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Synthesis and Hepatic Transport of Strongly Fluorescent Cholephilic Dipyrrinones

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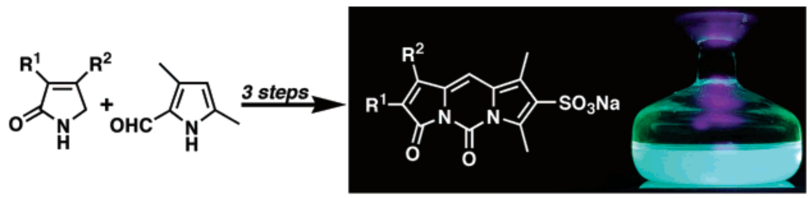

A new class of highly fluorescent (ϕ_F 0.3−0.8) low molecular weight water-soluble cholephilic compounds has been synthesized in two steps from dipyrrinones. The dipyrrinone nitrogens are first bridged by reaction with 1,1′-carbonyldiimidazole to form an *N*,*N*′-carbonyldipyrrinone (3*H*,5*H*-dipyrrolo[1,2-*c*:2′,1′-*f*]pyrimidine-3,5-dione) nucleus, and a sulfonic acid group is then introduced at C(8) by reaction with concd H_2SO_4. The resulting sulfonated *N*,*N*′-carbonyl-bridged dipyrrinones ("sulfoglows") are isolated as their sodium salts. When the alkyl substituents of the lactam ring are lengthened from ethyl to decyl, sulfoglows become increasingly lipophilic while maintaining water solubility. Low molecular weight sulfoglows were rapidly excreted intact in both bile and urine after intravenous infusion into rats, but higher molecular weight sulfoglows were excreted more selectively in bile. Hepatobiliary excretion of sulfoglows was partially, but not completely, blocked in mutant rats deficient in the multidrug-resistance associated transport protein Mrp2 (ABCC2). These observations point to the feasibility of developing simple sulfoglows with clinical diagnostic potential that are normally excreted in bile but appear in urine when hepatic elimination is impaired by cholestatic liver disease.

Introduction

Bilirubin (Figure 1A), the lipophilic yellow pigment of neonatal jaundice[1] is comprised of two *Z*-dipyrrinone chromophores.[2] In the absence of light-promoted $Z \rightarrow E$ photoisomerization,[3,4] it is unexcretable (across the liver into bile) in normal human metabolism, except following enzymic conjugation with glucuronic acid (Figure 1B).[1,4] Accumulation of bilirubin and its glucuronides leading to jaundice is a well-known sign of liver disease.[1] In contrast to bilirubin, xanthobilirubic acid[5] (Figure 1C), a polar, but water-insoluble synthetic analogue for one-half of bilirubin, is readily excreted intact in bile without the need for glucuronidation.[6] Both xanthobilirubic acid and bilirubin are essentially nonfluorescent at room temperature, e.g., with fluorescence quantum yields ϕ_F $<10^{-3}$ because of rapid $Z \rightarrow E$ isomerization in the excited states.[2,7] In earlier work, we demonstrated a dramatic

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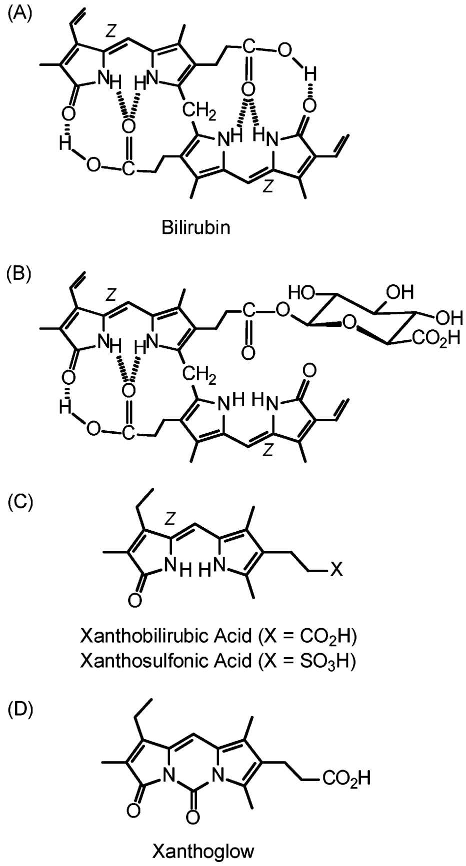
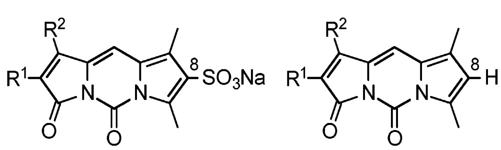


FIGURE 1. (A) Bilirubin and (B) one of its two monoglucuronides. (C) Xanthobilirubic acid, a dipyrrinone model for bilirubin, and its sulfonic acid analogue, xanthosulfonic acid. (D) A highly fluorescent (ϕ_F 0.80, cyclohexane) *N,N′*-carbonyl-bridged analogue of xanthobilirubic acid (xanthoglow).

increase of dipyrrinone fluorescence (to ϕ_F ~1) by bridging the two nitrogens of the dipyrrinone.[7] The easiest bridge to build, and most effective in enhancing fluorescence, is the carbonyl, from treatment of the dipyrrinone with 1,1′-carbonyldiimidazole (CDI) in CH_2Cl_2. Thus, xanthobilirubic acid was easily converted to xanthoglow (Figure 1D), with ϕ_F ~0.80 in cyclohexane at 25 °C for λ_{exc} 410 nm and λ_{em} 473 nm.[7c,8]

