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Synthesis, properties, and hepatic metabolism of strongly fluorescent fluorodipyrrinones

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Abstract—From non-fluorescent 8-*H* fluorophenyldipyrrinones, highly fluorescent (ϕ_F 0.4–0.6) analogs have been synthesized by reaction with 1,1′-carbonyldiimidazole to bridge the dipyrrinone nitrogens and form an *N*,*N*′-carbonyldipyrrinone (3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]-pyrimidine-3,5-dione). Amphiphilic, water-soluble 8-sulfonic acid derivatives are then obtained by reaction with concd H_2SO_4. The resulting fluorinated and sulfonated *N*,*N*′-carbonyl-bridged dipyrrinones, isolated as their sodium salts, are potential cholephilic fluorescence and ^{19}F MRI imaging agents for use in probing liver and biliary metabolism. After intravenous injection in the rat they were excreted rapidly and largely unchanged in bile. ^{19}F NMR spectroscopy of a pentafluorophenyl-tosylpyrrolinone synthetic precursor exhibited rarely seen diastereotopicity.

1. Introduction

The natural pigment bilirubin (Fig. 1A) is produced continuously in animals during normal catabolism of heme.[1] It is a bichromophoric structure comprised of two *Z*-dipyrrinones bearing intramolecularly hydrogen-bonded propionic acid side-chains.[2,3] It is lipophilic and has low solubility in water at physiologic pH. In humans accumulation of bilirubin is thwarted by its efficient clearance from the blood by the liver and elimination in bile as ester glucuronide conjugates of the propionic acid side-chains.[1,4] Formation of the glucuronides is catalyzed by a specific glucuronosyl transferase enzyme (UGT1A1)[5] and their excretion from the liver is mediated by a membrane transporter known as MRP2 (multidrug resistance associated protein 2).[6] These two proteins are important in the metabolism and hepatic elimination of a large number of xenobiotics in addition to bilirubin. Genetic deficiencies of either UGT1A1 or MRP2 and a variety of liver disorders can cause accumulation of bilirubin or its glucuronides and clinical jaundice.[5,6] In the absence of UGT1A1, elimination of bilirubin is almost totally impaired. If the concentration of unconjugated bilirubin in the circulation exceeds the binding capacity of serum albumin, movement of the pigment across the blood–brain barrier and deposition within the brain can cause toxicity.[1] In contrast to bilirubin, xanthobilirubic acid (Fig. 1B),[7] a polar but water-insoluble synthetic dipyrrinone analog for one-half of bilirubin, is readily excreted intact in bile without the need for glucuronidation.[8] Thus, bilirubin and its analogs are useful probes for hepatobiliary disfunction and mechanisms of glucuronidation and biliary excretion.

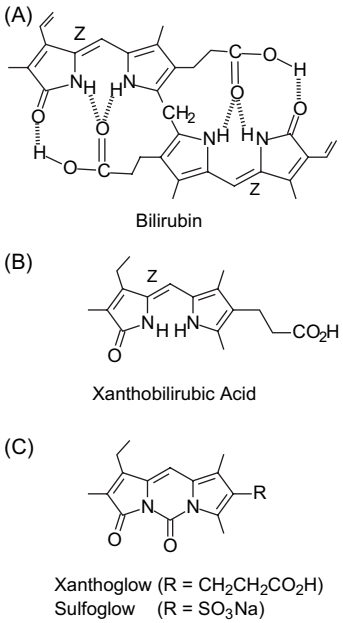

Figure 1. (A) Bilirubin. (B) Xanthobilirubic acid, a dipyrrinone model for bilirubin. (C) Xanthoglow, a highly fluorescent (ϕ_F 0.80, cyclohexane) *N*,*N*′-carbonyl-bridged analog of xanthobilirubic acid and sulfoglow, a water-soluble analog.

Keywords: Dipyrroles; Perfluorophenyl; ^{19}F NMR; Fluorescence.
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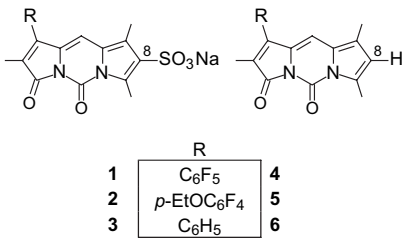
Though brightly colored, bilirubin and xanthobilirubic acid are essentially non-fluorescent at room temperature, e.g., with fluorescence quantum yields of $\phi_F<10^{-3}$ because of rapid $Z\rightarrow E$ isomerization in the excited states.[2,3,9] Restricting $Z\rightarrow E$ isomerization by bridging the two nitrogens of a dipyrrinone causes a dramatic increase in fluorescence (to $\phi_F\sim1$). Recently we found that a carbonyl group is a convenient bridge, easily inserted by reaction of the dipyrrinone with 1,1′-carbonyldiimidazole (CDI) in the presence of strong base (such as DBU) in CH_2Cl_2, and is very effective in enhancing fluorescence.[10] Thus, xanthobilirubic acid methyl ester was readily converted to xanthoglow (Fig. 1C) methyl ester, with $\phi_F=0.80$ in cyclohexane at 25 °C for λ_{exc} 410 nm and λ_{em} 473 nm.[10]

Seeking a water-soluble xanthoglow analog, we prepared the first 'sulfoglow' (Fig. 1C),[10a] a xanthoglow analog with the C(8) propionic acid replaced by sodium sulfonate. When injected intravenously into rats it was rapidly excreted intact into bile and urine, which became highly fluorescent.[11] These preliminary studies suggested that it might be possible to develop highly fluorescent cholephilic ('bile-loving') analogs useful for fluorescence imaging of renal and hepatic metabolism or for early detection of cholestatic liver disease. Were they to contain fluorine, such agents might also be useful for ^{19}F-magnetic resonance imaging. To this end we set out to synthesize cholephilic highly fluorinated sulfoglows ('fluoroglows'). From our experience and perspective, a logical target was pentafluoroglow **1**. In the course of this study, we learned, however, that its synthesis was compromised in the presence of nucleophilic base (ethoxide) in favor of *p*-ethoxytetrafluoroglow (**2**) and regioisomers. We also present the synthesis of pure **2** here because it provides a key intermediate for synthesizing *p*-alkoxy analogs of **2** of differing lipophilicity and hence regulating hepatic versus renal excreability.[11] In the following, we describe the syntheses and spectroscopic properties of new fluorinated, fluorescent dipyrrinones **4–6** and the sulfoglow sodium salts **1** and **2**, with pentafluoro- and tetrafluorophenyl groups on the lactam ring. We also present preliminary studies on the metabolism and hepatobiliary excretion of **1** and **2**. This work is part of a more comprehensive investigation of cholephilic fluorescent pharmacophores for understanding transhepatic uptake and transport and cholestatic disorders, particularly in the newborn.[11]

Target Compounds



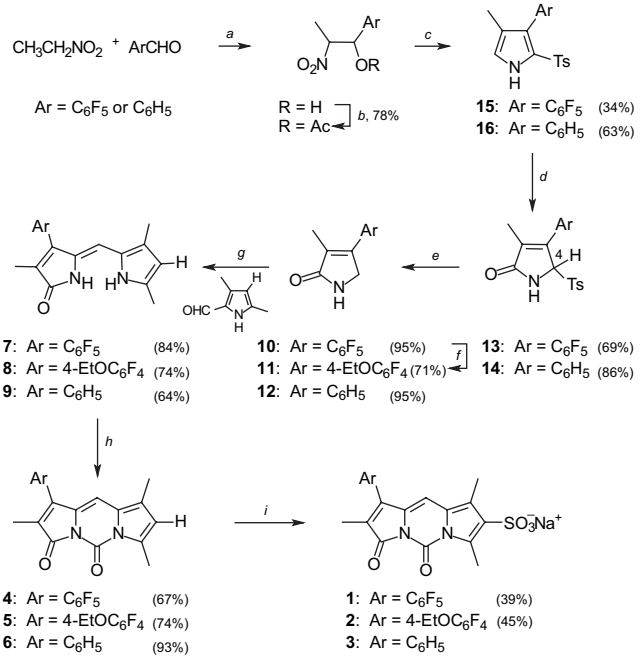
	R	
1	C_6F_5	4
2	*p*-$EtOC_6F_4$	5
3	C_6H_5	6

2. Results and discussion

2.1. Syntheses

The syntheses of **4** and **5** employed a Barton–Zard pyrrole-forming reaction sequence explored earlier with benzaldehyde.[12] Commercial pentafluorobenzaldehyde was condensed with nitroethane in the presence of DBU (Scheme 1), a Henry reaction that afforded an alcohol that was acetylated and used without purification in a Barton–Zard reaction with *p*-toluenemethylisocyanide (TosMIC) to give **15**. Replacing nitroacetate in Scheme 1 with a nitroalkene from a Knoevenagel reaction between pentafluorobenzaldehyde and nitroethane using ammonium acetate[13] might also have provided a suitable precursor to pyrrole **15**; however, this nitroalkene synthesis was unsuccessful. α-Bromination followed by treatment with TFA/H_2O is a typical way[12,14] of transforming an α-free pyrrole into a pyrrolinone (e.g., **13**), and this route was successfully applied in transforming **16** into **14**. The same approach was found earlier by our group to give unsatisfactory results with **15**. Therefore, direct oxidation of **15** to **13** using hydrogen peroxide was examined. This reaction was optimized over many runs, particularly by changing the contact time from 12 to 30 h. The yield seemed dependent also on the quality of the peroxide; an aged (lower percent peroxide?) reagent gave the best results. Chromatographic purification was necessary to obtain a 69% yield of **13**. Quantitative removal of the tosyl group to yield **10** was achieved using sodium borohydride in ethanol.[14]

Base-catalyzed condensation of **10** with 3,5-dimethyl-2-formyl-1*H*-pyrrole[15] should have led directly to **7**, but replacement of aromatic ring fluorine also occurred. Although substitution of the *para*-fluorine might have been expected, we observed a lack of regioselective replacement during test condensation reactions of pyrrolinone **10**. With a 2-fold excess of aldehyde versus pyrrolinone **10**, freshly prepared sodium methoxide or ethoxide, and a reaction time of 20 h at reflux, the yield of isolated yellow pigment from methanol was lower than from higher boiling ethanol, from which ~58% of a yellow pigment was obtained after



Scheme 1. Reagents and conditions: (a) DBU; (b) Ac_2O, DMAP; (c) TosMIC, TMG; (d) H_2O_2 or i: $PhNMe_3^+Br_3^-$, ii: TFA, H_2O; (e) $NaBH_4$; (f) NaOEt; (g) *N*-methylmorpholine; (h) CDI, DBU; (i) concd H_2SO_4, then Na_2CO_3.

chromatography and crystallization. Although the pigment from each solvent was crystalline and homogeneous by TLC, ^{1}H NMR spectroscopy revealed only ~80–85% *p*-alkoxy regioisomeric purity. Recrystallizations, even with ~50% loss, improved the purity of the ethoxy pigment **8** to only ~90%. ^{1}H NMR spectroscopy, in particular the C(5)-methine signal, was indicative of the presence of three different dipyrrinones, most likely due to *ortho*- as well as *para*-substitution and *ortho*, *para*-disubstitution of fluorine by ethoxy. The ^{13}C NMR spectral lines of the minor isomeric impurities, heavily split by the fluorines, were very weakly intense and were thus of little use in analyzing different substitution patterns. But ^{19}F NMR spectroscopy, with its wide dispersion range, revealed unequivocally the presence of the mono *ortho*-substituted analog of **8**, in addition to *para*.

