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Strongly fluorescent dipyrrinones. Internal quenching

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Abstract Yellow *N*,*N*′-carbonyl-bridged dipyrrinones can generally be prepared from dipyrrinones simply by reaction with *N*,*N*′-carbonyldiimidazole in the presence of a strong, non-nucleophilic base. They are typically intensely fluorescent, with fluorescent quantum yields approaching 1.0. In an effort to shift the excitation wavelength, and thus the fluorescence emissions, strongly to the red, we prepared bridged dipyrrinones conjugated with thiobarbituric acid and *Meldrum*'s acid substituents at C-9. Such conjugation causes the dipyrrinones to have a magenta color (absorption wavelength shifted from ~ 400 nm of a typical dipyrrinone to ~ 550 nm of the dipyrrinone conjugate). For comparison, we also prepared analogs with formyl, carboxyl, acrylate, and acetyl substituents at C-9. Unexpectedly and uniquely, the 9-CHO substituent caused the fluorescence quantum yield to drop to $\sim 10^{-3}$ while carboethoxy substituent exerted only a minor influence.

Keywords Pyrrole; Synthesis; Fluorescence.

Introduction

N,*N*′-Carbonyl-bridged dipyrrinones were first prepared only a few years ago [1] and were discovered to be intensely fluorescent [1, 2]. Shortly thereafter, it was shown that other carbonyl-bridged dipyrrinones [3] and verdins were intensely fluorescent [4], with fluorescence quantum yields (ϕ_F) approaching unity in organic solvents. The chromophore has been used to probe chirality in circular dichroism (*CD*) spectroscopy [5] and studies are currently in progress on their use in fluorescence-detected *CD*. Most recently, analogs with potential medical applications, to detect cholestasis, and in fluorescence imaging were prepared [6]. The majority of the bridged dipyrrinones exhibited intense fluorescence, in only a few instances did we find markedly reduced fluorescence – and then only with two substituents at a pyrrole β-position: $-CH{=}C(CN)CO_2CH_2CH_3$ and $-CH{=}C(CO_2CH_2CH_3)_2$ where ϕ_F dropped to ~ 0.1 in cyclohexane and $\sim 10^{-3}$ in *DMSO*.

Seeking strongly red-shifted fluorescence emitters, we turned our attention toward *N*,*N*′-carbonyl-bridging of the magenta-colored dipyrrinone derivatives obtained *via* a *Mannito–Monti* cleavage of biliverdinoids with thiobarbituric acid [7] (Fig. 1). Finding that we could not insert the bridge directly into an adduct like **A** of Fig. 1, we prepared a bridged 9-CHO dipyrrinone as a potential precursor to adducts like **C** of Fig. 1 to be obtained by a *Knövenagel* reaction at the final step. In the following, we describe the synthesis and characterization of wavelength shifted adducts **1** and **2**, obtained from **3**, and a collection of other 9-substituted bridged dipyrrinones (**4**–**9**) (Fig. 2), and we report on the surprising influence of the 9-substituents on the fluorescence properties of *N*,*N*′-carbonyl-bridged dipyrrinones.

Results and discussion

Manitto and *Monti* [7] showed nearly 3 decades ago that biliverdinoids can be cleaved with thiobarbituric acid (*TBA*) at C(10) to afford *TBA* adducts (*e.g.*, **A**,

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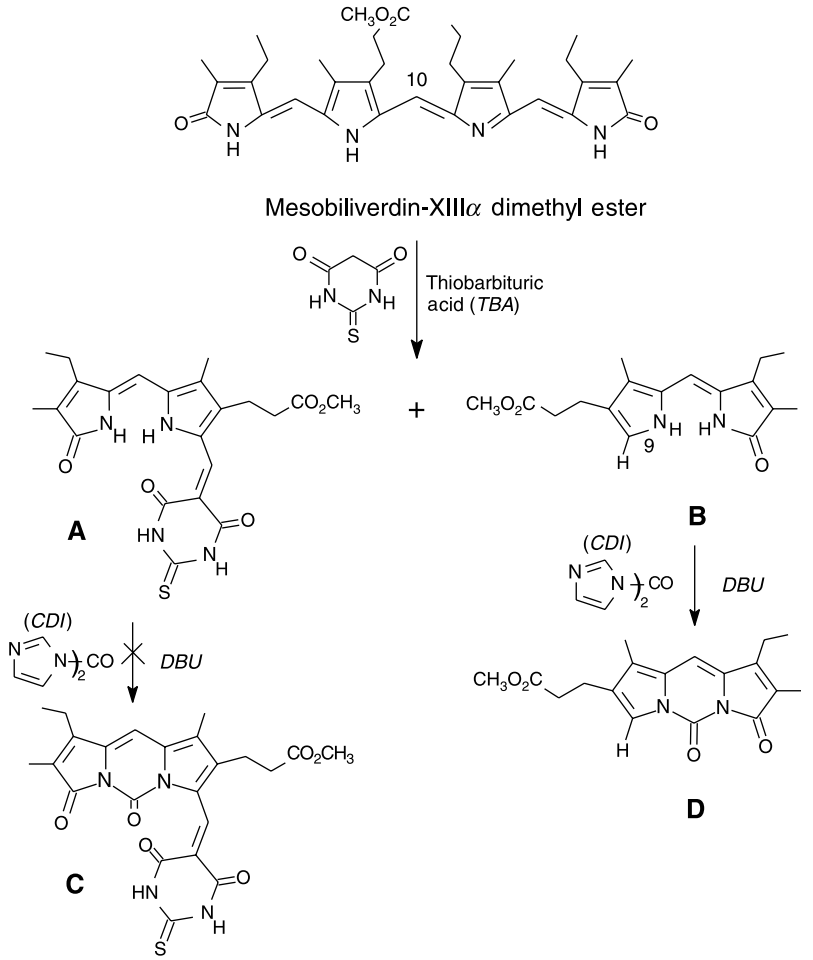


Fig. 1 *TBA*-initiated cleavage of mesobiliverdin-XIIIα dimethyl ester, as per *Manitto* and *Monti* [7] to give **A** and **B**, and then reaction with *N*,*N*′-carbonyl diimidazole (*CDI*) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (*DBU*). *TBA* adduct **A** fails to react; whereas, cyclization of **B** to **D** is facile [1]

Fig. 1) and 9-H dipyrrinones (*e.g.*, **B**, Fig. 1). We were attracted to the *TBA* adducts because of their intense magenta color (the dipyrrinone chromophore is yellow) and our interest in shifting the fluorescence emission from the blue toward the red spectral region by using appropriately-derivatized *N*,*N*′-carbonyl-bridged dipyrrinones, which fluoresce intensely in the 450–500 nm region. However, attempts to insert the carbonyl bridge into *TBA*-adduct **A** failed to give the carbonyl-bridged dipyrrinone **C**.

