Published in final edited form as:

Tetrahedron. 2009 January 3; 65(1): 77–82. doi:10.1016/j.tet.2008.09.082.

Carboxylic Acid to Thioamide Hydrogen Bonding

Suchitra Datta and David A. Lightner*

Department of Chemistry, University of Nevada, Reno, Nevada 89557

Abstract

The lactam groups of dipyrinones avidly engage in amide-amide hydrogen bonding to form dimeric association complexes in nonpolar solvents (in CHCl_3 , $K_D \sim 25,000 \text{ M}^{-1}$ at 22°C). The corresponding thioamides (dipyrinethiones), prepared from dipyrinones by reaction with Lawesson's reagent, also form intermolecularly hydrogen-bonded dimers in nonpolar solvents, albeit with much weaker association constants (in CHCl_3 , $K_D \sim 200 \text{ M}^{-1}$ at 22°C). When a carboxylic acid group is tethered to C(9) of the dipyrinone, as in the hexanoic acid of [6]-semirubin, tight intramolecular hydrogen bonding between the carboxylic acid group and the lactam moiety (intramolecular $K_{\text{assoc}} \gg 25,000$) is found in CHCl_3 with no evidence of dimers. In contrast, the analogous dipyrinethione, [6]-thiosemirubin, eschews intramolecular hydrogen bonds, as determined using NMR spectroscopy and vapor pressure osmometry, preferring to form intermolecularly hydrogen-bonded dimers of the thioamide-thioamide type.

Keywords

Pyrroles; dipyrinethiones; NMR; VPO

1. Introduction

Although not as well studied as amide-amide and carboxylic acid-carboxylic acid hydrogen bonding,¹ amides are known to form strong hydrogen bonds to carboxylic acids, as was first recognized in bilirubin (Fig. 1A),^{2,3} the yellow pigment of jaundice.⁴ There, nature designed a molecule whose lipophilic character and poor aqueous solubility ($K_{\text{sp}} \sim 4 \times 10^{-15} \text{ M}$ at 37°C)⁵ may be understood by considering the pigment's structure in which both propionic acids are tucked inward and firmly *intramolecularly* hydrogen-bonded to the opposing dipyrinones,^{2,3,6} the key factor in bilirubin's aqueous solubility and metabolism. In further illustration of this attraction, strong, selective *intermolecular* hydrogen bonding between a carboxylic acid and a pyridone lactam has also been reported,⁷ to the exclusion of carboxylic acid-carboxylic acid and amide-amide hydrogen bonding. The unusual type of hydrogen bonding seen in bilirubin is probably facilitated by the adjacency of the pyrrole hydrogen and has been replicated in simpler analogs such as [6]-semirubin (Fig. 1B)⁸ and related analogs where recent studies have shown that the dipyrinone moiety⁹ is an excellent receptor for a carboxylic acid group^{10,11} — and probably also a carboxylate anion.^{3c,6b}

The essential components for the hydrogen bonding seen in bilirubin are an amide (lactam) of a Z-configuration dipyrinone and a carboxylic acid chain of at least 6 carbons that is tethered

*Corresponding author: Tel +775-784-4980; fax +775-784-6804; email lightner @scs.unr.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

at C(9). This complementary combination was designed into the synthetic pigment model for one-half of bilirubin, [6]-semirubin (**1**) (Fig. 1B),⁸ whose dipyrinones, like those in bilirubin, have the *syn-Z* configuration,^{3,6,8} and which was shown to be a monomer in chloroform with its CO₂H group engaged tightly in intramolecular hydrogen bonding (Fig 1A,B).⁸ In contrast, the ethyl ester (**2**) of **1** and simple dipyrinones with an alkyl group at C(9) replacing the carboxylic acid chain, e.g., kryptopyrromethenone (**3**), were shown to form strongly intermolecularly hydrogen-bonded dimeric structures (Fig. 1C) in CDCl₃ with large association constants ($K_D \sim 25,000 \text{ M}^{-1}$ at 22°C)¹² — surprisingly, much larger than those of simple amides, whose K_D varies from ~ 30 –7,000.¹³ The observation that [6]-semirubin (**1**) eschews dipyrinone to dipyrinone dimer formation⁸ is consistent with the dipyrinone unit strongly favoring ($K_{\text{assoc}} \gg 25,000$) complexation to the carboxylic acid group over a second dipyrinone molecule. As such, **1** is an excellent model for exploring amide to carboxylic hydrogen bonding.

Though less well studied, thioamides, like amides, are known to dimerize in nonpolar solvents by forming intermolecular hydrogen bonds. The simple thiolactams, γ -butyrolactam and δ -valerolactam, were shown to exhibit dimerization equilibrium constants ($K_D = 278$ and 438 M^{-1} in CCl₄ at 25°C)^{13a,b} similar to the parent lactams.^{13a,b} But the thiolactam analogs of 2-pyridone were found to have variable, but always lower K_D values than the parent: 4100 M^{-1} vs 7100 M^{-1} in CCl₄,^{13c} 28 vs 2200 M^{-1} in benzene,^{13d} and $2.7 \pm 1.0 \times 10^3 \text{ M}^{-1}$ vs $2.5 \pm 1.0 \times 10^4 \text{ M}^{-1}$ in CHCl₃.¹⁴ To the best of our knowledge, hydrogen bonding between a thioamide and a carboxylic acid remains unknown.