Seeking a water-soluble analogue of xanthobilirubic acid, we prepared xanthosulfonic acid (Figure 1C),[9] which was found to be excreted *intact* by the liver in rats following intravenous injection of the pigment.[10] To examine whether a *water-soluble* fluorescent analogue might be similarly excreted, we synthesized the first "sulfoglow" (**1a**),[7c] a xanthoglow analogue with the C(8) propionic acid replaced by sulfonic acid. When injected intravenously into rats it was rapidly excreted intact into bile and urine, which became highly fluorescent.[10] These preliminary studies suggested that it might be possible to develop highly fluorescent cholephilic ("bile-loving") compounds that are excreted in urine only when hepatic elimination is impeded. Such compounds could provide a novel method for detecting cholestatic liver disorders by injecting a bolus dose of the fluorophore intravenously and examining urine visually or instrumentally for fluorescence. To test this hypothesis, we required more lipophilic analogues of **1a** that would normally be excreted preferentially and nearly exclusively by the hepatobiliary route but that would be excreted in urine when normal hepatic excretion is impaired (cholestasis). In the following text, we describe the syntheses, spectroscopic properties, and metabolic disposition of cholephilic sulfoglow sodium salts **1b**−**e** with alkyl groups of varying lengths on the lactam ring to mitigate the intrinsic high water solubility of the sulfonate group and modulate the balance of hepatic vs renal excretion. The synthetic study is the first part of a more comprehensive investigation of cholephilic fluorescent pharmacophores for detecting cholestatic disorders, particularly in the newborn.



	R^1	R^2	
1a	CH_3	CH_2CH_3	**2a**
1b	CH_3	$(CH_2)_3CH_3$	**2b**
1c	$(CH_2)_3CH_3$	CH_3	**2c**
1d	CH_3	$(CH_2)_6CH_3$	**2d**
1e	CH_3	$(CH_2)_9CH_3$	**2e**

Results and Discussion

Syntheses. The syntheses of **1a**−**e** are diagrammed in Figure 2A. The key starting materials are dipyrrinones **3a**−**e**, which are readily prepared by base-catalyzed condensation of 3,5-dimethylpyrrole-2-aldehyde (**5**)[11] with pyrrolinones **4a**−**e**. Three of the latter (**4a**,[12] **4b**,[13] and **4c**[13]**)** were available from Barton−Zard pyrrole syntheses[14] followed by appropriate oxidation steps, as previously published. The syntheses of **4d** and **4e** followed closely the method of synthesis of pyrroles with long alkyl chains,[15] followed by oxidation. Thus, as outlined in Figure 2B, 1-octanal or 1-undecylic aldehyde and nitroethane were condensed to afford the *β*-hydroxynitro product (**10d** or **10e**) in nearly quantitative yield, which (after acetylation to **9d** or **9e**) was reacted with *p*-toluenesulfonylmethyl isocyanide (TosMIC)[16] in the presence of tetramethylguanidine to afford tosylpyrrole **8d**

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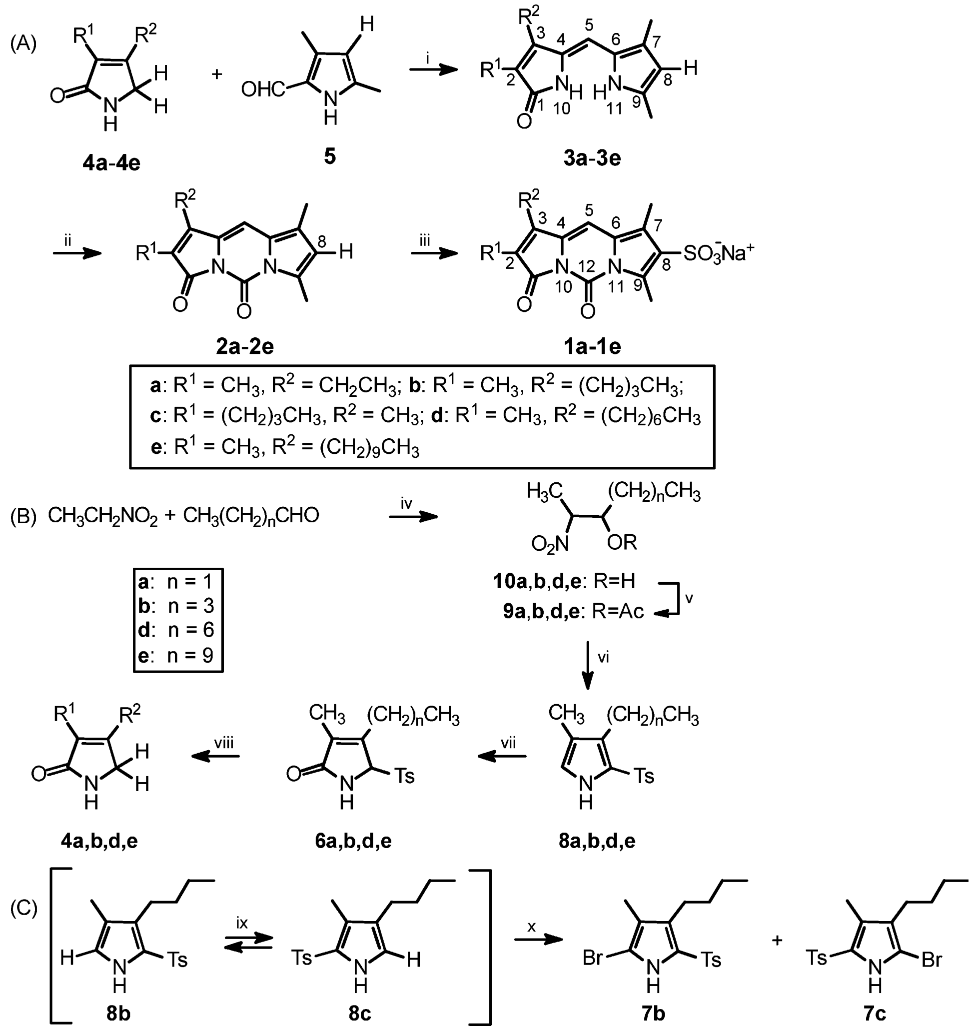