Since **C** is formally a *Knövenagel* adduct of a 9-CHO dipyrrinone with *TBA*, we explored a different route to it, one in which a 9-CHO *N*,*N*′-carbonyl-bridged dipyrrinone was reacted with *TBA*. Toward this end, we continued our investigation with simpler, more available dipyrrinone-forming starting materials **10** and **11**, as shown in Scheme 1. Thus, pyrrolinone **10** [8] and pyrrole aldehyde **11** [9], both prepared previously in our lab, were condensed to afford dipyrrinone acid **12** in 72% yield. Smooth decarboxylation to the 9-H dipyrrinone **13** was achieved in 73% isolated yield, and **13** was converted by reaction with *N*,*N*′-carbonyldiimidazole (*CDI*) catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (*DBU*) to the highly-fluorescent yellow parent dipyrrinone (**3**), with an *N*,*N*′-carbonyl bridge, in 92% yield. The last was formylated at C(9) by acid-catalyzed reaction with trimethyl orthoformate to yield **4** in 84%. The reverse synthetic order: formylation of **13** to give **14**, then cyclization from *CDI-DBU*, failed at the latter step. We noticed immediately, with considerable surprise, that while **3** was intensely fluorescent (confirmed by measuring $\phi_F \sim 0.3$–0.7), **4** exhibited no fluorescence, as detected by eye and confirmed by fluorescence spectroscopy ($\phi_F \sim 10^{-3}$).

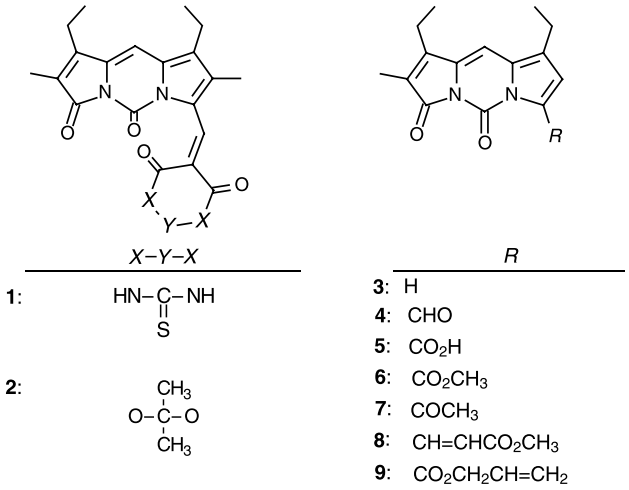
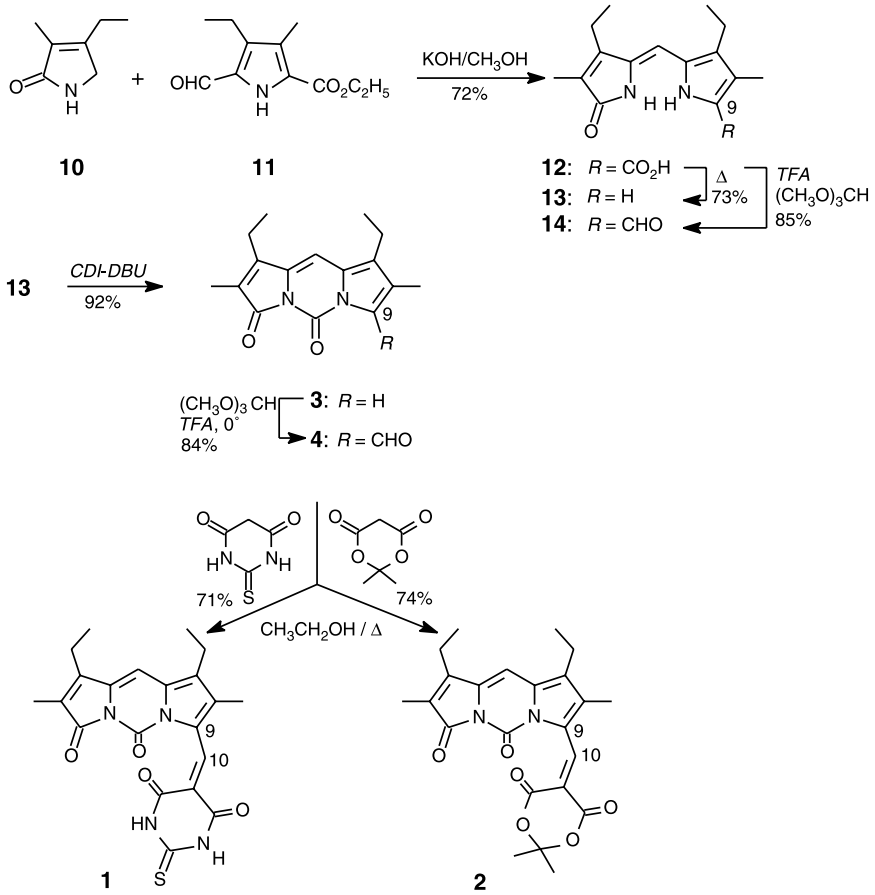


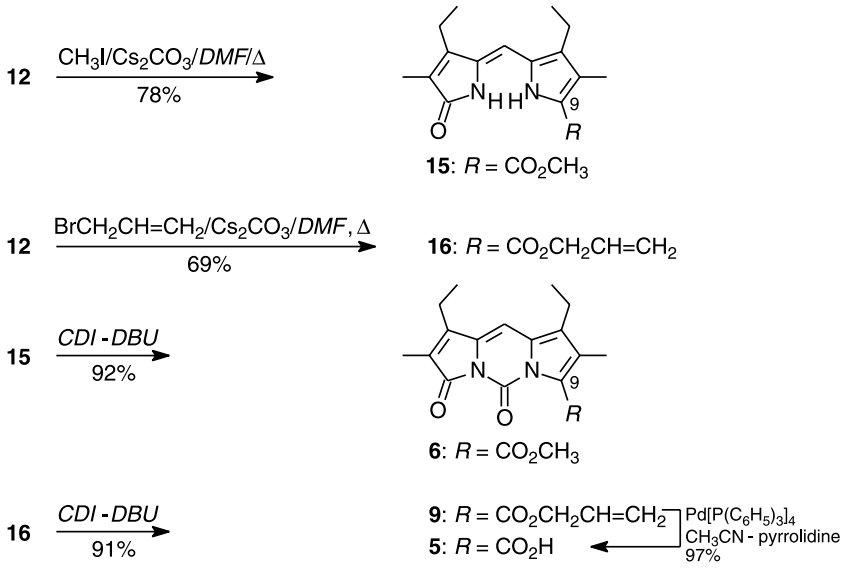
Fig. 2 The target 9-substituted *N*,*N*′-carbonyl-bridged dipyrrinones of this work

Preparation (Scheme 1) of magenta-colored target **1** proceeded smoothly *via* a *Knövenagel* condensation of aldehyde **4** with thiobarbituric acid (*TBA*) in 71% isolated yield. Adduct **1** also exhibited no detectable fluorescence. Confronted with this situation, we prepared the *Knövenagel* condensation product **2** using *Meldrum*'s acid and **4** in 74% yield. The orange-colored adduct **2** also exhibited no detectable fluorescence, thus indicating that it was not the greater conjugation *per se* that was responsible for the diminished fluorescence.