In order to explore and evaluate the potential for such hydrogen bonding we took advantage of the special ability of dipyrinones to sequester a carboxylic acid group. Using [6]-semirubin (**1**) as a model, we perturbed the dipyrinone by converting its lactam moiety to thiolactam to give the dipyrinithione, [6]-thiosemirubin (**4**). For comparison and evaluation of thiolactam-thiolactam association involving hydrogen bonding, we prepared the dipyrinithiones, methyl thioxanthobilirubinate (**5**),¹⁵ and kryptopyrromethenethione (**6**)¹⁵ (Fig. 2A), from which we could further evaluate the thioamide-thioamide hydrogen bonding and whose K_D values might serve as a reference value to be exceeded if intramolecular hydrogen bonding prevails in **4**. In the following, we describe the syntheses of **4**, its methyl ester (**8**), and dipyrinithiones **5** and **6** (Fig. 2), and we analyze their ability to engage in hydrogen bonding in terms of inter- and intramolecular hydrogen bonded structures.

2. Results and Discussion

2.1. Synthesis

The syntheses of **4**, **5**, **6** and **8** involve thionation of the lactam units of the corresponding dipyrinones using Lawesson's reagent.¹⁶ We had previously converted kryptopyrromethenone **3** to its thioamide analog (**6**) in 82% yield¹⁵ by treatment in dry THF with one equiv. of Lawesson's reagent for 2 h. Under similar reaction conditions, the known methyl xanthobilirubinate (**7**)¹⁷ was converted smoothly to the thioamide analog (**5**) in 83% yield. Synthesis of **4** and **8** (Scheme 1) required the known [6]-semirubin (**1**)⁷ and its methyl ester (**7**),⁷ both obtained following Friedel-Crafts acylation at carbon-9 of 2,3,7,8-tetramethyl-10*H*-dipyrinone.⁸ Unlike the formation of thiolactam **5** from **7**, conversion of the amide of [6]-semirubin methyl ester (**2**) to its thioamide (**8**) with Lawesson's reagent proceeded inefficiently (32% yield). The reason for this is unclear and, disappointingly, methyl ester **8** resisted conversion to the parent free acid (**4**). Numerous attempts at converting **8** to **4** led to decomposition, as did direct thionation of dipyrinone acid **1** to **4**. In order to circumvent these problems, especially the difficulties encountered in the methyl ester hydrolysis step (**8** $\rightarrow$ **4**), [6]-semirubin (**1**) was converted to its more easily deprotected *tert*-butyldiphenylsilyl ester (**9**) in good yield. Successful thionation of **9** using Lawesson's reagent, followed by fluoride

ion deprotection of the silyl ester group, afforded the desired [6]-thiosemirubin (**4**) in 32% isolated yield from **9**.

2.2. Constitutional Structures from NMR

The constitutional structures of **4** and **8** follow from the structures of their precursors, (**1** and **2** of Fig. 1 and Scheme 1), and from their ^{13}C -NMR spectra (Table 1). Thus, evidence for the loss of the lactam C=O at ~174 ppm and the appearance of the C=S carbon at ~187 ppm correlates with the C=S chemical shifts of dipyrinethiones **5** and **6**¹⁵ and the expectation of a more strongly deshielded carbon. Likewise, C(2) is deshielded to ~132 ppm in **4** and **8** (and **5** and **6**) relative to the lactam parents' ~123 ppm. Similar, but smaller deshieldings at C(4) and C(5) are found in **4**, **8** (**5** and **6**) relative to their parent dipyrinones. Interestingly, the usual small (0.1 ppm) deshielding of C(5) in the acid relative to the ester (**1** vs **2**) is much smaller than the (~0.6 ppm) shielding in thiosemirubins **4** and **8**.

The 4Z-configuration of dipyrinones (Figs. 1 and 2, and Schemes), as well as bilirubin, has been long established: 2 in the solid by X-ray crystallography;^{3,18,19} in solution by nuclear Overhauser effect (NOE) spectroscopy, where NOEs are found between C(5)-H and the C(3) and C(7) CH₃ groups, and between the two NHs.^{2,18,20} Thus, it came as no surprise that NOE studies of **4**, **6** and **8** indicated the favored *syn-Z* configuration (Fig. 3). *E*-configuration dipyrinones are accessible only by photoirradiation.²¹

2.3. Hydrogen Bonding

The NH chemical shifts of dipyrinones have frequently been used in ^1H -NMR to detect hydrogen bonding: intermolecular between two dipyrinones²⁰ and between a dipyrinone and a carboxylic acid.^{6,8-10} It seemed reasonable to assume that this structure-distinguishing probe might carry over to dipyrinethiones. Dipyrinones that engage exclusively in intermolecular hydrogen bonding of the dipyrinone to dipyrinone type in CDCl₃, as in **2**, **3** and **7** (Table 2), show lactam NH resonances near 11 ppm and pyrrole NHs near 10 ppm; whereas, with the presence of a carboxylic acid group, as in [6]-semirubin, the pyrrole NH moves upfield by ~1 ppm (to ~9 ppm), and the lactam NH also moves upfield (to ~10.4 ppm). In (CD₃)₂SO a leveling effect is exerted and the lactam NH signals of **1**, **2**, **3** and **7** are more shielded (to ~9.8 ppm) than the pyrrole NH (~10.2 ppm).