The pentafluorophenyl ring is known to be susceptible to aromatic nucleophilic substitution, and so we considered pentafluorobenzaldehyde[16] reactivity with nucleophiles to be a model for that of a pentafluorophenyl β-substituent of a dipyrrinone, where in both cases the carbon attached to the fluorinated benzene ring is sp^2 hybridized. Typical fluorine-replacing nucleophilic aromatic substitution reactions including the reaction of pentafluorobenzaldehyde with (i) sodium hydrosulfide in DMF and (ii) sodium thiophenolate in CH_3OH yielded predominantly *para*-thiophenol and *para*-phenylthioether, respectively.[17] A different *ortho*–*para* selectivity was found in the reaction of dimethylamine with pentafluorobenzaldehyde in diethyl ether at room temperature for 1 h: the *ortho*-amino isomer dominated over the *para* by a 3:2 ratio.[17] No clear-cut procedure has been reported for the reaction of pentafluorobenzaldehyde with sodium methoxide in methanol; however, the *para*-methoxy derivative has been isolated in low yield after careful distillation.[17] Similarly, methoxide ion replaced predominantly the *para*-fluorine in bromopentafluorobenzene after reflux in methanol for 8 h and isolation in 70% yield by preparative gas-chromatography.[16] Recent papers discussed the regioselectivity of nucleophilic replacement (including by ethoxide) in di-, tri-, tetra-, and pentafluoropyridines.[18] On the basis of the available literature and the preliminary work described above, we deemed it advantageous to find conditions for regioselective replacement of *para*-fluorine only.

Instead of synthesizing the dipyrrinone while simultaneously and non-selectively replacing fluorine with an ethoxy group, regioselective *para*-fluorine substitution was achieved in an earlier step, under milder conditions. A significant improvement in regioselectivity was achieved when the pyrrolinone **10** was treated at sub-ambient temperature with a freshly prepared solution of sodium ethoxide in ethanol. Optimized parameters included the exact amount of ethoxide and the duration of contact, with best results being obtained from 5 equiv of sodium ethoxide and a 1-h reaction time at ~10 °C to give a 71% yield of **11** from **10**. In light of recent literature data, the reaction might be successful even at a lower temperature. After optimization, similar conditions were also found to give good regioselectivity for *para*-substitution relative to *ortho* in recently published work.[18]

With *p*-ethoxypyrrolinone **11** now isolated in pure form, we examined ways to carry it forward to dipyrrinone **8** while avoiding strongly nucleophilic ethoxide/ethanol (or sodium hydroxide/ethanol) because we suspected that a high reaction temperature and a nucleophilic base[16–18] would facilitate further substitution of (*ortho*) fluorines. Thus, condensation using a non-nucleophilic base in a non-nucleophilic solvent was sought. With a strong non-nucleophilic base (DBU) in DMF or CH_3CN solvent at 85–90 °C, condensation of **11** with 2-formyl-3,5-dimethylpyrrole (Scheme 1) led mostly to decomposition products and no formation of dipyrrinone. Hünig's base (*N*,*N*-diisopropylethylamine) in CH_3CN led to no reaction, and the strong dibasic amine, 1,4-diazabicyclo[2.2.2]octane (DABCO) in CH_3CN at 90 °C resulted mostly in decomposition products. However, with *N*-methylmorpholine in dimethylformamide at 90 °C, a promising 31% yield of dipyrrinone was obtained. With a change of solvent to CH_3CN and increased reaction time to 84 h, **11** was converted smoothly into dipyrrinone **8** in 74% yield.

With the proper conditions for dipyrrinone formation from a fluorine-containing pyrrolinone having been found, they were successfully employed on the pentafluorophenyl substituted starting material (**10**). Using pure pyrrolinone **10** and 3 equiv of 3,5-dimethylpyrrole-2-aldehyde in presence of *N*-methylmorpholine in CH_3CN at 95 °C for 84 h, the desired dipyrrinone **7** bearing a C(3)-pentafluorophenyl group was isolated in 84% yield (which was even higher than that obtained with *p*-ethoxy analog **8**). The solubility of **7** in chloroform (or dichloromethane) is very low, however, in comparison with **8**.

Although the yield at the condensation step was higher for **7** than **8**, its conversion to the *N*,*N*′-carbonyl bridged fluorophore **4** was not as clean and high-yielding as is usually observed.[10,11] Only 67% of purified tricycle **4** was isolated from a reaction, which usually gives >90% of product. The starting material **7** was almost completely consumed, and a significant amount of rather polar side product was isolated. Its structure was deduced from ^{1}H and ^{13}C NMR spectra to be the analog of **5** with a 1-imidazolyl ring replacing ethoxy at the *para*-position. Thus, nucleophilic substitution of the *para*-fluorine cannot be completely suppressed even under the mild conditions used. The adventitious imidazole nucleophile apparently is released from CDI after the carbonyl transfer reaction that forms the bridged dipyrrinone.

For comparison of spectroscopic data, the non-fluorinated parent of **7** was prepared from the known pyrrolinone **12**.[12] Here, too, condensation with 3,5-dimethylpyrrole-2-aldehyde was conducted using *N*-methylmorpholine in CH_3CN at 95 °C for 48 h to give the then unknown C(3)-phenyl-substituted dipyrrinone (**9**) in 64% yield. The typical reaction conditions (KOH/EtOH) gave unacceptable results: a mixture of two products in low yield. Cyclization of **9**, unlike **7** or **8**, smoothly afforded tricyclic **6** in 93% yield.

Insertion of the carbonyl bridge by reacting **8** with CDI and DBU was sluggish and afforded the *N*,*N*′-carbonyl-bridged dipyrrinone **5**, which was isolated in moderate 74% yield. A similar slow carbonyl insertion and comparable product yield (67%) were found in the conversion of **7** to **4**. Sulfonation of **5** using concd H_2SO_4 at 0 °C, followed by an alkaline quench, gave sodium sulfonate **2**, which seemed to be less soluble in water than its C(3)-*n*-decyl analog

synthesized earlier.[11] After quenching the sulfonation reaction, the organic material separated as a solid from both aqueous and organic (chloroform) phases. More complete extraction into chloroform was achieved by addition of methanol. The crude product was purified by radial chromatography to afford a 45% yield of the yellow sulfonate salt. Its purity was determined by ^{1}H NMR to be >90%. Sulfonation of **4** (to **1**) was found to be more sluggish, and under the reaction conditions of **5**→**2**, no product (**1**) was found. At 25 °C for 4 h, however, **4** was transformed to **1** in 39% yield. In contrast to these two reactions, attempted conversion of **6** to **3** resulted in products that were not extractable, presumably due to multiple sulfonation, of the phenyl ring and other sites.

2.2. Constitutional structures

The constitutional structures of **1**, **2**, **4**–**16** follow from the method of synthesis and their ^{1}H and ^{13}C NMR spectra. Pyrrolinone **12** was reported previously.[12] The ^{19}F NMR spectra of **1**, **2**, **4**, **5**, **7**, **8**, **10**, **11**, **13**, and **15** were also obtained and correlated with the assigned structures. The ^{19}F NMR spectrum of **13** differed in significant ways from the other nine. In particular, whereas **10** showed three ^{19}F NMR signals with second order ^{19}F{^{19}F} couplings (−139.51 ppm, *ortho*; −152.89 ppm, *para*; −161.51 ppm, *meta*), **13** exhibited two sharp and two very broad signals at 298 K (Fig. 2). The unexpected appearance of four chemical shifts at ambient temperature was clarified by lowering the temperature to 223 K where five chemical shifts were found, with the two most downfield signals showing fine structure. From this apparently more complex spectrum, it became clear that a dynamic process occurs in **13**. Although the same process might occur in the five other pentafluorophenyl substituted compounds (**1**, **4**, **7**, **10**, and **15**), it was not detected by NMR. What makes **13** unique is the presence of a stereogenic center at C(4) bearing the tosyl group. Stereogenic centers are absent in **1**, **4**, **7**, **10**, and **15**—as well as in **2**, **5**, **8**, and **11** (the *p*-ethoxy analog of **13** was not prepared). The stereogenic center in **13**, combined with the axis of rotation defined by C(3)–C(*ipso*) bond (an axis of chirality), renders the *ortho*- and *meta*-fluorines diastereotopic. They lie in different electronic environments in the preferred conformation of **13**, and ^{19}F NMR is sufficiently sensitive to detect different chemical shifts even at room temperature. Rotation around the C(3)–C(*ipso*) bond is rather uninhibited at 298 K, and only the *ortho*-fluorines, being closest to the stereogenic center, are anisochronous. At lower temperature, the hindered rotation extends the diastereotopicity to meta-fluorines as well (at −160.57 ppm and −160.75 ppm). In principle, the phenyl *ortho* and *meta* hydrogens, as well as those from the tosyl group of **14** are diastereotopic, but the ^{1}H NMR spectrum was too complicated to resolve due to overlapping signals.

The coalescence temperature (T_c) of *ortho*- and *ortho*′-^{19}F NMR signals of **13** was 313 K in $CDCl_3$. The first order rate constant (k_c) of the exchange process (i.e., the rotation rate) at this temperature is given by: $k_c = \pi \times \Delta\nu/(2)^{1/2}$ where $\Delta\nu$ is the signal separation in hertz at stop-exchange regime.[19] From the low temperature experiments $\Delta\nu$=841 Hz, which gives a rate k_c=1870 s^{-1}. Dynamic ^{19}F NMR also allows for calculation of the activation barrier to rotation[19] at coalescence according to: $\Delta G^{\ddagger}=RT_c[22.96+\ln(T_c/\Delta\nu)]$. Substituting T_c and $\Delta\nu$ gives a barrier $\Delta G^{\ddagger}$=13.7 kcal mol^{-1}, which indicates only a moderately hindered atropisomerization process.