Given the observation that neither **1** nor **2**, nor the simpler **4** were fluorescent, we suspected that the presence of an sp^2-hybridized carbon attached to C(9) might be the origin of any fluorescence quenching. To examine this further, we prepared esters **6** and **9**, and acid **5**, as outlined in Scheme 2. Conversion of **12** to esters **15** and **16** followed by insertion of the bridging carbonyl using *CDI-DBU* in CH_2Cl_2. In fact, all three (**5**, **6**, and **9**) proved to be highly fluorescent ($\phi_F \sim 0.9$, Table 1), respectively, thus discounting the notion that an sp^2-hybridized carbon attached exocyclic to C(9) was a direct cause of the loss of fluorescence in **1**, **2**, and (especially) **4**.



Scheme 1



Scheme 2

Table 1 Solvent dependence of the fluorescence excitation (λ_{ex}/nm) and emission (λ_{em}/nm) wavelengths and quantum yields (ϕ_F) of **3**–**9**

Compound	Cyclohexane			C_6H_6			$CHCl_3$			CH_3OH			$(CH_3)_2SO$		
	λ_{ex}	λ_{em}	ϕ_F	λ_{ex}	λ_{em}	ϕ_F	λ_{ex}	λ_{em}	ϕ_F	λ_{ex}	λ_{em}	ϕ_F	λ_{ex}	λ_{em}	ϕ_F
3	397	454	0.68	398	468	0.62	411	482	0.55	411	518	0.27	411	495	0.57
4	424	450	1.56×10^{-3}	398	453	3.72×10^{-3}	410	450	3.64×10^{-3}	410	483	1.12×10^{-2}	419	479	5.80×10^{-2}
5	397	436	0.91	403	452	0.84	401	453	0.86	419	525	0.35	397	485	0.60
6	396	457	0.87	397	451	0.90	397	470	0.85	396	498	0.43	397	476	0.85
7	399	439	0.18	405	453	0.34	408	472	0.37	404	504	0.25	407	480	0.41
8	449	475	0.22	449	492	0.19	449	494	0.14	449	521	0.09	449	504	0.09
9	396	456	0.91	397	449	0.88	397	469	0.84	396	496	0.42	397	474	0.84
5-Ethyl ester	396	457	0.88	397	451	0.89	398	472	0.85	397	499	0.41	397	476	0.86

We suspected that orientation of the group attached to C(9) might offer an explanation. In earlier work involving 9-substituted dipyrrinones, we concluded on the basis of nuclear *Overhauser* effects (N*O*Es) that the 9-formyl hydrogen was oriented mainly *syn* to the pyrrole NH, and thus the aldehyde carbonyl adopted an *anti* orientation to the pyrrole nitrogen. In contrast, the carbonyl group of a C(9) butanoyl group was oriented *syn* to the pyrrole nitrogen [10]. In contrast, when the dipyrrinone is *N*′,*N*′-carbonyl-bridged as in **4**, the 9-formyl hydrogen shows an N*O*E with the C(8)–CH_3, and thus the aldehyde carbonyl is oriented with a slight preference toward *syn* to the pyrrole nitrogen (Fig. 3), estimated from the relative intensity of the N*O*Es. Similarly, N*O*Es are found between the C(8)–CH_3 and the C(10) methine hydrogens of **1** and **2** – data indicating the likely orientations of the groups attached to C(9), as shown in Fig. 3.

In order to pursue examining whether the orientation of the C(9) substitutent is the cause of fluorescence quenching, we synthesized an analog of **4** with an acetyl group (**7**) in place of formyl, and one with an acrylate ester group (**8**) at C(9) in place of the *TBA* and *Meldrum*'s acid conjugates of **1** and **2** (Scheme 3). Both syntheses were accomplished from **3**: acetyl derivative **7** from a careful *Friedel-Crafts* $SnCl_4$-catalyzed reaction with acetic anhydride; acrylate derivative **8** from acid catalyzed condensation with a mixed ester-acetal. Both **7** and **8** were less fluorescent ($\phi_F \sim 0.2$–0.4 for **7** and 0.1–0.2 for

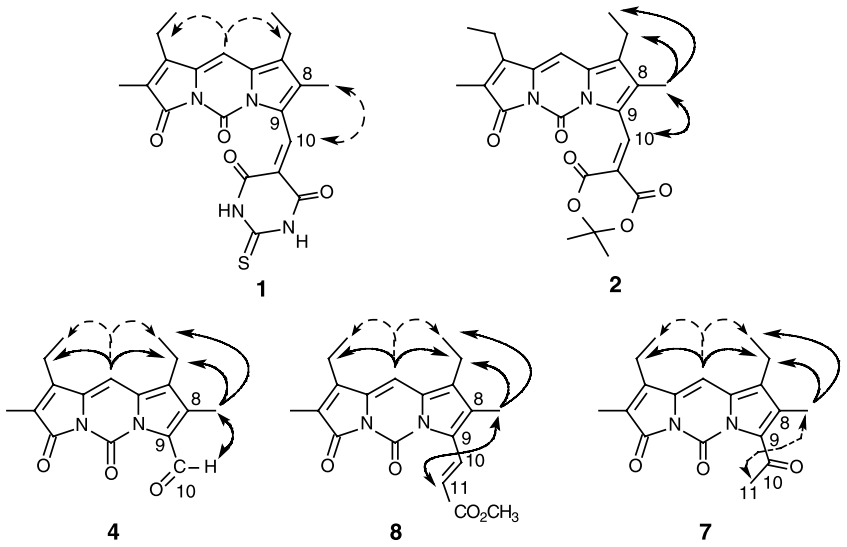
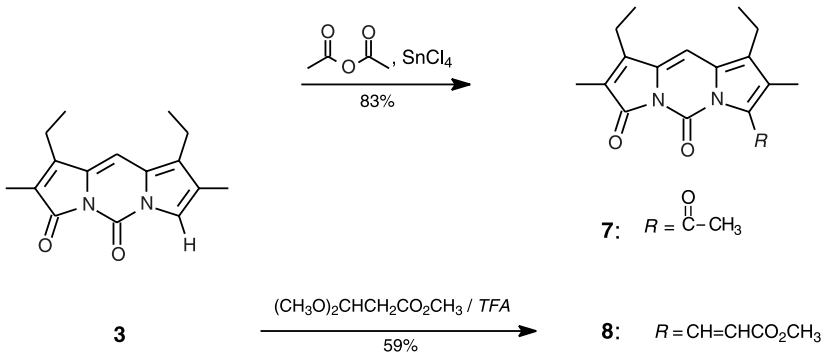