With dipyrinethiones **5** and **6** as reference standards for investigating the pyrrole and lactam NH chemical shift, we note that in CDCl₃, consistent with those of the parent dipyrinones (**7** and **3**), the lactam NH (~11.5 ppm) is more deshielded than the pyrrole NH (~10 ppm). And in fact the thiolactam and pyrrole NH chemical shifts (11.47 and 11.41, and 9.95 and 9.94 ppm) of **5** and **6** are not very different from the lactam (11.15 and 11.10) and pyrrole (10.02 and 10.05) NH chemical shifts of **3** and **7**. Remarkably, the thiolactam NH chemical shifts of **5** and **6** are nearly identical in CDCl₃ and (CD₃)₂SO, which is not seen in the parent lactams, **3** and **7**; yet the pyrrole NH chemical shifts of **5** and **6** in CDCl₃ are shielded by ~1 ppm from those in (CD₃)₂SO — a much larger difference than found in **3** and **7** (0.01-0.28 ppm). The reasons for this are unclear. Comparing **4** and **8** to **5** and **6**, we note that the NH chemical shifts of both **4** and **8** in CDCl₃ and (CD₃)₂SO are similar to those of **5** and **6** in the corresponding solvent. These data suggest similar environments for the dipyrinethiones in (CD₃)₂SO, as is typically found for this solvent. Unexpectedly, the data also suggest similar environments in CDCl₃ — a solvent where intramolecular hydrogen bonding between carboxylic acid and dipyrinethione might have been expected in **4**, and dipyrinethione to dipyrinethione hydrogen-bonded dimer formation expected only for **5**, **6** and **8** — analogous to **1** vs **2**, **3** and **7**. However these data alone do not disprove dipyrinethione to CO₂H hydrogen bonding in **4**.

2.4. NOE Measurements

One indicator of dimer formation lies with NOE data from ^1H -NMR spectroscopy in CDCl_3 . Thus, when dipyrinones form intermolecularly hydrogen-bonded dimers, an NOE is observed between the $\text{C}(2^1)$ hydrogens and those at $\text{C}(9^1)$, as has been noted for **3**, **7** and even **1**. In contrast, when the dipyrinone sequesters a CO_2H group through hydrogen bonds, an NOE is observed between the CO_2H hydrogen and the dipyrinone lactam NH — as is seen in [6]-semirubin (**1**), bilirubin, etc. For dipyrinethiones **5**, **6** and **8** we observe NOEs (Fig. 3) of the former type. For **4**, we also see the former type; not the latter. These data suggest that all of the dipyrinethiones of this study form predominantly *intermolecularly* hydrogen-bonded dimers in CDCl_3 . Again, however, although the failure to find a CO_2H to dipyrinethione NOE does not support carboxylic acid to dipyrinethione hydrogen bonding, it does exclude its possibility.

2.5. Beer's Law Behavior

Further evidence for a monomer $\rightleftharpoons$ dimer equilibrium may be found in the Beer's Law plots (Fig. 4) of the dipyrinethiones. The parent dipyrinone, [6]-semirubin (**1**) was found to obey Beer's Law, whereas its ester (**2**) diverges.⁸ Just as dipyrinones **2**, **3** and **7** (but not **1**) diverge from ideality with increasing concentration in CHCl_3 ,⁷ so do their thiolactam analogs **5**, **6**, and **8** — as might be expected. What was not originally expected was that **4**, like **1**, would not diverge; yet, its Beer's Law behavior, like that of **8**, is found to be consistent with dimer formation.

2.6. VPO Studies of Self-Association

In order to establish the presence of dimers in nonpolar solvents, we used vapor pressure osmometry (VPO) to analyze the molecular weights of **4**, **5**, **6** and **8** in CHCl_3 solution (Table 3). Such studies showed earlier that dipyrinones such as **3** and **7** were dimeric, that [6]-semirubin ester (**2**) was also a dimer, but [6]-semirubin (**1**) itself was a monomer. The data were part of the argument in favor of an intramolecularly hydrogen bonded structure for **1**.⁸ When VPO was applied to dipyrinethione esters **5**, **6**, and **8** we found that these compounds were *dimers in CDCl_3* , consistent with the behavior of their dipyrinone ester parents (**2**, **3** and **7**), and showing that their sulfur analogs were capable of intermolecular hydrogen bonding. Just how strong this preference might be remained to be determined. Especially revealing was the unexpected observation that **4**, unlike **1** also was a dimer in CHCl_3 — at least at the concentrations of the VPO measurement.

2.7. Dimeric Association

In an earlier study of dimerization of **3** and **7**, we determined the dimeric association constant (K_D) in CDCl_3 from the concentration dependence of the ^1H -NMR chemical shifts of the lactam and pyrrole NHs. The K_D values were large ($K_D \sim 25,000 \text{ M}$ at 25°C). Using the same method, in order to compare the tightness of hydrogen bonding in dipyrinones with their thiolactam analogs, we determined K_D for **5** and **6** in CDCl_3 and found the pyrrole and thiolactam NH chemical shifts to be more concentration-sensitive than the pyrrole and lactam NHs of the parent dipyrinones, **3** and **7**. For example, the thiolactam (δ_L) and pyrrole (δ_P) chemical shifts were observed at 11.41 and 9.94 ppm respectively at $1.33 \times 10^{-2} \text{ M}$ concentration of **6**, and at 10.27 and 9.06 ppm, respectively at $2.06 \times 10^{-3} \text{ M}$ — a difference ($\Delta\delta$) of 1.14 and 0.88 ppm, respectively. In contrast, δ_L and δ_P were observed at 11.15 and 10.25 ppm, respectively, for $1.41 \times 10^{-2} \text{ M}$ **3** and at 10.84 and 10.04 ppm, respectively, at $2.10 \times 10^{-3} \text{ M}$ — a $\Delta\delta$ of 0.31 and 0.21 ppm, respectively. This was a tip-off that the K_D of **6**, for example, might be substantially lower than that of **3**. This was realized from the K_D determinations, using the method described previously for **3** and **7**,¹⁰ where the upper plateau (Fig. 5, upper) and lower foot (Fig. 5, lower) of plots of NH chemical shifts vs pigment concentration give δ_D and δ_M for dimer and

monomer, respectively, for both the thiolactam and pyrrole NHs. Using the equations developed earlier that relate K_D to δ_D , δ_M , δ_{observed} and initial concentration of pigment, $[M_O]$, one extrapolates to K_D values that are a factor of 100 smaller than those of the dipyrinones (Table 4) — a clear indication that the thiolactam unit, unlike the lactam, in cooperation with the pyrrole behaves more or less like an ordinary thiolactam. The relative failing in this regard of the dipyrinrithione may be due to the longer C=S (vs C=O) bond that (in the dimer) increases the pyrrole $\text{NH}\cdots\text{S}=\text{C}$ distance relative to the $\text{NH}\cdots\text{O}=\text{C}$ distance.