Typically, when an AB-spin system is envisioned, methylene protons in the neighborhood of an element of chirality come first to mind. However, ^{19}F NMR, with intrinsically high receptivity of fluorine nuclei, has been used for stereochemical purposes and for testing basic NMR concepts chronologically in parallel to ^{1}H NMR. Yet, despite the enormous quantity of ^{19}F NMR spectral data accumulated,[20,21] including material clearly related to stereochemistry and conformational analysis,[19,22] there are few clear-cut examples of AB fluorine spectra.

The closest literature examples of diastereotopic fluorines in a CF_2 group are in perfluorinated compounds. Variable temperature ^{19}F NMR has been reported for 1-perfluorohexyl-1-phenylethanol,[23] which has one stereogenic center and five pairs of prochiral fluorines at increasing distance. At 353 K only the two nearest CF_2 show diastereotopicity and at 193 K only the central in the chain CF_2 continues to show a singlet with small satellites as a sign of nonequivalence. The structure of perfluoro 4-ethyl-3,4-dimethylhexan-3-yl carbanion has also been studied by dynamic ^{19}F NMR.[24] At 298 K only the two CF_2 groups furthest from the carbanionic center exhibit diastereotopicity (ABX_3 spin system). The CF_2 closest to the negative charge shows anisochronous fluorines below 193 K, and this was interpreted as an indication of frozen rotation around the relevant bonds at the carbanionic center. The only AB fluorine spin system from an intentionally labeled CF_2 was found in a difluoromethionine incorporated in three different sites in a recombinant protein.[25] The degree of chemical shift difference is small when the amino acid is at relatively free surface positions, but the anisochronicity is enhanced for a methionine incorporated in tightly packed protein core where there is less conformational freedom. Variable temperature ^{19}F NMR and ^{19}F{^{19}F} COSY experiments of free difluoromethionine have been also reported.[25]

Although difficult to perform at sub-ambient temperatures, a ^{19}F{^{19}F} COSY spectrum was acquired on a sample of pyrrolinone **13** in $CDCl_3$ at 223 K (Fig. 2). The experiment unequivocally confirmed the assignments made earlier on the ground of substituent chemical shift increments. At low temperature the two downfield signals with chemical shifts at −136.92 ppm and −138.71 ppm correlated with the most shielded fluorine nuclei at −160.57 ppm and −160.75 ppm. With scale expansion it becomes evident that the signal at −136.92 ppm correlates with that at −160.57 ppm, and the downfield signal at −138.71 ppm correlates with that at −160.75 ppm. Both upfield signals (−160.75 ppm and −160.57 ppm) correlate with the signal at −150.28 ppm but the latter did not show any other correlations. This means that the latter signal belongs to the fluorine nucleus attached to the *para*-position, which does not show long range (^4J) coupling to the *ortho*-fluorines. Such coupling (^4J=2.9 Hz), however, was detected at 323 K in the 1D high resolution ^{19}F NMR spectrum (Fig. 2A, upper middle trace). From the assignment of the *para*-fluorine signal, it follows that the signals at −160.75 ppm

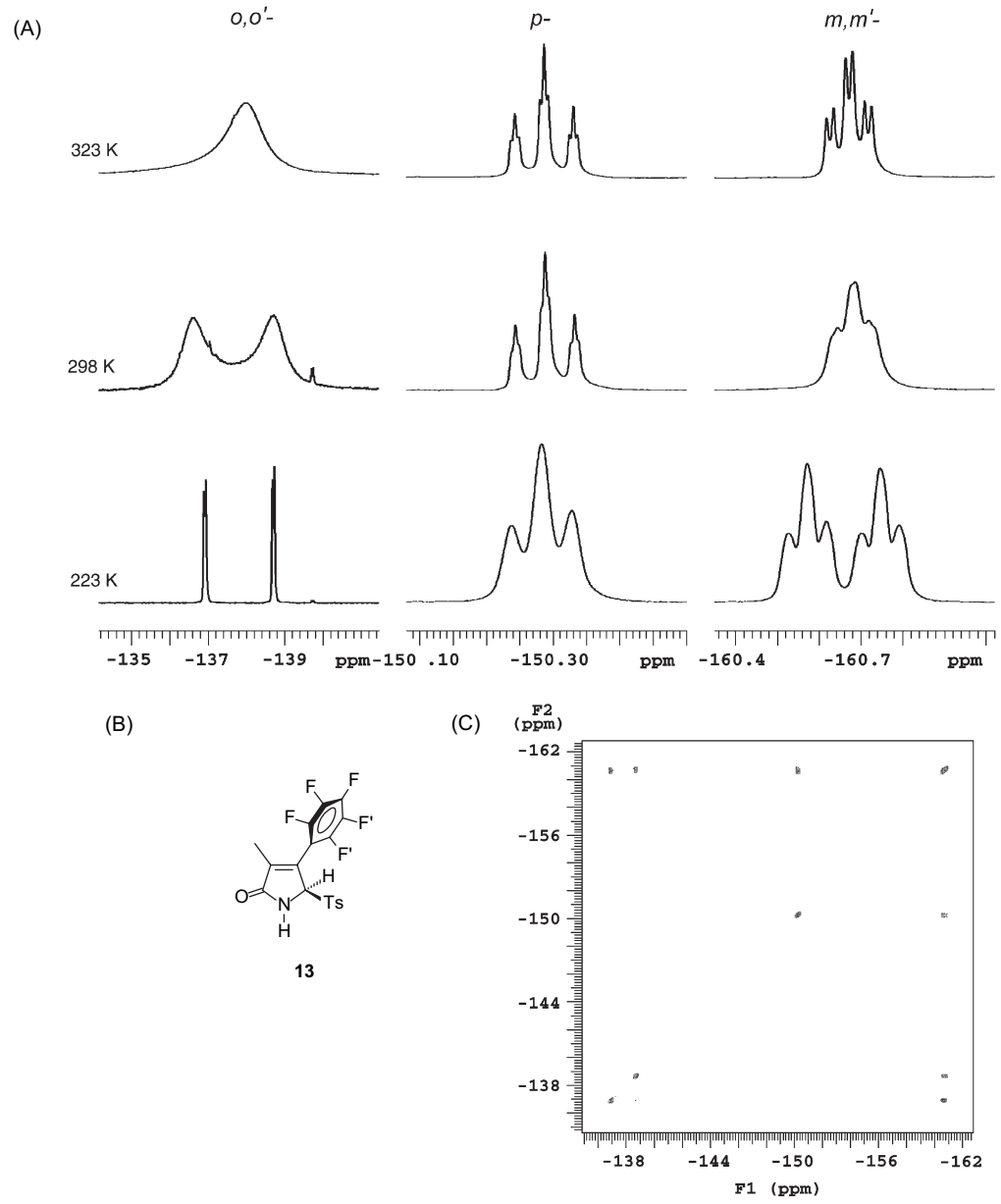


Figure 2. (A) Expanded partial ^{19}F NMR spectra of tosylpyrrolinone **13** in $CDCl_3$ at 323 K (upper traces), at 298 K (middle traces), and at 223 K (lower traces), referenced to external C_6F_6 in $CDCl_3$ at −162.90 ppm. (B) Stereochemical drawing of **13**. (C) Full $^{19}F\{^{19}F\}$ COSY spectrum of **13** in $CDCl_3$ at 223 K.

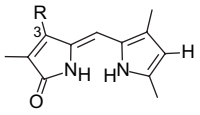
and −160.57 ppm are from *m*,*m*′-fluorines and those at −138.71 ppm and −136.92 ppm from *o*,*o*′-fluorines. In other words, only the most shielded signals (those assigned to *m*,*m*′-fluorines) exhibit a full complement of off-diagonal peaks, all of which are the result of spin–spin coupling via three bonds.

The structures of **4**–**6** also followed from the method of synthesis and from ^{13}C NMR spectra that were characteristic of dipyrrinones (**7**–**9**)[7,11] and *N*,*N*′-carbonyl-bridged dipyrrinones (**4**–**6**),[10,11] with the latter showing a urea-type carbonyl at ∼143 ppm. In the ^1H NMR, **7**–**9** exhibited more shielded C(5)-hydrogens compared to their C(3)-alkyl counterparts. Thus, C(3)-phenyl **9** showed this proton at 6.06 ppm, the pentafluorophenyl **7** at 5.78 ppm, and its *p*-ethoxy analog **8** at 5.82 ppm versus the normally encountered chemical shift, e.g., 6.13 ppm for the C(3)-*n*-decyl substituted dipyrrinone analog.[11] The shieldings in **7**–**9** are due to the influence of the aromatic π-system attached at C(3) of the dipyrrinone, because the aromatic ring faces the C(5)-hydrogen, as supported by molecular mechanics conformational analysis using PC Model.[26] PC Model revealed two isoenergetic minima when the aromatic ring of **7** was allowed to rotate with respect to the lactam, with dihedral angles C(2)–C(3)–C(*ipso*)–C(*ortho*-upper)=+65° and −65°.

2.3. ^1H NMR chemical shifts and hydrogen-bonded dimers

The dipyrrinone NH ^1H NMR chemical shifts in $CDCl_3$ reveal much about the extensively studied monomer⇆dimer equilibrium.[27] Comparison of dipyrrinone pyrrole and lactam NH ^1H NMR chemical shifts in **7**–**9** and in the

Table 1. Comparison of dipyrrinone ^{1}H NMR NH chemical shifts[a] for **7–9** and their C(3)-*n*-decyl analog in $CDCl_3$ at 25 °C



Chemical shift	M	**7** (R=C_6F_5)	**8** (R=4-EtOC_6F_4)	**9** (R=C_6H_5)	R=$(CH_2)_9CH_3$
Lactam NH	1×10^{-2}	11.97	11.91	11.78	11.30
	1×10^{-3}	11.84	11.76	11.53	11.08
	1×10^{-4}	11.61	11.51	11.02	10.58
Pyrrole NH	1×10^{-2}	10.51	10.51	10.56	10.44
	1×10^{-3}	10.42	10.40	10.43	10.30
	1×10^{-4}	10.22	10.17	10.11	9.97

[a] δ, ppm downfield from Me_4Si.