Fig. 3 N*O*Es (shown by arrows) found in $(CD_3)_2SO$ for *N*,*N*′-carbonyl-bridged dipyrrinones of this work. Weak N*O*Es are represented by dashed arrows. Since all experiments were done using identical parameters (within short elapse of time), the relative magnitudes of N*O*E obtained by irradiating C(8)–CH_3 were: **1** (almost not detectable) $\ll$**7** $<$ **4** $<$ **2** $\sim$ **8**. All N*O*E measurements were performed in $(CD_3)_2SO$ using two methods: classical, steady state, and transient N*O*E experiment



Scheme 3

8) than **3**, **5**, **6**, and **9**, but they were much more strongly fluorescent than **1**, **2**, or **4**.

We investigated the orientation of the acetyl and acrylate groups of **7** and **8** relative to the C(8)–CH_3 by means of N*O*E. In **7**, a weak N*O*E was found between the acetyl CH_3 and the C(8)–CH_3, suggesting a *syn* orientation of these groups and thus a *syn* orientation of the acetyl C=O and the pyrrole nitrogen – as in **4**. However, the N*O*E found here was weaker than that found in **4** between the aldehyde hydrogen and the C(8)–CH_3, indicating that the orientation of the acetyl group of **7** is freely rotating, a blend of *syn* and *anti*. In **8** we found a strong N*O*E between the acrylate α-H at C(11) and the C(8)–CH_3 – an indication of an *anti* configuration about the C(9)–C(10) bond.

Taken collectively, a correlation between the measured fluorescence and N*O*Es would suggest that when the exocyclic double bond at C(10) is oriented *syn* to the pyrrole nitrogen, fluorescence is reduced considerably (**1**, **2**, and **4**) from that of the parent, unsubstituted bridged dipyrrinone (**3**). Whether this reflects entirely an orientation effect of the group attached to C(9) of the dipyrrinone is unclear. One might expect differences in orientation to weigh in on the UV-Vis spectra, but a rationalization based on differences in the electronic spectra is problematic, because the nature of the functional group will, of course, affect the spectrum independent of orientation. Nonetheless, from the data of Table 2, comparing the formyl (**4**) to the acetyl (**7**) one notices hypsochromic and hypochromic shifts in λ_{max} and ε,

Table 2 Solvent dependence of the UV-Vis spectra of **1**–**9**

Compound	λ_{max}/nm (ε/dm^{3}mol^{-1}cm^{-1})				
	Cyclohexane	C_6H_6	$CHCl_3$	CH_3OH	$(CH_3)_2SO$
1	–	532 (34100) 499 (27700)^{sh} 343 (19300)	539 (32800) 507 (24500)^{sh} 342 (25100)	532 (30200) 341 (24300)	537 (31300) 345 (22300)
2	483 (19700) 318 (22100) 266 (9700)	497 (21300) 324 (22600)	502 (22200) 325 (23800) 270 (9700)	501 (23800) 324 (23200) 269 (9200)	508 (24400) 327 (21000) 270 (9400)^{sh}
3	420 (19200) 398 (19400) 268 (8900)	419 (18000) 404 (18000)	413 (17900) 319 (3200)^{sh} 272 (10100)	412 (17800) 317 (3400)^{sh} 271 (10800)	413 (17900) 317 (3200)^{sh} 269 (11400)
4	432 (24400) 406 (23900) 387 (14600)^{sh} 268 (24300)	437 (21500) 412 (21700) 392 (13400)^{sh}	439 (23200) 413 (23200) 392 (14100)^{sh} 273 (25300)	435 (22400) 411 (23300) 392 (14800)^{sh} 272 (26000)	439 (22800) 414 (23100) 394 (14100)^{sh} 273 (25100)
5	425 (19000) 399 (19500) 253 (18700)	429 (17800) 404 (18200)	428 (18700) 404 (19200) 258 (21300)	419 (17300) 402 (17100) 257 (15500)	421 (17200) 403 (17400)
6	421 (20000) 397 (20500) 252 (15100)	422 (18100) 399 (18900)	423 (18000) 401 (18800) 258 (15900)	416 (17600) 398 (18300) 257 (17000)	419 (17900) 400 (18800)
7	428 (20100) 403 (20400) 320 (2800) 263 (16800)	429 (18200) 407 (18700) 315 (2600)^{sh}	431 (18600) 408 (19300) 316 (3000)^{sh} 271 (17300)	425 (18200) 406 (18900) 316 (3200)^{sh} 270 (17800)	428 (18300) 408 (19000) 317 (3100)^{sh} 270 (18100)
8	452 (21500)^{sh} 435 (24000) 292 (23000)^{sh} 285 (23400)	466 (19500)^{sh} 443 (23700) 298 (22000) 290 (21300)^{sh}	465 (20600)^{sh} 445 (23900) 298 (24800) 253 (9900)	463 (21000)^{sh} 443 (24700) 293 (25700) 252 (9500)	467 (21100)^{sh} 445 (24600) 301 (23700) 293 (23100)^{sh}
9	421 (19600) 397 (20100) 252 (15400)	422 (17900) 400 (18700)	423 (17800) 401 (18700) 259 (16100)	417 (17800) 399 (18500) 258 (17700)	420 (17600) 400 (18500)

respectively, in **7** relative to **1**. And, one finds that the UV-Vis spectral data of **7** are closer to those of **5** (or **6** and **9**) than to **4**.