Although the ^1H -NMR study of the concentration dependence of the pyrrole and lactam chemical shifts in CDCl_3 , VPO measurements and Beer's Law plots of [6]-semirubin (**1**) differed uniquely from its methyl ester (**2**) as well as dipyrinones **3** and **7**, we failed to detect a similar difference in its thiolactam derivative **4** relative to thiolactams **5**, **6** and **8**. For **1**, VPO indicated monomers in CHCl_3 ; its NH chemical shifts were invariant over the concentration range, and Beer's Law was obeyed — all of which are consistent with an intramolecular hydrogen bonded monomer: dipyrinone to CO_2H . We expected **4** to behave like **1** in these studies, but its behavior was much more like its ester (**8**), **5** and **6**, which may be considered to be intermolecularly hydrogen-bonded dimers in chloroform.

2.7. Solution Properties and UV-visible Spectra

Dipyrinrithiones (**4**, **5**, **6**, **8**) are red solids that form reddish solutions with long wavelength UV-vis absorption bands centered near 490 nm. In contrast, the parent dipyrinones are yellow with typical intense long-wavelength UV-vis absorption near 410 nm. Although bathochromically shifted, the absorption bands of the thiolactam analogs have molar absorptivity constants nearly unchanged from those of the parents, and their solutions are more noticeably solvatochromic (Table 5) than those of the parent dipyrinones.

3. Concluding Comments

[6]-Thiosemirubin **4** is a dimer in CHCl_3 , as measured by VPO. Its pyrrole and lactam NH chemical shifts are concentration-dependent and it fails to obey Beer's Law. We conclude that the thiolactam unit of **4**, and indeed the entire dipyrinrithione, offers no special attraction to the carboxylic acid, that the unique ability of a dipyrinone to sequester a CO_2H group through hydrogen bonding is lost in dipyrinrithiones. Since the available evidence suggests a weaker K_D (~ 300 M) for the monomer $\rightleftharpoons$ dimer equilibrium in dipyrinrithiones relative to dipyrinones, ($K_D \sim 25,000$ M), we predict that any hydrogen bonding between the dipyrinrithione and CO_2H units of **4** would have an intermolecular association constant (K_{assoc}) $< \sim 200$ -300 M.

4. Experimental Section

4.1. General Procedures

Nuclear magnetic resonance (NMR) spectra, NOEs and T_1 measurements were obtained on a Varian Unity Plus 500 MHz spectrometer in CDCl_3 solvent (unless otherwise specified) at 25°C . Chemical shifts were reported in δ ppm referenced to the residual CHCl_3 ^1H signal at 7.26 ppm and ^{13}C signal at 77.0 ppm. A combination of heteronuclear multiple bond correlation (HMBC) spectra and $^1\text{H}\{^1\text{H}\}$ NOE data were used to assign ^1H and ^{13}C NMR spectra. UV-visible spectra were recorded on a Perkin-Elmer Lambda-12 spectrophotometer. Melting points were taken on a Mel Temp capillary apparatus and are corrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. High resolution mass spectra were determined at the Nebraska Center for Mass Spectroscopy, University of Nebraska, Lincoln. Analytical thin layer chromatography was carried out on J. T. Baker silica gel IB-F plates (125 μ layers). Radial chromatography was carried out on Merck silica gel PF₂₅₄ with gypsum preparative

layer grade, using a Chromatotron (Harrison Research, Palo Alto, CA). Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). The THF used was distilled from Na/benzophenone. Deuterated chloroform and dimethylsulfoxide were from Cambridge Isotope Laboratories. 2,3,7,8-Tetramethyl-(10*H*)-dipyrrin-1-one,^{8,10} [6]-semirubin (**1**),⁸ [6]-semirubin methyl ester (**2**),⁸ kryptopyrromethenone (**3**),²² kryptopyrromethenethione (**6**),¹⁵ methyl xanthobilirubinate (**7**),¹⁷ and methyl thioxanthobilirubinate (**5**)¹⁵ were prepared as described in the literature.

The Beer's Law measurements were prepared as follows:⁸ (1) measure a standard UV-vis spectrum and calculate $\epsilon^{\max}$ at $\lambda^{\max}$. (2) Using $\epsilon^{\max}$, calculate the highest concentration possible for the shortest cell pathlength available, usually 0.5–1.0 mm, to give an absorbance = 2. (3) Prepare a solution at the concentration determined in (2) and measure the spectrum. (4) Perform serial dilutions, e.g., 1:2, etc., until the lower limit of detection of the instrument is reached. (5) Normalize the absorbance data to a pathlength = 1 cm and plot. This procedure gave the plots of Fig. 4.