FIGURE 2. (A) Reaction scheme for the syntheses of dipyrrinones **3a**–**e** from pyrrole aldehyde **5** and pyrrolinones **4a**–**e**, conversion of **3a**–**e** to their *N*,*N*′-carbonyl-bridged analogues **2a**–**e**, and sulfonation of the latter to afford sulfoglows **1a**–**e**. (B) Synthesis scheme for **4a**–**e**. (C) Scrambling of **8b** and conversion to **7c**. Synthetic conditions: (i) KOH, CH_3CH_2OH; (ii) CDI, DBU in CH_2Cl_2; (iii) concd H_2SO_4, then Na_2CO_3; (iv) KF/CH_3CH_2OH or DBU; (v) Ac_2O, pyridine; (vi) TosMIC, TMG; (vii) H_2O_2, or (a) phenyltrimethylammonium tribromide (PTT) then (b) aq TFA; (viii) $NaBH_4$; (ix) TFA; (x) PTT.

or **8e** in 45% and 61% yield. The latter was converted to tosylpyrrolinone **6d** or **6e** by α-bromination of the pyrrole, followed by acid-catalyzed hydrolysis, and the tosyl group was cleaved smoothly by reduction with $NaBH_4$ to afford pyrrolinone **4d** or **4e**.

Dipyrrinone **3a** was known from earlier studies.[17] Dipyrrinones **3b**–**e** are new and were prepared in 48, 44, 42, and 68% yields, respectively, by condensing pyrrolinones **4b**–**e** with pyrrole aldehyde **5**[11] in the presence of ethanolic KOH. Dipyrrinones **3a**–**e** are bright yellow nonfluorescent pigments in organic solvents at room temperature. They were converted in excellent yield to their *N*,*N*′-carbonyl-bridged analogues (**2a**–**e**) by reaction with CDI in CH_2Cl_2 in the presence of DBU. The bridged dipyrrinones (**2a**–**e**) are much more soluble in organic solvents than their dipyrrinone precursors, and they fluoresce intensely with a yellow-green color. They could be sulfonated selectively at C(8) by reaction with concentrated H_2SO_4 to produce the desired sulfoglows **1a**–**e**, which, like **2a**–**e**, are also intensely fluorescent. The increase in lipophilicity from **1a** to **1b** and **1c** to **1d** and **1e** is very noticeable in the workup and manipulation of the sulfonic acid sodium salts. Thus, in the aqueous workup followed by evaporation of water, solid **1a**–**c** were separated from inorganic salts by washing into absolute ethanol. Pigments **1b** and **1c** were more soluble than **1a** in ethanol and could be further purified by radial chromatography. In contrast, **1d** and **1e** could be extracted directly into $CHCl_3$ from aqueous solution. Compared with **1a**–**c**,**d**,**e** are more amenable to chromatography and crystallization.

Barton–Zard-type syntheses[13–15] of tosylpyrroles **8a**,**b**,**d**,**e** proceeded smoothly from inexpensive starting materials; nitroethane and propionaldehyde (to give **8a**), valeraldehyde (to **8b**), caprylaldehyde (to **8d**), or 1-undecylic aldehyde (to **8e**), as outlined in Figure 2B. A similar route to **8c** required more expensive nitropentane,

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TABLE 1. Fluorescence[a] of *N*,*N*′-Carbonyl-Bridged Dipyrrinones 1 and 2 at ~10^{-6} M Concentration

bridged dipyrrinone	C_6H_6		$CHCl_3$		CH_3OH		$(CH_3)_2SO$		H_2O	
	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}	ϕ_F	λ_{em}
1a	0.54	487	0.48	494	0.30	517	0.51	502	0.30	529
1b	0.67	503	0.43	489	0.41	516	0.67	503	0.29	528
1c	0.81	504	0.55	493	0.48	515	0.81	504	0.27	523
1d	0.80	502	0.47	493	0.46	515	0.80	502	0.26	528
1e	0.50	482	0.49	492	0.45	517	0.74	503	0.24	527
2a	0.82	502	0.87	491	0.46	502	0.82	503	*b*	
2b	0.90	502	0.91	491	0.55	524	0.90	502	*b*	
2c	0.87	502	0.90	491	0.50	522	0.87	502	*b*	
2d	0.87	502	0.86	489	0.49	524	0.87	502	*b*	
2e	0.74	472	0.66	490	0.36	523	0.67	501	*b*	

[a] λ_{exc} = excitation wavelength, varying from 409 to 420 nm; λ_{em} = emission wavelength in nm; ϕ_F = fluorescence quantum yield. [b] Insoluble.

