Tetrahedron Vol. 47, No. 23, pp. 3719-3726, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00

RESTRICTED BOND ROTATION AND FLUORESCENCE FOLLOWING PHOTOEXCITATION OF DIPYRRINONES

Jin-Shi Ma[1a] and David A. Lightner*[1b]

Department of Chemistry, University of Nevada
Reno, Nevada 89557-0020 USA
and
The Institute of Photographic Chemistry
Academia Sinica, Beijing, P.R. China

(*Received in USA* 4 *February* 1991)

Summary: Weakly fluorescing ($\phi_F \simeq 10^{-4}$, hexane) dipyrrinone analogs of bilirubin, methyl xanthobilirubinate and kryptopyrromethenone, exhibit intense room temperature fluorescence ($\phi_F \simeq 0.85$, cyclohexane) after conversion to their N_{10}-N_{11} methano-bridged derivatives **1** and **2**.

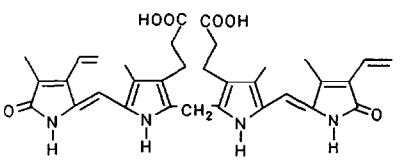
The fastest and most quantum efficient photochemical reaction of bilirubin is *Z*→*E* carbon-carbon double bond isomerization[2-4] — a process of considerable importance to the success of phototherapy for jaundiced newborn babies[5]. This efficient photochemical process involves an ultrafast (rate = 19 ± 2 ps^{-1}) double bond twisting as the principle relaxation mechanism at normal temperatures for the singlet photoexcited pigment[2-4,6,7] and has been the subject of many investigations concerned with improving the efficacy of phototherapy.[5,8] Its relatively high quantum yield in aqueous solutions of human serum albumin at room temperature ($\phi_{Z\rightarrow E} \simeq 0.22$ $\phi_{E\rightarrow Z} > 0.6$) correlates well with the lower efficiency of other deexcitation processes, such as fluorescence ($\phi_F \simeq 0.003$, $\tau_F = 18 \pm 3$ ps).[2] Similar results have been reported for organic solvents such as chloroform: $\phi_{Z\rightarrow E} \simeq 0.3$, $\phi_F \simeq 0.0002$.[2] However, as the viscous drag of the microenvironment increases, the efficiency of photoisomerization *decreases*: $\phi_{Z\rightarrow E} < 0.01$ at 77°K for bilirubin in 50% aqueous ethylene glycol + human serum albumin.[9] Reciprocally, when the microenviron-

Dedicated to Professor Kurt Schaffner on the occasion of his 60th birthday.

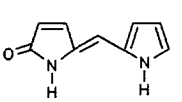
ment is made more rigid, as in polymers such as polymethylmethacrylate at room temperature or in 50% aqueous ethylene glycol + human serum albumin at 77°K, the fluorescence quantum yield increases dramatically: ϕ_F = 0.71 for the former (vs ϕ_F < 0.0005 in ethyl acetate); ϕ_F = 0.92 for the latter (vs ϕ_F = 0.006 at 22°C).[2]

When examined at the level of the isolated parent chromophore of bilirubin, it is not surprising that the $Z \rightleftarrows E$ photoisomerization is the most quantum efficient relaxation pathway for photoexcited dipyrrinones: $\phi_{Z\rightarrow E} \simeq 0.4$ in aqueous buffered human serum albumin at 22°C;[10] $\phi_{Z\rightarrow E}$ = 0.22, $\phi_{E\rightarrow Z}$ = 0.40 in EPA at 20°C.[6] Fluorescence quantum yields are correspondingly low: $\phi_F \simeq 0.003$ in aqueous buffered human serum albumin at 22°C;[10] $\phi_F \simeq < 10^{-3}$ in EPA.[6] And as with bilirubin, at very low temperatures (77°K) the dipyrrinone fluorescence quantum yield goes up ($\phi_F \simeq 0.33$ in EPA) and the $Z \rightleftarrows E$ quantum yields decrease ($\phi_{Z\rightarrow E} < 5\times10^{-4}$, $\phi_{E\rightarrow Z} < 5\times10^{-4}$ in EPA).