4.1.1 9-[5-Carbo(*t*-butyldiphenylsilyl)pentyl]-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-one ([6]-Semirubin *t*-butyldiphenylsilyl ester) (9**)**—One equivalent of [6]-semirubin (**1**) (100 mg; 0.303 mmol) and three equivalents of imidazole (61.8 mg, 0.909 mmol) were mixed in dry THF (40 mL). Then 2 equivalents of *t*-butyldiphenylsilyl chloride (166.5 mg $\equiv$ 0.16 mL, 0.606 mmol) were added to the reaction mixture and stirred at RT. After 4 days, 15 mL of water was added, and the reaction solution was extracted with CH₂Cl₂. The organic layer was removed and dried over anhyd. Na₂SO₄, and the solvent was evaporated (rotovap) to give a yellow solid. This was purified by radial chromatography on silica gel using (2:98 CH₃OH:CH₂Cl₂). The isolated yellow solid (71% yield) mp 144–146°C, was obtained after evaporation of CH₂Cl₂-CH₃OH solution and was treated directly with Lawesson's reagent. It had ¹H-NMR δ : 1.37–1.42 (m, 2H), 1.42 (s, 9H), 1.65–1.72 (m, 4H), 1.91 (s, 3H), 1.95 (s, 3H), 2.11 (s, 6H), 2.45 (t, 2H, *J* = 7.6 Hz), 2.75 (t, 2H, *J* = 7.2 Hz), 5.92 (s, 1H), 6.13 (s, 1H), 7.36 (t, 4H), 7.41 (d, 2H), 7.65 (d, 4H, *J* = 7.5 Hz), 10.15 (s, 1H), 11.30 (s, 1H) ppm; ¹³C-NMR δ : 9.3, 9.8, 10.4, 10.6, 19.4, 25.8, 26.9, 27.5 (x 3), 29.5, 30.8, 36.8, 101.2, 116.6, 122.9, 123.8, 126.0, 128.0, 128.9, 130.7, 132.7, 136.0, 136.6, 142.9, 173.6, 174.6 ppm.

Anal. Calcd for C₃₅H₄₄N₂O₃Si (568.8): C, 73.90; H, 7.80; N, 4.92

Found: C, 73.48; H, 7.73; N, 5.32

4.1.2. 9-(5-Carboxypentyl)-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-thione ([6]-Thiosemirubin) (4**)**—One equivalent (100 mg, 0.183 mmol) of *t*-butyldiphenylsilyl ester (**9**) from above and three equivalents of Lawesson's reagent (222.6 mg, 0.550 mmol) were mixed together in dry tetrahydrofuran (20 mL), and reaction mixture was stirred at room temperature for 2 days under an N₂ atmosphere. Then 2 mL (2.0 mmol) of a 1 M solution of tetrabutylammonium fluoride in THF was added to deprotect the silyl ester, and the reaction mixture was stirred for 12 h. The THF solvent was removed (rotovap), the residue was dissolved in CH₂Cl₂, and the solution was passed through a short column of silica gel (filtration chromatography), using CH₂Cl₂ as eluent. The product was further purified by radial chromatography using CH₂Cl₂ as eluent. The deep red-colored solid (**1**) (32% yield), mp 178–180°C was obtained after evaporation of the CH₂Cl₂. It was stored in the dark under N₂ at 0°C. It had ¹H-NMR δ : 1.38–1.43 (m, 2H), 1.57–1.66 (m, 2H), 1.71–1.74 (m, 2H), 1.90 (s, 3H), 1.94 (s, 3H), 2.09 (s, 3H), 2.11 (s, 3H), 2.48 (t, 2H, *J* = 7.2 Hz), 2.72 (t, 2H, *J* = 7.2 Hz), 6.11 (s, 1H), 8.96 (s, 1H), 10.49 (s, 1H), 13.5 (br s, 1H) ppm; ¹³C-NMR data in Table 1; UV-vis spectral data are in Table 5; HRMS (FAB, glycerol); calcd for [M + H⁺] C₁₉H₂₇N₂O₂S: 347.1793; found 347.1784.

4.1.3. 9-(5-Carbomethoxypentyl)-2,3,7,8-tetramethyl-(10H)-dipyrrin-1-thione ([6]-Thiosemirubin methyl ester) (8)—One equivalent (100 mg, 0.291 mmol) of [6]-semirubin methyl ester (**2**) and three equivalents of Lawesson's reagent (352 mg, 0.872 mmol) were mixed together in dry THF (40 mL), and the reaction mixture was stirred at room temperature for 36 h under N₂. The THF solvent was removed (rotovap), the residue was dissolved in CH₂Cl₂, and the solution was passed through a short column of silica gel (filtration chromatography) using CH₂Cl₂ as eluent. The solution was evaporated and the residue was purified by radial chromatography on silica gel using CH₂Cl₂ as eluent. The deep-red colored solid (**4**) (32% yield), mp 215–217°C, was obtained after evaporation of the CH₂Cl₂. It was stored the dark under N₂ at 0°C. It had ¹H-NMR δ: 1.22–1.28 (m, 2H), 1.49–1.54 (m, 4H), 1.86 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 2.24 (t, 2H, J = 7.9 Hz), 2.67 (t, 2H, J = 7.5 Hz), 3.59 (s, 3H), 6.19 (s, 1H), 9.48 (brs, 1H), 10.98 (brs, 1H) ppm; ¹³C-NMR data in Table 1; UV-vis spectral data may be found in Table 5.

Anal. Calcd for C₂₀H₂₈N₂O₂S (360.5): C, 66.63; H, 7.83; N, 7.77.
Found: C, 67.03; H, 7.88; N, 7.42.

Acknowledgments

This work was supported by the U.S. National Institutes of Health (HD17779). SD is an R. C. Fuson Graduate Fellow. We thank the U.S. National Science Foundation (CHE-0521191) for matching funds to acquire a 400 MHz NMR spectrophotometer and upgrade our 500 MHz NMR. We also thank Prof. T. W. Bell for use of the vapor pressure osmometer.