plus acetaldehyde.[13] A less expensive route was suggested in the work of Kohori et al.:[18] that tosylpyrrole **8b** might be equilibrated to a mixture of **8b** and **8c**, catalyzed by TFA (Figure 2C). In our hands, such isomerization gave a 90% recovery of isomerized tosylpyrroles in a 4:1 ratio favoring **8c**. The reaction darkens rapidly, producing byproducts, including a dark side product that is not easily removed by chromatography. An oily reaction mixture of crude **8b** + **8c** was clarified by repeated filtration chromatography and then brominated with *N*,*N*,*N*-trimethylphenylammonium tribromide (PTT) at the free α-site of the tosylpyrroles. The resulting bromotosylpyrroles (**7b** + **7c**) were crystalline, and pure **7c** was obtained by fractional crystallization in 28% overall yield from pure **8b**. Treatment of **7c** with TFA−H_2O afforded the tosylpyrrolinone **6c** in 39% yield, which gave **4c**, as previously described.[13] Unfortunately, this isomerization procedure, when applied to **8d** did not lead to crystalline products at the various reaction stages and so the *exo* analogue of **1d** was not prepared, nor was the *exo* analogue of **1e**.

Structures and Spectroscopy. The structures of **1a**−**e** follow logically from their synthetic precursors: **2a**−**e** and **3a**−**e**, and from characterization by NMR. Especially noticeable in converting **3a**−**e** into **2a**−**e** is the disappearance of the lactam and pyrrole NH signals from **2a**−**e** in the ^{1}H NMR, and the appearance in the ^{13}C NMR of a characteristic new signal at ~143 ppm from the new *N*,*N*′-imide carbonyl.[7c,8] But the most striking visible manifestation of the conversion of **3** → **2** is the emergence of strong fluorescence during the course of the reaction—an event that characteristically signals resistance to isomerization about the dipyrrinone C(4)−C(5) double bond.

As observed previously for *N*,*N*′-carbonyl-bridged *syn*-dipyrrinones,[7c,8] sulfonated analogues **1a**−**e** gave pronounced hypochromicity and a bathochromically shifted λ_{max} of the long-wavelength UV−vis transition relative to unbridged dipyrrinones **3**, with only a small influence due to changes in solvent type and polarity (Table S-1, Supporting Information). Solutions of **1** were visibly strongly fluorescent (Table 1). Excitation of the long wavelength band (409−420 nm) produced intense fluorescence between 490 and 530 nm, with a large Stokes shift. The relative fluorescence quantum yields at room temperature in C_6H_6, $CHCl_3$, CH_3OH, DMSO, and H_2O determined[8] versus 9,10-diphenylanthracene standard, $\phi_F = 0.90 \pm 0.02$,[19] were typically very large (ϕ_F 0.5−0.8) in organic solvents, but in water they were approximately halved (ϕ_F ~0.3). The strong fluorescence is consistent with radiative de-excitation being the dominant relaxation pathway for return to the ground state because nonradiative pathways cannot be accessed, e.g., photoisomerization from 4*Z* to 4*E*. The small values of ϕ_F in nonpolar solvents may be caused by dimer formation in these rather polar derivatives of *N*,*N*′-carbonyl dipyrrinones, which exhibited far greater solubility in all solvents tested and very high ϕ_F values (>0.65) in benzene and in cyclohexane than the analogous xanthoglows with carboxylic side chains.

Metabolism. The biliary excretion of small (~0.25 mg) intravenous bolus doses of **1a**−**d** was studied in normal (Sprague−Dawley) male rats and in homozygous male TR$^-$ and Gunn rats. TR$^-$ rats are a mutant strain that lacks the canalicular membrane transporter Mrp2 (ABCC2),[20,21] which is required for efficient excretion of bilirubin glucuronides in bile. Biliary excretion of bilirubin glucuronides and other endogenous cholephiles is markedly impaired in these animals compared to normal rats. Gunn rats are a mutant strain that is deficient in the UGT1A family of glucuronosyl transferases, including UGT1A1, the specific isozyme that catalyzes bilirubin glucuronidation.[22] Their bile is devoid of bilirubin glucuronides, and we used them for these studies to simplify the appearance and analysis of HPLC chromatograms of bile measured with diode-array detection in the 400−450 nm range. In terms of Mrp2 and canalicular transport they are not significantly different from normal rats.

Biliary excretion of each compound was studied in detail in four Mrp2-normal rats (including at least one Gunn and one Sprague−Dawley rat) and at least two TR$^-$ rats. Urine was collected for HPLC analysis during the experiments at irregular intervals whenever the animal spontaneously micturated and in some animals by aspiration of the urinary bladder at the end of the experiment.

Figure 3 shows HPLC chromatograms of bile from a Gunn rat, a Sprague−Dawley rat, and a TR$^-$ rat before and after intravenous injection of a bolus dose of **1b**, which has a butyl substituent at C(3). The chromatograms show clearly that the compound is taken up rapidly by the liver and excreted in bile predominantly in unchanged form. Compounds **1a** and **1c**−**e** behaved similarly. Metabolites of **1a**−**c** were not observed in bile or urine, but very minor amounts of more polar metabolites of the heptyl- and decyl-substituted analogues (**1d** and **1e**) were detected. These were not identified, but were clearly not UGT1A-formed glucuronides since they were produced in both Gunn and Sprague−Dawley rats.