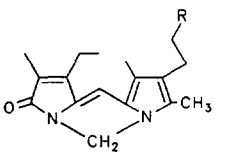
In the following, the interesting correspondence between the fluorescence emission and the $Z\rightarrow E$ configurational inversion deexcitation pathways is investigated further by synthesizing and studying dipyrrinones **1** and **2**, which an internal resistance to photoisomerization due to the presence of a $-CH_2-$ unit bridging the ring nitrogens. The parent compounds, xanthobilirubic acid methyl ester (**3**) and kryptopyrromethenone (**4**), from which **1** and **2**, respectively are prepared undergo a rapid, efficient $Z\rightarrow E$ isomerization, ($\phi_F \simeq 0.2$)[4] and exhibit essentially no fluorescence.[6]



Bilirubin



Dipyrrinone Chromophore



1: R=CO_2CH_3
2: R = H

Synthesis. Conversion of dipyrrinones **3** and **4** to their N-CH_2-N bridged analogs involved deprotonation of the lactam and pyrrole N-H groups with the strong base dimsyllithium in dimethylsulfoxide (DMSO) followed by reaction with diiodomethane.[11] The reaction proceeded best in oxygen-free, argon-saturated solvents, and a noticeable fluorescence developed in the reaction solution. But whether fluorescence was

due to the desired product, reaction by-products or deprotonated pigment was ascertained only upon isolation of the product. The product yields, though reproducible, were only modest, but the derived -CH_2- bridged dipyrrinones **1** and **2** were reasonably stable in the solid and only moderately reactive toward oxygen (and light) in solution.

Spectroscopic Properties. Most notably, solutions of **1** and **2** were strongly (blue-green) fluorescent to the naked eye — in marked contrast to the parent dipyrrinones **3** and **4** from which fluorescence is not detectable ($\phi_F < 10^{-4}$ in cyclohexane).[6] The fluorescence quantum yields of **1** and **2** at room temperature in cyclohexane, determined *vs* 9,10-diphenylanthracene standard ($\phi_F = 0.90$), were very large ($\phi_F \simeq 0.85$), consistent with fluorescence deexcitation being the major relaxation path for return of singlet excited **1** or **2** to the ground state. The fluorescence emission λ_{max} of **1** and **2** were centered near 485 nm in CH_2Cl_2 solvent, and near 505 nm in methanol (Fig. 1). The extremely large fluorescence quantum yields are unprecedented for dipyrrinones at room temperature and indicate only one major deexcitation pathway, as might be anticipated from the observation that neither **1** nor **2** can be photoisomerized from 4*Z* to 4*E* The behavior of **3** and **4** vs **1** and **2** is akin to that found by Saltiel *et al.*[12] for stilbene ($\phi_{t\to c} \simeq 0.5$, $\phi_F \simeq 0.05$) and its restricted rotation analog, indenoindene, ($\phi_F \simeq 1.0$) in methylcyclohexane at 298°K, or 1,2-diphenylcyclobutane ($\phi_F \simeq 1\ 0$, hexane).[13]

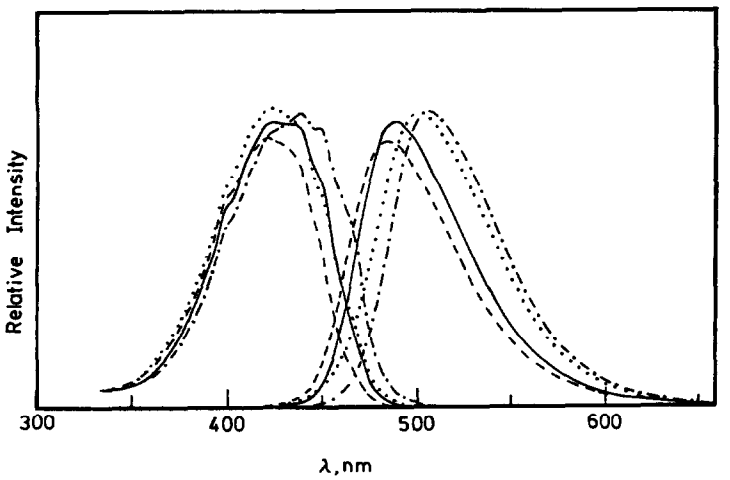
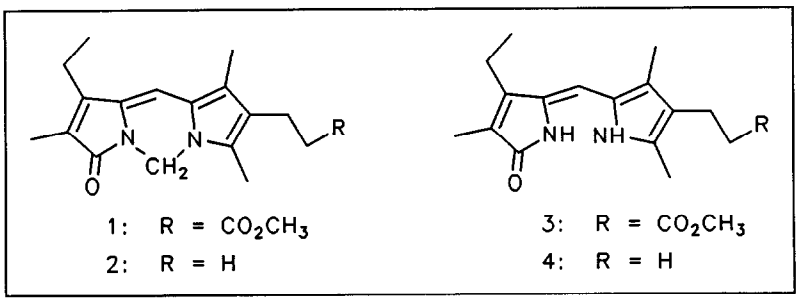