References

- (a) Pimentel, GC.; McClellan, AL. The Hydrogen Bond. W. H. Freeman & Co.; San Francisco: 1960. (b) Joelsten, MD.; Schaad, LJ. Hydrogen Bonding. Marcel Dekker; New York: 1974.
- (a) Falk, H. The Chemistry of Linear Oligopyrroles and Bile Pigments. Springer; Wien: 1989. (b) Lightner, DA. Bilirubin. Heirwegh, KPM.; Brown, SB., editors. Vol. 1. CRC Press; Boca Raton, FL: 1982. p. 1-58.
- (a) Bonnett R, Davies JE, Hursthouse MB, Sheldrick GM. Proc R Soc London Ser B 1978;202:249–268. [PubMed: 28528] (b) LeBas G, Allegret A, Mauguen Y, De Rango C, Bailly M. Acta Crystallogr 1980;B36:3007–3011. (c) Mugnoli A, Manitto P, Monti D. Acta Crystallogr 1983;C39:1287–1291.
- Chowdhury, JR.; Wolkoff, AW.; Chowdhury, NR.; Arias, IM. The Metabolic and Molecular Bases of Inherited Disease. Scriver, CR.; Beaudet, AL.; Sly, WS.; Valle, D., editors. Vol. II. McGraw-Hill Inc; NY: 2001. p. 3063-3101.
- Brodersen R. Bilirubin :75–123. Vol. 1 of ref 1(b)
- (a) Person RV, Peterson BR, Lightner DA. J Am Chem Soc 1994;116:42–59. (b) Nogales D, Lightner DA. J Biol Chem 1995;270:73–77. [PubMed: 7814422] (c) Boiadjev SE, Person RV, Puzicha G, Knobler C, Maverick E, Trueblood KN, Lightner DA. J Am Chem Soc 1992;114:10123–10133. (d) Puzicha G, Pu Y-M, Lightner DA. J Am Chem Soc 1991;113:3583–3592.
- Wash PL, Maverick E, Chiefari J, Lightner DA. J Am Chem Soc 1997;119:3802–3806.
- Huggins MT, Lightner DA. J Org Chem 2000;65:6001–6008. [PubMed: 10987933]
- For a review of dipyrinones, see Boiadjev SE, Lightner DA. Org Prep Proc Intl 2006;38:347–349.
- (a) Chen Q, Lightner DA. J Org Chem 1998;63:2665–2675. [PubMed: 11672135] (b) Tipton AK, Lightner DA. Monatsh Chem 1999;130:425–440. (c) Huggins MT, Lightner DA. Tetrahedron 2000;56:1797–1810.
- (a) Boiadjev SE, Anstine DT, Lightner DA. J Am Chem Soc 1995;117:8727–8736. (b) Boiadjev SE, Holmes DL, Anstine DT, Lightner DA. Tetrahedron 1995;51:10663–10678.
- Nogales DF, Ma J-S, Lightner DA. Tetrahedron 1993;49:2361–2372.
- For δ-valerolactam and γ-butyrolactam, K_D = 277 and 287 M⁻¹ at 25°C in CCl₄ by IR (a) Kulevsky N, Froehlich PM. J Am Chem Soc 1967;89:4839–4841. Lower values are reported (82 and 27 M⁻¹) from VPO studies in benzene (b) Montando G, Caccamuse S, Recca A. J Phys Chem 1975;79:1554–

1554. For 2-pyridone and 2-thiopyridone, $K_D = 7100 \text{ M}^{-1}$ and 4100 M^{-1} in CCl_4 at 25°C by IR (c) Kulevsky N, Reinecke W. *J Phys Chem* 1968;78:3339–3340. For 2-pyridone and 2-thiopyridone in benzene, $K_D = 2200 \text{ M}^{-1}$ and 28 M^{-1} at 37°C by VPO (d) Krackov MH, Lee CM, Mautner HG. *J Am Chem Soc* 1965;87:892–896.
14. For thiopyridone, $K_D = 2.7 \pm 1.0 \times 10^3 \text{ M}^{-1}$ in CHCl_3 at ambient T, and pyridone $K_D = 2.5 \pm 1.0 \times 10^4 \text{ M}^{-1}$ in CHCl_3 at 27°C by VPO Beak P, Covington JB, Smith SG, White JM, Ziegler JM. *J Org Chem* 1980;45:1354–1361.
15. Xie M, Lightner DA. *J Heterocyclic Chem* 1991;28:1753–1756.
16. Lawesson's reagent : 2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide, used as available from Aldrich Chemicals.
17. (a) Grunewald JO, Cullen R, Bredfeldt J, Strope ER. *Org Prep Proc Intl* 1975;7:103–110. (b) Shrout DP, Lightner DA. *Synthesis* 1990:1062–1065.
18. Cullen DL, Pèpe G, Meyer EF Jr, Falk H, Grubmayr K. *J Chem Soc Perkin II* 1979:999–1004.
19. Kaplan D, Navon G. *Israel J Chem* 1983;23:177–186.
20. Huggins MT, Lightner DA. *Monatsh Chem* 2001;132:203–221.
21. McDonagh AF, Agati G, Lightner DA. *Monatsh Chem* 1998;129:649–660.
22. Fischer H, Joshioka T, Hartmann P. Hoppe-Seyler's *Z Physiol Chem* 1932;212:146–156.

Figure 1.

(A) Bilirubin, (B) its intramolecularly hydrogen-bonded dipyrinone analog [6]-semirubin (**1**), and (C) intermolecularly hydrogen-bonded kryptopyrromethenone (**3**).

	X	R
1:	O	H
2:	O	CH ₃
4:	S	H
8:	S	CH ₃

	X	R
3:	O	CH ₂ CH ₃
5:	S	(CH ₂) ₂ CO ₂ CH ₃
6:	S	CH ₂ CH ₃
7:	O	(CH ₂) ₂ CO ₂ CH ₃

Figure 2.

The thiolactam analogs (**4**, **5**, **6**, **8**) of [6]-semirubin (**1**) and its methyl ester (**2**), methyl xanthobilirubinate (**7**) and kryptopyrromethenone (**3**).

Figure 3.

Key NOEs observed for **4** ($R^1 = H$, $R^2 = \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), **8** ($R^1 = H$, $R^2 = \text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{CH}_3$) and **6** ($R^1 = \text{CH}_3$, $R^2 = H$) in CDCl_3 are shown by double-headed arrows. The dashed arrows indicate weak NOEs. No NOEs were observed between the CO_2H and the lactam or pyrrole NH.