The biliary excretion profile for **1c**, with an *exo* *n*-butyl substituent at C(2), in Gunn/Sprague−Dawley rats,

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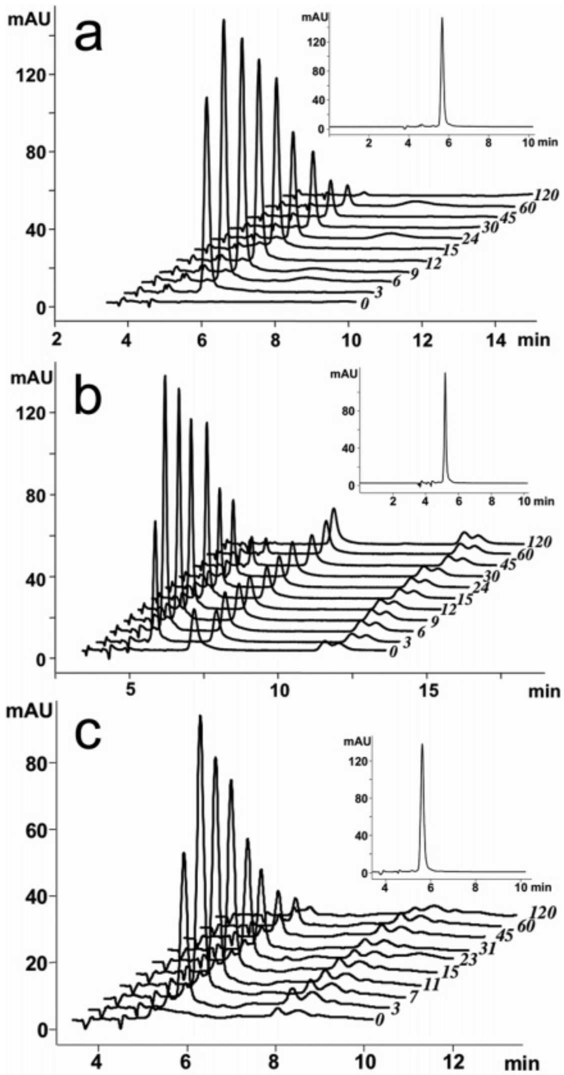

FIGURE 3. HPLC chromatograms of bile from rats before (0 min) and at indicated times (3−120 min) after intravenous injection of 0.25 mg of **1b**: (a) Gunn rat; (b) Sprague−Dawley rat; (c) TR$^-$ rat. The inset in each panel shows a chromatogram of the serum solution of **1b** that was injected into the animal. Peaks eluting after the main **1b** peak in b are di- and monoglucuronides of bilirubin; those eluting after the main **1b** peak in c are acyl-migration isomers of bilirubin glucuronides. HPLC conditions used for each panel were similar but not identical.

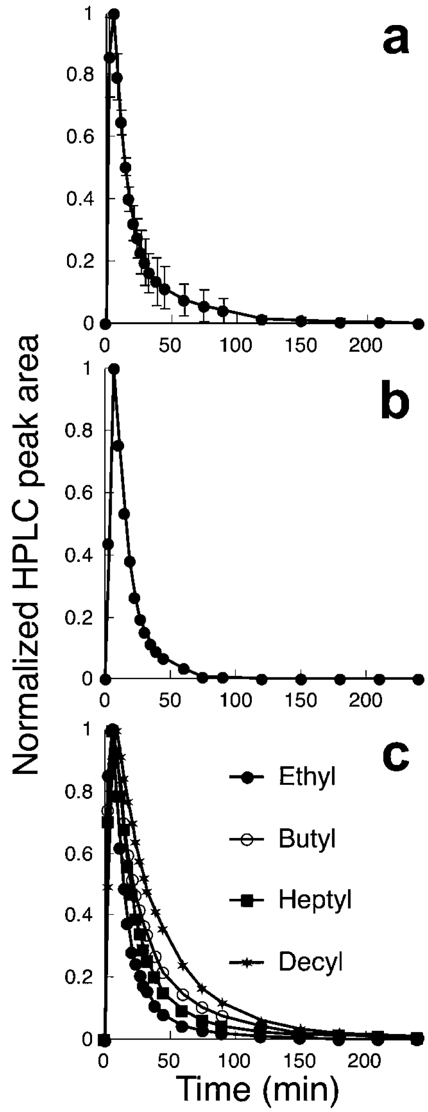

FIGURE 4. Biliary excretion profiles of **1a**−**e** in rats. Panel a: Mean data for the excretion of **1c** in three Gunn and two Sprague−Dawley rats; error bars show standard deviation. Panel b: Mean data for the excretion of **1c** in two TR$^-$ rats. Panel c: Comparative biliary excretion profiles of **1a**, **1b**, **1d**, and **1e** in Gunn/Sprague−Dawley rats. Each line represents mean data from at least four experiments. Error bars omitted for clarity.

based on the HPLC data, is shown in Figure 4a and for all four *endo*-alkyl-substituted compounds (**1a**, **1b**, **1d**, and **1e)** in Figure 4c. The biliary excretion profiles for the four compounds were similar, with rapid appearance of each compound in bile within minutes of injection and complete disappearance within ~3 h. As expected, excretion of the *endo*-ethyl congener (**1a**) was fastest and excretion of the *endo*-*n*-decyl analogue (**1e**) slowest, with the *endo*-*n*-butyl (**1b**) and *endo*-*n*-heptyl (**1d**) derivatives intermediate. However, counterintuitively, the *endo*-*n*-heptyl compound (**1d**) appeared to be excreted somewhat faster than the *endo*-*n*-butyl derivative (**1b**). Excretion of the latter was slightly slower than its *exo*-substituted counterpart (**1c**). The fraction of the injected dose excreted in bile in 4 h in Gunn and Sprague−Dawley rats was similar for all five compounds studied with a mean value of 0.6 ± 0.2. In TR$^-$ rats the kinetic profiles of biliary excretion (e.g., Figure 4b) were similar to those in Gunn and Sprague−Dawley rats, but the fraction of the injected dose that was excreted was reduced to a mean value of 0.3 ± 0.1.