Concluding comments

In our attempts to prepare *N*,*N*′-carbonyl-bridged dipyrrinones with strong fluorescence at $\lambda_{em} > 550$ nm, we prepared such analogs from magenta-colored thiobarbituric acid and orange-colored *Meldrum*'s acid adducts **1** and **2**. Unexpectedly, they were nonfluorescent at room temperature. Their precursor, dipyrrinone 9-aldehyde **4** similarly was nonfluorescent. Yet the parent bridged dipyrrinone **3** and derivatives with carbonyl groups at C-9, such as CO_2H, CO_2CH_3, $COCH_3$, and $CO_2CH_2CH{=}CH_2$, and even the conjugate $CH{=}CHCO_2CH_3$ were all strongly fluorescent. It is unclear why **1** and **2** are nonfluorescent, and it is surprising that **4** was also. Orientation of the group attached to C(9) may play a role: the *syn* appears to be preferred in **1**, **2**, and **4**, but deexcitation of the long wavelength excited state of **4** may occur from facile motion about the C(9)–C(10) bond.

Experimental

All fluorescence spectra were measured on a Jobin Yvon Fluorolog 3 model FL 3-22 instrument by using constant spectral parameters: step resolution (increment) of 1 nm, both excitation and emission slits of 2 nm and integration time of 0.5 sec and were uncorrected. The UV-Vis spectra were recorded on a Perkin-Elmer Lambda 12 spectrophotometer. NMR spectra were acquired on a Varian Unity Plus spectrometer at 11.75 T magnetic field strength operating

at ^{1}H frequency of 500 MHz and ^{13}C frequency of 125 MHz in solutions of $CDCl_3$ (referenced at 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C) or $(CD_3)_2SO$ (referenced at 2.49 ppm for ^{1}H and 39.50 ppm for ^{13}C). *J*-modulated spin-echo (Attached Proton Test) and gHMBC experiments were used to assign the ^{13}C NMR spectra. Radial chromatography was carried out on Merck silica gel PF_{254} with $CaSO_4$ binder preparative layer grade, using a Chromatotron (Harrison Research Inc., Palo Alto, CA) with 1, 2, or 4 mm thick rotors and analytical thin-layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125 μm layer). Melting points were determined on a Mel-Temp capillary apparatus and are corrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ, and found to be within $\pm$0.3% of theoretical values.

The spectral data were obtained in spectral grade solvents (Aldrich or Fischer) which were distilled under Ar stream just prior to use. Before the distillation $CHCl_3$ was passed through a basic alumina column. Distillation of $(CH_3)_2SO$ solvent was carried out at 0.5 mm Hg vacuum collecting the solvent at 0°C and thawing it under Ar. The starting compounds: 4-ethyl-3-methyl-3-pyrrolin-2-one (**10**) [8] and ethyl 4-ethyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylate (**11**) [9] were prepared as described in the literature.

*General procedure for syntheses of conjugates **1** and **2***

A mixture of 0.50 mmol tricyclic aldehyde **4**, together with 0.55 mmol of the corresponding C–H acid component, and 5 cm^3 absolute ethanol was stirred at 60°C for 2 h [11]. After cooling and keeping at 0°C for 2 h, the precipitated crude product was collected by filtration and purified by re-precipitation from *DMF*-anhydrous diethyl ether in the case of **1** or by radial chromatography (hexane:ethyl acetate:$CHCl_3$ = 7:2:1 to 4.5:5.5:2) and recrystallization from C_2H_5OH–CH_2Cl_2 in the case of **2**.

1,9-Diethyl-2,8-dimethyl-7-[4′,6′-dioxo-2′-thioxo-(1′H,3′H,5′H)-pyrimidin-5′-ylidene]methyl-(3H,5H)-dipyrrolo[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**1**, $C_{21}H_{20}N_4O_4S$)

Yield 71%; mp 294–297°C (with decomposition); ^{1}H NMR ($CDCl_3$): δ = 1.21 (3H, t, *J* = 7.6 Hz), 1.25 (3H, t, *J* = 7.6 Hz), 2.01 (3H, s), 2.16 (3H, s), 2.60 (2H, q, *J* = 7.6 Hz), 2.63 (2H, q, *J* = 7.6 Hz), 6.51 (1H, s), 8.78 (1H, s), 8.85 (1H, br, s), 8.94 (1H, br, s) ppm; ^{13}C NMR ($CDCl_3$): δ = 8.7, 12.0, 13.6, 14.9, 17.3, 18.0, 95.0, 113.1, 121.8, 126.4, 128.7, 129.6, 136.9, 139.2, 141.3, 142.4, 146.7, 160.6, 161.6, 166.0, 175.9 ppm; ^{1}H NMR ($(CD_3)_2SO$): δ = 1.11 (3H, t, *J* = 7.6 Hz), 1.15 (3H, t, *J* = 7.6 Hz), 1.89 (3H, s), 2.00 (3H, s), 2.63 (2H, q, *J* = 7.6 Hz), 2.66 (2H, q, *J* = 7.6 Hz), 7.26 (1H, s), 8.60 (1H, s), 12.18 (1H, s), 12.37 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$): δ = 8.2, 12.0, 13.5, 14.9, 16.3, 17.2, 96.8, 114.6, 127.5, 127.9, 128.5, 134.1, 135.4, 138.4, 140.7, 141.4, 147.6, 159.2, 161.5, 166.7, 178.1 ppm.

1,9-Diethyl-2,8-dimethyl-7-[2′,2′-dimethyl-4′,6′-dioxo-1′,3′-dioxan-5′-ylidene]methyl-(3H,5H)-dipyrrolo[1,2-c:2′,1′-f]-pyrimidine-3,5-dione (**2**, $C_{23}H_{24}N_2O_6$)

Yield 74%; mp 242–244°C (with decomposition); ^{1}H NMR ($CDCl_3$): δ = 1.20 (3H, t, *J* = 7.7 Hz), 1.24 (3H, t, *J* = 7.7 Hz), 1.87 (6H, s), 1.98 (3H, s), 2.21 (3H, s), 2.58 (2H, q, *J* = 7.7 Hz), 2.61 (2H, q, *J* = 7.7 Hz), 6.50 (1H, s), 8.60 (1H, s) ppm; ^{13}C NMR ($CDCl_3$): δ = 8.6, 11.3, 13.6, 15.0, 17.3, 18.0, 27.7, 95.1, 104.7, 111.7, 127.1, 128.2, 129.0, 134.8, 136.0, 139.3, 140.3, 141.4, 146.6, 161.0, 163.4, 166.9 ppm; ^{1}H NMR ($(CD_3)_2SO$): δ = 1.10 (3H, t, *J* = 7.5 Hz), 1.15 (3H, t, *J* = 7.5 Hz), 1.77 (6H, s), 1.89 (3H, s), 2.09 (3H, s), 2.63 (2H, q, *J* = 7.5 Hz), 2.66 (2H, q, *J* = 7.5 Hz), 7.28 (1H, s), 8.65 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$): δ = 8.2, 11.6, 13.5, 14.9, 16.2, 17.2, 27.0, 96.6, 104.0, 109.9, 126.7, 127.8, 128.4, 135.0, 135.7, 138.7, 140.8, 141.2, 147.6, 160.0, 162.4, 166.6 ppm.