Figure 4. Beer's Law Plot for (A) thiosemirubin (**4**) and (B) its methyl ester (**8**) showing deviation from ideality. The actual absorbance measured for the highest concentration = 2.0 (see Experimental). For the contrasting plots of **1**, which obeys Beer's Law, and **2**, which does not, see ref. ⁸.

Figure 5.

(A) Plots of pyrrole and lactam ^1H -NMR chemical shifts vs concentration in CDCl_3 of thiosemirubin (**4**) (A, upper) and methyl thioxanthobilirubin (**5**) (B, upper) and comparison of data from the low concentration of **4** (A, lower) and **8** (B, lower) plotted as NH chemical shift vs log concentration at 25°C and 56°C. Similar plots were found for **6** and **8**.

Scheme 1.

^a *t*BuPh₂SiCl + imidazole/dry THF, RT, 4 days; ^b CH₃OH/H₂SO₄; ^c Lawesson's reagent/dry THF, RT, 36 h; ^d (*n*Bu)₄NF/THF – 5% H₂O, 12 h, RT.

Table 1

¹³C-NMR chemical shifts (δ)^a and assignments of **1-8** in CDCl₃.

		X	R ¹	R ²	R ³				
		1	O	(CH ₂) ₅ CO ₂ H	CH ₃				
		2	O	(CH ₂) ₅ CO ₂ CH ₃	CH ₃				
		3	O	CH ₃	CH ₂ CH ₃				
		4	S	(CH ₂) ₅ CO ₂ H	CH ₃				
		5	S	CH ₃	(CH ₂) ₂ CO ₂ CH ₃				
		6	S	CH ₃	CH ₂ CH ₃				
		7	O	CH ₃	(CH ₂) ₂ CO ₂ CH ₃				
		8	S	(CH ₂) ₅ CO ₂ CH ₃	CH ₃				
Carbon		1	2	3	4	5	6	7 ^b	8
1	C = CX	174.7	173.9	174.1	186.8	186.7	185.9	174.1	186.7
2	=C-	123.6	123.2	122.2	132.2	131.5	131.0	122.4	133.5
2 ¹	CH ₃	8.2	8.6	9.5	11.1	10.4	10.4	9.6	9.6
3	=C-	142.2	142.2	148.2	138.0	146.1	146.0	148.4	140.1
3 ¹	CH ₂ /CH ₃	9.8	9.9	15.4	12.6	18.4	18.4	18.0	11.3
3 ²	CH ₃	—	—	17.5	—	15.1	17.3	15.0	—
4	=C-	128.7	128.3	127.0	131.4	132.3	132.0	127.2	133.2
5	=CH-	101.3	101.2	101.2	103.4	103.9	104.2	101.4	104.0
6	=C-	122.4	122.2	122.9	122.9	129.3	129.8	122.4	124.1
7	=C-	125.5	125.2	122.2	122.9	121.3	123.5	119.1	130.5
7 ¹	CH ₃	9.7	9.7	11.5	12.3	11.9	11.9	11.5	11.0
8	=C-	135.8	135.8	124.6	138.0	123.6	123.5	124.6	140.6
8 ¹	CH ₂ /CH ₃	8.8	9.1	18.0	12.0	19.7	18.0	19.9	10.6
8 ²	CH ₂ /CH ₃	—	—	15.1	—	34.7	15.1	35.2	—
8 ³	CO ₂ R	—	—	—	—	173.5	—	174.1	—
9	=C-	116.7	115.9	131.1	117.0	136.1	136.0	131.6	118.8
9 ¹	CH ₂ /CH ₃	23.0	25.7	8.5	26.1	9.8	9.7	8.5	26.7
9 ^{2-9⁵}	CH ₂	<i>c</i>	<i>d</i>	—	<i>e</i>	—	—	—	<i>f</i>
9 ⁶	CO ₂ H/CO ₂ CH ₃	180.8	174.2	—	178.7	—	—	—	174.2

		X	R ¹	R ²	R ³
1	O	(CH ₂) ₅ CO ₂ H	CH ₃	CH ₃			
	2	O	(CH ₂) ₅ CO ₂ CH ₃	CH ₃	CH ₂ CH ₃			
	3	O	CH ₃	CH ₂ CH ₃	CH ₂ CH ₃			
	4	S	(CH ₂) ₅ CO ₂ H	CH ₃	CH ₃			
	5	S	CH ₃	(CH ₂) ₂ CO ₂ CH ₃	CH ₂ CH ₃			
	6	S	CH ₃	CH ₂ CH ₃	CH ₂ CH ₃			
	7	O	CH ₃	(CH ₂) ₂ CO ₂ CH ₃	CH ₂ CH ₃			
	8	S	(CH ₂) ₅ CO ₂ CH ₃	CH ₃	CH ₃			
Carbon	1	2	3	4	5	6	7 ^b	8
CO ₂ CH ₃	—	51.4	—	—	51.7	—	51.2	52.4

^aIn ppm downfield from (CH₃)₄Si for 2 × 10⁻² M solutions in (CD₃)₂SO at 25°C.

^bValues taken from ref. 15.

^cR¹: 21.4 (9² - CH₂), 26.2 (9³ - CH₂), 28.2 (9⁴ - CH₂), 32.6 (9⁵ - CH₂) ppm.

^dR¹: 24.8 (9² - CH₂), 28.7 (9³ - CH₂), 29.9 (9⁴ - CH₂), 34.0 (9⁵ - CH₂) ppm.

^eR¹: 27.6 (9² - CH₂), 30.3 (9³ - CH₂), 30.9 (9⁴ - CH₂), 35.9 (9⁵ - CH₂) ppm.