For compounds **1a**−**c**, with ethyl and butyl substituents, substantial amounts of unchanged compound were detected by HPLC in urine collected during or at the end of the experiments. In contrast, compounds **1d** and **1e**, with heptyl and decyl substituents, were undetectable or present in only trace amounts in urine following intravenous injection.

The in vivo experiments show that **1a**−**e** are taken up efficiently by the liver and excreted rapidly in bile following intravenous injection. Their fractional excretion in bile was greatly diminished in the Mrp2-deficient TR$^-$ animals, but this membrane protein is clearly not essential for their biliary excretion since substantial biliary excretion was observed in TR$^-$rats. In this respect, they behave somewhat differently from xanthosulfonic acid[10] (Figure 1C) and the ditaurine conjugate of bilirubin,[23] which are excreted almost as efficiently in TR$^-$rats as in normal rats or Gunn rats and do not require Mrp2 for efficient efflux from liver to bile. The Mrp2-independent pathway by which these sulfonated pigments are secreted into bile is not known; possibilities are active transport by Bsep (the bile salt export pump) or Bcrp1 (breast-cancer resistance protein 1) which are present in the canalicular membrane of the liver.[24] The mechanism of uptake of these compounds into the liver, and the identity of any transport proteins involved, is also unknown.

On the basis of the limited number of in vivo experiments reported here, increasing the length of the *endo*-alkyl substituent from ethyl to *n*-decyl did not markedly increase the fraction of the dose eliminated through the liver into bile. However, from HPLC analysis of urine samples, renal excretion of the *n*-heptyl and *n*-decyl compounds (**1d** and **1e**) was negligibly low compared to that of the ethyl- and butyl-substituted analogues. In preliminary experiments, we have observed that renal excretion of the decyl derivative **1e** remains barely detectable in rats with acute experimental cholestasis caused by ligation of the common bile duct, whereas, in contrast, renal excretion of the heptyl sulfoglow **1d**, and more polar fluorescent metabolites, was readily detectable under the same conditions. Thus, **1d** appears to be on the border between cholephilic sulfoglows that undergo some renal excretion and those that do not, and in its behavior close to the desired goal of a fluorescent probe that shows renal elimination only when hepatic elimination is blocked.

Experimental Section

For general procedures, see refs 8−10 and 13. Di-*n*-octylamine, used for preparing HPLC solvent, was obtained from a commercial supplier. Fluorescence measurements were done on solutions prepared as reported previously.[7c,8] Fluorescence quantum yields at 20 °C were determined as reported[8] by relating the quantum yield of the sample to that of a reference standard, 9,10-diphenylanthracene ($\phi_F = 0.90 \pm 0.02$ in cyclohexane[19]). The equation used to relate these quantum yields is given by

$$\phi_s = [(A_r F_s n_s^2)/(A_s F_r n_r^2)]\ \phi_r$$

where the subscript s refers to the sample and the subscript r refers to the reference standard; ϕ is quantum yield, A is the absorbance at the excitation wavelength, F is the integrated emission band, and n is the index of refraction (at the sodium D line) of the solvent containing the sample and the reference standard.

Pyrrolinones **4a**−**c**,[12,13] aldehyde **5**,[11] dipyrrinone **3a**,[17] *N,N'*-carbonyl-bridged dipyrrinone **2a**,[7c] and its sulfonated derivative **1a**[7c] were synthesized according to previously published methods. See the Supporting Information for the syntheses of compounds **1c**, **2c**, and **3c**, **1d**; **2d**, **3d**, **4d**, **6d**, **7d**, **8d**, **9d**, **10d**; and **1e**, **2e**, **3e**, **4e**, **6e**, **7e**, **8e**, **9e**, and **10e**.

5-Bromo-4-*n*-butyl-3-methyl-2-*p*-toluenesulfonyl-1*H*-pyrrole (7c).[13] 3-Butyl-4-methyl-2-*p*-toluenesulfonyl-1*H*-pyrrole (**8b**) (5.00 g, 17.2 mmol) was dissolved into 100 mL of a solution of 10% TFA in CH_2Cl_2 (v/v). The resulting solution was stirred 24 h before being diluted with an additional 100 mL of CH_2Cl_2, and then it was washed with 4 × 200 mL of water, 1 × 200 mL of saturated aqueous Na_2CO_3, and 1 × 200 mL of brine solution. The dark organic layer was then dried over anhyd Na_2SO_4, and the solvent was removed by roto-evaporation to give a crude deep-blue oil. The oil (**8b** + **8c**) was rectified by repeatedly passing it through a small column of TLC-grade silica under reduced pressure (using CH_2Cl_2 as an eluent). After purification, a light yellow oil (3.63 g, 12.3 mmol) was obtained and taken up in 32 mL of CH_2Cl_2. The stirred solution was chilled to 0 °C, and then a solution of 6.93 g (18.5 mmol, 1.5 equiv) of phenyl-*N,N,N*-trimethylammonium tribromide (PTT) in 60 mL of CH_2Cl_2 was added dropwise. The resulting deep brown solution was stirred at 0 °C for 2 h before being brought up to a total volume of 100 mL by adding CH_2Cl_2. It was then washed with $Na_2S_2O_3$ solution (2 × 100 mL), water (1 × 100 mL), and brine solution (1 × 100 mL). The organic layer was dried over anhyd Na_2SO_4, and after removal of the solvent the resulting dark brown oil was purified by filtration through TLC-grade silica. The purified oil (**7b** + **7c**) crystallized with the addition of hexane to give grayish crystals that could be recrystallized from CH_2Cl_2−hexane to give 1.75 g (4.73 mmol) of pure bromopyrrole (**7c**) (28%): crystals; mp 174−176 °C (lit.[13] mp 177−178 °C); ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, J = 7.3 Hz), 1.26−1.50 (4H, m), 2.17 (3H, s), 2.30 (2H, t, J = 7.3 Hz), 2.41 (3H, s), 7.30 (2H, d, J = 8.4 Hz), 7.76 (2H, d, J = 8.4 Hz), 8.78 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 9.9, 14.0, 21.7, 22.6, 24.7, 32.1, 104.9, 125.6, 126.1, 126.9, 130.0, 140.0, 144.0 ppm. Bromopyrrole **7c** was converted to tosylpyrrolinone **6c** and pyrrolinone **4c** as reported previously.[13]