FIGURE 1. Fluorescence emission (right) and excitation (left) spectra in dichloromethane for **1** (- - - -) and **2** (——), and in methanol for **1** (• • •) and **2** (- • - •) at 20°C The fluorescence λ_{max} are: 484 (**1** in CH_2Cl_2), 488 (**2** in CH_2Cl_2), 502 (**1** in CH_3OH) and 506 (**2** in CH_3OH)

The UV-visible spectra of **1** and **2** are also different from the parent dipyrrinones **3** and **4** (Table 1) The absorption maxima are bathochromically shifted in the bridged dipyrrinones and the ϵ values are reduced

Molecular models indicate that the π-systems of **1** and **2** are held planar. In contrast, the parent dipyrrinones do not necessarily adopt planar conformations and may be rotated about the C_5-C_6 bond. In fact, through LIS-NMR studies in dilute solutions, Falk *et al.*[6,14] have shown that dipyrrinones like **3** and **4** adopt twisted conformations with the C_5-C_6 bond rotated by about $\sim 40°$ in non-polar solvents such as $CDCl_3$. Their preferred conformation in polar solvents such as DMSO is unclear, although solvent-solute hydrogen bonding[15] may play a role in stabilizing a planar dipyrrinone conformation, such as attends the self-association of dipyrrinones through intermolecular hydrogen bonding in nonpolar solvents and in the crystal.[6,16] The unexpectedly reduced ϵ values of the bridged dipyrrinones apparently emanate from the fact that the π-systems of the methylene-bridged dipyrrinones are planar. Whether this can be predicted from molecular orbital calculations remains to be examined. The bathochromically shifted λ_{max} may also have their origin in the planarity of dipyrrinones **1** and **2**; however, methyl-substitution of π-systems is known to produce bathochromic shifts, e.g., 2,3,8-triethyl-7,9-dimethyl-(10*H*)-dipyrrin-1-one (λ_{max} = 417 in CH_3OH)[17] *vs* 2,3-diethyl-9-methyl-(10*H*)-dipyrrin-1-one (λ_{max} = 400 in CH_3OH) and N-methylpyrrole (λ_{max} = 216 in CH_3OH) *vs* pyrrole (λ_{max} = 211 in CH_3OH).[18]

TABLE 1. UV-Visible Spectral Data for 1.5×10^{-5} *M* Dipyrrinones at 20°C



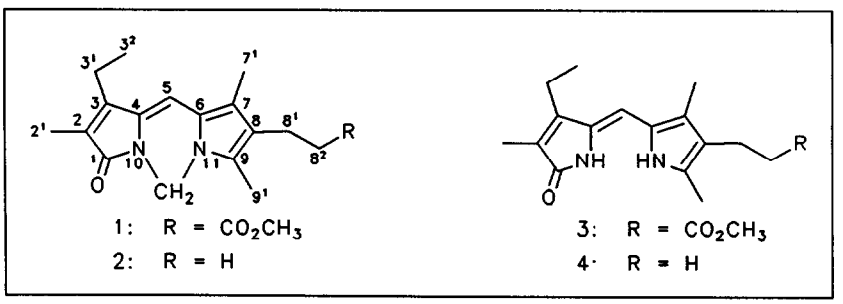
1: R = CO_2CH_3 3: R = CO_2CH_3

2: R = H 4: R = H

	CH_2Cl_2		CH_3OH	
Compound	ϵ^{max}	λ	ϵ^{max}	λ
1	19,700	414	18,900	424
2	18,400	429	19,200	432
3	34,000	406	37,700	411
4	36,150	410	38,000	415