C(3)-decyldipyrrinone synthesized earlier[11] was made at the same sample concentration (Table 1). In all four examples, evidence of intermolecular hydrogen bonding is evident from the deshielded NH resonances at the typical chemical shifts.[27] A 10-fold dilution, from 10^{-3} to 10^{-4} M causes the expected slight upfield shift, again a shift that correlates with a monomer⇆dimer equilibrium. The C(3)-*n*-decyl substituted dipyrrinone exhibited somewhat less deshielded NH chemical shifts than **7–9**, suggesting slightly stronger intramolecular hydrogen bonding in the latter.

If the aromatic substituents at C(3) increase the donor ability of the lactam NH (or the acceptor ability of the carbonyl oxygen), then the dimerization constant is expected to increase. In fact, the lactam NH appears to be more sensitive to C(3) substituent and concentration than the pyrrole NH. The largest lactam NH deshielding difference ($\Delta\delta\sim0.5$ ppm) occurs when changing from C(3)-*n*-decyl to C(3)-phenyl and not between, e.g., phenyl and pentafluorophenyl, suggesting that the relative NH deshielding has an origin other than simply stronger association. A deshielding edge effect from the aromatic ring might be felt by the NH, a view supported by the gradual increase of chemical shift in changing the unsubstituted phenyl to *p*-ethoxytetrafluorophenyl and to pentafluorophenyl. Based on data at 10^{-3} M concentration, the electronegative fluorinated rings of **7** and **8** are more effective in deshielding the lactam NH to 11.84 ppm and 11.76 ppm than the unsubstituted phenyl ring (11.53 ppm). The calculated distances, lactam NH to *ortho*-F, are 4.8–5.0 Å (PC Model).[26] It seems probable that both the shielding of C(5)-H and the deshielding of lactam NH are expressions of the same magnetic anisotropy of the aryl ring on C(3).

2.4. UV–vis absorption and fluorescence emission

All the dipyrrinones and *N*,*N*′-carbonyl-bridged dipyrrinones are yellow compounds forming yellow solutions. The latter are highly fluorescent. Comparison of their UV–vis data (Table 2), particularly those in chloroform (Fig. 3), shows only small perturbations of λ_{max} and ε_{max} due to the C(3)

Table 2. Comparison of UV–vis spectral data of *N*,*N*′-carbonyl-bridged dipyrrinones **1–2**, **4–6**, and **7–9**[a]

Pigment	ε^{max} (λ^{max}, nm)					
	Cyclohexane	C_6H_6	$CHCl_3$	CH_3OH	$(CH_3)_2SO$	H_2O
1		17,600 (447)	17,700 (437)	18,600 (433)	18,400 (447)	17,700 (433)
		10,700 (282)	12,300 (278)	12,900 (275)	12,100 (281)	22,900 (271)
2		13,500 (440)	13,700 (436)	14,400 (432)	13,800 (444)	13,400 (431)
		10,800 (283)	11,500 (280)	11,600 (276)	11,300 (281)	12,000 (273)
4	20,400 (451)	19,200 (449)	19,200 (447)	19,400 (443)	19,300 (445)	
	20,000 (432)					
	10,900 (277)	sh 19,000 (439)	12,300 (279)	13,000 (277)	12,400 (276)	
5	20,100 (448)	19,500 (444)	19,000 (443)	19,200 (441)	19,100 (443)	
	20,200 (428)					
	13,600 (278)	13,600 (281)	14,000 (281)	14,200 (279)	13,500 (279)	
6	19,200 (437)	18,200 (433)	18,000 (433)	17,900 (431)	18,200 (433)	
	19,500 (419)					
	sh 12,900 (281)		13,800 (279)	13,600 (278)	13,600 (278)	
7	sh 24,900 (453)	sh 22,000 (456)	39,200 (425)	38,200 (431)	35,800 (429)	
	48,000 (427)	42,300 (429)				
	6800 (299)	6200 (299)	6200 (290)	6000 (290)	sh 6100 (287)	
8	sh 24,700 (447)	sh 23,000 (452)	37,200 (423)	37,700 (429)	35,300 (429)	
	46,100 (423)	42,000 (427)				
	7600 (297)	7000 (298)	7400 (285)	6900 (286)	7400 (282)	
9	sh 22,600 (439)	sh 22,400 (441)	35,700 (411)	36,900 (417)	34,400 (417)	
	43,600 (412)	40,700 (415)				
	7500 (290)	7100 (292)	7200 (282)	6800 (282)	7200 (286)	

[a] Concentration range $2.3–3.8\times10^{-5}$ M at 20 °C.

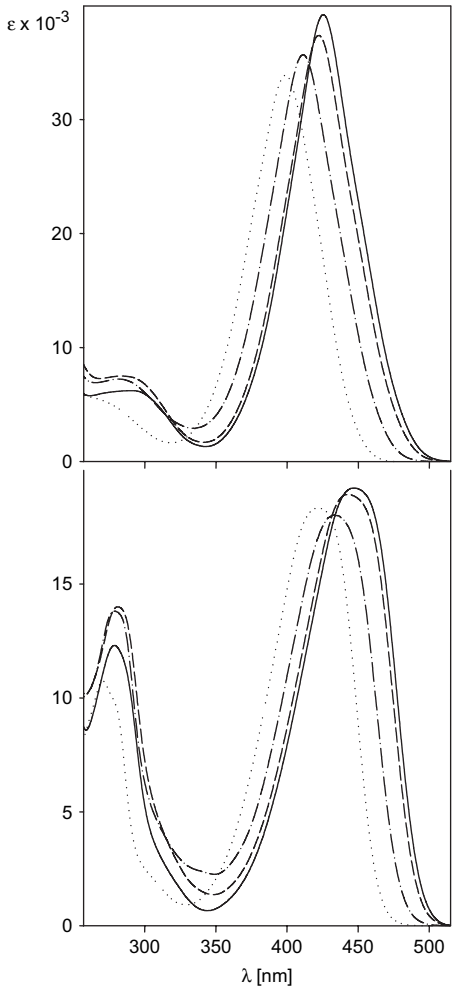


Figure 3. Comparison of UV–vis spectra in chloroform of dipyrrinones (upper panel) and their *N*,*N*′-carbonyl-bridged derivatives (lower panel) substituted at C(3) with: *n*-decyl (dotted line), phenyl (dash–dotted line), *p*-ethoxytetrafluorophenyl (dashed line), and pentafluorophenyl (solid line).

substituents. The parent dipyrrinone **9** in $CHCl_3$ has ε=35,700 (411 nm), the tetrafluorinated **8** has ε=37,200 (423 nm), and the pentafluorinated analog **7** has ε=39,200 (425 nm); and in DMSO **9** has ε=34,400 (417 nm), tetrafluorinated **8** has ε=35,300 (429 nm), and the pentafluorinated **7** has ε=35,800 (429 nm)—evidence that the electron-withdrawing phenyl substituents exert a significant bathochromic shift (of 12–14 nm) and that non-polar solvents cause a gradual but considerable hyperchromic shift, compared to the C(3)-*n*-decyl substituted dipyrrinone in chloroform: ε=33,800 (399 nm) (Fig. 3). A significant (11–23 nm) bathochromic shift is seen in DMSO, as compared to C(3)-*n*-decyl dipyrrinone, ε=34,600 (406 nm).

The influence of the nature of the *para*-substituent (F vs EtO vs H) on the absorption wavelength suggests (at least partial) conjugation between the dipyrrinone π-system and the C(3)-aryl group π-system. Similar effects are observed in the *N*,*N*′-carbonyl-bridged dipyrrinones (Table 2 and Fig. 3) and correlate directly with a decreased reactivity of **4** versus **5** in the sulfonation step.

Fluorescence of *N*,*N*′-carbonyl-bridged dipyrrinones **4**–**6** was measured in five solvents, and the intensity of fluorescence as fluorescence quantum yields (ϕ_F) was quantified using 9,10-diphenylanthracene standard as described previously.[10c] Their excitation, emission wavelengths, and calculated quantum yields in cyclohexane, benzene, chloroform, methanol, and dimethylsulfoxide are given in Table 3. These data might be compared with similar dipyrrinones described earlier,[10,28] but the best reference compound is the dipyrrinone analog with C(8)-free and C(3) substituted with C(3)-*n*-decyl, which showed ϕ_F=0.66 and emission λ_{max}^{em} = 490 nm in chloroform whereas the **6** analog showed ϕ_F=0.57 and λ_{max}^{em} = 502 nm. The corresponding *p*-ethoxytetrafluorophenyl tricycle **5** exhibited ϕ_F=0.59 with λ_{max}^{em} = 508 nm, and the pentafluorophenyl analog **4** had ϕ_F=0.57 and maximum emission at λ_{max}^{em} = 509 nm (Fig. 4). These data show that the quantum yields are of similar magnitude whether the C(3) substituent is aryl, fluorinated aryl or alkyl, and the emission wavelength $\left(\lambda_{max}^{em}\right)$ follows the same trend observed in the UV spectra: a significant bathochromic shift accompanies the replacement of C(3)-*n*-decyl with phenyl group, and an additional but smaller bathochromic shift occurs with fluorine substitution on the aryl group.

Sodium sulfonates **1** and **2** exhibited similar high fluorescence quantum yields in organic solvents: ϕ_F~0.4–0.6. Even in non-polar benzene, the values are in agreement with those of **4**–**6**, thus indicating that aggregation or solubility issues are not influencing the emission. In dimethylsulfoxide solvent somewhat enhanced fluorescence of **1** and **2** was detected in comparison to **4**–**6**. In aqueous solutions the fluorescence efficiency is diminished to about 0.3 but is still sufficient for detection in biological samples—and this solvent offers the largest Stokes shift. The fluorescence emission wavelength from **1** to **2** is not affected significantly by

Table 3. Fluorescence data for *N*,*N*′-carbonyl-bridged dipyrrinones[a] of this work and their C(3)-*n*-decyl analog

Pigment	Cyclohexane		C_6H_6		$CHCl_3$		CH_3OH		$(CH_3)_2SO$	
	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}
1[b]	—	—	0.51	516	0.43	513	0.40	536	0.67	537
2[c]	—	—	0.52	513	0.57	510	0.41	534	0.62	532
4	0.58	480	0.58	508	0.57	509	0.39	541	0.56	533
5	0.67	475	0.61	504	0.59	508	0.39	539	0.59	530
6	0.60	466	0.60	494	0.57	502	0.39	533	0.56	521
C(3)-n-decyl	0.78	463	0.74	472	0.66	490	0.36	523	0.67	501

[a] Concentration range 7.7–9.0×10^{-7} M; excitation λ_{ex}=413–450.
[b] In H_2O, ϕ_F=0.30, λ_{em}=546 nm.
[c] In H_2O, ϕ_F=0.29, λ_{em}=542 nm.

