, N. Aoyama, T. Masunari, and A. Miike, *Tetrahedron Lett.*, 1995, **36**, 8641; b) Y. Tominaga, N. Yoshioka, and S. Kataoka, *Heterocycles*, 1996, **43**, 1597; c) Y. Tominaga, N. Yoshioka, H. Minematsu, and S. Kataoka, *Heterocycles*, 1997, **44**, 85.
3. a) T. G. Burdo and W. Rudolf Seitz, *Anal. Chem.*, 1975, **47**, 1639; b) R. B. Brundrett, D. F. Roswell, and E. H. White, *J. Am. Chem. Soc.*, 1972, **94**, 7536; c) C. C. Wei and E. H. White, *Tetrahedron Lett.*, 1971, 3559; d) M. Ii H. Yoshida, Y. Aramaki, H. Masuya, T. Hada, M. Terada, M. Hatanaka, and Y. Ichimori, *Biochem. Biophys. Res. Comm.*, 1993, **193**, 540.
4. a) W. Flitsch, "Bicyclic 5-6 System with One Ring Junction Nitrogen Atom: No Extra Heteroatom" in "Comprehensive Heterocyclic Chemistry II", Vol. 8, ed. by A. R. Katritzky, C. W. Rees, and E. F. V. Scriven, Pergamon Press, Oxford, 1993, p. 237; b) W. Flitsch, "Pyrroles with Fused Six-membered Heterocyclic Rings: (i) a-Fused, in Comprehensive Heterocyclic Chemistry", 1st edn., Vol. 4, ed. by A. R. Katritzky, C. W. Rees, and E. F. V. Scriven, Pergamon Press, Oxford, 1984, p. 443; c) Y. Tominaga, Y. Shiroshita, and A. Hosomi, *Heterocycles*, 1988, **27**, 2251.
5. 2: This compound was prepared along with ethyl 7-dimethylamino-1-nitro-2-methylthioindolizine-3-carboxylate by reaction of 1-ethoxycarbonylmethylpyridinium bromide with 1,1-bis(methylthio)-2-nitroethylene in the presence of potassium carbonate in DMSO. cf. Y. Matsuda, K. Katou, H. Matsumoto, S. Ide, K. Takahashi, K. Torisu, K. Furuno, and S. Maeda, *Yakugaku Zasshi*, 1992, **112**, 42.
6. 3: mp 63-64°C, colorless needles. ¹H-NMR(CDCl₃)δ: 1.37(3H, t, J=7.1 Hz, CH₂-Me), 2.99(6H, s, NMe₂), 4.32(2H, q, J=7.1 Hz, CH₂), 6.11(1H, d, J=4.3 Hz, 1-H), 6.45(1H, J=2.5 Hz, 8-H), 6.51(1H, dd, J=2.5, 8.0 Hz, 6-H), 7.42(1H, d, J=4.3 Hz, 2-H), 9.24(1H, d, J=8.0 Hz, 9-H).
7. 4: mp 66-68°C, tan needles. ¹H-NMR(CDCl₃)δ: 2.87(6H, s, NMe₂), 6.08(1H, dd, J=1.0, 3.6 Hz,

- 1-H), 6.29(1H, dd, $J=2.5, 7.7$ Hz, 6-H), 6.42(1H, d, $J=2.5$ Hz, 8-H), 6.66(1H, dd, $J=2.9, 3.6$ Hz, 2-H), 7.05(1H, dd, $J=1.0, 2.9$ Hz, 3-H), 7.74(1H, d, $J=7.7$ Hz, 5-H).
8. **5**: mp 125-126°C, orange needles. $^1\text{H-NMR}(\text{CDCl}_3)\delta$: 3.20(6H, s, NMe₂), 3.99(3H, s, OMe), 4.04(3H, s, OMe), 7.17(1H, d, $J=4.8$ Hz, 4-H), 7.35(1H, s, 5-H), 7.56(1H, d, $J=4.8$ Hz, 3-H), 7.65(1H, s, 7-H).
9. The deesterification of **2** with PPA gave a mixture of 7-dimethylamino-2-methylthioindolizine (**6**) and 7-dimethylamino-2-ethylthioindolizine (ratio: 3 : 1 by NMR) which was used in the next step without separation. The reaction of this mixture with DMAD afforded a mixture of **7** and dimethyl 3-ethylthio- 6-dimethylamino[2.2.3]cyclozine-1,2-dicarboxylate which are readily separated by silica gel column chromatography using toluene as an eluent. The yields were 24% and 8%, respectively.