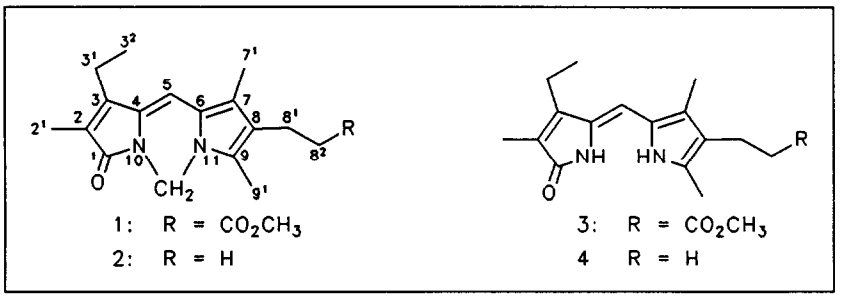
^{1}H-NMR and ^{13}C-NMR spectra (Tables 2 and 3) of **1** and **2** both indicate new signals consistent with the presence of the unique new N-CH_2-N group. Except for the expected loss of the N-*H* signals of **3** and **4** in going to **1** and **2**, the remaining signals in the ^{1}H-NMR spectra do not differ in a major way among the dipyrrinones. The most noticeable differences are found in the ^{13}C-NMR spectra. With the π-system held planar in **1** and **2**, the C_5 carbon resonances are shielded by ~4 ppm, indicating the special sensitivity of this carbon to conjugation effects or internal angle distortion due to the -CH_2- bridge. Ring carbon atoms flanking the nitrogens are also shifted, with major (2-5 ppm) shieldings seen at C_2 and C_9. Whether these, too, are due to the planarization of the π-system or to changes accommodating an inability to self-associate through intermolecular hydrogen bonding (**3** and **4** have a $K_A \simeq 1700$ for self-association as an intermolecularly hydrogen-bonded dimer)[6] is unclear. The C_9-CH_3 carbon resonance exhibits the only major shift among the peripheral groups — probably due mainly to the inability of **1** and **2** to self-associate. A small shielding for this CH_3 group is also seen in the ^{1}H-NMR spectra.

TABLE 2 ^{1}H-NMR Spectral Data for 10^{-2} *M* Dipyrrinones in Deuteriochloroform at 21 °C



Position	1	2	3	4
2^1	1.95	1.94	1.95	1.95
3^1	2.54 (q, J = 7.5 Hz)	2.51 (q, J = 7.5 Hz)	2.55 (q, J = 7.5 Hz)	2.55 (q, J = 7.5 Hz)
3^2	1.18 (t, J = 7.5 Hz)	1.17 (t, J = 7.5 Hz)	1.18 (t, J = 7.5 Hz)	1.17 (t, J = 7.5 Hz)
5	6.14	6.15	6.15	6.15
7^1	2.10	2.09	2.14	2.14
8^1	2 47 (t, J = 7.2 Hz)	2.39 (q, J = 7.5 Hz)	2.42 (t, J = 8.0 Hz)	2.42 (q, J = 7.5 Hz)
8^2	2.72 (t, J = 7.2 Hz)	1.06 (t, J = 7.5 Hz)	2.77 (t, J = 8.0 Hz)	1.07 (t, J = 7.5 Hz)
8^4	3.68	—	3.68	—
9^1	2.24	2.20	2.41	2 41
10	—	—	11.22	11.23
11	—	—	10.31	10.30
N-CH_2-N	5.52	5.51	—	—

TABLE 3. ^{13}C-NMR Spectral Data for 10^{-2} *M* Dipyrrinones in Deuteriochloroform at 21°C



Position	1	2	3	4
1	169.06	169.10	173.92	174.05
2	125.92	124.90	124.57	124 61
2^1	8.85	8.84	8.53	8.52
3	143.43	143.41	148 29	148 23
3^1	17.82	17.86	17.96	17 46
3^2	14.67	14.78	15.08	15 07
4	128.69	128.11	127.10	126.86
5	97.04	97.34	101.04	101 24
6	123.11	124.26	122 43	122 91
7	118.64	118.73	119 03	122 17
7^1	9.51	9.47	9.60	9.52
8	120.39	122.90	122.40	122 22
8^1	19 72	17.39	19.88	17 98
8^2	35.07	15.50	35.13	15 44
8^3	173.41	—	173 59	—
8^4	51.54	—	51.56	—
9	129.43	129.13	131.65	131 13
9^1	8.37	8.42	11.57	11.50
N-CH_2-N	54.15	54.18	—	—

Concluding Comments. Dipyrrinones with -CH_2- groups connecting N_{10} and N_{11} cannot undergo facile *Z*→*E* double bond configurational isomerization at C_4 and the excited states relax by strong fluorescence emission, $\phi_F \simeq 0.85$.