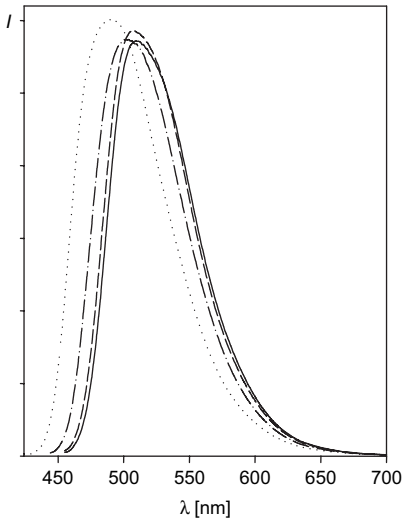


Figure 4. Comparison of fluorescence emission band in chloroform of *N*,*N*′-carbonyl-bridged dipyrrinones substituted at C(3) with: *n*-decyl (dotted line), phenyl (dash–dotted line), *p*-ethoxytetrafluorophenyl (dashed line), and pentafluorophenyl (solid line). The vertical axis plots relative intensity (*I*) of fluorescence emission.

the presence of the C(8) sulfonate group as compared to the C(8)-free parents **4**–**6**.

2.5. Hepatic excretion

When **1**, dissolved in serum, was injected intravenously as a bolus in the rat, the endogenous fluorescence of bile rapidly increased and a prominent peak, followed by a smaller broader peak, was detected in bile by HPLC within 3 min of the injection (Fig. 5a). The prominent peak was identified as unchanged **1**, based on its absorption spectrum and retention time. The highest concentration of **1** in bile was observed in the first sample collected, 3 min after the intravenous injection. Since bile flows only slowly through the short biliary cannula, onset of the efflux of **1** from the liver must occur within less than 3 min. The smaller less polar peak in the chromatograms is yet to be identified. Its normalized absorption spectrum in the HPLC mobile phase is almost superimposable on that of **1**, suggesting no major structural modification. Although it could be a metabolite of **1**, Phase I and Phase II metabolic reactions in the liver generally generate products *more* polar than the precursor. The shape of the peak suggests that it might also be a chromatographic artifact caused by colorless co-migrating constituents of bile. Figure 5b shows a biliary excretion curve for **1**, obtained by plotting HPLC peak areas of **1** in bile normalized to the sample with the maximum peak area. Most pigment was excreted within the first 30 min after injection and the fraction of the administered dose excreted unchanged in bile in two animals in 4 h was 0.5. Unchanged **1** and its possible metabolite was also detectable by HPLC and by fluorescence in urine samples collected intermittently during the experiment. Thus, **1** is cleared rapidly and preferentially from the circulation by the liver and eliminated predominantly by biliary excretion, with a smaller fraction appearing in urine.

The tetrafluorophenyl analog **2** behaved qualitatively similarly to **1**, being excreted rapidly in bile in unchanged form after intravenous injection, with an apparently smaller fraction appearing in urine. However, significant amounts of metabolites of **2**, especially less polar metabolites, were not detected. Determination of the fraction of the dose of **2** excreted in bile was hampered by poor recoveries in the analyses of **2** in the serum injectate. The reason for this is presently unclear, but may be related to the high affinity of **2** for serum albumin. However, it was clear from the bile chromatograms that **2** is excreted preferentially via the liver into bile. Thus, both **1** and **2**, like the sulfoglows reported previously, are cholephilic. They appear to be taken up rapidly by hepatocytes in vivo in the rat, and then excreted rapidly across the biliary canalicular membrane into bile.

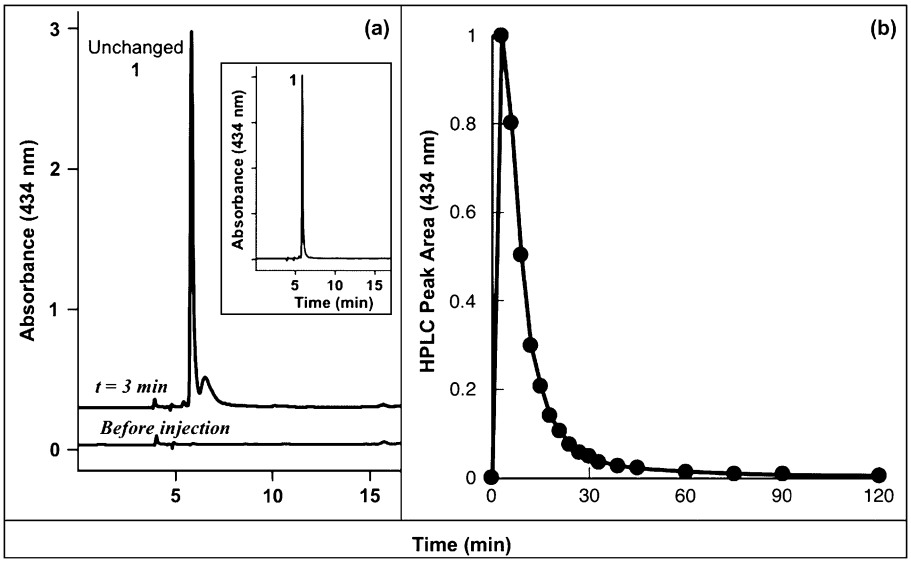


Figure 5. Hepatobiliary excretion of **1** in the homozygous Gunn rat. (a) HPLC chromatograms of bile collected just before (lower trace) and 3 min after (upper trace) intravenous injection of ~0.25 mg **1** dissolved in 1 mL rat serum. The inset shows a chromatogram of a sample of the serum solution of **1** that was injected. (b) Biliary excretion curve for **1** generated by plotting HPLC peak areas of unchanged **1** in bile before and after its injection. Values were normalized to the maximum peak area (at 3 min).

3. Concluding comments

Two new, highly fluorinated (perfluorophenyl and *p*-ethoxytetrafluorophenyl substituents) *N*,*N*′-carbonyl-bridged dipyrrinones were converted to amphiphilic derivatives by sulfonation. The highly fluorescent sulfoglows (**1** and **2**) so obtained are excreted rapidly and principally in unchanged form in bile in the rat after intravenous infusion. Some excretion in urine also was observed. These compounds may form the basis for development of ^{19}F MRI and fluorescence agents for probing liver metabolism and disease.

4. Experimental

4.1. General procedures

Nuclear magnetic resonance spectra were acquired on a Varian Unity Plus spectrometer at 11.75 T magnetic field strength operating at an ^{1}H frequency of 500 MHz, ^{13}C frequency of 125 MHz, and ^{19}F frequency of 470 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). All ^{13}C NMR spectra were broadband ^{1}H decoupled, and *J* constants indicated for some signals are from ^{13}C–^{19}F coupling. The ^{19}F NMR spectra were referenced to external C_6F_6 in $CDCl_3$ at −162.90 ppm. UV–vis spectra were recorded on a Perkin Elmer Lambda 12 spectrophotometer. All fluorescence spectra were measured on a Jobin Yvon FluoroMax 3 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 s. Radial chromatography was carried out on Merck preparative layer grade silica gel PF_{254} with $CaSO_4$ binder, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2 or 4 mm thick rotors. 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 uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. High resolution mass-spectra were obtained from Nebraska Center for Mass Spectrometry, Lincoln, NE.

Spectral data were obtained in spectral grade solvents (Aldrich or Fischer), which were distilled under Ar just prior to use. Before distillation, $CHCl_3$ was passed through a basic alumina column (Woelm, Eschwege, Act. 0). Distillation of $(CH_3)_2SO$ solvent was carried out under vacuum (0.5 mmHg) collecting the solvent at 0 °C and thawing it under Ar. Pyrrole **16**,[12] tosylpyrrolinone **14** (derived from **16**),[12] and pyrrolinone **12**,[12] as well as 3,5-dimethyl-2-formyl-1*H*-pyrrole,[15] were synthesized according to previously published methods.

The concentration study (Table 1) of dipyrrinones **7**–**9** NH ^{1}H NMR chemical shifts was conducted as follows. All solutions were prepared in $CDCl_3$ that had been freshly treated (freshly distilled in two cases) by passing through Woelm Activity 0 basic alumina. Stock solutions concentrated to approximately the upper limit of solubility at 25 °C were prepared (in the case of **9** this limit was 20 mM and for pentafluorophenyl **7** it was only 10 mM) then consecutively diluted in 10-fold increments to 10 μM. The ^{1}H NMR spectra were acquired using a high sensitivity probe for indirect detection. The pulse width was set at about the optimum 65° flip angle and the total repetition time was kept at 8–10 times the *T*1 values of NH signals to allow for complete relaxation. Artificial high line broadening was used in the processing to smoothen the low intensity signals, in particular at low concentrations. Overall, meticulously identical experimental conditions with samples at the same concentrations were used and all spectra were referenced to the residual $CHCl_3$ signal at 7.260 ppm. This precision was necessary to measure the chemical shifts with 10^{-3} ppm confidence.

4.1.1. 4-Methyl-3-pentafluorophenyl-2-(4-toluenesulfonyl)-1*H*-pyrrole (15).

4.1.1.1. 2-Nitro-1-pentafluorophenylpropanol. To a solution of 23.53 g (120 mmol) of perfluorobenzaldehyde and 11.26 g (150 mmol) of nitroethane in 12 mL of anhyd CH_3CN was slowly added 1.8 mL (12 mmol) of DBU, and the mixture was stirred for 30 h at room temperature. The mixture was diluted with 200 mL of CH_2Cl_2, washed with 3% aq HCl (100 mL), H_2O (3×100 mL), dried over anhyd $MgSO_4$, filtered, and the solvent was evaporated under vacuum (1 h at 40 °C). The crude nitropropanol is a 1:1 mixture of diastereomers: ^{1}H NMR *δ*: 1.40, 1.76 (2×1.5H, 2×d, *J*=6.9 Hz), 5.03–5.08 (1H, m), 5.43–5.52 (1H, m) ppm; ^{13}C NMR *δ*: 16.1, 19.5, 61.2, 67.3, 85.0, 86.0, 112.4 (m), 136.1 (dm, ^{1}J=250.7 Hz), 143.4 (dm, ^{1}J=249.8 Hz), 146.7 (dm, ^{1}J=250.2 Hz) ppm and was used directly in the next step.