^fR¹: 25.1 (9² - CH₂), 29.2 (9³ - CH₂), 30.0 (9⁴ - CH₂), 24.5 (9⁵ - CH₂) ppm.

Table 2

Comparison of the pyrrole, lactam and carboxylic acid ^1H -NMR chemical shifts^a of **1-8** in CDCl_3 and $(\text{CD}_3)_2\text{SO}$.^b

Compound	Lactam NH	Pyrrole NH	CO ₂ H
1	10.42 ^c	8.98 ^c	13.22 ^c
	<i>9.81</i>	<i>10.12</i>	<i>11.95</i>
2	11.17 ^c	10.11 ^c	—
	<i>9.81</i>	<i>10.12</i>	—
3	11.10	10.05	—
	<i>9.83</i>	<i>10.33</i>	—
4	11.05 ^d	9.55 ^d	13.06 ^d
	<i>11.92</i>	<i>10.98</i>	<i>12.62</i>
5	11.47	9.95	—
	<i>11.44</i>	<i>10.94</i>	—
6	11.41 ^e	9.94 ^e	—
	<i>11.43</i>	<i>10.91</i>	—
7	11.15	10.25	—
	<i>9.72</i>	<i>10.26</i>	—
8	11.26 ^f	9.71 ^f	—
	<i>11.59</i>	<i>10.91</i>	—

^a δ , ppm downfield from $(\text{CH}_3)_4\text{Si}$ for 3×10^{-3} M solutions in CDCl_3 and $(\text{CD}_3)_2\text{SO}$ at 25°C.

^b The data from $(\text{CD}_3)_2\text{SO}$ are shown in italics, conc = 4×10^{-2} M.

^c 1×10^{-3} M.

^d 8×10^{-3} M at 3×10^{-2} M, $\delta_{\text{lactam}} = 11.77$ and $\delta_{\text{pyrrole}} = 9.88$ ppm;

^e Conc. 1.33×10^{-2} M; at 3.09×10^{-3} M, $\delta_{\text{lactam}} = 10.27$ and $\delta_{\text{pyrrole}} = 9.06$ ppm.

^f 1×10^{-2} M.

Table 3

Molecular weights of dipyrrothiones and dipyrroinones **1-8** determined by vapor pressure osmometry^a at 45°C in CHCl₃ solution.

Dipyrrothione	Formula Weight (g/mol)	Molecular Weight by VPO (g/mol)	Concentration range (mol/kg)
1	330	337 ±20 ^b	2.1-6.6 × 10 ⁻³
2	344	584 ±30 ^b	1.7-6.1 × 10 ⁻³
3	258	509 ±20 ^c	2.0-12.8 × 10 ⁻³
4	346	689 ± 7	1.0-6.5 × 10 ⁻³
5	384	654 ±20	0.72-2.2 ×10 ⁻³
6	274	535 ±12	4-7 × 10 ⁻³
6	274	416 ±21	4-7 × 10 ⁻⁴
7	316	579 ±25 ^c	1.5-6.5 × 10 ⁻³
8	360	700 ±30	1.6-5.8 × 10 ⁻³

^aCalibrated with benzil (FW = 210, measured MW = 208 ±5).

^bData from ref. 7.

^cData from ref. 15.

Table 4

Extrapolated dimerization association constants (K_D , M) and dimer (δ_D , ppm) and monomer (δ_M , ppm) NH chemical shifts of dipyrinethiones (**4**, **5**, **6**, **8**) at 25°C compared with dipyrinones **3** and **7** and **8** at 25°C.

	3	4	5	6	7	8
K_D^a	22,000	160	315	238	28,600	208
K_D^b	23,000	103	292	198	24,800	162
δ_D^c (HN-C=X)	11.42	11.78	11.78	11.83	11.39	11.52
δ_D^d (pyrrole)	10.44	9.98	10.17	10.21	10.43	10.02
δ_M^c (HN-C=X)	7.75	8.21	8.65	8.65	7.75	8.25
δ_M^d (pyrrole)	8.10	7.61	7.81	7.73	8.10	7.52

^aFrom thiolactam or lactam NH data.

^bFrom pyrrole NH data.

^cThiolactam (X=S) or lactam (X=O) NH NMR chemical shift of monomer (δ_M) and dimer (δ_D).

^dPyrrole NH NMR chemical shift of monomer (δ_M) and dimer (δ_D).

Table 5

Comparison of UV-vis spectral data of **1-8**.^a

Pigment	$\epsilon_{\text{max}} (\lambda_{\text{max}}, \text{nm})$			
	C_6H_6	CHCl_3	CH_3CN	CH_3OH $(\text{CH}_3)_2\text{SO}$
1	27,200 (426)	28,200 (421)	26,600 (411)	31,500 (416) 30,400 (413)
2	33,200 (411)	28,800 (407)	33,600 (413)	33,500 (415) 33,200 (411)
3	36,100 (412)	32,200 (408)	32,000 (406)	39,400 (417) 35,600 (415)
4	28,400 (492) 26,200 (513) ^{sh}	29,100 (495) 27,800 (515) ^{sh}	28,000 (490)	29,400 (490) 28,300 (493)
5	26,200 (484)	32,500 (489)	28,500 (480)	32,000 (481) 31,100 (482)
6	34,800 (484)	32,200 (493)	31,200 (484)	34,500 (485) 33,700 (484)
7	26,500 (412)	34,000 (408)	28,900 (400)	37,700 (411) 34,600 (410)
8	38,500 (487)	38,300 (492)	42,350 (485)	46,000 (488) 42,600 (494)

^aSolutions were $\sim 1 \times 10^{-5}$ M at 22°C; ϵ in $\text{L} \cdot \text{mol} \cdot \text{cm}^{-1}$.