General Procedure for Condensation to Dipyrrinone. A solution of 2.0 mmol of pyrrolinone **4a**−**e** and 2.5 mmol of 3,5-dimethyl-2-formyl-1*H*-pyrrole (**5**)[11] in 8 mL of ethanol and 2.5 mL of 4 M KOH was heated at reflux for 1−2 days. The mixture was cooled and poured into 200 mL of ice−water. The precipitated yellow product was collected by filtration, washed with water (3 × 50 mL), and dried under vacuum (P_2O_5) to give crude dipyrrinones **3**. Alternatively, **3d**,**e** were extracted into CH_2Cl_2. The extracts were washed with H_2O, and after removal of solvent, the residue was purified by radial chromatography. The products **3a**−**e** were recrystallized from CH_3OH or CH_2Cl_2−hexane to give pure dipyrrinones.

3-*n*-Butyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (3b). The general procedure gave the desired dipyrrinone from 0.25 g (1.63 mmol) of 4-*n*-butyl-3-methylpyrrolin-2-one (**4b**) in 48% yield (0.20 g, 0.78 mmol): mp 140−141 °C; IR (thin film) ν 3349, 2929, 1671, 1636, 1582, 1477, 1380, 1264 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (3H, t, J = 7.3 Hz), 1.39 (2H, m), 1.55 (2H, m), 1.94 (3H, s), 2.18 (3H, s), 2.45 (3H, s), 2.54 (2H, t, J = 7.3 Hz), 5.83 (1H, s), 6.13 (1H, s), 10.48 (1H, br.s), 11.35 (1H, br s) ppm; ^{13}C NMR ($CDCl_3$) δ 8.6, 11.4, 13.4, 13.8, 22.5, 24.3, 32.6, 101.3, 110.1, 123.1, 123.2, 126.3, 126.3, 127.6, 134.2, 147.0, 174.1 ppm. Anal. Calcd for $C_{16}H_{22}N_2O$ (258.4): C, 74.38; H, 8.56; N, 10.84. Found: C, 74.02; H, 8.36; N, 10.37.

General Procedure for Inserting Bridging Carbonyl. Dipyrrinone **3a**−**e** (0.50 mmol) was dissolved in 35 mL of dry CH_2Cl_2. To this solution was added 0.41 g (2.50 mmol, 5 equiv) of 1,1′-carbonyldiimidazole, 0.38 g (2.50 mmol, 5 equiv) of DBU, and 0.50 g of 4 Å molecular sieves. The solution was allowed to reflux with magnetic stirring for 19 h before being cooled and filtered to remove the molecular sieve debris. The filtered solution was then washed sequentially with water (2 × 50 mL) and brine (50 mL) and then dried over anhyd $MgSO_4$. The organic solvent was removed (rotovap) to give a crude brownish oil, which was purified by radial chromatography (using 1% MeOH in CH_2Cl_2 as eluent) to give bright yellow crystals of the pure product.

3-*n*-Butyl-2,7,9-trimethyl-*N,N'*-carbonyl-(10*H*)-dipyrrin-1-one (2b). Using the general procedure for inserting a bridging carbonyl, 0.25 g (1.00 mmol) of **3b** gave 0.18 g (0.63 mmol, 63%) of a product (**2b**): mp 150−152 °C; IR (thin film)

(23) Jansen, P. L. M.; Vanklinken, J. W.; Vangelder, M.; Ottenhoff, R.; Oude Elferink, R. P. J. *Am. J. Physiol.* **1993**, *265*, G445−G452.
(24) Chandra, P.; Brouwer, K. L. *Pharm. Res.* **2004**, *21*, 719−735.

ν 2927, 1760, 1684, 1521, 1308 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, J = 7.3 Hz), 1.25−1.60 (4H, m), 1.96 (3H, s), 2.15 (3H, s), 2.52 (2H, t, J = 7.3 Hz), 2.68 (3H, s), 5.83 (1H, s), 6.12 (1H, s) ppm; ^{13}C NMR ($CDCl_3$) δ 10.2, 13.5, 13.8, 15.6, 22.5, 23.3, 30.6, 97.2, 117.2, 120.8, 126.9, 131.4, 131.5, 135.0, 140.7, 143.5, 167.3 ppm. Anal. Calcd for $C_{17}H_{20}N_2O_2$ (284.4): C, 71.81; H, 7.09; N, 9.85. Found: C, 71.89; H, 7.09; N, 9.48.