Acknowledgement. We thank the National Institutes of Health (HD-17779) for generous support of this research. J.S. Ma thanks the Institute of Photographic Chemistry, Academia Sinica, Beijing, PRC for a leave of absence during which this research was carried out.

EXPERIMENTAL PART

General. Fluorescence spectra were recorded on a Perkin-Elmer MPF-44A fluorescence instrument using 9,10-diphenylanthracene as a reference for quantum yield determinations UV-visible spectra were determined on a Cary 219 spectrophotometer, and IR spectra were determined on a Perkin-Elmer Model 1610 FTIR spectrometer. NMR spectra were recorded on a GE QE-300 spectrometer, and the data are referenced downfield from tetramethylsilane ($\delta = 0.0$). High resolution mass measurements were determined at the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, Nebraska and the Institute of Chemistry, Academia Sinica, Beijing. Dimethylsulfoxide and methyllithium were from Aldrich. The former was dried over CaH_2, degassed with Ar and stored over 3Å molecular sieves.

N_{10}-N_{11}-Methano-xanthobilirubic acid methyl ester **(1)**: Xanthobilirubic acid methyl ester **(3)**[19] (100 mg, 0.32 mmol) was dissolved in dry dimethylsulfoxide (250 mL) under argon and stirred for 0.5 h. Then 1.4 *M* methyllithium (1.0 mL, 1.4 mmol) in ether was added. The solution was heated to 100°C then diiodomethane (0 06 mL, 0.72 mmol) was added, and the mixture turned brown. Reaction was continued for 3 h until the solution became green, then it was quenched by pouring into ice-cold 5% aqueous ammonium sulfate solution (700 mL). The mixture was extracted with chloroform (3 x 100 mL), and the combined chloroform extracts were washed with 5% aqueous ammonium sulfate (3 x 100 mL), dried over anhydrous sodium sulfate and evaporated. The residue was chromatographed on Woelm TLC grade silica gel (20 mm diameter x 200 mm column), eluting with dichloromethane then dichloromethane-methanol (50:1 v/v) A fluorescent fraction eluted with the latter solvent and was rechromatographed by preparative TLC (1000 μ layer) using dichloromethane-methanol as irrigant. The main fluorescent band was collected and rechromatographed by TLC as above to give 14.5 mg (14% yield) of yellow product with mp 97-100° and IR (KBr) 1736, 1708, 1630 cm^{-1}; high resolution EI-MS, *m/z* (rel. intens.): 328.1782 (100%) [$M^{+\bullet}$], 327.1682 (15%), 326.1625 (14%), 314.1621 (18%), 313.1553 (42%), 281.1301 (12%), 255.1489 (86%), 242 1415 (25%), 137.1327 (14%) amu. UV and NMR spectral data may be found in Tables 1-3.

Anal. Calcd for $C_{19}H_{24}N_2O_3$ (328.178682). Found: 328.1778.