4.1.1.2. 1-Acetoxy-2-nitro-1-pentafluorophenylpropane. To a solution of the alcohol (26.1 g) from above in 70 mL of anhyd CH_2Cl_2 and 50 mg of DMAP was added acetic anhydride (18.1 mL, 193 mmol) during 10 min, and the mixture was stirred for 16 h. Methanol (25 mL) was added during 15 min, and after 1 h stirring the mixture was diluted with 200 mL of CH_2Cl_2 and then carefully poured into 150 mL of satd aq $NaHCO_3$. The organic layer was washed with H_2O (3×100 mL), dried over anhyd $MgSO_4$, filtered, and the solvent was evaporated under vacuum to give 29.3 g (~78%) of a 1:1 diastereomeric mixture of nitroacetates. ^{1}H NMR *δ*: 1.46, 1.48 (2×1.5H, 2×d, *J*=6.8 Hz), 2.04, 2.21 (2×1.5H, 2×s), 4.91–4.99 (1H, m), 5.19–5.29 (1H, m) ppm; ^{13}C NMR *δ*: 14.6, 14.7, 20.0, 21.8, 67.4, 67.7, 83.8, 84.0, 108.5 (m), 136.0 (dm, ^{1}J=250.9 Hz), 143.6 (dm, ^{1}J=248.7 Hz), 145.9 (dm, ^{1}J=251.1 Hz), 167.0, 168.1 ppm. This mixture was used directly in the next step without further purification.

4.1.1.3. Pyrrole ring formation. To a solution of 24.40 g (125 mmol) of 4-toluenesulfonylmethyl isocyanide (TosMIC)[29] and 25 mL (200 mmol) of tetramethylguanidine in 100 mL of a mixture anhyd THF/isopropyl alcohol=1:1 (v/v) kept at 0 °C was added over 1 h a solution of the nitroacetate from above dissolved in 25 mL of the same solvents. The mixture was warmed slowly to room temperature and stirred for 28 h. Then it was diluted with 400 mL of CH_2Cl_2, poured into 300 mL of ice-5% aq HCl, and the organic layer was washed with 5% aq $NaHCO_3$ (100 mL) and H_2O (3×100 mL). The extract was dried (anhyd $MgSO_4$), filtered through a silica pad, evaporated, and the residue was recrystallized from ethyl acetate/hexane to afford

16.38 g (34% based on perfluorobenzaldehyde) of pyrrole **15**. It had mp 253–255 °C (dec); ^{1}H NMR δ: 1.87 (3H, s), 2.40 (3H, s), 6.89 (1H, d, J=1.8 Hz), 7.23 (2H, m, ^3J=8.3 Hz), 7.50 (2H, m, ^3J=8.3 Hz), 9.24 (1H, br s) ppm; ^{1}H NMR ($(CD_3)_2SO$) δ: 1.79 (3H, s), 2.34 (3H, s), 7.09 (1H, dq, ^3J=2.2 Hz, ^4J=0.8 Hz), 7.35 (2H, m, ^3J=8.4 Hz), 7.55 (2H, m, ^3J=8.4 Hz), 12.61 (1H, br s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.5, 20.9, 108.3 (td, ^2J=19.6 Hz, ^4J= 3.5 Hz), 111.6, 120.6, 123.1, 125.7, 126.4, 129.8, 136.9 (dm, ^1J=249.4 Hz), 138.9, 140.2 (dm, ^1J=251.4 Hz), 143.9, 144.2 (dm, ^1J=248.6 Hz) ppm. ^{19}F NMR δ: −163.4 (m), −154.8 (m, ^3J=21.0 Hz), −138.7 (m, ^3J=22.9 Hz) ppm.

Anal. Calcd for $C_{18}H_{12}F_5NO_2S$ (401.3): C, 53.86; H, 3.01; N, 3.49.

Found: C, 53.71; H, 2.96; N, 3.41.

4.1.2. 3-Methyl-4-pentafluorophenyl-5-(4-toluenesulfonyl)-3-pyrrolin-2-one (13). A mixture of 2.41 g (6 mmol) of pyrrole **15**, 240 mL of acetic acid, 120 mL of CH_2Cl_2, and 120 mL of 30% H_2O_2 was heated at reflux for 24 h. The mixture was cooled, diluted with 200 mL of H_2O, and solid NaCl (~50 g) was added. The product was extracted with CH_2Cl_2 (5×70 mL), and the combined extracts were washed with 5% aq $NaHCO_3$ (100 mL) and H_2O (2×100 mL). The solution was dried (anhyd $MgSO_4$), filtered, and the residue, after evaporation of solvent, was purified in three portions by radial chromatography eluting with a gradient of 1–4% CH_3OH in CH_2Cl_2 (v/v). The pure fractions, after evaporation, were crystallized from ethyl acetate/hexane to afford 1.73 g (69%) of **13**. It had mp 205–206 °C (dec); ^{1}H NMR δ: 1.77 (3H, q, J=1.4 Hz), 2.44 (3H, s), 5.72 (1H, br t, J=1.5 Hz), 7.31 (2H, m, ^3J=8.4 Hz), 7.58 (2H, m, ^3J=8.4 Hz), 7.66 (1H, br s) ppm; ^{13}C NMR δ: 10.9 (t, J=2.9 Hz), 21.7, 76.9 (t, J=3.2 Hz), 106.7, 129.4, 129.9, 130.5, 130.9, 137.9 (dm, ^1J=251.4 Hz), 141.8, 142.1 (dm, ^1J=250.7 Hz), 144.0 (dm, ^1J=247.6 Hz), 146.5, 171.3; ^{19}F NMR δ: −160.9 (m, ^3J=20.5 Hz), −150.9 (m, ^3J=21.1 Hz), −138.5 (v br s), −136.4 (v br s) ppm.

Anal. Calcd for $C_{18}H_{12}F_5NO_3S$ (417.3): C, 51.80; H, 2.90; N, 3.36.

Found: C, 52.25; H, 2.93, N, 3.23.

4.1.3. 3-Methyl-4-pentafluorophenyl-3-pyrrolin-2-one (10). To a solution of 417 mg (1.0 mmol) of toluenesulfonylpyrrolinone **13** in 60 mL of abs ethanol under N_2 was added sodium borohydride (114 mg, 3.0 mmol) during 10 min. The mixture was stirred for 10 more minutes, the solvent was evaporated under vacuum (<35 °C), and the residue was partitioned between 100 mL of 1% aq HCl and 100 mL of $CHCl_3$. The organic layer was washed with 3×50 mL of H_2O, dried over anhyd Na_2SO_4, filtered, and solvent was evaporated under vacuum. The crude material was purified by radial chromatography eluting with 1–2% CH_3OH in CH_2Cl_2 (v/v), and the polar band, after evaporation, was crystallized from ethyl acetate/hexane to afford 249 mg (95%) of pyrrolinone **10**. It had mp 133–134 °C; ^{1}H NMR δ: 1.88 (3H, m), 4.22 (2H, m), 7.78 (1H, br s) ppm; ^{13}C NMR δ: 10.2 (t, J=2.0 Hz), 48.4 (t, J=2.6 Hz), 108.4 (td, ^2J=18.2 Hz, ^4J=3.9 Hz), 135.7, 137.5, 137.9 (dm, ^1J=253.8 Hz), 141.4 (dm, ^1J=256.7 Hz), 143.9 (dm, ^1J=250.3 Hz), 174.4 ppm; ^{19}F NMR δ: −161.5 (m), −152.9 (m, ^3J=20.8 Hz), −139.5 (m, ^3J=21.7 Hz) ppm.

Anal. Calcd for $C_{11}H_6F_5NO$ (263.2): C, 50.20; H, 2.29; N, 5.31.

Found: C, 50.01; H, 2.59; N, 5.40.

4.1.4. 3-Methyl-4-(4-ethoxytetrafluorophenyl)-3-pyrrolin-2-one (11). To a solution of 789 mg (3.0 mmol) of pentafluorophenylpyrrolinone **10** in 10 mL of abs ethanol (cooled to 10 °C) was added during 1 h a freshly prepared solution of 15.0 mmol sodium ethoxide (from 345 mg of sodium) in 15 mL of abs ethanol. The mixture was stirred for an additional 1 h, before being diluted with 100 mL of $CHCl_3$ and poured into 100 mL of 1% aq HCl. The organic layer was washed with H_2O (3×50 mL), dried over anhyd Na_2SO_4, and filtered. The solvent was evaporated under vacuum and the residue was purified by radial chromatography eluting with 0.5–1.5% CH_3OH in CH_2Cl_2 (v/v). After solvent evaporation, the combined pure fractions were crystallized from ethyl acetate/hexane to give 617 mg (71%) of pyrrolinone **11**. It had mp 128–129 °C; ^{1}H NMR δ: 1.45 (3H, t, J=7.0 Hz), 1.87 (3H, s), 4.20 (2H, br s), 4.36 (2H, q, J=7.0 Hz), 7.58 (1H, br s) ppm; ^{13}C NMR δ: 10.3 (t, J=2.4 Hz), 15.4, 48.4 (t, J=2.9 Hz), 71.0 (t, J=3.6 Hz), 106.4 (t, ^2J=18.3 Hz), 136.5, 136.8 (t, ^3J=1.0 Hz), 137.9 (tt, ^2J=12.1 Hz, ^3J=3.6 Hz), 141.3 (dm, ^1J=248.2 Hz), 144.0 (dm, ^1J=248.2 Hz), 174.6 ppm; ^{19}F NMR δ: −157.4 (m, ^3J=20.4 Hz), −141.6 (m, ^3J= 20.4 Hz) ppm.

Anal. Calcd for $C_{13}H_{11}F_4NO_2$ (289.2): C, 53.98; H, 3.83; N, 4.84.

Found: C, 53.75; H, 4.07; N, 4.86.

4.2. General procedure for syntheses of dipyrrinones

A mixture of 1 mmol of the corresponding pyrrolinone, 369 mg (3 mmol) of 3,5-dimethyl-2-formyl-1*H*-pyrrole,[15] 2.5 mL of anhyd CH_3CN, and 1.1 mL (10 mmol) of *N*-methylmorpholine was heated under Ar at 90–95 °C in a sealed thick wall tube for 84 h (48 h for **9**). After being cooled and opened to atmospheric pressure, the mixture was chilled at −20 °C for 3 h, and the separated product was collected by filtration. It was washed on the filter with CH_3CN/H_2O/AcOH (5 mL/2 mL/2 mL), then dried under vacuum, and purified in several portions by radial chromatography eluting with gradient 1–3% CH_3OH in CH_2Cl_2 (v/v). The fractions containing pure pigment were combined, evaporated under vacuum, and the residue was crystallized from CH_3OH/CH_2Cl_2 to give pure bright yellow dipyrrinone.