1,9-Diethyl-2,8-dimethyl-(3H,5H)-dipyrrolo[1,2-c:2′,1′-f]-pyrimidine-3,5-dione (**3**, $C_{16}H_{18}N_2O_2$)

To a solution of 1.22 g (5.0 mmol) dipyrrinone **13**, 4.05 g (25.0 mmol) *CDI*, and 400 cm^3 anhydrous CH_2Cl_2 was added 3.75 cm^3 (25.0 mmol) *DBU*, and the mixture was heated at reflux under nitrogen for 6 h. After cooling, the mixture was washed with 200 cm^3 2% aqueous HCl, then with H_2O (3×100 cm^3), and dried over anhydrous Na_2SO_4. After filtration and removal of the solvent under vacuum, the residue was purified by radial chromatography (eluent: 0.5–1.5% CH_3OH in CH_2Cl_2) and recrystallized from hexane-ethyl acetate to give **3**. Yield 1.24 g (92%); mp 196–197°C; ^{1}H NMR ($CDCl_3$): δ = 1.17 (3H, t, *J* = 7.6 Hz), 1.22 (3H, t, *J* = 7.6 Hz), 1.95 (3H, s), 2.09 (3H, s), 2.54 (2H, q, *J* = 7.6 Hz), 2.56 (2H, q, *J* = 7.6 Hz), 6.43 (1H, s), 7.44 (1H, s) ppm; ^{13}C NMR ($CDCl_3$): δ = 8.4, 10.3, 13.7, 15.2, 17.6, 18.0, 96.8, 117.7, 125.6, 126.1, 126.5, 128.4, 131.3, 141.6, 147.2, 167.6 ppm.

1,9-Diethyl-2,8-dimethyl-7-formyl-(3H,5H)-dipyrrolo-[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**4**, $C_{17}H_{18}N_2O_3$)

To 12 cm^3 trifluoroacetic acid (cooled to 0°C) was added 810 mg (3.0 mmol) tricyclic **3**, followed by 6 cm^3 (55 mmol) trimethyl orthoformate, and the mixture was stirred at 0°C for 1 h. It was then poured into 200 cm^3 ice-water, and the product was extracted into $CHCl_3$ (3×100 cm^3). The combined extracts were washed with H_2O (4×50 cm^3), dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum, and the residue was crystallized from ethyl acetate-hexane to give **4**. Yield 756 mg (84%); mp 214–215°C; ^{1}H NMR ($CDCl_3$): δ = 1.15 (3H, t, *J* = 7.6 Hz), 1.24 (3H, t, *J* = 7.6 Hz), 1.99 (3H, s), 2.40 (3H, s), 2.58 (2H, q, *J* = 7.6 Hz), 2.59 (2H, q, *J* = 7.6 Hz), 6.49 (1H, s), 10.87 (1H, s) ppm; ^{13}C NMR ($CDCl_3$): δ = 8.6, 11.3, 13.6, 15.0, 16.8, 18.0, 95.5, 128.0, 128.6, 130.5, 131.5, 134.3, 136.3, 142.2, 147.0, 167.1, 184.2 ppm.

3,7-Diethyl-2,8-dimethyl-(10H)-dipyrrin-1-one (**13**, $C_{15}H_{20}N_2O$)

A mixture of 4.18 g (20 mmol) ethyl 4-ethyl-5-formyl-3-methyl-1*H*-pyrrole-2-carboxylate [9], 2.50 g (20 mmol) 4-ethyl-3-methyl-1,5-dihydro-(2H)-pyrrol-2-one [8], 105 cm^3 methanol, and a solution of 16.8 g (300 mmol) KOH in 70 cm^3 H_2O was heated at vigorous reflux for 3 h. After cooling, the methanol was evaporated under vacuum, the residue was diluted with 50 cm^3 H_2O, cooled in an ice bath and slowly acidified with conc. HCl, to $pH < 3$. After further stirring for 30 min,

the product was collected by filtration, washed with H_2O ($2 \times 10\,cm^3$) and anhydrous diethyl ether ($3 \times 20\,cm^3$), dried under vacuum (P_2O_5) to give acid **12**, which was pure enough for use in the next step. Yield 4.15 g (72%); ^1H NMR ($(CD_3)_2SO$): $\delta = 1.01$ (3H, t, $J = 7.5$ Hz), 1.09 (3H, t, $J = 7.6$ Hz), 1.79 (3H, s), 2.20 (3H, s), 2.49 (2H, q, $J = 7.6$ Hz), 2.51 (2H, q, $J = 7.5$ Hz), 5.91 (1H, s), 10.53 (1H, s), 10.96 (1H, s), 12.32 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$): $\delta = 8.1$, 10.0, 14.6, 15.8, 16.9, 17.2, 95.6, 121.8, 125.2, 125.6, 126.7, 129.5, 132.7, 147.6, 162.3, 172.7 ppm. A sample of **12** was converted into its methyl ester **15** and analyzed as such (*vide infra*).

To 1.15 g (4.0 mmol) crude acid **12** was added a solution of $3\,cm^3$ conc. H_2SO_4 in $50\,cm^3$ ethanol, and the mixture was heated under nitrogen at reflux for 35 min. After cooling, it was diluted with $300\,cm^3$ $CHCl_3$ and washed consecutively with $100\,cm^3$ H_2O, $100\,cm^3$ 5% aqueous $NaHCO_3$, and H_2O ($2 \times 100\,cm^3$). The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum, the residue was purified by radial chromatography (eluent: 1–2.5% CH_3OH in CH_2Cl_2), and recrystallized from CH_3OH to give 9-H dipyrrinone **13**. Yield 713 mg (73%); mp 206–208°C; ^1H NMR ($CDCl_3$): $\delta = 1.14$ (3H, t, $J = 7.6$ Hz), 1.18 (3H, t, $J = 7.6$ Hz), 1.95 (3H, s), 2.07 (3H, s), 2.55 (2H, q, $J = 7.6$ Hz), 2.58 (2H, q, $J = 7.6$ Hz), 6.16 (1H, s), 6.83 (1H, d, $J = 2.0$ Hz), 10.43 (1H, br, s), 11.05 (1H, br, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.0$, 10.0, 14.9, 16.2, 17.8, 18.0, 101.2, 118.6, 121.6, 123.2, 123.6, 128.3, 131.0, 148.4, 174.3 ppm.