N_{10}-N_{11}-Methano-kryptopyrromethenone **(2)**: Kryptopyrromethenone **(4)**[20] (100 mg, 0.38 mmol) was dissolved in dry dimethylsulfoxide (300 mL) under an argon atmosphere. After stirring at room temperature for 0.5 h, 1.4 *M* methyllithium in ether (1.0 mL, 1.4 mmol) was added, and the solution turned a deep yellow then red The temperature was raised to 100°C, and diiodomethane (0.06 mL, 0.74 mmol) was added to the solution A few minutes later a fluorescent green circle appeared on the surface of the reaction solution After reaction for 3 h, the green fluorescent solution was poured into ice-cold 20% aqueous sodium chloride (700 mL) and extracted with dichloromethane (3 x 100 mL). After washing the combined dichloromethane extracts with 20% aqueous sodium chloride (3 x 100 mL), they were dried (sodium sulfate) and evaporated to dryness under vacuum at 40°C. The residue was chromatographed on a short column of Woelm TLC grade silica gel using chloroform-methanol (50:0, 50:1, 50:2 v/v successively) as eluent. A fluorescent green pigment was eluted with chloroform and was purified by preparative TLC on silica gel (1000 μ layer) using chloroform-methanol (50:1 v/v) as irrigant. After rechromatography by TLC, crystallization afforded 15.9 mg (15% yield) of yellow **2**, mp 125-127°C; IR 1667 cm^{-1}; high resolution EI-MS, *m/z* (rel. intens.): 270.1734 (100%) [$M^{+\bullet}$], 269.1648 (19%), 255.1492 (54%), 241.1383 (6%), 165.0779 (18%), 111.1171 (21%) amu. UV and NMR spectral data may be found in Tables 1-3.

Anal. Calcd for $C_{17}H_{22}N_2O$ (270.173204). Found: 270.1725.

REFERENCES AND NOTES

1. (a) Institute of Photographic Chemistry
(b) University of Nevada

2. For review of the photophysical processes of bilirubin, see Lamola, A.A., in *Optical Properties and Structure of Tetrapyrroles* (G. Blauer and H. Sund, eds.) W. de Gruyter & Co., NY **1985**, pp 311-326.

3. McDonagh, A.F.; Agati, G.; Fusi, F.; Pratesi, R. *Photochem. Photobiol.*, **1989**, *50*, 305-319.

4. Agati, G.; Pratesi, R.; McDonagh, A.F., Lightner, D.A. *Photochem. Photobiol.*, **1990**, 102S.

5. McDonagh, A.F.; Lightner, D.A. *Seminars in Liver Disease*, **1988**, *8*, 272-283.

6. Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*, Springer-Verlag Wien, **1989**.

7. The quantum yield of triplet states from photoexcited bilirubin is thought to be ≤ 0.08 at 77°K and < 0.01 at room temperature (ref. 2).

8. Ennever, J.F. *Photochem. Photobiol.*, **1988**, *47*, 871-876.

9. Greene, B.I.; Lamola, A.A.; Shank, C.V. *Proc. Natl. Acad. Sci., U.S.A.*, **1981**, *78*, 2008-2012.

10 Lamola, A.A.; Braslavsky, S.E.; Schaffner, K.; Lightner, D.A. *Photochem. Photobiol.*, **1983**, *37*, 263-270.

11. Falk, H.; Thirring, K. *Tetrahedron*, **1981**, *37*, 761-766, used potassium hydroxide in DMSO to link N_{21} and N_{24} of etiobiliverdin-IVγ by $-CH_2-$.

12. Saltiel, J.L.; Zafiriou, O.C.; Megarity, E.D.; Lamola, A.A. *J. Am. Chem. Soc.*, **1968**, *90*, 4759-4760.

13. DeBoer, C.D.; Schlessinger, R.H. *J. Am. Chem. Soc.* **1968**, *90*, 803-804.

14. Falk, H.; Grubmayr, K.; Herzig, J.; Hofer, O. *Tetrahedron Lett.*, **1975**, 559-562.

15. Trull, F.R.; Ma, J-S.; Landen, G.L.; Lightner, D.A. *Isr. J. Chem.*, **1983**, *23*, 211-218.

16. (a) Cullen, D.L.; Black, P.S.; Meyer, E.F., Jr.; Lightner, D.A.; Quistad, G.B.; Pak, C-S. *Tetrahedron*, **1977**, *33*, 477-483.
(b) Cullen, D.L.; Pepe, G.; Meyer, E.F., Jr.; Falk, H.; Grubmayr, K. *J. Chem. Soc. Perkin Trans II*, **1981**, 1525-1528.

17 Lightner, D.A.; Park, Y-T. *Tetrahedron*, **1979**, *35*, 463-471.

18 Jaffé, H.H.; Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy*, J. Wiley, New York, **1962**.

19 Shrout, D.P ; Lightner, D.A. *Synthesis*, **1990**, 1062-1065.

20. Trull, F.R.; Franklin, R.W.; Lightner, D.A. *J. Heterocyclic Chem.*, **1987**, *24*, 1573-1579.