4.2.1. 3-Pentafluorophenyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (7). This compound was isolated in 84% yield. It decomposed without melting at >321–324 °C; ^{1}H NMR δ: 1.94 (3H, s), 2.05 (3H, s), 2.46 (3H, s), 5.78 (1H, s), 5.86 (1H, d, ^4J=1.8 Hz), 10.51 (1H, br s), 11.97 (1H, br s) ppm; ^{13}C NMR δ: 9.8 (br), 11.5, 13.6, 105.0, 107.3 (td,

^2J=19.2 Hz, ^4J=3.3 Hz), 111.1, 123.2, 124.9, 128.2, 129.4, 130.8 (m), 136.4, 137.8 (dm, ^1J=252.5 Hz), 141.6 (dm, ^1J=250.1 Hz), 144.2 (dm, ^1J=248.5 Hz), 172.3 ppm; ^{1}H NMR ($(CD_3)_2SO$) δ: 1.76 (3H, s), 1.92 (3H, s), 2.22 (3H, s), 5.60 (1H, s), 5.74 (1H, d, ^4J=0.9 Hz), 10.48 (1H, s), 10.55 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.2 (br), 11.1, 12.9, 101.0, 106.8 (td, ^2J=19.4 Hz, ^4J=3.3 Hz), 110.3, 122.4, 125.4, 125.7, 129.3, 129.9 (t, ^3J=1.9 Hz), 133.8, 137.5 (dm, ^1J=250.3 Hz), 140.9 (dm, ^1J=251.8 Hz), 143.6 (dm, ^1J=245.1 Hz), 170.1 ppm; ^{19}F NMR δ: −161.6 (m), −153.0 (m, ^3J=21.0 Hz), −138.4 (m, ^3J=22.8 Hz) ppm.

Anal. Calcd for $C_{18}H_{13}F_5N_2O$ (368.3): C, 58.70; H, 3.56; N, 7.61.

Found: C, 58.46; H, 3.94; N, 7.54.

4.2.2. 3-(4-Ethoxytetrafluorophenyl)-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (8). This pigment was obtained in 74% yield. It had mp 287–289 °C (dec); ^{1}H NMR δ: 1.49 (3H, t, J=7.1 Hz), 1.94 (3H, s), 2.05 (3H, s), 2.46 (3H, s), 4.42 (2H, q, J=7.1 Hz), 5.82 (1H, s), 5.84 (1H, d, ^4J=2.3 Hz), 10.54 (1H, br s), 11.96 (1H, br, s) ppm; ^{13}C NMR δ: 9.8, 11.4, 13.5, 15.5, 71.0, (t, J=3.5 Hz), 104.9, 105.2 (t, ^2J=19.1 Hz), 110.9, 123.3, 125.3, 127.9, 128.9, 132.0 (t, ^3J=2.0 Hz), 136.1, 137.9 (tt, ^2J=12.1 Hz, ^3J=3.4 Hz), 141.3 (dm, ^1J=248.1 Hz), 144.3 (dm, ^1J=247.8 Hz), 172.6 ppm; ^{19}F NMR δ: −157.5 (m, ^3J=21.3 Hz), −140.5 (m, ^3J=21.3 Hz) ppm.

Anal. Calcd for $C_{20}H_{18}F_4N_2O_2$ (394.4): C, 60.91; H, 4.60; N, 7.10.

Found: C, 60.99; H, 4.88; N, 7.11.

4.2.3. 3-Phenyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (9). This compound was isolated in 64% yield. It had mp 293–295 °C (dec); ^{1}H NMR δ: 2.01 (3H, s), 2.02 (3H, s), 2.47 (3H, s), 5.82 (1H, d, ^4J=1.8 Hz), 6.06 (1H, s), 7.37 (2H, m), 7.44 (1H, m), 7.49 (2H, m), 10.61 (1H, br s), 11.81 (1H, br s) ppm; ^{13}C NMR δ: 9.4, 11.4, 13.5, 105.5, 110.4, 123.4, 123.6, 127.3, 127.8, 128.2, 128.3, 129.8, 132.7, 135.1, 146.7, 173.3 ppm.

Anal. Calcd for $C_{18}H_{18}N_2O$ (278.3): C, 77.67; H, 6.52; N, 10.06.

Found: C, 77.76; H, 6.28; N, 10.20.

4.3. General procedure for insertion of *N*,*N*′-carbonyl bridge

A mixture of 1 mmol of dipyrrinones **7**–**9**, 1,1′-carbonyldiimidazole (0.81 g, 5 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.75 mL, 5 mmol), and 80 mL of anhydrous CH_2Cl_2 was heated under N_2 at reflux for 16 h. After cooling, the mixture was washed with 100 mL of 1% aq HCl, then with H_2O (3×100 mL), and the solution was dried over anhyd $MgSO_4$. After filtration and evaporation of the solvent under vacuum, the residue was purified by radial chromatography on silica gel eluting with gradient 0.2–0.8% CH_3OH in CH_2Cl_2 (v/v). The fractions containing non-polar fluorescent band were combined, the solvents were evaporated under vacuum, and the residue was crystallized from ethyl acetate/hexane or methanol/water to afford pure bright yellow-orange tricyclic compounds.

4.3.1. 1-Pentafluorophenyl-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (4). This tricycle was isolated in 67% yield. It had mp 255–256 °C; ^{1}H NMR δ: 1.98 (3H, s), 2.10 (3H, s), 2.70 (3H, s), 6.06 (1H, br s), 6.16 (1H, s) ppm; ^{13}C NMR δ: 10.0 (br), 10.8, 15.7, 99.7, 105.3 (td, ^2J=18.7 Hz, ^4J=4.0 Hz), 117.9, 123.2, 126.9, 128.1, 129.0 (br), 132.5, 136.4, 138.1 (dm, ^1J=250.3 Hz), 142.1 (dm, ^1J=252.8 Hz), 143.1, 144.1 (dm, ^1J=250.5 Hz), 165.9 ppm; ^{19}F NMR δ: −160.4 (m), −151.1 (m, ^3J=21.0 Hz), −137.8 (m, ^3J=21.0 Hz) ppm.

Anal. Calcd for $C_{19}H_{11}F_5N_2O_2$ (394.3): C, 57.87; H, 2.81; N, 7.10.

Found: C, 57.84; H, 3.19; N, 7.13.

4.3.2. 1-(4-Ethoxytetrafluorophenyl)-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (5). This compound was obtained in 74% yield. It had mp 192–193 °C; ^{1}H NMR δ: 1.49 (3H, t, J=7.1 Hz), 1.97 (3H, s), 2.10 (3H, s), 2.69 (3H, s), 4.42 (2H, q, J=7.1 Hz), 6.04 (1H, br s), 6.18 (1H, s) ppm; ^{13}C NMR δ: 10.0 (t, J=1.6 Hz), 10.8, 15.5, 15.6, 71.1 (t, J=3.6 Hz), 99.7, 102.9 (t, ^2J=18.6 Hz), 117.7, 122.7, 126.9, 128.4, 130.2 (t, ^3J=2.2 Hz), 132.0, 136.1, 138.9 (tt, ^2J=11.9 Hz, ^3J=3.5 Hz), 141.4 (dm, ^1J=249.1 Hz), 143.2, 144.1 (dm, ^1J=249.1 Hz), 166.2 ppm; ^{19}F NMR δ: −156.5 (m, ^3J=20.1 Hz), −139.9 (m, ^3J=20.1 Hz) ppm.

Anal. Calcd for $C_{21}H_{16}F_4N_2O_3$ (420.4): C, 60.00; H, 3.84; N, 6.66.

Found: C, 59.61; H, 4.16; N, 6.68.

4.3.3. 1-Phenyl-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione (6). This tricycle was isolated in 93% yield. It had mp 196–197 °C; ^{1}H NMR δ: 2.04 (3H, s), 2.08 (3H, s), 2.70 (3H, s), 6.03 (1H, br s), 6.34 (1H, s), 7.42 (2H, m), 7.53 (3H, m) ppm; ^{13}C NMR δ: 9.4, 10.7, 15.6, 99.9, 117.4, 121.7, 126.9, 127.1, 128.8, 128.9, 129.4, 130.3, 130.5, 135.3, 143.5, 143.8, 167.3 ppm.

Anal. Calcd for $C_{19}H_{16}N_2O_2$ (304.3): C, 74.98; H, 5.30; N, 9.21.

Found: C, 75.00; H, 5.42; N, 9.29.

4.4. Sodium 2,7,9-trimethyl-1-pentafluorophenyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione-8-sulfonate (1)

Finely powdered bridged dipyrrinone **4** (100 mg, 0.25 mmol) was added to 6 mL of concd H_2SO_4, and the mixture was stirred at 25 °C for 4 h. Then the temperature was lowered from −10 °C to −15 °C, and the solution was neutralized to pH 7–8 with satd aq Na_2CO_3 while introducing a stream of air in order to reduce foaming. When foaming became excessive, methanol was added in 1 mL portions on four occasions. After dilution with H_2O (50 mL), the mixture was

extracted with $CHCl_3/CH_2Cl_2$ 1:1 (7×50 mL), adding 10 mL portions of CH_3OH after each extraction. The combined extracts were evaporated under vacuum, and the residue was purified by radial chromatography by eluting with gradient 5–20% CH_3OH/CH_2Cl_2 (v/v). The polar band was collected, evaporated to dryness, and triturated with ethyl acetate. The solid product was collected by filtration to afford 49 mg (39%) of sulfonate **1**. It decomposed at >277–285 °C; ^{1}H NMR ($(CD_3)_2SO$) δ: 1.87 (3H, s), 2.19 (3H, s), 2.83 (3H, s), 6.88 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.5, 10.2, 13.5, 100.7, 104.9 (m, ^2J=18.7 Hz), 121.8, 125.9, 128.2, 128.8, 131.5, 132.7, 134.6 (br), 137.5 (dm, ^1J=251.4 Hz), 141.3 (dm, ^1J=250.5 Hz), 142.6, 143.8 (dm, ^1J=248.9 Hz), 165.3 ppm; ^{19}F NMR ($(CD_3)_2SO$) δ: −161.6 (m), −153.8 (m, ^3J=22.1 Hz), −138.2 (m, ^3J=22.1 Hz) ppm. FAB-HRMS (3-NBA+Na) calcd for $C_{19}H_{10}F_5N_2Na_2O_5S$ $(M+Na)^+$, *m/z*: 519.0026, found: 519.0021, Δ=0.5 mDa, error 1.0 ppm.

