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

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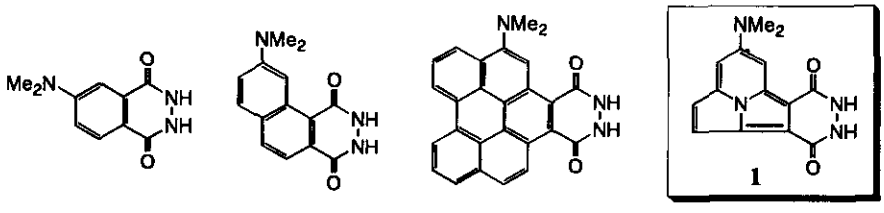
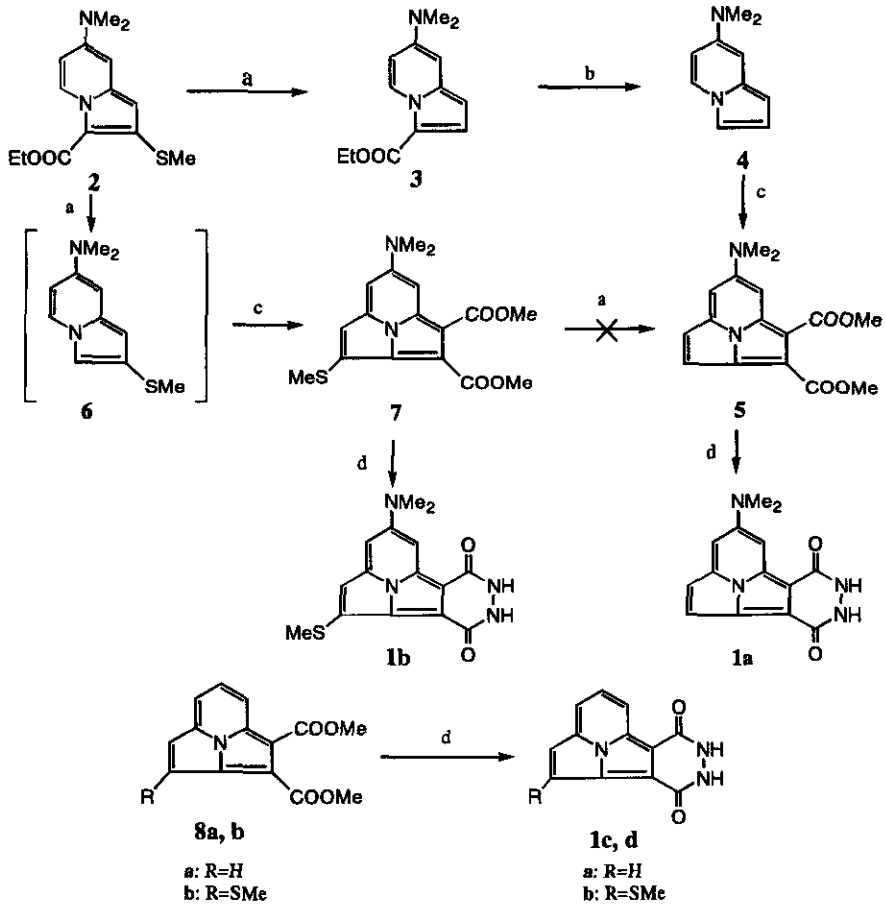
Abstract-----Some pyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-diones (**1a-d**) as a luminescent compound were synthesized *via* several steps from indolizine derivatives. The key intermediates, dimethyl 6-dimethylamino[2.2.3]cyclazine-1,2-dicarboxylates (**5, 7**) were synthesized by the [8 + 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 10π-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 **2**[5] 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, mp125-126°C, in 32% yield. This compound (**5**) was not obtained by the desulfurization of

dimethyl 6-dimethylamino-3-methylthio[2.2.3]cyclazine-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 acetylene-dicarboxylate) reflux for 5 h in toluene; d: excess of 80% NH_2NH_2 H_2O

Scheme 1

The expected 6-dimethylaminopyridazino[4,5-*a*][2.2.3]cyclazine-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]cyclazine-1,4(2*H*,3*H*)-diones (**1c, d**)[15, 16] were also readily prepared from the corresponding dimethyl [2.2.3]cyclazine-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(2*H*,3*H*)-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 H_2O_2 ($2.5x10^{-9}$ mol/L as final concentration).

In conclusion, 8-dimethylaminopyridazino[4,5-*a*][2.2.3]cyclazine-1,4(2*H*,3*H*)-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.

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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. ^{1}H-NMR($CDCl_3$)δ: 1.37(3H, t, J=7.1 Hz, CH_2-Me), 2.99(6H, s, NMe_2), 4.32(2H, q, *J*=7.1 Hz, CH_2), 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. ^{1}H-NMR($CDCl_3$)δ: 2.87(6H, s, NMe_2), 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}H-NMR($CDCl_3$)δ: 3.20(6H, s, NMe_2), 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]cyclazine-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}H-NMR($CDCl_3$)δ: 2.43(3H, s, SMe), 2.84(6H, s, NMe_2), 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}H-NMR($CDCl_3$)δ: 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}H-NMR(DMSO-d_6)δ: 3.21(6H, s, NMe_2), 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}H-NMR(DMSO-d_6)δ: 2.69(3H, s, SMe), 3.19(6H, s, NMe_2), 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]cyclazine-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}H-NMR(DMSO-d_6)δ: 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}H-NMR(DMSO-d_6)δ: 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.
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18. Cl intensity was measured with a Magic Lite Analyzer of CIBA-CONING.

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