10. In the case of the use of methyl 7-dimethylamino-2-methylthioindolizine-3-carboxylate, which was obtained by reaction of 1-methoxycarbonylmethylpyridinium chloride with 1,1-bis(methylthio)-2-nitroethylene, the compound (**6**) was only obtained in 27% yield in a similar manner to that the described for the above treatment with PPA. **6**: tan crystals, mp 62-64°C. $^1\text{H-NMR}(\text{CDCl}_3)\delta$: 2.43(3H, s, SMe), 2.84(6H, s, NMe₂), 6.05(1H, s, 1-H), 6.21(1H, dd, $J=2.5, 7.6$ Hz, 6-H), 6.27(1H, d, $J=2.5$ Hz, 8-H), 6.94(1H, s, 3-H), 7.58(1H, d, $J=7.6$ Hz, 5-H).
11. **7**: Yield 16%, mp 125-126°C, orange needles. $^1\text{H-NMR}(\text{CDCl}_3)\delta$: 2.65(3H, s, SMe), 3.17(3H, s, NMe), 3.18(3H, s, NMe), 3.97(3H, s, OMe), 4.05(3H, s, OMe), 6.75(1H, s, 4-H), 7.11(1H, d, $J=1.7$ Hz, 5-H), 7.42(1H, d, $J=1.7$ Hz, 7-H).
12. **1a**: mp >360°C, orange red leaflets. $^1\text{H-NMR}(\text{DMSO-d}_6)\delta$: 3.21(6H, s, NMe₂), 7.29(1H, d, $J=4.5$ Hz, 6-H), 7.73(1H, d, $J=1.5$ Hz, 7-H), 7.77(1H, $J=4.5$ Hz, 5-H), 7.77(1H, d, $J=1.5$ Hz, 9-H), 11.50(2H, s, NH). Cl spectrum (DMSO + 5% NaOH): λ 519nm.
13. **1b**: mp >360°C, orange crystals. $^1\text{H-NMR}(\text{DMSO-d}_6)\delta$: 2.69(3H, s, SMe), 3.19(6H, s, NMe₂), 6.96(1H, br s, 6-H), 7.51(2H, br s, 7, 9-H), 11.40(2H, br s, NH). Cl spectrum (DMSO + 5% NaOH): λ 513 nm.
14. When the compound (**5**) was allowed to react with 80% hydrazine hydrate 10 min in refluxing in methanol, monohydrazide derivative, 1-or 2-methoxycarbonyl-6-dimethylamino[2.2.3]cyclozine-1-or 2-hydrazide was obtained as orange leaflets, mp 250-270°C (decomp), in 88% yield.
15. **1c**: mp >360°C, yellow needles. $^1\text{H-NMR}(\text{DMSO-d}_6)\delta$: 7.65(1H, d, $J=4.5$ Hz, 6-H), 7.99(1H, d, $J=4.5$ Hz, 5-H), 8.14(1H, dd, $J=7.8, 7.8$ Hz, 8-H), 8.50(1H, $J=7.8$ Hz, 7-H), 8.59(1H, d, $J=7.8$ Hz, 9-H), 11.77(2H, br s, NH). Cl spectrum (DMSO + 5% NaOH): λ 470 nm.
16. **1d**: mp >360°C. yellow needles. $^1\text{H-NMR}(\text{DMSO-d}_6)\delta$: 2.74(3H, s, SMe), 7.33(1H, s, 6-H), 8.03(1H, dd, $J=7.8, 7.8$ Hz, 8-H), 8.19(1H, dm $J=7.8$ Hz, 7-H), 8.39(1H, d, $J=7.8$ Hz, 9-H), 11.70(2H, s, NH). Cl spectrum (DMSO + 5% NaOH): λ 463 nm.
17. a) Y. Tominaga, Y. Shiroshita, T. Kurokawa, H. Gotou, Y. Matsuda, and A. Hosomi, *J. Heterocycl. Chem.*, 1989, **26**, 477; b) A. Galbraith, T. Small, R. A. Barnes, and V. Boeckelheide, *J. Am. Chem. Soc.*, 1961, **83**, 452.
18. Cl intensity was measured with a Magic Lite Analyzer of CIBA-CONING.