4.5. Sodium 1-(4-ethoxytetrafluorophenyl)-2,7,9-trimethyl-3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione-8-sulfonate (2)

Finely ground bridged dipyrrinone **5** (105 mg, 0.25 mmol) was added to 5 mL of concd H_2SO_4 precooled at 0 °C, and the magenta colored mixture was stirred for 2 h. Then the temperature was lowered from −10 °C to −15 °C, and the solution was neutralized to pH 7–8 with concd aq Na_2CO_3 while introducing a stream of air in order to reduce foaming. Methanol was added in 1 mL portions on four occasions to wash the foam down. After dilution with H_2O (50 mL), the mixture was extracted with $CHCl_3/CH_2Cl_2$ 1:1 (7×50 mL) adding 10 mL portions of CH_3OH after each extraction. The combined extracts were evaporated under vacuum and the residue was purified by radial chromatography eluting with gradient 5–20% CH_3OH in CH_2Cl_2 (v/v). The polar band was collected, evaporated to dryness, and triturated with CH_2Cl_2/hexane. The solid product was collected by filtration to give (after drying under vacuum at 80 °C) 59 mg (45%) of sulfonate **2**. It had mp 258–261 °C (dec); ^{1}H NMR ($(CD_3)_2SO$) δ: 1.39 (3H, t, J=7.0 Hz), 1.86 (3H, s), 2.20 (3H, s), 2.83 (3H, s), 4.40 (2H, q, J=7.0 Hz), 6.87 (1H, s) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 9.5, 10.2, 13.5, 15.3, 71.1, 100.7, 102.7 (t, ^2J=18.9 Hz), 121.7, 125.9, 128.4, 129.8 (br t), 131.0, 132.6, 134.7 (br), 137.9 (m), 141.1 (dm, ^1J=246.6 Hz), 142.7, 143.9 (dm, ^1J=243.3 Hz), 165.4 ppm; ^{19}F NMR ($(CD_3)_2SO$) δ: −156.7 (m, ^3J=21.9 Hz), −140.1 (m, ^3J=21.9 Hz) ppm. FAB-HRMS (3-NBA+Na) calcd for $C_{21}H_{15}F_4N_2Na_2SO_6$ $(M+Na)^+$, *m/z*: 545.0383; found: 545.0393, Δ=−1.0 mDa, error −1.9 ppm.

4.6. Metabolism studies

The procedure for metabolism studies in the rat has been described in detail elsewhere.[11,12] Solutions of **1** and **2** in 1 mL rat serum, prepared by dissolving ~0.25 mg of each pigment in 0.1 mL $(CH_3)_2SO$ and diluting this solution slowly into 1 mL rat serum, were injected intravenously as a bolus into the femoral vein and bile was collected from a short indwelling cannula in frequent 20-μL aliquots under safelights. Bile samples were flash frozen at once in dry-ice and stored at <−50 °C until analyzed by HPLC as previously described[11,12] using absorbance at 434 nm for detection. Studies were conducted in adult male homozygous Gunn rats. Gunn rats were used to simplify the appearance and integration of chromatograms since these rats lack UGT1 isozymes and do not excrete bilirubin glucuronides in bile.[1,5] No metabolism of **1** or **2** by UGT1 isozymes would be expected and control experiments indicated that hepatic metabolism and excretion of **2** was qualitatively similar in homozygous, heterozygous, and wild-type rats.

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References and notes

1. Chowdhury, J. R.; Wolkoff, A. W.; Chowdhury, N. R.; Arias, I. M. *The Metabolic and Molecular Bases of Inherited Disease*; Scriver, C. R., Beaudet, A. L., Sly, W. S., Valle, D., Eds.; McGraw-Hill: New York, NY, 2001; Vol. II, pp 3063–3101.
2. (a) Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*; Springer: Wien, 1989; (b) Lightner, D. A. *Bilirubin*; Heirwegh, K. P. M., Brown, S. B., Eds.; CRC: Boca Raton, FL, 1982; Vol. 1, pp 1–58.
3. McDonagh, A. F.; Palma, L. A.; Trull, F. R.; Lightner, D. A. *J. Am. Chem. Soc.* **1982**, *104*, 6865–6867.
4. (a) McDonagh, A. F.; Lightner, D. A. *Semin. Liver Dis.* **1988**, *8*, 272–283; (b) McDonagh, A. F.; Lightner, D. A. *Pediatrics* **1985**, *75*, 443–455.
5. Owens, I. S.; Basu, N. K.; Banerjee, R. *Methods Enzymol.* **2005**, *400*, 1–22.
6. Leslie, E. M.; Deeley, R. G.; Cole, S. P. *Toxicol. Appl. Pharmacol.* **2005**, *204*, 216–237.
7. (a) Lightner, D. A.; Ma, J. S.; Adams, T. C.; Franklin, R. W.; Landen, G. L. *J. Heterocycl. Chem.* **1984**, *21*, 139–144; (b) Shrout, D. P.; Lightner, D. A. *Synthesis* **1990**, 1062–1065; (c) Grunewald, J. O.; Cullen, R.; Bredfeldt, J.; Strope, E. R. *Org. Prep. Proced. Int.* **1975**, *7*, 103–110.
8. McDonagh, A. F.; Lightner, D. A. *Hepatic Metabolism and Disposition of Endo and Xenobiotics*; Bock, K. W., Gerok, W., Matern, S., Eds.; Falk Symposium No. 57; Kluwer: Dordrecht, The Netherlands, 1991; Chapter 5, pp 47–59.
9. (a) Ma, J. S.; Lightner, D. A. *Tetrahedron* **1991**, *47*, 3719–3726; (b) Hwang, K. O.; Lightner, D. A. *Tetrahedron* **1994**, *50*, 1955–1966.
10. (a) Brower, J. O.; Lightner, D. A. *J. Org. Chem.* **2002**, *67*, 2713–2717; (b) Pavlopoulos, T. G.; Lightner, D. A.; Brower, J. O. *Appl. Opt.—LP* **2003**, *42*, 3555–3557; (c) Boiadjiev, S. E.; Lightner, D. A. *J. Phys. Org. Chem.* **2004**, *17*, 675–679.
11. Woydziak, Z. R.; Boiadjiev, S. E.; Norona, W. S.; McDonagh, A. F.; Lightner, D. A. *J. Org. Chem.* **2005**, *70*, 8417–8423.
12. Brower, J. O.; Lightner, D. A.; McDonagh, A. F. *Tetrahedron* **2001**, *57*, 7813–7827.

13. Weber, L. M.; Cook, P. D.; Elslager, E. F.; Hung, J. H.; Johnson, J. L.; Kesten, S. J.; McNamara, D. J.; Ortwine, D. F.; Worth, D. F. *J. Med. Chem.* **1986**, *29*, 924–939.
14. Kinoshita, H.; Hayashi, Y.; Murata, Y.; Inomata, K. *Chem. Lett.* **1993**, 1437–1440.
15. (a) de Groot, J. A.; Gorter-LaRoy, G. M.; van Koeveringe, J. A.; Lugtenburg, J. *Org. Prep. Proced. Int.* **1981**, *13*, 97–101; (b) Smith, K. M.; Langry, K. C.; Minnetian, O. M. *J. Org. Chem.* **1984**, *49*, 4602–4609.
16. White, W. L.; Filler, R. *J. Chem. Soc. C* **1971**, 2062–2068.
17. Aroskar, E. V.; Brown, P. J. N.; Plevey, R. G.; Stephens, R. *J. Chem. Soc. C* **1968**, 1569–1575.
18. (a) Schlosser, M.; Bobbio, C.; Rausis, T. *J. Org. Chem.* **2005**, *70*, 2494–2502; (b) Bobbio, C.; Schlosser, M. *J. Org. Chem.* **2005**, *70*, 3039–3045; (c) Sandford, G.; Slater, R.; Yufit, D. S.; Howard, Y. A. K.; Vong, A. *J. Org. Chem.* **2005**, *70*, 7208–7216.
19. Günter, H. *NMR Spectroscopy*, 2nd ed.; Wiley: Chichester, UK, 1994; pp 335–389.
20. Emsley, J. W.; Phillips, L. *Prog. Nucl. Magn. Reson. Spectrosc.* **1971**, *7*, 1–526.
21. (a) Jones, K.; Mooney, E. F. *Annu. Rep. NMR Spectrosc.* **1970**, *3*, 261–421; (b) Jones, K.; Mooney, E. F. *Annu. Rep. NMR Spectrosc.* **1971**, *4*, 391–495.
22. Thomas, W. A. *Annu. Rep. NMR Spectrosc.* **1970**, *3*, 91–147.
23. Ute, K.; Kinoshita, R.; Matrui, K.; Miyatake, N.; Hatada, K. *Chem. Lett.* **1992**, 1337–1340.
24. Gross, U.; Pfeifer, D. *J. Fluorine Chem.* **2002**, *113*, 17–26.
25. Vaughan, M. D.; Cleve, P.; Robinson, V.; Duewel, H. S.; Honek, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 8475–8478.
26. PCModel version 9.0 from Serena Software, Bloomington, IN (http://www.serenasoft.com), 2004.
27. (a) Nogales, D. F.; Ma, J.-S.; Lightner, D. A. *Tetrahedron* **1993**, *49*, 2361–2372; (b) Huggins, M. T.; Lightner, D. A. *Monatsh. Chem.* **2001**, *132*, 203–221.
28. (a) Boiadjiev, S. E.; Lightner, D. A. *J. Heterocycl. Chem.* **2004**, *41*, 1033–1037; (b) Boiadjiev, S. E.; Lightner, D. A. *J. Org. Chem.* **2005**, *70*, 688–691.
29. (a) Chen, Q.; Huggins, M. T.; Lightner, D. A.; Norona, W.; McDonagh, A. F. *J. Am. Chem. Soc.* **1999**, *121*, 9253–9264; (b) Hoogenboom, B. E.; Oldenziel, O. H.; van Leusen, A. M. *Org. Synth. Coll.* **1988**, *6*, 987–990.