3,7-Diethyl-2,8-dimethyl-9-allyloxycarbonyl-(10H)-dipyrrin-1-one (**16**, $C_{19}H_{24}N_2O_3$)
To a solution of 2.88 g (10.0 mmol) crude acid **12** in $60\,cm^3$ anhydrous *DMF* under nitrogen was added 3.26 g (10.0 mmol) Cs_2CO_3 followed by $2.6\,cm^3$ (30.0 mmol) allyl bromide. The flask was closed tightly and stirred at 80–85°C for 18 h. After cooling, the mixture was diluted with $400\,cm^3$ $CHCl_3$ and washed with 3% aqueous HCl ($150\,cm^3$) and water ($4 \times 100\,cm^3$). After drying over anhydrous Na_2SO_4 and filtration, the solvent was evaporated under vacuum, and the residue was recrystallized from CH_3OH–CH_2Cl_2 to give allyl ester **16**. Yield 2.27 g (69%); mp 192–193°C; ^1H NMR ($CDCl_3$): $\delta = 1.11$ (3H, t, $J = 7.5$ Hz), 1.18 (3H, t, $J = 7.6$ Hz), 1.95 (3H, s), 2.31 (3H, s) 2.52 (2H, q, $J = 7.5$ Hz), 2.53 (2H, q, $J = 7.6$ Hz), 4.74 (2H, ddd, $^3J = 5.7$ Hz, $^4J = 1.4$, 1.5 Hz), 5.21 (1H, ddt, $^3J = 10.4$ Hz, $^2J = 1.2$ Hz, $^4J = 1.4$ Hz), 5.33 (1H, ddt, $^3J = 17.3$ Hz, $^2J = 1.2$ Hz, $^4J = 1.5$ Hz), 5.96 (1H, ddt, $^3J = 5.7$, 10.4, 17.3 Hz), 5.99 (1H, s), 9.68 (1H, br, s), 9.76 (1H, br, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.3$, 10.4, 14.6, 15.7, 17.5, 17.9, 64.8, 97.3, 117.9, 121.5, 127.0, 127.7, 130.3, 132.5, 134.2, 147.9, 161.1, 174.3 ppm.

7-Allyloxycarbonyl-1,9-diethyl-2,8-dimethyl-(3H,5H)-dipyrrolo[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**9**, $C_{20}H_{22}N_2O_4$)
To a solution of 3.28 g (10.0 mmol) dipyrrinone **16**, 8.11 g (50.0 mmol) *CDI*, and $750\,cm^3$ of anhydrous CH_2Cl_2 were added $7.50\,cm^3$ (50 mmol) *DBU*, and the mixture was heated at reflux under nitrogen for 14 h. After cooling, the mixture was washed with $2 \times 200\,cm^3$ 2% aqueous HCl, then with H_2O ($4 \times 100\,cm^3$), and dried over anhydrous $MgSO_4$. After filtration the solvent was removed under vacuum, the residue was purified by radial chromatography (eluent: 1–3% CH_3OH in CH_2Cl_2), and recrystallized from hexane-ethyl acetate to give tricycle **9**. Yield 3.23 g (91%); mp 127–128°C; ^1H NMR ($CDCl_3$): $\delta = 1.13$ (3H, t, $J = 7.5$ Hz), 1.20 (3H, t, $J = 7.6$ Hz), 1.94 (3H, s), 2.14 (3H, s), 2.52 (2H, q, $J = 7.5$ Hz), 2.54 (2H, q, $J = 7.6$ Hz), 4.83 (2H, ddd, $^3J = 5.8$ Hz, $^4J = 1.4$, 1.5 Hz), 5.25 (1H, ddt, $^3J = 10.4$ Hz, $^2J = 1.2$ Hz, $^4J = 1.4$ Hz), 5.38 (1H, ddt, $^3J = 17.3$ Hz, $^2J = 1.2$ Hz, $^4J = 1.5$ Hz), 6.03 (1H, ddt, $^3J = 5.8$, 10.4, 17.3 Hz), 6.41 (1H, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.4$, 9.5, 13.6, 15.0, 17.2, 17.9, 66.4, 95.9, 118.8, 122.7, 127.1, 127.6, 128.3, 130.1, 132.1, 133.2, 140.7, 146.9, 161.8, 167.2 ppm.

7-Carboxy-1,9-diethyl-2,8-dimethyl-(3H,5H)-dipyrrolo-[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**5**, $C_{17}H_{18}N_2O_4$)
Following a procedure outlined earlier [12], to a solution of 1.77 g (5.00 mmol) allyl ester **9** in $20\,cm^3$ anhydrous acetonitrile under argon was added 290 mg (0.25 mmol) tetrakis-(triphenylphosphine)palladium(0) and 130 mg (0.50 mmol) triphenylphosphine, followed by a solution of $0.46\,cm^3$ (5.50 mmol) pyrrolidine in $5\,cm^3$ acetonitrile, and the mixture was stirred at ambient temperature for 6 h. It was diluted with $400\,cm^3$ CH_2Cl_2 and washed with $100\,cm^3$ 1% HCl and H_2O ($3 \times 100\,cm^3$). Then the organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum, the residue was purified by radial chromatography (eluent: 2.5–5.0% CH_3OH in CH_2Cl_2), and recrystallized from ethyl acetate-CH_2Cl_2 to give acid **5**. Yield 1.52 g (97%); mp 180–181°C (with decomposition); ^1H NMR ($CDCl_3$): $\delta = 1.16$ (3H, t, $J = 7.7$ Hz), 1.26 (3H, t, $J = 7.7$ Hz), 2.02 (3H, s), 2.47 (3H, s), 2.61 (2H, q, $J = 7.7$ Hz), 2.62 (2H, q, $J = 7.7$ Hz), 6.58 (1H, s), 14.05 (1H, br, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.7$, 12.6, 13.5, 14.9, 17.1, 18.1, 96.4, 123.4, 128.7, 128.9, 130.5, 133.1, 141.9, 146.1, 147.9, 159.4, 166.3 ppm.

3,7-Diethyl-2,8-dimethyl-9-methoxycarbonyl-(10H)-dipyrrin-1-one (**15**, $C_{17}H_{22}N_2O_3$)
Following the same procedure for synthesis of **16** and using methyl iodide, 10 mmol of acid **12** were converted into methyl ester **15**. Yield 78%; mp 231–232°C; ^1H NMR ($CDCl_3$): $\delta = 1.11$ (3H, t, $J = 7.5$ Hz); 1.18 (3H, t, $J = 7.6$ Hz), 1.95 (3H, s), 2.30 (3H, s), 2.52 (2H, q, $J = 7.5$ Hz), 2.53 (2H, q, $J = 7.6$ Hz), 3.83 (3H, s) 5.99 (1H, s), 9.37 (1H, br, s), 9.48 (1H, br, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.3$, 10.2, 14.6, 15.7, 17.5, 17.9, 51.2, 97.3, 121.5, 127.1, 127.2, 127.6, 130.3, 134.3, 148.0, 161.7, 174.1 ppm.