General Procedure for Sulfonation to 1b−e. Finely powdered solid bridged dipyrrinone **2b−e** (0.40 mmol) was placed in a flask immersed in a cooling bath at 0 °C, and 5 mL of concd H_2SO_4 (precooled to 0 °C) was added. The solid slowly dissolved to give a magenta solution, which was stirred for 2 h at 0 °C. The temperature was then lowered to −5 to −10 °C, and the solution was slowly neutralized with saturated aqueous Na_2CO_3 while introducing a stream of air in order to reduce foaming. After dilution with water to ~150 mL, extraction with CH_2Cl_2 resulted in removal of the starting material in the case of **1b** and **1c**. In the case of **1d** and **1e**, the product was also extracted. The latter two, after evaporation of CH_2Cl_2, were purified by radial chromatography. In the case of **1b** and **1c**, the yellow alkaline aqueous solution was evaporated to dryness and the pigment was removed from the solid mixture with Na_2SO_4 by extraction with absolute EtOH. After evaporation of the ethanol, the residue was purified by radial chromatography.

Sodium 3-*n*-Butyl-2,7,9-trimethyl-*N*,*N*′-carbonyl-(10*H*)-dipyrrin-1-one-8-sulfonate (1b). Following the general procedure, tricyclic **2b** (100 mg, 0.35 mmol) was converted to 88 mg (65%) of yellow salt **1b**: mp >300 °C; IR (thin film) ν 2927, 1769, 1684, 1317 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, J = 7.5 Hz), 1.25−1.60 (4H, m), 1.81 (3H, s), 2.26 (3H, s), 2.58 (2H, t, J = 7.5 Hz), 2.77 (3H, s), 6.96 (1H, s) ppm; ^{13}C NMR (DMSO-d_6) δ 8.3, 10.1, 13.5, 13.8, 22.0, 23.5, 31.0, 98.3, 120.0, 125.6, 125.7, 130.8, 131.5, 134.4, 142.9, 145.9, 166.9 ppm; HRMS (FAB, 3-NBA) calcd 365.1171 ($C_{17}H_{21}N_2O_5S$, M + H$^+$), found 365.1186, Δ = 1.5 mDa, error 4.1 ppm.

Metabolism Studies. Sprague−Dawley rats were from local suppliers and Gunn and TR$^-$ rats from our own colonies. For excretion studies, the femoral vein and common bile duct of an adult male (>250 g) were cannulated under ketamine anesthesia and a liposomal solution containing phosphatidylcholine (1.5 g), cholesterol (62 mg), sodium cholate (12.95 g), and taurine (3.75 g) in 1 L of water was infused (2 mL/h) through the femoral catheter to maintain hydration and bile flow. The total length of the biliary cannula was 7.5 cm. The animal was placed in a restraining cage under an infrared heating lamp and, once bile flow and body temperature were stable (~30−60 min), pigment (0.25 mg), dissolved in normal rat serum (1 mL) with the aid of a small volume (0.1 mL) of DMSO, was infused via the femoral vein as a bolus over a period of 20−45 s. Bile was collected in 20-μL aliquots into micropipets from the tip of the bile duct cannula immediately before injection of pigment, 3 min after injection, and at frequent intervals thereafter. Samples were flash frozen immediately in dry ice and then kept at −70 °C until analyzed by HPLC. Collection of each bile sample took <1 min for Sprague−Dawley and Gunn rats but often exceeded 1 min for TR$^-$ rats because of their relatively slow bile flow rates. Consequently, the biliary excretion curves for experiments in TR$^-$ rats are less precise than those for the other two strains. Bile flow rates were measured gravimetrically by periodically collecting timed 5-min aliquots of bile into tared Pasteur tubes. Urine samples were collected in 20-μL aliquots into micropipets from the tip of the penis or by aspiration of the urinary bladder at the end of each experiment. For HPLC, frozen bile samples (20 μL) were mixed with 80 μL of ice-cold 0.1 M methanolic di-*n*-octylamine acetate, microfuged for 30 s, and the supernate (20 μL) was injected onto the column. Isocratic HPLC analyses were run using a Beckman-Altex Ultrapshere-IP 5 μm C-18 ODS column (25 × 0.46 cm) fitted with a similarly packed precolumn (4.5 × 0.46 cm) and Hewlett-Packard multiwavelength diode array detector. Compounds were monitored at their absorbance maxima and peak areas measured using HP ChemStation software. The elution solvent was 0.1 M di-*n*-octylamine acetate in 5−8% aqueous methanol, flow rate 0.75 mL/min, and column temperature 35−38 °C. Biliary excretion curves were derived by plotting integrated HPLC peak areas normalized to the maximum peak area. The fraction of the injected dose excreted was estimated by comparing the area under the biliary excretion curve (HPLC peak area versus time), adjusted for total bile volume excreted, with the HPLC peak area of the pigment in a 20-μL sample of the original serum solution injected into the rat. Areas under biliary excretion curves were determined using Un-Scan-It software (Silk Scientific, Inc., Orem, UT). Except for the animal surgery, metabolic procedures were done under orange or red safelights in a darkroom.

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Supporting Information Available: The syntheses of **1c−3c**, **1d−4d**, **6d−10d**, **1e−4e**, and **6e−10e** are described. UV−vis spectral data from compounds **1−3** are available in Table S-1. This material is available free of charge via the Internet at http://pubs.acs.org.

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