1,9-Diethyl-2,8-dimethyl-7-methoxycarbonyl-(3H,5H)-dipyrrolo[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**6**, $C_{18}H_{20}N_2O_4$)
Following the same procedure as above for **9**, 7 mmol of **15** were converted into tricycle **6**. Yield 92%; mp 176–177°C; ^1H NMR ($CDCl_3$): $\delta = 1.16$ (3H, t, $J = 7.6$ Hz), 1.22 (3H, t, $J = 7.6$ Hz), 1.97 (3H, s), 2.16 (3H, s), 2.55 (2H, q, $J = 7.6$ Hz), 2.56 (2H, q, $J = 7.6$ Hz), 3.93 (3H, s), 6.42 (1H,

s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.5$, 9.5, 13.7, 15.1, 17.3, 18.0, 52.6, 95.9, 122.8, 127.1, 127.8, 128.3, 130.1, 133.2, 140.9, 146.8, 162.7, 167.3 ppm.

1,9-Diethyl-2,8-dimethyl-7-(methoxycarbonyl-methylidene)-methyl-(3H,5H)-dipyrrolo[1,2-c:2′,1′-f]-pyrimidine-3,5-dione (**8**, $C_{20}H_{22}N_2O_4$)

To a solution of 270 mg (1.0 mmol) tricycle **3** in 3 cm^3 trifluoracetic acid kept under argon, were added 0.57 cm^3 (4.0 mmol) methyl 3,3-dimethoxypropionate and after stirring for 5 min another portion of the same size was added. After stirring an additional 15 min, the mixture was poured into a well-stirred mixture of 100 cm^3 ice-cold water and 100 cm^3 $CHCl_3$. The organic layer was washed with 100 cm^3 5% aqueous $NaHCO_3$, and H_2O (3×50 cm^3), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under vacuum, the residue was purified by radial chromatography (eluent: 1–3% CH_3OH in CH_2Cl_2 and recrystallized from ethyl acetate-hexane to give acrylate **8**. Yield 209 mg (59%); mp 171–172°C. ^{1}H NMR ($CDCl_3$): $\delta = 1.16$ (3H, t, $J = 7.6$ Hz), 1.23 (3H, t, $J = 7.6$ Hz), 1.97 (3H, s), 2.22 (3H, s), 2.56 (2H, q, $J = 7.6$ Hz), 2.58 (2H, q, $J = 7.6$ Hz), 3.78 (3H, s), 6.10 (1H, d, $J = 16.2$ Hz), 6.43 (1H, s), 8.77 (1H, d, $J = 16.2$ Hz) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.5$, 11.8, 13.7, 15.2, 17.2, 17.9, 51.6. 95.9, 118.6, 127.5, 128.6, 128.9, 129.3, 129.5, 132.6, 134.3, 142.8, 146.5, 167.3, 167.4 ppm.

3,7-Diethyl-2,8-dimethyl-9-formyl-(10H)-dipyrrin-1-one (**14**, $C_{16}H_{20}N_2O_2$)

To 8 cm^3 trifluoroacetic acid, kept under argon was added 577 mg (2.0 mmol) acid **12**, and the mixture was heated to 60°C followed by slow cooling to ~35°C. The heating-cooling cycle was repeated three times during one hour. Finally, after cooling to room temperature, 3 cm^3 (28 mmol) trimethyl orthoformate were added at once, and the mixture was stirred for 5 min. Then it was poured into a well-stirred mixture of 50 cm^3 ice-water and 50 cm^3 $CHCl_3$. After dilution with 250 cm^3 $CHCl_3$, the organic layer was washed with H_2O (4×100 cm^3), dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum; the residue was purified by radial chromatography (eluent: 1–3% CH_3OH in CH_2Cl_2), then recrystallized from CH_3OH in CH_2Cl_2 to give aldehyde **14**. Yield 465 mg (85%); mp 255–257°C; ^{1}H NMR ($CDCl_3$): $\delta = 1.13$ (3H, t, $J = 7.6$ Hz), 1.20 (3H, t, $J = 7.6$ Hz), 2.00 (3H, s), 2.34 (3H, s), 2.55 (2H, q, $J = 7.6$ Hz), 2.56 (2H, q, $J = 7.6$ Hz), 5.98 (1H, s), 9.74 (1H, s), 10.76 (1H, br, s), 10.95 (1H, br, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.4$, 8.8, 14.6, 15.6, 17.3, 17.9, 95.7, 127.5, 130.9, 131.4, 131.9, 132.5, 135.4, 147.8, 174.2, 177.4 ppm.

7-Acetyl-1,9-diethyl-2,8-dimethyl-(3H,5H)-dipyrrolo-[1,2-c:2′,1′-f]pyrimidine-3,5-dione (**7**, $C_{18}H_{20}N_2O_3$)

To a solution of 135 mg (0.5 mmol) tricycle **3** in 30 cm^3 of CH_2Cl_2, kept under argon at 0°C were added 4.8 cm^3 (50 mmol) acetic anhydride followed by 2.0 cm^3 (18 mmol) tin(IV) chloride. After stirring at 0°C for 1.5 h, the mixture was poured into a vigorously-stirred mixture of 100 cm^3 ice-cold 5% aqueous HCl and 100 cm^3 CH_2Cl_2. The organic layer was washed with H_2O (4×50 cm^3), dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under vacuum, the residue was purified by radial chromatography (eluent: 0.5–1.5% CH_3OH in CH_2Cl_2), and recrystallized from ethyl acetate-hexane to give acetyl derivative **7**. Yield 129 mg (83%); mp 171–172°C; ^{1}H NMR ($CDCl_3$): $\delta = 1.15$ (3H, t, $J = 7.6$ Hz), 1.23 (3H, t, $J = 7.7$ Hz), 1.97 (3H, s), 2.10 (3H, s), 2.50 (3H, s), 2.55 (2H, q, $J = 7.6$ Hz), 2.56 (2H, q, $J = 7.7$ Hz), 6.45 (1H, s) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 8.5$, 9.6, 13.6, 15.0, 17.3, 18.0, 31.7, 96.2, 127.8, 127.88, 127.90, 129.9, 131.7, 133.2, 141.7, 146.9, 167.2, 194.1 ppm.

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